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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry in the Graduate School of Duke University

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ABSTRACT


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Abstract

Modern patterning and fabrication techniques provide powerful opportunities for the preparation of micro- and nanostructured objects with applications in fields ranging from drug delivery and bioimaging to organic based electronic devices and real time biochemical sensors. In this thesis we report a systematic study focused on the development of new unconventional patterning and fabrication techniques with applications in the preparation of functional micro- and nanostructured devices.

Catalytic microcontact printing is a powerful technique that offers a simple and effective methodology for patterning chemically-functionalized surfaces with sub-100 nm accuracy. By avoiding diffusive mechanisms of pattern replication it effectively obviates the most significant limitation of traditional microcontact printing – lateral molecular ink diffusion. Moreover, catalytic microcontact printing significantly expands the diversity of patternable surfaces by using prefunctionalized substrates and gives rapid facile access to chemically discriminated surfaces that can be further functionalized with organic and biological molecules. We have developed several catalytic microcontact printing techniques that transfer pattern from an elastomeric stamp bearing an immobilized catalyst to a preformed functionalized self-assembled monolayer. By avoiding diffusive pattern transfer we were able to replicate features with sub-50 nm edge resolution. We also demonstrated that catalytic printing can be expanded to technologically important substrates not accessible through conventional soft lithography, by patterning reactive organic monolayers grafted to chemically passivated silicon.
The non-symmetric structure of Janus particles produces novel physical properties and unusual aggregation behavior that makes these materials attractive candidates for drug delivery and as nano-sensors and nano-probes, SERS and PEF imaging agents, small molecules carriers, and switchable devices. We have developed a new protocol for preparation of non-spherical inorganic Janus particles comprising metallic and semiconductor layers. The method allows for precise control over the composition, shape and size and permits fabrication of non-symmetrical particles, the opposite sides of which can be orthogonally functionalized using well-established organosilane and thiol chemistries.
Dedicated to my family.
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List of Abbreviations

µCP – microcontact printing
PUA – polyurethane acrylate
PDMS – polydimethylsiloxane
h-PDMS – hard polydimethylsiloxane
SAM – self-assembled monolayer
SERS – surface enhanced Raman spectroscopy
PEF – plasmon enhanced fluorescence
Fmoc – 9H-fluoren-9-ylmethoxycarbonyl
Boc – tert-butoxycarbonyl
TBS – tert-butyl dimethyl silyl
TMS – trimethyl silyl
NHS – N-hydroxy-succinimide
GFP – green fluorescent protein
XPS – X-ray photoelectron spectroscopy
AFM – atomic force spectroscopy
LFM – lateral force spectroscopy
DDT – 1-dodecanethiol
SEM – scanning electron microscopy
IC – integrated circuit
UV – ultra violet
m.p. – melting point
DNA – deoxyribonucleic acid

ss-DNA – single stranded deoxyribonucleic acid

ds-DNA – double stranded deoxyribonucleic acid

PEG – polyethylene glycol

Exo – exonuclease

TFA – trifluoroacetic acid

TFT – thin-film transistor

DBU – 1,8-diazabicyclo[5.4.0]undec-7-en

Rq – root mean square roughness

Ra – arithmetic average roughness

Rmax – maximum roughness depth

NTA – nitrilotriacetic acid

DMF – dimethylformamide

NMR – nuclear magnetic resonance

IPA – isopropyl alcohol

MIBK – methyl isobutyl ketone

THF – tetrahydrofuran

DMAP – 4-dimethylaminopyridine

PTFE – polytetrafluoroethylene

E-beam – electron beam
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1. Introduction

1.1 Overview

Throughout history changes in the nature of human society have always been linked to the development of new patterning techniques. The oldest preserved human-made paintings, which were discovered in the Chauvet-Pont-d'Arc Cave in southern France, date to the Aurignacian culture that existed more than 32,000 years ago. Although the exact purpose of cave paintings is still a subject of debate, it is generally accepted that they were not merely accidental or decorative elements of habited areas, but were the first symbols that served an important communication and/or ceremonial purpose. 9,000 years ago during the Neolithic period cave drawings gradually evolved into early proto-writing systems, which during the Bronze Age transformed into true writing that has served us since then as a mean for recording and storing information. The invention of phonetic writing marks the dawn of the first technological revolution that completely changed the face of human history.

However, copying scholarly texts by hands in order to preserve and pass information was a tedious and time-consuming process, which significantly hindered the spread of knowledge. Only much later, around 220 in China, woodblock printing techniques were developed for printing text, images, and patterns on textiles. And even before that, around 3000 BC, Mesopotamians used cylinder seals as the first parallel patterning tools to impress and transfer images on clay tablets. Eventually the development of a printing press by Johannes Gutenberg in 1454 and lithography by
Aloys Senefelder in 1796\textsuperscript{4} gave way to modern printing techniques that are used today to produce all high-volume texts worldwide. The invention of the printing press completely changed our ways of preserving, storing, and spreading information. It led to increased literacy among people and helped establish scientific communities that could easily communicate their discoveries through scientific journals. For the first time in history knowledge became available to many individuals, helping to bring the second scientific revolution.

The invention of the first field effect transistor in 1925 by Julius Edgar Lilienfeld\textsuperscript{5} and later work of William Shockley on semiconductor materials brought to life one of the greatest inventions of the twentieth century: the semiconductor transistor. By offering many advantages over the vacuum (electron) tube, such as smaller size, lower operation voltage, greater energy efficiency, and much higher reliability, transistors quickly replaced tubes as key active components in all modern electronics. However, in the middle of the last century, with the rapid increase in size and complexity of electrical circuits, scientist and engineers were faced with the problem of making increasingly complicated circuits to carry out more complex functions. Since all electrical components at that time had to be interconnected manually in a slow serial process, the problem of making more complex electronics was almost impossible to overcome without new means to pattern materials onto surfaces in a parallel fashion. The first integrated circuit – a miniaturized electronic device manufactured on the surface of a semiconductor material – was developed by Jack Kilby and Robert Noyce\textsuperscript{6} in the late 50s. By depositing, patterning, and etching different materials onto a single
semiconductor surface, all components of electronic devices could be constructed and interconnected concurrently. The simultaneous integration of multiple transistors into a single chip was a tremendous step forward over the slow manual assembly of individual electronic components. With significantly reduced costs and increased performance, integrated circuits completely replaced discrete circuits and formed the basis for the continuous miniaturization of electronic devices.

In many ways all advances in modern electronic technologies are tightly connected to developments in photolithography, which remains the patterning method of choice for production of all ICs on the planet. Photolithography dramatically decreases the cost of electronic components by simultaneously patterning millions of objects on a single semiconductor substrate. Although throughout the past four decades photolithography has been tremendously successful in increasing the number of transistors in a single chip from 2,300 in 1971 to 781,000,000 in 2008 while simultaneously reducing the size of an individual transistor from 10 µm to 45 nm, its resolution is intrinsically limited by optical diffraction, which significantly increases the development and production cost of every new photolithographic system. Despite the prediction that photolithography will remain as the primary patterning tool in state-of-the-art IC technologies, it currently remains too expensive and incompatible with too many materials to be used in such growing fields as (bio)organic sensing, hybrid organic-semiconductor interfaces, and bioelectronic systems, all of which require patterned integration of ordered molecular systems with conventional inorganic semiconductor materials.
Microcontact printing (µCP) was first introduced by Whitesides and coworkers in 1993 as a new flexographic technique for patterning self-assembled monolayers (SAMs) on metal surfaces.\(^7\) It represents the prototypical embodiment of a family of related patterning techniques, collectively termed soft lithography,\(^8\) that make use of conformal contact between a substrate and a flexible stamp as a means of pattern reproduction. One of the original goals of µCP was to reproduce objects similar to ones generated by photolithography using much faster and less expensive methods.\(^7, 9-13\) For example, by using patterned SAMs on gold as a mask in alkaline cyanide wet etching, it was demonstrated that this novel soft lithographic method can be used to pattern metal objects on semiconductor surfaces.\(^7, 13\) Because µCP does not depend on light to generate patterns, its resolution is not limited by optical diffraction. This important property of µCP created the perception that it could potentially replace photolithography in IC production. However, due to recent advances in photolithography and for reasons which will be discussed bellow it soon became apparent that µCP cannot achieve the same level of resolution and integration with existing IC technologies as conventional photolithography in the foreseeable feature. Nonetheless, the value of µCP in patterning surfaces for other applications quickly became apparent. During the past 20 years µCP has been successfully utilized to pattern small organic compounds, inorganic substances, biomolecules, and even living cells on a variety of hard materials.\(^14-15\) Here we review the operating principals of traditional µCP, discuss its potential applications and limitations, and provide an overview of novel techniques that successfully avoid the major limitations of traditional µCP.
### 1.2 Principles of microcontact printing

Traditional microcontact printing is a remarkably simple protocol that allows patterning of various substrates with SAMs. It is reasonably flexible with regard to the number and type of SAM-substrate systems, and it is often used to create patterned surfaces terminated with different chemical functionalities. In traditional μCP an inflexible master is first used as a template to make an elastomeric stamp with an inverted pattern. In this step, a prepolymer is poured onto the patterned master, where it is polymerized at elevated temperatures or under UV light to make a solid but elastic stamp (Figure 1).

![Figure 1. Traditional microcontact printing](image)

Figure 1. Traditional microcontact printing

After polymerization the stamp is peeled off the master, cut to the appropriate size, and inked with a solution of molecular ink. Subsequently, the stamp is dried and applied to a corresponding substrate. During stamping the molecular inks migrate from the stamp to the substrate and form SAMs in the areas of conformal stamp-substrate contact. The voids on the stamp surface serve as transport barriers and prevent SAM
formation in non-contacted areas. The stamping yields a substrate that bears a pattern of SAMs cognate to the features on the corresponding master. After printing, the stamp can be washed to remove remaining molecular inks and then applied again to pattern another substrate with the same or different ink.\textsuperscript{7, 10-11}

\textbf{Figure 2. Arrangement of decanthiolates on gold (111) lattice.}

The most common SAM-substrate system in traditional µCP is monolayers of thiolates on gold.\textsuperscript{14} Gold binds thiols with high affinity\textsuperscript{16} without undergoing any unusual reactions. Long-chain aliphatic thiols form ordered and stable close-packed SAMs on gold that can withstand relatively harsh reactive environments and elevated temperatures (Figure 2).\textsuperscript{17} The high affinity of thiols for gold also helps to displace adventitious physisorbed materials from the surface, allowing formation of SAMs on contaminated substrates. Moreover, because thiolated SAMs can serve as masks during cyanide-based wet etching of gold,\textsuperscript{9} the unprotected areas on patterned gold substrates can be etched away, while the areas covered with SAMs remain intact.
An elastomeric stamp with patterned relief structures is the key element of all soft-lithographic techniques, including µCP. The majority of stamps used in µCP are made of polydimethylsiloxane (PDMS), which is one of the most popular materials in all soft lithographic techniques. PDMS is often used in nanoimprint lithography owning to its low surface energy and high elasticity. PDMS molds are chemically inert, easily form spontaneous conformal contact with most substrates, and can be nondestructively removed from molded structures. Liquid PDMS prepolymer adheres with very high fidelity to patterned masters, while polymerized PDMS can support very small (~5 nm) features. The resolution in such molds is strongly influenced by the density of the cross-links in the polymer (Figure 3).

Microcontact printing is an inherently parallel process that can be used to pattern large substrates in a single stamping step. It is inexpensive and flexible, and
does not rely on energy-consuming or complicated instruments. However, traditional µCP suffers from a set of limitations that preclude accurate replication of features with sub-300 nm dimensions, and even after 15 years traditional µCP still cannot be utilized to directly pattern many technologically important substrates.

1.2 Limitation of microcontact printing

Immediately following its invention, due to low cost and simplicity, µCP inspired interest in patterning a broader range of substrates with small features. However, despite the fact that traditional µCP was already routinely used to create patterns of microscopic objects, limitations associated with ink diffusion and stamp deformation constrained its use for the replication of sub-300 nm features. Furthermore, the vast majority of all known µCP techniques were limited to less technologically relevant metal and oxide surfaces, and could not be easily applied to substrates such as silicon or germanium, which do not react readily with organic materials. These limitations precluded further investigation of µCP as a potential alternative for photolithography and restricted its implementation in other areas of material science that utilize oxide-free inorganic semiconductors.

1.2.1 Deformations in elastomeric stamps

PDMS elastomers have a number of properties that make them very attractive materials for traditional µCP. PDMS stamps can form spontaneous conformal contact with large substrate, they are homogenous and transparent, they have low surface energy (21.6×10⁻³ J m⁻²) and are chemically inert. However, PDMS has relatively poor
elasto-mechanical properties, which form the basis of the serious technical problems that limit its application in the replication of complex patterns containing multiple features with different morphologies.

The elastic deformations in stamp materials distort edges of the printed features and limit the resolution of µCP. Therefore, in order to accurately reproduce complex sub-micron patterns, several factors must be considered when choosing an elastomer. First, due to the elevated stress caused by gravity, adhesion, and capillary forces the features in elastomeric stamps often collapse to each other (Figure 4).\textsuperscript{27} The pairing of features can be prevented either by using more rigid stamp materials or by reducing the aspect ratio of the features. Although the elasticity of PDMS can be fairly easy controlled by polymerization conditions, very rigid PDMS molds tend to have poor mechanical properties and are often too brittle to be applied over large substrates.\textsuperscript{34-35} Therefore, the aspect-ratio of the relief structures in elastic PDMS mold should be smaller than 2 in order to produce defect-free stamps.\textsuperscript{27} Second, stamps with low aspect ratio features and with widely separated features can sag and adhere to the substrates under their own weight or under the compressive forces applied during the printing. For PDMS, it was demonstrated that the aspect ratio of the features should be greater than 0.2 and that the feature separation \(d\) should be smaller than \(20h\) in order to prevent stamps from sagging (Figure 4).\textsuperscript{21} Third, it is important for the stamps in µCP not to contaminate the substrates with the polymer materials and to maintain their morphological properties when exposed to chemicals (organic solvent, aqueous solution, molecular ink solutions) or harsh environments (oxygen plasma, UV irradiation, vacuum).
Unfortunately, PDMS molds swell in most organic solvent\textsuperscript{33, 36-38} and tend to leave low-molecular-weight contaminants on the surfaces of substrates.\textsuperscript{39-40}

\textbf{Figure 4. Deformations in elastomeric stamps}

Moreover, due to the intrinsic hydrophobic nature of PDMS, it can only be inked with non-polar molecules, restricting its application in patterning biological molecules and inorganic complexes.\textsuperscript{15} In general, the mechanical and physical properties of the PDMS elastomer permit its routine application in patterning hydrophobic molecular inks with micrometer resolution; however, the accurate replication of sub-500 nm features normally requires careful consideration in pattern design and material selection.
1.2.2 Molecular ink diffusion

In traditional µCP ordered SAMs are formed as the result of ink diffusion from the elastomeric stamp to the substrate in areas of conformal contact.\textsuperscript{41-42} However, lateral diffusion of the molecules along the substrate surface often causes the spreading of molecular inks to non-contacted areas.\textsuperscript{22, 24} This spreading results in the enlargement of the patterned features and distortion of their edges. Lateral ink diffusion significantly limits the resolution of traditional µCP and precludes accurate replication of sub-micrometer features. The extent of lateral diffusion depends on the printing time and ink volume on the stamp. Many elastomeric stamp materials (including PDMS) act as sponges and can take up large volumes of liquids into their bodies during the inking step.\textsuperscript{22} Although, the stamps are dried before printing, they can still retain large quantities of ink in their capillary structures, providing a continuous supply of diffusive molecules. It was demonstrated that during traditional µCP with alkanethiols on gold ink diffusion is responsible for at least 50 nm of enlargement at the feature edges independent of the stamping time or ink concentration; the same study showed that eicosanethiol, which is a solid at room temperature, is capable of forming ordered defect-free SAMs only with 150 nm enlargement of the features.\textsuperscript{22} The large volume of molecular ink in the stamp was suggested as the main reason for inaccurate feature replication, suggesting that ink diffusion can be limited either by using less porous stamp materials or by modifying inking methods.

Another mechanism for transport of molecules to non-contacted regions of the substrate is gas diffusion either mediated by the surface or through the ambient
between the stamp and the substrate. The extent of gas diffusion is proportional to the vapor pressure of molecular inks and their reactivity with the surface; however, it was demonstrated that even hexadecanethiol (m.p. 18 °C) and eicosanethiol (m.p. 46 °C) both diffuse via the gas phase when used in traditional μCP. Another possible mechanism of ink spreading is diffusion of ink molecules along the surface from their initial attachment points. However, due to the relatively strong Au-S bond this mechanism does not play important role in traditional μCP.

**Figure 5. Ink diffusion in traditional μCP**

Figure 5 summarizes possible mechanisms of ink spreading in traditional μCP, specifically lateral diffusion (pathways 4,1,2), gas diffusion (pathway 2), and diffusion of bound molecules (pathway 3). Overall, several investigations have determined that μCP with alkanethiols on gold can be used to accurately replicate features as small as 250 nm; however, this resolution was feasible with only a limited number of molecular inks and only after careful optimization of all printing conditions such as stamping time,
inking method and thiol concentration.\textsuperscript{21-26} Features below 250 nm can also be replicated, but to date their shape and size are always distorted from the features on the corresponding stamp. It was also demonstrated that in almost any cases diffusion is responsible for at least 50 nm of edge distortion,\textsuperscript{22} suggesting that in routine applications traditional µC can only be used for replication of micrometer-size features.

1.2.3 Limitations of stamp-substrate systems

Traditional µCP is restricted to surfaces that undergo rapid irreversible reactions with molecular inks. Therefore, it is largely restricted to thiol/metal and silane/oxide systems, and cannot pattern many important hard substrates, such as polycrystalline silicon or germanium.\textsuperscript{8, 14} The formation of monolayers on such surfaces usually requires prolonged reaction times and harsh reacting conditions (high temperature, inert atmosphere, UV irradiation), which are incompatible with traditional stamp materials and µCP conditions. Although during the past 20 years µCP has been successfully adapted to pattern objects such as inorganic metals, organic molecules and polymers, proteins, DNAs, and even living cells, the variety of patternable substrates has not significantly expanded beyond a narrow range of metals, oxides, and plastics.

1.3 Unconventional microcontact printing

To overcome the diffusive and deformation limitations of traditional µCP a number of alternative techniques have been developed, which either modify the stamp materials and printing conditions to limit ink diffusion and avoid elastomer deformations or use entirely different patterning approaches that do not rely on ink
self-assembly. Furthermore, significant attention has been giving to broadening the variety of available µCP substrates and molecular inks in order to expand the utility of µCP in biological, biochemical and bioengineering fields.

### 1.3.1 Avoiding stamp deformations

In order to accurately replicate sub-micrometer features stamp materials should be relatively rigid. At the same time, stamps must be elastic in order to conform to the substrates and form nanoscale contacts in all areas of pattern transfer despite substrate roughness. These two contradicting conditions require a careful balance between elasticity and stiffness in the stamp polymers that are used in µCP. In addition, the mechano-elastic properties of the stamps must be also considered, as the mechanical stability of many polymers significantly degrades with increasing in stiffness and degree of cross-linkage.

#### 1.3.1.1 Positive microcontact printing

As previously mentioned, the replication of patterns from stamps bearing separated high-aspect-ratio features by traditional µCP is problematic due to poor the mechanical stability of common stamp materials. Nonetheless, in cases when µCP is used to pattern etch-resistant SAMs on gold, at least some of these problems can be alleviated by using positive microcontact printing, which makes use of stamps with mechanically more stable inverted patterns. Instead of directly printing stable etch-resistant SAMs on gold, this method first prints monolayers, which provides only minor gold protection (e.g. a polydisperse mixture of mercaptoundecylocta(ethylene glycol)),
and then backfills noncontacted areas with molecules that form ordered protective monolayers (e.g. octadecanethiol). A subsequent wet etching step dissolves gold areas covered by printed SAMs but does not etch regions covered with octadecanethiol (Figure 6).\textsuperscript{43-44}

\textbf{Figure 6. Traditional (left) and positive (right) microcontact printing}

Although this technique obviates at least some of the deformation problems of traditional µCP; its resolution is still limited by ink diffusion and by the molecular exchange between printed and backfilled SAMs, which is particularly fast near the pattern edges where the number of SAM defects is the greatest.\textsuperscript{17}

\textbf{1.3.1.2 Composite stamps with rigid back support}

To improve the stability of PDMS stamps in general and to reduce sagging in particular new bicomponent stamps have been developed, in which a thin PDMS layer is attached to a rigid polymeric or inorganic support.\textsuperscript{45-47} Such bicomponent stamps can be used to pattern arrays of proteins in which individual 20 µm features were isolated from
each other, a task considered impossible with normal PDMS stamps.\textsuperscript{47} In later studies ultra-thin PDMS stamps (thickness below 1 µm) supported on glass and silicon substrates were used to accurately replicate sub-micrometer features of alkanethiols on gold. It was shown that these stamps can be used in multilevel lithography with alignment and that they have significantly enhanced mechanical properties compared to conventional PDMS stamps.\textsuperscript{45-46} Unfortunately, such composite PDMS stamps did not overcome deformation problems associated with buckling and collapsing of high-aspect-ratio features, which are highly desirable in traditional µCP for avoiding gas diffusion of molecular inks from the voids between features.

1.3.1.3 Hard PDMS stamps

The most common PDMS stamp formulation cannot successfully support high-aspect-ratio structures on size scales below 500 nm due to its low elastic modulus and high compressibility factor. In order to increase resolution in traditional µCP a new hard PDMS (h-PDMS) was developed which, due to its high modulus (\textasciitilde 9 N/mm$^2$), is capable of supporting patterns with \textasciitilde 100 nm features.\textsuperscript{34} This polymeric composite is based on vinyl and hydrosilane end-linked polymers and can replicate high-density features at the 100 nm scale. However, in this study ink diffusion was identified as the primary cause of feature distortion. h-PDMS stamps were also brittle and hard to handle, demonstrating a large number of cracks on their surfaces and requiring additional pressure to achieve conformal contact. These problems resulted in printing defects and long-range non-uniform distortion of the patterns. To improve the utility of h-PDMS a composite PDMS / h-PDMS stamp was developed.\textsuperscript{35} This stamp was able to support small dense high-
aspect ratio features in its thin rigid h-PDMS layer (30–40 µm), which was attached to a thick and elastic PDMS slab (~3 mm). These stamps were to handle than h-PDMS stamps and released from the corresponding masters without difficulty. It was shown that such stamps can support 50 nm features and can be used in phase-shifting photolithography. One of the drawbacks of h-PDMS stamps was thermal shrinking upon cooling resulting in the creation of the residual stress in the stamp structure. To overcome the shrinking problem and increase elongation at break of the h-PDMS material, a UV-curable h-PDMS was developed. This new material showed lower shrinkage than h-PDMS and was able to support and replicates dense sub-micron lines in traditional μCP.48

1.3.1.4 Chemically patterned flat stamps

Nearly all problems associated with stamp deformations are a direct consequence of the inclusion of voids in the topographically patterned stamp, which are necessary as transport barriers for molecular inks. By using chemically patterned flat stamps almost all deformation problems of the PDMS-based techniques can be eliminated. Flat chemically patterned stamps completely avoid such problems as stamp sagging and buckling and pairing of features, and also prevent gas diffusion of ink molecules from the areas between the features. However, μCP with flat stamps creates a new challenge of chemically modifying stamp surfaces in a pattern-specific manner.

The first example of μCP with the stamps relied on forming a chemical pattern on a featureless PDMS surface by contact inking with a patterned inker pad (Figure 7). The accuracy of the developed method was largely defined by the stability of the
pattern on the inked flat stamp. As a result, the protocol was only useful for patterning non-diffusive molecules such as Pd complexes and proteins. Highly diffusive alkanethiols were too labile to remain confined to the inked regions of the stamps. The technique did however, simplify stamp fabrication and was able to replicate protein micropatterns with high fidelity.\textsuperscript{49}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.png}
\caption{\textit{\mu}CP with chemically patterned flat stamp}
\end{figure}

In similar study a flat PDMS stamp was chemically patterned using Dip-Pen Nanolithography. The technique demonstrated successful replication of small (sub-500 nm) separated features of alkanethiols, gold nanoparticles, and antibodies. The main drawback of this method was the prerequisite of a slow serial patterning technique for an every new stamp application.\textsuperscript{50}

To stabilize ink patterns on the surfaces of flat stamps another approach to PDMS functionalization was developed. It was shown that flat PDMS can be patterned by oxidation with an oxygen plasma through a mask followed by chemical stabilization of oxidized regions with silanes. The silane molecules form densely packed SAMs on oxidized PDMS providing an effective ink barrier.\textsuperscript{51-53} Using this approach, the hydrophobic properties of the oxidized areas can be easily controlled by choosing hydrophobic or hydrophilic silanes, providing a flexible pattern-specific inking method.
for flat stamps in which silanized areas serve as ink barriers to either hydrophilic or hydrophobic molecules (Figure 8).

**Figure 8. Patterned functionalization of flat stamps with organosilanes**

This method was used to pattern a variety of materials including alkanethiols, proteins, and polar Pd/Sn colloids on glass and gold surfaces; however, only features large than 500 nm were attempted. This technique completely avoids µCP problems associated with deformation of elastic materials and also prevents gas diffusion of the ink molecules from the voids between the features, however it does not prevent the lateral spreading of the molecular inks, and also introduces new potential problems associated with ink diffusion on the stamp surfaces.

A similar strategy was used to prepare bifunctional, chemically patterned flat stamps. By simultaneously patterning flat stamps with hydrophilic and hydrophobic SAMs, it was possible to prepare functionalized PDMS stamps capable of transferring
either hydrophilic or hydrophobic molecules without losing their physical integrity over multiple applications and extended periods of time. Moreover, it was suggested that such a bifunctional design might eliminate ink diffusion due to stronger interactions between transferring inks and SAMs on the stamp and prevent stamps from overloading by absorbing only a monolayer of inks on the stamp surface.\textsuperscript{53}

A significant drawback of the described PDMS patterning techniques is the use of shadowmasks to define chemical patterns on stamp surfaces. Commercially available shadowmasks usually have features greater than 350 nm. Moreover, they require conformal contact between the stamp and the mask during oxidation or silane deposition, which is hard to maintain with features smaller than 500 nm. These limitations were eliminated by using nanoimprint lithography to define chemical patterns on flat PDMS stamps.\textsuperscript{54} In this technique a perfluorinated silane is deposited on the stamp surface using a polymer mask created on the flat stamp with nanoimprint lithography. After the removal of a residual nanoimprint resist, the silanized areas serve as efficient ink barriers during replication of small (sub-100 nm) gold lines, which were manufactured by patterning etch-resistant SAMs and wet etching unprotected gold regions. Although the patterned gold features were approximately 1.7 times bigger than the corresponding stamp objects, it was suggested that the increase in line width could be attributed to the wet-etching of gold and not to the ink spreading.

A very simple recently developed inking and printing method permits sub-100 nm patterning of proteins on silicon. The approach replicates features by transferring a
pattern of proteins from a nanotemplate to a substrate using a flat stamp as a transfer vehicle (Figure 9).

**Figure 9. Subtraction of proteins from the flat stamp using a nanotemplate.**

In this method a planar PDMS stamp is inked with a protein solution, after which absorbed proteins are subtracted from the stamp using a silicon nanotemplate with a surface energy higher than PDMS. Subsequently, the remaining protein pattern is transferred from the stamp onto a flat silicon surface. This method allows accurate replication of small protein patterns on silicon, requiring a nanotemplate as the only key element of the pattern reproduction.\(^5^5\)

Although PDMS can be functionalized via plasma oxidation and silanization, this procedure only modifies the elastomer surface and does not permit pattern-specific incorporation of desired chemical functionalities into the elastomer body. The first example of such functionalization relied on self-assembly to selectively modify flat PDMS through minimization of interfacial free energy.\(^5^6\) In this approach patterning of the PDMS surface was achieved by self-assembly of functional molecules in an
unpolymerized PDMS precursor near the polymer-substrate interface by mirroring the distribution of surface energy in the patterned SAMs on a substrate.

![Diagram of chemical patterned PDMS via self-assembly of precursor molecules]

**Figure 10. Chemically patterned PDMS via self-assembly of precursor molecules**

As such, SAMs of alkanethiols on gold were prepared to serve as a flat chemical master. Subsequently, a mixture of PDMS and alkenes with different functional groups was applied against the SAM master and polymerized. The alkene molecules preferentially accumulated near the SAM areas with complementary surface energies to minimize unfavorable interfacial interactions. During polymerization the PDMS cross-linking agent reacted with alkenes, “freezing” the chemical pattern into the PDMS structure and producing a flat stamp with a chemically patterned surface (Figure 10). The chemical patterning of the PDMS elastomer was confirmed by introducing alkenes with functional groups capable of surface-initiated polymerization, and by growing polymer brushes on the elastomer in a pattern-specific manner.
1.3.1.5 Other elastomeric materials

To improve the resolution of traditional μCP materials with elasto-mechanical properties superior to PDMS have been developed. These new materials offer such advantages as a decrease polymer impurities, better control over the total amount of molecular inks on the stamp surface and its capillary network, tunable surface energies and improved long-term stability. For example, the higher stiffness of thermoplastic block-copolymers such as poly(styrene-\textit{block}-butadiene-\textit{block}-styrene) and poly(styrene-\textit{block}-ethylene-co-butylene-\textit{block} styrene) have been exploited in traditional μCP of thiols on gold. Special attention in this study was given to replication of widely separated low-aspect-ratio features. It was demonstrated that both polymers can successfully replicate patterns of thiols on gold. Both elastomers possess a high modulus and toughness in comparison to PDMS, decreasing stamp deformation during printing and permitting replication of features with extremely low aspect ratios.\textsuperscript{57} In a subsequent study a multiblock copolymer comprising poly(tetramethylene glycol) and poly(butylene terephthalate) fragments was used to prepare hydrophilic stamps, which could be inked with polar molecules. The printed hydrophilic SAMs had well-defined features, which were more resistant towards etching, than SAMs printed with oxidized PDMS.\textsuperscript{58}

To improve the hydrophilic properties of μCP stamps and to make them compatible with biological molecules, a relatively rigid self-supporting hydrophilic hydrogel stamp was prepared by cross-linking high density acrylate monomers with PEG diacrylates in the presence of water or buffer. It was demonstrated that the water-
containing hydrogel stamps permit replication of active biological molecules by keeping them in an aqueous environment through the entire inking–stamping process.\textsuperscript{59}

A polyurethane acrylate UV-curable mold material was recently developed and used in replica molding and microcontact printing. It was demonstrated that this new material can successfully replicate SAMs of alkanethiols on gold with 250 nm resolution. The mechanical properties of the polymer could be tuned by varying the ratio of high- and low-molecular weight acrylates. Because the developed material was UV-curable and amenable to polymerization at room temperature, it did not show any pattern shrinkage as opposed to almost all PDMS-based protocols.\textsuperscript{60}

\subsection*{1.3.2 Avoiding ink diffusion}

\subsubsection*{1.3.2.1 Contact inking of stamps}

In contact inking \(\mu\)CP a flat PDMS substrate impregnated with molecular inks serves as an inking pad for transferring molecules onto a patterned stamp. During the inking step molecules migrate from the flat pad to the protruding areas on the stamp leaving voids between the features free of molecular inks. In the subsequent printing step the inked stamp is used to transfer molecules from the surfaces of the protruding features onto a substrate.\textsuperscript{23} Because the stamp does not contain inks in the areas between the features gas diffusion is almost completely eliminated. However, both the ink concentration on the inking pad and stamping time significantly affect the quality and morphology of the printed features, suggesting that lateral ink spreading still plays a significant role in this variation of traditional \(\mu\)CP.
1.3.2.2 µCP with low-diffusion inks

One of the most effective ways to limit diffusion and increase the resolution of µCP is to use printing compounds with low diffusion coefficients. For example, by using inks with high molecular weights or molecules that can form multiple attachment points on a substrate it is possible to significantly reduce the rate of lateral spreading of ink molecules. However, high molecular weight compounds and molecules that form several bonds with the substrate do not usually form ordered tightly-packed monolayers that can protect underlying metals from etching or oxidation.61-62

Nonetheless, the low diffusion ink approach can be used to pattern ordered SAMs of thiols on gold if combined with previously described positive µCP techniques.43 In this combined method low diffusion inks are printed first onto a gold substrate. Because of the suppressed ink spreading, the first stamping step can achieve sub-micrometer resolution, but does not form tightly-packed SAMs. In the subsequent step the entire substrate is submerged into a solution of another alkanethiol that form stable ordered monolayers. These monolayers are formed in the areas between the printed features ideally achieving the same resolution as the first printing step. Finally, because of their disordered nature, the initial printed SAMs can be selectively disorbed from the substrate either at elevated temperatures or electrochemically.63-67 It was demonstrated that thiolated PEGs can be used as heavy-weight inks in positive µCP, however this approach did not achieve high resolution because of the relatively fast lateral spreading of PEG molecules.44 In contrast, poly(propylene imine) dendrimers with thioether end groups have been used as excellent non-diffusive inks for positive µCP. Even at high
contact times these materials showed almost no surface spreading bringing the potential resolution of positive µCP close to a 100 nm region.\textsuperscript{68}

### 1.3.2.3 Decal transfer and nanotransfer lithographies

By substituting liquid molecular inks on solid polymers or inorganic metals it is possible to completely avoid all diffusive limitations of traditional µCP. Decal transfer lithography allows patterning of silicon, glass, quartz, and silicon oxide substrates with polymeric resists.\textsuperscript{69-70} This technique is capable of simultaneously patterning submicron polymeric features with high accuracy over large substrate areas. The printed features on silicon oxide can then be used as masks in dry or wet etching, making this protocol compatible with modern IC technologies.

In the simplest variation of decal transfer lithography a PDMS stamp is first oxidized using UV/ozone and then reacted with the oxidized inorganic substrate at 70 °C for 20 minutes. During the reaction the strength of interfacial bonds between the oxidized PDMS and the substrate gradually increase, making their attachment irreversible. Subsequently, the elastomer pattern is transferred by peeling off the PDMS pad and breaking of the substrate-bound features from the bulk PDMS material (Figure 11).\textsuperscript{70} Although this method is simple, it works well only for the patterns with small features. Moreover, the physical breakage of features from the PDMS stamp contaminates the substrate surface and the approach requires a new PDMS stamp for each application.
To obviate the design and mechanical limitations of these methods, a slightly different approach was developed. In selective pattern release decal transfer lithography an additional interface is engineered between the patterned features and the supporting PDMS pad. This interface has a lower surface energy than the feature-substrate interface and, therefore, it allows controlled release and deposition of the polymeric features on a variety of inorganic substrates. The features in this method are not restricted by the design requirements and can have a broad range of precisely designed morphologies.\(^\text{70}\)
In the selective pattern release method a PDMS pre-polymer is first spin-casted on a patterned silicon master just below the height of the features on the master and then oxidized with UV/ozone. The oxidized PDMS features are immediately treated with perfluorinted silanes to reduce the surface energy of their exposed facets. Subsequently, another layer of PDMS is deposited on top of the features and cured at elevated temperature. The composite PDMS replica is then extracted from the master, oxidized with UV/ozone and reacted with the inorganic substrate at elevated temperature. After the reaction, the bulk PDMS pad can be easily removed from the substrate leaving an accurate pattern of PDMS decals (Figure 12). Although the protocol requires the manufacture of a new PDMS replica for an every single application, it permits very accurate replication of resist materials on a variety of inorganic substrates with submicron resolution. These properties make decal transfer lithography potentially
useful in microfabrication processes, especially in combination with other traditional micromachining techniques. For example, it was demonstrated that patterned PDMS decals can be used as masks to prepare arrays of silicon dots by dry etching with SF$_6$ and as metal lift-off layers to pattern gold objects on silicon substrates.

**Figure 13. Formation of the closed patterns using decal transfer lithography**

The described protocol was also used to prepare closed PDMS patterns on silicon, which were used as membrane-sealed microfluidic reactor systems. As such, sealed microfluidic PDMS channels were prepared on a silicon substrate, using a modified methodology for selective pattern release lithography (Figure 13). Subsequently, the channel system was filled with a solution of chloroplatinic acid. Exposure of the system to a hydrogen atmosphere triggered the deposition of a thin platinum film selectively on the bottom of the silicon channels, living PDMS and PDMS-
contacted silicon areas unmodified. In general, decal transfer lithography combines many of the attractive features of traditional µCP such as simplicity, accuracy, affordability and ability to pattern non-planar substrates with new and unique characteristics such as broader feature design rules, high fidelity and, most importantly, compatibility with many inorganic substrates and traditional IC technologies.

Another soft-lithographic methodology that substitutes organic molecular inks for solid inorganics in order to avoid ink diffusion is nanotransfer printing.71-73 This technique uses SAMs as covalent “glues” to transfer materials from the relief structures on a stamp to a substrate. This approach can reproduce nanoscale patterns of single or multilayered materials over large substrate areas in a single additive step. In contrast to traditional µCP, this method can directly pattern functional materials such as metal films on inorganic substrate without relying on post-printing etching or deposition steps. Moreover, by transferring solid inorganic objects it completely avoids all problems associated with ink diffusion and the distortion of pattern edges in the printed features. It does however rely on functional SAMs, which must form strong covalent bonds with the transferring materials, making this protocol somewhat limited to a narrow range of SAM-substrate systems capable of supporting dense reactive functional groups on the top of SAM structures.
In a typical example of nanotransfer printing a silicon substrate is first chemically oxidized to form a layer of native oxide and then functionalized with mercapto-propyl-trimethoxysilane to form a thiol-terminated SAM on its surface. This thiolated SAM is then used to react and transfer thin gold films deposited on the patterned surface of an elastomeric stamp (Figure 14). It was demonstrated that this method can correctly reproduce very small (sub-100 nm) gold features on silicon substrates with remarkably high fidelity, uniformity, and edge resolution (~15 nm).  

In another instance nanotransfer printing was used to transfer multilayered Au/Ti features on an oxidized silicon substrates. In this example, layers of gold and titanium were deposited on an elastomeric stamp via physical vapor deposition after which the exposed titanium layer was oxidized with an oxygen plasma. Subsequently, the stamp was brought into contact with oxidized silicon promoting a condensation reaction that produces –Ti–O–Si– bonds, which facilitated material transfer from the stamp to the substrate. Nanotransfer printing was also expanded to pattern other
substrates such as gallium arsenide making this method a useful nanofabrication tool with a relatively broad applicability.\textsuperscript{73}

1.3.2.4 High-speed, submerged, microdisplacement, and other \textmu CP techniques

By decreasing the stamping time by three orders of magnitude to the range of milliseconds it was possible to significantly improve the reproducibility and fidelity of the printed features in traditional \textmu CP with alkanethiols on gold.\textsuperscript{74} However, in order to achieve ordered densely-packed SAMs within such short periods of time it was necessary to use relatively high concentrations of ink molecules. As such, it was demonstrated that the best results in terms of SAM order and feature resolution can be achieved using stamping times between 1 and 8 ms and ink concentrations between 20 and 40 mM. At such fast contact times ink diffusion by both lateral spreading and gas diffusion was avoided, while the high concentrations of ink solutions ensured formation of ordered SAMs in the areas of conformal stamp-substrate contact. One of the drawbacks of the described technique was the requirement for an automated piezoelectric actuator to control position, printing, and retraction of the inked PDMS stamp.

Gas diffusion of the alkanethiols in traditional \textmu CP can be significantly suppressed by printing in a liquid medium that does not mix with hydrophobic thiolates. By printing alkanethiols under water,\textsuperscript{75-77} the vapor transfer of the ink molecules from the voids between the features to the substrate surface is significantly limited due to the incompressibility of water. This feature significantly expands possible pattern designs and facilitates the use of stamps with very small aspect ratios. The surface
spreading of inks in this method can be controlled by varying stamping times, allowing replication of submicrometer features.

The lateral spreading of ink molecules can also be significantly hindered by replacing preformed labile SAMs on a gold surface with more stable and ordered SAMs during printing.78-79 As such, 1-decanethiol and 11-mercaptoundecanoic acid can be used to replace a SAM of 1-adamantanethiol from a gold surface during µCP with a PDMS stamp (Figure 15). In this method the original adamantane SAM limits the lateral spreading of molecules permitting replication of SAM features with inks that are otherwise too labile to pattern by traditional µCP.

Figure 15. Microdisplacement printing

Another very effective way to prevent lateral diffusion of molecular inks on the surface is to limit the total volume of molecules on the stamps by avoiding the wet inking step. For example, in traditional µCP of silanes on silicon oxide the stamps are
inked with solutions of alkoxysilanes. Under such conditions silanes tend to react with themselves to form undesired oligomers and multilayered films on both the stamp and the receiving substrate, making the patterning process less reliable and reproducible.\textsuperscript{14, 80-83} On the other hand, by exposing a PDMS stamp to a saturated silane atmosphere in a reaction chamber, the PDMS stamp can be loaded with volatile molecules due to the inherent capillary structure and permeability of the stamp to gases. In the next step, the silanized stamp is brought into contact with the oxidized silicon substrate and the silanes are transferred from the stamp to the areas on the substrate not contacted by PDMS by gas diffusion (Figure 16).\textsuperscript{84}

![Figure 16. Gas-phase soft lithography](image)

The described technique has several attractive features which theoretically allow it to achieve very high (submicron) resolution. First, molecular deposition occurs only in the cavities between the stamp features avoiding spreading of the inks outside the pattern. Second, the technique does not rely on the controlled distribution of forces during the stamp application making it more reliable and reproducible. Third, gas-phase
deposition of silanes yields more ordered SAMs with a smaller number of defects compared to solution deposited molecules. The drawback of the methodology is the possibility of SAM formation in the contacted regions due to the less favorable gas diffusion of silanes to the PDMS-silicon oxide interface. Nonetheless, it was demonstrated that the reaction of silanes at the PDMS/silicon oxide interface can be suppressed by adjusting the stamping time.84

1.3.2.5 Catalytic µCP

The diffusive limitation of traditional µCP can be entirely eliminated by avoiding the use of molecular inks as means for pattern replication. This approach could be realized by making the surface of a stamp catalytically active towards a functionalized SAM immobilized on a substrate. In catalytic µCP pattern replication is achieved via a specific chemical or biochemical reaction between the stamp material and the functionalized substrate. Because all components of such a system are either covalently or specifically immobilized and cannot spread laterally or diffuse via the gas phase, the ultimate resolution of inkless µCP is only limited by deformations in elastomeric stamps and by the physical nature of the substrate surface.
The first example of catalytic µCP was reported by Reinhoudt and coworkers in 2003, who used an oxidized PDMS stamp to deprotect TMS- and TBS- protected SAMs of hydroxydisulfides immobilized on gold.\textsuperscript{85} The acidic nature of UV or plasma oxidized PDMS was used to hydrolyze acid-labile protection groups of the corresponding SAMs in the places of conformal stamp substrate contact (Figure 17). Because all components of the described system were either covalently linked to the substrate or incorporated into the bulk stamp material, the method successfully reproduced submicrometer features with ~ 50 nm edge resolution. However, according to the XPS analysis, the technique was able to achieve only 30 % deprotection for the more labile TMS group even after 30 minutes of contact time. In all experiments the oxidized stamps have to be used immediately after preparation since catalytic efficiency was lost after a short period of time. Moreover, each new printing experiment required a new PDMS stamp, since both...

**Figure 17. Catalytic µCP with oxidized PDMS stamps**

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UV/ozone and plasma treated patterned stamps showed significant changes in feature morphology and had many defects and cracks induced by oxidation.

The second example of catalytic \(\mu\)CP was developed in our lab. This approach utilized a biocatalytic reaction between an immobilized substrate and a catalytic stamp for pattern replication. Enzymes offer several attractive attributes for \(\mu\)-lithographic techniques, including a tremendous diversity of reactivity and extraordinary rates of catalysis. To achieve enzymatic modification of a surface a uniform SAM of organic molecules was created first on the underlying gold or glass substrate. This modification is fundamentally different from the typical mode of pattern replication by \(\mu\)CP: rather than transfer inks to a hard surface through diffusive migration off an elastomeric stamp, a preformed uniform monolayer would be modified by an immobilized catalyst through a non-diffusive process. This approach facilitates extraordinary fidelity of pattern replication over very short distances, and permits patterning on surfaces otherwise inaccessible by \(\mu\)CP. Moreover, the technique offers almost unlimited versatility considering the broad range of chemistries available through biological catalysis and the extraordinarily catalytic efficiencies of enzymes.

In this example of biocatalytic \(\mu\)CP the action of exonuclease I (ExoI), immobilized on a poly(acrylamide) stamp was examined on single-stranded DNA immobilized on both glass and gold surfaces. Good pattern transfer was achieved, although the resolution was constrained by deformation and distortion of the acrylamide stamp, which lacked the mechanical integrity necessary to support and transfer sub-micron features. One of the most distinctive features of the developed
technique was a specific immobilization of histidine-tagged ExoI on the nitrilotriacetic acid-terminated stamp surface via Ni$^{2+}$ complexation. Such non-covalent immobilization potentially allows for a reversible immobilization of different enzymes on the same stamp.

In another embodiment of inkless µCP developed by Reinhoudt and coworkers heterogeneous catalysis mediated by a Cu-modified stamp was used to replicate chemical patterns on gold. In this approach µCP was used to promote the spatially selective reaction between a functionalized SAM on gold and a molecular ink physisorbed on the stamp surface.$^{87}$ It was demonstrated that the Cu-catalyzed azide-alkyne cycloaddition reaction between the immobilized azide molecules and the pendant alkynes can be promoted by a Cu-coated PDMS stamp (Figure 18). It was estimated via electrochemical measurements that the reaction achieves almost complete conversion and that the best results are achieved when the Cu-modified stamps were aged for at least 24 hours, presumably due to the increased presence of the surface-bound catalytically active Cu$_2$O species. This method was also used to prepare patterned substrates functionalized with chemical fluorophores, however only large (~5 µm) features were used.
Another interesting variation of catalytic µCP uses a flat PDMS stamp to physically push together reacting molecules in order to promote amide-bond formation. In this technique an oxidized flat PDMS stamp with physisorbed N-protected amino acids is pressed against an NH$_2$-terminated SAM immobilized on gold, thus forcing the ink molecules into van der Waals contacts with the substrate.\textsuperscript{88} It was speculated that such artificial nanoscale confinement of the inks at the stamp-SAM interface, together with the preorganization of the SAM molecules, facilitates the formation of the covalent amide bonds (Figure 19). It was demonstrated that the same PDMS stamp can be used to synthesize short peptide chains in a stepwise manner with an estimated 90 % efficiency for a single step. However, this method only used flat stamps and did not attempt replication of any patterns, leaving unclear the applicability of the approach for the creation of chemically distinctive patterns of peptides on inorganic substrates.

\textbf{Figure 18. Heterogeneous catalysis through µCP}

Another interesting variation of catalytic µCP uses a flat PDMS stamp to physically push together reacting molecules in order to promote amide-bond formation. In this technique an oxidized flat PDMS stamp with physisorbed N-protected amino acids is pressed against an NH$_2$-terminated SAM immobilized on gold, thus forcing the ink molecules into van der Waals contacts with the substrate.\textsuperscript{88} It was speculated that such artificial nanoscale confinement of the inks at the stamp-SAM interface, together with the preorganization of the SAM molecules, facilitates the formation of the covalent amide bonds (Figure 19). It was demonstrated that the same PDMS stamp can be used to synthesize short peptide chains in a stepwise manner with an estimated 90 % efficiency for a single step. However, this method only used flat stamps and did not attempt replication of any patterns, leaving unclear the applicability of the approach for the creation of chemically distinctive patterns of peptides on inorganic substrates.
In an approach toward sub-100 nm patterning of organic SAMs via catalytic μCP, a PDMS stamp bearing random arrays of flat Pd nanoparticles was used to catalyze hydrosilylation of alkenes on H-terminated silicon. In this method, arrays of flat Pd nanoparticles presynthesized on silicon oxide were transferred onto a surface of PDMS via a peel-off procedure. Subsequently, the NP-patterned stamp was used to catalyze a reaction between alkene molecules physisorbed on its surface and an H-terminated silicon substrate (Figure 20). Because the reaction is catalyzed by solid NPs molded into the PDMS stamp, pattern replication in this technique is not affected by ink diffusion or stamp deformation.
The method can achieve high (sub-100 nm) resolution, although the fidelity and uniformity of the printed features was very poor compared to cognate objects on the PDMS stamp. Moreover, the protocol creates SAMs on H-silicon only in areas contacted by NPs, thus failing to protect the rest of the passivated substrate from oxidative degradation. Most importantly, the described technique is severely limited by pattern design and can only replicate patterns of random NP arrays.
Figure 21. Electrochemical nanoimprinting

In one of the most unusual variations of catalytic µCP a pattern replication on metallic films is achieved via electrochemical nanoimprinting with a completely solid non-elastic stamp.\(^9\) This technique uses a patterned solid electrolyte or superionic conductor as a stamp to etch a metallic film by an electrochemical reaction. Although such method can hardly be classified as a soft-lithographic technique, because it uses a solid nonelastic stamp; it still can be categorized as a catalytic µCP method, since it relies on a conformal stamp-substrate contact for pattern replication.

In this protocol a solid superionic conductor with a mobile cation (e.g. silver sulfide) is first patterned by focused ion-beam milling or by direct embossing against silicon molds, and then contacted with the corresponding substrate (e.g. silver metal). On application of an electrical bias a solid-state electrochemical reaction occurs at the
contact points of the stamp-substrate interface (Figure 21). In this step the anodic
dissolution progressively removes a metallic layer of the substrate at the contact areas,
generating shapes in the silver substrate complimentary to the stamp features. It was
demonstrated that this method can replicate small (~ 100 nm) three-dimensional
objects with extreme precision and uniformity, achieving less than 10 nm mismatch
between the features on the stamp and the substrate. Moreover this method works
under ambient conditions and does not require complex process steps or expensive
equipment making it extremely useful in patterning metallic films with small (sub-100)
nm features.

1.3.3 Avoiding limitations of the stamp-substrate system

Direct SAM formation by traditional µCP sets several requirements on potential
SAM-substrate systems that cannot be easily met by many inorganic substrates. For
example, such technologically important materials as H-terminated silicon, germanium,
and diamond form SAMs on their surfaces only under extreme conditions, and thus
cannot be easily patterned by traditional µCP. On the other hand, many inorganic oxides
tend to rapidly form covalent bonds with various organic materials, thus requiring
carefully controlled conditions in order to form uniform and ordered monocomponent
SAMs. In many cases such conditions cannot be achieved by traditional µCP, which in
general requires prior inking of the stamps in solutions of printing materials. To avoid
the aforementioned limitations and to expand possible applications of µCP, several
other approaches towards pattern replication by soft-lithographic printing have been developed in the past.

1.3.3.1 Reactive µCP

Reactive µCP represents a viable alternative to the direct patterning of SAM by traditional printing. In this method a uniform reactive SAM is first formed on the corresponding substrate and then patterned by µCP, which locally transfers the reagent to the functionalized SAM promoting chemical reaction in the places of conformal stamp-substrate contact (Figure 22). This method can be used to modify SAMs grafted to surfaces otherwise incompatible with traditional µCP, while at the same time permitting direct formation of patterned bi-functional SAMs on the same substrate without an additional backfilling step.

![Figure 22. Reactive µCP](image)

The main difference of reactive µCP from previously described catalytic or nanoconfined µCP is that the SAM–reagent reaction in this case can also proceed in solution under the same conditions without requiring any action from the stamp, which
is required in this method only as a mean of localized reagent transfer, whereas in previously described techniques (sub-chapter 1.3.2.5) the stamp is used either to catalyze a chemical reaction or to force molecules into a reactive conformation by placing them together in a nanoconfined environment.

In the first example of reactive µCP SAMs, activated carboxylic acids on gold and silver were reacted in a patterned manner with amines and poly(ethylene imines). This technique was also used to create nanostructured biointerfaces by reacting functionalized block-copolymer films with trifluoroacetic acid absorbed in the patterned stamp. In this protocol TFA on the stamp was used for the localized deprotection of the tert-butyl acrylate side chains in the polymer, making them reactive towards amine-terminated biomolecules. Surprisingly, this method achieved relatively good printing fidelity despite the highly diffusive nature of TFA. Presumably, highly volatile TFA diffused too quickly to react with the areas outside of the conformal stamp-substrate contact. In another study amino-functionalized PEG molecules were printed on a reactive film of poly (N-hydroxysuccinimidyl methacrylate) to serve as a passivating layer and to prevent non-specific absorption of biomolecules to the printed regions. Such reactive reagents as diazonium salts can also be used in µCP, where they were reacted with pyrolized photoresist films to create amino- or carboxylic acid-terminated patterns.

Reinhoudt and coworkers investigated reversible reactions of aldehyde-terminated SAMs and aliphatic amines. It was demonstrated that reactive µCP can be successfully used to promote imine formation between the SAM and the amines on the
stamp to prepare chemically functionalized patterns, which could be completely erased by acid-catalyzed imine hydrolysis. “Click” chemistry was also investigated in reactive μCP to pattern acetylenes on azido-terminated SAMs.\textsuperscript{102} It was shown that such approach can be successfully used to create carbohydrate arrays on glass and silicon.\textsuperscript{103}

In another unusual variation of reactive μCP an acid stamp was used to physically etch an underlying substrate in areas of conformal contact. As such, a high-gel-strength agarose stamp was used as a reservoir for hydrofluoric acid, which was used to dissolve glass and silicon substrates placed on top of the inverted patterned stamp.\textsuperscript{104-105} Due to the diffusive nature of the HF solution the substrates were successfully etched in a patterned manner by the stamp creating physical three-dimensional features.

1.3.3.2 Supramolecular μCP

A highly specific and reversible binding nature of supramolecular host–guest partners offers enormous flexibility in manipulation, transferring, and positioning. Such properties make supramolecular systems especially attractive for various printing techniques that rely on the controlled release and migration of organic and biological molecules from one surface to another.\textsuperscript{106} In a typical supramolecular μCP experiment a functionalized stamp is used to selectively adsorb molecules from a solution and then transfer them to a substrate functionalized with complementary binding partners (Figure 23).
It was demonstrated that a stamp functionalized with single stranded DNA can be used to selectively absorb complimentary ss-DNA molecules bearing reactive chemical groups from the DNA solution, and then used to print functionalized DNAs on the corresponding chemically modified substrate by dissociating ds-DNAs.\textsuperscript{107-111} Similarly, specific proteins can be selectively extracted from crude biological mixtures and then printed on corresponding substrates using carefully designed supramolecular \( \mu \text{CP} \) systems.\textsuperscript{112} Reinhoudt and coworkers have used supramolecular \( \mu \text{CP} \) to print various organic monovalent and multivalent compounds on substrates functionalized with receptor molecules to study the kinetic and thermodynamic properties of surface-immobilized molecular systems.\textsuperscript{106, 113-117}

### 1.3.3.3 Edge-transfer and edge-spreading lithographies

In edge-transfer lithography an elastomeric stamp is inked with a solution that dewets the stamp material.\textsuperscript{118} Such dewetting confines ink molecules to the voids
between the stamp features, leaving the top surface of the features free of molecular inks. During printing molecules migrate from the recessed areas of the stamp to the substrate surface and spread laterally. At the same time areas of conformal stamp-substrate contact remain unmodified due to the repelling forces between the stamp material and the molecular inks. By controlling the lateral spreading of the molecular inks with the stamping time and the ink concentration, this method can be used to print relatively small features along the edges of the contacted areas.

Figure 24. Edge spreading lithography

Edge-spreading lithography uses similar approach to pattern small features on inorganic substrates, but instead of using the dewetting properties of the stamp material to confine molecular inks on the substrate, it combine elements of traditional μCP with nanosphere lithography to transfer inks along the edges of the silica beads immobilized on silicon (Figure 24). In this technique, silica bead are first physisorbed on the silicon surface. Subsequently, a flat stamp inked with molecules is used to transfer the inks along the bead surfaces to the underlying substrate on which inks spread laterally creating circular features. This technique is very effective for
creating small features with submicron resolution and can be used to prepare bifunctional patterns where two stamps with different inks are used in a sequence.\textsuperscript{119}
2. Catalytic microcontact printing

2.1 Overview

Since the initial report in 1993 by Whitesides and coworkers\(^7\) µCP has emerged as a powerful and versatile tool to pattern diverse inorganic, organic, and biological materials on a variety of substrates.\(^8, 15, 18, 122\) By combining high throughput, low cost, and operational simplicity µCP has become the method of choice for low cost high fidelity patterning in such diverse fields as microelectronics,\(^10-12, 21, 42, 123-128\) (bio)chemical sensing,\(^129-130\) organic TFT and photovoltaic devices,\(^131-134\) tissue engineering and cell signaling,\(^14, 135-140\) and DNA/protein array fabrication.\(^47, 55, 99, 141-150\)

Although traditional µCP can be routinely used to create arrays of microscopic objects, two important limitations diminish the broad applicability of the approach. First, ink diffusion\(^15, 22, 41\) and stamp deformation\(^27-28\) degrade the feature edge resolution, and preclude accurate replication of patterns with features below roughly 300 nm.\(^23-24\)

Second, because the approach relies on a rapid, reaction between a diffusible liquid ink and the underlying solid surface the vast majority of µCP techniques are restricted to a small group of less technologically relevant metal and metal oxide surfaces\(^8, 15, 18, 122\) and cannot be easily applied to such important substrates as silicon, which does not react readily with organic materials.\(^91\)

At the same time, organic-semiconductor interfaces have gained increased attention in such fields as electronics, molecular recognition, and biosensing. The demand for continuous miniaturization of electronic components brings an especially great interest in new methods for integration of ordered molecular systems with
conventional inorganic semiconductors. Furthermore, advances in bio(organic) sensing have raised questions such as how to incorporate biological materials with microelectronics to develop true hybrid bioelectronic devices. In recent studies, patterned organic monolayers were suggested as an ideal mean to achieve integration between microelectronic materials and organic and biological systems. By covalently grafting stable ordered molecular systems to inorganic substrates, it is not only possible to protect and preserve the electronic properties of the inorganic semiconductors, but also to tune and adjust them to fit the needs of specific applications.

Here we describe several soft-lithographic techniques methods that employ chemical catalysts bound to polymeric stamps to reproduce patterns on functionalized self-assembled monolayers (SAMs). During our study, we have undertaken a systematic exploration of inkless µCP methods for accurate and uniform replication of nanoscale features on functionalized metal and semiconductor substrates. We have shown that the technique completely avoids the diffusive limitations of traditional µCP and accurately replicates nano-size patterns through a specific chemical reaction between a surface-immobilized substrate and a complementary stamp-bound catalyst. We have utilized several chemical catalyst-substrate systems, developed new stamp materials that provide outstanding fidelity in pattern transfer and exceptional flexibility in catalyst design, developed new extremely effective catalysts, and extended our approach to materials that cannot be patterned by traditional µCP methodologies. The developed methodologies are operationally straightforward and generally applicable, greatly extending the utility of soft lithography.
2.2 Substrate – catalyst system: working principal

A typical catalytic µCP process is shown on Figure 25. In this technique a patterned elastomeric stamp bears a covalently attached reagent, which is either copolymerized with the stamp polymer or immobilized on the surface via a chemical reaction. The reagent on the stamp catalyzes a reaction with a substrate, which is immobilized on a flat surface usually as a self-assembled monolayer. During the stamp-substrate reaction, the catalyst on the stamp modifies the corresponding substrate on a surface in places of conformal contact, while the rest of the substrate stays unchanged. After reaction, the stamp leaves a pattern of unaffected and catalytically modified SAMs, with features identical in shape and size to those on the stamp.

![Figure 25. Catalytic microcontact printing](image)

Catalytic µCP achieves pattern reproduction via a specific chemical reaction and offers many advantages over the traditional µCP, including higher resolution and accuracy, compatibility with a greater variety of surfaces, and instant access to bi-
functional substrates that can be chemoselectively functionalized in a pattern-specific manner.

### 2.3 Substrate – catalyst system: requirements

Figure 26 summarizes the requirements of an ideal catalytic µCP system for the stamp and substrate materials. Optimization of such properties as the chemical selectivity of the stamp-substrate reaction, the amenability of the stamp material towards functionalization, and the ability of inorganic surface to support functional SAMs are required for all catalytic µCP methods.

<table>
<thead>
<tr>
<th>Polymeric catalytic stamps</th>
<th>Inorganic substrates</th>
<th>Stamp-substrate reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>– must support covalent or specific attachment of a catalyst</td>
<td>– support attachment of functionalized SAMs</td>
<td>– specific, should not lead to unwanted side reactions</td>
</tr>
<tr>
<td>– should be chemically and mechanically stable</td>
<td>– should not degrade or change properties with time</td>
<td>– fast to avoid accidental stamp deformations</td>
</tr>
<tr>
<td>– flexible enough to achieve uniform conformal contact</td>
<td>– should be atomically flat to achieve high resolution</td>
<td>– should proceed at ambient or close to ambient conditions</td>
</tr>
<tr>
<td>– relatively rigid to avoid unwanted deformations</td>
<td>– should not react with or hinder the reactivity of terminal functional groups in SAMs</td>
<td>– should not require other reagents than catalyst</td>
</tr>
<tr>
<td>– should not contaminate SAMs with unpolymerized components</td>
<td>– should undergo complete irreversible conversion</td>
<td>– should form stable species</td>
</tr>
<tr>
<td>– should have low surface energy to alleviate release</td>
<td>– should not react with the stamp materials</td>
<td>– should form bi-functional patterned SAMs</td>
</tr>
<tr>
<td>– should not degrade with time</td>
<td></td>
<td>– should not degrade SAMs or SAM-surface interfaces</td>
</tr>
<tr>
<td>– transparent to simplify applications and alignment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– should not shrink or swell in liquids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 26. Stamp, substrate, and catalytic reaction requirements**
Here we have undertaken a systematic exploration of catalytic µCP methods and developed a universal patterning technique that complies with all of the requirements summarized in Figure 26. We now describe in detail our results and discuss their potential significance.

2.4 Catalytic µCP on SAMs of Fmoc-protected aminothiols

The first developed catalytic µCP protocol achieves complete transfer of patterns with sub-micron features using an alkaline chemical catalyst bound to a rigid polymeric stamp. The approach reproduces patterns by deprotecting Fmoc-modified SAMs on gold using an elastomeric polyurethane acrylate stamp modified with a piperidine catalyst (Figure 27 A). The 9-fluorenylmethoxycarbonyl (Fmoc) group is among the most frequently-used amino-protection groups.\textsuperscript{151-154} It is easily introduced by the reaction of a free amine with 9-fluorenylmethyl chloroformate (Fmoc-Cl) or 9-fluorenylmethyl-\textit{N}-hydroxysuccinimide (Fmoc-NHS) and selectively cleaved under mildly basic nonhydrolytic conditions using aliphatic amines such as piperidine, morpholine and, 8-diazabicyclo[5.4.0]undec-7-ene (DBU).
In our inkless µCP, the stamp containing polymerized piperidin-4-ylmethanamine (1) is brought into contact with the gold surface functionalized with a SAM of (9H-fluoren-9-yl)methyl 11-mercaptoundecylcarbamate (2), promoting catalytic cleavage of the Fmoc groups (Figure 27 B). This technique has several advantages over traditional µCP. First, it does not depend on chemical ink for diffusive pattern transfer, utilizing instead a catalytic reaction between piperidine, covalently immobilized on the stamp, and an Fmoc-protected amine on a gold surface to chemically modify the surface in the places of conformal contact. As a result, the limitations of the traditional µCP associated with diffusion\textsuperscript{15, 22, 41} are removed. Second, the use of polyurethane acrylate polymer, which was recently utilized to make highly accurate patterned molds with high aspect ratios,\textsuperscript{60, 155-156} eliminates some of the problems of traditional µCP related to
deformation and collapse of the elastomeric stamps prepared from PDMS.\textsuperscript{27-28} Third, our stamping method allows fast subsequent functionalization of the printed surfaces, providing a route to the patterned SAMs with various chemical and physical properties. For instance, this technique can be used in a range of bio-related applications, where patterning of various biomolecules through covalent attachment to the surface is required.\textsuperscript{140}

2.4.1 SAM materials

To form Fmoc-protected SAMs on gold we synthesized protected aminothiol 2 from 11-aminoundecanoic acid (3) in eight steps following in part a previously reported procedure.\textsuperscript{157} Acid 3 was reduced with LiAlH\textsubscript{4} to the corresponding alcohol 4, protected with di-t-butyl dicarbonate, and converted to bromide 6 with NBS and PPh\textsubscript{3}. Subsequently, compound 6 was converted into thiol ester 7 and reduced to give desired Boc-protected aminothiol 8. Fmoc-protected aminothiol 2 was prepared from 8 in three steps. Thiol 8 was oxidized to the disulfide and deprotected with 4M HCl in dioxane to give diamine 10, which was then reacted with Fmoc-NHS to produce 11. Finally, compound 11 was reduced with Zn dust and TFA in dichloromethane-methanol solution at room temperature\textsuperscript{158} to give (9H-fluoren-9-yl)methyl 11-mercaptoundecylcarbamate (2)\textsuperscript{159} (Figure 28). We also prepared the hydrochloride salt of deprotected aminothiol 11 from Boc-aminothiol 6.\textsuperscript{157}
Figure 28. Synthesis of (9H-fluoren-9-yl)methyl 11-mercaptoundecylcarbamate (2) and 11-aminoundecane-1-thiol (11).

Synthesized thiols 2 and 11 were used to prepare the Fmoc- and NH₂-terminated SAMs on gold surfaces, by soaking pre-cleaned gold substrates in a 1mM ethanolic solution of 2 or 11 for at least two hours (Substrates A1 and B0 respectively). After modification, the surfaces were washed with ethanol and water and dried under a stream of filtered nitrogen.

2.4.2 Materials for acrylamide and acrylate stamps

To covalently immobilize a piperidine moiety on the stamp for use in μCP, we required a polymer that is sufficiently elastic to ensure conformal contact between the stamp and the substrate, sufficiently rigid to avoid collapse and deformation near pattern edges, and amenable to polymerization with piperidine-modified monomers. Acrylate stamps have been previously used in soft lithography. Their prepolymeric mixtures can be functionalized through the covalent attachment of primary amines via Michael addition. However, a significant limitation to the use of acrylamide stamps was
reported previously\textsuperscript{86}: while these materials are easily functionalized, they lack the mechanical rigidity necessary for high fidelity transfer at short length scales. To alleviate this limitation we utilized a polyurethane acrylate copolymer, which was previously used to prepare molds with densely arrayed nanopatterns of sub-100nm features with high aspect ratios for use in replica molding. PUA produces highly accurate defect-free molds with densely arrayed features that do not collapse laterally.\textsuperscript{60,155-156}

PUA monomer 16 was prepared from isophorone diisocyanate 12. The reaction of 12 with 0.5 equivalents of polyethylene glycol 13 (av. Mw 400 g/mol) for 5 hours at 55 °C in the presence of a urethane polymerization catalyst and radical suppressant produced intermediate 14, which was immediately reacted with an isomeric mixture of hydroxypropyl acrylate 15 for 4 hours at 85 °C to produce the target monomer 16 (Figure 29).\textsuperscript{161}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure29.png}
\caption{Preparation of the tetraurethane diacrylate monomer 20.}
\end{figure}

Acrylate 16 was diluted by 30 % with trimethylolpropane ethoxylate triacrylate (17, av. MW 912 g/mol) to reduce viscosity. To the mixture were added photoinitiators 18 and 19, and the resulting solution was polymerized between two glass microscope
slides (Stamp I) or a glass slide and a silicon master containing ~14 μM features (Stamp II) by exposure to UV light for 2 hours at room temperature (Figure 30). The unfunctionalized stamps were somewhat less elastic than the acrylamide stamps previously investigated in our laboratory; nevertheless, the flexibility of the PUA stamps was dramatically improved at 50 °C.

The piperidine derivative was covalently immobilized to the polyurethane acrylate stamp through a Michael addition reaction. A prepolymeric mixture, containing 16 (67 wt%), 17 (30 wt%), 18 (1.5 wt%), and 19 (1.5 wt%), was combined with 2-aminomethyl piperidine (1) (8 v/v %), heated at 60 °C in vacuum for 10 minutes, flushed with argon, and polymerized between two glass slides under UV light (2 hours, room temperature) to give featureless PUA stamps (Stamp III) modified with piperidine fragment. Stamps IV and V containing 7.5 μm and 14 μm features respectively were prepared by polymerizing the prepolymeric mixture containing 1 between the corresponding silicon masters and glass slides. Prepared stamps were thoroughly rinsed with EtOH and H₂O, and dried under filtered nitrogen.
To confirm that the prepared stamps are sufficiently elastic to make conformal contact with the substrate, traditional $\mu$CP with 1-dodecanethiol (DDT) was performed with **Stamp II** to produce a uniform pattern of SAMs on gold. In this experiment, the stamp was soaked in a 10mM solution of DDT in ethanol for 30 sec, dried under a stream of nitrogen for 30 seconds, and brought into contact with the clean gold substrate for 10 seconds, transferring DDT from the stamp to the gold places of conformal contact. The produced pattern was analyzed using contact mode AFM (Figure 31), to confirm successful DDT transfer. The shape and size of the features on piperidine-modified **Stamps IV** and **V** were identical to those of the corresponding masters, were unaffected during storage at room temperature for several days, and retained their integrity even after heating to 70 °C and cooling to room temperature (Figure 31).
Figure 31. A lateral AFM friction image (left side) of the DDT patterned with polyurethane acrylate stamp on the gold surface. Images A and C: optical micrographs of the silicon masters containing 7.5 µm and 14 µm features. Images B and D: optical micrographs of stamps IV and V after keeping at room temperature for one week, heating to 70 °C, and cooling to room temperature.

2.4.3 Stamping experiments with featureless stamps

Functionalized PUA stamps were used to deprotect Fmoc-modified SAMs of aminothiols on gold. Two different approaches were investigated. In the first set of experiments, Fmoc-protected SAMs on gold were formed by immersing clean gold substrates in 1mM EtOH solution of 2 for at least two hours at room temperature (Substrate A1). Amino-terminated SAMs were prepared from Substrate A1 by deprotection with a 1M piperidine solution in DMSO for 45 minutes at room temperature (Substrate A2).162 Featureless Stamps I and III were used to deprotect Fmoc-modified SAMs on gold and to estimate the extent of Fmoc removal. Substrate A1 was placed on the top of the piperidine modified Stamp III preheated to 50 °C, and permitted to react for three hours at 50 °C to produce Substrate A3. After the reaction, Stamp III was soaked in EtOH for one hour, rinsed with EtOH and H2O, dried with filtered nitrogen, and used again with new Substrate A1 under the same conditions to
confirm that the same PUA stamp can be used several times (Substrate A4). To ensure specificity of the piperidine-modified stamp, another Substrate A1 was placed on the top of empty Stamp I without immobilized piperidine, and held in contact for three hours at 50 °C (Substrate A5) (Figure 32).

**Figure 32.** Experiments with Fmoc-protected SAMs prepared from (9H-fluoren-9-yl)methyl 11-mercaptoundecylcarbamate (2).

The extent of Fmoc-group removal from Substrates A3–A5 by piperidine-modified Stamp III and empty Stamp I was determined by comparing water contact angles of Substrates A1–A5 and ratios of the C 1s and Au 4p signals in their XPS spectra (Table 1). C1s/Au4p signal ratio of Substrate A1 was used as a reference for 100 % Fmoc-protected sample, whereas C1s/Au4p ratio of Substrate A2 was assigned to completely deprotected substrate. Based on these values, we estimated that piperidine-modified Stamp III nearly completely deprotected Fmoc-protected SAMs on gold in both experiments (Substrates A3 and A4), while unmodified Stamp I did not change the
chemical composition of Substrate A1 during the reaction. These findings were also supported by the water contact angle measurements of Substrates A1–A5 (Table 1).

Table 1. C1s/Au4p XPS signal ratios, Fmoc-group fractions, and water contact angle of Substrates A1–A5.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Au 4p</th>
<th>C 1s</th>
<th>C 1s / Au 4p</th>
<th>Fmoc %</th>
<th>Water contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>23934.2</td>
<td>10562.5</td>
<td>5.03</td>
<td>100</td>
<td>78 °</td>
</tr>
<tr>
<td>A2</td>
<td>32414.7</td>
<td>6745.6</td>
<td>2.37</td>
<td>0</td>
<td>65 °</td>
</tr>
<tr>
<td>A3</td>
<td>35545.4</td>
<td>7566.8</td>
<td>2.42</td>
<td>1.9</td>
<td>64 °</td>
</tr>
<tr>
<td>A4</td>
<td>33141.2</td>
<td>6859.7</td>
<td>2.38</td>
<td>0.4</td>
<td>64 °</td>
</tr>
<tr>
<td>A5</td>
<td>24534.9</td>
<td>10783.9</td>
<td>5.01</td>
<td>99.2</td>
<td>77 °</td>
</tr>
</tbody>
</table>

In a second set of experiments, similar surface modifications were performed on Substrates B1, which was prepared by reacting an NH$_2$-terminated monolayer of aminothiol 11 (Substrate B0) with an Fmoc-NHS solution for 45 minutes at room temperature (Figure 33). First, Substrate B1 was placed on the top of the preheated to 50 °C piperidine-functionalized PUA Stamp III and was permitted to react with the stamp for 3 hours at 50 °C (Substrate B3). After the experiment Stamp III was washed in EtOH for at least 1 hour, and used again with new Substrate B1 to produce Substrate B4. Similarly, another Substrate B1 was placed on the top of an empty PUA Stamp I
lacking immobilized piperidine, and held in contact for 3 hours at 50 °C to ensure specificity of the piperidine-modified stamp (Substrate B5). Finally, Substrate B1 was soaked in a 1M DMSO solution of piperidine for 45 minutes at room temperature to ensure the ability of the piperidine solution deprotect Fmoc-functionilized SAMs (Substrate B2).

Figure 33. Experiments with Fmoc-protected SAMs prepared from 11-aminoundecane-1-thiol (11).

Substrates B0–B5 were analyzed by XPS to estimate the extent of Fmoc removal by Stamps III and I (Table 2). Similarly to Substrates A1–A5, piperidine-modified PUA Stamp III produced completely deprotected Substrates B3 and B4, whereas unfunctionalized Stamp I did not modify Substrate B1.
Table 2. C1s/Au4p XPS signal ratios and Fmoc-group fractions of Substrates B0–B5.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Au 4p</th>
<th>C 1s</th>
<th>C 1s / Au 4p</th>
<th>Fmoc %</th>
</tr>
</thead>
<tbody>
<tr>
<td>B0</td>
<td>27982.6</td>
<td>7470.7</td>
<td>3.00</td>
<td>0</td>
</tr>
<tr>
<td>B1</td>
<td>25467.0</td>
<td>10666.5</td>
<td>4.77</td>
<td>100</td>
</tr>
<tr>
<td>B2</td>
<td>31092.1</td>
<td>8153.6</td>
<td>2.99</td>
<td>-0.6</td>
</tr>
<tr>
<td>B3</td>
<td>30888.4</td>
<td>7923.0</td>
<td>2.92</td>
<td>-4.5</td>
</tr>
<tr>
<td>B4</td>
<td>31676.7</td>
<td>8117.1</td>
<td>4.72</td>
<td>97.2</td>
</tr>
<tr>
<td>B5</td>
<td>26476.4</td>
<td>10964.5</td>
<td>4.72</td>
<td>97.2</td>
</tr>
</tbody>
</table>

By comparing XPS C 1s / Au 4p signal ratios of Substrates A1, A2, B0, and B1 we estimated that the NH2-terminated SAM on Substrate B0 is 1.27 times denser then the analogous monolayer on Substrate A2, and that the surface reaction of Substrate B0 with the solution of Fmoc-NHS achieves only 75 % yield (Figure 34). These findings suggest that the monolayer density on Substrates A1 is mainly defined by the packing arrangement of the bulky terminal Fmoc-groups rather than by hydrophobic interactions of the aliphatic chains, and that the corresponding deprotected amino-terminated SAM on Substrate A2 is less ordered than its analogue prepared directly from aminothiol 11 (Substrate B0). However, since denser and, presumably, better
ordered monolayer on Substrate B0 can yield only partial reaction with the bulky Fmoc-NHS, in our future stamping experiments we decided to use 100 % Fmoc-protected Substrates A1, which should also achieve higher conversion ratios when subsequent chemical modification of the deprotected surfaces is required.

**Figure 34. Fractions of Fmoc groups in Substrates A1 and B1, and relative density of SAMs in Substrates A2 and B0.**

### 2.4.4 Optimization of the stamping conditions

We also examined the temperature dependence of the deprotection reaction. Fmoc-modified Substrates A1 and piperidine-functionalized flat polyurethane acrylate Stamps II were held together at 23 °C, 40 °C, 50 °C, and 60 °C for various intervals of time (Figure 35) to determine optimal stamping conditions. After a reaction stamps and substrates were separated and cooled to room temperature. The substrates were washed with ethanol and water, dried under filtered nitrogen, and analyzed by XPS. At 60°C complete deprotection is achieved in 30 minutes, while at lower temperatures significantly longer reaction times are required. Because B0 SAM is only 1.27 times...
denser than A2 it is reasonable to suggest that the terminal Fmoc-groups in A1 achieve a relatively tight packing arrangement. This suggests that the Fmoc-deprotection reaction in A1 proceeds via the two-step E1cB elimination mechanism with the formation of a smaller carbanion intermediate, rather than the concerted E2 mechanism, which advances through a bulky bi-molecular transition state. Yet, the extent to which steric constraints of the closely packed substrates affects the rate of reaction and the exact mechanism of the fluorenlyl methylene proton abstraction are unclear, and await further study.

Figure 35. Deprotection of Substrates A1 with piperidine-modified Stamps II at 23, 40, 50, and 60 °C.

2.4.5 Stamping experiments with micrometer patterned stamps

To transfer a pattern from a PUA stamp on a gold substrate we used piperidine-modified stamps IV and V containing 7.5 μm and 14 μm features to selectively deprotect
SAMs of \((9H\text{-fluoren-9-yl})\text{methyl 11-mercaptoundecylcarbamate (Z)}\) on gold in the places of conformal contact. Fmoc-modified \textbf{Substrates A1} were placed on top of \textbf{Stamps IV} and \textbf{V} preheated to 50 °C and held in contact for three hours at 50 °C to produce \textbf{Substrates A6 (stamp IV)} and \textbf{A7 (stamp V)}. After the reaction, \textbf{A6} and \textbf{A7} were thoroughly washed with \text{EtOH} and \text{H}_{2}\text{O}, dried with filtered nitrogen, and analyzed by lateral mode AFM for height and friction differences (Figure 36).

![Figure 36](image)

**Figure 36.** Contact mode AFM images of Substrates A6 (A) and A7 (B) showing height and friction differences between protected and deprotected regions.

As is evident from Figure 36, piperidine-modified PUA stamps successfully transferred pattern features on gold substrates, by selectively deprotecting SAMs of thiol \textbf{Z} in the places of contact (darker regions on Figure 36). \textbf{Stamps IV} and \textbf{V} produced patterns consistent across the entire areas of \textbf{Substrates A5} and \textbf{A6} with features identical in shape and size to features of the corresponding silicon masters. Patterns on \textbf{A5} and \textbf{A6} were uniform across the feature, showing clear height and friction differences between deprotected and protected regions.
2.4.6 Stamping experiments with sub-micrometer patterned stamps

Figure 37. Tapping mode AFM and SEM images of Stamp VI.

The goal of this work was to provide a methodology for pattern transfer that alleviates the diffusive resolution limit of conventional µCP. To evaluate this capability, we prepared catalytic stamp VI bearing 620 nm lines separated by 380 nm with aspect ratio of 0.15 (Figure 37), which was used to selectively deprotect SAMs of (9H-fluoren-9-yl)methyl 11-mercaptopoundecylcarbamate (1) on gold. An Fmoc-modified substrate was placed on top of patterned stamps preheated to 50 °C and held in contact for three hours (Substrate 7). Following reaction, the substrate was washed with EtOH and H₂O, dried with filtered nitrogen, and analyzed by contact mode lateral AFM and SEM.¹⁶³-¹⁶⁴ Figure 38 clearly shows the efficiency of the catalytic stamp in nanoscale pattern fabrication, producing patterns consistent across the entire substrate surface and generating features identical in shape and size to those of the corresponding silicon-PMMA master. Produced pattern shows a height difference of approximately 0.65 nm between deprotected and protected regions, which correlates well with the size of the fully extended Fmoc-group (~9 Å), and a friction difference of approximately 14 mV.
Printed features demonstrate edge resolution less than 50 nm (Figure 39), indicating diffusion-free process.

Figure 38. SEM image (bottom), and height (top) LFM images of patterned Substrate 7

In conclusion, we have demonstrated that piperidine-modified polyurethane acrylate stamps effectively transfer patterns in an inkless variant of µCP. We showed that piperidine-bearing PUA stamps completely deprotect the Fmoc-modified gold surfaces, whereas the unfunctionalized empty stamp does not change the chemical composition of the substrates. The technique offers several advantages over traditional µCP. Most significantly, the approach obviates the diffusive resolution limitation of µCP, and is constrained now only by the mechanical properties of the stamp material. The use of polyurethane acrylate polymer, which was recently utilized to make highly
accurate patterned molds with high aspect ratios, eliminates some of the problems of traditional μCP related to deformation and collapse of the elastomeric stamps prepared from PDMS. Finally, the method permits rapid subsequent functionalization of the printed surfaces, providing a route to the patterned SAMs with a range of chemical and physical properties.

Figure 39. Top: lateral AFM friction images of Substrate 7 showing 620 nm wide lines of the deprotected regions separated by 380 nm wide lines of the Fmoc-protected areas. Bottom: friction profiles of the features indicating less than 50 nm edge resolution.

2.5 Catalytic μCP on SAMs of Boc- and TBS-protected thiols

Although the catalytic μCP method described above significantly improves on traditional approach, at least some limitations remain, including prolonged reaction times and the requirement for elevated temperatures to achieve complete deprotection.
of Fmoc-groups by the alkaline stamp. To shorten reaction times, we decided to consider common acid-labile protection groups that can be removed from the functionalized SAMs using stamps functionalized with acid catalysts. Both tert-butyl carbamate- (Boc) and tert-butyldimethylsilyl- (TBS-) protection groups are commonly used in chemistry and biochemistry. They can be easily installed to protect free amines and hydroxyl groups and removed quickly under acidic conditions. By immobilizing these protection groups on the gold substrate and exploiting their lability at low pH, we developed a new inkless catalytic µCP technique that achieves accurate, fast, and complete pattern reproduction on SAMs of Boc- and TBS-protected thiols immobilized on gold using a polyurethane-acrylate stamp functionalized with covalently bound sulfonic acid moieties (Figure 40). The protocol achieves complete ablation at room temperature just after one minute of contact and renders sub-200 nm size structures of chemically discriminated SAMs. The strategy permits direct functionalization of the printed surfaces, providing a convenient method for chemical and biochemical modifications of surfaces in a spatially controlled fashion.

Figure 40. Components of the catalytic stamp and functionalized SAMs on gold
2.5.1 Silicon-PMMA masters with sub-micrometer features

In order to demonstrate that our patterning technique completely avoids the diffusive limitations of traditional µCP and to confirm that the catalytic PUA stamps show significantly diminished mechanical deformations compared to PDMS stamps, we required a patterned master with various low-aspect-ratio nanofeatures. Such a master was prepared by depositing a 125 nm layer of PMMA on a silicon chip and writing a predesigned pattern in the PMMA layer using E-beam lithography.

Figure 41. SEM images of silicon-PMMA masters

Figure 41 shows SEM images of the produced pattern on the Si-PMMA substrate. The features were accurately and uniformly reproduced on this substrate from the corresponding CAB file, creating a master suitable for preparation of nanopatterned catalytic PUA stamps.
2.5.2 Catalytic PUA stamps

Our approach utilized an elastomeric stamp comprising monomers 16 and 17, and 2-mercaptoethanesulfonic acid (20) as a reactive element to promote catalytic pattern-specific cleavage of Boc- and TBS-protection groups from SAMs of tert-butyl 11-mercaptoundecylcarbamate (8) and 11-(2,3,3-trimethylbutan-2-yloxy)undecane-1-thiol (21) chemisorbed on gold (Figure 42).

![Figure 42. SEM images of patterned acid-functionalized stamps](image)

In order to moderate the deformation and distortion associated with many stamp materials used in µCP, we again employed a rigid polyurethane-acrylate polymer as the stamp support. We have previously demonstrated that this polymer can be utilized for sub-micron µCP and can be easily functionalized prior to polymerization through covalent incorporation of nucleophiles to the acrylate monomer. To produce
stamps bearing acidic moieties, polyurethane-acrylate monomer 16 was first mixed with triacrylate 17 and photoinitiators and allowed to react at 50 °C in the presence of thiolated sulfonic acid 20. The resulting mixture was deoxygenated under vacuum, cooled to room temperature, and polymerized between two glass plates or between a glass plate and a patterned master to produce flat or patterned acidic stamps, respectively. The stamps were easily demolded from the Si-PMMA masters without degrading the PMMA layer. Figure 42 shows SEM images of stamps, prepared with 6.5 µm size dots and with various sub-micron features (down to ~170 nm lines). The features on stamps were identical to the ones on the corresponding masters, indicating high fidelity of the molding technique.

2.5.3 SAM materials

Boc- and TBS-protected SAMs on gold were formed by immersing freshly prepared or oxygen plasma cleaned gold chips in 1 mM solutions of 8 or 21 in EtOH at room temperature for at least 20 hours. Contact angle measurements of the resulting monolayers ( 8: θ_A = 89°, θ_R = 65°; 21 θ_A = 108°, θ_R = 95°) showed significant hysteresis indicating the formation of loosely ordered low-density hydrophobic SAMs, presumably due to the presence of bulky protection groups, and were consistent with previous reports.168 XPS spectra also confirmed monolayer formation and indicated the presence of thiolated hydrocarbons on the gold surface (Figure 43).
Figure 43. XPS spectra of Boc- (top) and TBS- (bottom) protected SAMs on gold
2.5.4 Deprotection efficiency with featureless catalytic stamps

A featureless acidic polyurethane-acrylate stamp was used to deprotect Boc- and TBS-modified monolayers. Freshly prepared SAMs of 8 and 21 were brought into conformal contact with flat acidic stamps at room temperature for intervals ranging from 1 to 40 minutes. The treated gold substrates were rinsed with ethanol and water and analyzed by XPS to determine deprotection efficiencies. Table 3 shows C1s/Au4p and O1s/Au4p XPS signal ratios of Boc-protected SAMs and C1s/Au4d signal ratios of TBS-protected SAMs before and after stamping. On the basis of these data it is apparent that the acidic polyurethane-acrylate stamp effects complete deprotection of both Boc- and TBS-modified SAMs after 1 minute, and no significant change in signal ratios occur beyond this time for either monolayer. The carbon concentration (C1s/Au4p and C1s/Au4d ratios) in the stamped substrates correlates with calculated XPS carbon signals for 100% deprotected samples (Table 3), suggesting a high degree of deprotection for both Boc- and TBS-functionalized SAMs. Boc- and TBS-protected substrates were also treated with 1:1 TFA:CH₂Cl₂ for 1 hour at room temperature to produce fully deprotected reference surfaces. These substrates however, showed a significantly lower carbon signal than predicted for fully deprotected surfaces, presumably as a result of trifluoroacetic acid mediated desorption through oxidative degradation of the loosely ordered Boc- and TBS-protected monolayers.
Table 3. Deprotection of Boc- and TBS-modified SAMs with the featureless acidic polyurethane-acrylate stamp.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Boc SAMs (C\textsubscript{1s}/Au\textsubscript{4p})</th>
<th>Boc SAMs (O\textsubscript{1s}/Au\textsubscript{4d})</th>
<th>TBS SAMs (C\textsubscript{1s}/Au\textsubscript{4d})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.164</td>
<td>0.458</td>
<td>2.606</td>
</tr>
<tr>
<td>1</td>
<td>2.25</td>
<td>0.313</td>
<td>1.429</td>
</tr>
<tr>
<td>2</td>
<td>2.18</td>
<td>0.284</td>
<td>1.427</td>
</tr>
<tr>
<td>5</td>
<td>2.178</td>
<td>0.299</td>
<td>1.435</td>
</tr>
<tr>
<td>10</td>
<td>2.203</td>
<td>0.278</td>
<td>1.35</td>
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<tr>
<td>20</td>
<td>2.254</td>
<td>0.335</td>
<td>1.311</td>
</tr>
<tr>
<td>40</td>
<td>2.185</td>
<td>0.296</td>
<td>1.275</td>
</tr>
<tr>
<td>Depr(TFA)\textsuperscript{1}</td>
<td>1.054</td>
<td>0.148</td>
<td>0.6</td>
</tr>
<tr>
<td>Depr(calc)\textsuperscript{2}</td>
<td>2.175</td>
<td>–</td>
<td>1.686</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Deprotection with a TFA/CH\textsubscript{2}Cl\textsubscript{2} solution

\textsuperscript{2} Calculated for 100% deprotected substrates

2.5.5 Stamping experiments with patterned stamps

To evaluate the ability of the acidic stamp to transfer patterns to functionalized SAMs we prepared a reactive polyurethane-acrylate stamp containing ~ 6.5 \( \mu \)m dots.

The stamp was placed in conformal contact with Boc- or TBS-protected SAMs for 4 minutes at room temperature under only the weight of the stamp. Following reaction, substrates were rinsed with ethanol and water, dried under a stream of nitrogen, and
analyzed by contact mode lateral AFM to determine tribological and topological properties.

Figure 44. AFM friction images of the patterned Boc- (left) and TBS- (right) modified substrates.

Figure 44 clearly demonstrates the effectiveness of the protocol for the fabrication of uniform patterns on both surfaces. The patterns produced on both Boc- and TBS-functionalized SAMs are consistent across the entire substrate area and showed friction differences of ~6 mV and ~4.5 mV, respectively, with very small height differences between protected and deprotected regions (Figure 45), indicating formation of chemically distinctive features.\textsuperscript{168-169} As predicted, given the catalytic nature of pattern transfer, a single patterned stamp produced identical features during multiple applications.
The efficiency of our patterning technique was also analyzed by SEM. Following AFM experiments, the same patterned stamp (6.5 µm dots) was placed on top of functionalized SAMs and held in contact for intervals ranging from 2 to 40 minutes with no external load. Following reaction, the substrates were rinsed with ethanol and water, and analyzed by SEM. Figure 46 again demonstrates that the acidic polyurethane-acrylate stamp successfully deprotected SAMs of thiols 8 and 21 in the areas of conformal contact. The patterns produced on all substrates were identical regardless of protection group or stamping time, suggesting a truly catalytic pattern transfer free of diffusive spreading and the resulting degradation of feature contrast. Notably, a single catalytic stamp was used to transfer patterns on all TBS- and Boc-functionalized substrates, once more confirming the truly catalytic nature of the pattern transfer. The patterned acidic stamp did not lose its catalytic efficiency even after two months, and showed no evidence of polymer degradation over this time. Control stamps bearing no sulfonic acid moiety failed to produce any detectable change in surface character or morphology.
During SEM analysis of the patterned Boc-substrates we also encountered areas where catalytic stamp was slightly shifted either during the initial application or during the subsequent stamp release. Figure 47 demonstrates that the shift trajectory of the stamp was accurately reproduced on SAM substrates, signifying a remarkable efficiency of the developed method. This accidental discovery not only proves that the catalytic stamp almost instantly deprotect Boc-modified SAMs, but also suggests that the described technique can be used to pattern non-planar surfaces in a roll-to-roll fashion with catalytic stamps wrapped around cylindrical supports.
To further demonstrate the extent to which catalytic printing protocols eliminate the diffusive limitations of conventional µCP, a TBS-protected substrate was reacted for 4 minutes with the acidic polyurethane-acrylate stamp containing a series of low aspect ratio features ranging in sizes from 170 nm to 3000 nm. SEM images of the resulting surfaces demonstrate the accuracy and precision of the transfer of small densely populated structures, as well as large deprotected areas and micrometer size features, a task that remains one of the most challenging for traditional µCP (Figure 48). The transferred pattern contains $3000 \times 600$ nm rods separated by 2000 nm and were produced from stamp structures with 0.042 aspect ratio; these features were accurately and uniformly reproduced over the entire stamped region, indicating the absence of gas diffusion and suggesting an exceptionally low deformation in the patterned acidic stamp. In traditional µCP diffusive wetting results in at least 50 nm ink spreading, even under optimal conditions. Here, a patterned acidic stamp transferred 240 nm lines separated by 170 nm on TBS-protected substrate. Figure 48 clearly show that the printed lines and other features have dimensions identical to those of the corresponding Si-PMMA master demonstrating the powerful capabilities of pattern transfer by non-diffusive means.
Figure 48. SEM images of the patterned TBS-protected substrates

The edge resolution of the printed features was in a sub 50-nm region (Figure 49), suggesting that the ultimate resolution of this method is only limited by the structure of the underlying substrate (in this case polycrystalline granular gold (111)).
In conclusion, we have demonstrated that catalytic μCP with functionalized polyurethane stamps effectively transfer patterns on both Boc- and TBS-functionalized SAMs on gold. In this inkless variant of μCP an acidic polyurethane-acrylate stamp catalytically deprotects acid-labile functional groups with complete conversion after just one minute of contact. The approach limits the edge resolution to the size of gold grains (15-30 nm) and allows fast and accurate reproduction of small features completely obviating the limitations of both gas diffusion and diffusive wetting inherent to traditional μCP. Our method also permits defect-free replication of features with very low aspect ratio (to 0.042) eliminating the deformation behavior of many PDMS-based techniques.

Figure 49. Edge resolution of the patterned TBS-modified substrates
2.6 Advantages over conventional microcontact printing

The developed catalytic protocols for patterning Fmoc-, Boc-, and TBS-modified SAMs offer several advantages over the traditional µCP and permit accurate replication of nano-features with sub-50 nm edge resolution.

2.6.1 Catalytic µCP avoids diffusive limitations of traditional µCP

Figures 39 and 49 demonstrate that both piperidine- and sulfonic acid-modified stamps reproduced patterns on corresponding SAMs on gold with sub-50 nm edge resolution, a task impossible in traditional µCP where diffusive wetting results in at least 50 nm of ink spreading. Additionally, we have demonstrated that the acidic catalytic stamp produces identical features on both Boc- and TBS-monolayers, which do not show evidence of edge blurring with increased stamping time (Figure 46). Finally, both catalytic stamps reproduced sub-400 nm patterns (170 nm lines in case of acidic catalytic stamp, Figures 38 and 48), once again confirming a truly catalytic nature of the developed methods.

2.6.2 PUA stamps avoid deformation problems of PDMS-based techniques

We have also demonstrated that both catalytic PUA stamps avoid the deformation problems of PDMS-based techniques. As such, the piperidine-modified stamp successfully reproduced 380 nm lines separated by 620 nm on Fmoc-modified SAMs from the corresponding stamp features with 0.15 aspect ratio (Figure 37). Furthermore, the sulfonic acid-modified stamp simultaneously transferred large (3000 × 600 nm) rods separated by 2000 nm together with small (170 nm) lines on TBS-modified
SAM (Figure 48). Taking into account that some of the features in the stamp pattern had aspect ratios as small as 0.042, such a remarkable example of patterning large unmodified and deprotected areas together with small densely populated objects clearly points out superior mechanical properties of the PUA stamps compare to PDMS materials. These results suggest exceptionally low deformation in the patterned PUA stamps and the absence of any diffusive pattern transfer trough the gas phase.

2.7 Self-assembled molecular systems on semiconductor surfaces

H-terminated polycrystalline silicon is the most common starting point for the construction of electronic devices. The ability to functionalize and pattern silicon with organic monolayers without even partial oxidation is imperative for the fabrication of functional organic-semiconductor devices and silicon-based bio(chemical)sensors. A variety of methods have been developed to protect silicon from degradation and preserve its electronic properties. In particular, the formation of Si-C bonds, which are more chemically stable and less susceptible to nucleophilic substitution than Si-O and Si-N bonds, is exceptionally effective for the protection of silicon surfaces (Figure 50).
Figure 50. Common functionalization protocols of oxide-free silicon

One of the most effective approaches for the covalent attachment of organics to silicon is a two-step chlorination/Grignard process. This approach can be used to prepare highly chemically and electrically passive surfaces,\textsuperscript{170-173} where all surface-exposed Si atoms are terminated with methyl groups. Another commonly used protocol reacts hydrogen-passivated silicon with alkene molecules using different activating agents. In this case, a silicon substrate is first passivated in HF (2 – 10\%) or NH\textsubscript{4}F (40\%) solutions and then reacted with alkenes via peroxide, metallo-organic, UV light, or thermally activated reactions.\textsuperscript{91} Although, the second method does not usually achieve complete termination of all surface exposed silicon atoms with Si–C bonds, it can nonetheless protect silicon from oxidation and the Si-SAM interface from degradation during prolonged periods of time.\textsuperscript{178-182}

2.8 Application of catalytic μCP on oxide-free silicon

Traditional μCP methods are largely restricted to thiol/metal and silane/oxide systems, and cannot pattern many technologically important hard substrates, such as polycrystalline silicon or germanium. The formation of monolayers on these surfaces
typically requires prolonged reaction times and harsh conditions (high temperature, inert atmosphere, UV irradiation), which are incompatible with the traditional stamp materials and µCP conditions. Despite the advantages of our inkless soft lithography, the technique remains limited by the use of gold as an underlying SAM substrate, a material which supports only relatively unstable monolayers and limits the resolution of printed features to the grain size of evaporated gold.\textsuperscript{17} To expand the utility of inkless µCP we sought to extend the methodology to flat polycrystalline surfaces that support chemically robust, stable SAMs\textsuperscript{91}: simple, fast, reliable strategies for soft lithography on such surfaces are largely lacking. As such, to further enhance the resolution of catalytic µCP and to extend its applicability to a broader group of underlying substrates, including silicon, we sought to immobilize Boc-functionalized molecules on polycrystalline silicon and transfer pattern to this surface using an acidic PUA stamp (Figure 51).

![Figure 51. Catalytic µCP on Boc-modified SAMs on silicon](image)

### 2.8.1 SAM Materials

SAMs covalently bound to silicon have gained significant attention for (bio)chemical sensing and electronic applications due to their exceptional stability and
their ability to passivate silicon surfaces towards chemical oxidation.\textsuperscript{175} Most chemical approaches to silicon functionalization are based on the hydrosilation of Si-H groups with alkenes, a process catalyzed by heat, peroxides, metalloorganic catalysts, or UV light.\textsuperscript{178-182} To form Boc-modified SAMs on silicon, we prepared Boc-protected aminoalkene 26 from 11-bromo-1-undecene in four steps (Figure 52). As such, alkene bromide 22 was reacted with HO-PEG\textsubscript{5}-OH to produce hydroxyalkene 23, which was converted into azide 24 using diphenyl phosphorazidate. Azide 24 was reduced with triphenylphosphine to give aminoalekene 25, which was Boc-protected with di-\textit{tert}-butyl dicarbonate to give 26.

![Figure 52. Synthesis of Boc-protected aminoalkene 26](image)

Subsequently, a (111) silicon surface was oxidized in Nanostrip at 75 °C for 15 minutes to remove all organic contaminants and submerged in 5% aq. HF solution for 5 minutes to remove native oxide. A freshly prepared H-terminated silicon surface was reacted with a mixture of Boc-modified and blank alkenes 26 and 27 (1/4 v/v ratio) for 2 hours under UV light in a nitrogen atmosphere (Figure 53). The terminal Boc-group in 26 does not allow formation of highly ordered SAMs, because it hinders noncovalent chain–chain interactions.\textsuperscript{168} We decided to use mixed monolayers to separate Boc-
protected molecules further apart and to achieve higher density and better association between the SAM components to help protect the underlying passivated silicon from oxidation.

Figure 53. Preparation of Boc-protected SAMs on silicon and XPS spectra of Boc-modified and H-terminated surfaces

XPS spectra of H-terminated and Boc-modified substrates support formation of SAMs directly grafted to silicon through Si-C bonds. The Si2p signals in both substrates completely lack the silicon oxide peak at 103 eV indicative of native oxide layer. XPS C1s signal of the Boc-modified surfaces indicates the presence of both PEG and alkyl carbons, while the H-terminated surface shows only a small residual C1s peak (Figure 53).
2.8.2 Catalytic PUA stamps

Previously, we demonstrated that a sulfonic acid-modified polyurethane-acrylate stamp effectively transfers both micro- and nano-scale patterns to Boc-functionalized SAMs on gold by selectively deprotecting Boc moieties within the contact area. We have also shown that the use of the polyurethane stamp material eliminates the deformation behavior of PDMS-based stamps, permitting defect-free replication of features with very low aspect ratios (0.042). Here we utilized an identical acidic stamp to selectively deprotect Boc-modified SAMs on passivated silicon and to prepare patterns of protected and free amino regions. The stamp was prepared by reacting a mixture containing monomers 16 and 17 with 2-mercaptoethanesulfonic acid (20) at 50 °C followed by deoxygenation and UV-induced polymerization of the resulting solution either between two glass plates or between a glass plate and a patterned master to produce flat or patterned acidic stamps, respectively (Figure 54). An unfunctionalized flat polyurethane-acrylate stamp comprising only monomers 16 and 17 was also prepared as an inactive control.
2.8.3 Stamping experiments with featureless catalytic stamps

To establish the ability of a sulfonic-acid modified stamp to deprotect Boc-modified monolayers on silicon and to determine the efficiency of deprotection, a single H-terminated silicon chip was functionalized with a mixture of alkenes 26 and 27 (1:4 (v:v) ratio) and reacted in several areas with both flat acidic and blank (unfunctionalized) stamps (Figure 55).
The resulting surface was analyzed by XPS at six different points to determine both the uniformity of the monolayer across the sample and the efficiency of Boc ablation by the acidic PUA stamp. Following XPS analysis, the substrate was treated with TFA/CH$_2$Cl$_2$ solution (1/3 v/v ratio) at room temperature for 30 minutes to completely remove Boc-groups from all locations, and the resulting surface was reanalyzed by XPS to determine the carbon concentration in a fully deprotected substrate.

Figure 55. Reactions of the Boc-modified silicon surface with catalytic and inactive stamps

1, 3, 6 - unmodified Boc-protected SAM
2 - Boc SAM treated with acidic PUA stamp at r.t. for 5 min
4 - Boc SAM treated with acidic PUA stamp at r.t. for 40 min
5 - Boc SAM treated with inactive PUA stamp at r.t. for 40 min
6' - Boc SAM treated with TFA/CH$_2$Cl$_2$ solution at r.t. for 30 min
Figure 56. XPS analysis of the reactions of the Boc-modified silicon surface with catalytic and inactive stamps

Figure 56 shows that the carbon concentration in the Boc-protected areas decreases slightly from point 1 (C\textsubscript{1s}/Si\textsubscript{2p}=2.046) to 6 (C\textsubscript{1s}/Si\textsubscript{2p}=1.908), in observation that can be rationalized by a somewhat uneven distribution of light intensity across the substrate surface reaching from the single UV light source. XPS analysis also revealed that inactive stamp produces no change in the Boc-substrate (point 5, C\textsubscript{1s}/Si\textsubscript{2p}=1.909). On the other hand, the acidic stamps decreased the carbon concentrations in areas 2 and 4 (5 and 40 min reaction times) by approximately the same amount as treatment with TFA/CH\textsubscript{2}Cl\textsubscript{2} solution, demonstrating that an acidic PUA stamp completely
deprotects Boc-functionalized monolayers on passivated Si in as little as 5 minutes at room temperature.

2.8.3 Stamping experiments with patterned catalytic stamps

To evaluate the effectiveness of the sulfonic acid-modified PUA stamp for catalytic pattern transfer, we reacted both acid-functionalized and control PUA stamps containing 6.5 µm features with Boc-functionalized SAMs for 5 minutes at room temperature. The resulting substrates were analyzed by SEM to evaluate chemical differences between protected and deprotected (patterned) regions. While Boc-modified monolayer surfaces exposed to inactive PUA stamp showed no discernable features, those exposed to acidic stamps showed feature patterns cognate to the patterns of the corresponding Si-PMMA master (Figure 57). The acidic stamp selectively deprotected SAMs in regions of conformal contact, creating distinctive areas of Boc-protected and free amine groups. The pattern was uniform across the entire stamped area. Contrast between the protected and deprotected regions was relatively low, presumably due to the low surface population of Boc-groups.

![Figure 57. SEM images of the patterned Boc-protected substrates](image)

Figure 57. SEM images of the patterned Boc-protected substrates
In conclusion, we have demonstrated that sulfonic acid-modified PUA stamp effectively deprotects Boc-modified SAMs on passivated silicon, creating uniform patterns of protected and free amine functionalities. The protocol makes use of a highly stable SAM directly grafted to silicon through Si-C bonds and allows for a direct attachment of desirable chemical or biological functionalities to the patterned substrate. This protocol is, to the best of our knowledge, the first example of a soft lithographic printing technique that creates regions of chemically distinctive SAMs on oxide-free silicon substrates.

2.9 Universal bi-layered patterning technique

The ability to integrate ordered molecular systems with conventional microelectronic materials should facilitate the continuous miniaturization of electronic components and the development of true bioelectronic devices for the application in molecular recognition and sensing.\textsuperscript{183-184} Such hybrid devices rely on electron tunneling through the organic-semiconductor interface, thus requiring a novel method for patterning two dimensional ordered molecular assemblies. Inkless catalytic μCP obviates most of the limitations of traditional μCP and can achieve very high (sub-50 nm) feature edge resolution. By relying on a specific chemical or biochemical reaction between a surface-immobilized substrate and a complementary stamp-bound catalyst to replicate patterns, the approach completely avoids the use of molecular inks.\textsuperscript{85-86, 169, 185} Moreover, catalytic μCP significantly expands the diversity of patternable surfaces by utilizing substrates already functionalized with SAMs. Until now, however, inkless μCP
has only been used to pattern relatively disordered reactive SAMs, which do not protect underlying surfaces from chemical degradation or oxidation.

Monolayers comprising simple alkyl chains can act as impervious barriers to both organic and aqueous solutions due to their highly ordered structures and hydrophobic nature. However, reactive functional SAMs directly attached to inorganic substrates are generally ineffective for the protection of the underlying SAM-surface interfaces due to the relatively low order resulting from steric constraints induced by the presence of terminal functional groups.\textsuperscript{17, 122, 167, 186} The degree of order in such systems can be increased either by backfilling grafted SAMs\textsuperscript{17, 122, 187} with smaller molecules or by using mixed solution to simultaneously deposit functional and spacer species.\textsuperscript{188-190} Both approaches have significant limitations. Backfilling can cause desorption of functional SAMs,\textsuperscript{63, 188, 191-198} while mixed deposition can result in formation of phase-separated domains.\textsuperscript{188, 190, 199-201} The functionalization of highly ordered primary SAMs with secondary reactive overlayers presents a different approach for achieving simultaneous passivation and functionalization of inorganic substrates.\textsuperscript{174-175} The optimal structure of such functional bi-layered molecular system is shown in Figure 58. Ideally, the initial primary SAM should achieve complete termination of all surface-exposed reactive atoms, forming an ordered close-packed system capable of protecting an underlying surface from degradation. This primary SAM should be chemically inert, undergoing only very specific transformation. The secondary over-layer should contain terminal functional units whose reactivity can be either masked or enhanced via additional chemical transformation. In order to be stable, the overlayer must form covalent bond
(preferably C–C bond) with the primary SAM and should not contain any chemically unstable moieties except for the terminal functional group.

By introducing different functionalities to the described bi-layered systems we can significantly expand the conceivable range of catalyst – substrate systems for the implementation of inkless μCP and, in theory, realize multistep patterning of the bifunctional mixed systems through sequential application of two or more catalytic stamps, where each stamp selectively modifies only one complementary component at a time. Moreover by varying the size of molecules in the reactive overlayer and by using mixtures of blank and reactive compounds we can gain control over the spatial distribution of the functional groups on the surface, which plays a crucial role in the immobilization of biological molecules and heterobifunctional linkers.

**Figure 58. Structure and characteristics of the bi-layered molecular system**

Here we demonstrate that a bi-layered NHS-modified substrate, containing a highly ordered protective primary SAM and an NHS-modified secondary overlayer, can
be hydrolyzed in a pattern-specific manner with a sulfonic acid-modified PUA stamp, producing chemically distinct patterns of activated and free carboxylic acids. We also demonstrate the selective functionalization of patterned substrates with small molecules and proteins. In this embodiment of inkless μCP, a catalytic stamp was used to promote a pattern-specific hydrolysis of \( N \)-hydroxysuccinimide-activated acids immobilized on silicon (Figure 59). A subsequent modification of the resulting chemically discriminated patterns provided the ability to chemoselectively anchor biomolecules to the silicon surface. To the best of our knowledge, this is the first example of a soft-lithographic technique that completely protects silicon from chemical oxidation, allows for a precise control over the shape and size of the patterned features in a sub-micrometer region, and gives rapid, facile access to chemically discriminated patterns that can be further functionalized with organic and biological molecules. We anticipate that a similar strategy can be used to passivate and pattern other semiconductor materials. Such a demonstration of catalytic μCP on diverse semiconductor surfaces holds a great promise for applications in biomolecular recognition, chemical and biological sensing, organic electronic devices, and biomedical engineering.\textsuperscript{184, 202-205}
2.9.1 SAM Materials

In order to protect silicon from oxidation and degradation and to retain its desirable electronic properties, we required a highly ordered molecular system that produces complete Si-C termination of all surface-exposed atoms. Concurrently, we required a SAM that supports attachment of relatively loosely packed NHS esters. To this end, we decided to chlorinate H-terminated silicon with PCl₅ and alkylate it with a Grignard reagent to form a close-packed propylene-terminated SAM. Subsequently, we planned to react this primary surface with a carbene donor containing activated acids to prepare an NHS-modified sample. Such molecular system, containing an inert close-packed primary monolayer and a reactive NHS-terminated secondary overlayer, should simultaneously protect silicon from oxidation and SAM degradation and provide the reactive sites required for selective attachment of (bio)organic molecules.
To prepare NHS-modified SAMs on silicon we synthesized an NHS-diazirine carbene donor 37 from (4-bromophenyl)methanol (28) in 9 steps (Figure 60). Alcohol 28 was protected with TDS-Cl and acetylated with methyl trifluoroacetate to give trifluoroacetophenone 30. Ketone 30 was converted into ketoxime 31, which was O-tosylated to produce 32. Diaziridine 33 was prepared by reacting 32 with liquid ammonia at -30 °C for 4 hours. Subsequently, 33 was oxidized with iodine to give diazirine 34, which was deprotected under acidic conditions to give free alcohol 35. Alcohol 35 was oxidized with potassium permanganate to prepare carboxylic acid 36, which was coupled with N-hydroxysuccinimide to give NHS-diazirine 37.

Figure 60. Synthesis of 2,5-dioxopyrrolidin-1-yl 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate (37).

The reactive NHS-modified bi-layered SAMs were grafted to oxide-free silicon surfaces via a three-step chlorination-alkylation-carbene addition pathway. As such, a (111) silicon surface was first oxidized with Nanostrip to remove organic contaminants.
and then treated with 5% aq. HF solution to give an oxide-free H-terminated substrate. The prepared surface was reacted with a saturated solution of PCl$_5$ in chlorobenzene for 1 h at 105 °C to produce a chlorinated silicon surface (Figure 61). The XPS analysis of the chlorinated substrate showed the presence of chlorine atoms on the surface and also revealed the presence of a thin native oxide layer indicating high susceptibility of the chlorinated sample toward oxidation at ambient conditions.

![Figure 61. XPS spectra of the chlorinated silicon substrate](image)

After chlorination, the substrate was briefly washed with chlorobenzene and then immediately reacted with propenylmagnesium bromide in a sealed tube for at least 24 hours at 135 °C. The resulting methyl-terminated SAM showed very small contact angle hysteresis indicating formation of a highly ordered close-packed molecular system (Figure 62). The substrate showed no evidence of oxidation or degradation even after two weeks at ambient conditions and heating at 140 °C for more than 1 hour (by XPS). The lateral AFM analysis supported formation of a uniform SAM with very low surface roughness (Rq=0.366 nm, Ra=0.287 nm, Z range=3.34 nm).
Figure 62. XPS spectra and an LFM height image of the primary protective SAM

The protective primary SAM on oxide-free silicon was reacted with a 0.1 M solution of diazirine 37 in carbon tetrachloride under UV light at room temperature for 1 hour to form a secondary reactive overlayer containing NHS-activated acids. Following the reaction, the functionalized substrate was thoroughly washed with dichloromethane and isopropanol and kept under argon to prevent NHS hydrolysis. According to the AFM analysis, the prepared surface was uniform and showed very low roughness (Rq=0.320 nm, Ra=0.256 nm, Z range=2.42 nm). A contact angle hysteresis of 22° indicated the formation of a relatively loose-packed overlayer. Another possible origin of the observed hysteresis is the NHS-hydrolysis by water droplets during goniometry measurements. XPS analysis revealed the presence of fluorine atoms on the surface, confirming attachment of diazirine molecules to the primary protective SAM. The XPS Si 2p spectrum of the NHS-modified substrate showed no noticeable oxide peaks, again confirming remarkable stability of primary SAMs toward degradation and desorption (Figure 63).
The prepared molecular system, containing an inert close-packed primary monolayer and a reactive NHS-terminated secondary overlayer, simultaneously protects silicon from oxidation and SAM degradation and provides reactive sites for the selective attachment of (bio)organic molecules. According to literature data, this functionalization method achieves nearly complete termination of all surface-exposed Si atoms with Si-C bonds preventing oxygen and water migration towards the Si-SAM interface.  

2.9.2 Chemical functionalization of the NHS-modified SAMs

N-hydroxysuccinimide (NHS) is one of the most common activating reagents for carboxylic acids in chemistry and biochemistry. It is often used to conjugate diverse organic and biological substrates to chemically modified inorganic surfaces. By utilizing NHS ester moieties attached to the initial highly stable and dense SAM, we can passivate underlying Si-C interface towards chemical oxidation and protect all successive pattern modifications from degradation. The ability to directly pattern desirable chemical or biological functionalities to a stable polycrystalline silicon surface can be applied to
numerous areas in (bio)chemical sensing where the constant exposure of biological samples to aqueous solutions is required.\textsuperscript{91}

To demonstrate that NHS-modified substrates can be further derivatized with functional organic molecules, we reacted a freshly prepared NHS-SAM with mono Boc-protected ethanediamine. The reaction was carried out in dichloromethane solution for two hours at room temperature. After reaction, the Boc-modified substrate was extensively washed with dichloromethane and ethanol, and then analyzed by XPS to determine elemental composition. The XPS C 1s signal of the Boc-modified substrate showed a narrow peak at 286.5 eV, which corresponds to the presence of single C-C, C-O, C-N bonds in the surface-grafted SAM (Figure 64). At the same time, the intensity of the 286.5 eV peak in the initial NHS-modified substrate, which has a significantly smaller number of sp3 hybridized carbons, was significantly lower.
Deprotection of the Boc-modified substrate with a 25 % TFA solution in dichloromethane resulted in the reduction of the sp3 C1s peak intensity to a level intermediate between Boc- and NHS-functionalized substrates, suggesting selective removal of the Boc-group by the TFA solution. These secondary modifications of the NHS-modified SAMs did not result in silicon oxidation and formation of the native oxide layer, suggesting excellent chemical resistance and complete passivation of the underlying Si-SAM interface by the primary protective SAM (Figure 64). These results represent a significant improvement in the stability of the bi-layered SAMs grafted to
oxide-free silicon compared to previously described Boc-protected monolayers on gold, which show significant susceptibility towards desorption when exposed to TFA.

2.9.3 Si/SiO$_2$ masters and catalytic PUA stamps

The Si/SiO$_2$ master containing a pattern of 8 µm squares was prepared using photolithography. As such, a layer of silicon oxide (1000 nm) was deposited on a silicon wafer using plasma-enhanced chemical vapor deposition. Subsequently, a negative photoresist (Futurex NR9, 1600 nm) was spin-cast on the substrate, pre-baked at 155 °C for 70 seconds, and patterned by photolithography using a glass-Cr photomask. The patterned photoresist layer was then used as a mask in reactive ion etching to pattern the underlying silicon oxide layer. After RIE the remaining photoresist was removed with Nanostrip to give a patterned Si/SiO$_2$ master (Figure 65).
Figure 65. SEM images of the patterned sulfonic acid-modified stamp and the corresponding Si/SiO₂ master bearing 8 µm squares.

Patterned catalytic PUA stamps were prepared using the above described protocol. Briefly, components of the pre-polymeric mixture were reacted with 2-mercaptoethanesulfonic acid at 50 °C for 5 minutes, and then deoxygenated under vacuum at room temperature. The resulting mixture was cast against the Si/SiO₂ master, covered with a transparent glass slide, and polymerized under UV light at room temperature for 2 hours. Following polymerization, the stamp was peeled off from the master, washed extensively with ethanol and water, dried under a stream of filtered nitrogen, and kept at ambient temperature. Figure 65 shows SEM images of the patterned stamp and the master, indicating very high fidelity of the developed molding
technique. Featureless stamps were prepared in a similar manner by polymerizing pre-polymeric mixture between two flat glass slides. Catalytically inactive stamps were prepared by polymerizing initial PUA mixtures without reacting them with 2-mercaptoethanesulfonic acid.

2.9.4 Hydrolyzing efficiency of the catalytic PUA stamps

We have previously demonstrated that the sulfonic acid-modified PUA stamp almost instantly deprotects Boc-modified SAMs on gold and silicon with nearly 100 % efficiency. To determine the efficiency of the acidic stamp in hydrolysis of the NHS-modified SAMs, we reacted featureless catalytic and inactive stamps with NHS-terminated monolayers at room temperature for various intervals of time. Following reaction, the substrates were rinsed with isopropanol, dried with filtered argon, and analyzed by XPS. As is evident from Figure 66, the catalytic stamp decreased carbon concentrations in all reacted substrates by approximately the same proportion, whereas the inactive stamp did not bring any changes to the SAM composition. Moreover, the catalytic stamp achieved the same level of hydrolysis as a 1M HCl solution within just 5 minutes of stamp-substrate contact, indicating exceptionally high NHS-hydrolysis efficiency. The fluorine concentration in all analyzed samples remained constant and did not change from the concentration showed by the initial NHS-modified substrate, suggesting that all transformations induced by the catalytic stamp and the HCl solution were specific to the NHS groups and did not affect other components of the bi-layered molecular system.
Figure 66. XPS analysis of the NHS-hydrolysis efficiency with featureless catalytic stamps: direct measurements.

The hydrolysis efficiency of the catalytic PUA stamp was also evaluated via secondary reactions of the NHS-modified SAMs with perfluorinated alkyl amines (Figure 67). Stamp and HCl-hydrolyzed samples and unmodified NHS-SAM were reacted with a dichloromethane solution of pentadecafluorooctan-1-amine for 2 hours at room temperature. Subsequently, the carbon and fluorine concentrations of the samples were analyzed by XPS. Figure 66 and 67 show that all stamp- and HCl-modified SAMs showed essentially identical carbon and fluorine concentrations before and after reaction with perfluorinated amine, whereas the NHS-SAM showed a significant increase in both fluorine and carbon concentrations after the reaction with the fluorinated amine (Figure 67). These results not only confirm that the catalytic PUA stamp induces the same changes in the NHS-SAMs as does HCl solution, but also that neither stamp- or HCl-
hydrolyzed free acid SAMs react with primary amines without coupling reagents. As it will be demonstrated later, the observed difference in chemical reactivities of free acid- and NHS-terminated SAMs can be effectively used to selectively functionalize patterned NHS-substrates with heterobifunctional linkers and biomolecules, providing a very simple and efficient method for patterning and functionalizing passivated oxide-free silicon with a vast variety of functional organic and biological molecules.

Figure 67. XPS analysis of the NHS-hydrolysis efficiency with featureless catalytic stamps: secondary functionalization measurements.

The difference in reactivities of surface bound free acids and activated NHS esters was also demonstrated qualitatively by reacting the corresponding substrates with mono Boc-protected ethanediamine. As such, freshly prepared NHS-SAMs were first hydrolyzed with either a flat sulfonic acid-modified stamp or an aqueous HCl solution, and then reacted with a solution of mono Boc-protected diamine in
dichloromethane for 2 hours at room temperature. Subsequently, the C1s XPS spectra of the prepared samples were compared to the C1s spectrum of the Boc-terminated SAM prepared by reaction of NHS-SAMs with the same Boc-amine. Figure 68 shows that reaction of the surface-bound free acids did not bring about any qualitative changes in the XPS C1s spectra compared to the NHS-SAM, whereas the Boc-modified substrate showed a new peak at 286.5 eV that corresponds to the presence of sp3-hybridized carbon. Moreover, a control sample, prepared by sequentially reacting the propylene-terminated SAM with carboxylic acid-diazirine \textsuperscript{36} and mono Boc-protected ethanediamine, did not showed the 285.5 eV peak in the C1s spectrum and had identical features with the C1s spectra of other COOH-terminated SAMs. These results suggest that carboxylic acid-terminated SAMs, prepared either by hydrolyzing NHS-modified substrates or by directly functionalizing primary SAMs with carboxylic acid-diazirine molecules, do not react with primary amines without coupling reagents, whereas the NHS-terminated surfaces readily undergoes acyl addition/elimination transformation when exposed to solutions of free amines.
2.9.5 Patterned NHS-modified SAMs on passivated silicon

Previously, we have demonstrated that sulfonic acid-modified stamps can reproduce features on Boc- and TBS-functionalized gold substrates with sub-50 nm edge resolution, obviating the most significant limitations of PDMS-based methods related to stamp deformation and diffusive spreading. To evaluate the ability of a catalytic PUA stamp to replicate features on NHS-functionalized oxide-free silicon, we reacted a single catalytic stamp containing an array of 8 µm squares with NHS-terminated SAMs at room temperature for intervals of 1 to 90 minutes. During the stamp-substrate reaction, catalyst on the stamp hydrolyzed NHS groups in the places of conformal contact yielding patterned bifunctional substrates containing areas of activated and free carboxylic acids. Following each reaction, the stamp was washed with ethanol and water, dried under argon, and kept at ambient temperature prior to the next application. The
patterned silicon substrates were rinsed with isopropanol, dried under argon, and analyzed by SEM.

Figure 69 shows the efficiency of our technique during production of uniform bi-functional patterns on passivated silicon. The patterns were uniformly reproduced on all substrates using a single catalytic stamp, which did not lose its efficiency even after multiple applications. The generated features show evidence of edge distortion and were identical to each other despite differences in the stamping time, confirming a truly catalytic and specific nature of the developed patterning technique. The size and the shape of replicated features were identical to the size and the shape of features on the corresponding Si/SiO₂ master and the catalytic stamp, suggesting a very low degree of deformation in the catalytic PUA stamp.
Figure 69. SEM images of the patterned NHS-modified SAMs after reacting with the patterned catalytic stamp for 1, 5, and 30 minutes.

Patterned silicon substrates were also analyzed by lateral AFM. An NHS-SAM on silicon was reacted with the patterned catalytic stamp for 30 minutes at room temperature, rinsed with isopropanol, dried under a stream of filtered argon, and examined for tribological and morphological properties by lateral contact mode atomic force microscopy. Although AFM did not reveal any significant height differences between hydrolyzed and unmodified areas, it clearly established an apparent friction disparity between the NHS and free carboxylic acid regions (Figure 70), indicating a
chemical difference in the produced pattern and confirming a specific nature of the stamp-substrate reaction.

![Image](image.jpg)

**Figure 70. Lateral AFM images of the patterned NHS-modified SAMs after reacting with the patterned catalytic stamp for 30 minutes.**

Previously, we have demonstrated that NHS-modified SAMs on silicon readily react with primary amines and that the COOH-terminated SAMs do not undergo such transformation without coupling reagents. We therefore anticipated that patterned NHS-substrates would be amenable to chemoselective functionalization with organic molecules in a pattern-specific manner. This assumption was corroborated by reacting a patterned NHS SAM on silicon with mono Boc-protected ethandiamine at room temperature for two hours and by analyzing the resulting surface by SEM. Figure 71 shows that the substrate features did not change their morphology after reaction with amine, confirming pattern-specific attachment of primary amines selectively to the NHS-modified areas.
Figure 71. Secondary functionalization of the patterned NHS-modified SAMs and specificity of the catalytic stamp.

To further demonstrate that the acidic stamp selectively hydrolyzes NHS-groups and does not bring about any other changes to the bi-layered SAM on silicon, we conducted two sets of printing experiments with the patterned catalytic stamps. As such, we successively reacted the NHS-modified SAM first with the patterned catalytic stamp and then with a 1M HCl solution. Concurrently, another patterned catalytic stamp was applied to a free acid-terminated SAM, prepared by reacting a propylene-terminated monolayer with carboxylic acid-diazirine 36 (Figures 68). Figure 71 clearly shows that the stamp-induced pattern was completely erased with the HCl solution, while the catalytic stamp on the free acid-terminated SAM failed to replicate any
features. These results again suggest that the catalytic stamp selectively hydrolyzes NHS esters and does not affect other functional groups in the bi-layered SAM.

Previously, we have determined that the sulfonic acid-modified stamp can deprotect Boc-modified SAMs on gold and silicon. To demonstrate that the acidic catalytic stamp can be universally applied to pattern any Boc-modified surface, we reacted a multilayered Boc-terminated substrate (Figure 64) with a catalytic patterned stamp at room temperature for 30 minutes. Figure 72 shows that the stamp successfully transferred its pattern on the silicon surface by selectively deprotecting Boc-terminated SAMs in the places of conformal contact. The pattern was uniformly reproduced over the entire substrate area, creating features identical in size and shape to those on the corresponding Si/SiO₂ master. This experiment shows remarkable specificity and

Figure 72. SEM images of the Boc-protected SAMs patterned with the catalytic PUA stamp

Previously, we have determined that the sulfonic acid-modified stamp can deprotect Boc-modified SAMs on gold and silicon. To demonstrate that the acidic catalytic stamp can be universally applied to pattern any Boc-modified surface, we reacted a multilayered Boc-terminated substrate (Figure 64) with a catalytic patterned stamp at room temperature for 30 minutes. Figure 72 shows that the stamp successfully transferred its pattern on the silicon surface by selectively deprotecting Boc-terminated SAMs in the places of conformal contact. The pattern was uniformly reproduced over the entire substrate area, creating features identical in size and shape to those on the corresponding Si/SiO₂ master. This experiment shows remarkable specificity and
universality of the sulfonic acid-modified PUA stamp, proving that it can be used to rapidly and accurately reproduce bi-functional chemical patterns on a variety of surfaces and molecules, starting from simple monocomponent SAMs on gold to highly ordered and complex multilayered molecular systems grafted to inert oxide-free silicon.

2.9.6 Nano-patterned NHS-modified SAMs on passivated silicon (in collaboration with Briana N. Vogen)

To demonstrate that the proposed method obviates diffusive limitations of traditional µCP, we reacted a patterned catalytic stamp containing 250 and 500 nm lines with the NHS-modified silicon chips. The features were accurately transferred to the silicon surfaces, which would not be possible in the conventional µCP where the ink spreading is responsible for at least 50 nm enlargements of the feature edges. Figure 73 shows the efficiency of the developed technique to produce patterns across the entire substrate surface and generate nanoscale features identical in size to those on the PUA stamp (250 and 500 nm). Printed features have very high edge resolution (sub-50 nm), confirming the truly catalytic nature of inkless µCP. Notably, the same acidic stamp can be used multiple times without losing its catalytic efficiency.
2.9.7 Patterned functionalization of passivated oxide-free silicon with organic and biological molecules (in collaboration with Carleen J. Morris).

The main goal of this work was to develop a chemoselective method for patterning passivated silicon with (bio)organic materials. This task was achieved by selectively attaching nitrilotriacetic acid-terminated (NTA) heterobifunctional linkers to the patterned NHS-SAM using a difference in reactivities between the activated and free acids; and by utilizing the prepared surface as a template for the selective attachment of histidine-tagged proteins (Figure 74). A silicon surface was first oxidized in Nanostrip and then exposed to HF to give passivated H-terminated silicon (2). Substrate 2 was subsequently chlorinated and reacted with propenyl magnesium bromide to form a protective SAM 4. After functionalization with the NHS-diazirine and patterning with the catalytic PUA stamp, resulting surface 6 was reacted with a DMF-water solution of...
lysine-$N,N$-diacetic acid for 1 hour at room temperature. The prepared patterned NTA surface 7 was briefly exposed to a NiSO$_4$ solution and then to a 40 µm GFP solution for 1 hour at 0 °C to give a passivate silicon substrate patterned with GFP molecules. Figure 74 demonstrates the successful modification of the patterned substrates with the hexahistidine-tagged green fluorescent protein (GFP). The fluorescent micrograph clearly shows a difference in fluorescent intensity between the GFP-modified regions and free carboxylic acid regions, confirming the remarkable specificity and accuracy of the developed technique. Because specifically bound His-tagged protein can be removed from the surface with imidazole, in theory this process allows for the reversible immobilization of different histidine tagged proteins on the same NTA-patterned substrate. The protocol is not limited to His-tagged protein, but in combination with other heterobifunctional linkers can be used to pattern other biomolecules including DNA and antibodies.
Figure 74. Soft-lithographic patterning and functionalization of passivated silicon with organic and biological molecules (top). Fluorescent images of the GFP patterned substrate showing differences in fluorescent intensity between GFP-modified and free carboxylic acid areas.

The successful demonstration of catalytic µCP on oxide-free silicon provides great promise for applications in biomolecular recognition, chemical and biological sensing, organic electronic devices, and biomedical engineering. Conceptually, the described technique presents a universal method for patterning semiconductor surfaces with a broad range of inorganic, organic, biological, and polymeric materials. It provides precise control over the type and spatial distribution of the terminal functional groups in
the patterns by varying heterobifunctional linkers and carbene donors in the reactive overlayer, and simultaneously passivate an underlying inorganic surface with the highly ordered protective SAM. By successfully and accurately replicating uniform 100 nm features on oxide-free silicon, our method approaches resolution of the current photolithographic techniques, without relying on extremely expensive and delicate instruments. The ability to create patterned self-assembled organic-semiconductor interfaces without relying on expensive or energy-consuming devices will not only have a profound effect in fields such as electronics, nanotechnology, biochemistry and biophysics, but will also facilitate the understanding of fundamental issues of molecular self-assembly and the means by which individual molecules function in ordered two-dimensional systems.

3 Multicomponent inorganic Janus particles with controlled compositions, morphologies, and dimensions

3.1 Overview

Recently, Janus particles (JPs) have emerged as new colloidal structures with anisotropic properties that can be effectively used in a variety of applications. The non-symmetric structure of JPs brings novel physical properties and unusual aggregation behavior that makes these materials attractive candidates for pre-programmed self-assembled superstructures. JPs have been used as unique nano-sensors and nanoprobes, and as efficient microscopic mixers and emulsifiers. Bi-colored JPs have been applied to prepare switchable display devices; while small nanoscopic Janus particles have been proposed for applications in targeted drug delivery and advanced
bioimaging. In the past, morphologically anisotropic micrometer- and nanometer-size particles were used to produce highly ordered superstructures with properties precisely defined by non-covalent particle-particle interactions and by particle dimensions and shapes. It was also proposed that the assembly of these complex structures can be controlled through selective orthogonal functionalization of the particle’s non-symmetrical sides.

The synthesis of Janus particles with diverse shapes and compositions remains a challenging problem. Most approaches deposit metals on the top surface of polymeric beads immobilized on a flat substrate. Microfluidic polymerization systems and approaches based on the self-organization of triblock terpolymers are also used to prepare particles comprised of two or more different polymers. Unfortunately, most current synthetic techniques do not provide simple access to fully inorganic non-spherical Janus particles with precisely controlled compositions, dimensions, and morphologies. Considering the limited number of methods available for preparation of highly adjustable Janus particles, a new protocol that allows precise control over the particle’s shape and size and permits facile orthogonal functionalization of the particles with various organic materials on all non-symmetrical sides is needed.

3.2 Flat gold-silicon oxide Janus particle

Here we demonstrate that inorganic gold-oxide Janus particles, containing metallic and semiconductor layers on opposite sides, can be produced by a combination of simple photolithographic and etching techniques. The proposed pathway permits very good control over the particle composition and can be used to prepare particles
with anisotropic magnetic and/or light reflecting properties. At the same time, the size and the shape of the particles are controlled by the deposition conditions and by the photolithographic mask (Figure 75). The opposite sides of these particles can be orthogonally functionalized with organic, polymeric, and biological molecules using established surface-grafting protocols to achieve desired physical/chemical properties, while the shape and size of the particles, as well as the composition of the inner core, can all be easily controlled.

![Possible shapes of magnetic Au-oxide Janus particles](image)

**Figure 75. Gold-oxide Janus particles**

The principle of this approached is illustrated in Figure 76. First, an array of gold-nickel-titanium structures is fabricated on an oxidized silicon wafer using photolithography and physical vapor deposition. These structures are then covered with
a silicon oxide layer, which is subsequently patterned by reactive ion etching using a positive photoresist as an etching mask. The exposed gold-oxide particles can be easily removed from the wafer by sonication in ethanol because of the low adhesion force between the gold and the native oxide layer on the silicon wafer.²²⁹ The use of photolithography in this fabrication protocol inherits all advantages of this well-developed technique and permits preparation of Janus particles with virtually unlimited morphologies and compositions in a high precision, high throughput manner. Notably, the protocol produces ordered arrays of particles immobilized on a flat surface with their silicon oxide side facing up. This not only significantly alleviates orthogonal functionalization of the particles, but also allows for their selective patterning and labeling through various lithographic techniques, and for the precise compartmentalization of the JPs for applications in switchable devices.
Figure 76. Preparation of gold-oxide Janus particles

Figure 77 (top) shows an array of the gold-oxide particles on a silicon wafer after reactive ion etching and positive resist removal. These particles are 8 µm squares comprising layers of gold (500 nm), nickel (75 nm), titanium (75 nm), and silicon oxide (150 nm). The nickel layer gives these particles magnetic properties, while the titanium layer serves as an adhesion layer for the silicon oxide. As it can be seen from the SEM images the produced particles were 100% monodisperse and were prepared without any noticeable defects, moreover all particles remained intact on the wafer and were not disturbed or shifted during the etching.
The prepared particles were removed from the wafer by sonication in ethanol. Figure 77 confirms that all particles were successfully transferred from the wafer to the ethanol solution leaving behind small silicon posts resulting from the overetching. The particles in solutions can be easily washed, concentrated, and manipulated, due to the intrinsic magnetic properties of the nickel layer. Figure 77 shows gold-oxide particles on a clean gold surface. The images clearly indicate a difference between the gold and oxide sides and demonstrate uniform morphology and monodisperse size distribution of the particles after they were removed from the wafer.
### 3.3 Bent gold-silicon oxide Janus particles

The proposed method can also be adjusted to prepare bent particles. Intrinsic surface-induced stress of the nickel metal, which remains in the film after physical vapor deposition, can be used to control curvature of the disk-like gold-oxide Janus particles. The residual stress in the nickel films depends strongly on the thickness of the film and on the deposition rate. As such, by increasing the thickness of the nickel layer in our Janus particles and by using a higher deposition rate we were able to produce bent particles comprising gold, nickel, titanium, and silicon oxide layers (Figure 78).

![Diagram of preparation process](image)

**Figure 78. Preparation of bent gold-oxide Janus particles**

Figure 79 shows an array of the particles on the wafer after silicon oxide deposition. These particles are comprised of gold (300 nm), nickel (100 nm), titanium (100 nm), and silicon oxide (330 nm) layers. Due to the induced curvature, the particles
can be released from the wafer immediately after the deposition of oxide, with no additional photolithographic patterning and etching of the oxide layer required. The SEM image of the released particle (Figure 79) clearly demonstrates bent morphology of the particles and the difference between the gold and silicon oxide sides. After particle removal, the residual oxide layer on the supporting wafer (Figure 79) can be etched away and the wafer can be reused.

![Figure 79](image)

**Figure 79.** (Top) An array of the bent gold-oxide particles on a silicon wafer; (bottom left) silicon wafers after removal of the particles; (bottom right) detached bent gold-oxide particles.

### 3.4 Gold-titanium oxide Janus particles

On advanced instruments a photolithographic alignment can be used to create sub 100 nm objects, however in the majority of routine applications it restricts the
resolution to a micrometer region. To obviate this limitation we required a method for gold-oxide particle fabrication that does not rely on photolithographic alignment. Many metal oxides can support well ordered and robust self-assembled monolayers. Simple multicomponent metal-oxide particles can be produced on the silicon wafer by first, patterning multilayered metallic structures with the gold-silicon contact on the bottom and an aluminum or titanium layer on top, and then by oxidizing the top metallic layer with an oxygen plasma (Figure 80). This protocol does not require photolithographic alignment and gives a simple pathway towards submicron gold-oxide particles with precisely controlled morphologies and compositions.

Figure 80. Preparation of gold-titanium oxide Janus particles

Figure 81 shows 8 µm particles comprised of gold (500 nm), nickel (75 nm), and titanium (75 nm) layers on the supporting silicon wafer and on the gold chip. The top titanium layer of the particles was oxidized with an oxygen plasma. Subsequently, all particles were removed from the wafer by sonication in ethanol. Because this method
does not deposit silicon oxide on the wafer surface the wafer can be reused again without using a complicated cleaning procedure.

**Figure 81. Gold – titanium oxide Janus particles on a silicon wafer and on a gold chip.**

### 3.5 Orthogonal functionalization of metal-oxide Janus particles

Self-assembled monolayers can be effectively used to modify the chemical and physical properties of the surfaces supporting them. Silicon oxide and gold can support well ordered SAMs of organosilanes and thiols respectively. To orthogonally modify prepared Janus particles with two different SAMs on opposite sides, the silicon oxide side of the particles was first modified with a fluorinated silane through vapor deposition before the particles were released from the wafer. Subsequently, after removing the particles from the wafer, their gold side was reacted with a solution of
mercaptoundecanoic acid (Figure 82). This functionalization protocol was successfully applied to both gold-silicon oxide and gold-titanium oxide particles and produced particles with opposite hydrophilic and hydrophobic sides. Considering a large variety of commercially and synthetically available thiols and silanes this strategy can be applied to prepare particles bearing almost any given chemical functional group on either side.

To confirm that the oxide layers on both types of particles can support silane SAMs, we compared XPS carbon and fluorine signals from the wafers with and without particles after they were modified with a fluorinated silane. Table 4 shows that the fluorine and carbon signals decreased by approximately one quarter after the particles were removed from the wafer, which suggests that the deposited silicon oxide and the oxidized titanium layer can both support SAMs similar to the well-studied monolayers of silanes on native silicon oxide.

**Figure 82. Functionalization of gold-oxide particles.**

To confirm that the oxide layers on both types of particles can support silane SAMs, we compared XPS carbon and fluorine signals from the wafers with and without particles after they were modified with a fluorinated silane. Table 4 shows that the fluorine and carbon signals decreased by approximately one quarter after the particles were removed from the wafer, which suggests that the deposited silicon oxide and the oxidized titanium layer can both support SAMs similar to the well-studied monolayers of silanes on native silicon oxide.
Table 4. XPS fluorine and carbon signals of silane modified gold-silicon oxide and gold-titanium oxide particles supported on a silicon wafer.

<table>
<thead>
<tr>
<th></th>
<th>Wafer with Au-SiO&lt;sub&gt;2&lt;/sub&gt; particles</th>
<th>Wafer without Au-SiO&lt;sub&gt;2&lt;/sub&gt; particles</th>
<th>Wafer with Au-TiO&lt;sub&gt;2&lt;/sub&gt; particles</th>
<th>Wafer without Au-TiO&lt;sub&gt;2&lt;/sub&gt; particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;1s&lt;/sub&gt; / Si&lt;sub&gt;2p&lt;/sub&gt;</td>
<td>0.4591 / 100%</td>
<td>0.3389 / 74%</td>
<td>0.5681 / 100%</td>
<td>0.405 / 71%</td>
</tr>
<tr>
<td>C&lt;sub&gt;1s&lt;/sub&gt; / Si&lt;sub&gt;2p&lt;/sub&gt;</td>
<td>0.6351 / 100%</td>
<td>0.4259 / 67%</td>
<td>0.5292 / 100%</td>
<td>0.3594 / 68%</td>
</tr>
</tbody>
</table>

The ability to produce inorganic Janus particles with precisely controlled shape, size, and composition will give us an opportunity to develop several applications based on the non-symmetric structure of these particles. One of the most useful characteristics of the Janus particles is the ability to control chemical and physical properties of the opposite sides. By functionalizing sides with different molecules we can design methods for the side- and area-specific attachment of the gold-oxide JPs to various surfaces patterned with SAMs. Such methods, in combination with the ability to control particle assembly, will ultimately provide a pathway for the incorporation of the practical JPs into the desired functional systems.

The ability to produce and functionalize gold-oxide Janus nanoparticles should open a large area of potential applications in the target-specific imaging and molecule delivery. By functionalizing the gold side of the nanoparticles with the Raman-reporting or fluorescent molecules and their oxide side with the target-binding substrates it will be possible to image specific targets with such techniques as surface enhanced Raman spectroscopy and the plasmon enhanced fluorescence, which take advantage of both
the nanoscale size and the metallic composition of the gold-oxide JPs. Such research can potentially yield very useful bioimaging and small molecule delivery agents.
4. Experimental

4.1 Catalytic μCP on SAMs of Fmoc-protected aminothiols

All reagents and solvents were purchased from Sigma-Aldrich. Tetrahydrofuran, dichloromethane, and benzene were dried on the Aldrich solvent purification system; all other reagents and solvents were used as supplied. Thin-layer chromatography was performed on Merc Silica Gel 60 F254 aluminium plates using iodine vapors or CAM stain for spot visualization. Column chromatography was performed using Silicycle Silica-P Flash Silica Gel. $^1$H and $^{13}$C NMR spectra were recorded on a Varian 300 (300 MHz) spectrometer. XPS spectra were recorded on a Kratos Axis Ultra XPS spectrometer equipped with a mono-Al X-ray source. Gold substrates (~ 1 cm x 1 cm) were manufactured by coating silicon wafers with a 70Å chromium adhesion layer followed by a 230Å gold layer using an electron-beam metal evaporator (CHA Industries). Gold substrates were rinsed twice with water and absolute ethanol, and dried under a stream of filtered nitrogen prior to use. Patterned gold substrates were visualized by contact mode lateral force microscopy on a Veeco D3100 microscope using Veeco DNP-S silicon nitride probes with a 0.32 N/m spring constant (scan rate 0.996 Hz). Patterned polyurethane acrylate stamps were visualized by tapping mode atomic force microscopy on the Veeco D3100 microscope using Veeco TESP silicon probes with a 42 N/m spring constant (scan rate 0.996 Hz). Water contact angles were measured on a Rame-Hart NRL contact angle goniometer. SEM images were recorded on a FEI XL30 SEM-FEG microscope detecting secondary electrons at a 6 cm working distance (accelerating voltages: 2 kV for SAMs on gold and 1 kV for polyurethane acrylate stamps).
**Silicon-PMMA master fabrication.**

950 PMMA C2 (Microchem) was spun to a clean silicon chip at 3000 rpm for 40 seconds. Resulting substrate was baked on a digital hot plate at 160°C for 1 hour to produce a Si-PMMA chip with a 125-nm layer of the polymerized PMMA. Electron beam lithography was performed on this chip with the FEI XL30 SEM-FEG microscope equipped with a Nanometer Pattern Generation System at the following conditions:

- array 9*9: 100 µm*100 µm pattern
- magnification: 700
- center to center distance: 10 nm
- line spacing: 20 nm
- spot size: 3
- beam current: 137.7 pA
- dose value: 400 µC/cm²
- accelerating voltage: 30 kV
- working distance: 7.5 cm

After e-beam exposure, the substrate was developed in 1:3 MIBK:IPA mixture for 1 min and 20 sec, followed by a rinse in pure IPA and a rinse in water for 20 sec each to produce the silicon-PMMA master.

**Materials for SAM substrates.**

*Tert*-butyl 11-mercaptoundecylcarbamate (8) was synthesized from 11-aminoundecanoic acid (3) in five steps following a previously reported procedure.\(^{157}\) In short, acid 3 was reduced with LiAlH\(_4\) to the corresponding alcohol 4, protected with di-
t-butyldicarbonate, and converted to bromide 6 with NBS and PPh3. Subsequently, compound 6 was converted into thiol ester 7 and reduced to give desired Boc-protected aminothiol 8.

**11-Aminoundecanol (4).** 11-Aminoundecanoic acid (23.6 g, 117 mmol) was added slowly at -20 °C to a solution of LiAlH4 (154 mmol) in freshly distilled THF (250 mL) and the reaction mixture was stirred for 6 hours at 67 °C. The solution was cooled to 0 °C, treated with a NaOH solution (10%, 10 mL) and H2O (20 mL) and stirred for 30 min at 23 °C. The reaction mixture was filtered, dried over MgSO4, and concentrated under reduced pressure to provide the desired 11-aminoundecanol (6.4 g) in 30 % yield. 1H NMR: δ = 1.24–1.35 (m, 14H), 1.40 (m, 2H), 1.54 (quin, 2H, J=6.46 Hz), 2.64 (t, 2H, J=6.94 Hz), 3.59 (t, 2H, J=6.59 Hz).

**Tert-butyl 11-hydroxyundecylcarbamate (5).** Triethylamine (4.80 mL, 34.5 mmol) and di-tert-butyl dicarbonate (7.5 g, 34.4 mmol) were added at 0 °C to a solution of 11-aminoundecanol (6.4 g, 34.2 mmol) in freshly distilled CH2Cl2 (150 mL) and the reaction mixture was stirred for 1.5 hours at 0 °C and another 6 hours at 23 °C. The resulting solution was washed with water (100 mL) and sat. NaCl solution (100 mL). The organic phase was dried over MgSO4, filtered and concentrated under reduced pressure. Purification of the crude material by flash chromatography, eluting with 30% AcOEt in hexanes, gave 89% yield of tert-butyl 11-hydroxyundecylcarbamate (8.4 g). 1H NMR: δ = 1.24–1.35 (m, 15H), 1.42–1.47 (m, 10H), 1.57 (quin, 2H, J=6.87 Hz), 1.70 (s, 1H), 3.09 (q, 2H, J=6.59 Hz), 3.63 (t, 2H, J=6.59 Hz), 4.58 (s, 1H).
**Tert-butyl 11-bromoundecylcarbamate (6).**¹⁵⁷ *N*-Bromosuccinimide (5.22 g, 29.3 mmol) and triphenylphosphine (7.73 g, 29.5 mmol) were added to a solution of tert-butyl 11-hydroxyundecylcarbamate (8.35 g, 29.1 mmol) in freshly distilled benzene (110 mL) and the reaction mixture was stirred for 11 hours at 23 °C. The resulting solution was washed with water (100 mL) and sat. NaCl solution (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography, eluting with 10% AcOEt in hexanes, gave 77% yield of tert-butyl 11-bromoundecylcarbamate (7.82 g). ¹H NMR: δ = 1.16–1.25 (m, 12H), 1.31–1.42 (m, 13H), 1.78 (quin, 2H, J=7.28 Hz), 3.02 (q, 2H, J=6.32 Hz), 3.33 (t, 2H, J=6.87 Hz), 4.51 (s, 1H).

**(S)-11-(Tert-butoxycarbonyl)undecyl-ethanethioate (7).**¹⁵⁷ Thioacetic acid (2.15 mL, 30.1 mmol), triethylamine (4.1 mL, 30.9 mmol) and 4-DMAP (0.27 g, 2.21 mmol) were added to a solution of tert-butyl 11-bromoundecylcarbamate (7.77 g, 22.2 mmol) in freshly distilled CH₂Cl₂ (150 mL) and the reaction mixture was stirred for 24 hours at 22 °C. The solution was washed with a saturated Na₂CO₃ solution (100 mL), water (100 mL), and sat. NaCl solution (100 mL). The organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure. Purification of the crude material by flash chromatography, eluting with 10% AcOEt in hexanes and subsequent crystallization (n-pentane), gave 80% yield of (S)-11-(tert-butoxycarbonyl)undecyl-ethanethioate (6.1 g). ¹H NMR: δ = 1.22–1.36 (s, 14H), 1.41–1.48 (s, 11H), 1.57 (quin, 2H, J=7.28 Hz), 2.32 (1, 3H), 2.86 (t, 2H, J=7.55 Hz), 3.10 (q, 2H, J=6.59 Hz), 4.51 (s, 1H).
**Tert-butyl 11-mercaptopoundecylcarbamate (8).** A hydrazine solution (1M in THF, 100 mL, 0.1 M) was added at 0 °C to a solution of 7 (4.00 g, 11.6 mmol) in CH₂Cl₂ (80 mL) and the reaction mixture was stirred for 5 hours at 23 °C. The solvent was concentrated under reduced pressure and the residue dissolved in CH₂Cl₂ (200 mL). The resulting solution was washed with a saturated NH₄Cl solution (200 mL) and water (50 mL). The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure. Purification by column chromatography on silica gel, eluting with 10 % AcOEt in hexane, gave 95 % yield of pure tert-butyl 11-mercaptopoundecylcarbamate (3.34 g). ¹H NMR: δ = 1.19–1.53 (m, 25H), 1.61 (quin, 2H, J=7.25 Hz), 2.52 (q, 2H, J=7.28 Hz), 3.10 (q, 2H, J=6.50 Hz), 4.60 (s, 1H).

Fmoc-protected aminothiol 2 was prepared from 8 in three steps. Thiol 8 was oxidized to the disulfide and deprotected with 4M HCl in dioxane to give diamine 9, which was then reacted with Fmoc-NHS to produce 10. Compound 10 was reduced with Zn dust and TFA in dichloromethane-methanol solution at room temperature to give (9H-fluoren-9-yl)methyl 11-mercaptopoundecylcarbamate (2).

**11,11'-Disulfanediyldiundecan-1-amine (9).** A 10 M solution of iodine in MeOH was slowly added to a solution of 8 (0.303 g, 1 mmol) and KI (0.015 g) in MeOH (10 ml) at room temperature with stirring until a brown color persisted. The resulting mixture was stirred at room temperature for 30 min. A Na₂SO₃ 10 % aq. solution was added to the reaction mixture until it turned colorless. Solvents were evaporated under reduced pressure. The remaining residue was dissolved in CH₂Cl₂ (20 ml) and washed with aqueous solutions of 1M HCl (2×20 ml), 10 % KHCO₃ (2×20 ml), and brine (20 ml). The
organic fraction was dried over MgSO₄ and evaporated under reduced pressure to give an intermediate Boc-protected disulfide, which was reacted with 4M HCl in dioxane (7 ml). The dioxane solution was stirred at room temperature for 2 hours, dioxane was evaporated under reduced pressure, and the resulting salt was washed with Et₂O (2×10 ml) and n-pentane (2×10 ml) to give 9 (0.15 g, 0.31 mmol), which was used in the next step without purification. Yield: 31 %.

(9H-Fluoren-9-yl)methyl 11-mercaptoundecylcarbamate (2). Fmoc-NHS (0.184 g, 0.546 mmol) and Et₃N (0.18 ml, 1.3 mmol) were added to a solution of 9 (0.13 g, 0.273 mmol) in CH₂Cl₂ (15 ml). The reaction mixture was stirred at room temperature for 4 hours, then washed with aqueous solutions of 1M HCl (2×20 ml), 10 % KHCO₃ (2×20 ml), and brine (20 ml). The organic fraction was dried over MgSO₄ and evaporated under reduced pressure to give 10, which was used in a subsequent reaction without additional purification.

Disulfide 10 was reduced to thiol 2 following previously published protocol. In short, 10 was dissolved in a mixture of CH₂Cl₂ (8 ml), MeOH (25 ml), and TFA (0.842 mmol, 0.842 ml). Zn dust (0.546 mmol, 0.039 g) was added to the resulting solution and the reaction mixture was stirred overnight at room temperature. The solution was filtered to remove the zinc metal and concentrated under reduced pressure. The residue was dissolved in EtOAc (20 ml) and washed with aqueous solutions of 1M HCl (3×15 ml), 10 % KHCO₃ (3×15 ml), and brine (20 ml). The organic fraction was dried over MgSO₄ and concentrated under reduced pressure. Purification by column chromatography on silica gel, eluting with 30 % AcOEt in hexane, gave pure 2 (0.073 g, 0.1 mmol). Yield 32 %, mp
69-70 °C. $^1$H NMR: $\delta = 1.37$ (m, 17H), 1.61 (quintet, 2H, $J=6.59$ Hz), 2.51 (q, 2H, $J=7.28$ Hz), 3.18 (q, 2H, $J=7.55$ Hz), 4.22 (t, 1H, $J=6.87$ Hz), 4.41 (d, 2H, $J=6.87$ Hz), 4.73 (br. s, 1H), 7.31 (t, 2H, $J=7.55$ Hz), 7.40 (t, 2H, $J=7.42$ Hz), 7.59 (d, 2H, $J=7.28$ Hz), 7.77 (d, 2H, $J=7.42$ Hz).

11-Aminoundecane-1-thiol hydrochloride (11). $^{157}$ Compound 8 (1.00 g, 3.30 mmol) was dissolved in a HCl solution in dioxane (4 M, 20.0 mL, 80 mmol) and the reaction mixture was stirred for 3 hours at room temperature. The solvent was evaporated under reduced pressure and the solid washed with diethyl ether (50 mL) and $n$-pentane (50 mL) to provide pure 11-aminoundecan-1-thiol hydrochloride (0.78 g) in 99 % yield. $^1$H NMR: $\delta = 1.17–1.40$ (m, 14H), 1.52 (m, 4H), 2.20 (t, 1H, $J=7.42$ Hz), 2.45 (q, 2H, $J=7.14$ Hz), 2.73 (m, 2H), 7.91 (br. s, 3H).

Materials for polyurethane acrylate (PUA) stamps.

Diacrylate 16. $^{161}$ Polyethylene glycol 13 (av. Mw = 400, 35.46 ml, 0.1 mol) was slowly added dropwise at 50 °C to a solution of isophorone diisocyanate (12, 41.89 ml, 0.2 mol), tin octoate (0.064 ml), and 2,2'-methylene-bis(4,6-di-tert-butylphenol) (0.09 g). The reaction mixture was stirred for 5 hours at 55 °C. The temperature of the reaction was raised to 85 °C and hydroxypropyl acrylate (15, mixture of isomers, 25.55, ml, 0.205 mol) was added dropwise. The reaction mixture was stirred for additional 4 hours at 85 °C to give diacrylate 16.

Prepared acrylate 16 was diluted by 30 % with trimethylolpropane ethoxylate triacrylate (17, av. MW 912 g/mol) to reduce viscosity. Photoinitiators 18 and 19 were
added to the reaction mixture, and the resulting solution was polymerized between two
glass microscope slides (Stamp I) or a glass slide and a silicon-PMMA master containing
~14 μM features (Stamp II) by exposure to UV light for 24 hours at room temperature.
After polymerization, stamps were washed with EtOH for at least one hour, rinsed with
EtOH and H₂O, and dried with filtered nitrogen. The unfunctionalized stamps were fairly
rigid; nevertheless, the flexibility of the stamps was dramatically better at 50 °C.

Piperidine-functionalized PUA stamps were prepared in a similar manner. Five
milliliters of the prepolymeric mixture, containing 16 (67 wt%), 17 (30 wt%), 18 (1.5
wt%), and 19 (1.5 wt%), were combined with 400 µL of 2-aminomethyl piperidine (1),
heated with stirring at 60 °C for 10 minutes, and polymerized between two glass slides
under UV light (24 hours) to give featureless PUA Stamp III modified with piperidine
fragment. Stamp IV-VI containing micron- and submicron features were prepared by
polymerizing the prepolymeric mixture containing 1 between the corresponding silicon-
PMMA masters and a glass slide. Prepared piperidine modified stamps were washed
with EtOH for at least one hour, rinsed with EtOH and H₂O, and dried with filtered
nitrogen. The shape and size of the stamp features were identical to those of the
corresponding masters, were unaffected during storage at room temperature for
several days, and retained their integrity even after heating to 70 °C and cooling to
room temperature.
**SAMs formation and stamping experiments.**

**Substrate A1** (Au-S-(CH$_2$)$_{11}$-NH-Fmoc). A pre-cleaned gold surface was soaked at room temperature in a 1 mM EtOH solution of Fmoc-protected aminothiol 2 for at least two hours. The prepared substrate was washed with EtOH, H$_2$O, EtOH, and dried with filtered nitrogen.

**Substrate A2** (Au-S-(CH$_2$)$_{11}$-NH$_2$).$^{232}$ Fmoc-modified **Substrate A1** was soaked in a 1M DMSO solution of piperidine for 45 minutes. The prepared substrate was washed with DMSO, EtOH, H$_2$O, EtOH, and dried with filtered nitrogen.

**Substrate A3–A5**, general procedure. Fmoc-modified **Substrate A1** and a corresponding polyurethane acrylate stamp were preheated on a hot plate to 50 °C. The gold substrate was placed on the top of the stamp, and they were held together for 3 hours at 50 °C. After the reaction the stamp and the substrate were separated and cooled down to room temperature. The substrate was washed with DMSO, EtOH, H$_2$O, EtOH, and dried with filtered nitrogen. The stamp was washed in EtOH for at least one hour, rinsed with EtOH, H$_2$O, EtOH, dried with filtered nitrogen, and kept at room temperature.

**Substrate B0** (Au-S-(CH$_2$)$_{11}$-NH$_2$).$^{232}$ Pre-cleaned gold surface was soaked at room temperature in a 1 mM EtOH solution of aminothiol 11 for at least two hours. Prepared substrate was washed with EtOH, H$_2$O, EtOH, and dried with filtered nitrogen.

**Substrate B1** (Au-S-(CH$_2$)$_{11}$-NH-Fmoc).$^{232}$ Fmoc-protection of the NH$_2$-terminated monolayer was achieved by exposing **Substrate B0** to an Fmoc-NHS solution (3 mM in 1:1 DMSO:100mM triethanolamine) for 45 minutes at room temperature. Prepared
substrate was washed in DMSO for 5 minutes, rinsed with EtOH and H2O, and dried with filtered nitrogen.

**Substrates B2–B5** were prepared by following procedures for the preparation of corresponding **Substrates A2–A5**.

### 4.2 Catalytic μCP on SAMs of Boc- and TBS-protected thiols

All reagents and solvents were purchased from Sigma-Aldrich. Tetrahydrofuran, dichloromethane, and benzene were dried on the Aldrich solvent purification system; all other reagents and solvents were used as supplied. Thin-layer chromatography was performed on Merc Silica Gel 60 F254 aluminium plates using iodine vapors or CAM stain230 for spot visualization. Column chromatography was performed using Silicycle Silica-P Flash Silica Gel. ¹H and ¹³C NMR spectra were recorded on a Varian 300 (300 MHz) spectrometer. XPS spectra were recorded on the Kratos Axis Ultra XPS spectrometer equipped with a mono-Al X-ray source. Gold substrates (~ 1 cm x 1 cm) were manufactured by coating silicon wafers with a 70Å chromium adhesion layer followed by a 230Å gold layer using an electron-beam metal evaporator (CHA Industries). Prior to use gold substrates were cleaned with oxygen plasma for 3 minutes at 100 watts and 6×10⁻¹ mbar O₂ pressure (Emitech K-1050X plasma asher). Patterned gold substrates were visualized by the contact mode lateral force microscopy on the Veeco D3100 microscope using Veeco DNP-S silicon nitride probes with a 0.32 N/m spring constant. Water contact angles were measured on the Rame-Hart NRL contact
angle goniometer. SEM images were recorded on the FEI XL30 SEM-FEG microscope detecting secondary electrons at 6 cm working distance.

**Silicon-PMMA master fabrication**

950 PMMA C2 (Microchem) was spun on a clean silicon chip at 3000 rpm for 40 seconds. Resulting substrate was baked on a digital hot plate at 180°C for 2 minutes to produce a Si-PMMA chip with a 125-nm layer of the polymerized PMMA. Electron beam lithography was performed on the Elionix ELS-7500 EX lithography system at 50 kV and 50 pA on a 60000 dots 300 µm field at a dose value of 350 µC/cm². After e-beam exposure, the substrate was developed in 1:3 MIBK:IPA mixture for 1 min and 20 sec, followed by a rinse in pure IPA and water (20 sec each) to produce a patterned silicon-PMMA master.

**Synthesis of Boc- and TBS-functionalized thiols and SAMs formation**

*Tert*-butyl 11-mercaptopoundecylcarbamate (8) was synthesized from 11-aminoundecanoic acid in five steps following a previously reported procedure.\(^\text{157}\)

11-(2,3,3-Trimethylbutan-2-yloxy)undecane-1-thiol (21).\(^\text{233}\) A solution of 11-mercaptopoundecan-1-ol (0.408 g, 2 mmol), *t*-butyldimethylsilyl chloride (0.301 g, 2 mmol), triethylamine (0.4 ml, 2.87 mmol), and 4-dimethylaminopyridine (0.042 g, 0.34 mmol) in dichloromethane (5 ml) was stirred at room temperature for 4 hours. The reaction mixture was poured on diethyl ether (40 ml), washed with 10% aq. NaHSO\(_4\) (2×10 ml) and 10% aq. K\(_2\)CO\(_3\) (10 ml), dried with MgSO\(_4\), and concentrated under reduced pressure. The resulting crude product was purified on a silica gel column using
an ethyl acetate – hexane mixture (1:9) as an eluent (RF value = 0.85). Yield 87% (0.553 g, 1.74 mmol), colorless liquid. $^1$H NMR (acetone d$_6$): δ = 0.04 (s, 6H), 0.89 (s, 9H), 1.30 – 1.66 (m, 19H), 2.50 (q, 2H, J=7.55 Hz), 3.62 (t, 2H, J=6.18 Hz). $^{13}$C NMR (acetone d$_6$): -5.23, 24.74, 26.22, 26.49, 28.99, 29.25, 29.51, 29.76, 30.02, 30.19, 30.28, 30.53, 34.81, 63.52.

**Monolayer formation.** Freshly prepared or oxygen plasma cleaned gold surfaces were soaked at room temperature in a 1 mM ethanol solution of Boc- or TBS-protected thiols for at least 20 hours. The prepared substrate was thoroughly rinsed with ethanol and water, and dried under a stream of filtered nitrogen.

**Preparation of acidic polyurethane–acrylate stamps**

Sodium 2-mercaptoethanesulfonate (0.2 g, 1.22 mmol) was added to a 4N HCl solution in dioxane (10 ml), and the reaction mixture was stirred at room temperature for 30 minutes. Sodium chloride was filtered off first through a fine glass filter and then through a 0.2 μm PTFE membrane syringe filter to afford clear solution of 2-mercaptoethanesulfonic acid (20) in dioxane. Dioxane was evaporated under reduced pressure and the resulting sulfonic acid 20 was reacted with 2 ml of the polyurethane-acrylate prepolymeric mixture at room temperature and then under vacuum at 50 °C. After the mixture was free from trapped air bubbles, the resulting solution was cooled down to room temperature and polymerized between two glass microscope slides or a glass slide and a master by exposure to UV light for 2 hours at room temperature. After polymerization, the stamps were washed with EtOH for at least 30 minutes, rinsed with
EtOH and H₂O, and dried with filtered nitrogen. Inactive polyurethane-acrylate stamps were prepared in a similar manner by directly polymerizing prepolymeric mixture of 16, 17, 18, and 19 without sulfonic acid 20.

**Stamping protocol**

A corresponding polyurethane-acrylate stamp was placed on the top of the Boc- or TBS-modified substrate at room temperature for a required interval of time with no external load to hold them together. After the reaction the stamp and the substrate were separated. The substrate was washed with EtOH, H₂O, EtOH, and dried with filtered nitrogen. The stamp was rinsed with EtOH, H₂O, EtOH, dried with filtered nitrogen, and kept at room temperature before the next application.

**4.3 Application of catalytic µCP on oxide-free silicon**

All reagents and solvents were purchased from Sigma-Aldrich and were used as supplied. Thin-layer chromatography was performed on Merc Silica Gel 60 F₂₅₄ aluminium plates using iodine vapors or CAM stain²³⁰ for spot visualization. Column chromatography was performed using EMD Silica Gel 60 40–63 μM. ¹H and ¹³C NMR spectra were recorded on a Varian 300 (300 MHz) spectrometer. XPS spectra were recorded on the Kratos Axis Ultra XPS spectrometer equipped with a mono-Al X-ray source. SEM images were recorded on the FEI XL30 SEM-FEG microscope detecting secondary electrons at 6 cm working distance.
Synthesis of alkene 26 and SAMs formation

**tert-Butyl 3,6,9,12,15-pentaoxahexacos-25-enylcarbamate (26).** NaH (381.2 mg of a 60% suspension in oil, 9.53 mmol) was added to a THF solution (30 mL) of pentaethylene glycol (10.09 ml, 47.7 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min and then held at 80 °C for 2 h under an argon atmosphere. 11-Bromo-1-undecene (1.88 ml, 8.58 mmol) was added to the solution, and the reaction mixture was stirred at 80 °C for 12 h. The resulting solution was cooled to room temperature, concentrated under vacuum, diluted with hexane (300 mL), and washed with brine (2×100 ml). The hexane layer was dried over MgSO₄, concentrated in vacuum, and purified by column chromatography (elution with 5% methanol in hexane) to give product 23 in 73% yield. ¹H NMR: δ = 5.90-5.74 (m, 1H), 5.05-4.90 (m, 2H), 3.77-3.55 (m, 20H), 3.44 (t, 2H, J=6.73 Hz), 2.54 (t, 1H, J=6.46 Hz), 2.10-1.98 (m, 2H), 1.64-1.50 (m, 2H), 1.44-1.20 (m, 12H).

Diphenyl phosphorazidate (0.864 g, 4 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.597 ml, 4 mmol) were added to a DMF solution (15 ml) of alcohol 23 (0.78g, 2 mmol) and the solution was stirred at room temperature for 2 hours. Subsequently, sodium azide (0.325 g, 5 mmol) was added to the reaction mixture, which was allowed to react at 60 °C overnight. The precipitate was filtered off, the resulting solution was concentrated in vacuum, and the residue was redisolved in ethyl acetate (150 ml). The organic solution was washed with brine (4×100 ml), dried over MgSO₄, and concentrated in vacuum. The crude product was purified by column chromatography (elution with 1:1 ethyl acetate:hexane mixture) to give 24 in 50% yield. ¹H NMR: δ =
A THF solution (1 ml) of 24 (0.387 g, 0.932 mmol), triphenylphosphine (0.262 g, 1 mmol), and water (0.027 ml, 1.5 mmol) was stirred at room temperature for 4 hours. Subsequently, the reaction mixture was concentrated in vacuum and redisolved in a small amount of dichloromethane (2 ml). The resulting mixture was transferred on a short dry silica gel column, which was subsequently eluted with dichloromethane (60 ml). The column was dried, washed with an ethyl acetate–hexane mixture (1:1 v/v ratio, 60 ml), and dried again. The product was eluted from the column with a dichloromethane–methanol solution (1:1 v/v ratio, 60 ml), which was concentrated in vacuum to give a mixture of triphenylphosphine and compound 25 in 2/3 ratio. The product was used in the next step without further purification. 

**1H NMR:** δ = 5.85-5.67 (m, 1H), 5.05-4.84 (m, 2H), 3.70-3.56 (m, 16H), 3.45 (t, 2H, J=5.36 Hz), 3.45 (t, 2H, J=6.73 Hz), 2.87 (t, 2H, J=5.36 Hz), 2.10-1.98 (m, 2H), 1.64-1.50 (m, 2H), 1.45-1.16 (m, 12H).

Amine 25 (0.866 mmol) and di-tert-butyl dicarbonate (0.218 g, 1 mmol) were added to a solution of triethylamine (0.139 g, 2 mmol) in dichloromethane (5 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and then at room temperature for additional 1.5 hours. The resulting organic solution was washed with saturated NH₄Cl solution (15 ml), water (15 ml), and brine (15 ml), and was subsequently dried over MgSO₄ and concentrated in vacuum. The crude product was purified by column chromatography (elution with 1:1 ethyl acetate:hexane mixture) to give 26 in 89% yield. 

**1H NMR:** δ = 5.90-5.74 (m, 1H), 5.12-4.88 (m, 3H), 3.76-3.50 (m, 18H), 3.45 (t, 2H, J=5.36 Hz), 2.87 (t, 2H, J=5.36 Hz), 2.10-1.98 (m, 2H), 1.64-1.50 (m, 2H), 1.45-1.16 (m, 12H).
J=6.87), 3.36-3.26 (m, 2H), 2.09-1.98 (m, 2H), 1.64-1.51 (m, 2H), 1.49-1.24 (m, 21H). 13C NMR 156.18, 139.42, 114.30, 79.29, 71.72, 70.79, 79.71, 70.41, 70.23, 40.55, 33.98, 29.84, 29.80, 29.71, 29.64, 29.61, 29.30, 29.10, 28.61, 26.26.

**Monolayer formation.** A silicon (111) chip (10×10 mm) was consecutively soaked in a Nanostrip solution at 75 °C for 15 min, in a 4% HF solution at room temperature for 4 minutes and then again in the Nanostrip solution at 75 °C for 15 min to remove all organic components and to produce uniform layer of native oxide. Subsequently, the surface was rinsed with water and soaked in the 4% HF solution at room temperature for 4 minutes to remove the native oxide layer and produce the H-terminated substrate. The surface was immediately covered with the mixture of alkenes 26 and 27 (1/4 v/v ratio, 10 µL) and transferred into a nitrogen-filled glove box, were it was irradiated with UV light for 2 hours at room temperature (UVP 11sc lamp, 4400 µC/cm² at 2 cm distance). After the reaction, the substrate was rinsed with EtOH and CH₂Cl₂, sonicated in CH₂Cl₂ for 5 minutes, rinsed again with EtOH and H₂O, and dried under the stream of filtered argon.

**Acidic polyurethane-acrylate stamps**

Patterned and featureless catalytic polyurethane-acrylate stamps were prepared following the previously described protocol (see sub-chapter “3.2 Catalytic µCP on SAMs of Boc- and TBS-protected thiols”).
**Stamping protocol**

A corresponding polyurethane-acrylate stamp was placed on top of a Boc-modified substrate at room temperature for a required interval of time with no external load to hold them together. After the reaction the stamp and the substrate were separated. The substrate was washed with EtOH, H₂O, EtOH, and dried with filtered argon. The stamp was rinsed with EtOH, H₂O, EtOH, dried with filtered argon, and kept at room temperature before the next application.

**4.4 Universal bi-layered patterning technique**

All reagents and solvents were purchased from Sigma-Aldrich and used as supplied. Ethanol, isopropanol and deionized water were filtered through 0.2 µm filter before use. Thin-layer chromatography was performed on Merc Silica Gel 60 F₂₅₄ aluminium plates using iodine vapors or CAM stain²³⁰ for spot visualization. Column chromatography was performed using EMD Silica Gel 60 40–63 µM. ¹H and ¹³C NMR spectra were recorded on a Varian 300 (300 MHz) spectrometer. XPS spectra were recorded on the Kratos Axis Ultra XPS spectrometer equipped with a mono-Al X-ray source. Water contact angles were measured on the Rame-Hart NRL contact angle goniometer. Patterned substrates were visualized by the contact mode lateral force microscopy on the Veeco D3100 microscope using Veeco DNP-S silicon nitride probes with a 0.32 N/m spring constant. SEM images were recorded on the FEI XL30 SEM-FEG microscope detecting secondary electrons at 6 cm working distance. GFP-patterned substrates were imaged using a Zeiss Axio Imager widefield fluorescence microscope.
with a mercury arc lamp (Zeiss HBO100). Images were taken using a green filter cube set and a 40x/0.75 DIC objective with 4 second exposure time. The plasmid pEGFP was purchased from BD Biosciences. Restriction enzymes, Antartic phosphatase and T4 Ligase were purchased from New England Biolabs. BL21(DE3) singles, pET30b and Paint Pellet were obtained from Novagen. XL10 Gold cells were purchased from Stratagene. Qiaquick gel extraction kit and Miniprep kit were purchased from Qiagen. Dialysis tubing and Bradford Plus reagent was purchased from Pierce. Nickel chelating resin was manufactured by GE Healthcare. The lysis was performed by the Emulsiflex C-5 high pressure homogenizer. The protein was purified on the Amersham Bioscience AKTA FPLC.

**Master fabrication**

Silicon-PMMA masters containing 1000, 500 and 250 nm lines were prepared following the previously described protocol (see sub-chapter “3.2 Catalytic µCP on SAMs of Boc- and TBS-protected thiols”).

**Stamp fabrication**

Patterned and featureless catalytic polyurethane-acrylate stamps were prepared following the previously described protocol (see sub-chapter “3.2 Catalytic µCP on SAMs of Boc- and TBS-protected thiols”).
NHS-modified diazirine synthesis

2,5-Dioxopyrrolidin-1-yl 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate was prepared from (4-bromophenyl)methanol in 9 steps following the previously published protocol.234-235

\[ \text{(4-Bromobenzyl)oxy}(2,3\text{-dimethylbutan-2-yl})\text{dimethylsilane} \quad (29). \]

Bromobenzylalcohol (28) (49.8 g, 266 mmol) was slowly added to a stirred solution of thexyldimethylsilyl chloride (TDSCI) (50 g, 280 mmol) and imidazole (27.2 g, 400 mmol) in anhydrous DMF (115 mL). The reaction mixture was stirred for 3 hour at room temperature, quenched with ice-cold water (160 mL), and extracted with hexane (4×100 mL). The extracts were washed with water (3×80 mL) and brine (80 mL), and dried over anhydrous sodium sulfate. The reaction mixture was concentrated under vacuum and then dried under high vacuum to give 29 (90.2 g), which was used for the next step without purification

1-(4-(((2,3-Dimethylbutan-2-yl)dimethylsilyl)oxy)methyl)phenyl)-2,2,2-trifluoroethanone (30). An n-BuLi solution (2.5 M in hexane) (103 mL, 216 mmol) was added dropwise to a solution of 29 (58.93 g, 181 mmol) in anhydrous THF (245 mL) at –78°C. The reaction mixture was stirred at –78°C for 2 hours and then methyl trifluoroacetate (25 g, 195 mmol) added dropwise. The reaction was stirred at at –78°C for additional 2 hours and was then allowed to reach –30°C. Saturated NH₄Cl solution (360 mL) was added to the reaction mixture at –30°C. The lower aqueous layer was extracted with ether (2×180 mL) and combined with the previously separated upper organic layer. Combined organic extracts were washed with saturated NH₄Cl solution
(2×90 mL) and brine (2×90 mL), and dried (Na₂SO₄). The reaction was concentrated under vacuum to give 30 (58.6 g, 171 mmol), which was used for the next step without further purification.

1-(4-(((2,3-Dimethylbutan-2-yl)dimethylsilyl)oxy)methyl)phenyl)-2,2,2-trifluoroethanone oxime (31). Hydroxylamine hydrochloride (35.6 g, 512 mmol) was added to a solution of 30 (58.6 g, 171 mmol) in dry pyridine (90 mL). The reaction mixture was refluxed at 118 °C for 2 hours. The resulting biphasic solution was concentrated under vacuum to give a yellow suspension, which was first treated with 0.2N aqueous citric acid (130 mL) and then was extracted with ethyl acetate (3×55 mL). The extracts were washed with water (3×45 mL) and brine (45 mL), dried over sodium sulfate, and concentrated under vacuum to give oxime 31 as a yellow oil (64.1 g), which was used for the next step without further purification.

1-(4-(((2,3-Dimethylbutan-2-yl)dimethylsilyl)oxy)methyl)phenyl)-2,2,2-trifluoroethanone O-tosyl oxime (32). p-Toluenesulfonil chloride (33.9 g, 178 mmol) was added at room temperature during a 40 minute period to a stirred solution of 31 (64.1 g, 176 mmol), triethylamine (37.1 mL, 266 mmol), and 4-(dimethylamino)-pyridine (33.8 g, 178 mmol) in dry dichloromethane (190 mL). The reaction mixture was stirred at room temperature for 1 hour and the resulting suspension was filtered off, rinsing the filter cake with dichloromethane (35 mL). The combined filtrates were treated with 0.2N aqueous citric acid (140 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (2×35 mL). The combined dichloromethane fractions were washed with water (90 mL) and brine (90 mL) and dried (Na₂SO₄). The solvent was
removed under vacuum to give 32 (89.55 g) as a brown oil, which was used for the next step without purification.

3-(4-((((2,3-Dimethylbutan-2-yl)dimethylsilyl)oxy)methyl)phenyl)-3-(trifluoromethyl)diaziridine (33). Compound 32 (89.55 g, 174 mmol) was dissolved in anhydrous dichloromethane (260 mL) in a 1L three-neck round bottom flask, equipped with a thermometer and a dry ice condenser. The solution was cooled down to -40 °C with a dry ice/i-PrOH bath and gaseous ammonia was slowly introduced into the reaction flask until ~ 80 mL of liquid ammonia was accumulated in the flask. The reaction mixture was stirred, maintaining the inner temperature between -35 °C – -25 °C (important), for approximately 4 hours until all the starting material was consumed (TLC monitoring). The dry ice condenser was refilled with dry ice and the reaction mixture was allowed to warm up to room temperature overnight. The resulting suspension was filtered off, washing the filter cake with dichloromethane (2×50 mL). The combined filtrates were washed with water (50 mL) and brine (50 mL) and dried (Na₂SO₄). The solvent was evaporated under vacuum, the resulting suspension was redisolved in hexane (200 mL) and the precipitated was filtered off. Hexane was evaporated under reduced pressure to give 33 (58.35 g) as a brown oil, which was used for the next step without purification.

3-(4-((((2,3-Dimethylbutan-2-yl)dimethylsilyl)oxy)methyl)phenyl)-3-(trifluoromethyl)-3H-diazirine (34). Solid iodine (41.2 g, 162.3 mmol) was added at a rate ~2g/min to a stirred solution of 33 (58.35 g, 162 mmol) and triethylamine (44.86 mL, 322.5 mmol) in anhydrous methanol (115 mL). Temperature of the reaction mixture was kept below 40
°C during the addition. After the red color of iodine persisted, the reaction mixture was stirred at room temperature for 30 min and then treated with saturated solution of citric acid (190 mL). The mixture was extracted with diethyl ether (4x190 mL), the organic layer was washed with saturated solution of sodium hydrogensulfite (2x100mL), water (2x100 mL) and brine (100 mL) and dried (Na₂SO₄). The solvent was evaporated under vacuum to give 34 (54.0 g) as a brown oil, which was used for the next step without purification.

(4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)phenyl)methanol (35). To a solution of 34 (54.0 g, 151 mmol) in methanol (185 mL) concentrated HCl (20.1) was added. The reaction mixture was stirred at room temperature for 90 min (monitored by TLC). Methanol was evaporated under reduced pressure and the resulting residue was dissolved in diethyl ether (220 mL). The organic layer was separated and washed with water (2x75 mL), saturated NaHCO₃ (75 mL) and brine (2x75 mL) and dried over sodium sulfate. The crude product was concentrated first by rotary evaporation and then under vacuum (2mmHg) for 1h. The resulting oil was dissolved in hexane, cooled down to -65 °C with isopropanol/dry ice bath, and kept at this temperature for 1 hour or until small crystals formed. Subsequently, the flask with the product was kept in the -20 °C freezer overnight, yielding white precipitate. Hexane was decanted from the solid and kept in a separate flask. The solid was redissolved in hexane (30 mL) and flask was left in -20 °C freezer overnight. The hexane was decanted and combined with the previously decanted hexane solution. The crystals were washed with minimal amount of hexane and dried under house vacuum for 15 min at -65 °C to give 35. The combined hexane
solutions were reduced to ~ 70 mL and then low-temperature recrystallization procedure was repeated. Compound 35 was obtained as white crystals (18.7 g).

4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)benzoic acid (36). Potassium permanganate (12.9 g, 81.4 mmol) was added portionwise at room temperature to a stirred solution of 35 (12.6 g, 58.3 mmol) and tetraethylammomium hydrogen sulfate (0.25 g, 1.1 mmol) in 0.2N aqueous KOH. The reaction mixture was stirred at room temperature for 2 hours. The resulting suspension was filtered through a celite pad, washing the filter cake with hot water (40-50 °C) (2x35mL). Aqueous filtrates were combined and washed with methyl tert-buthyl ether(MTBE) (3x110mL). The aqueous solution was acidified with concentrated HCl (8.4 mL) to pH=1, and the resulting suspension was extracted with MTBE (3x110 mL). The MTBE extracts were washed with water (70 mL) and brine (70 mL) and dried (Na2SO4). Resulting solid was recrystallized from hexanes/ethyl acetate to give 36 (4.7g) as a white solid.

2,5-Dioxopyrrolidin-1-yl 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate (37).  To the reaction mixture containing 36 (4.7 g, 20 mmol), N-hydroxysuccinimid (2.3 g, 20 mmol), dioxane (150 mL) and ethyl acetate (150 mL) was added a solution of N,N'-dicyclohexylcarbodiimide (4.1 g, 20 mL) in dioxane (150 mL). The reaction mixture was stirred for 6 hours at room temperature, the precipitated dicyclohexylurea was removed by filtration, and the filtrate was concentrated under vacuum. The product was purified by column chromatography (ethyl acetate/hexane 1:1) and then recrystallized from ethyl acetate/hexane to give pure 37 in 54 % yield (3.5 g, 10.7 mmol). 1H NMR: δ = 2.91 (s, 4H), 7.31 (d, 2H, J=8.4 Hz), 8.15 (d, 2H, J=8.4 Hz). 19F NMR: δ = -64.8.
**Si (111) functionalization**

A silicon (111) chip (~1×1 cm) was washed with ethanol and water and blow-dried with filtered argon to remove dust particles. The substrate was then oxidized in Nanostrip solution (cyantek inc) at 75 °C for 15 min to remove all organic contaminants. The native oxide film was etched from the surface with 5% aq. HF at room temperature for 4 minutes yielding oxide-free polycrystalline surface displaying Si-H bonds. The silicon chip was transferred from the HF solution to a saturated phosphorus pentachloride solution in chlorobenzene containing small amount of benzoyl peroxide (0.1 % m/v) and heated in this solution for 1 hour at 105 °C. The chlorinated substrate was quickly washed with chlorobenzene, dried under a stream of argon, and immediately transferred into a 0.5 M THF solution of 1-propenylmagnesium bromide. Subsequently, the surface was reacted in a sealed vial with the Grignard at 135 °C for at least 24 hours to produced stable close-packed propylene-terminated SAMs on oxide-free silicon. After the reaction, the surface was thoroughly rinsed with ethanol and dichloromethane and dried first under a stream of argon and then on a hot plate at 75 °C for 10 minutes. The propylene-functionalized chip was subsequently covered with ~ 50 µL of a 0.1 M solution of 2,5-dioxopyrrolidin-1-yl 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoate in CCl₄ and reacted under UV light for 1 hour at room temperature. After the reaction, the NHS-functionalized surface was thoroughly washed with dichloromethane and isopropanol, dried under a stream of argon and used immediately.
Stamping protocol

A corresponding polyurethane-acrylate stamp was placed on the top of the NHS-modified substrate at room temperature for 4 minutes if not indicated otherwise with no external load to hold them together. After the reaction the stamp and the substrate were separated. The substrate was washed with isopropanol, and dried with filtered argon. The stamp was rinsed with EtOH, H₂O, EtOH, dried with filtered argon, and kept at room temperature before the next application.

Preparation of pET30b-EGFP plasmid

pET30b-EGFP plasmid was prepared using standard DNA techniques. The final construct encodes EGFP with an in-frame N-terminal hexahistidine tag.

GFP Purification

EGFP-pET30b Plasmid DNA was transformed into BL21(DE3) cells and a single colony was used to start overnight cultures. One liter of TB and kanamycin were inoculated with the overnight culture and induced with 1mM IPTG at OD₆₀₀=0.6. Cells incubated for an additional 3 hours. Harvested cells were lysed by multiple passages over the Emulsiflex between 15,000 and 20,000 psi. Following centrifugation, the supernatant containing His₆-GFP was loaded onto a pre-equilibrated column containing charged nickel chelating resin. The column was subjected to a wash in lysis buffer and a linear gradient of increasing imidazole. Fractions were analyzed by SDS-PAGE and those exhibiting EGFP were pooled. The EGFP was subjected to four rounds of dialysis, the first containing 5mM EDTA, 50mM sodium phosphate pH=7.6, and 300mM NaCl and the
remaining containing all components except for the EDTA. The protein concentration was quantified by an Edelhoch assay.

**GFP patterned substrates**

The NHS-patterned bifunctional substrate presenting an array of hydrolyzed 8 µm squares was submerged in Lysine-N,N-diacetic acid (20 mM) and Et₃N (100 mM) in DMF:H₂O (1:1) at room temperature for 1 hr and then rinsed with water and ethanol. Theses substrates were subsequently incubated in a 50 mM NiSO₄ solution for 5 min at room temperature. The chelated substrates were then rinsed excessively with water and binding buffer (20 mM NaP, 250 mM NaCl, 10mM imidazole, pH 7.5) and submerged in a filtered GFP solution (~40 µM) for 1 hr at 0°C. Substrates were immediately rinsed with binding buffer followed by PBS (pH 7.4). Substrates were kept hydrated in PBS at 0°C until they were ready for analysis.

**4.5 Multicomponent inorganic Janus particles with controlled compositions, morphologies, and dimensions**

All reagents and solvents were purchased from Sigma-Aldrich and were used as supplied. XPS spectra were recorded on the Kratos Axis Ultra XPS spectrometer equipped with a mono-Al X-ray source. Gold substrates (~ 1 cm x 1 cm) were manufactured by coating silicon wafers with a 70Å chromium adhesion layer followed by a 230Å gold layer using an electron-beam metal evaporator (CHA Industries). SEM images were recorded on the FEI XL30 SEM-FEG microscope detecting secondary electrons.
Gold-silicon oxide Janus particles.

A 3 inch silicon wafer (prime grade, N/Phos, <1-0-0>, 1-10 Ω-cm, 381±25 µm, Silicon Quest Int.) was oxidized with oxygen plasma for 10 minutes at 100 watts and 6×10⁻¹ mbar O₂ pressure (Emitech K-1050X plasma asher). Subsequently, it was treated with Nanostrip solution (Cyantek co.) for 10 minutes at 75 °C, washed with DI water, and blown dry with nitrogen. NR9-1500PY negative photoresist (Futurex) was spun on the wafer at 4000 rpm for 40 seconds. The resulting substrate was baked on a digital hot plate at 155°C for 130 seconds. Photolithography was performed on a Karl Suss MA6/BA6 mask alignment system with 365 nm light source at 12 mw/cm² for 15 seconds using soda lime photomask bearing 8 µm dark squares (Photo Sciences). After exposure the wafer was post baked at 105 °C for 1 minute and 10 seconds and was developed in RD6 developer (Futurex) for 11 seconds. Subsequently, it was rinsed with water, blown dry with nitrogen, and oven dried at 105 °C for 5 minutes. The wafer was then treated with oxygen plasma for 45 seconds at 100 watts and 6×10⁻¹ mbar O₂ pressure to remove undeveloped resist. Layers of gold (500 nm, 5 Å/sec), nickel (75 nm, 0.8 Å/sec), and titanium (75 nm, 2 Å/sec) were evaporated on the patterned wafer in an electron-beam metal evaporator (Kurt Lesker PVD 75). Subsequently, the negative photoresist was lifted off in RR4 resist remover (Futurex) at 55 °C. The substrate was then immersed in acetone (55 °C), isopropanol (55 °C), and water (55 °C), and was oven dried at 115 °C for 5 minutes. A silicon oxide layer (150 nm) was deposited on the substrate in a plasma enhanced chemical vapor deposition system (Advanced Vacuum Vision 310) at 250 °C. Subsequently, P20 adhesion promoters was spun on the wafer at
3000 rpm for 30 seconds, followed by 1813 PR photoresist (Shipley) at 3000 rpm for 30 seconds. The substrate was baked on a digital hot plate at 117°C for 70 seconds and was subjected to a second round of photolithography on the Karl Suss MA6/BA6 mask alignment system with 365 nm light source at 12 mw/cm² for 6 seconds using the same photomask and a 6 µm alignment gap. The exposed substrate was developed in MF319 developer for 35 seconds, rinsed with water, blown dry with nitrogen, and oven dried 80°C for 10 minutes and at 110°C for 20 minutes. The exposed silicon oxide layer between the metal-oxide structures was dry etched using a reactive ion etching system (Trion Technology Phantom II) for 21 minutes with 3 minute brakes after every 3 minutes of etching at 150 mT, 100 W RIE, CF₄ 30 sccm, CHF₃ 15 sccm, O₂ 5 sccm. The remaining photoresist was removed from the top of the metal-oxide structures first in the reactive ion etching system (Trion Technology Phantom II) 2 minutes, 500 mT, 25 W RIE, 500 W ICP, O₂ 50 sccm, and then in warm acetone (55 °C, 5 inutes) and Nanostrip solution (room temperature, 20 seconds). The resulting wafer bearing metal-oxide structures was dipped in DI water and in isopropanol, and was oven dried at 110°C for 2 minutes. The gold-oxide particles were released from the substrate by sonicating the wafer in ethanol at room temperature for approximately 10 – 15 minutes.

**Bent gold-silicon oxide Janus particles.**

A 3 inch silicon wafer (prime grade, N/Phos, <1-0-0>, 1-10 Ω-cm, 381±25 µm, Silicon Quest Int.) was oxidized with oxygen plasma for 10 minutes at 100 watts and 6×10⁻¹ mbar O₂ pressure (Emitech K-1050X plasma asher). Subsequently, it was treated
with Nanostrip solution (Cyantek co.) for 10 minutes at 75 °C, washed with DI water, and blown dry with nitrogen. NR9-1500PY negative photoresist (Futurex) was spun on the wafer at 4000 rpm for 40 seconds. Resulting substrate was baked on a digital hot plate at 155°C for 130 seconds. Photolithography was performed on a Karl Suss MA6/BA6 mask alignment system with 365 nm light source at 12 mw/cm² for 15 seconds using soda lime photomask bearing 8 µm dark squares (Photo Sciences). After exposure the wafer was post baked at 105 °C for 70 seconds and was developed in an RD6 developer (Futurex) for 11 seconds. Subsequently, it was rinsed with water, blown dry with nitrogen, and oven dried at 105 °C for 5 minutes. The wafer was then treated with oxygen plasma for 45 seconds at 100 watts and 6×10⁻¹ mbar O₂ pressure to remove undeveloped resist. Layers of gold (300 nm, 10 Å/sec), nickel (100 nm, 2 Å/sec), and titanium (100 nm, 5 Å/sec) were evaporated on the patterned wafer in an electron-beam metal evaporator (CHA). Subsequently, the negative photoresist was lifted off in RR4 resist remover (Futurex) at 55 °C. The substrate was then immersed in acetone (55 °C), isopropanol (55 °C), and water (55 °C), and was oven dried at 115 °C for 5 minutes. A silicon oxide layer (330 nm) was deposited on the substrate in a plasma enhanced chemical vapor deposition system (Advanced Vacuum Vision 310) at 250 °C. Due to the induced bend in the resulting gold-oxide structures we were able to release them from the wafer by sonication in ethanol without etching the surrounding silicon oxide layer.
Gold-titanium oxide Janus particles.

A 3 inch silicon wafer (prime grade, N/Phos, <1-0-0>, 1-10 Ω-cm, 381±25 μm, Silicon Quest Int.) was oxidized with oxygen plasma for 10 minutes at 100 watts and $6 \times 10^{-1}$ mbar O$_2$ pressure (Emitech K-1050X plasma asher). Subsequently, it was treated with Nanostrip solution (Cyantek co.) for 10 minutes at 75 °C, washed with DI water, and blown dry with nitrogen. NR9-1500PY negative photoresist (Futurex) was spun on the wafer at 4000 rpm for 40 seconds. The resulting substrate was baked on a digital hot plate at 155 °C for 130 seconds. Photolithography was performed on a Karl Suss MA6/BA6 mask alignment system with 365 nm light source at 12 mw/cm$^2$ for 15 seconds using soda lime photomask bearing 8 μm dark squares (Photo Sciences). After exposure the wafer was post baked at 105 °C for 70 seconds and was developed in RD6 developer (Futurex) for 11 seconds. Subsequently, it was rinsed with water, blown dry with nitrogen, and oven dried at 105 °C for 5 minutes. The wafer was then treated with oxygen plasma for 45 seconds at 100 watts and $6 \times 10^{-1}$ mbar O$_2$ pressure to remove undeveloped resist. Layers of gold (500 nm, 5 Å/sec), nickel (75 nm, 0.8 Å/sec), and titanium (75 nm, 2 Å/sec) were evaporated on the patterned wafer in an electron-beam metal evaporator (Kurt Lesker PVD 75). Subsequently, the negative photoresist was lifted off in RR4 resist remover (Futurex) at 55 °C. The substrate was then immersed in acetone (55 °C), isopropanol (55 °C), and water (55 °C), and was oven dried at 115 °C for 5 minutes. The titanium layer on the metallic structures was oxidized with oxygen plasma for 10 minutes at 100 watts and $6 \times 10^{-1}$ mbar O$_2$ pressure. The gold-titanium
oxide particles were released from the substrate by sonicating the wafer in ethanol at room temperature for approximately 10 – 15 minutes.

**Orthogonal functionalization of the gold-oxide particles (general procedure).**

The gold-oxide particles supported on the silicon wafer were exposed to vapors of 1H,1H,2H,2H-perfluorooctyltriethoxysilane at room temperature for 2 hours under argon. The substrate was then left to cure at room temperature for 2 days under argon. The particles were released from the wafer by sonication, and were filtered on the 0.45 μm teflon membrane filter (Whatman), where they were washed several times with water, ethanol, acetone, and hexane. The particles were transferred from the filter to a 20mL vial and their gold side was reacted with 1 mM ethanol solution of mercaptoundecanoic acid for 2 hours at room temperature. Subsequently, they were collected and held on the bottom of the vial with a magnet, while they were washed several times with ethanol.
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Biographical sketch

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PUBLICATIONS


MANUSCRIPTS IN PREPARATION


6. “Multicomponent Inorganic Janus Particles with Precisely Controlled Compositions, Morphologies, and Dimensions”, Alexander A. Shestopalov, Robert L. Clark, Eric J. Toone, pre-submission

POSTERS AND PRESENTATIONS

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