Bioactive Surfaces

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Reactive Polymer Coatings that "Click"**

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Future advances in the design of biologically active interfaces require novel strategies for the robust and specific attachment of biological ligands onto surfaces.[1] Herein, a new type of biofunctional surface based on alkyne-containing vapor-deposited polymer coatings is reported. These reactive coatings are applicable to a wide range of substrates and can be modified by subsequent spatially directed "click chemistry". Click chemistry represents a family of powerful and efficient chemical reactions, which are modular, widely applicable, relatively insensitive to solvents and pH value, while resulting in stereoselective conversions with high to very high yields.[2] The most widely used click reaction is the Huisgen 1,3-dipolar cycloaddition between azides and terminal alkynes.[3] This coupling reaction has been employed for drug discovery applications[4] and for the target-guided synthesis of enzyme-inhibitors.[5,6] Moreover, Huisgen 1,3-dipolar cycloadditions of azide- and alkyne-functionalized self-assembled monolayers (SAMs) have been used as a versatile tool for tailoring surface functionalities.[7–11]Their bioorthogonality, that is, the dependence on proximity and proper alignment of the reactants make click reactions promising candidates for biointerface design.[12] However, further use is currently hampered by the availability of polymer coatings that can undergo surface-directed dipolar cycloadditions. In the past, chemical vapor deposition (CVD) polymerization of substituted [2,2]paracyclophanes has been instrumental in creating a wide array of functionalized poly-(*p*-xylylenes) with a diverse class of functional groups, such as amines,[13,14] esters,[15–17] aldehydes,[18] and alcohols,[19–21] which enable the immobilization of biomolecules.

To further extend the concept of click-chemistry-based immobilization, we use CVD polymerization for synthesizing alkyne-containing polymer coatings (Scheme 1). Moreover, we demonstrate the applicability of these reactive coatings by

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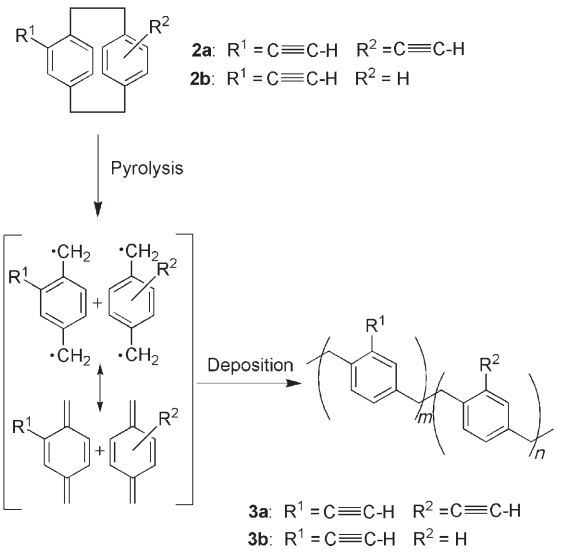
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 1. Synthesis of alkyne-containing polymers by CVD polymerization of diethynyl[2,2]paracyclophane (**2a**) and 4-ethynyl-[2,2]paracyclophane (**2b**).

conducting spatially directed Huisgen 1,3-dipolar cycloaddition on a surface.

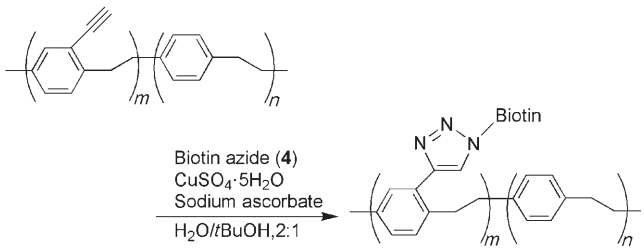
Prior to polymer deposition using CVD polymerization, the starting materials diethynyl[2,2]paracyclophane (**2a**) and ethynyl[2,2]paracyclophane (**2b**) were prepared from the commercially available [2.2]paracyclophane (**1**), according to a synthesis recently reported by Hopf and co-workers.[22] Under these conditions, pseudo-*ortho* and pseudo-*meta* derivatives have been reported as the major isomers,[22] but in this case they were not separated for subsequent CVD polymerization.

First, we attempted the CVD polymerization of the dialkyne **2a**, which was expected to yield poly(diethynyl-*p*-xylylene) (**3a**). For this purpose, **2a** was sublimed at 90–110 °C and a reduced pressure of 0.5 mbar. The reactants were then transported into pyrolysis chamber (650 °C) and then into a deposition chamber (15 °C), where the spontaneous formation of a polymer film was observed. However, the FTIR spectrum of polymer films formed under these conditions did not show the band for the alkyne C–H stretch around 3200 cm^{-1}, indicating the absence of alkyne groups. Instead, several side products were formed, which were not further characterized, but could potentially be due to an alkyne–vinylidine rearrangement.[23] Alteration of process conditions, (e.g., pyrolysis temperatures below 550 °C), resulted in alkyne-functionalized polymers, with typical ellipsometric thicknesses of about 50 nm (for 50 mg of the precursor polymerized onto a 4-inch wafer). Nevertheless, these polymer films showed little reactivity, underwent thermal decomposition, and generally had quite poor stability towards organic solvents (see Supporting Information). Thus, they were not pursued further.

Instead, we shifted our focus to the CVD polymerization of the monoalkyne **2b**. In sharp contrast to the dialkyne-containing polymer **3a**, poly(4-ethynyl-*p*-xylylene-co-*p*-xylylene) (**3b**) was prepared without appreciable side reactions, even under very typical CVD conditions[20] (pressure of 0.5 mbar, sublimation, pyrolysis, and substrate temperatures of 90–110 °C, 680 °C, and 15 °C, respectively). Moreover, the FTIR spectrum of **3b** revealed a strong band at 3286 cm^{-1} for the alkyne C–H stretch and a signal at 2100 cm^{-1}, which can be attributed to the carbon–carbon triple bond. Evidence from the FTIR data was reaffirmed by X-ray photoelectron spectroscopy (XPS), which was used to quantify the surface elemental composition of **3b**. The analysis revealed that **3b** contained 1.3 % oxygen, which may be due to contaminations during CVD polymerization or subsequent sample handling. The high resolution C1s spectrum of **3b** further revealed a symmetric and narrow peak centered at 285.6 eV with a full width at half maximum (FWHM) of 1.13 eV. This peak is associated exclusively with the presence of carbon that is bound to carbon or hydrogen.[24] The C1s peak spectrum further showed a smaller signal centered at 291.7 eV, which can be attributed to a π–π^* shake-up signal characteristic of aromatic π electrons. This signal has been observed for similar polymer systems.[19] For polymer **3b**, ellipsometry gave a thickness of 91.81 ± 0.03 nm (for 50 mg of precursor); **3b** is stable in aqueous solutions and organic solvents such as acetone, ethanol, methanol, and chloroform. Probing the adhesiveness of **3b** using the scotch tape test[18] showed that the film had good adhesion to a wide variety of substrates such as glass, poly(dimethylsiloxane) (PDMS), silicon, and gold.

A spectrofluorometer study of the polymer **3b** showed a characteristic excitation peak at 380 nm and a characteristic emission peak at 450 nm. These peaks disappeared after heating the polymer to 150 °C for 3 h, presumably as a result of cross-linking of the polymer (see Supporting Information). To assess the thermal stability of polymer **3b**, we compared the FTIR spectra of samples that were stored at 20 °C, 80 °C, 150 °C, and 250 °C, respectively. The C–H stretch at 3283 cm^{-1} continuously decreased with increasing temperature and was absent in samples stored at 250 °C (Supporting Information). Again, this result suggests that the polymer has limited thermal stability, most likely arising from cross-linking of the ethynyl groups.

To assess whether the reactive coating **3b** can be used for heterogeneous click reactions, its reactivity against azides was studied. Specifically, the Huisgen 1,3-dipolar cycloaddition between **3b** and an azide-containing biotin-based ligand **4** in the presence of copper(II) sulfate and sodium ascorbate was examined (Scheme 2). As described for solvent-based systems, this coupling reaction yielded triazoles.[25] Sodium



Scheme 2. Huisgen 1,3-dipolar cycloaddition between biotin-based azide-ligand **4** and the polymer **3b**.

ascorbate acts as a reductant, generating Cu^{I} ions in situ, which then function as the catalyst.[25] The biotin azide **4** was chosen as the representative ligand in this study, because biotin forms a strong noncovalent interaction with streptavidin (which has been widely used for binding biotinylated biomolecules).[15]

To ensure spatial control over the cycloaddition reaction, a microcontact printing (μCP) approach was chosen. For this approach to be successful, the Cu^{I} catalyst and the reductant have to be used independently of each other. Therefore a thin layer of **4** and sodium ascorbate was spread onto a film of **3b** and dried using N_2. Next, a patterned PDMS stamp was inked with a solution of $CuSO_4$ and kept in contact with the substrate for 12–18 h (Figure 1). After rinsing, the patterned

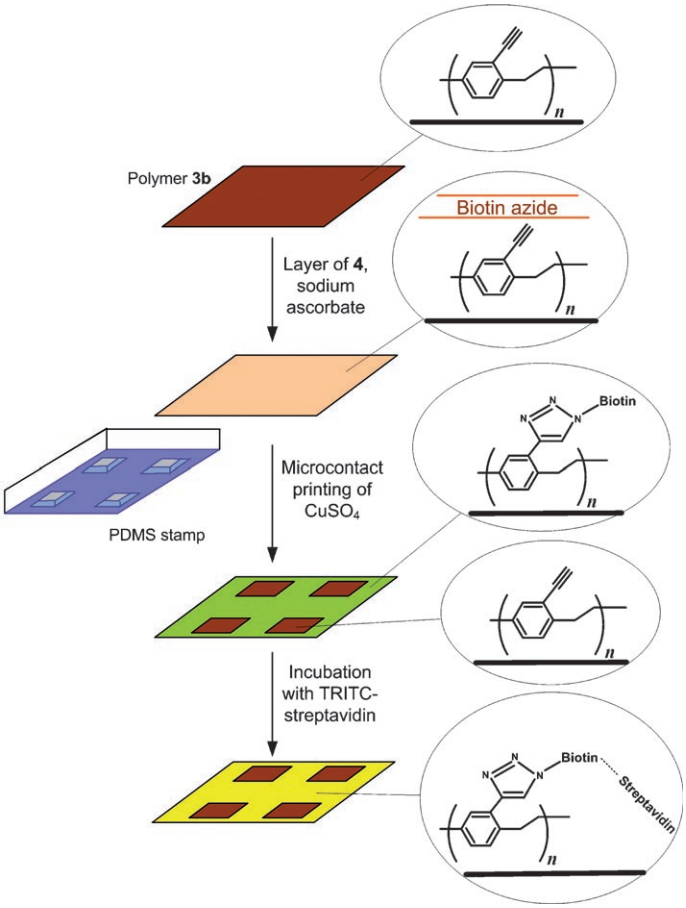


Figure 1. Immobilization of azide-containing ligand on the reactive polymer coating **3b**. Subsequently the $CuSO_4$ catalyst precursor is microcontact printed on a preadsorbed layer of biotin azide ligand **4** on the reactive polymer **3b**.

substrate was incubated with an aqueous solution of rhodamine-labeled streptavidin. Fluorescence microscopy was used to assess the immobilization of **4** onto polymer **3b**. The fluorescence micrographs shown in Figure 2a,c confirm selective protein coupling in the regions where the $CuSO_4$ solution was microcontact printed, thus demonstrating the spatially directed binding of **4** to polymer **3b**. This result shows that the alkyne groups on the polymer surface are

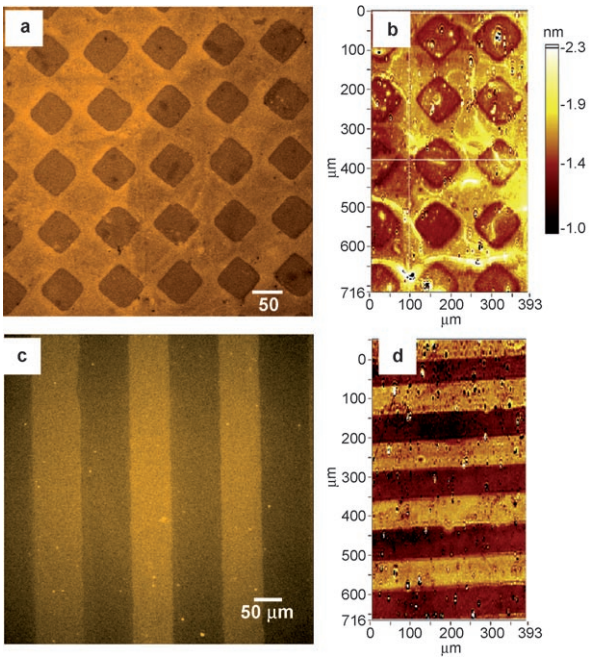


Figure 2. a),c) Fluorescence micrographs showing the binding of TRITC-streptavidin to patterns of biotin azide, b),d) Corresponding ellipsometric images for determining layer thickness. TRITC = tetramethylrhodaminisothiocyanate, PDMS = poly(methylsiloxane).

reactive and can be effectively used as anchoring sites for surface modifications. The two-step approach was found to be superior to the concurrent microcontact printing of catalyst and azide.

To complement the fluorescence study, patterned surfaces were further analyzed by imaging ellipsometry (Figure 2b and d) which revealed protein patterns, which are inline with the corresponding fluorescence patterns. The observed thickness differences of about 1–2 nm between the biotinylated and the non-biotinylated regions after incubation with streptavidin are comparable with the thickness of a protein monolayer.[26]

In conclusion, the alkyne-containing polymer **3b** has been found to show remarkable reactivity towards azides through the chemoselective Huisgen 1,3-dipolar cycloaddition. In contrast to the dialkyne containing polymer **3a**, reactive coating **3b** showed excellent adhesion and stability at elevated temperatures and in solvents. Further work will be directed towards the elucidation of the CVD polymerization mechanism as well as its scope with respect to different cycloadditions. The development of bioactive surfaces is an important step towards advanced biomaterials and biointerfaces. Our regioselective immobilization strategy could be applicable in the design of biofunctional surfaces for diagnostics (e.g. microarrays), biosensors, and biomedical device coatings.

Experimental Section

CVD: **2a** (50 mg) was sublimed at 90–110 °C and 0.5 mbar and carried into the pyrolysis chamber by argon at a flow rate of 20 sccm. After pyrolysis (at different pyrolysis temperatures T_{pyr}), the polymer was

deposited on the substrate at 15 °C. $T_{pyr} = 650$ °C. IR (grazing angle of 85°): $\tilde{\nu} = 837$, 1039, 1150, 1439, 1505, 1624, 1694, 1910, 2916, 3010, 3048 cm^{-1}. XPS signal (%; referenced to aliphatic C at 285.0 eV): C 96.9, O 3.1; $T_{pyr} = 550$ °C. IR (grazing angle of 85°): $\tilde{\nu} = 839.35$, 877, 922, 1036, 1261, 1439, 1623, 1913, 2100, 2848, 2921, 3013, 3052, 3285 cm^{-1}. XPS signal (%; referenced to aliphatic C at 285.0 eV): C 93.7, O 6.3.

2b: CVD as described above. IR (grazing angle of 85°): $\tilde{\nu} = 833$, 894, 1158, 1251, 1411, 1454, 1493, 1513, 1605, 1699, 1900, 2102, 2859, 2926, 3015, 3286 cm^{-1}; XPS signal (%; referenced to aliphatic Cat 285.0 eV): C 98.7, O 1.3; XPS signals: 285.6 eV (C1 s); 291.7 eV (π–π^*).

Surface Characterization: IR spectroscopy: Nicolet 6700 spectrometer. XPS elemental analyses: Axis Ultra X-ray photoelectron spectrometer (Kratos Analyticals, UK) equipped with a monochromatized $Al_{K\alpha}$ X-ray source.

Height analysis data were recorded using an EP^3-SW imaging ellipsometer (Nanofilm AG, Germany) at a wavelength of 532 nm. Both, nulling (four zones) and mapping experiments were performed at an angle of incidence of 65°. An anisotropic Cauchy parameterization model was used for curve fitting. For the mapping mode, data was recorded by an imaging scanner with a lateral resolution of 1 μm with a field of view of about 100 μm × 500 μm.

Patterning of **4** on polymer films of **3b**: Patterned PDMS stamps were created as described elsewhere.[14] A thin layer of solution of ligand **4** (Photoprobe biotin, Vector labs, 10 $\mu g\,mL^{-1}$) and sodium ascorbate (1 mM) in a 2:1 mixture of water and *tert*-butyl alcohol was spread on **3b** and dried using N_2. The patterned PDMS stamp was oxidized for 20 min using UV-ozone cleaner (Jelight) and inked with $CuSO_4$ solution (1 mM in methanol) adopting an approach reported by Abbott et al.[27]. The stamp was then kept in contact with the polymer substrate for 12–18 h. After stamp removal, the patterned substrate was incubated with rhodamine-labeled streptavidin (50 $\mu g\,mL^{-1}$ in aqueous phosphate buffer PBS containing 0.02% (v/v) Tween 20 and 0.1% (w/v) bovine serum albumin (BSA)) for 1 h. The substrate was then repeatedly washed with the incubating buffer, PBS and finally rinsed with deionized water. The fluorescence micrographs were captured using a Nikon TE200 fluorescence microscope.

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