Surface Patterning

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Immobilization of Liposomes and Vesicles on Patterned Surfaces by a Peptide Coiled-Coil Binding Motif**

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Coiled-coil motifs are abundant in proteins where they exhibit an array of functions like gene regulation,[1] cell signaling,[2] transport of small molecules,[3] and membrane fusion.[4] Native SNARE proteins control fusion processes between and within cells (for example, exocytosis). The common feature of all these coiled-coils is that at least two α-helical peptide strands bind, thereby acting as molecular Velcro. The specific molecular recognition between helices has enabled scientists to develop self-assembled, highly structured materials based on the coiled-coil motif.[5–7] Herein we describe a completely new function for the coiled-coil peptide binding units, namely their application in materials science and surface modification. We report that an α-helical coiled-coil pair exclusively forms parallel heterodimers, denoted "peptide E" $(EIAALEK)_3$ and "peptide K" $(KIAALKE)_3$ and acts as selective recognition unit through which liposomes and cyclodextrin (CD) vesicles can be selectively immobilized in surface patterns obtained using microcontact printing.

The immobilization of vesicles and liposomes by recognition units, such as complementary DNA strands,[8] electrostatic interactions[9] and protein–ligand pairs,[10] has attracted increasing attention in recent years. By using these recognition units, it is possible to attach liposomes and vesicles to a variety of substrates, to prepare microarrays of liposomes,[11,12] to construct sensing platforms,[13–15] and to investigate reactions in immobilized liposomes,[16–19] including single-molecule reactions.[20]

In this study we used microcontact printing to produce well-defined patterns of peptide E (**1**) by the formation of a covalent bond (i.e. a triazole unit) between an azide self-assembled monolayer (SAM) and the alkyne-terminated peptide E in the presence of Cu^I. Surface patterning by microcontact chemistry has been widely studied by several groups during the last years, and it was successfully applied for the preparation of various functional surfaces, including carbohydrate,[21,22] DNA,[23] and peptide microarrays.[24] Peptide E is able to bind to the complementary peptide K by forming a coiled-coil binding motif, which has previously been used to induce liposomal fusion processes in buffered aqueous media.[25–27] The main benefits of this complementary peptide binding motif include its simplicity, selectivity, pH and temperature stability, and low cost.

Figure 1 describes the process of liposome and vesicles immobilization on patterned surfaces, and Scheme 1 shows the molecular structures of the key components. After functionalization of a glass or silicon slide with an azide SAM (see the Supporting Information), patterns of the alkyne-terminated peptide E (**1**) were prepared by inducing the Cu^I-catalyzed azide–alkyne cycloaddition (CuAAC) with a structured polydimethylsiloxane (PDMS) stamp. The interspace was passivated with the alkyne–tetraethylene glycol derivative **2**. After incubation with either liposomes decorated with peptide K **4** or CD vesicles[28] functionalized by host–guest complexation with peptide K **5**, patterns of liposomes/CD vesicles were obtained. The noncovalent, reversible nature of liposome immobilization was checked by washing the liposome surface with ethanol, or with an excess of a buffered β-CD solution for the immobilized CD vesicles.

The immobilization of peptide E (**1**) results in a significant increase in the wettability of the surface, which is consistent with the hydrophilic nature of peptide E. This behavior was observed by water condensation on the printed surface, which shows clear dot patterns of water at the hydrophilic islands (Supporting Information, Figure S1). After CuAAC using flat PDMS stamps, the static water contact angle decreased from around 83° for the azide SAM to around 50° for the surface immobilized peptide E (Supporting Information, Figure S1).

Furthermore, the presence of carbonyl carbon atoms and amide groups was verified by X-ray photoelectron spectroscopy (XPS). After printing by using a flat PDMS stamp, an additional band (288.5 eV) in the C1s region belonging to the

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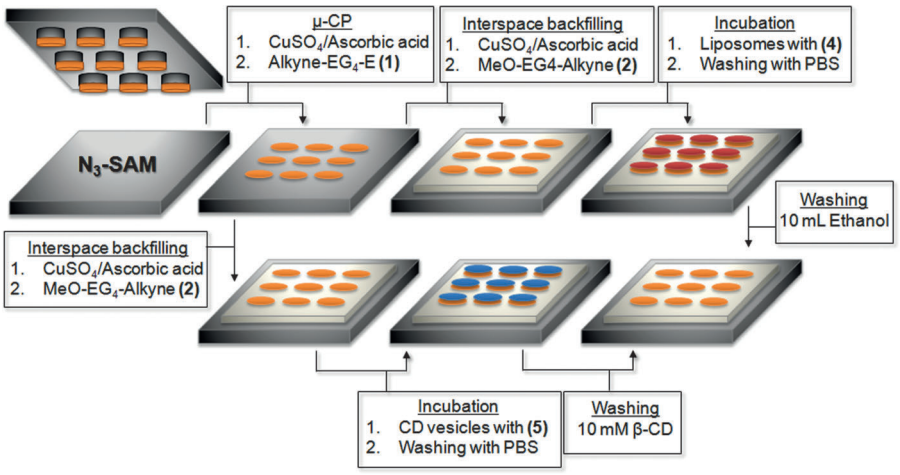
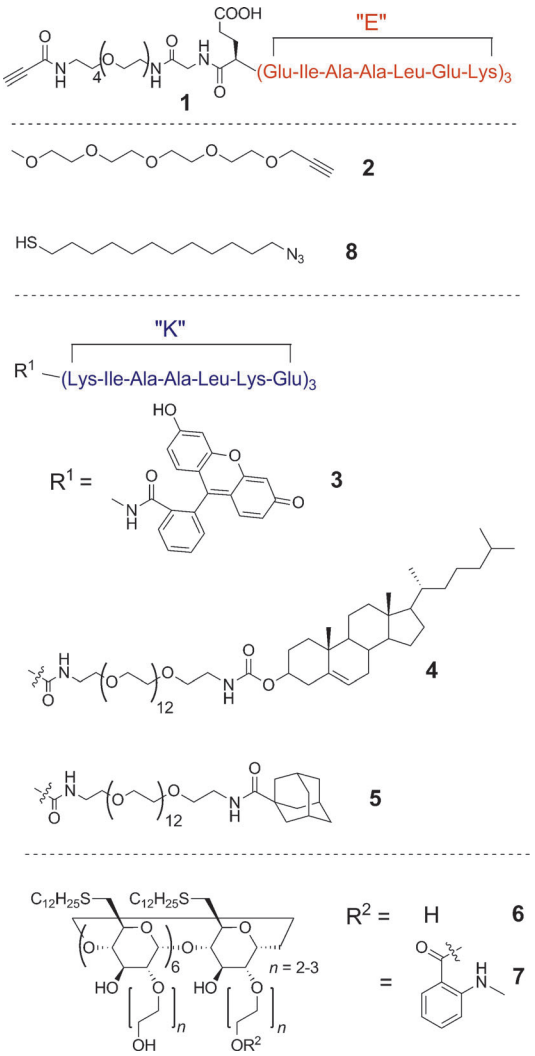


Figure 1. The printing process and the immobilization of liposomes or vesicles using a coiled-coil binding motif.



Scheme 1. Chemical structures of the compounds used in this work. **1**: Alkyne–peptide E conjugate, **2**: alkyne–tetraethylene glycol monomethyl ether, **3**: fluorescein-peptide K conjugate, **4**: cholesterol–peptide K conjugate, **5**: adamantane–peptide K conjugate, **6**: amphiphilic β-CD, **7**: anthranilic acid-labeled amphiphilic β-CD, **8**: Thiol–azide adsorbate.

amide carbons appeared (Figure 2A). As expected, the azide SAM reveals two nitrogen peaks (terminal and internal nitrogen atoms). It is clear from Figure 2B that after printing only one single nitrogen peak in the N1s region remains owing to a conversion of the azides to triazole rings. The amide nitrogen atoms overlap with the triazole nitrogen atoms, which explains the single remaining peak in Figure 2B.

To verify the patterning of peptide E (**1**)in well-defined dot structures, time-of-flight secondary ion mass spectrometry (ToF-SIMS) was carried out (Figure 2E,F). The expected characteristic fragments (predominantly $C_xH_xO_x$ and $C_xH_xN_x$) in the positive- and negative-ion mode were detected, indicating the presence of a peptide in the printed regions. It was also observed that the printed peptide **1** shields the SiO_2 surface, which was indicated by a weaker ion count (SiO_2^-) in the patterned regions (Supporting Information, Figure S2). Additionally, the immobilization of **1** was verified by AFM measurements. The pattern caused a significant increase in height (0.7–1 nm; Supporting Information, Figure S3) and a clear contrast in the phase image (Figure 2C,D).

After detailed analysis of the printing process, we investigated the binding properties of the surfaces to binding partners modified with peptide K. In the first experiment, the patterned surface was incubated with a carboxyfluorescein-labeled peptide K **3** (that is, without liposomes or lipid anchor) to determine whether the immobilized peptides were still able to form a coiled-coil complex. Figure 3A clearly shows a green dot pattern, indicating successful recognition of peptide K on the printed islands of peptide E.

In the next step, peptide K was immobilized by a lipid anchor into liposomes consisting of DOPC, DOPE, and cholesterol (50:25:25 mol%). To achieve this, the peptide was terminated with a cholesterol unit, which is known to bind to bilayer membranes by hydrophobic interactions. The interspace between the patterns of peptide E was passivated with alkyne-terminated tetraethylene glycol monomethyl ether **2** using CuAAC in solution to avoid nonspecific interactions of the liposomes with the hydrophobic azide-functionalized alkyl chains of the azide SAM (Supporting Information, Figure S4). A lipid film consisting of the lipid composition and 1 mol% of the peptide K **4** was obtained by drying a solution in MeOH and $CHCl_3$ in a stream of argon. This film was hydrated using 100 mM PBS buffer. After sonication for 5–10 min unilamellar liposomes with an average diameter of 150 nm with the K peptide at the surface were obtained. The final liposomal concentration was set to 0.1 mM.

To visualize the immobilization of the liposomes at the patterned surface by fluorescence microscopy, DOPE-LR at a concentration of 1 mol% was added to the lipid mixture.

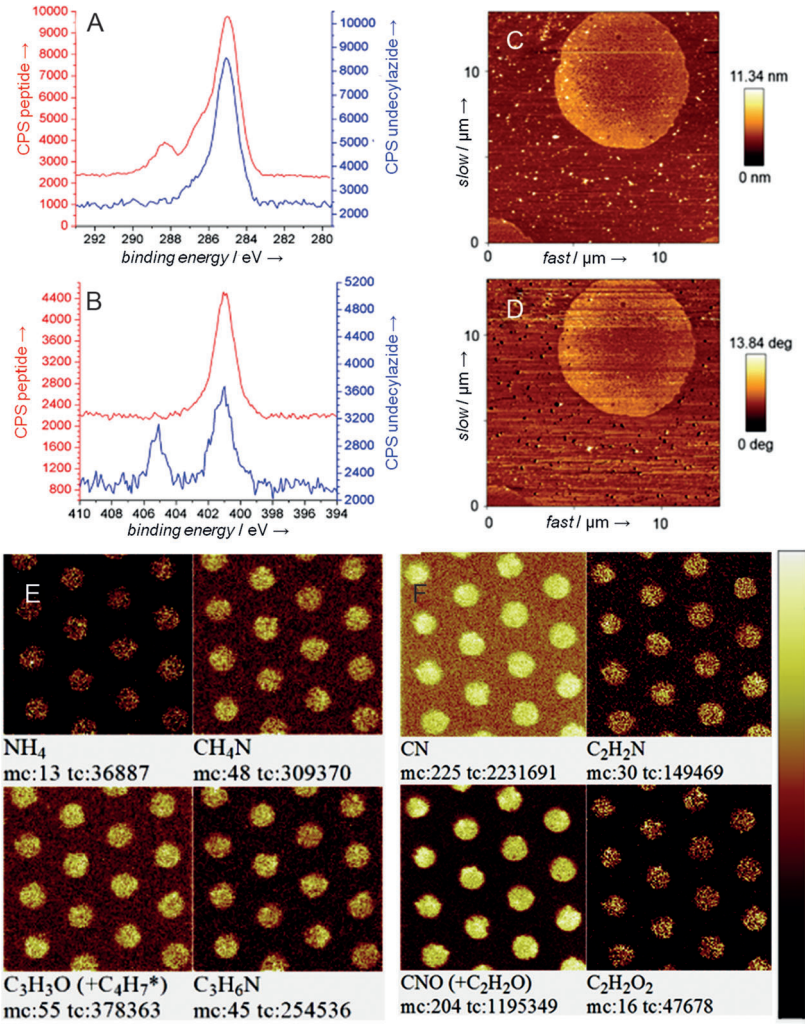


Figure 2. A) XPS analysis of the C1s region of an undecylazide monolayer on silicon before and after immobilization of peptide E (**1**). B) XPS analysis of the N1s region of the azide SAM and of the same monolayer after immobilization of **1**. C) AFM height image of **1** printed on silicon. D) AFM phase image of **1** printed on silicon. E), F) Selected secondary ions generated by ToF-SIMS measurements in the positive- (E) and negative-ion mode (F) (field of view: 50×50 µm). Scale bar in panels (C)–(F): 10 µm.

Incubation of the patterned surface for 15 min with the liposomal solution, followed by careful washing with 10 mL of 100 mM PBS solution, resulted in well-defined fluorescent dot patterns, indicating the selective interaction between the complementary peptides E and K (Figure 3B). The red dot patterns persists even after extensive washing with PBS (Supporting Information, Figure S5). Furthermore, the liposomes could easily be removed by washing the patterned surface with 10 mL of ethanol, which breaks the coiled-coil binding motif as well as the cholesterol anchoring in the membrane (Supporting Information, Figure S5).

To ensure that the liposomes remain intact and did not rupture immediately at the glass surface, sulphorhodamine B at a concentration of 20 mM in HEPES buffer was encapsulated inside liposomes modified with **4**. Any non-encapsulated dye was removed using size-exclusion chromatography by Sephadex G50. After incubation of the patterned surface with these liposomes for 15 min and several washing steps with HEPES buffer solution, a well-defined red dot pattern was obtained as visualized by fluorescence microscopy (Supporting Information, Figure S6). This clearly shows that the liposomes remain intact upon immobilization.

To obtain further insight regarding the immobilized liposomes, surface plasmon resonance (SPR) and quartz crystal microbalance with dissipation monitoring (QCM-D) measurements were conducted. To this end, gold chips (SPR chips and gold-coated quartz crystals) were incubated with **8** overnight followed by CuAAC-mediated immobilization of peptide E (**1**). Unreacted azides were blocked with **2** to avoid unspecific binding. Figure 4A shows that the binding between the complementary peptides E and K can be followed by SPR measurements. The binding of liposomes decorated with 1% of **4** at a surface functionalized with **1** gives a significant increase in the milli refractive index units (mRIU), whereas plain liposomes and decorated liposomes with **4** on a bare ethylene glycol surface composed of **2** give only a weak signal increase. This shows that the docking of K-decorated liposomes through binding with the surface modified with peptide E is selective and can be monitored by SPR.

To judge whether the immobilized liposomes remain intact at the surface, additional QCM-D measurements were performed to study the shape of liposomes at surfaces.[29] The incubation of a gold surface, modified with peptide E (**1**), with liposomes decorated with 1% of peptide K **4** leads to a significant increase in the dissipation (Figure 4B) and a decrease in the frequency (Figure 4C) of the quartz crystal. This indicates a mass adsorption on the surface owing to the tethering of an energy dissipative liposome layer, which is characteristic for intact anchored liposomes (whereas a lipid bilayer should not give a significant increase in the dissipation).[29] Upon washing the surface with PBS buffer, no changes in the binding of the liposomes were detected (blue arrow). Addition of Triton X leads to a spontaneous solubilization of the immobilized liposomes (black arrow). Plain liposomes only show negligible binding at the modified gold surface, which demonstrates the specificity of the heterodimerization of the coiled-coil binding motif. From the blank measurement, it can be calculated that roughly 7% of the tethered vesicles are immobilized by unspecific binding. Taken together, these findings lead to the conclusion that intact liposomes can be immobilized by the coiled-coil binding motif, as indicated by fluorescence images (Figure 3B; Supporting Information, Figure S6) as well as SPR and QCM-D data (Figure 4).

Nevertheless, the liposomes eventually collapse, spread, and form a supported bilayer upon prolonged storage. This was shown by AFM measurements after 3–4 h in solution on

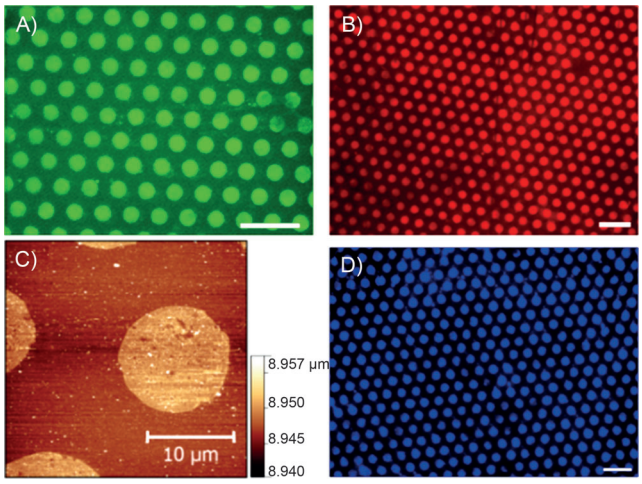


Figure 3. A) Fluorescence microscopy image of a dot pattern of peptide E (**1**) incubated with peptide K **2** for 15 min. B) Fluorescence microscopy analysis of a pattern of **1** incubated with liposomes decorated with peptide K **3** and DOPE-LR. C) AFM height image of liposomes decorated with **3** immobilized on dot patterns of **1**. D) Fluorescence microscopy image of the pattern of **1** incubated with CD vesicles decorated with peptide K **5**. Scale bar in panels (A), (B), and (D): 40 µm.

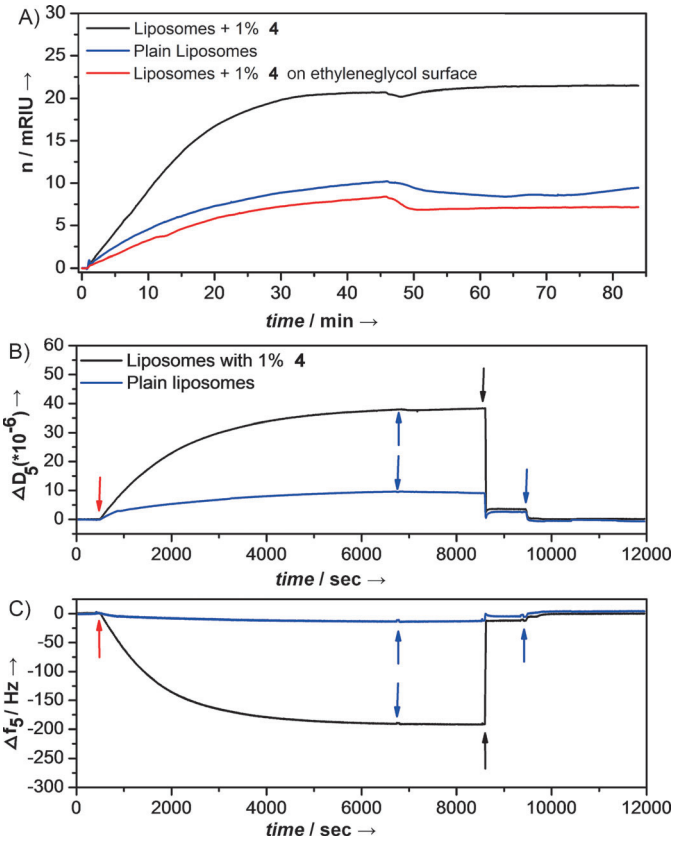


Figure 4. A) SPR sensogram of liposomes decorated with 1% of peptide K **4** as well as two blank measurements; [liposomes] = 0.2 mM. B), C) QCM-D measurements: Normalized dissipation (B) and normalized frequency of the fifth overtone (C), incubation with: liposomes (0.1 mM) with (black curve) and without peptide K **4** (blue curve); red arrows: incubation with liposomes, blue arrows: rinsing steps with PBS, black arrows: incubation with Triton X (0.1 wt%).

liposomes decorated with **4**, which were attached to a silicon surface patterned with **1** (Figure 3C). It can be clearly seen that lipidic material is located on the dot patterns. The measurements reveal structures with an average height of around 5–6 nm (Supporting Information, Figure S3), which is in good agreement with the dimensions of a lipid bilayer at the silicon surface. This finding is easy to understand, because it is likely that multiple coiled-coils will be formed by the complementary peptides E and K, each with an association constant $K_a \approx 10^7\,\mathrm{L\,mol^{-1}}$. The resulting strong adhesion constant forces the liposomes over time to flatten and form a supported bilayer at the surface.

To investigate the scope of the system, β-CD vesicles were used to combine their ability of forming stable inclusion complexes with hydrophobic guest molecules, such as adamantane, with the formation of coiled-coils consisting of peptides E and K. To this end, an adamantane-functionalized peptide K **5** was synthesized. Upon mixing of **5** with CD vesicles consisting of amphiphilic CD **6**, which were synthesized according to a literature procedure,[28] vesicles with peptide K at the surface were obtained by the noncovalent interaction between CD and adamantane. To visualize their attachment to a surface patterned with peptide E (**1**), methylanthranilic acid was introduced at the ethylene glycol units of the amphiphilic CD **6**, yielding the fluorescent CD **7**. CD vesicles were prepared by drying a stock solution of **6** and **7** (9:1) in $CHCl_3$ in a stream of argon to obtain a thin lipid film. This film was hydrated with PBS and sonicated for 10 min, yielding CD vesicles with an average diameter of 150 nm. After addition of 1% peptide **5** in PBS to the CD vesicles, CD vesicles decorated with peptide K were obtained. After incubation of the surface patterned with peptide E with the functionalized CD vesicles for 15 min, a blue dot pattern was observed, indicating the successful immobilization of the structures at the glass surface (Figure 3D), even though the interaction between adamantane derivatives and β-CD is rather low ($K_a \approx 1 \times 10^4\,\mathrm{L\,mol^{-1}}$).[28b] This observation is based on the multivalent binding of the CD vesicles to the surface, which substantially enhances the binding affinity compared to a monovalent adamantane–β-CD binding. To verify whether the vesicles adhered owing to host–guest complexation of the hydrophobic adamantane moiety of **5** into the CD cavities of the CD vesicles, the patterned surface with attached CD vesicles was washed with an aqueous solution of β-CD (10 mM). After washing, the patterns were completely removed and no significant fluorescence was detected, indicating a disruption of the host–guest complexes owing to competitive binding with the excess of free β-CD (Supporting Information, Figure S7).

In summary, we have described a novel biomimetic noncovalent method for the immobilization of liposomes and the formation of supported bilayers by using a coiled-coil binding motif, which was printed using microcontact printing-induced click chemistry. It was possible to obtain well-defined structures of liposomes and supported bilayers. This technique shows great potential to study fluidity and recognition processes of supported lipid bilayers. Further experiments will be conducted in the future to

investigate orthogonality of the coiled-coil binding motifs as well as the production of lipid arrays consisting of different lipid compositions.

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