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Recognition of Patterned Molecular Ink with Phage Displayed Peptides

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The development of a reliable chemical method for patterning and recognizing molecular inks could impact areas such as localization of biomolecules,[1] directing the growth of cells,[2,3] self-assembling nano- and microelectronic devices,[4,5] and developing microcontrolled wettability.[6] These patterning inks are a diverse class of materials including small organic molecules (e.g., octyltrimethoxysilane, octadecanethiol), polymers (e.g., poly(methyl methacrylate), polystyrene), and even metallic salts (e.g., $GaCl_3$, $In(NO_3)_3$).[7] Peptides are robust biorecognition molecules that can be chemically engineered to bind specific targets and have broad chemical diversity (acidity, hydrophobicity, etc.) within a relatively compact size.[8] Peptides can be synthetically linked with materials to form conjugates, such that the retained binding capabilities of the peptide can lead to further self-assembly of various targets into complex structures.[9−12] For example, we have recently shown that theoretically tailored peptide sequences can be chemically bound to nanowire sensors, enabling high selectivity toward small molecule analytes.[13] Phage display has emerged as a powerful method for identifying peptide motifs that possess enhanced selectivity and binding affinity toward a variety of substrates, including metals,[12] semiconductors,[14] polymers,[11] and small molecular crystals.[15] In the phage display protocol, a library of peptide (or protein) variants is expressed on the coat of a phage virion, while the genetic material encoding each variant resides on the inside. This creates a physical linkage between the variant peptide sequence and the DNA encoding it, which allows for rapid, combinatorial partitioning of binding affinities toward given targets via in vitro selection processes. Here we report for the first time the patterning and identification of a small molecular ink with screened phage displayed peptides. Specifically, our approach combines a comprehensive phage displayed peptide screening process with novel microcontact printing or standard microfabrication techniques.

In contrast to solid inorganic or bound protein targets, it should be noted that the target molecules of interest here exist at room temperature as liquids (e.g., octane) or gases and, thus, are not readily available as facile substrates for the phage display process. Therefore, achieving recognition toward these molecular inks relies on their immobilization on a stable host substrate, which must then be *negatively* screened to collect only those phages that bind to the target ink. Our chosen ink, octyltrimethoxysilane (C8), was selected for its exceptional stability upon binding to silicon (Si) host substrates (see Supporting Information (SI)). The general strategy is shown in Figure 1. An aliquot of phage display library (New England BioLabs, Ph.D. 7) (Figure 1a) was incubated with a C8-functionalized Si surface (Figure 1b) and then eluted from this surface to collect the bound phage (Figure 1c). To obtain only those specific phage displayed peptides which identify the C8 ink, the eluted phages were subsequently incubated with a Si substrate (Figure 1d), to screen out phages that bind to the background Si (Figure 1e). The collected phage particles were then amplified in *E. coli* (ER 2738) (Figure 1f), followed by repeated centrifugation and precipitation. This "biopanning" selection process was repeated

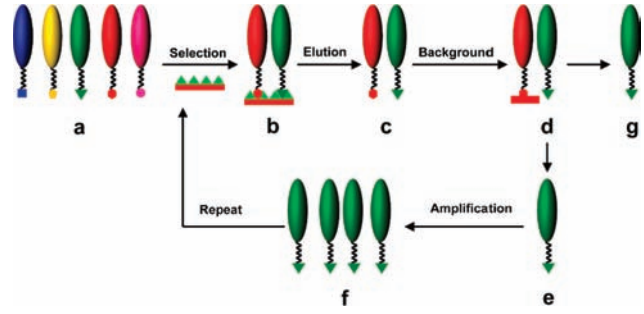

Figure 1. Schematic illustration of the generalized screening protocol for identifying phage displayed peptides binding to molecular inks. (a) A phage library is incubated with a substrate containing a molecular ink, whereupon (b) phages bind to the substrate and ink. (c) These phages are eluted and (d) exposed to the bare background substrate for negative screening and elimination. (e) Surviving screened phages are amplified (f), and the process is repeated to screen for the strongest binders. (g) Finally, phages which recognize only the molecular ink are collected.

Table 1. Summary of Phage Displayed Peptides Binding to C8

Num	Freq	Sequence	Average Hydrophilicity	Hydrophobic Ratio
1	4/16	HAIYPRH	−0.4	86%
2	2/16	SILPYPY	−1.1	86%
3	2/16	TTYSRFP	−0.3	71%
4	2/16	QILAFNS	−0.8	57%
5	1/16	AYSTLWP	−1.2	86%
6	1/16	LPIWRDF	−0.5	71%
7	1/16	GETRAPL	0.5	71%
8	1/16	GIRHTNP	0.1	71%
9	1/16	VYPHPER	0.2	71%
10	1/16	GNTPSRA	0.4	57%

up to six times to obtain those phage displayed peptides having the highest binding affinities to C8 ink (Figure 1g). Finally, the refined libraries were prepared for DNA sequencing, which allowed for identification of the C8-binding peptides. Table 1 shows the peptide sequences obtained from six rounds of biopanning. Interestingly, the peptides with the highest frequency of occurrence have negative average hydrophilicity (Hopp−Woods scale), with high ratios of hydrophobic to total number of residues.

One of the C8-binding phage displayed peptides (SILPYPY) was isolated as a single colony and selected for further investigation. First, fluorescent characterization for the binding of this phage to C8 and Si substrates was investigated. This was accomplished by exposing the substrates sequentially to (1) amplified single-colony phage displayed peptide, (2) blocking buffer, (3) antiphage biotin conjugated antibody, and (4) avidin-FITC, with washing steps in between. The color intensity of FITC on the surface, which is proportional to the binding of the phage displayed peptides, was observed by a fluorescence microscope. Significantly, as shown in Figure 2a, the C8 ink shows much higher fluorescence intensity relative to the bare Si substrate due to the binding of the phage

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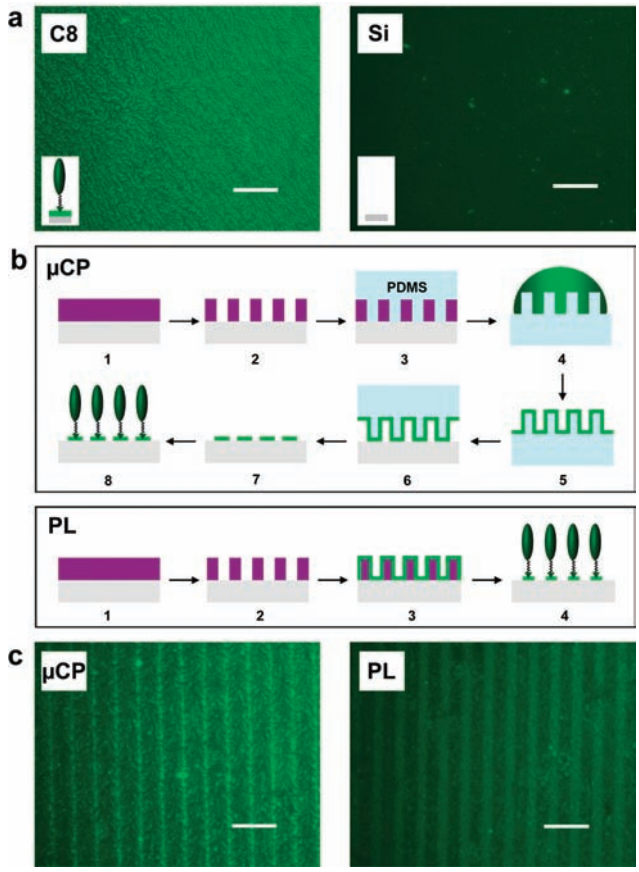

Figure 2. Identification of patterned C8 molecular ink with phage displayed peptides. (a) Fluorescent characterization of the binding of phage displayed peptides to C8-functionalized Si, and to bare Si. Inset: schematic of the phage binding. Gray is Si; green is the C8 ink. (b) Schematic illustrations of the micropatterning and identification of molecular inks with phage displayed peptides by μCP and PL methods. Purple is photoresist; blue is the PDMS stamp. (C) Fluorescent characterizations of the binding of phage displayed peptides on molecular inks micropatterned via μCP and PL. All scale bars: 20 μm.

displayed peptides. This fluorescent differential is much larger than control experiments involving either unselected phage, or native M13 phage *sans* peptide (see SI).

As an ultimate test of the ability for the phage displayed peptides to recognize molecular inks, C8 was micropatterned by both microcontact printing (μCP) and photolithographic (PL) methods. Schematic illustrations of the micropatterning processes (5 μm line width with 10 μm center-to-center spacing) are shown in Figure 2b (see SI). In μCP, a PDMS stamp was brought into contact with a clean Si wafer to generate the micropatterned C8 ink on the Si surface. Indeed, previous work has shown that nanoscale confinement by PDMS stamps is sufficient for forming covalent linkages to Si substrates.[16] By introducing appropriate phage displayed peptides, the phages bind to the specific locations mapped by the micropatterned ink. An alternative method involves the use of PL for patterning the ink.

Accordingly, C8 molecular ink was patterned according to both the μCP and PL schemes outlined and exposed to single colonies of the aforementioned phage displayed peptide (SILPYPY). Fluorescent characterizations for the binding of these peptide phages to the ink are shown in Figure 2c. Significantly, whether the ink was patterned by μCP or PL, stronger fluorescent signals were observed localized on the molecular inked stripes, relative to the background Si substrate. This result clearly indicates that the microstructured C8 ink imparts enhanced binding affinity toward the phage displayed peptides over the host Si substrate.

We have demonstrated a rational and general scheme for the patterning and identification of molecular inks via screened phage displayed peptides. Our approach has as its foundation several key steps, including (1) immobilization of the molecular ink on a stable host substrate; (2) comprehensive positive and negative screening of the target ink and host substrate, respectively; (3) identification and isolation of single colony binders; and (4) micropatterning of the ink and exposure to phage displayed peptides, for localized binding. The approach we describe here opens new avenues for applications in various fields, including cell biology, nanomaterials self-assembly, and selective sensors, by using the screened peptides as linkers for recognizing inks. Most critically, the breadth in surface chemistry for immobilizing small molecules (silane, thiol, amide, etc.), combined with the use of novel micropatterning techniques, suggests that this technique could form the basis for on-chip, multiplexed recognition of molecular inks by selective peptide binders.

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Supporting Information Available: Full experimental details, including phage selection and sequencing, peptide analyses, and ellipsometric, stability, and fluorescent characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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