Available online at www.sciencedirect.com
SCIENCE DIRECT®
Synthetic Metals 153 (2005) 21–24
SYNTHETIC METALS
www.elsevier.com/locate/synmet

Microcontact printing of metalloproteins

Adriana Biasco *, Dario Pisignano, Blandine Krebs, Roberto Cingolani, Ross Rinaldi

NNL, National Nanotechnology Laboratory of Istituto Nazionale di Fisica della Materia (INFM), c/o Dipartimento di Ingegneria dell'Innovazione, Università di Lecce, via Arnesano, I-73100 Lecce, Italy

Abstract

The morphological investigation by scanning probes techniques, and especially Atomic Force Microscopy (AFM) allow to measure the surface roughness of immobilized proteins with unequalled vertical spatial resolution. This is particularly important, since the biological role of proteins is closely related to their physiological folding architectures. In particular, metalloproteins, such as blue copper proteins, revealed to be good candidates for biomolecular electronics by virtue of their natural electron transfer activity, which is exploitable in molecular switches whose conduction state can be controlled by gating their redox state. The interest in these molecules is indeed motivated by the possibility of eliciting current flow through the redox level of single molecules. Printing metalloproteins can be an advantageous approach for the easy realization of bioelectronic circuits. We implemented and developed the selective deposition of the metalloprotein Azurin (Az) from *Pseudomonas aeruginosa* by microcontact printing, and investigated by AFM the surface morphology of the printed Az layers.

Keywords: Metalloproteins, microcontact printing, self-assembly using surface chemistry, atomic force microscopy,

1. Introduction

Several applications in biology require proteins immobilized on substrates. Among them, particular interest is being aroused by microarray fabrication [1, 2] and biomolecular electronics [3, 4] . In both these fields, the possibility of patterning proteins within specific locations in two-dimensional structures, possibly with μm-scale or sub-μm-scale controlled resolution, is a desirable goal [5, 6], in order to control the array distribution in protein chips.

Proteins are biological macromolecules constructed from one or more chains of amino acids linked by peptide bonds and folded into a specific three-dimensional configuration (tertiary structure). High-throughput protein-based technologies have to preserve this three-dimensional structure and, therefore, the activity and specificity of the employed biomacromolecules. We underline that, for instance, the fabrication of protein chip requires much more efforts, than DNA chip; in fact, while the DNA is a relatively robust molecule, consisting of only four different building blocks, proteins are typically more difficult to handle and a narrower range of environmental conditions (temperature, pH, solvent ionic force, etc.) are needed to deliver them as functional entities [7].

By virtue of their structural and functional properties, proteins are also considered good candidates as building blocks in the bottom-up fabrication of biosensors and hybrid molecular electronic devices. In particular, metalloproteins, such as blue copper proteins, revealed to be good candidates for biomolecular electronics because of their natural electron transfer activity, which is exploitable in molecular switches whose conduction state can be controlled by gating their redox state [8]. The interest in these molecules is indeed motivated by the possibility of eliciting current flow through the redox level of single molecules. Therefore, selectively patterning metalloproteins can be an advantageous approach for the easy realization of bioelectronic circuits.

For all these reasons, specific processing and patterning techniques are required, preserving proteins and preventing them from binding in a non-specific way, which would lead to protein chips with a high signal-to-noise ratio. So far, several techniques have been developed for patterning proteins on pre-modified substrates, such as photochemical methods [9], photografting, inkjet printing [10] and printing robots [11]. Nevertheless, soft lithography [12] is by far considered the most convenient approach

* Also at ISUFI, Università di Lecce, via Arnesano, I-73100 Lecce, Italy. Corresponding author. Tel: +39-0832-298378; fax: +39-0832-298238; E-mail: adriana.biasco@unile.it

doi:10.1016/j.synthmet.2005.07.232

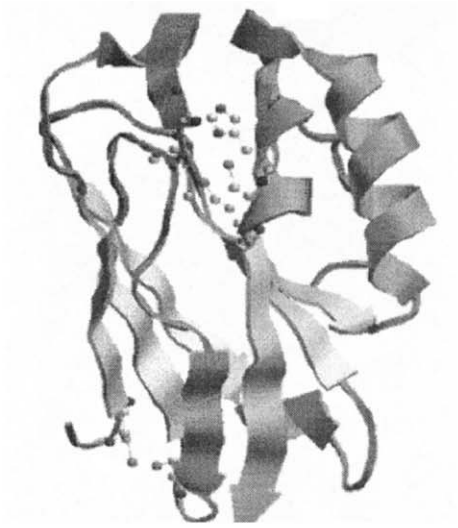

Fig. 1 Molecular structure of the Az protein.

because of its experimental simplicity, low cost, and extreme flexibility in terms of the employable substrates, solvents, and deliverable molecules. However, although much work has been done to achieve high-throughput direct patterning of biomolecules onto surfaces, the generality of the approach to produce patterns of azurin by microcontact printing (μCP) is still unknown.

In this work, we implemented and developed the selective deposition of the metalloprotein Azurin (Az, molecular structure in Fig. 1) from *Pseudomonas aeruginosa* by microcontact printing. We assessed the successful transfer of the biomolecules by immunofluorescence experiments, and investigated the surface morphology of the printed Az layers by atomic force microscopy (AFM). Our approach is based on the modification of the substrate surface properties by introducing a surface linker (-SH functional group) that allows a site-specific binding of the protein.

2. Experimental

Az and the anti-Az serum (primary antibody) were kindly supplied by the metalloprotein groups, Gorlaeus Laboratory, Leiden, The Netherlands. It was diluted in 20 mM Hepes buffer, pH 4.6, to a final concentration of 10^{-8} M and 10^{-5} M. 3-Mercapto Propyl Trimethoxysilane (3-MPTS) was purchased from Sigma and was diluted immediately prior to use to 2% (v/v) in absolute ethanol. Secondary antibody (1:32 rhodamine-labelled goat anti-rabbit IgG antibody) was purchased from Sigma-Aldrich.

All the microfabrication steps were performed in a 1000-class clean room. SiO_2 wafers were cleaved into 1 by 1 cm^2 pieces; they were pre-cleaned using a 3:1 mixture of H_2SO and H_2O_2 in order to remove organic contaminants and sonicated three times for 5 minutes in water at 25°C. The substrates were then dried with a blast of N_2 gas to remove the bulk water thus preventing undesirable polymerization of the 3-MPTS. All the surfaces were used immediately after cleaning. To introduce -SH functional groups on the surface, silanization was carried out in a 2% (v/v) solution of 3-MPTS in ethanol at 25 °C for 15 minutes. Sonication in ethanol was then performed for 1 minute, to remove eventually non-covalently adsorbed silane compounds, and then dehydration was carried out with a N_2 flux. The so treated substrates were stored under vacuum until the contact with protein.

The performed μCP lithography process is schematized in Fig. 2. The master patterns, made by parallel stripes of periodicity between 20 and 30 μm were fabricated by photolithography on Si/SiO_2 (SiO_2 thickness ≅ 100 nm) substrates. After the deposition of standard photoresist (Clariant AZ5214E:AZ EBR Solvent 1:1), UV-exposure (about 8 minutes at 45 W) and developing, we etched the

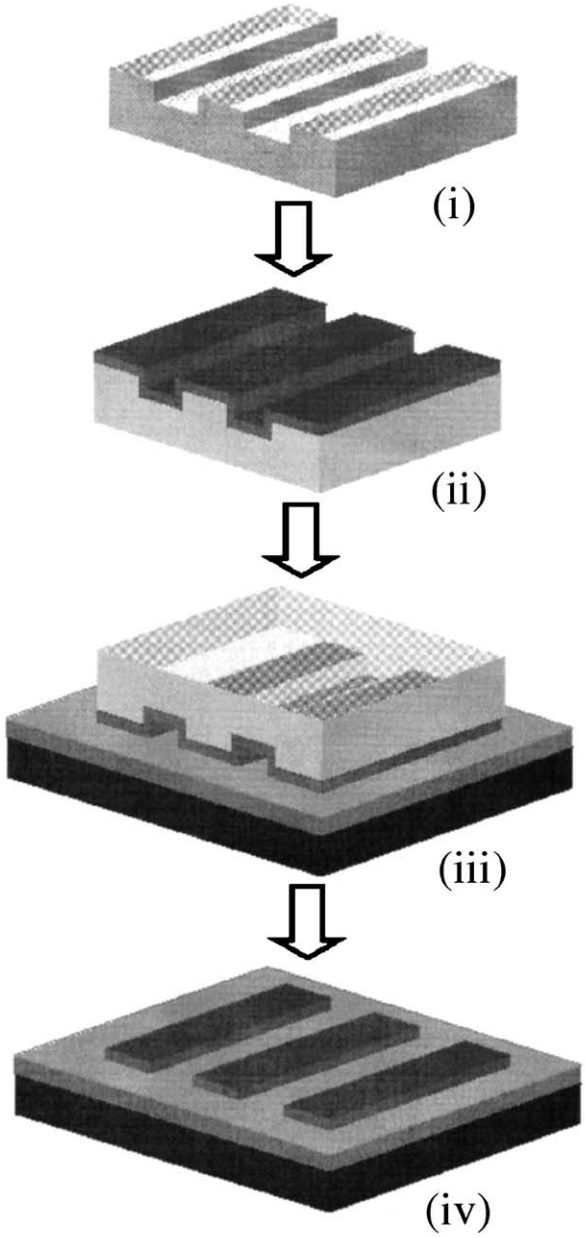

Fig. 2 Scheme of the μCP. The stamp, obtained by casting the elastomer over master (i), is inked by the protein solution (ii). It is then placed onto the functionalised substrate (iii), and then peeled off, thus forming a protein layer on the substrate surface (iv).