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Patterned gallium surfaces as molecular mirrors.

Alessandra Bossi[1]*, Claudio Rivetti[2], Laura Mangiarotti[2], Michael J. Whitcombe[3], Anthony P.F. Turner[3], Sergey A. Piletsky[3]

[1] Dipartimento Scientifico e Tecnologico, Università di Verona, 37134 Verona, Italy;

[2] Dipartimento di Biochimica e Biologia Molecolare, Università di Parma, Parco Area delle Scienze 23/A, 43100 Parma, Italy;

[3] Cranfield Health, Cranfield University at Silsoe, MK45 4DT, Silsoe, Bedfordshire, UK

Corresponding author: Alessandra Bossi, Dipartimento Scientifico e Tecnologico, Università di Verona, 37134 Verona, Italy; Phone +39-045-8027946;Fax: +39-045-8027929; email: alessandra.bossi@univr.it

Abstract

An entirely new means of printing molecular information on a planar film, involving casting nano-scale impressions of the template protein molecules in molten gallium, is presented here for the first time. The metallic imprints not only replicate the shape and size of the proteins used as template; they also show specific binding for the template species. Such a simple approach to the creation of antibody-like properties in metallic mirrors can lead to applications in separations, microfluidic devices and the development of new optical and electronic sensors and will be of interest to chemists, materials scientists, analytical specialists and electronic engineers.

Keywords: protein imprint, nano-scale impressions, metal, gallium, sensing, recognition

1. Introduction

The patterning of a material's surface is a generic tool for altering its physical and chemical characteristics i.e. texture, optical and electromagnetic properties, recognition abilities and responsiveness to the environment (Chailapakul and Crooks, 1993; Appell, 2002; Assender et al., 2002). Thus considerable effort has been expended in the development of methods for the deposition of materials encoding two- and three-dimensional information on the nanoscale level (Liu and Amro, 2002; Zhang, 2003). Methods for molecular patterning include: ordered deposition of nucleic acids, fusion proteins and self-assembled monolayers (Seeman, 1999; Niemeyer, 2000; Moll et al., 2002; Ulman, 2001; Jerome et al., 1987); microlithography and microcontact printing technology (Whitesides et al., 1991; Whitesides et al., 2001; Kumar and Whitesides, 1993; Kumar et al., 1994; Chou et al., 2005). Here we present a different approach to the nano-patterning of metal surfaces which does not involve the physical transfer of material from the template to the patterned surface. Instead, the pattern, in the form of molecular imprints, has been created by solidifying molten gallium in the presence of immobilised protein templates. The template is mechanically removed from the gallium leaving cavities resembling "negative" mirror images of the template proteins, capable of their selective rebinding. The approach resembles molecular imprinting in organic polymer and inorganic sol-gel matrices in the sense that molecular templates determine the shape of the cavities created (Wulff, 1995; Mosbach and Ramstrom, 1996) but opens up an entirely new class of material for electronic sensor design due to conductive nature of material used.

2 Materials and Methods

2.1. Surface preparation.

Template surfaces were prepared by physical adsorption of the following proteins onto freshly cleaved ruby mica: horseradish peroxidase (HRP), purified bovine serum albumin (BSA), RNA polymerase, urease from Jack Bean, Dps (**D**NA-binding **p**roteins from **s**tarved cells) from E. coli. All proteins were deposited onto mica from a 6 nM solution in deposition buffer (4 mM Hepes, 10 mM NaCl, 2 mM $MgCl_2$ pH 7.4). The deposition drop was incubated onto the surface for 3 minutes, rinsed with water milli-Q and dried in a nitrogen stream.

2.2. Casting of the metal surfaces.

Cassettes for casting the metal surfaces were prepared from a borosilicate glass slide and a mica slide, separated with a Teflon spacer of 50 mm thickness. Pure molten gallium (T = 37 0C) was poured into the cassette, and left to solidify at room temperature for 30 min in a humid controlled chamber. Finally the metal surface was peeled off the template.

2.3. Atomic force microscopy analysis.

Atomic force microscopy imaging was performed with a Nanoscope IIIA (Veeco Instruments), equipped with a type E scanner and operating in a “tapping mode”, using diving-board cantilevers mounted with a pyramidal tip (Veeco Instruments). All images were acquired “in air” on dried samples with a scan rate of ~3 lines/s and a

scan size of 2 mm. Image inspection and analysis was carried out with the Nanoscope software.

2.4. Dissociation constant calculation.

Metal surfaces printed with HRP, albumin printed metal surfaces and control gallium foils were cut into circles of 4 mm diameter, each placed into a microtiter plate well. Gallium foils were incubated at room temperature for 30 minutes with protein at a range of concentrations, varying from 0.23 nM to 23 mM, dissolved in 50 mM MES buffer (pH 6.0). Non-specifically bound protein was removed by a double washing with Milli-Q water followed by a washing step in MES buffer containing 0.05 % Tween-20. Gallium surfaces were dried in a nitrogen stream. The quantity of adsorbed protein was measured using 3,3',5,5'-tetramethylbenzidine (TMB) assay according to Porstman and Kiessig (1992). Dissociation constants were calculated from data linearised with a Scatchard-Rosenthal plot. Replica printing was tested by repeating the process of solidifying gallium on the same mica slide with immobilised HRP. Binding analysis was performed as described previously.

3. Results and discussion

Gallium was selected for investigation as a printable material due to its low melting temperature (~30°C), which seems particularly convenient for the imprinting of biological molecules. Several proteins, varying in molecular mass from 44 to 500 kDa, were immobilised on the surface of mica slides for use as templates. The molten metal was brought into contact with the template-coated surface and allowed to solidify between two slides at room temperature (Gilfillan and Bent Jr., 1934). Mica

slides were peeled from the gallium films to reveal flat foils bearing imprints on the surface. The morphology of the printed metal surfaces, analysed by atomic force microscopy (AFM), showed indentations of nanometre dimensions, complementary in average density distribution and size to the template (see AFM images in Figures 1a, 1b and Figure 2). The control metal foil, formed between two mica slides in the absence of protein template had a very flat surface without any structural features (Figure 1c). Atomic unevenness of the gallium surface manifested itself at a scale below 2 nm. As a result it was difficult to visualise relatively small imprints (~ 5 nm) formed by the templates with molecular masses below 60 kDa.

No protein molecules were detected by AFM on the printed surfaces which indicated that the mechanical separation procedure for removal of the template had been successfully achieved. The non-transfer of template material onto the printed surface was confirmed independently by an assay for enzymatic activity performed on gallium foils printed with horseradish peroxidase (HRP), which showed no activity.

The printing of metal surfaces resulted in mirror images of the template molecules, with consistent reproduction of the template pattern. There was a correlation between the size of the template and the size of the imprints formed (Table 1). Thus, printed metal surfaces have the potential to mirror nano-scale templates such proteins, fibrils or synthetic motifs in a desired two dimensional geometry, as suggested by Liu and Amro (2002). Despite a full understanding of the nature of the metal printing process is still to be found, it seems that casting nano-sized impressions on gallium foils has striking resemblance to the molecular imprinting process, thus suggesting the term ‘metal imprinting’ for the observed phenomenon.

An important question was whether the prints created in gallium would recognise their respective molecular templates in a similar way to imprints formed in molecularly imprinted organic polymers (Bossi et al., 2001). This possibility was investigated by an analysis of the binding properties of gallium surfaces to HRP. The dissociation constants were calculated for the printed and non-printed surfaces. The HRP-printed metal surface possesses more than ten times higher affinity to the template protein HRP compared with a non-printed surface or surface printed with albumin (see Table 2). Printed surfaces also possessed a significantly greater quantity of binding sites, compared with their non-printed counterparts. The enhanced binding of template protein to printed metal surfaces supports the hypothesis that shape-controlled recognition, generally associated with imprinted polymers (Wulff, 1995; Mosbach and Ramstrom, 1996; Bossi et al., 2001) and synthetic hosts (Rebek, 1985) is responsible for the increased affinity of the templated metal surfaces. A greater number of binding sites was observed in case of albumin printed gallium challenged with HRP, due probably to an increase in the roughness of the surface, respect the control gallium.

The printing process can be repeated using the same parent template layer with the recognition ability of the second replica surface being very similar to the first. The process however becomes progressively less efficient after the third and subsequent printing cycles, presumably due to the denaturation of protein templates as a result of mechanical and thermal stresses.

4. Conclusions

The casting of structures in metal, as coins or bronze statues, is an ancient art, but can metals be used to replicate molecular-scale objects, a million times smaller than the

average coin? Results here proposed with the low melting point metal (gallium) just support that, using protein molecules fixed to a surface as the "mould". The metallic prints not only replicate the shape and size of the proteins used as template; they also show specific binding for the template species. In our view this is the first successful demonstration of the creation of molecular imprints, using a microcontact method, and a material that differs significantly from the traditional approaches, involving organic or inorganic polymers. The conditions for metal printing could be further optimised using alloys with different composition and melting points. Apart from their academic interest “molecular mirrors”, which combine conductive properties with molecular selectivity, may open new avenues in optical and electronic sensor design. In particular it is possible to envisage the development of electrochemical sensors based on imprinted metal surfaces.

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Figure Captions.

Figure 1. Creating molecular mirrors. AFM image of: (a) the globular protein Dps deposited on mica; (b) metal surface printed with Dps protein and (c) control (non-printed) metal surface. Marks in (a) and (b) highlight the profile analysis performed on the master and on the printed metal. Image analysis performed along the lines indicates the dimensions of created structural features. The average dimensions of Dps are: height, 1.8 nm, width, 38 nm; the dimensions of the Dps imprints are: depth, 1.5 nm, width, 19 nm. Note: The AFM tip dimensions introduces distortions in the two-dimensional images resulting in overestimation of the size of the deposited proteins and underestimation of the size of the prints.

Figure 2. Molecular mirror created using RNA-polymerase (300 kDa) as template. Three-dimensional model generated from AFM data: AFM image of RNA-polymerase deposited on mica (A) and metal surface printed with RNA-polymerase (b). The average dimensions of RNA-polymerase are: 3.7 nm height, 40 nm width; the average dimensions of RNA-polymerase prints are: 3.6 nm depth, 25 nm width.

Figure 1. Bossi et al.

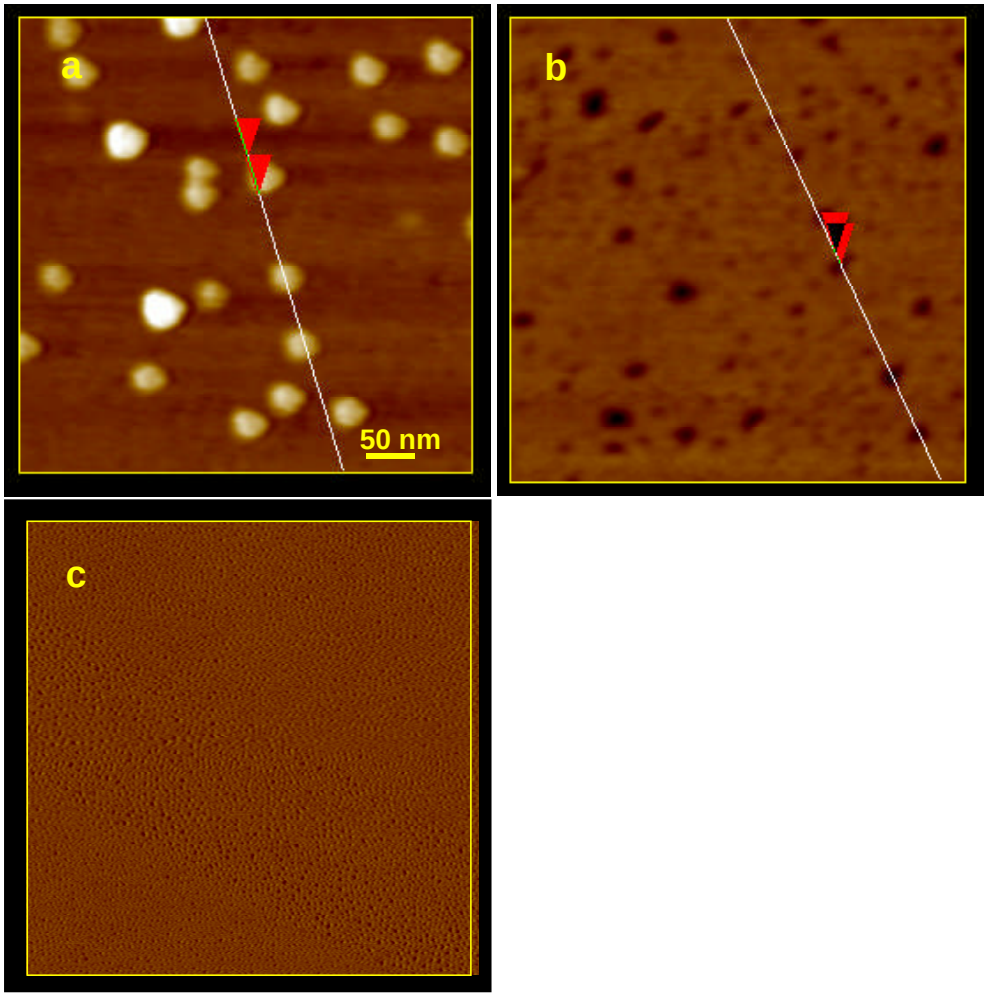

Figure 2. Bossi et al.

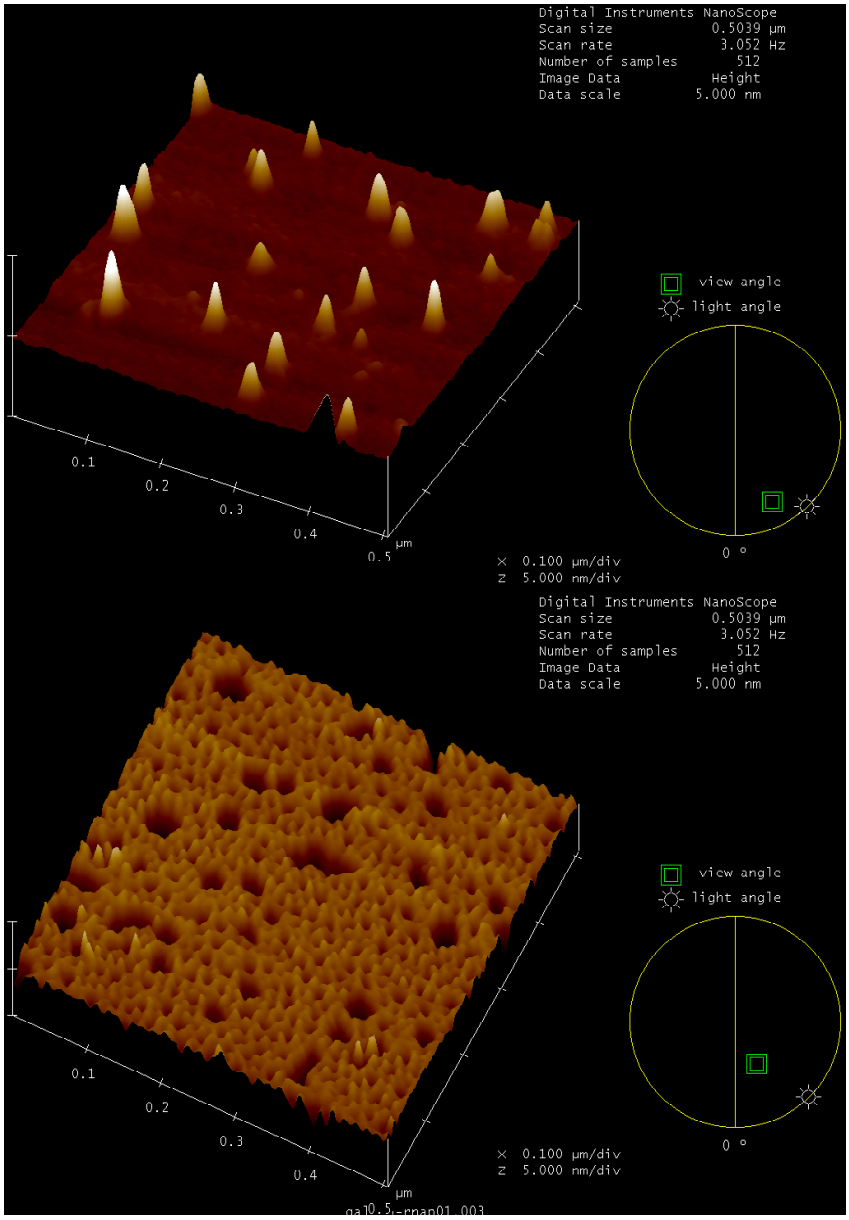

Table 1. Influence of the size of printed molecules on the diameter of prints formed on gallium surface.

Template	Molecular weight, Da	Diameter of cavities, nm	Depths of cavities, nm
RNA polymerase	500.000	25 ± 2	3.6 ± 0.5
Dps[a]	95.000	19 ± 2	1.5 ± 0.3
Bovine serum albumin	68.000	7 ± 1	1.1 ± 0.3

Horseradish peroxidase	44.000	5 ± 2	0.7 ± 0.2

[a] DNA-binding proteins from starved cells.

Table 2. Recognition properties of the metal surfaces. Rebinding experiments were conducted with HRP as challenging protein on all the type of surfaces.

Type of surface	Dissociation constant, mol/L	Quantity of binding sites, $fmol/cm^2$
HPR-printed gallium	$11 \pm 2\ 10^{-9}$	3
Control gallium	$184 \pm 18\ 10^{-9}$	0.7
Albumin-printed gallium	$205\ 10^{-9}$	1.3

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Bossi, Alessandra

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