COMMUNICATIONS

Fabricating Microarrays of Functional Proteins Using Affinity Contact Printing**

Jean Philippe Renault, André Bernard, David Juncker, Bruno Michel, Hans Rudolf Bosshard, and Emmanuel Delamarche*

Phenomena involving the binding between biomolecules are ubiquitous in biology and are essential for cell growth, signal transmission, and immune defense. In the latter system, the binding between antibody and antigen has already been exploited technologically to perform affinity purifications on columns and immunoassays on surfaces.[1] Recently, the fabrication of microarrays of proteins which require the immobilization of a large number of receptors on a surface have fueled the invention of novel patterning techniques such as pin-spotting and drop-on-demand.[2] Microarrays of proteins may find utility in proteomics, immunoassays, or for screening libraries of (bio)chemicals. It is at present not clear which patterning method will be the one best suited to pattern proteins on surfaces, but classical lithography does not seem capable of fabricating microarrays of proteins. Soft lithography[3] offers the possibility of manipulating proteins and other biomolecules by printing them from a micropatterned stamp to a surface[4] or by depositing them from a liquid using microfluidic networks (µFNs).[5] Affinity microcontact printing (αCP)[6] is a refined soft-lithographic technique that uses an elastomeric stamp made of polydimethylsiloxane (PDMS) and derivatized with binding biomolecules to extract corresponding binding partners from an impure, dilute source for placing them on a surface with spatial control.

Herein, we describe, by using one particular example, how specific binding between biomolecules provides a unique opportunity to make use of self-assembly processes in technology: we propose different variants of αCP to pattern surfaces with ensembles of biomolecules where the pattern on the affinity stamp (α-stamp) is not determined by its topography but by the position of various proteins covalently linked to a planar α-stamp (Figure 1). This modified surface enables the simultaneous capture of different target proteins on the α-stamp from a complex solution (Figure 1 A). Thus, the capture step (Figure 1 B) directs the assembly of an array of target molecules on the stamp (Figure 1 C), which can be

[*] Dr. E. Delamarche, Dr. A. Bernard, D. Juncker, Dr. B. Michel
IBM Research
Zurich Research Laboratory
Säumerstrasse, 4, 8803 Rüschlikon (Switzerland)
Fax: (+41) 1-724-8952
E-mail: emd@zurich.ibm.com

Dr. J. P. Renault, Prof. H. R. Bosshard
Dept. of Biochemistry
University of Zurich
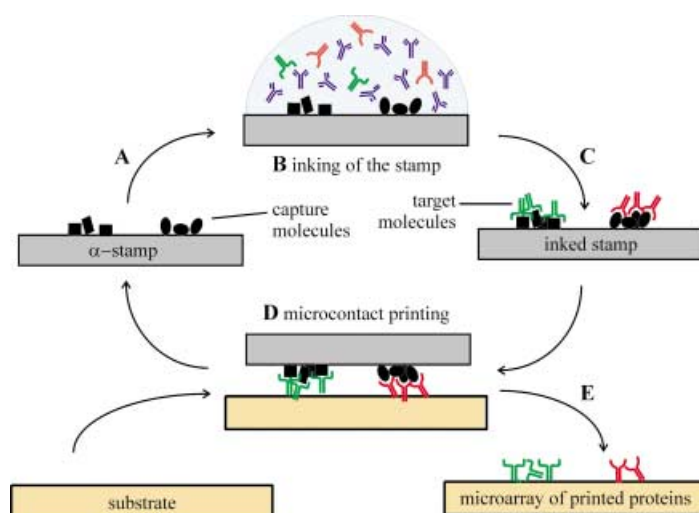Winterthurerstrasse 190, 8057 Zurich (Switzerland)

Figure 1. Microarrays of proteins on surfaces can be fabricated using an α-stamp derivatized with various capture sites that can extract target biomolecules from a complex solution and release them on a surface in a single microcontact-printing step. The α-stamp can be reused for several inking and printing cycles.

microcontact-printed onto a substrate in one step (Figure 1 D). The α-stamp is recovered at the end of this process, and can be reused.[6] We opted for protein antigens (entire immunoglobulin G) as capture molecules and antibodies as targets because these binding partners are very specific, can be readily conjugated with fluorescent markers, and of course play an important role in heterogeneous immunoassays.

Affinity stamps are prepared by immobilizing the capture molecules on an "activated stamp" that is reactive towards NH_2 groups of proteins. An activated stamp is made in three steps from a planar layer of PDMS (see the Supporting Information for experimental details). First, silanol groups are created at the PDMS surface using an O_2 plasma.[7] These silanol groups are subsequently treated with 3-aminopropyl-triethoxysilane to create an amino-derivatized surface. These amines are then treated with a homo-bisfunctional cross-linker (BS³) to produce the activated surface.[7] The stamps produced in this way are stable for several hours in a dry environment such as a dessicator, and can immobilize monolayers of proteins under mild chemical conditions.[8, 9] Activated stamps are hydrophilic, with an advancing contact angle with water of approximately 30°, and therefore cannot be locally derivatized at high resolution with solutions of proteins by pin-spotting or ink-jet methods.[2, 10] Our aim was to prepare α-stamps having arbitrary patterns with dimensions as small as a few micrometers, and we developed various methods to achieve this goal. The first method relies on coupling proteins to small areas of an activated stamp using microwells[11] (µ-wells; Figure 2). The microwells are aniso-tropically etched through a 525-µm-thick Si wafer, and can be placed in contact with the activated stamp (Figure 2 a). This contact is conformal and seals each microwell individually. Pipetting the desired amount of protein solution into all or a subset of the microwells determines the array of capture molecules formed on the α-stamp (Figure 2 b, c). The hydrophobization of the top and bottom faces of the array of

a) Fabricating the μ-wells and activating the PDMS stamp

b) Placing the μ-wells and filling them with solutions of antigens

c) Removing the μ-wells under a buffer and rinsing the stamps

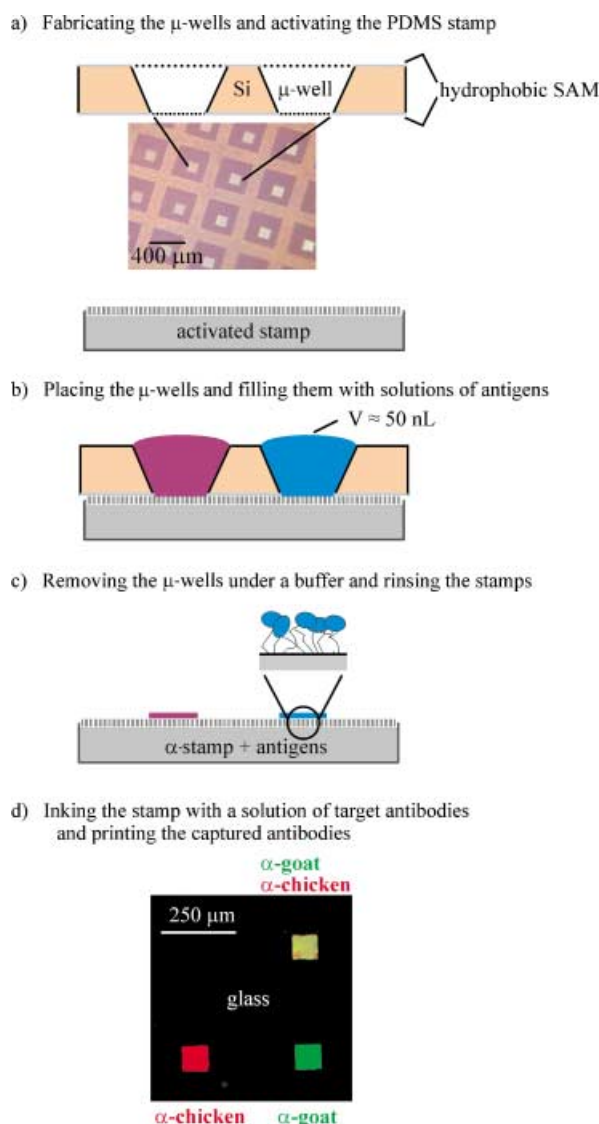## d) Inking the stamp with a solution of target antibodies and printing the captured antibodies

Figure 2. Preparation of a microarray of proteins using an α-stamp patterned with microwells. a) The microwells are formed in a Si wafer, and the PDMS stamp is derivatized with cross-linkers for proteins. b) The contact of the microwells with the activated stamp localizes the attachment of capture molecules from solution to the area of the stamp exposed in each microwell. c) After separating it from the microwells, rinsing and drying, the α-stamp is ready for use. d) The α-stamp in this example had an empty capture site and sites with covalently attached anti-chicken antigens, anti-goat antigens, and protein A. Inking the α-stamp consists of the binding of antibodies (here tagged fluorescently) from solution to their specific antigens on the surface of the stamp. After rinsing and drying the inked α-stamp, the antibodies can be printed onto a glass surface and visualized by fluorescence microscopy.

microwells by using a perfluorinated silane prevents leakage of liquid across the wells.[12] In addition, the truncated pyramidal shape of the microwells makes it possible to fill them readily. The microwells used here can hold up to 50 nL of solution, and their drying could be controlled on the timescale needed for the coupling reaction. Affinity–capture sites based on the immobilization of protein A, and mouse, goat, and chicken antigens were patterned on an α-stamp by using 100×100 μm^2 wells (Figure 2d). This α-stamp is inked with a solution of anti-species antibodies (fluorescein iso-

thiocyanate labeled (FITC) anti-goat and tetramethylrhodamine B isothiacyanate linked (TRITC) anti-chicken antibodies) containing a large amount of bovine serum albumin (BSA). Each type of antibody binds in parallel to its specific antigen on the α-stamp during this inking step, and protein A captures both types of antibodies whereas BSA adsorbs elsewhere and prevents nonspecific adsorption on the α-stamp. The target molecules are transferred from the α-stamp to a glass slide during a printing step, and can be visualized as a result of their fluorescence label. The pattern in Figure 2d reveals the expected fluorescence pattern in which the target molecules are placed with high accuracy and contrast on their final substrate.

The preparation of the α-stamp is probably the most critical part of the αCP technique, and dispensing the solution of proteins into the microwells limits the practical resolution of αCP. This limitation can be circumvented by using microfluidic networks (μFNs) to prepare the α-stamp. We take advantage of the sealing between the channels of a μFN and a PDMS surface to deposit capture proteins on an intermediate stamp from the microchannels of the μFN (Figure 3a). This stamp is then contacted with the activated stamp for 10 min. The capture antigens transfer and bind covalently to the surface of the activated stamp in this step (Figure 3b). We verified that the transfer was complete and that it did not alter the pattern by using fluorescently tagged antigens. The fluorescence microscopy images in Figure 3c reveal that the α-stamp prepared with this method can extract an ensemble of fluorescently tagged antibodies and then release them by printing onto a surface several times. The affinity site of line d in this example comprises protein A, for which we noticed that fewer FITC anti-goat antibodies were captured and printed after the third cycle. We speculate that during these consecutive cycles protein A captures both types of antibodies present in the ink with differing efficiencies. The high-resolution potential of μFNs[13] is conserved in the fabricated array of antibodies, and the investment in preparing the α-stamp is compensated by reusing it for several cycles of capture and release.[6]

μCP is an efficient and low-cost method for patterning proteins with submicrometer resolution.[14] Since μCP uses the deposition of proteins from bulk solution on stamps and prints them on a substrate, liquids can be handled simply by manual pipetting without the need for a particular dispensing device. αCP can be extended by using μCP to form very high-resolution arrays of proteins in the following way. First, a layer of capture antigens is deposited from solution onto a hydrophobic PDMS stamp (Figure 4a). The contact between a patterned Si substrate and the inked stamp releases the proteins from the stamp to the Si surface in the areas of contact (Figure 4b).[15] This operation is a subtractive transfer of proteins, and does not require structured PDMS stamps; it is therefore insensitive to mechanical deformations as can occur in conventional μCP.[16] The patterned antigens are transferred to an activated PDMS stamp in a printing step (Figure 4c). Repeating these steps with careful alignment enables the formation of ensembles of arrays on the α-stamp, each containing one type of capture protein (Figure 4d). The stability of the activating layer on the stamp means there is no
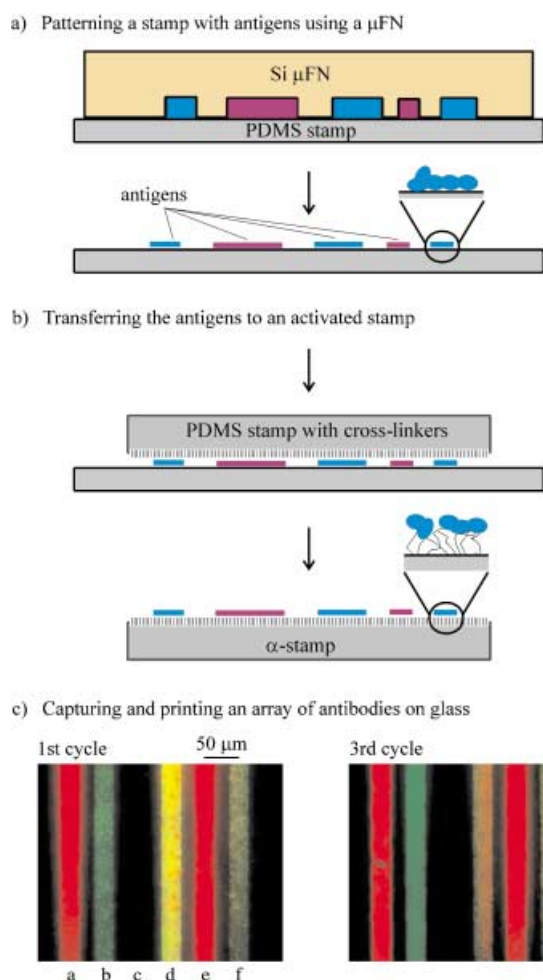
a) Patterning a stamp with antigens using a μFN

b) Transferring the antigens to an activated stamp

c) Capturing and printing an array of antibodies on glass

Figure 3. Patterning lines of proteins on a surface with an α-stamp prepared using a μFN. The μFN localizes the deposition of capture antigens on an intermediate, nonmodified PDMS stamp (a), which can then transfer and attach the proteins to an activated PDMS stamp (b). The α-stamp is used to capture fluorescently tagged antibodies, and print them as lines onto a glass surface (c). The captured molecules on the α-stamp were chicken IgGs (lines a and e), goat IgGs (lines b and f), protein A (line d), and mouse IgGs (line c). Inking this stamp was done by exposing it to a solution containing BSA (1 %), FITC-anti-goat antibodies, and TRITC-anti-chicken antibodies. The fluorescence microscope images reveal that the capture was specific, and the release efficient in providing a high-resolution pattern of printed antibodies even after the α-stamp had been used several times.

need to reactivate the α-stamp between printing steps of the capture proteins if the overall process is shorter than about 2 h. The pattern on the glass substrate in Figure 5a involved two printing steps (done manually) to prepare the α-stamp, and one inking and printing cycle using the α-stamp to yield the "microarray". Specifically, two different antigens (IgGs) from goat and chicken were immobilized on an activated stamp, and used as antigens to extract their respective target antibodies simultaneously from a solution containing FITC-anti-goat and TRITC-anti-chicken antibodies. The captured antibodies were then printed onto the glass substrate in $3 \times 3\ \mu m^2$ areas. This microarray has a density of approximately 10^4 spots of proteins per mm^2 with two types of proteins. The ability of the printed anti-goat antibodies to bind to goat antigens is seen in the AFM image of Figure 5b.

a) Inking a PDMS stamp with antigens

b) Removal of some of the antigens by substractive printing

c) Transferring the antigens to an activated stamp

d) Repeating steps (a) to (c) with a second type of antigen

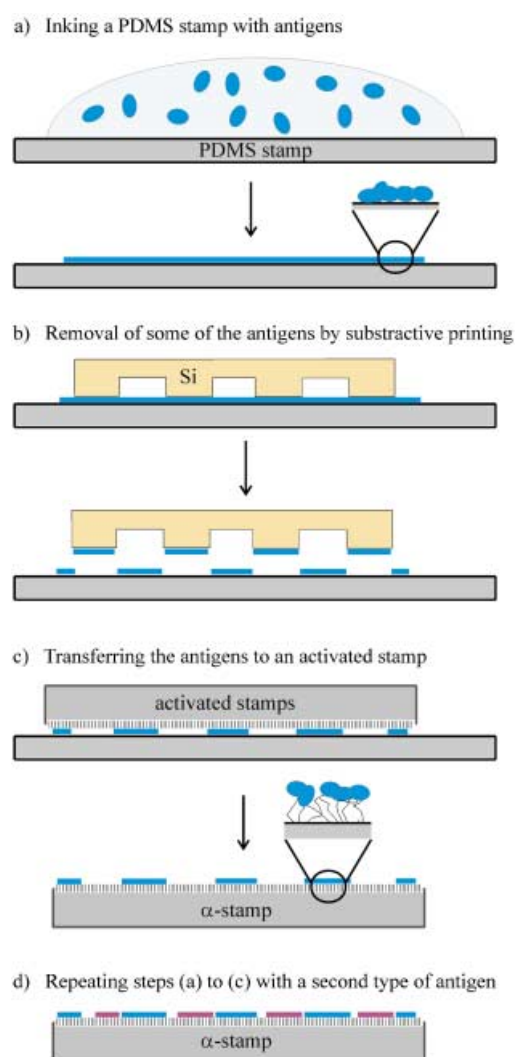Figure 4. Preparation of an α-stamp by using the deposition of capture proteins from solution and subtractive μCP. A layer of capture antigens is deposited from solution onto a PDMS stamp (a), and patterned by removing the antigens in some regions of the stamp by subtractive printing (b). The remaining capture antigens are transferred onto an activated stamp (c). Repeating these steps enables several arrays of capture proteins to be successively added to the α-stamp (d).
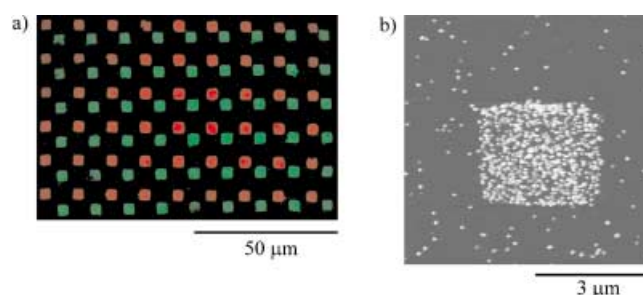

Figure 5. Arrays of anti-chicken antibodies and anti-goat antibodies printed using an αCP having 10^4 equivalent capture sites per mm^2 that consist of immobilized chicken and goat antigens. a) Fluorescence microscope image showing the placement of the TRITC-anti-chicken and FITC-anti-goat antibodies from a stamp onto a glass substrate. b) AFM image obtained on a spot of the array in which the printed anti-goat antibodies bound to Au-labeled goat antigens presented in solution. Detection of this binding was done by staining the Au labels with electroless-deposited silver particles of an average diameter of 80 nm.

The fluorescence data in our experiments indicated that the surface coverage of the final printed layer for each of the three patterning methods presented here is nearly equivalent and reaches about 60% of the surface coverage obtained by direct deposition of the antibodies from solution. As already described for μCP and αCP of proteins, the printing process does not compromise the binding efficiency of the printed antibody. This strategy might not be suitable for patterning a large number of different proteins on a surface. However, it can place a few different proteins as adjacent high-density arrays on a surface. Such arrays could find an application for high-throughput screening in which a large number of analytes could be spotted using a subset of the patterned areas. Another possibility for creating high-density immuno-assays on planar surfaces is by performing surface immuno-assays using many different analytes and capture sites, such as shown in Figure 5. The main limiting factor in using the prepared microarrays for diagnostic purposes could be mis-placement of target molecules during the inking of the α-stamp. Such a misplacement, which may induce false positive reactions, can arise from cross-reactions of the target mole-cules with different capture proteins and/or from nonspecific adsorption on the α-stamp. The former is limited by biological specificity of affinity extraction. The latter can be limited by the systematic use of blocking agents such as BSA. Indeed, for the recognition of goat antigen by the printed array shown in Figure 5a, the recognition signal in the areas with printed anti-chicken antibodies was only 5% of that in the areas with printed anti-goat antibodies.

In summary, we have illustrated how αCP can complement different patterning methods to produce repeatedly, and in parallel, high resolution arrays of proteins in three simple steps: 1) "inking", 2) rinsing, and 3) printing the stamp on the substrate. Since α-stamps carry the complementary pattern of binding partners specific to the target proteins on their surface, the proteins self-assemble into the predefined array on the stamp surface during inking in solution, and dissociate upon printing. Hence, the (re)production of the target protein arrays is fast and easy. The initial production of the α-stamp is a one-time burden only. We thus believe that the methodology presented is powerful and versatile, and should be useful in detection and fabrication strategies that are based on arrays of proteins.

[1] *Immonuassay* (Eds.: E. P. Diamandis, T. K. Christopoulos), Academic Press, San Diego, CA, **1996**.
[2] *DNA Microarrays: A Practical Approach* (Ed.: M. Schena), Oxford University Press, Oxford, UK, **1999**.
[3] a) Y. Xia, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568–594; *Angew. Chem. Int. Ed.* **1998**, *37*, 550–575; b) G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D. E. Ingber, *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335–373.
[4] A. Bernard, E. Delamarche, H. Schmid, B. Michel, H. R. Bosshard, H. Biebuyck, *Langmuir* **1998**, *14*, 2225–2229.
[5] E. Delamarche, A. Bernard, H. Schmid, B. Michel, H. Biebuyck, *Science* **1997**, *276*, 779–781.
[6] A. Bernard, D. Fitzli, P. Sonderegger, E. Delamarche, B. Michel, H. R. Bosshard, H. Biebuyck, *Nat. Biotechnol.* **2001**, *19*, 866–869.
[7] C. Donzel, M. Geissler, A. Bernard, H. Wolf, B. Michel, J. Hilborn, E. Delamarche, *Adv. Mater.* **2001**, *13*, 1164–1167.
[8] S. C. Lin, F. G. Tseng, H. M. Huang, C. Y. Huang, C. C. Chieng, *Fresenius J. Anal. Chem.* **2001**, *371*, 202–208.
[9] A. Bernard, H. R. Bosshard, *Eur. J. Biochem.* **1995**, *230*, 416–423.
[10] a) G. MacBeath, S. L. Schreiber, *Science* **2000**, *289*, 1760–1763; b) H. Zhu, M. Bilgin, R. Bangham, D. Hall, A. Casamayor, P. Bertone, N. Lan, R. Jansen, S. Bidlingmaier, T. Houfek, T. Mitchell, P. Miller, R. A. Dean, M. Gerstein, M. Snyder, *Science* **2001**, *293*, 2101–2105.
[11] a) J. Hyun, A. Chilkoti, *J. Am. Chem. Soc.* **2001**, *123*, 6943–6944; b) J. S. Hovis, S. G. Boxer, *Langmuir* **2001**, *17*, 3400–3405; c) U. Schobel, I. Coille, A. Brecht, M. Steinwand, G. Gauglitz, *Anal. Chem.* **2001**, *73*, 5172–5179.
[12] D. Juncker, H. Schmid, A. Bernard, I. Caelen, B. Michel, N. de Rooij, E. Delamarche, *J. Micromech. Microeng.* **2001**, *11*, 532–541.
[13] E. Delamarche, A. Bernard, H. Schmid, A. Bietsch, B. Michel, H. Biebuyck, *J. Am. Chem. Soc.* **1998**, *120*, 500–508.
[14] A. Bernard, J. P. Renault, B. Michel, H. R. Bosshard, E. Delamarche, *Adv. Mater.* **2000**, *12*, 1067–1070.
[15] Z. Yang, A. M. Belu, A. Liebmann-Vinson, H. Sugg, A. Chilkoti, *Langmuir* **2000**, *16*, 7482–7492.
[16] A. Bietsch, B. Michel, *J. Appl. Phys.* **2000**, *88*, 4310–4318; M. Geissler, A. Bernard, A. Bietsch, H. Schmid, B. Michel, E. Delamarche, *J. Am. Chem. Soc.* **2000**, *122*, 6303–6304.

Au-Nanoparticle Nanowires Based on DNA and Polylysine Templates

Fernando Patolsky, Yossi Weizmann, Oleg Lioubashevski, and Itamar Willner*

The assembly of ordered nanoparticle architectures is a challenging topic in nanotechnology directed to the construc-tion of nanoscale devices.[1] Within this broad subject, the conjugation of biomaterials and nanoparticles to yield or-dered architectures is a promising route to tailor future sensing and catalytic devices, nanocircuitry, or nanodevices, for example transistors, and computing devices.[2] DNA is an attractive biomaterial for use as a template in programmed nanoparticle structures. The ability to synthesize nucleic acids of predesigned shapes and composition, the versatile biocat-alytic transformations that can be performed on DNA, for example, ligation, scission, or polymerization, enable "cut and paste" procedures to be carried out on the template DNA, thus enabling us to design and manipulate the DNA "mold". Furthermore, the association of metal ions to the DNA phosphate units, or the intercalation of transition-metal complexes or molecular substrates into the DNA provide a means to functionalize the DNA-template and to initiate further chemical transformations on the mold. Nanoparticle–DNA assemblies were organized by the hybridization of nucleic-acid-functionalized metal[3] or semiconductor nano-

[*] Prof. I. Willner, F. Patolsky, Y. Weizmann, O. Lioubashevski
Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904 (Israel)
Fax: (+972) 2-652-7715
E-mail: willnea@vms.huji.ac.il