16

Nanosystems for Biosensing

Multianalyte Immunoassay on a Protein Chip

Eiichi Tamiya, Zheng-liang Zhi, Yasutaka Morita, and Quamrul Hasan

Summary

This chapter describes the construction of addressable two-dimensional (2D) microarrays via the random fluidic self-assembly of metallic particles and the use of these arrays as platforms for constructing protein chips for bioassays. These arrays will be useful as platforms for constructing protein chips for bioassays in a broad range of applications. The basic units in the assembly are microfabricated particles, which carry a straightforward visible code, and the corresponding array template patterned on a glass substrate. On one face, the particles consist of a hydrophobic and magnetic Ni-polytetrafluoroethylene (Ni-PTFE) composite layer; the other face has a gold layer that was modified for biomolecular attachment. We use photoresist patterning to create an array template with spatially discrete microwells into which an Ni-PTFE hydrophobic composite layer and a hydrophobic photoadhesive coating are electrodeposited. After biomaterial attachment and binding processes in bulk, the particles are randomly self-assembled onto the lubricated bonding sites on the chip substrate. This self-assembly process is driven by a combination of magnetic, hydrophobic, and capillary interactions. The encoding symbol carried by each particle is used to identify the target attached to the particle surface. This model system demonstrates the utility of the protein chip array for conducting simultaneous multianalyte immunoassays of human immunoglobulins (IgA, IgG, and IgM).

Key Words: Protein chip; biochip; nanosystem; biosensing; microparticle array; self-assembly; encoding; multianalyte immunoassay.

1. Introduction

The fabrication of high-throughput and cost-effective bioassay systems for solution-phase multianalyte parallel detection is critically dependent on arrays of biofunctional biomolecular features arranged on a highly integrated chip ***(1)***. Generally, conventional techniques for biomaterial patterning on a biochip

From: *Methods in Molecular Biology, vol. 300:*
Protein Nanotechnology, Protocols, Instrumentation, and Applications
Edited by: T. Vo-Dinh © Humana Press Inc., Totowa, NJ

involve direct application of the biocomponents onto the substrate surface using any one of several techniques: microcontact printing ***(2–4)***, robotic spotting ***(5–7)***, and site-selective photodeposition synthesis ***(8–10)***. However, these procedures require sophisticated high-precision devices and are both costly and time-consuming. Typical planar arrays also have several technical drawbacks, such as slow diffusion of targets to the binding surface and nonuniform size of the arrayed features. In an effort to overcome these problems, Goodey et al. ***(11)***, Christodoulides et al. ***(12)***, and Curey et al. ***(13)*** developed a versatile platform that uses an on-chip, microcavity-accommodated microbead array for the parallel detection of multiple components in a complex fluid. In this implementation, however, the array was fabricated by sequentially placing individual beads into the on-chip microcavities, one at a time. Practically, this methodology is extremely cumbersome, particularly when the application calls for the assembly of high-density arrays of small beads. Michael et al. ***(14)*** and Walt ***(15)*** proposed another method, in which a random and high-density optical biosensor array is created by randomly dispersing encoded microspheres at the distal end of the fiber bundles. This approach, although overcoming the typical shortcomings associated with the planar-surface technique, requires an optical-encoding chemistry for individual microspheres in order to identify each sensor settled in the array.

Several articles have reported on the development of large-scale integrated picoliter microchamber arrays for polymerase chain reaction ***(16)***, the introduction of a novel nanoliter dispensing system suitable for DNA amplification on a microchamber array chip ***(17)***, the development of a new approach for manufacturing encoded microstructures used as versatile building blocks for miniaturized multiplex bioassays ***(18)***, and the construction of a protein chip array ***(19)***.

This chapter describes a two-step methodology for constructing a protein chip array. First, biocomponents are immobilized individually on micromachined, encoded microcarriers (rectangular metallic particles bearing an engraved signature), and specific binding to multiple targets is carried out in a bulk solution. Second, the particles are arranged onto the complementary binding sites on an array template and subsequently detected. The encoding symbol carried by the microparticles is a straightforward visible feature, introduced while the particles were being manufactured. Thus, this approach does not require additional sophisticated chemistry to incorporate an encoding element. The encoding symbol is used as the signature of the individual arrayed particle and identifies the target/compound attached to each particle surface.

Self-assembly is used to arrange the organized structures on a chip substrate, and to construct the particle array. Generating high-dimensional arrays

of structures using self-assembly of small objects is a common technique in biology, chemistry, and physics ***(20)***. The components to be assembled can be either spheres ***(21)*** or nonspheres ***(22–29)***, with a wide range of sizes, from a few nanometers ***(21)*** to several millimeters ***(24–29)***. So far, self-assembly techniques have been developed for a range of practical applications, including fabrication of microelectronic device elements ***(24,30,31)***, photonic band-gap structures ***(32)***, and other three-dimensional structures from colloid particles ***(33,34)***. Various kinds of weak interactions, such as electrostatic, magnetic, hydrophobic, and capillary, can drive the assembly ***(22,23)***. To promote the self-assembly process for larger objects, researchers have typically exploited capillary interactions in combination with a lubrication process ***(22–30)***.

For the process described here, we introduce hydrophobic and magnetic Ni-polytetrafluoroethylene (Ni-PTFE) composite layers in the interaction areas of both the assembling pieces and the spatially discrete microwells on the array template. The individual particles carrying immobilized biocomponents are then allowed to assemble randomly onto the array template, promoted by a combination of selective short-range forces including magnetic, hydrophobic, and capillary interactions. This approach combines the advantages of a typical microsphere-based array, providing high flexibility in array implementation and use ***(15)***, with the simplicity of the interrogation technologies available for a planar chip array. The utility of the constructed particle array platform is shown here as a model system for simultaneous multianalyte immunoassays of human immunoglobulins (IgA, IgG, and IgM) ***(19)***.

2. Materials

For the study described here, negative photoresist XP SU-8 50, developer, Nano Remover PG photoresist remover, and XP SU-8 release layer were purchased from Microchem (Newton, MA). Supersignal enzyme-linked immunosorbent assay femto maximum sensitivity substrate was obtained from Pierce (Rockford, IL). All the antibodies (developed in goat), antigens (human IgA, IgG, and IgM), and peroxidase-conjugated antibodies were obtained from Sigma (St. Louis, MO) and used as received. Metaflon FS Ni-PTFE dispersion electroplating solution, containing nickel sulfamate (Ni^{2+} 100 g/L) plus fine PTFE particles (diameter: approx 0.2 μm; concentration: 50 g/L), was purchased from C. Uyemura & Co (Osaka, Japan). 16-Mercaptohexadecanoic acid (90%) was obtained from Aldrich (Milwaukee, WI). 1-Hexadecanethiol was obtained from Wako (Osaka, Japan). Other chemicals used in this study were of reagent grade and purchased from local commercial sources. The photomasks were designed with Illustrator 9.01 (Adobe) and printed on a transparency film using a high-resolution laser printer system.

3. Methods

3.1. Photolithographic Patterning

Cleaned glass slides (38 × 26 mm) served as the substrate for the deposition by evaporation of 20 nmol of Cr and then 200 nmol of Cu that was used as a conducting seed layer and later also as the sacrificial release layer. A Karl Suss MJB 3 mask aligner (Zeiss, Oberkochen, Germany) in contact mode, powered by a 350-W mercury ultraviolet (UV) light source, was used for contact photolithography. The metal-coated substrates were first spin coated with a 2-μm XP SU-8 release layer, according to the protocol provided by the manufacturer (http://www.microchem.com). Next, they were spin coated with an approx 50-μm layer of SU-8 50 photoresist thick film and structured using standard photolithographic procedures. Two kinds of resist patterning templates were fabricated, one for particle preparation and another for the array template (chip substrate); the template fabrication process is described in **Subheading 3.7.**

For the particle fabrication template, a 48 × 24 microwell array with a 400 × 200 μm electrochemically active area in each well and a 50-μm separation space between the wells was designed on a 30 × 20 mm substrate area. The coding symbol was located at an upright area of each microwell. The array template had a 20 × 20 mm effect area consisting of 21 × 21 microwells in shapes and sizes that complemented the fabricated pieces.

3.2. Electrodeposition

The photoresist-patterned substrates were cleaned for 2 min using reactive-ion etching with an O_2 plasma. This treatment oxidized the hydrophobic SU-8 photoresist surface, thus making it hydrophilic (contact angle: <20°), as was required for the selective binding of the particles to the microwells on the array template. Electrodeposition of an Ni-PTFE composite layer in the microwell areas was carried out using an electroplating bath at 50°C for 30 min for particle preparation and 1 h for array template preparation, with a current density of approx 0.1 mA/mm^2 based on the active area of the substrate. The microwell areas prepared using this procedure had a smooth, hydrophobic, and magnetic surface, in contrast to the other areas, which remained hydrophilic.

3.3. Particle Fabrication

Following electrodeposition of the Ni-PTFE composite layer (approx 6 μm thick), the patterned particle fabrication and array templates were deposited sequentially on top of the photoresist by evaporation with a 0.02-μm layer of Cr and a 0.3-μm layer of Au. The metallized resist pattern on the chip was then lifted after immersion in an SU-8 Remover PG solution at 80°C for 10 to 20 min. The particles were finally liberated from the underlying substrate by dissolving the sacrificial Cu layer with an etchant containing sodium hypochlorite (0.2 *M*),

ammonia (0.7 *M*), and ammonium carbonate (2.6 *M*). The particles were transferred and stored in an Eppendorf-type tube filled with ethanol. They exhibited an instantaneous attraction when a magnet was moved close to the tube containing the particle suspension, thus confirming their magnetic properties.

3.4. On-Particle Immunoassays for IgA, IgG, and IgM

The fabricated particles were coated with a self-assembled monolayer (SAM) by soaking them for 24 h in a 1 m*M* solution of 16-mercaptohexadecanoic acid in ethanol. The particles carrying the carboxylic acid–terminated thiol monolayer were washed using ethanol and transferred into a 0.5-mL Eppendorf-type tube. Four sets of particles were then treated with 100 µL of a solution containing 2 µg/mL of antibodies against either IgA, IgG, IgM, or 2% casein (as a negative control) in 50 m*M* phosphate-buffered saline (PBS) with 138 m*M* NaCl (pH 7.4) for 3 h at room temperature with rotating agitation. They were subsequently passivated with 2% casein (sodium salt) for 1 h to prevent nonspecific adsorption of proteins during subsequent steps. Finally, the particles were rinsed three times with 10 m*M* PBS (pH 7.4) containing 0.01% Tween-20.

The sets, each containing 10–20 particles with immobilized individual antibodies, were combined in a 0.5-mL Eppendorf-type tube. A 100-µL sample containing a mixture of IgA, IgG, and IgM was added and incubated for 1 h. The particles were subsequently rinsed, and a 100-µL mixture of three peroxidase-conjugated reporter antibodies, each with a concentration of 4 µg/mL, was added for 1 h to form sandwich immunobinding complexes on the particles and rinsed. The immunotreated particles were then applied onto the array template and finally detected, as described in **Subheadings 3.5.** and **3.6.**

For calibration purposes, six standards with antigen concentrations between 0.001 and 10 µg/mL were tested individually, each with one set of particles. A calibration with several standards on a single experiment was also tested. In this case, three sets of particles were first immobilized with the same antibody and blocked in separate tubes; standards containing three different concentrations of the analyte were then added to the particles individually and incubated. The particles were washed and combined, and peroxidase-conjugated reporter antibody was added to form the sandwich immunobinding complexes.

3.5. Assembly of Particle Array

An SU-8 photoresist–patterned array template with an electrodeposited Ni-PTFE layer on the surface of its microwells was magnetized, using a permanent magnet, for 2 h. The template was then coated with an SAM of 16-hexadecanethiol for 12 to 24 h and lubricated with a thin hydrophobic photocurable adhesive film following the procedure described by Srinivasan et al. ***(30)***.

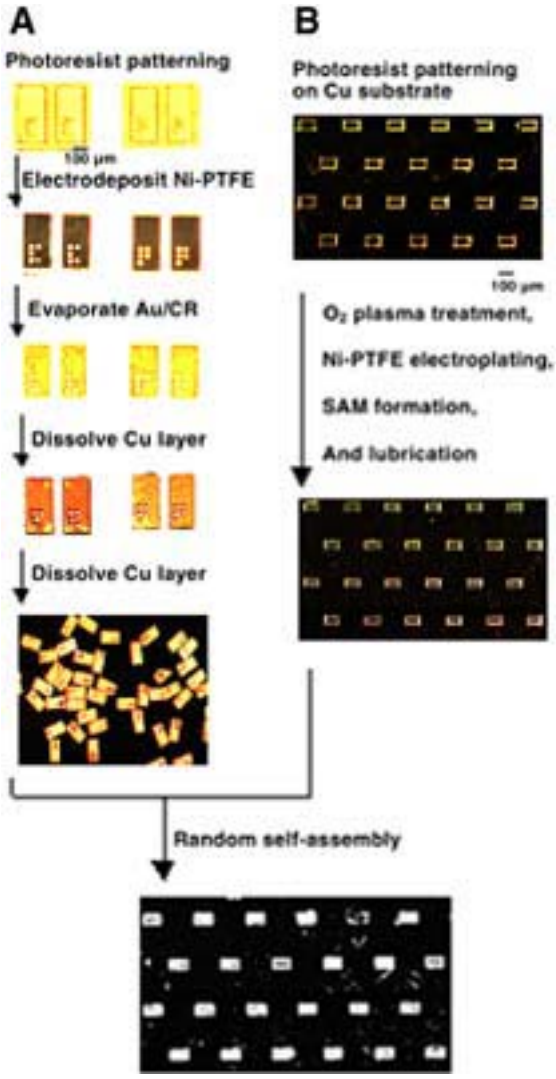

Fig. 1. Protocol used for fabrication and self-assembly of particle array on a protein chip substrate. **(A)** Fabrication of encoded multilayer particles. SU-8 50 photoresist was patterned as a template on a Cu-coated glass substrate, and an Ni-PTFE layer (approx 6 μm thick) was electrodeposited on the open area of the microwells. After evaporation of Cr (20 nm) and Au (300 nm) on the entire chip surface, the metallized SU-8 photoresist was lifted off using SU-8 Remover PG solution. Finally, the particles were released from the underlying substrate by dissolving the Cu sacrificial layer. Only two types of encoded particles are shown here as examples. **(B)** Fabrication of a microwell array template on a chip substrate. An SU-8 photoresist (approx 50 μm thick)–patterned microwell array on a Cu-covered glass slide was treated with O_2 plasma,

The photocurable adhesive consisted of 96:2:2 (w/w/w) dodecylmethacrylate/benzoin isobutyl ether/1,6-hexanediol diacrylate ***(22,23)***. After rinsing in distilled water, the chip was transferred to a 100-mL vessel; a continuously flowing water stream (containing 0.1 *M* NaCl) was maintained over the chip surface with the aid of a pump. Particles were then added slowly, using a Pasteur pipet, and settled onto the surface of the chip substrate. The pieces moved across the surface and finally settled onto the microwell sites covered with the photoadhesive layer, forming a particle array. After exposure to UV radiation (100 W) for 20 min, the pieces were fixed in place. Excess particles that were not bound to the microwells were removed by washing.

3.6. Detection of Chemiluminescence Signal

The chemiluminescence from multiple particle spots located on the substrate was magnified by an MZFLIII microscope (Leica, Heerbrugg, Switzerland) and imaged using a charge-coupled device camera detector (ZVS-3C75DE; Carl Zeiss, Oberkochen, Germany). The images were then analyzed using IPLab Spectrum P software. The chemiluminescence signals from the microscopic particle zones were captured with a 30-s acquisition time.

To initiate the luminescence reaction, 0.1 mL of a mixture of the chemiluminescence substrates comprising luminol and H_2O_2 was pipeted onto the particle-settled substrate. The peroxidase-conjugated antibodies, fixed on the particle surface through immunobinding, emitted localized chemiluminescent signals. By reading the encoding signature of the particle at a given location on the substrate, we were able to identify the targets bound onto the individual particles.

3.7. Fabrication and Assembly of Particle Array on a Protein Chip Substrate

Micromachining techniques were employed to manufacture the encoded multilayer particles and the corresponding array templates. Primarily, these techniques involved a combination of photolithography and lift-off, electroplating-through-mask ***(35)***, metal thermal evaporation, and wet etching. The typical process for the fabrication of the particles and the array template on a chip substrate is outlined in **Fig. 1**.

(Fig. 1. caption continued) and an Ni-PTFE layer (~6 µm thick) was electrodeposited on the open area of the microwells. The hydrophobic Ni-PTFE structures were then magnetized with a permanent magnet and further coated with a thin photoadhesive film through hydrophobic interactions. The chip substrate was next immersed in a fluid water system, and the particle suspension was added slowly to allow the self-assembly process. Finally, curing the adhesive by exposure to UV radiation provided mechanically stable particle arrays that could be used for detection.

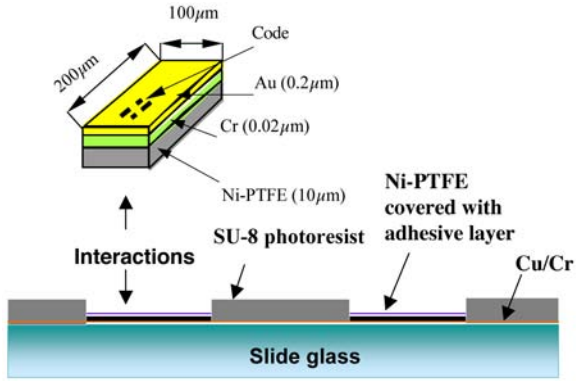

Fig. 2. Schematic representation of interaction of encoded particles with microwell surface of array template on a protein chip substrate.

To fabricate the array template, the surface of the substrate was patterned into defined hydrophobic (in microwells) and hydrophilic surface regions (the remaining area). An O_2 plasma etching for the photolithographically patterned resist layer was found to render the surface hydrophilic. Then, an electrodeposited Ni-PTFE layer (approx 6 μm thick) on the microwell areas on the array template made these areas hydrophobic. These areas were further coated with an SAM of 16-hexadecanethiol, which rendered the microwell surface ultrahydrophobic. With such a process, the microwell areas on the substrate became hydrophobic (also magnetic) while the other regions remained hydrophilic, thus facilitating the further coating of a hydrophobic, photoadhesive thin layer onto the microwell areas.

Figure 2 illustrates the principles underlying the self-assembly process, based on a combination of magnetic, hydrophobic, and capillary interactions.

3.8. Summary of Multianalyte Immunoassay Process

The multianalyte capacity of the present approach was tested and demonstrated for parallel determination of three independent analyses of human immunoglobulins (IgA, IgG, and IgM). The sandwich immunoassay format uses particles with immobilized antibodies, peroxidase-labeled reporter antibodies, and the luminol-based chemiluminescence detection system. The particle surface of gold when passivated with an SAM of 16-mercaptohexadecanoic acid is easily compatible with available biomolecular coupling chemistries (by adsorption) and luminescence-based detection techniques ***(35)***. The typical assay protocol for the particle-based multianalyte immunoassay is outlined in **Fig. 3**.

Calibration curves for the three antigens were obtained individually by using mean values of triplicates of integrated particle light intensities plotted against the antigen concentration values, as shown in **Fig. 4**.

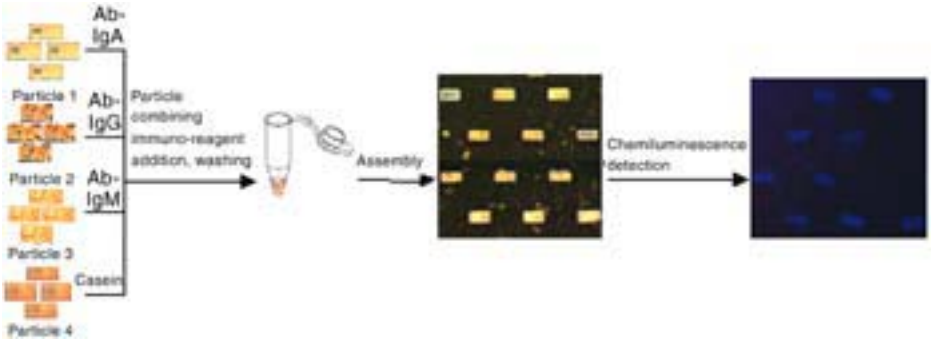

Fig. 3. Process leading to multianalyte immunoassays in parallel with arrayed particles on a protein chip substrate. Separate subsets of particles bearing an identifiable code were each tagged with a specific antibody. After combining the sets into a plastic tube, immunoreagents for a sandwich assay were added sequentially, and the particles were then assembled onto an array template in a random fashion. The encoding signature identified the individual particle registration and, subsequently, the assay target at each given location on the substrate.

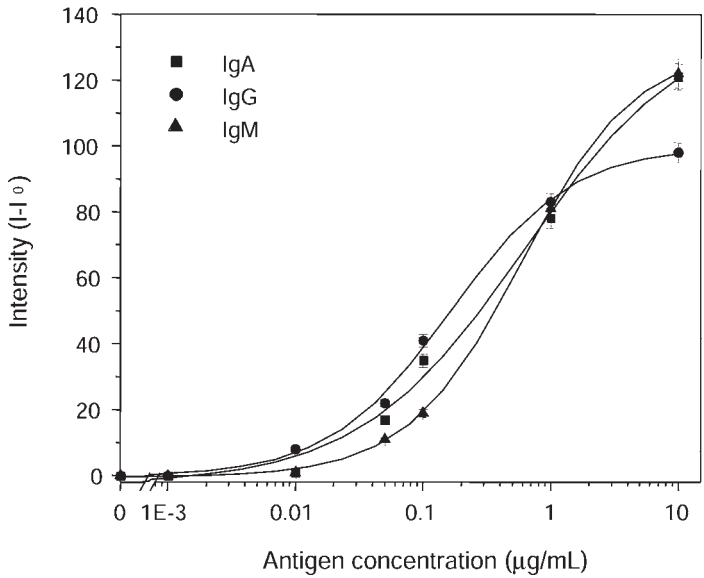

Fig. 4. Calibration curves for IgA, IgG, and IgM multianalyte immunoassays on microfabricated particles with chemiluminescent detection. The intensity data derived from the individual particles were subtracted from a background value.

All the curves show a sigmoidal binding behavior. The determination ranges for IgA, IgG, and IgM were typically from 10 ng/mL to 1 μg/mL, and the detection limits were approx 10 ng/mL. The detection limits achieved in this work mainly depended on the affinity of antibodies and antigens, as happens in conventional immunoassays.

For quantitative analysis, it is necessary to carry out calibration with several standards on a single chip. To accomplish this goal, the experimental protocol was altered slightly: different sets of the particles were first immobilized with the same antibody and blocked in separate tubes; standards containing a certain concentration of the analyte were then added to the particles individually and incubated. Peroxidase-conjugated reporter antibody was subsequently added to form the sandwich immuno–binding complexes. The procedure used for the particle fabrication by micromachining techniques is amenable to mass production at low cost and is thus suitable for applications that extend beyond the laboratory.

4. Conclusion

This chapter has outlined the template-directed self-assembly of a randomly ordered, addressable particle array that could be used for miniaturized multianalyte immunoassays. The approach described here provides a versatile building block for parallel bioassays in a combinatorial fashion. The benefits of this method include improved flexibility in assay implementation, and the ability to avoid overlap or particle aggregation between adjacent particles during detection. Moreover, including multiple copies of each kind of particle and control particles in a single operation improves precision through signal averaging and simultaneously minimizes the probability of false results (either positive or negative) that might occur if a single sensor was used in the assay. Because of the unique fabrication procedure and potentially high level of encoding capability, the particles fabricated by this method—once optimized regarding array-assembling efficiency—could also be widely applied in genomics, proteomics, combinatorial chemistry, and other high-throughput bioscreening and bioassay applications.

Acknowledgments

We thank Prof. R. B. DiGiovanni and J. A. Steeh for editing assistance. This work was partially funded by a grant-in-aid for Scientific Research Priority Area (A) of MEXT, Research for Future Program from the JSPS, and the 21st Century Center of Excellence Program on Technology Creation Based on Knowledge Science at JAIST.

References

1 Sanders, G. H. W. and Manz, A. (2000) Chip-based microsystems for genomic and proteomic analysis. *Trends Anal. Chem.* **19,** 364–378.

2 Jackman, R. J., Wilbur, J. L., and Whitesides, G. M. (1995) Fabrication of submicrometer features on curved substrates by microcontact printing. *Science* **269,** 664–666.

3 Bernard, A., Fitzli, D., Sonderegger, P., Delamarche, E., Michel, B., Bosshard, H. R., and Biebuyck, H. (2001) Affinity capture of proteins from solution and their dissociation by contact printing. *Nat. Biotechnol.* **19,** 866–869.

4 Okamoto, T., Suzuki, T., and Yamamoto, N. (2000) Microarray fabrication with covalent attachment of DNA using bubble jet technology. *Nat. Biotechnol.* **18,** 438–441.

5 MacBeath, G. M. and Schreiber, S. L. (2000) Printing proteins as microarrays for high-throughput function determination. *Science* **289,** 1760–1763.

6 Wiese, R., Belosludtsev, Y., Powdrill, T., Thompson, P., and Hogan, M. (2001) Simultaneous multianalyte ELISA performed on a microarray platform. *Clin. Chem.* **47,** 1451–1457.

7 Lee, K. B., Park, S. J., Mirkin, C. A., Smith, J. C., and Mrksich, M. (2002) Protein nanoarrays generated by dip-pen nanolithography. *Science* **295,** 1702–1705.

8 Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270,** 467–470.

9 Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T., and Solas, D. (1991) Light-directed, spatially addressable parallel chemical synthesis. *Science* **251,** 767–773.

10 Fodor, S. P. A., Rava, R. P., Huang, X. C., Pease, A. C., Holmes, C. P., and Adams, C. L. (1993) Multiplexed biochemical assays with biological chips. *Nature* **364,** 555, 556.

11 Goodey, A., Lavigne, J. J., Savoy, S. M., et al. (2001) Development of multianalyte sensor arrays composed of chemically derivatized polymeric microspheres localized in micromachined cavities. *J. Am. Chem. Soc.* **123,** 2559–2570.

12 Christodoulides, N., Tran, M., Floriano, P. N., Rodriguez, M., Goodey, A., Ali, M., Neikirk, D., and McDevitt, J. T. (2002) A microchip-based multianalyte assay system for the assessment of cardiac risk. *Anal. Chem.* **74,** 3030–3036.

13 Curey, T. E., Salazar, M. A., Oliveira, P., Javier, J., Dennis, P. J., Rao, P., and Shear, J. B. (2002) Enzyme-based sensor arrays for rapid characterization of complex disaccharide solutions. *Anal. Biochem.* **303,** 42–48.

14 Michael, K. L., Taylor, L. C., Schultz, S. L., and Walt, D. R. (1998) Randomly ordered addressable high-density optical sensor arrays. *Anal. Chem.* **70,** 1242–1248.

15 Walt, D. R. (2000) Bead-based fiber-optic arrays. *Science* **287,** 451, 452.

16 Nagai, H., Murakami, Y., Morita, Y. Yokoyama, K., and Tamiya, E. (2001) Development of a microchamber array for picoliter PCR. *Anal. Chem.* **73,** 1043–1047.

17 Matsubara, Y., Kobayashi, M., Morita, Y., and Tamiya, E. (2002) Application of a microchamber array for DNA amplification using a novel dispensing method. *Arch. Histol. Cytol.* **65(5),** 481–488.
18 Zhi, Z. L., Morita, Y., Hasan, Q., and Tamiya, E. (2003) Micromachining microcarrier-based biomolecular encoding for miniaturized and multiplexed immunoassay. *Anal. Chem.* **75,** 4125–4131.
19 Zhi, Z. L., Murakami, Y., Morita, Y., Hasan, Q., and Tamiya, E. (2003) Multianalyte immunoassay with self-assembled addressable microparticle array on a chip. *Anal. Biochem.* **318,** 236–243.
20 Whitesides, G. M. and Grzybowski, B. (2002) Self-assembly at all scales. *Science* **295,** 2418–2421.
21 Boal, A. K., Ilhan, F., DeRouchey, J. E., Thurn-Albrecht, T., Russell, T. P., and Rotello, V. M. (2000) Self-assembly of nanoparticles into structured spherical and network aggregates. *Nature* **404,** 746–748.
22 Clark, T. D., Tien, J., Duffy, D. C., Paul, K. E., and Whitesides, G. M. (2001) Self-assembly of 10-µm-sized objects into ordered three-dimensional arrays. *J. Am. Chem. Soc.* **123,** 7677–7682.
23 Clark, T. D., Ferrigno, R., Tien, J., Paul, K. E., and Whitesides, G. M. (2002) Template-directed self-assembly of 10-µm-sized hexagonal plates. *J. Am. Chem. Soc.* **124,** 5419–5426.
24 Gracias, D. H., Tien, J., Breen, T. L., Hsu, C., and Whitesides, G. M. (2000) Forming electrical networks in three dimensions by self-assembly. *Science* **289,** 1170–1172.
25. Terfort, A., Bowden, N., and Whitesides, G. M. (1997) Three-dimensional self-assembly of millimetre-scale components. *Nature* **386,** 162–164.
26 Grzybowski, B. A., Stone, H. A., and Whitesides, G. M. (2000) Dynamic self-assembly of magnetized, millimeter-sized objects rotating at a liquid-air interface. *Nature* **405,** 1033–1036.
27 Tien, J., Breen, T. L., and Whitesides, G. M. (1998) Crystallization of millimeter-scale objects with use of capillary forces. *J. Am. Chem. Soc.* **120,** 12,670–12,671.
28 Breen, T. J., Tien, J., Oliver, S. R. J., Hadzic, T., and Whitesides, G. M. (1999) Design and self-assembly of open, regular, 3D mesostructures. *Science* **284,** 948–951.
29 Oliver, S. R. J., Clark, T. D., Bowden, N., and Whitesides, G. M. (2001) Three-dimensional self-assembly of complex, millimeter-scale structures through capillary bonding. *J. Am. Chem. Soc.* **123,** 8119, 8120.
30. Srinivasan, U., Liepmann, D., and Howe, R. T. (2001) Microstructure to substrate self-assembly using capillary forces. *J. Microelectromech. Syst.* **10,** 17–24.
31 Gittins, D. I., Bethell, D., Schirin, D. J., and Nichols, R. J. (2000) A nanometerscale electronic switch consisting of a metal cluster and redox-addressable groups. *Nature* **408,** 67–69.
32 Joannopoulos, J. D. (2001) Self-assembly lights up. *Nature* **414,** 257, 258.

33. Li, M., Schnablegger, H., and Mann, S. (1999) Coupled synthesis and self-assembly of nanoparticles to give structures with controlled organization. *Nature* **402,** 393–395.
34. Cheng, Z., Russel, W. B., and Chaikin, P. M. (1999) Controlled growth of hardsphere colloidal crystals. *Nature* **401,** 893–895.
35. Nicewarner-Pena, S. R., Freeman, R. G., Reiss, B. D., He, L., Pena, D. J., Walton, I. D., Cromer, R., Keating, C. D., and Natan, M. J. (2001) Submicrometer metallic barcodes. *Science* **294,** 137–141.