($I \geq 2\sigma(I)$)), 275 parameters, 6 restraints, numerical absorption correction, $\mu = 5.468\ mm^{-1}$, $T_{min} = 0.5802$, $T_{max} = 0.8524$. Single crystals of **10** were obtained by dissolving the product in a slightly acidic EtOH/water mixture and slow evaporation of ethanol. Data were collected on a Stoe IPDS diffractometer using graphite-monochromated $Mo_{K\alpha}$ ($\lambda = 0.71073$ Å) radiation, and were corrected for Lorentz, polarization, and absorption effects. The structure was solved by direct methods with SHELXS97 and refined with SHELXL97 on F^2 using all data with all non-hydrogen atoms anisotropically defined.[23] The hydrogen atoms were placed in calculated positions and isotropically refined with thermal parameters at $1.5 \times U(eq)$ of the parent carbon atom, except for the piperazyl-N(H) which was found in the difference Fourier map and isotropically refined. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-160745 (**9**) and CCDC-160746 (**10**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[19] E. Keller, SCHAKAL97, University of Freiburg, **1997**.

[20] E. O. Fischer, W. Fellmann, *J. Organomet. Chem.* **1963**, *1*, 191–199.

[21] W. P. Hart, D. W. Macomber, M. D. Rausch, *J. Am. Chem. Soc.* **1980**, *102*, 1296–1298.

[22] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.

[23] G. M. Sheldrick, SHELXS97, University of Göttingen, **1990**; G. M. Sheldrick, SHELXL97, University of Göttingen, **1997**.

A Chemosensing Ensemble for Selective Carbonate Detection in Water Based on Metal–Ligand Interactions**

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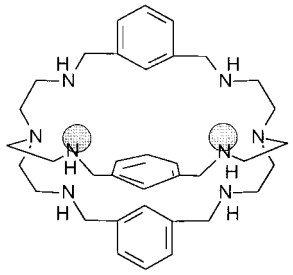
The classical approach to the design of a fluorescent sensor involves the covalent linking of a fluorescent fragment (the "signaling unit") to a receptor, which displays specific binding tendencies towards a given analyte. In order to communicate the occurrence of the recognition event to the outside world, the receptor/analyte interaction must affect, to some extent, the emission properties of the signaling unit—either quenching the fluorescence through a defined mechanism (usually energy or electron transfer) or enhancing it by suppressing a pre-existing quenching process.[1]

Recently, Anslyn and co-workers[2] have taken inspiration from antibody-based biosensors in immunoessays and developed an efficient "competition" approach to the design of chemosensors: according to the so-called "chemosensing ensemble" approach, the plain receptor interacts in solution with a colorimetric or fluorescent indicator, namely, a highly colored or light-emitting substrate. Two main requirements have to be fulfilled: the receptor/indicator interaction must not be too strong and the indicator must show significantly different optical properties when bound to the receptor and when dispersed in solution. Thus, the indicator is displaced from the host cavity on titration with the desired analyte and released to the solution, where it displays drastically different optical features. Hence, the occurrence of the recognition event is communicated by either a substantial color change or a dramatic modification of the light emission. In their competition studies Anslyn and co-workers considered anion receptors capable of interacting with the analyte through formation of hydrogen bonds. Typical investigated analytes were citrate, tartrate, malate, and inositol triphosphate. The procedure of competitive spectrophotometry has been treated in detail by Connors.[3] Ueno and co-workers later extended the approach by covalently linking the dye to the receptor.[4]

We now extend the competition approach to the fluorescent sensing of anions by making use of a different type of receptor–analyte interaction: metal–ligand (coordinative) interactions. Coordinative interactions present some substantial advantages compared to hydrogen bonding and, in general, to electrostatic interactions.[5] In fact, they can be highly energetic as a result of the strong ligand-field stabilization energy effects that may be induced by coordination. Moreover, transition metal ions present definite stereochemical preferences which can be addressed to impart selective binding tendencies towards anions of a given shape. In order to benefit from these features, metal–ligand interactions have been utilized in the design of anion receptors operating in highly polar media, including pure water. In this connection, we have shown that the dicopper(II) cage complex **1** is capable of including polyatomic anions



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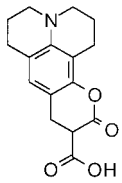

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within its intermetallic cavity in an aqueous solution buffered at pH 8. The ion selectivity is determined by the bite length of the anion, that is, the distance between two consecutive donor atoms.[6] In particular, receptor **1** showed a definite preference towards the triangular HCO_3^- ion[7] and the two linear triatomic ions N_3^- and NCO^-, whose bite length range between 2.28 and 2.42 Å. The intrinsic limit of receptor **1** as a proper molecular sensor is that only small changes in the absorption spectra and in the color are observed following anion recognition. For example, in the case of HCO_3^- ions, the color turns from the pale blue of the void receptor to the pale

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green of the inclusion complex. The sole exception is represented by the inclusion of the N_3^- ion, which induces the development of a rather intense ligand-to-metal charge transfer band centered at 400 nm. Thus, we considered the opportunity to teach the "old" receptor **1** new ways to act as a sensor, in particular, as a fluorescent sensor, by taking advantage of the chemosensing ensemble approach.

As a fluorescent indicator we chose coumarine 343 (**2**), which possesses a carboxylate function capable of bridging the two Cu^{II} centers of receptor **1**. It is also strongly fluorescent in its anionic form, which is the dominating species in an aqueous solution adjusted to pH 7. Complete quenching of the coumarine emission was observed ($\lambda_{exc} = 424$ nm, $\lambda_{em} = 487$ nm) on titrating a degassed solution of **2** (10^{-7} M), buffered to pH 7 (HEPES; 0.05 M), with a standard solution of receptor **1**.

Curve fitting of the titration profile (fluorescent intensity versus concentration of **1**; Figure 1) by a nonlinear least-squares procedure was consistent with the formation of an

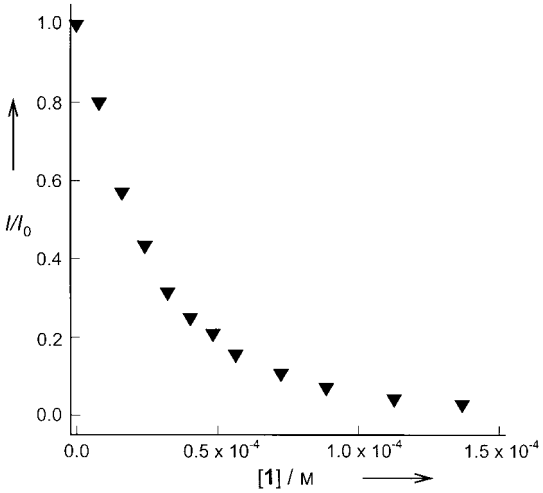

Figure 1. Titration of a solution of the fluorescent indicator coumarine 343 (**2**) with the dimetallic receptor **1** in an aqueous solution buffered to pH 7. A 1:1 adduct forms, whose association constant is 4.8 ± 0.1 log units. Quenching is ascribed to the occurrence of an intramolecular electronic energy transfer which involves the photoexcited coumarine subunit and the Cu^{II} center(s) within the **1**:**2** adduct.

adduct of 1:1 stoichiometry, whose association equilibrium constant K_{ass} was 4.8 ± 0.1 log units.[8] Fluorescence quenching is ascribed to an intracomplex electronic energy transfer process involving the photoexcited coumarine fragment and the Cu^{II} center(s). The occurrence of a transfer of energy rather than a transfer of electrons was demonstrated by the fact that quenching persisted even when an ethanolic solution of the **1**:**2** adduct was vitrified at 77 K.[9]

A chemosensing ensemble was then generated by making a degassed aqueous solution containing 2×10^{-4} M **1** and 10^{-7} M **2** buffered to pH 7 with HEPES (0.05 M). Titration of the resulting chemosensing ensemble solution with a standard solution of carbonate (Figure 2, ▲) resulted in an almost complete recovery of the coumarine emission. This result indicates there is successful competitive binding of the HCO_3^- ions and displacement of the indicator from the host cavity.

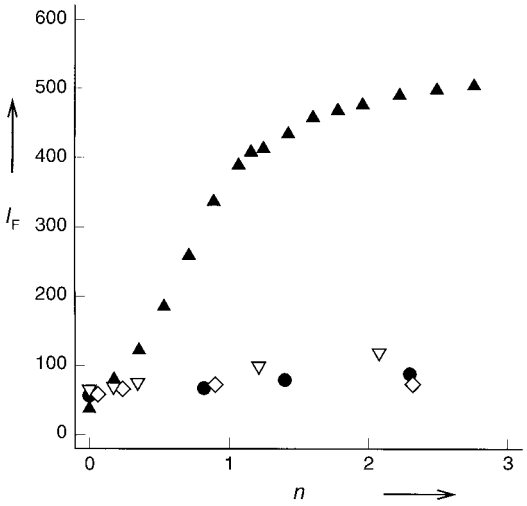

Figure 2. Competitive titration of an aqueous solution of 2×10^{-4} M **1** and 10^{-7} M **2**, buffered to pH 7, with standard solutions of selected anions. HCO_3^- (▲) is able to displace the indicator **1** from receptor **2**. The indicator is released into the solution and displays its full emission. Other anions, for example, phosphate (▼), acetate (●), and sulfate (◇) do not compete successfully with **2** for **1** and induce only a slight fluorescence enhancement. n = anion equivalents.

Full regeneration of the coumarine emission was observed also when the solution of the chemosensing ensemble was titrated with N_3^- and NCO^- ions. However, titration with a variety of ambidentate anions (NCS^-, NO_3^-, SO_4^{2-}, HPO_4^{2-}, $HCOO^-$, CH_3COO^-) induced only a slight enhancement of the fluorescence (Figure 2).

The selectivity pattern described above could be tentatively explained by assuming that HCO_3^-, N_3^-, and NCO^- ions exhibit association constants with **1** that are distinctly larger than that of the coumarine indicator (4.8 log units), whereas all the other investigated anions must show substantially lower $\log K_{ass}$ values. This hypothesis was confirmed through direct spectrophotometric titration experiments, that is, by adding a standard solution of the anion to a solution of **1** buffered at pH 7 and looking at the development/modification of the pertinent charge-transfer absorption bands. Association constants for the formation of 1:1 receptor:anion adducts were determined through a least-squares procedure,[8] and their values plotted versus the anion bite length (Figure 3). A selectivity pattern was observed, which is similar to that previously found in somewhat different conditions (aqueous solution buffered to pH 8 with 0.1M lutidine).[6] Figure 3 shows that the HCO_3^-, N_3^-, and NCO^- ions exhibit association constants with **1** higher than that of coumarine 343 (whose value is indicated as a dashed horizontal line), and are therefore able to displace the fluorescent indicator from the receptor cavity. The remaining anions, however, whose association constants are well below that of coumarine 343, cannot compete successfully with the indicator and cannot, therefore, be detected spectrofluorimetrically through the chemosensing ensemble approach.

We validated the approach by determining the $\log K_{ass}$ value for the association equilibrium of HCO_3^- ions with **1** from the combination of the spectrofluorimetric titration data (direct titration of Figure 1 and competitive titration of Figure 2), by

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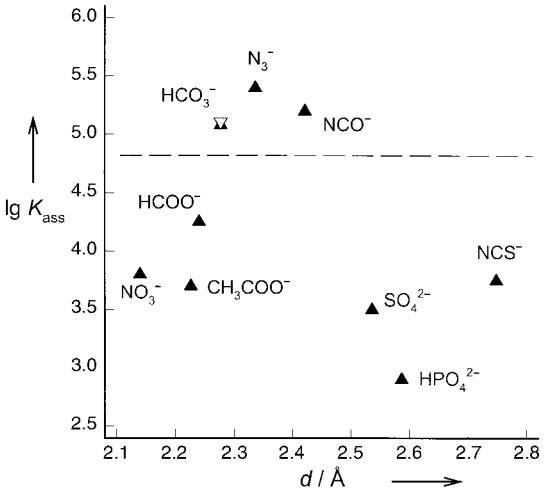

Figure 3. Selectivity pattern for the inclusion of polyatomic anions into the dimetallic receptor **1**: ▲ indicates the $\log K_{ass}$ value for the association equilibrium at pH 7 determined through spectrophotometric titrations. The dashed horizontal line corresponds to the $\log K_{ass}$ value for the association of **1** with the fluorescent indicator **2**. Only the anions whose $\log K_{ass}$ value is above the horizontal line are able to displace **2** from **1** and can be detected spectrofluorimetrically through the "chemosensing ensemble" method. d = bite length.

following a published calculation procedure.[3] The $\log K_{ass}$ value obtained from a competition spectrofluorimetric titration (gray triangle in Figure 3) is coincident with that obtained by direct spectrophotometric titration.

Of the anions that could be detected spectrofluorimetrically by the "chemosensing ensemble" method, carbonate is surely the most relevant from the point of view of practical applications. Thus, a calibration curve was obtained over the range $2\times10^{-5}-2\times10^{-4}$ M (Figure 4) and subsequently used for the quantification of carbonate in samples of commercial mineral water. In a typical measurement, a small amount of the sample (10–500 μL) was added to the chemosensing ensemble solution (10 mL, 2×10^{-4} M **1** and 10^{-7} M **2**). The fluorescence intensity was measured and the amount of carbonate was then calculated from the calibration curve. A substantial range of carbonate concentration can be explored by varying the volume of the sample used. For example, by taking a sample of 10–500 μL, it is possible to span a $4\times10^{-4}-2\times10^{-1}$ M range of HCO_3^- ions (corresponding to 24–1222 mg L^{-1} of hydrogencarbonate), which covers all the commercially available mineral waters. The present procedure seems easier and more selective than that currently used to determine the concentration of HCO_3^- ions in water which is based on volumetric titration of the alkalinity with standard hydrochloric or sulfuric acid:[10] in fact, the latter procedure does not distinguish HCO_3^- ions from other bases present in solution.

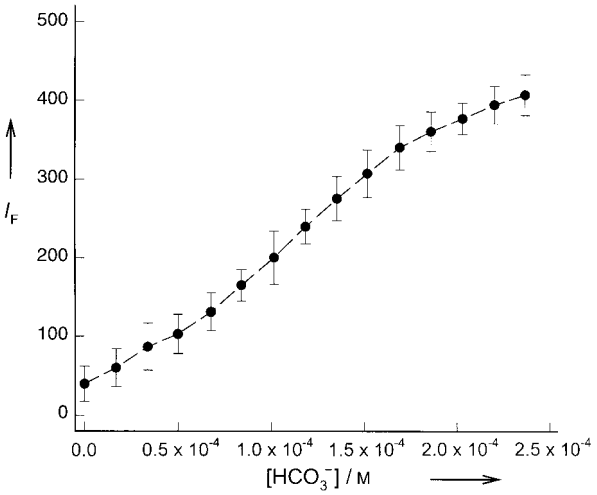

Figure 4. Calibration curve for the determination of the concentration of HCO_3^- ions by measurement of the fluorescent intensity of a solution containing the "chemosensing ensemble" **1**:**2**.

The marriage of a dimetallic cage and the chemosensing ensemble approach seems very promising for the fluorimetric determination of anions. The utilization of a smaller cavity cage may, for example, allow the fluorimetric determination of monoatomic anions such as halides.

Experimental Section

Complex **1** was prepared as described elsewhere.[6, 7] Compound **2** and other chemicals were purchased from Aldrich – Fluka and used without any further purification.

UV/Vis determinations of association constants between anions and **1** were performed in water buffered at pH 7 with 2-[4-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES; 0.05 M). Degassed standard solutions of anions were added to a 5×10^{-4} M degassed solution of **1**. Data fitting was performed on absorbance data at the appropriate wavelength.

The determination of the association constants between **1** and **2** was performed in degassed water solution buffered at pH 7 with HEPES (0.05 M) and containing 10^{-7} M **2**. Aliquotes of a fresh and degassed standard solution of **1** were added, and the emission spectra of **2** were recorded ($\lambda_{exc}=424$ nm, $\lambda_{em}=487$ nm). The chemosensing ensemble solutions for competition assays were prepared by adding **1** to a degassed solution of **2** in water buffered at pH 7 with HEPES (0.05 M), to avoid any carbonate contamination. The chemosensing ensemble solution (**1**: 2×10^{-4} M, **2**: 10^{-7} M) was titrated, under a nitrogen atmosphere, with standard solutions of anions. Points on the calibration curve for carbonate detection were obtained from four competition assays and the mean value calculated for every concentration used. Error bars were calculated as the double of the standard deviations of the values.

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[1] a) R. A. Bissel, A. P. de Silva, H. Q. N. Guaratne, P. L. M. Lynch, G. E. M. Maguire, K. R. A. S. Sandanayake, *Chem. Soc. Rev.* **1992**, *21*, 187; b) A. P. de Silva, H. Q. N. Guaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515; c) L. Fabbrizzi, A. Poggi, *Chem. Soc. Rev.* **1995**, *24*, 197; d) G. De Santis, L. Fabbrizzi, M. Licchelli, A. Poggi, A. Taglietti, *Angew. Chem.* **1996**, *108*, 224; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 202; e) L. Fabbrizzi, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, *Chem. Commun.* **1997**, 581; f) S. Weidner, Z. Pikramenou, *Chem. Commun.* **1998**, 1473.

[2] a) K. Niikura, A. Metzger, E. V. Anslyn, *J. Am. Chem. Soc.* **1998**, *120*, 8533; b) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682; *Angew. Chem. Int. Ed.* **1998**, *37*, 649; c) J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666.

[3] K. A. Connors, *Binding Constants, the Measurements of Molecular Complex Stability*, Wiley, New York, **1987**.

[4] a) K. Hamasaki, A. Ueno, F. Toda, *J. Chem. Soc. Chem. Commun.* **1993**, 331; b) K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki, T. Osa, *J. Am. Chem. Soc.* **1993**, *115*, 5035.

  1433-7851/01/4016-3068 $ 17.50+.50/0

[5] a) L. Fabbrizzi, M. Licchelli, G. Rabaioli, A. Taglietti, *Coord. Chem. Rev.* **2000**, *205*, 85; b) L. Fabbrizzi, I. Faravelli, G. Francese, M. Licchelli, A. Perotti, A. Taglietti, *Chem. Commun.* **1998**, 971.
[6] a) L. Fabbrizzi, P. Pallavicini, A. Perotti, L. Parodi, A. Taglietti, *Inorg. Chim. Acta* **1995**, *238*, 5; b) V. Amendola, L. Fabbrizzi, C. Mangano, P. Pallavicini, A. Poggi, A. Taglietti, *Coord. Chem. Rev.*, in press.
[7] R. Menif, J. Reibenspies, A. E. Martell, *Inorg. Chem.* **1991**, *30*, 3446.
[8] The HYPERQUAD program was used: A. Sabatini, A. Vacca, P. Gans, *Coord. Chem. Rev.* **1992**, *120*, 389.
[9] G. L. Gaines III, M. P. O'Neill, W. A. Svec, M. P. Niemczyk, M. R. Wasielewski, *J. Am. Chem. Soc.* **1991**, *113*, 719.
[10] *Standard Methods For the Examination of Water and Wastewater*, 16th ed., APHA, AWWA, WPCF, Washington, **1985**.

Combinatorial Templates Generated by Dip-Pen Nanolithography for the Formation of Two-Dimensional Particle Arrays**

Linette M. Demers and Chad A. Mirkin*

A general method for organizing micro- and nanoparticles on a substrate could facilitate the formation and study of photonic band-gap arrays for analysis of the relationship between pattern structure and catalytic activity and enable formation of arrays of single protein molecules for proteomics research. While several methods have been reported for assembling collections of particles onto patterned surfaces,[1, 2] a major challenge lies in the selective immobilization of single particles into predetermined positions with respect to adjacent particles.

A strategy for chemically and physically immobilizing a wide variety of particle types and sizes with a high degree of control over particle placement calls for a soft lithographic technique capable of high-resolution patterning, as well as one with the ability to form patterns of one or more molecules with precise alignment. Dip-pen nanolithography (DPN) has emerged as one such tool.[3] DPN is a scanning probe nanopatterning technique in which an atomic-force microscopy (AFM) tip is used to deliver molecules to a surface through a water meniscus, which naturally forms in the ambient atmosphere. Significantly, DPN also can be used to generate many customized templates formed from the same or different chemical inks which can be screened under identical conditions for a particular application. Herein, we report an example of this new combinatorial approach, and focus on the problem of particle assembly in the context of colloidal crystallization.

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Recently, conventional sedimentation methods for preparing colloidal crystals consisting of close-packed layers of polymer or inorganic particles have been combined with polymer templates, fabricated by electron-beam lithography, to form high-quality single-component structures.[1] However, sedimentation or solvent evaporation routes do not offer the element of chemical control over particle placement.[4] Herein, we describe a DPN-based strategy for generating charged chemical templates to study the assembly of single particles into two-dimensional square lattices.

Our general method (Figure 1) is to form a pattern on gold composed of an array of dots of a "molecular ink" which will attract and bind a specific type of particle. For these studies we

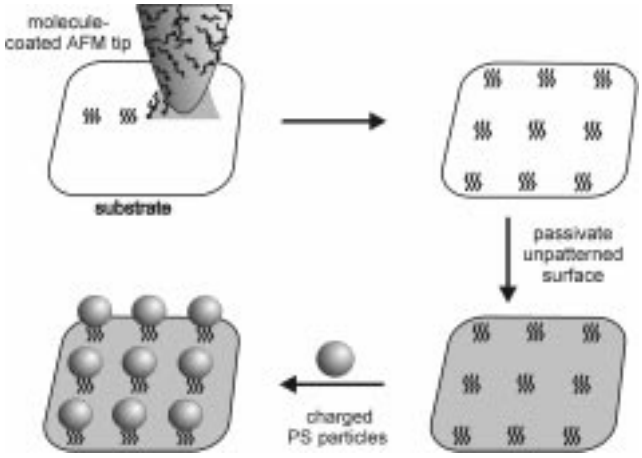

Figure 1. A schematic representation of the DPN-based particle organization strategy.

used 16-thiohexadecanoic acid (16-mercaptohexadecanoic acid, MHA) to make templates, and positively-charged protonated amine- or amidine-modified polystyrene (PS) spheres as particle building blocks. The unpatterned regions of the gold were passivated by immersing the substrate in a 1 mM ethanolic solution of another alkanethiol, such as 18-octadecanethiol (ODT), or cystamine for 9 min. Minimal, if any, exchange takes place between the MHA molecules and the ODT or cystamine in solution during this treatment, as evidenced by lateral force microscopy of the substrate before and after treatment with ODT (no change). Finally, particle assembly was accomplished by placing a 20-μL droplet of dispersed particles (10 % wt/vol in deionized water) onto the horizontal substrate in a humidity chamber (100 % relative humidity). Gentle rinsing with deionized water completes the process. In this particular study, it is important to note that the carboxylic acid groups in the MHA patterns are deprotonated, thus providing an electrostatic driving force for particle assembly.[5]

In a typical experiment involving 0.93 μm diameter particles, multiple templates are monitored simultaneously for particle assembly by optical microscopy. In these experiments, the diameter of the template dot is varied to search for optimal conditions for particle–template recognition (Figure 2). After 1 h of particle assembly, the substrates are rinsed with deionized water, dried under ambient laboratory conditions, and then imaged by optical microscopy (Figure 3).

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