# EXPERIMENTAL SECTION

## DNA-based Programmed Assembly of Gold Nanoparticles on Lithographic Patterns with Extraordinary Specificity

### MATERIALS AND REAGENTS

4" N-type silicon wafers were purchased from the Berkeley Microfabrication Laboratory. 2-[methoxy(polyethyleneoxy)propyl]trimethoxysilane (henceforth referred to as PEG silane) was purchased from Gelest, Inc. (Morrisville, PA.) 10 nm (nominal diameter) and 15 nm (nominal diameter) gold nanocrystals were purchased from Ted Pella, Inc. (Redding, CA.) B*is(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt* (henceforth referred to as Phosphine) was purchased from Cardinal Industries (Milwaukee, WI.) All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA.) at the 1 micromole scale in a lyophilized (powder) form. The names and base sequences of the complementary DNA molecules are as follows (note that the first 20 bases from the 5' end function as spacers and do not hybridize):

**Substrate Oligo**: 5'- SH-C6-TTTTTTTTTTTTTTTTTTTTAATATTGATAAGGAT
**NC Oligo**: 5'-SH-C6- TTTTTTTTTTTTTTTTTTTTATCCTTATCAATATT

### PREPARATION OF SUBSTRATES

Silicon wafers were used as the starting substrates. Approximately 10 nm of chromium adhesion layer was first deposited on the wafer using the Veeco 401 vacuum system evaporator, followed by the deposition of a gold film approximately 25 nm thick. The substrate was patterned using standard photolithography using a Karl Suss MA6 Mask Aligner. After development of the photoresist patterns, the exposed gold film was etched using gold etchant (saturated solution of KI in $H_2O$), followed by removal of exposed chrome film using CR-7 chromium etchant (Cyantek Corporation). A protective layer of photoresist was then coated on the wafer before dicing the wafer into 4mm x 4mm chips using the DISCO automatic dicing saw. After removing the photoresist film on the chip using acetone, the chip was washed in water and put in a solution of Piranha (70:30 (v/v) concentrated sulfuric acid: 30% hydrogen peroxide.) (*CAUTION: Piranha solution reacts violently with organic material and should be handled with extreme caution.)* for 20 minutes to make the silicon surface hydrophilic as well as to clean any photoresist residues off the gold patterns. The chips were then subsequently washed 3 times (for 2 minutes each wash) in de-ionized water (Resistivity > 18.2 MΩ-cm) and dried at 90 $^{o}$C for 5-10 minutes to completely dry them.

### FUNCTIONALIZATION OF SILICON WITH PEG GROUPS

We adopted the protocol of Papra *et. al.*[1] for functionalizing the chips with PEG silane. PEG silane and concentrated HCl were mixed in toluene in a freshly cleaned glass beaker with 100 µL PEG and 40 µL HCl present in 50 mL of toluene. The beaker was then placed in a sonicator for 10 minutes after which, it contents were transferred to another clean glass beaker. The dry chips were placed in the PEG-toluene solution for approximately 1 hour followed by washing once in toluene, once in ethanol and finally in

de-ionized water, each wash being of 2 minutes duration. These chips can now be stored under a dry $N_2$ environment for further use.

## PREPARATION OF THIOLATED DNA AND DNA-FUNCTIONALIZED GOLD NANOCRYSTALS

*(a) Preparation of thiolated DNA*

The thiolated DNA, purchased in powder form, is dissolved in 0.1 mL of a 0.1M solution of Dithiothreitol (DTT) and kept at room temperature for approximately 12 hours in order to break the disulfide bonds. The solution is then diluted to a final volume 1mL by adding de-ionized water. This 1mL solution of DNA and DTT is immediately run through a NAP-10 column (what kind of column is this, where did you buy it from?) to separate the DNA from the DTT, the DNA being eluted first and collected in a final volume of 1.5mL. This DNA is then frozen immediately for further use to prevent the formation of disulfide bonds.

*(b) Preparation of Au nanocrystals*

The gold nanocrystals, as purchased, have citrate ligands on their surface, making them negatively charged and thereby providing stability to the colloidal suspension due to electrostatic repulsion. To increase the stability of the nanoparticles, the ligand phosphine is dissolved in the nanocrystal solution at a concentration of 25-50 mg per 100 ml of solution. This solution is then stirred for approximately 12 hours at room temperature to allow for sufficient adsorption of the ligand onto the nanoparticle surface.

*(c) Preparation of DNA-nanoparticle conjugates*

Once the phosphine ligand has been coated onto the nanoparticles, they are ready to be reacted with the thiolated olignucleotides. Corresponding to each DNA strand that needs to be conjugated to the nanoparticles, we start with a 2.5 mL of the nanoparticle solution. 10 nm Au nanoparticles are at an initial concentration of ~30 nM and 15 nm particles at a concentration ~8 nM. The nanoparticle solutions are first concentrated by a factor of 100 by precipitating them at 14,000 RPM using a microcentrifuge, discarding the supernatant and resuspending the particles in approximately a 1X phosphine solution (1X phosphine contains 25-50 mg of phosphine ligand per 100 mL of de-ionized water) of the appropriate volume. (It should be mentioned that 0.3 mL of 10 nm nanoparticles takes approximately 45 minutes to precipitate fully, while the same volume of 15 nm particles takes approximately 10 minutes for precipitation.) This process of concentrating the nanoparticle solution is expected to accelerate its reaction with the thiolated DNA molecules.

A highly concentrated solution of DNA molecules (prepared in part (a)) is mixed with this concentrated solution of nanoparticles and the reaction is allowed to proceed at room temperature for approximately 12 hours. For this reaction, a DNA-nanoparticle ratio of 150:1 is employed for 10 nm particles, while a ratio of 250:1 is used for the 15 nm particles. While these numbers are far beyond of what is predicted to be on the nanoparticle surface, they ensure maximum surface coverage of the nanoparticle surface by the oligonucleotides. After 12 hours, NaCl is added to the DNA-nanoparticle solution to a final concentration of 50 mM, a process that apparently aids the oligonucleotides to stand up on the nanoparticle surfaces rather than lie down on them. After an additional 24 hours, the DNA-nanoparticle mixture is centrifuged using a microcentrifuge at 14,000

RPM to precipitate the nanoparticles (with oligonucleotides on them), the supernatant containing the excess DNA molecules. After removing the supernatant, the precipitate is resuspended in 1X phosphine and the centrifugation process repeated 1-2 more times to ensure as complete a removal of excess oligonucleotides as possible. The nanoparticles are now resuspended in a final volume of 0.5 mL, with the solution also containing final concentrations of 10 mM Phosphate Buffer (PB) and 0.3M NaCl. These nanoparticle-DNA conjugates are stored in glass vials (the nanoparticles stick to the walls of Eppendorf microcentrifuge tubes after a few days) at $4^{o}$C and they stay viable for approximately 6 months.

**IMMOBILIZATION OF SINGLE STRANDED DNA ON GOLD PATTERNS**

For immobilization, single-stranded thiolated oligonucleotide is dissolved in a 1.0M solution of $KH_2PO_4$ to obtain a final concentration of 1 μM of DNA, following the work of Herne and Tarlov[2]. The high ionic concentration of the solution is supposed to minimize the repulsive electrostatic interactions between DNA strands, resulting in a high surface density of DNA molecules on the gold surface. The chips containing the Au patterns are immediately immersed in this solution and the reaction is allowed to proceed at room temperature for about 4-6 hours. The chips are then thoroughly washed in a 0.1% (v/v) solution of Tween-20 in de-ionized water, followed by rinsing in de-ionized water, with each rinse being done for 2 minutes in order to remove a majority of non-specifically adsorbed DNA. The chips are subsequently placed in an aqueous solution of 1 mM Mercaptohexanol (MCH) in order to further remove the non-specifically bound oligonucleotides and also to enable the single-stranded DNA to stand up straight on the gold surface, an important requirement for highly efficient hybridization. After about 1 hour, the chips are washed twice in de-ionized water for 2 minutes each and are dried under a continuous flow of dry $N_2$. The chips are now ready to be used for assembling nanoparticle multilayers.

**ASSEMBLY OF MULTILAYERS OF AU NANOCRYSTALS**

The chips with a layer of single stranded DNA are now reacted with a solution containing gold nanoparticles conjugated to the complementary oligonucleotide, reaction being allowed to proceed for approximately 4 hours are room temperature. After the reaction, the chips are washed for 2 minutes each in a solution of 0.1% Tween-20 in 10 mM PB and 0.3M NaCl (to retain the hybridization of the DNA strands), followed by washing in a 10 mM PB, 0.3 M NaCl solution, to remove the non-specifically bound nanoparticles. The chips are then dried under a $N_2$ gun, after which they are placed in a solution of nanoparticles to which are attached DNA complementary to that on the first layer of nanoparticles. After a similar washing process to remove non-specific interactions, the last two steps are repeated until the desired number of layers is built. Following Mirkin and co-workers, after the final layer is assembled the chips are washed in a solution of 0.3 M solution of ammonium acetate. Ammonium acetate is a volatile salt; washing the chips in this solution keeps the multilayer assembly intact, while preventing crystalline salt residues (as NaCl does) when the chips are dried for imaging in the SEM.

**IMAGING THE ASSSSMBLIES**

All imaging is performed using a Hitachi S-5000 Field Emission SEM (FESEM) (Robert D. Ogg Electron Microscopy Center, UC Berkeley), with the accelerating voltage set to 10 kV. The chips are first stuck to a double-sided sticky carbon tape and then mounted

onto copper chips before imaging. The chips need to be completely dry before being imaged to achieve the best results.

REFERENCES

1. Papra, A., Gadegaard, N., Larsen, N. B., 2001**,** *Langmuir***,** *17,* 1457
2. Herne, T. M., Tarlov, M. J., *Journal of the Americal Chemical Society*, 1997, 119, 8916