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Optimizing Gold Nanoparticle Seeding Density on DNA Origami

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Electronic Supplementary Information

Experimental

Materials. M13mp18 and streptavidin-coated magnetic beads were obtained from New England Biolabs. DNA origami staple strands (100 μM in TE buffer) were purchased from Eurofins MWG Operon. PCR primers were ordered from either Integrated DNA Technologies or Eurofins MWG Operon and diluted to 1 μg/μL in water. Single-stranded DNA thiol was purchased from Eurofins MWG Operon with PAGE purification and diluted to 1 mM in water. PCR purification kits were purchased from Qiagen. *Taq* DNA polymerase and PCR buffers were obtained from Invitrogen. 30 kDa Amicon ultra 0.5 mL centrifugal filters were acquired from Millipore. Au NPs (5 nm) were ordered from Ted Pella. Bis(p-sulfonatophenyl)-phenylphosphine dihydrate dipotassium salt (BSPP) was acquired from Strem Chemicals. Silicon (100) wafers (p-type) with native oxide were purchased from Silicon Wafer Enterprises, and p-type silicon (100) wafers with a 200 nm oxide layer were obtained from MicroSil. Ultrapure, 18.3 MΩ water used for experiments was produced by an EasyPure UV/UF water purification system.

DNA Origami Scaffold Preparation. The scaffold for the "T" DNA origami structure was prepared as reported previously,[1] except PCR was adjusted for use with *Taq* polymerase instead of Pfx polymerase. *Taq* polymerase (2.5 units) was added to a

solution containing primers (0.5 μg each), M13mp18 template (20 ng), a mixture of dNTPs (200 μM), and 1x *Taq* polymerase buffer in a 100 μL volume. The PCR program was modified to have an initial denaturing step at 95 °C of 30 sec and denaturing steps within the cycles of 30 sec at 95 °C. The program was as follows: 95 °C for 30 sec, 30 cycles of 95 °C for 30 sec, 59 °C for 45 sec, and 68 °C for 3 min, with a final extension at 68 °C for 5 min and a hold temperature of 4 °C. The PCR purification and streptavidin-coated magnetic bead separation steps were performed as reported previously.[1] For the "T" DNA origami structure 20 μL of purified PCR product was used, and the samples were mixed with the beads for 30 min to allow them to bind together. The product from bead separations was purified with spin columns (QIAquick PCR purification kit).

DNA Origami Folding. Staple strands modified with 10 adenine nucleotides on the 3' end were used for the entire top section of the "T" structure (67 modified staple strands total) as described in previous work.[2] The DNA origami structures were folded with a 1:10 molar ratio of scaffold strand to staple strands in 1x TAE-Mg^{2+} buffer with a final scaffold concentration of 5 nM and volume of 100 μL. The structures were folded as reported,[2] by denaturing (95 ºC for 3 min) and slowly annealing from 95 ºC to 4 ºC in 90 min. The DNA origami structures were then filtered to remove excess staple strands using 30 kDa Amicon ultra 0.5 mL centrifugal filters. Each DNA origami sample was filtered by spinning for 10 min at 13,000 rpm (14550 rcf), rinsed three times with 1x TAE-Mg^{2+} buffer (500 μL) by spinning for 10 min at 13,000 rpm, and then recovered with a spin at 3,500 rpm (1055 rcf) for 3 min. The DNA concentration was measured with a Nanodrop 1000 spectrophotometer, and then the solution was diluted to 1 nM in 1x TAE-100 mM Mg^{2+} buffer. For the Mg^{2+} concentration variation experiments the

DNA origami was diluted and adjusted to be in 1x TAE buffer with either 40, 70, 100, or 130 mM Mg^{2+}.

Au NP Preparation. The Au NPs were prepared either as reported previously[2] or with larger volumes as follows: 20 mL of Au NPs (83 nM) were mixed with 10 mg of BSPP and shaken overnight. Solid NaCl was added until the color changed to brown and NaCl became harder to mix in. The solution was spun to pellet the Au NPs for 30 min at 3660 rpm (~1600 rcf) with a ss-34 rotor in a Sorvall RC 5C Plus Superspeed centrifuge and with an Eppendorf 5418 centrifuge for 10 min and 5 min at 5,000 rpm (2150 rcf) to help the Au NPs settle better. The solution was split into several smaller 1.5 mL centrifuge tubes for the 5,000 rpm step. The supernatant was removed and the Au NPs were combined and resuspended in an aqueous BSPP solution (500 μL, 2.5 mM). Methanol was added (500 μL), the solution was mixed, and then centrifuged for 30 min at 5,000 rpm with an Eppendorf 5418 centrifuge. The supernatant was removed and the Au NPs were resuspended with BSPP solution (400 μL, 2.5 mM). Methanol was again added (400 μL), the solution was mixed, and then centrifuged for 40 min at 5,000 rpm. The supernatant was removed and the AuNPs were resuspended with BSPP solution (400 μL, 2.5 mM). The concentration of the Au NPs was measured at 520 nm (using 12.05 $\mu M^{-1}cm^{-1}$ for the extinction coefficient)[3] using a Nanodrop 1000 spectrophotometer.

Au NP-DNA Preparation. The Au NP-DNA conjugates were prepared similarly to what was reported previously.[2] A solution containing a 1:200 ratio of Au NPs to thiolated DNA oligonucleotides was allowed to react for at least 60 hrs. The Au NP-DNA conjugates were filtered with 30 kDa Amicon ultra 0.5 mL centrifugal filters. Each sample was filtered by spinning for 10 min at 13,000 rpm (14550 rcf), rinsed three times

with 0.5x TBE buffer by spinning for 10 min at 13,000 rpm, and then recovered with a spin at 3,500 rpm (1055 rcf) for 3 min. The concentration of the Au NP-DNA conjugates was measured at 520 nm, and the solutions were diluted to the desired concentrations (5, 10, 25, and 50 nM) with water.

Attachment of Au NP-DNA Conjugates to DNA Origami Structures. Sample hybridization was performed following the process reported by Pilo-Pais et al.[4] using freshly washed and plasma cleaned (1 min, 18 W, with a Harrick Plasma Cleaner) silicon wafer pieces with either a native oxide surface or a 200 nm thermal oxide layer; thermal oxide surfaces were used only for the 10 and 20 min hybridization time samples. First, the "T" DNA origami (5 μL, 1 nM) was deposited on the silicon oxide surfaces for 5 min in a humid chamber at room temperature, followed by addition of Au NP-DNA solution (20 μL, 10 nM; for tests varying Au NP concentrations, 5, 10, 25, or 50 nM was used). After the designated time period, the silicon pieces with the DNA and Au NPs were dipped into a Petri dish with water for 10 sec and dried with a stream of compressed air.

Supplementary Figures:

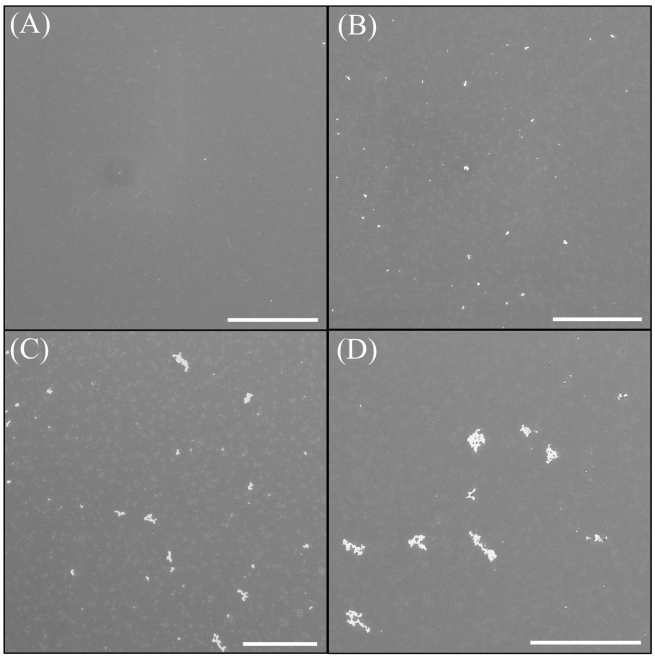

Figure S1. SEM images of aggregation of Au NPs with increasing Mg^{2+} concentration and hybridization time. Mg^{2+} concentrations and hybridization times for the samples were: (A) 40 mM and 10 min, (B) 70 mM and 30 min, (C) 100 mM and 90 min, and (D) 130 mM and 90 min. Scale bars are 5 μm.

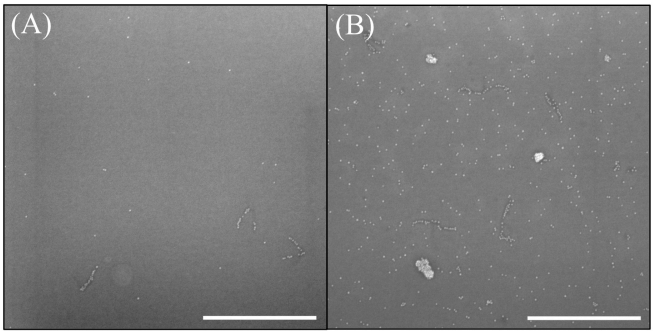

Figure S2. Background Au NP deposition with increasing Au NP concentration. SEM images of samples with (A) 4 nM and (B) 40 nM Au NPs. Scale bars are 500 nm.

Tables of Data Used in Selected Figures in the Text:

Table S1. Data for different hybridization times, used in Fig. 4.

	NP Density:		Largest Gap: (nm)				
Time (min)	NPs in a line	Total NPs	Min	Q1[1]	Median	Q3[2]	Max
10	12 ± 4	13 ± 5	9	38	54	60	98
20	12 ± 2	13 ± 2	29	37	47	61	69
30	16 ± 3	16 ± 3	16	28	34	40	53
60	17 ± 3	19 ± 4	14	23	28	37	63
90	24 ± 2	29 ± 4	6	12	15	18	35
150	24 ± 3	31 ± 6	5	11	14	17	36
210	26 ± 3	36 ± 7	5	8	11	12	21

[1] Q1 = 25th percentile, or middle value between min and median.
[2] Q3 = 75th percentile, or middle value between median and max.

Table S2. Data for different Mg^{2+} concentrations and hybridization times, used in Fig. 6.

		NP Density:		Largest Gap: (nm)				
[Mg^{2+}]	Time (min)	NPs in a line	Total NPs	Min	Q1	Median	Q3	Max
40 mM	10	16 ± 3	18 ± 5	12	24	32	45	58
	30	19 ± 3	25 ± 6	6	13	17	24	51
	90	23 ± 2	28 ± 4	6	11	14	17	29
70 mM	10	21 ± 2	23 ± 4	7	14	20	25	39
	30	22 ± 2	25 ± 5	7	15	17	20	51
	90	22 ± 2	25 ± 4	7	11	14	16	31
100 mM	10	21 ± 3	24 ± 5	10	17	25	33	41
	30	22 ± 2	25 ± 4	9	14	18	22	36
	90	25 ± 2	33 ± 5	7	10	12	16	22
130 mM	10	21 ± 3	23 ± 4	10	21	23	27	45
	30	22 ± 3	25 ± 4	15	19	23	32	50
	90	23 ± 2	26 ± 4	10	11	15	17	26

Table S3. Data for various Au NP concentrations, used in Fig. 7.

	NP Density:			Largest Gap: (nm)				
[Au NP] (nM)[1]	NPs: attachment location	NPs in a line	Total NPs	Min	Q1	Median	Q3	Max
4	0.9:1	17 ± 2	19 ± 3	16	25	31	35	57
8	1.8:1	19 ± 2	22 ± 3	5	17	22	30	43
20	4.5:1	21 ± 2	23 ± 4	12	20	23	28	36
40	9.1:1	22 ± 4	25 ± 5	11	16	20	27	69

[1] Concentration of Au NPs after addition with DNA origami solution. DNA origami was held at the same concentration for all samples (0.2 nM after Au NP addition).

Table S4. Data for Au NP solutions filtered at various times before use, for Fig. 8.

	NP Density:		Largest Gap: (nm)				
NP solution age (days)	NPs in a line	Total NPs	Min	Q1	Median	Q3	Max
0.2	17 ± 3	19 ± 4	14	23	28	37	63
0.3	19 ± 2	22 ± 3	5	17	22	30	43
1	22 ± 2	24 ± 3	8	14	17	20	32
66	23 ± 2	28 ± 4	7	12	15	19	29
89	22 ± 2	31 ± 5	4	10	13	17	21

References

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