Supporting Information

Title: "Spectroscopic studies of nucleic acid additions during seed-mediated growth of gold nanoparticles"

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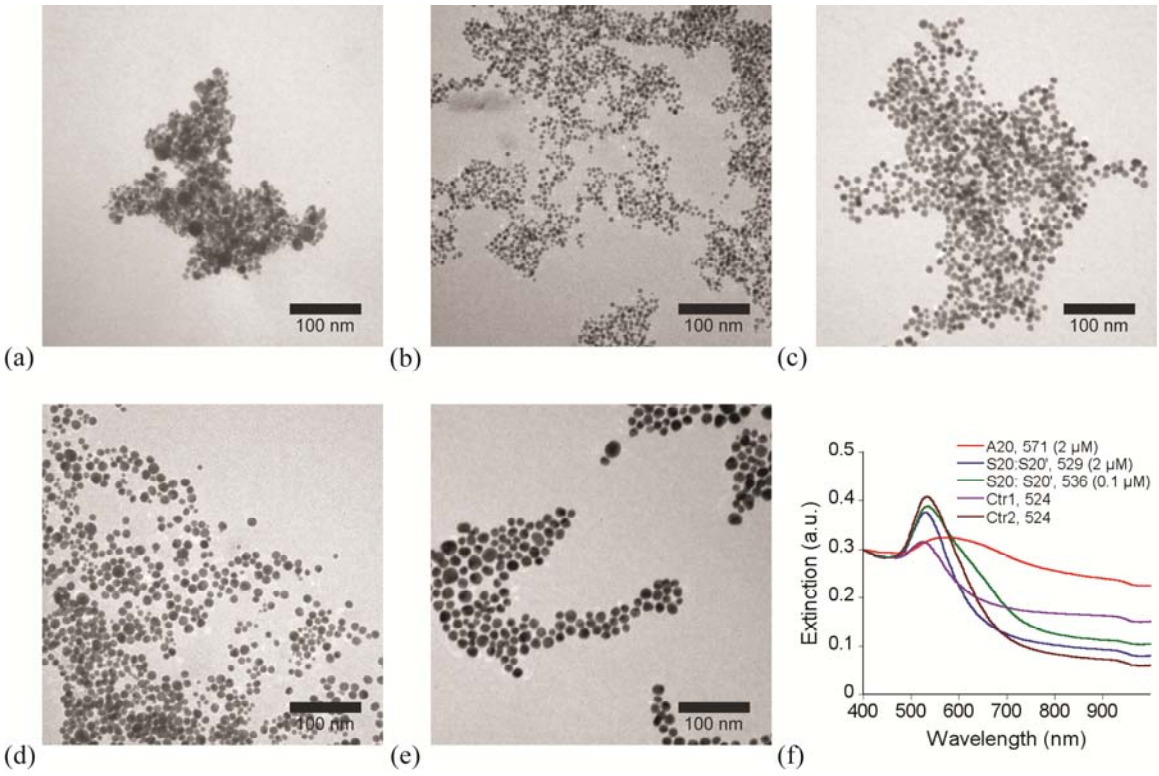

FIG. S1. TEM micrographs of gold seed aged for 7 days in the presence of added (a) **A20** at 2 μM (b) **S20:S20′** at 2 μM (c) **S20:S20′** at 0.1 μM (d) 2 μL Tris HCl (Ctr1) (e) 4 μL Tris HCl (Ctr2) and (f) accompanying UV-Vis spectra of each suspension used for TEM studies with the resulting peak wavelength values included in the legend.

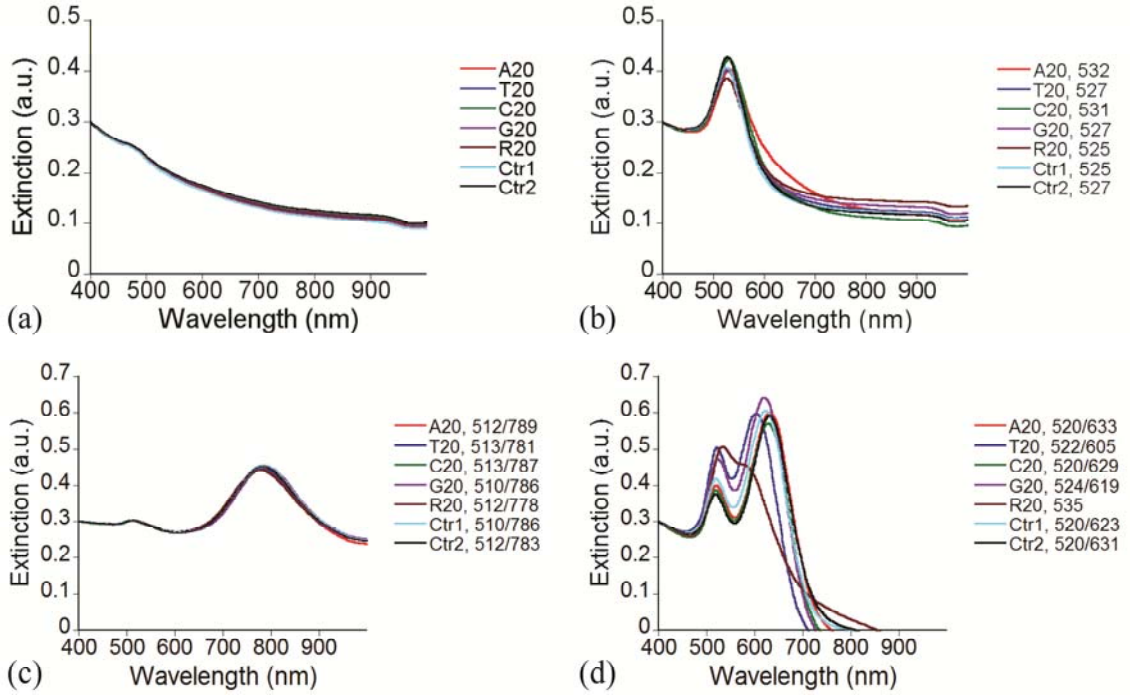

FIG. S2. UV-Vis spectra of (a,b) gold seed and (c,d) gold seed in AuNR growth solution following incubation with various 20 base-long homopolymers (**A20**, **T20**, **C20**, **G20**) and random (**R20**) sequences at 0.1 µM for 2 h (left) and 7 d (right). Controls involve the addition of Tris HCl (Ctr1= 2 µL Tris HCl) or nanopure water (Ctr2= 2 µL 18 MΩ-cm water) in the absence of DNA. The resulting peak wavelength values are included in the legend.

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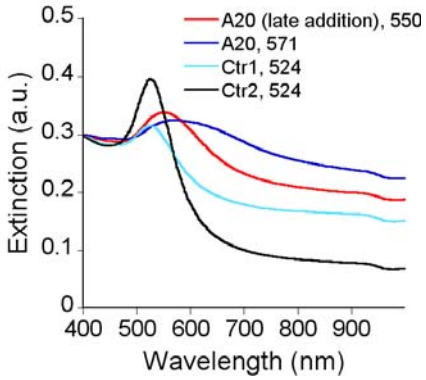

FIG. S3. UV-Vis spectra of gold nanoparticle suspensions in which (i) the gold nanoparticle seed solution was aged for 7 d prior to the addition and brief incubation of the aged seed solution with **A20** (2 μM) (late addition) or (ii) following a 7 d incubation in which **A20** (2 μM) was immediately added to a freshly-prepared gold nanoparticle seed solution. Controls involve the addition of Tris HCl (Ctr1= 2 μL Tris HCl) or water (Ctr2= 2 μL 18 MΩ-cm water) in the absence of DNA. The resulting peak wavelength values are included in the legend.

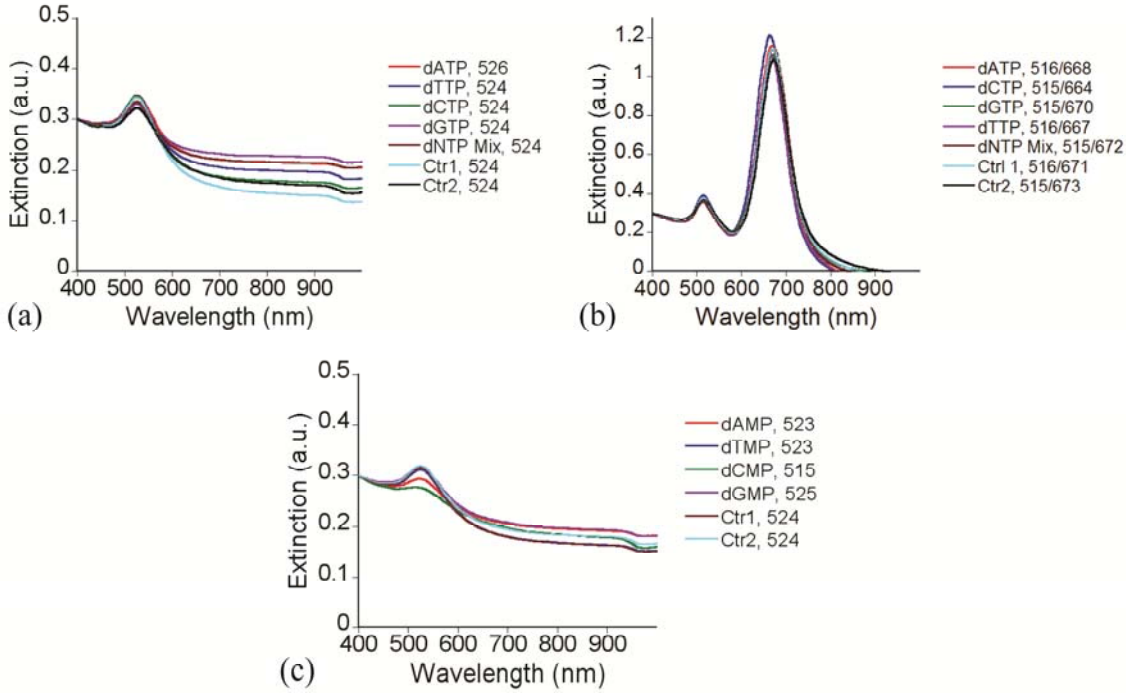

FIG. S4. UV-Vis spectra of (a) aged gold seed; (b) gold seed in AuNR growth solution following incubation with various dNTPs (dATP, dTTP, dCTP, dGTP, dNTP Mix); and (c) aged gold seed following incubation with various dNMPs (dAMP, dTMP, dCMP, dGMP) at 2 μM for 7 d. Controls involve the addition of Tris HCl (Ctr1= 2 μL Tris HCl) or water (Ctr2=2 μL 18 MΩ-cm water) in the absence of DNA. The resulting peak wavelength values are included in the legend.

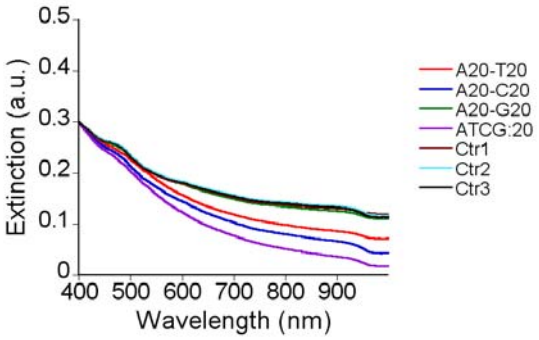

FIG. S5. UV-Vis spectra of gold seeds after a 2 h incubation in the presence of various homopolymer mixtures of two or four sequences at 2 μM. Controls involve the addition of Tris HCl (Ctr1= 4 μL Tris HCl, Ctr2= 8 μL Tris HCl) or water (Ctr3= 4 μL 18 MΩ-cm water) in the absence of DNA.

Confirming duplex formation is possible in gold nanoparticle seed solution

To prepare gold nanoparticle seed solution, 10 mL of 0.5 mM $HAuCl_4$ and 10 mL of 0.2 M CTAB were mixed on a stir plate for 50 min. After mixing, 1.2 mL of chilled 0.01 M $NaBH_4$ was added to the solution and mixed for 2 min. This solution was then used as the incubation buffer for the primary hybridization study carried out with 1.1 μM carboxylated polystyrene beads (PS). Beads were coupled to aminated **S20*** and primary hybridization was conducted with fluorescently tagged complementary and non-complementary targets, following the protocol described by Hardin and Milam[1] using sequences listed in Table S1 for the suspension conditions listed in Table S2). Duplex densities were evaluated by measuring the average fluorescence of the microsphere population and converting these average fluorescence intensities to duplex densities using calibration standards (Bangs Laboratories) in conjunction with BD FACSDiva software on a BD-LSR II flow cytometer as detailed by Hardin and Milam.[1]

Table S1. List of sequences used for flow cytometry study to verify duplex formation possible in gold nanoparticle seed solution conditions.

Function	**Sequence**
Probe	**S20*=** 3'-TAG TCG GCG TTA GGT TTT TT /NH_2/-5'
Complementary Target	**S15'**= 3'-ACC TAA CGC CGA CTA/ FITC/- 5'
Noncomplementary Target	**NC14**= 3'-GGA TTG CGG CTG AT/ FITC/- 5'

Table S2. Tabulated description of samples analyzed via flow cytometry to verify duplex formation between complementary **S20** and **S15′** sequences.

Sample	**Description**
Bare Beads (BB)	Bare Polystyrene (PS) Bead
BB + S20*	Bare PS bead + **S20*** probe functionalized with amine
BB+NC14	Bare PS bead incubated with **NC14**
BB+S15’	Bare PS bead incubated with **S15'**
CB	**S20***-conjugated PS bead alone
CB+NC14	**S20***-conjugated PS bead incubated with noncomplementary **NC14**
CB+S15'	**S20***-conjugated PS bead incubated with complementary **S15'**

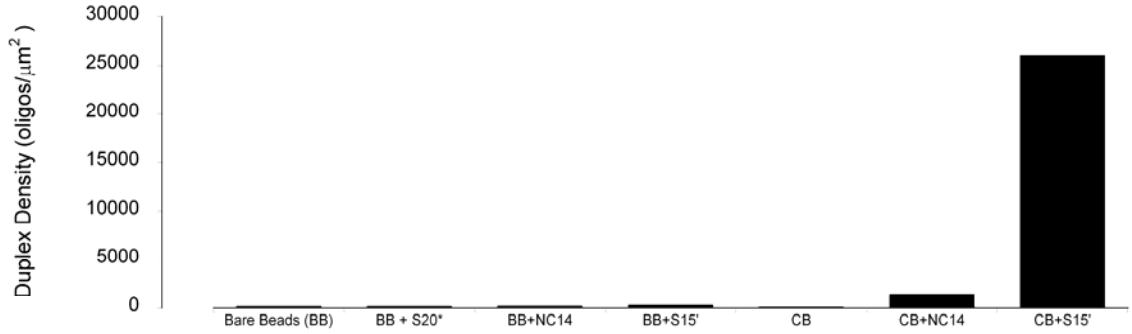

FIG. S6. Duplex density per μm^2 for controls (BB, BB+20*, BB+**NC14**) and complementary target cases (CB+**S15'**)

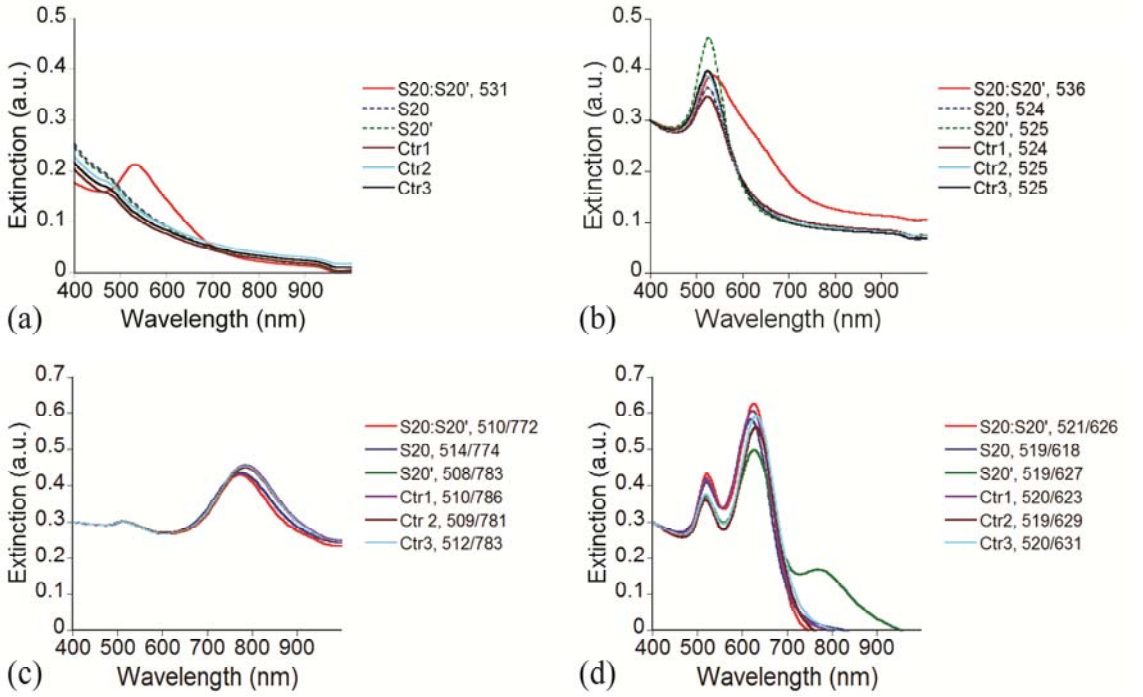

FIG. S7. UV-Vis spectra of (a,b) gold seeds and (c,d) gold seeds in gold nanorod growth solution following incubation with various complementary ssDNA alone (**S20** or **S20'**) and mixed together (**S20:S20'**) at 0.1 μM for 2 h (left) and 7 d (right). Controls involve the addition of Tris HCl (Ctr1= 4 μL Tris HCl, Ctr2= 2 μL Tris HCl) or water (Ctr3= 4 μL 18 MΩ-cm water) in the absence of DNA. The resulting peak wavelength values are included in the legend.

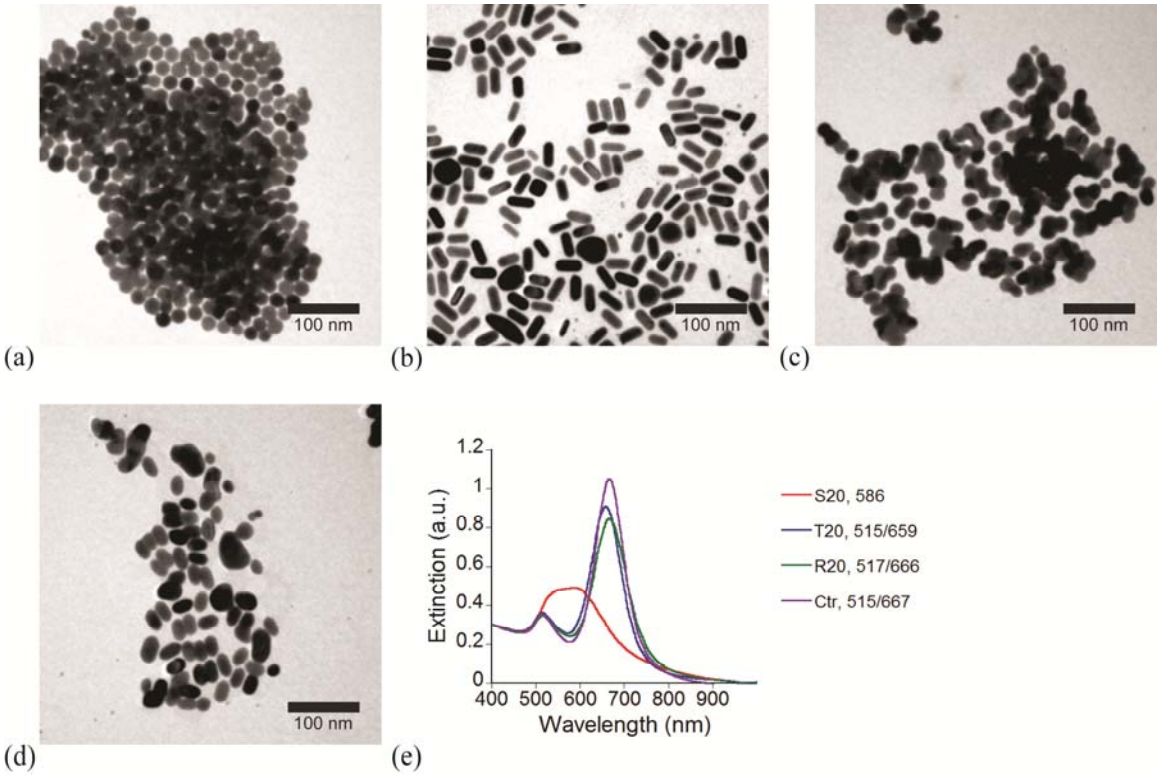

FIG. S8. TEM micrographs of 2 μM incubation samples after 3 days (a) **S20** (b) **T20** (c) **R20** (d) Control= 2 μL Tris HCl and (e) accompanying UV-Vis spectra of all incubation samples. The resulting peak wavelength values are included in the legend.

References

1. J. O. Hardin and V. T. Milam: Measuring in situ primary and competitive DNA hybridization activity on microspheres. *Biomacromolecules* **14**, 986 (2013).