Supporting Information

Efficient Selection of Biomineralizing DNA Aptamers Using Deep Sequencing and Population Clustering

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[‡]These authors contributed equally to this work

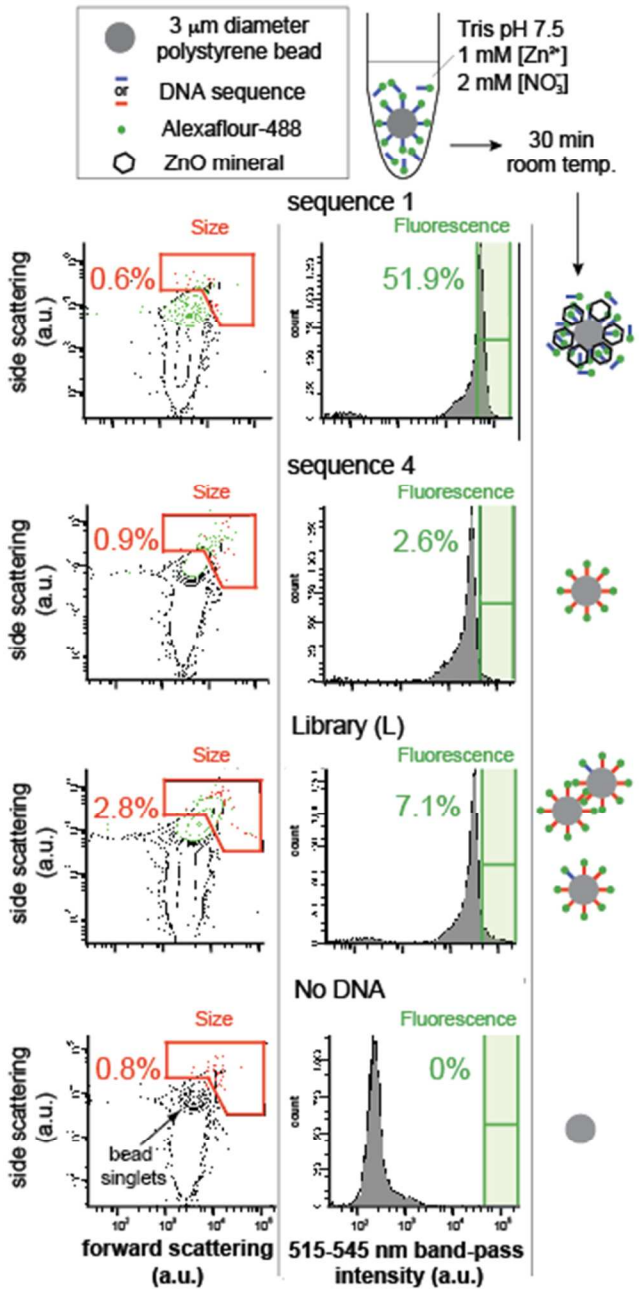

Figure S1. Flow cytometry results of a mineralization assay with biotin- and fluorophore-conjugated DNA. Either aptamers from sequence families 1 and 4, or the starting library (L) were assayed. DNA was pre-incubated with ProMag™ streptavidin-coated polystyrene beads in binding buffer (including 20 mM Tris-HCl, see main text), and $Zn_2(NO_3)_2$ was then added to the solution. Following a 15 min incubation for mineralization and washing of the beads to remove unreacted precursor, the beads were analyzed by flow cytometry. Shown in the left column are plots of light-scattering intensities from the reacted bead populations (each point represents the forward- and side-scattering signal from a single bead). Increased side-scattering signal indicates an increase in bead surface roughness, and increased forward-scattering indicates an increase in bead size. Shown in the middle column are histograms of the fluorescence intensities from the same bead populations as those shown in the left column. The right column shows schematic interpretation of critical characteristics of the reacted bead populations that

may be drawn from the displayed flow cytometry data. Note that for sequence 1 a large increase in fluorescence but not scattering (relative to the no DNA control) suggests nanoparticle synthesis on the bead surfaces (vs. growth of extensive agglomerates, which would yield increased light scattering); this is consistent with the results shown in Figure 4.

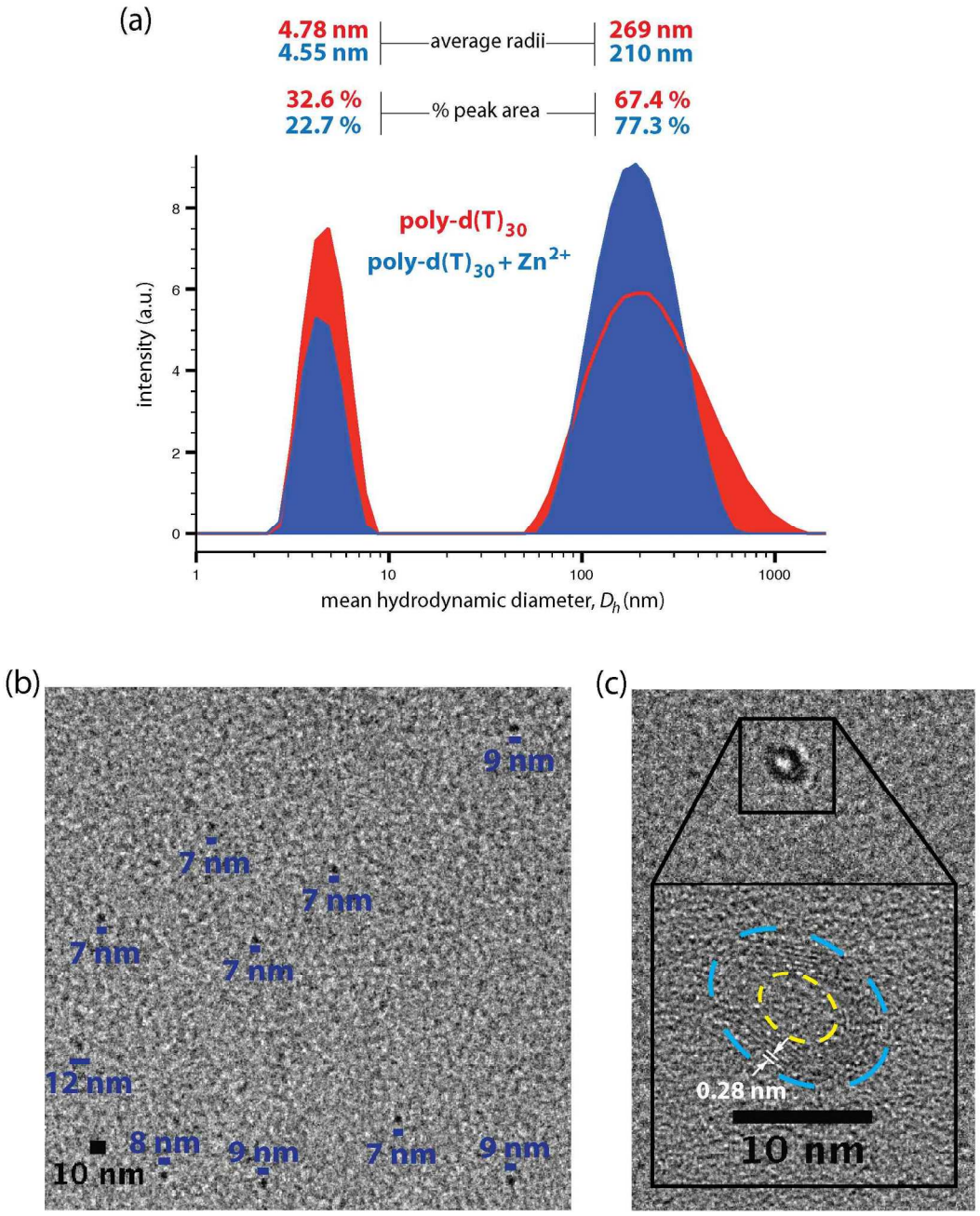

Figure S2. Examination of poly-d(T)$_{30}$ ssDNA size under conditions relevant to poly-d(T)$_{30}$–controlled zinc-based mineralization. (a) Dynamic light scattering of ~100 μM poly-d(T)$_{30}$ in binding buffer, either in the absence (red) or presence (blue) of 10 mM $Zn(NO_3)_2$. (b) TEM analysis of products captured on formvar films of TEM grids after 45 min of reaction between 1 μM poly-d(T)$_{30}$ and 10 mM $Zn(NO_3)_2$. (c) In addition to the majority reaction products shown in *b*, in some cases core-shell amorphous-hexaganol-ZnO nanoparticles, as shown here, are also found on the formvar films. These are similar to the nano-shells produced in higher yield after longer reaction times (24 h; see Figure 4a), suggesting that observed amorphous nanoparticles shown in *b* are precursor structures to ZnO nano-shells.

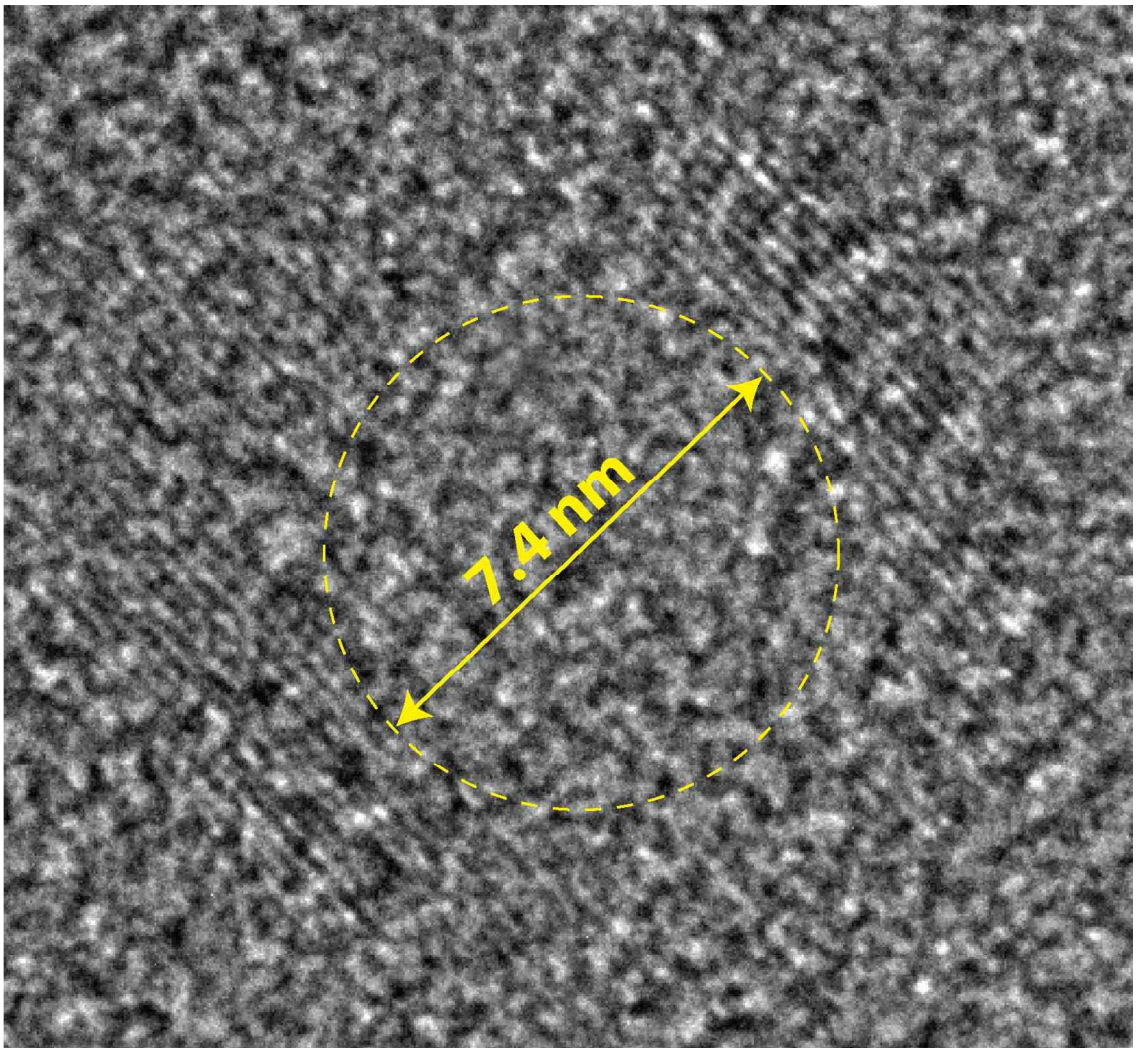

Figure S3. ZnO nanoshell structure shown in Figure 4, here indicating the specific size of the inner amorphous region for one of the core-shell nanoparticles.

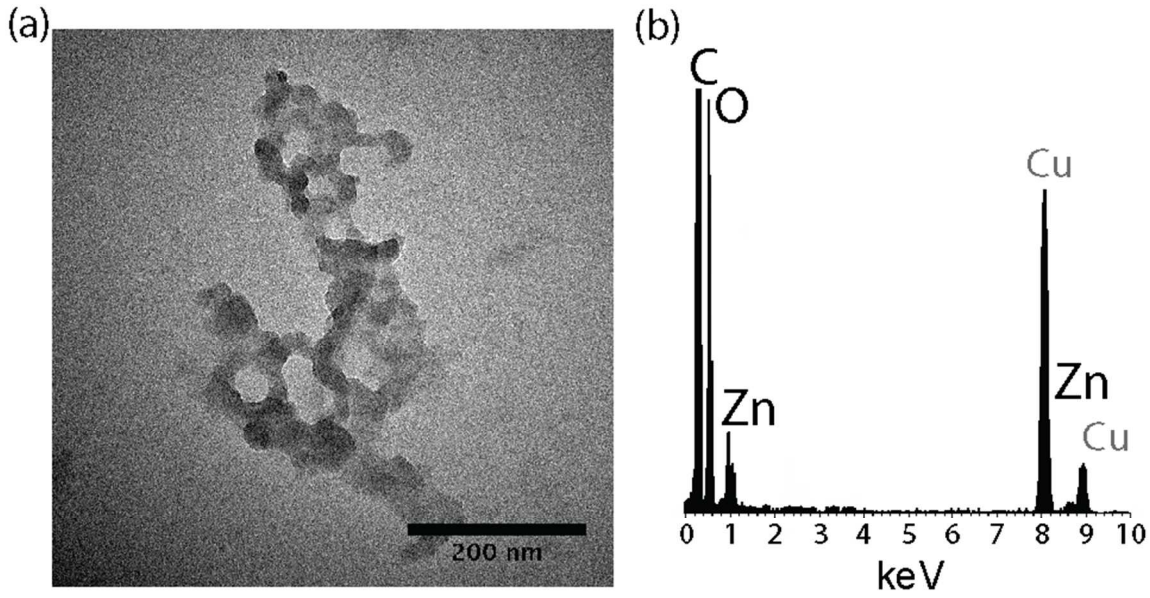

Figure S4. Amorphous zinc-containing precipitates shown by (a) TEM and (b) EDS. These products were captured on formvar films of TEM grids when 1 μM poly-d$(T)_{30}$ is reacted with 10 mM $Zn(NO_3)_2$ in the presence of 100 mM HEPES (pH 7.6) buffer instead of binding buffer (300 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris-HCl pH 7.6). Other than the change in buffer, the reaction was conducted exactly as described in the main text for the data presented in Figure 4a,b. This result shows that buffer conditions are critical to poly-d$(T)_{30}$–controlled ZnO crystallization.

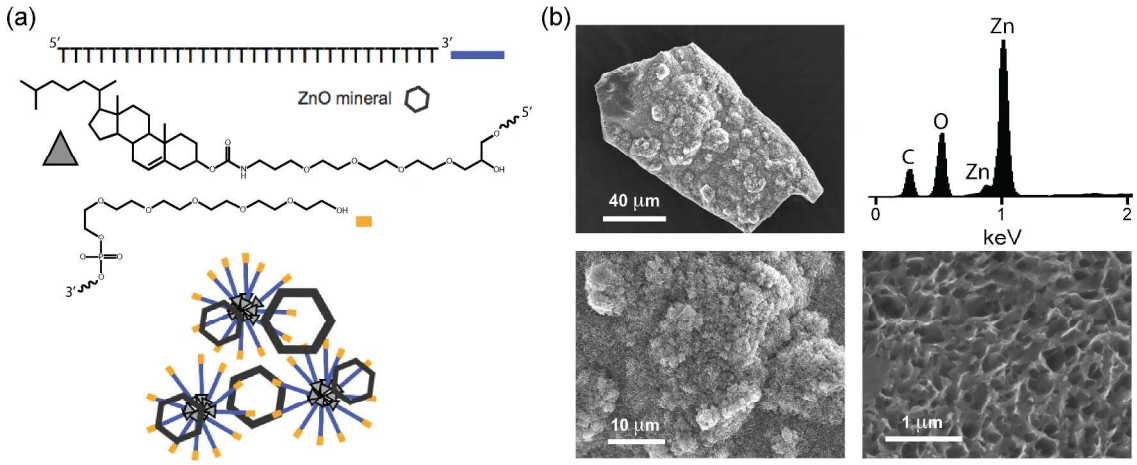

Figure S5. Large amorphous zinc-containing minerals are formed when self-assembling moieties are covalently attached to the ZnO-binding aptamer poly-d$(T)_{30}$ prior to the mineralization reaction, confirming that the efficiently identified ZnO aptamers are flexible material-synthesizing biomolecular tools. (a) A schematic of the chemical groups added to the 5'- and 3'-ends of the poly-T_{30} aptamer, which include cholesterol moieties to contribute to hydrophobic-driven aptamer assembly. The overall chemically-modified aptamer was prepared commercially by Integrated DNA Technologies. 100 μM of the sequence was reacted with 100 mM $Zn(NO_3)_2$ in binding buffer at room temperature for 17 h. A schematic description of the suggested mineralized structure is also presented (bottom). (b) Mineralization products were recovered by pelleting by centrifugation in 1.5 mL eppendorf tubes at 12 krpm, and were washed with 70% ethanol several times *via* centrifigation and then evaporated onto carbon tabs for imaging by scanning electron microscopy (SEM). Resulting SEM images are shown here. No reaction products were found in a control reaction with cholesterol only.

Supporting Experimental Methods

PCR recovery of screened library

The entire eluted library was used as template for PCR amplification, partitioned into 10 μL template aliquots introduced to separate 57 μL PCR reactions. The forward primer used was 5'-CCA CTA CGC CTC CGC TTT CCT CTC TAT-3' and the reverse primer was 5'-CTG CCC CGG GTT CCT CATTCT-3'. Each PCR reaction included 0.9 μM of each primer and 45 μL of Invitrogen PCR SuperMix (containing nucleotides, buffer and requisite ions, and *Taq* DNA polymerase). The following thermocycling schedule was applied: 95 ˚C for 5 min; 16 cycles of (95 ˚C, 15 sec; 62 ˚C, 15 sec; 70 ˚C, 1 min); 70 ˚C, 5 min. Amplicons were purified from the PCR reaction using Qiagen's MinElute PCR purification kit according to the manufacturer's instructions.

Estimate of number of DNA molecules in the eluate

In trial PCR runs, 16 cycles were determined to be optimum as the minimum number of cycles to produce a distinct single band of amplified product. We used a Lonza gel system to analyze the appearance of DNA amplicons. According to the manufacturer, the lower limit for DNA band detection is 5ng (1). Dividing this value by 65,000 (*i.e.* 2^{16}-fold amplification from 16 rounds of PCR) gives ~7.5×10^{-6} ng of eluted DNA. Using an approximate DNA molecular mass of 23,750 g/mol (for 78 nt, including the 30N region plus the priming regions), ~7.5×10^{-6} ng gives ~191,000 template molecules in one PCR reaction. Ten PCR reactions were used, so the total eluate is estimated to be composed of ~1.9 million DNA molecules. Thus, ~16% of eluate was recovered for analysis *via* high-throughput sequencing and population clustering. This type of population coverage is intractable with traditional sequencing approaches.

Surface binding assay

Silica surfaces for surface binding assays were prepared from a 500 μm-thick Si wafer, which was scribed into rectangular strips ~0.5x2 cm. The pieces were prepared for Au deposition by washing 10 min in acetone in a sonication bath, rinsing with 70% ethanol, and drying with N_2. Gold films were then deposited by plasma sputtering. These gold-coated silicon strips were then used as substrates for the electrochemical deposition of ZnO films. For this deposition, each strip was clamped at one end, attaching it to a voltage source to allow the Au surface to serve as a negative electrode. The strip was immersed into a solution of 0.1 M $Zn(NO_3)_2$ at 70 ˚C. The counter electrode (Al) was then inserted into the same solution and 1 V was applied to the system for 5 min. Successfully grown ZnO films were observed as uniform white coatings on the Au surfaces. The strips were then scribed into smaller rectangular pieces (average surface area 6.5 mm^2).

For each surface-binding assay, a 5 μL droplet of a given DNA sequence (at 10 μM DNA in binding buffer) was applied to the ZnO surface in a moist atmosphere to prevent droplet evaporation. After a 90 min incubation period the droplet was aspirated from the surface by pipetting followed by a gentle burst of air to remove residual liquid. Surface-bound DNA was extracted by transferring the mineral substrate to a 100 μL solution of binding buffer with 3 μm-diameter streptavidin-coated ProMag™ beads (Bang's Laboratories; binding capacity 0.65 μg-FITC/mg microspheres) at a concentration of 1.66 x 10^4 beads/μL. Streptavidin on the ProMag™ bead surfaces captured the biotin handle of the DNA sequences during an incubation period of 30 min with gentle mixing on a tube turner. A strong magnet was then used to pull the beads to the bottom of the test tube; the supernatant was removed, and the beads were washed once with 1 mL H_2O, twice with 1 mL PBS/T (phosphate buffered saline, 0.1% tween-20), and resuspended in 1 mL PBS/T. Beads were then analyzed by flow cytometry (BD FACS-Aria) to measure

DNA-fluorophore fluorescence through the 515-545 nm band-pass channel under excitation at 488 nm, with untreated beads used to set the detector voltages of the instrument before each measurement.

Mineralization assays

For mineralization reactions in the presence of ProMag™ beads, 1.66 x 10^6 beads from the stock solution were added to a 50 μL mixture of 1x binding buffer and 1 μM DNA of the sequence to be assayed. The mixture was incubated for 15 min with gentle shaking, after which 5 μL of 100 mM $Zn(NO_3)_2$ was added, and the reaction incubated for 30 min at room temperature with gentle shaking. A strong magnet was used to wash the beads 1x with 1 mL H_2O and 3x with PBS/T. The washed beads were then resuspended in 1 mL of PBS/T and analyzed by flow cytometry with a BD FACS-Aria instrument. For mineralization reactions in the absence of beads, 50 μL reactions were prepared with 1x binding buffer, 10 mM $Zn(NO_3)_2$ and 2 μM DNA, or as indicated in the main text.

1. Lonza FlashGel™ System Product Document ©2009 Lonza Rockland, Inc.