# Selected DNA aptamers influence kinetics and morphology in calcium phosphate mineralization

*Jacob Shlaferman; Alexander Paige; Krista Meserve; Jason A. Miech; Aren E. Gerdon**

Department of Chemistry and Physics, Emmanuel College, 400 The Fenway, Boston, MA 02115

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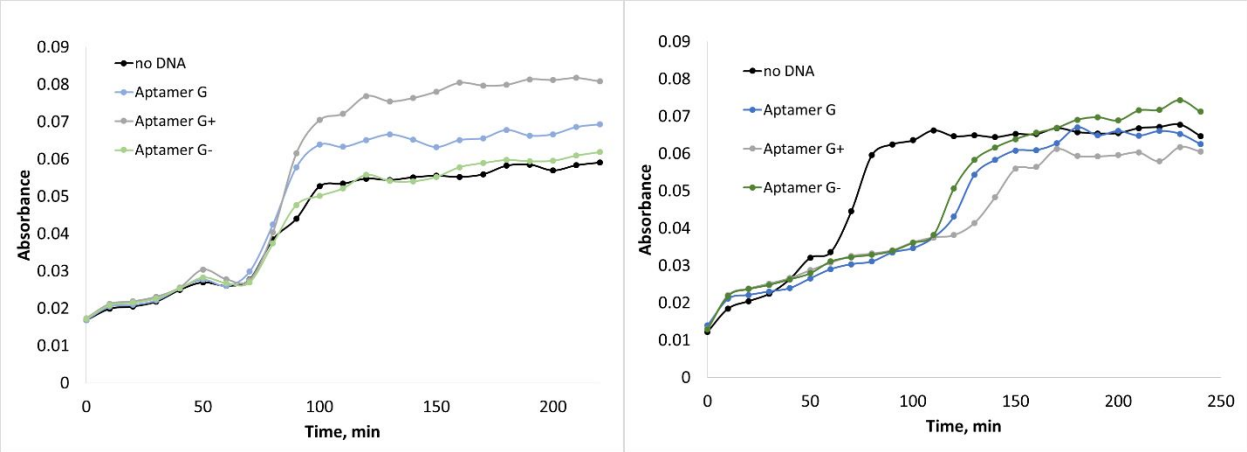
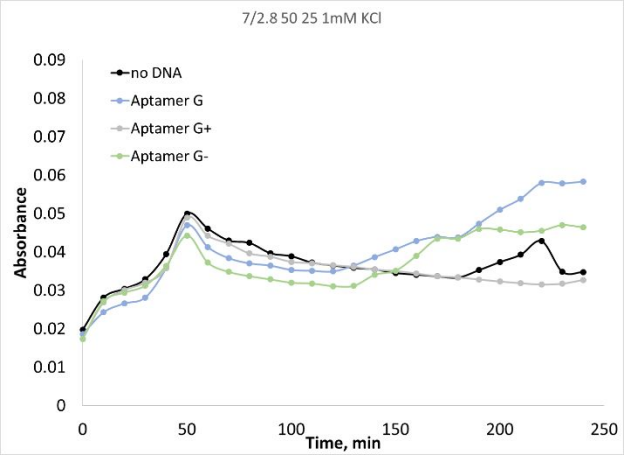
**Supporting Information**

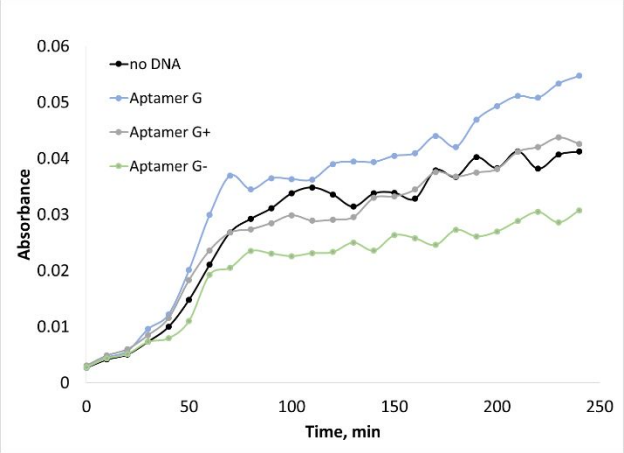
**S1. Homogenous mineralization under various reaction conditions.** Homogenous mineralization reactions were completed with an additional 10 mM Tris buffer at pH 7.4. Because the pH was held constant, optical density at 630 nm was measured rather than an absorbance change due to pH changes at 420 nm. Reactions were run at 25 nM (left) and 500 nM (right) aptamer.




Homogenous mineralization reactions were completed with 25 nM aptamer with the addition of 1 mM KCl to solution and measured in the standard format.



Homogenous mineralization reactions were completed where the 25 nM DNA aptamer was initially dissolved in a calcium precursor solution, then phosphate was spiked in to initiate the reaction. Due to the sharp change in solution pH upon the addition in the phosphate, optical density at 630 nm was measured rather than an absorbance change due to pH changes at 420 nm.




In addition to these experiments, a range of supersaturation ratios were tested using from 5 to 10 mM calcium chloride and from 2 to 4 mM sodium phosphate pH 7.4. The conditions of 7 mM calcium chloride and 2.8 mM sodium phosphate were ultimately selected to provide the greatest analytical sensitivity in these experiments.

**S2. Microfluidic device design and fabrication**. In order to control reactant mixing, microfluidic flow cells were used and constructed. Three or four holes corresponding to the inlets and outlets were drilled into a double-width (75 x 50 mm) glass microscope slide (Ted Pella Inc.) using a Dremel and a 1 mm diamond drill bit. The flow cell design was cut out of Secure Seal doubled-sided adhesive tape, 0.36 mm thick (Grace Bio-labs) using a Silhouette Cameo Cutter and Silhouette Studio software (Silhouette). The flow cell design used here is shown in Figure SI 2-1.

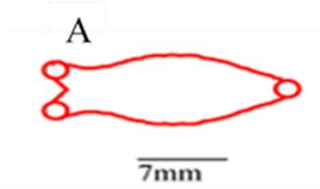



**Figure SI 2-1.** Pattern used for flow cell cut from double-sided tape.

Tubing with a length of 43 cm (Cole Parmer, OD: 0.07 in, ID: 0.04 in) was inserted in tubing with a length of 1.5cm (Cole Parmer, OD: 0.188 in, ID: 0.062 in) and was attached to the glass slide using 5 min epoxy before attaching the double-sided adhesive. For QCM experiments, a quartz crystal (Stanford Research Systems, 1 in. 5 MHz) was attached to the bottom of the flow cell in conjunction with the tape, while for morphology experiments a gold slide was attached (1 mm thick Biogold substrate, Electron Microscopy Sciences, coating of gold on the wafer was 1200 angstroms with purity of 99.999%). Additional epoxy was added to ensure no air leaked into the flow cell. A typical finished microfluidic flow cell is shown in Figure SI 2-2. A new device was used for each condition, with the same device being reused for all similar experiments.

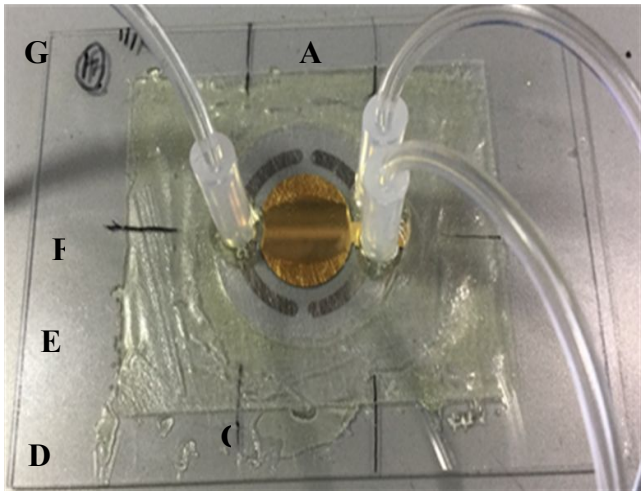


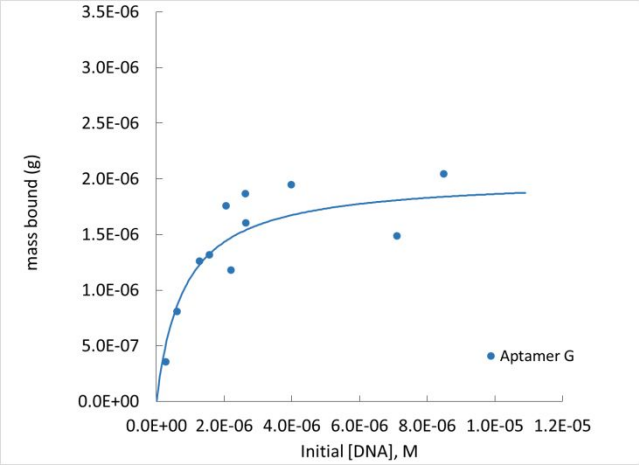

**Figure S 2-2.** Finished Fish Microfluidic Device. **A** is the microfluidic channel of the Fish design. **B** is the Tygon inlet tubing. **C** is the QCM crystal. **D** is the 0.36 mm double sided tape. **E** shows the quick dry epoxy. **F** shows the Tygon outlet tubing. **G** shows the glass slide.

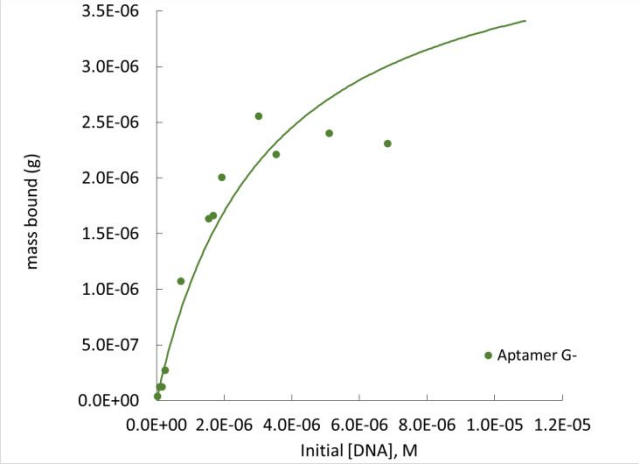
*Microfluidic Device QCM Calibration*. The microfluidic QCM sensor was calibrated in solution conditions where only density and viscosity changes are expected. The flow cell was attached to QCM electrodes with binder clips and the capacitance was adjusted until the QCM locked. Then deionized water was pumped into the flow cell via the outlet by a syringe pump at 25.00 uL/min. Next the solution was changed to 25% w/w sucrose and the response of the QCM was measured. The negative change in frequency is plotted against the change in resistance to provide the calibration factor for the QCM's response to density and viscosity changes (data not shown).

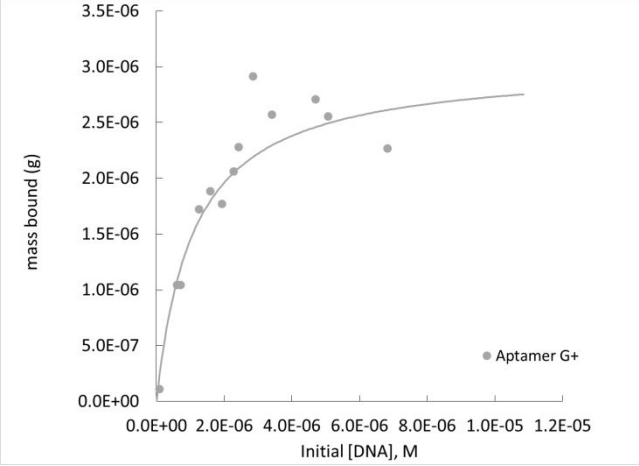
*Mineralization Procedure*. During calcium phosphate mineralization, QCM data and optical measurements of the mineral were collected. Measurements were made using a quartz crystal

microbalance (RQCM, Inficon) and a microscope (Motic, SMZ-171-TLED) with digital camera attached (Motic, Moticam 580). For control experiments, 10 mM phosphate 10 mM sodium chloride buffer (pH 7.40) was pumped through the outlet for even filling of the flow cell. After the flow cell was filled, sodium phosphate (pH 7.40) was introduced into the inlets. The two inlets were pumped by a dual syringe pump (GenieTouch Infusion/Withdrawal Dual Syringe Pump, Kent Scientific) at 25.20 uL/min. The flow-rates were calibrated by measuring the mass of water pumped over a set amount of time. Once a baseline was established, the top was replaced with 12.5 mM sodium chloride for a few seconds and then calcium chloride and allowed to react for two hours. Pictures were taken every 5 minutes. At the end of the two hours the flow cell was cleaned out with 0.5 M acetic acid. With the presence of an aptamer or linker, the linker and/or aptamer was pumped through the outlet, and after filling the flow cell it was allowed to sit and react with the gold surface for an hour. After an hour, the linker and/or aptamer was pushed out of the flow cell with 10 mM phosphate 100 mM sodium chloride buffer (pH 7.40) and the procedure for control experiments was followed.

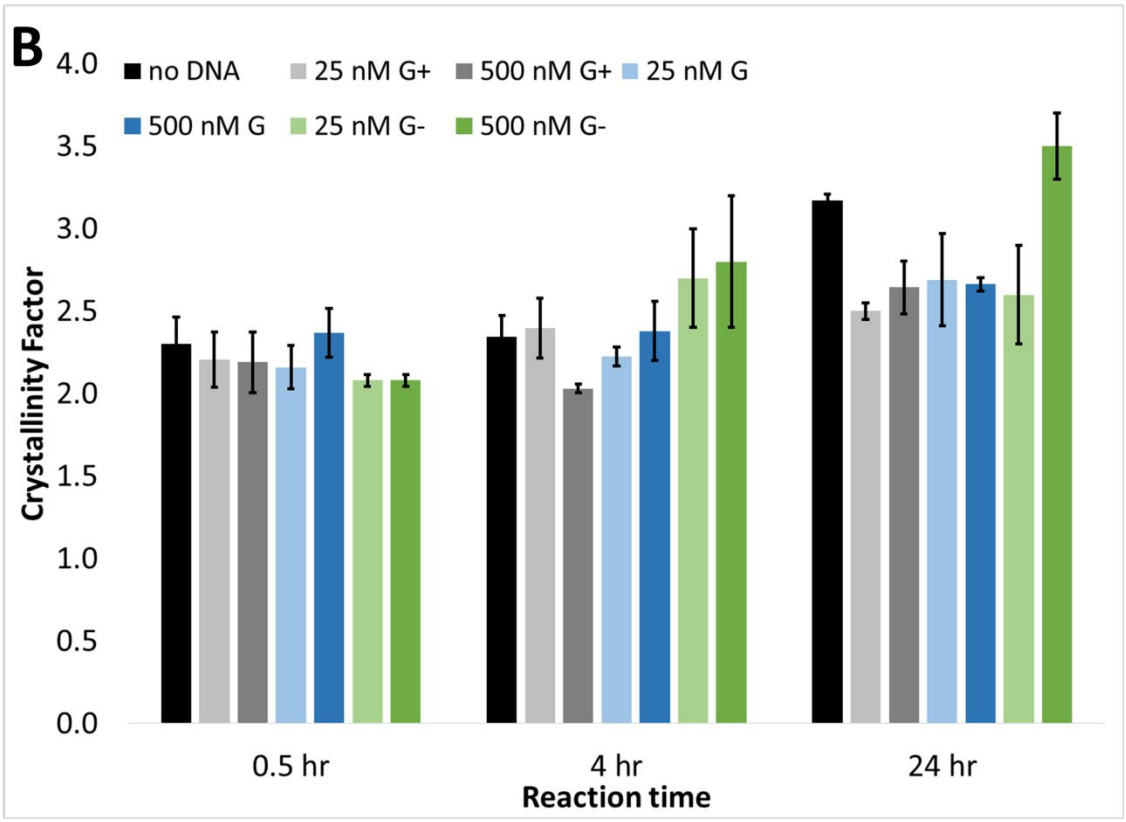
**S3. DNA aptamer affinity to hydroxyapatite.** Binding curves and Langmuir Isotherm fits for DNA aptamer interaction with HAP are provided below.



**S4. Crystallinity factor, Cf, calculated from FT-IR measurements.** Cf is given for all aptamer strands at both 25 nM and 500 nM aptamers concentrations.



**S5.** TEM images of mineral that include images at 0.5 hr and 25 nM concentrations of aptamers