

# DNA-FUNCTIONALIZED MICROPARTICLES

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## RATIONALE/OBJECTIVES

DNA-coated microparticles can allow for the assembly of a variety of bulk crystals using colloids of differing sizes. This procedure has proved to be more difficult than functionalizing DNA onto nanoparticles for one main reason: thin coating. Since the length of DNA is about 1% of the particle size, this results in a greater sensitivity to particle roughness and other characteristics. A subsequent issue is “hit and stick” binding, where particles are unable to rearrange to form equilibrium structures that minimize free energy. The objectives of this experiment are to create smooth surfaces on the colloid microparticles, and attach a dense layer of ssDNA using a strain-promoted alkyne-azide cycloaddition reaction (SPAAC). This methodology should work for many microparticles (PS, silica, TPM, titania, etc), resulting in the possibility of numerous crystalline lattice structures.

## MATERIALS

### Particle purification

- TPM particles (3-(trimethoxysilyl)propyl methacrylate): *an organo-silica hybrid material*
- deionized water
- ammonium hydroxide
- CHPMA (3-chloro-2-hydroxypropyl methacrylate)
- SDS (sodium dodecyl sulfate): *acts as a stabilizing agent*
- AIBN (azobis(isobutyronitrile) ): *initiates polymerization*

### Azide functionalization

- F127 solution
- $\text{NaN}_3$
- potassium iodide
- Triton X-100 solution: *nonionic surfactant*

### DNA preparation/DBCO conversion

- single-stranded oligonucleotides, with sticky ends @ 5' end
- DBCO-sulfur-NHS
- PBS: 10 mM, pH 7.4, 100 mM sodium chloride
- Cy3, Cy5 fluorescent dye
- Filtration columns

### DNA grafting

- Pluronic F-127 (20% solution in DMSO)

## METHODS

### TPM Particles

Chlorinated TPM particles are fabricated by copolymerizing 3-(trimethoxysilyl)propyl methacrylate (TPM) with 3-chloro-2-hydroxypropyl methacrylate (CHPMA)

- 200  $\mu$ L of TPM + 20 mL of deionized water + 20  $\mu$ L of ammonium hydroxide (1% w/w)  
[stirred for 4 h @ room temp] = producing monodisperse TPM emulsions
- + 40  $\mu$ L of CHPMA: allowed to diffuse into the emulsion droplets  
[30 min later] + 5 mL of an aqueous SDS (5% w/w)  
[10 min later] + 10 mg of azobis(isobutyronitrile) (AIBN)  
[stir for 20 min] then temp rapidly raised  $\rightarrow$  80  $^{\circ}$ C  
[4 h later] Reaction quenched, cooled to room temp = TPM particles  $\sim$ 1 $\mu$ m in diameter
- Purified by repeated centrifugation/redispersion

Particles of different sizes can be synthesized by varying the amount of TPM and adjusting pH levels

### Azide Functionalization of Particles

Particles with halogen groups on the surface are treated with sodium azide ( $\text{NaN}_3$ ) to obtain azide functional groups

- 20 mL of particle suspension ( $\sim$ 1% w/w) in aqueous F127 solution (0.2% w/w) + 500mg of  $\text{NaN}_3$  and trace/catalytic amount of potassium iodide
- heated at 70  $^{\circ}$ C for 12h
- washing by centrifugation/redispersion
- stored in deionized water

### DBCO-DNA

Single-stranded oligonucleotides with a sticky end are used in this study. 5'- $\text{NH}_2$ -DNAs are purchased (Integrated DNA Technologies, USA), and the amine group is converted to a dibenzyl cyclooctyne (DBCO)

- treat DNA w/ DBCO-sulfo-NHS (Click Chemistry Tool) in phosphate buffered saline (PBS), 10 mM, pH 7.4, 100 mM sodium chloride
- DNA internally fluorescent labeled with Cy3 or Cy5, respectively, used self-complementary, palindrome (P - CGCG) & complementary (A - GCAG / B - CTGC) // sticky end containing four bases @ 3' end// 61-bp 'poly-T' spacer

Sequences:

- A4: 5'-/DBCO/(T)<sub>20</sub>-Cy3-(T)<sub>41</sub>-GCAG-3'
- B4: 5'-/DBCO/(T)<sub>20</sub>-Cy5-(T)<sub>41</sub>-CTGC-3'
- P4: 5'-/DBCO/(T)<sub>20</sub>-Cy5-(T)<sub>41</sub>-CGCG-3'
- P8: 5'-/DBCO/(T)<sub>20</sub>-Cy5-(T)<sub>41</sub>-CGTATACG-3'
- 33.3  $\mu$ L of amine ssDNA (300  $\mu$ M) + 50  $\mu$ L of 1 mM DBCO-sulfo-NHS in PBS (freshly prepared)

- [vigorously stirred overnight]
- purified by passing through a MicroSpin G-25 columns (GE), diluted to 100  $\mu\text{M}$ , and stored in PBS at  $-20\text{ }^{\circ}\text{C}$

### **DNA Grafting (SPAAC)**

- Azide-functionalized particles + 400  $\mu\text{L}$  of PBS containing Triton X-100 (0.1% w/w) w/ particle concentration  $\sim 0.1\%$  w/w
- + 20  $\mu\text{L}$  of DBCO-DNA (100  $\mu\text{M}$ )
- stirred at  $55\text{ }^{\circ}\text{C}$  for 24 h
- washed and stored in PBS containing 1% w/w Pluronic F127

To determine the number of DNA strands functionalized per particle, flow cytometry is utilized. Roughly speaking, the fluorescent intensity data will be measured and collected, then converted into the approximate number for DNA grafted on each particle.

### *DATA ANALYSIS*

#### **Crystallization**

The particles of interest are combined, mixed according to the stoichiometry of the target crystalline structure

- particles transferred to a hydrophobic glass capillary tube (2 mm  $\times$  100  $\mu\text{m}$   $\times$  10 cm, pretreated with oxygen plasma and exposed to hexamethyldisilazane vapor)

[This pretreatment causes some of the F127 dissolved in the particle suspension to adhere to the glass tube thus forming a dense steric layer that prevents particles from sticking to the glass]

- capillary tube is sealed and attached to a microscope glass slide using wax
- slide is then mounted on a homemade microscope thermal stage with a temperature gradient

*Crystallization:* sample heated above the melting temperature ( $T_m$ ) to melt any aggregates

- temperature range of the stage is set such that the lower limit is about  $5^{\circ}$  below  $T_m$ , while the upper limit is about  $5^{\circ}$  above  $T_m$ . Crystallization occurs in minutes to hours, but in this study, the samples are typically allowed to anneal overnight before microscopic imaging
- crystal structures determined by initial particle size, stoichiometry, and sticky ends sequences
- thickness determined by initial particle concentration, size and density

Predictions:  $\sim 115,000$  strands on each 1.0  $\mu\text{m}$  particle, density of 1/27 strand/ $\text{nm}^2$

FCC and CsCl crystals

## *PRINCIPLE REFERENCES*

Wang, Yu, et al. "Crystallization of DNA-coated colloids." *Nature communications* 6 (2015).

Wang, Yufeng, et al. "Synthetic strategies toward DNA-coated colloids that crystallize." *Journal of the American Chemical Society* 137.33 (2015): 10760-10766.