Teathering Microparticles to Gold Surfaces using Oligos

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handle the crystals only with tweezers
crystal degregation

1 Cleaning and Preparing the Crystals

The quartz crystals must be cleaned prior to use to remove surface contaminants and allow the chemistry to function. There are two methods which seem to work more or less equivalently.

1.1 Method 1: Piranha Solution

A solution of fresh piranha will both remove organic contaminates and hydroxylate the surface, rendering it hydrophobic and immediately suitable for surface chemistry.

1. Immerse crystals in a fresh piranha (3:1 mixture of 97 % H_2SO_4 and 30 % H_2O_2) for 5 min.
2. Remove and rinse liberally with pure water. The crystals are clean and ready for chemistry.
3. If the crystals are to be stored and not used immediately, subject them to either oxygen/plasma cleaning for 5 min or to UV/ozone cleaning for 20 min prior to chemistry. This will remove organic contaminants and make the surface hydrophilic.

1.2 Method 2: Sonication and Plasma Cleaning

If the crystal is more or less clean, suitable cleaning may be achieved with the following method.

1. Sonicate the crystal in acetone for 10 min at room temperature.
2. Rinse liberally with water,

3. Dry crystal surfaces under a stream of dry N_2.
4. Subject crystals to either oxygen/plasma cleaning for 5 min or to UV/ozone cleaning for 20 min. This will remove organic contaminants and make the surface hydrophilic.

Acetone is used because it is a very polar solvent which has the ability to dissolve dissolves most organic compounds. It is additionally easily miscible with water, so it washes away easily.

2 Preparation of Buffers

The following buffers are used in oligo attaching procedure.

DBFR 1 M potassium phosphate (PBS) buffer, pH 3.8. This buffer has a high salt concentration which accelerates the binding of thiol to gold.

MCH 1 mM 6-Mercapto-1-hexanol. Dilute with water. This ostensibly causes the oligos to stand "straight up".

HBFR 1 M NaCl with 10 mM Tris buffer, pH 7.4 and 1 mM EDTA. This is the hybridization buffer, not sure what it does.

The pH of both DBFR and HBFR must be corrected after preparation.

3 Attaching Oligos to a Gold Surface

The oligo used to attach to the gold surface was 5'-ThioMC6-TTT TTT TTT CAC TAA AGT TCT TAC CCA TCG CCC-3' from IDT.

1. Using a crystal whose surface is immediately hydrophobic from one of the two cleaning procedures above, immerse each surface in a 1 µM solution of oligos in DBFR and wait for 1 h.
2. Gently rinse the surface of unattached oligos with water and immerse in 1 mM MCH for 1 h.
3. Rinse surfaces again and hybridize in HBFR.

4 Attaching Oligos to Streptavidin Coated Particles

Complementary to the oligo which attaches to the gold, 5'-biotin-CT CAC TAT AGG GCG ATG GGT AAG AAC TTT AGT-3' was attached to 20 µm streptavidin coated polystyrene particles using the following procedure:

1. Aliquot 100 µL of streptavidin coated particles into a microcentrifuge tube.
2. Wash two times in a 100 µL solution of HBFR by centrifuging at 5000 RPM for 3 min and decanting the supernatant.
3. Resuspend particles in 20 µL of HBFR and add 10 µg oligo.
4. Incubate for 15 min at room temperature on a vortexer.
5. Wash two times again in a 100 µL solution of HBFR by centrifuging at 5000 RPM for 3 min and decanting the supernatant.
6. Resuspend in 100 µL HBFR.

5 Attaching the Modified Particles to the Modified Surface

Attaching the modified particles to the surface is straightforward. Carefully inject whatever amount of particles you desire on the surface of the crystal in HBFR and wait about 15 min for the oligos to bind to each other. The longer one waits the more oligos attach and the stronger the particles are bound to the surface.

6 Releasing the Beads

Inject the release strand and away we go.

7 List of Oligos

name	description	sequence
Goldlink	attach to gold surface	5'-ThioMC6-TTT TTT TTT CAC TAA AGT TCT TAC CCA TCG CCC-3'
Goldlink	attach to gold surface	5'-ThioMC6-TTT TTT TTT CAC TAA AGT TCT TAC CCA TCG CCC-3'-Alexa FluorTM 488 dyes (Ex. 492 and Em. 517)
Beadlink	attach to streptavidin coated PS beads	5'-biotin-CT CAC TAT AGG GCG ATG GGT AAG AAC TTT AGT-3'
Release	release beads by toehold exchange	5'-CACTAAAGTTCTTACC CATCGC CCTATAGTGAGTCGTATTAAT-3'

8 Equipment

item	manufacturer	part number
UV/ozone cleaner	Bioforce Nanosciences	UV/Ozone ProCleaner
5 MHz QCM crystal, 1 in, Gold, Cr polished.	Stanford Research Systems	