Protocol for the Arrangement of F-Actins *Tracks* on Au Surface:

1. Stamp ODT on gold surface and backfill the gap with cysteamine. Stamp size is 10/20 μm (10 μm for stamped molecules, 20 μm for backfilled molecules).

2. Build the flow cell.

3. Rinse with deionized (dI) H_2O, 200 μl.

4. Flow in NHS-biotin (0.1 mM in DMSO/H_2O), 200 μl, incubate for 20 min.

5. Wash with dI H_2O, 200 μl.

6. Flow in streptavidin (1 mg/ml), 2 × 100 μl, incubate for 15 min.

7. Wash with dI H_2O, 200 μl.

8. Flow in the complex of biotin-gelsolin and labeled F-actin slowly and continuously, 100 μl, followed with rinsing with the motility buffer.

9. Observe the binding behavior of actin on the gold surface under fluorescence microscope.

Protocol for Alignment of F-actins on Coverslip Surfaces:

1. Modified the glass surface between Au microelectrodes with APTES by immersing coverslip into APTES solution under absolutely dry atmosphere.

2. Build microelectrodes as shown in Scheme 3A.

3. Flow in NHS-biotin solution in DMSO/H_2O at 0.1 mM, incubate for 15 min.

4. Wash with dI H_2O.

5. Flow in streptavidin at 1mg/ml in d H_2O, incubate for 10 ~ 15 min.

6. Wash with dI H_2O.

7. Flow in the labeled biotin-gelsolin-actin complex slowly and continuously, and incubate for 2 min.

8. Check binding behavior under fluorescence microscope with and without electric field.

Quantification of Biotin per Gelsolin

Procedures for Biotinylation of Gelsolin:

1. Dissolve gelsolin in M buffer and get the concentration at 0.1 mg/ml, which is 1.10×10^{-6} M.

   mole gelsolin/ml = (0.1/91,000 MW) = 1.10×10^{-6} M.

2. Mix NHS-biotin/DMSO/M buffer with gelsolin solution with a concentration ratio at 9:1 and incubate for 2 hours at room temperature.

3. Dialyze the reaction mixture in cold room for 24 hours and separate the biotinylated gelsolin from the mixture. The membrane size is 10,000 MW.

4. Result: biotinylation yield at 23.03 biotin/gelsolin.

Procedures to Quantify Biotin per Gelsolin:

1. Absorbance factor at 500 nm in UV spectroscopy:

   Reference: 0.9619; Sample: 0.8662

2. Calculation:

a) $\Delta A_{500} = 0.9 (0.9619 - 0.8662) = 0.08613$

[0.9 = dilution factor of NHS-biotin with sample]

b) μmole biotin/ml = $(\Delta A_{500}/34)(10) = 0.0253$

[34 = mM extinction coefficient at 500 nm, 10 = dilution factor of sample into cuvette]

c) μmole gelsolin/ml = 1.10 μM X $10^{-3} = 0.0011$

d) mole biotin/mole gelsolin = (μmole biotin/ml sample)/(μmole gelsolin/ml sample)

=(0.0253)/(0.0011) = 23.03