Detection of Actin Polymerization with Pyrene Actin

Materials

1. Polymerization buffer: 6 mM $MgCl_2$, 300 mM KCl, 3 mM ATP, 75 mM imidazole, pH 7.0. This may be tailored for the specific purpose of experiments. Need 500 µl per sample.

2. Microcuvette for spectrofluorimeter (600 µl capacity).

3. Stopwatch.

Procedure

1. Dialyze pyrene actin and unlabeled actin for 2-3 days with 2-3 exchanges of buffer 1.

2. Centrifuge the actins in a 42.2Ti rotor at 35,000 rpm, 4^oC for 2 hr. Collect only the upper portion of the supernatant if the detection of nucleation phase is important.

3. Warm up the spectrofluorimeter. Set the excitation wavelength at 365 nm and emission wavelength at 407 nm.

4. Perform Lowry assay for the concentrations of pyrene actin and unlabeled actin.

5. Mix pyrene actin with unlabeled actin at 1:20 molar ratio. Dilute with buffer 1, which may contain factors to be tested, to obtain a net actin concentration of 0.1 mg/ml. Each sample should be at least 300 ul in volume.

6. At time 0, add 1/2 volume of polymerization buffer, mix by short pulses of vortexing, and load immediately into the cuvette. Record fluorescence intensity as a function of time. For steady state readings, the samples are incubated for 15 hr at room temperature.
