Purification and Pyrene Labeling of Actin

Actin was purified as follows: actin powder (average 5-6 g) was washed with 20 ml/g actin ice cold G-buffer (2 mM Tris pH 7.7, 0.2 mM $CaCl_2$, 0.2 mM ATP, 2 mM DTT) and was stirred on ice for 40 min. After stirring actin was filtered using filter paper. 2 mM $MgCl_2$ and 50 mM KCl were added to the filtered solution and was incubated at room temperature for 30 min. After incubation solution was stirred on ice and KCl was added to the final concentration 0.5 M and was stirred for more 60 min. Solution was then ultracentrifuged (40,000 rpm, 90 min, 4°C) and pellet was homogenized in G-buffer and was dialyzed against G-buffer 2 rounds. After dialysis solution was ultracentrifuged (40,000 rpm, 90 min, 4°C). The supernatant is the clear G-actin which can be dialyzed against F-buffer to obtain F-actin.

Pyrene labeling of actin was carried out as follows: actin was dialyzed against DTT free F-buffer (4 mM HEPES pH 7.1, 2 mM $MgCl_2$, 3 mM NaN_3) to 1 mg/ml concentration. PIA was solved in DMSO and was added to F-actin in 10-fold molar excess. It was vortexed immediately for 30 s and was incubated on a swinging table at room temperature overnight, wrapped into foil. After incubation it was centrifuged briefly in a Janetzky K-23D centrifuge (1000 rpm, 2 min, 4°C). The supernatant was then diluted with F-buffer containing 1 mM DTT to 0.5 mg/ml concentration and was ultracentrifuged (40,000 rpm, 90 min, 4°C). Pellet was homogenized in G-buffer (2 mM Tris pH 7.7, 0.1 mM $CaCl_2$, 0.5 mM ATP, 2.5 mM DTT) and dialyzed against G-buffer 2 rounds (wrapped into foil). After dialysis it was ultracentrifuged (40,000 rpm, 90 min, 4°C) and 2 mM $MgCl_2$ and 100 mM KCl were added to the supernatant. After 30 min incubation on room temperature it was ultracentrifuged (40,000 rpm, 90 min, 4°C) and pellet was resuspended in F-buffer with 1 mM DTT and was dialysed against F-buffer with 1 mM DTT 2 rounds (both overnight). After measuring the concentration and the labeling ratio equimolar phalloidin was added to stabilize pyrene-labeled F-actin.