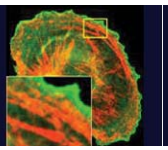
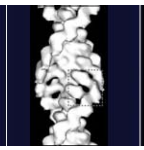
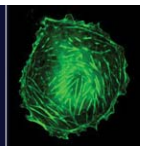

Myosin-I Motility Method – For use with biotinylated myosin-I isoforms.
Adapted from, Lin and Ostap (2005) *J. Biol. Chem.* 280:41562-41567.

1. Prepare nitrocellulose-coated coverslip chamber
2. Flow in 50 µL 0.1 mg/ml Streptavidin, incubate 1 min
3. Flow in 50 µL 1.0 mg/ml BSA, 2 x 1 min
4. Flow 50 µL 250 nM Myo1C (diluted in 1x MB), 1 x 2 min
5. Wash with 50 µL 1x MB that included 5 mM Mg-ATP
6. Wash with 50 µL 1x MB, 3 times
7. Flow 50 µL 40 nM Rhodamine Phalloidin Labeled Actin
8. Add 1ul 100x-GOC to 99 µL Activation Buffer,
9. Flow in 50 ul Activation Buffer, check the motility immediately at 37 °C.

5x MB
50 mM Mops, pH 7.0
125 mM KCl
5 mM EGTA
5 mM $MgCl_2$
- When making 1x MB, add DTT to 1 mM

100X GOC
20 mg/ml Glucose oxidase
(G-7016 Sigma, 50,000 U)
4 mg/ml Catalase
(Roche)

Activation Buffer
1x MB
5 mM MgATP
50 mM DTT
5 mg/mL Glucose
10 µM Calmodulin
0.5 % Methyl Cellulose

Nitrocellulose-Coated Coverslip
Coat the surface of microscope cover glass (Cat#12-544-B) with 20 ul nitrocellulose (Superclean from ERNEST F. FULLAM, INC., Cat# 11180). Dry for > 20min before using.

Notes:
- Make sure calmodulin is included in the activation buffer
- We do not have to do a “black-actin” blocking step.
- The 0.1 mg/ mL streptavidin concentration is much higher than required. This can be decreased substantially, if necessary.