

Surface initiated actin polymerization from top-down manufactured nanopatterns

Branden Brough,^{†a,b,e} Karen L. Christman,^{†a,c,d} Tak Sing Wong,^{a,b} Christopher M. Kolodziej,^{a,c,d} Jeffrey G. Forbes,^e Kuan Wang,^{*e} Heather D. Maynard^{*a,c,d} and Chih-Ming Ho^{*a,b}

^a Center for Scalable and Integrated NanoManufacturing, University of California, Los Angeles, CA, USA.

^b Department of Mechanical and Aerospace Engineering, University of California, Los Angeles, School of Engineering and Applied Science, Engineering IV, Room 38-137J, 420 Westwood Plaza, Los Angeles, CA 90095-1597, USA. Tel: +1 310 825 9993; E-mail: chihming@ucla.edu

^c Department of Chemistry and Biochemistry, 607 Charles E. Young Dr. East, University of California, Los Angeles, CA 90095-1569, USA. Tel: +1 310 267 5162; E-mail: maynard@chem.ucla.edu

^d California NanoSystems Institute, University of California, Los Angeles, CA, USA

^e Muscle Proteomics and Nanotechnology Section, Lab of Muscle Biology B50, Rm1140, NLM/NIH, Bethesda, MD 20892, USA. Tel: +1 301 496 4097; E-mail: wangk@exchange.nih.gov

† These authors contributed equally to this work.

SUPPORTING INFORMATION

X-ray photoelectron spectroscopy

XPS analysis was performed by the UCSB Materials Research Laboratory on a Kratos Axis Ultra using monochromated Al k-alpha x-radiation. Survey scans were performed at a pass energy of 160 eV with a 0.5 eV channel width, while high-resolution scans were performed at 20 eV pass energy with a 0.05 eV channel width. Charge compensation was achieved using low-energy electrons from a filament, and peak positions were calibrated by adjusting aliphatic carbon to 284.8 eV.

Atomic Force Microscopy

AFM images were acquired on air dried samples with a multimode Nanoscope IIIA system (Digital Instruments) operated in tapping mode using a rotated monolithic silicon probe with a nominal spring constant of 40 N/m and a tip radius <10 nm (model BS-Tap300, Nanoscience Instruments, Inc.). Images were acquired as 512 x 512 pixels with a scan rate of 2.00 Hz.

FX-45 biotinylation

FX-45 was dialyzed against 50 mM KCl, 20 mM NaPi at pH 7.0 overnight at 4°C, follow by a centrifugation at 65,000 g for 20 minutes. Protein concentration was measured spectrophotometrically using FX45 extinction coefficient $\text{Abs}_{280}, 0.1\% = 1.201$. Biotinylation was accomplished by mixing 0.95 mg of FX45 in 50 mM KCl, 20 mM NaPi at pH 7.0 with 16 μl of Sulfo-NHS-Biotin (Pierce) (0.75mg/ml in water) and incubating at room temperature. The coupling reaction was monitored and 1 ml aliquots at time points 0, 10, 20, 40 and 80 min were taken and quenched with 10 μl of 1M Ethanolamine-HCl at pH 8.5. Excess reagents were removed by gel filtration using HiTrap Desalting columns in 50 mM KCl, 20 mM NaPi at pH 7.0 (Amersham Biosciences). An average of 2-3 biotin groups per FX45 were incorporated as determined by MALDI mass spectrometry.