Supporting Information for:

Epithelial-Mesenchymal Transition Enhances Nano-scale Actin Filament Dynamics of Ovarian Cancer Cells

Sunyoung Lee[1†], Yang Yang[2†], David Fishman[3], Mark M. Banaszak Holl[4], and Seungpyo Hong[*2]*

[1]Elmhurst Hospital Center, Icahn School of Medicine at Mount Sinai, Elmhurst, NY 11373

[2]Department of Biopharmaceutical Sciences, University of Illinois, Chicago, IL 60612

[3]Department of Obstetrics, Gynecology, and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY 10029

[4]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

[†]Both authors contributed equally to this work.

*All correspondence should be addressed to:

Prof. Seungpyo Hong, Ph.D.
Department of Biopharmaceutical Sciences
College of Pharmacy
The University of Illinois at Chicago
833 S. Wood St. Rm 335
Chicago, IL 60612
Phone: 312-413-8294
Fax: 312-996-0098
E-mail: sphong@uic.edu

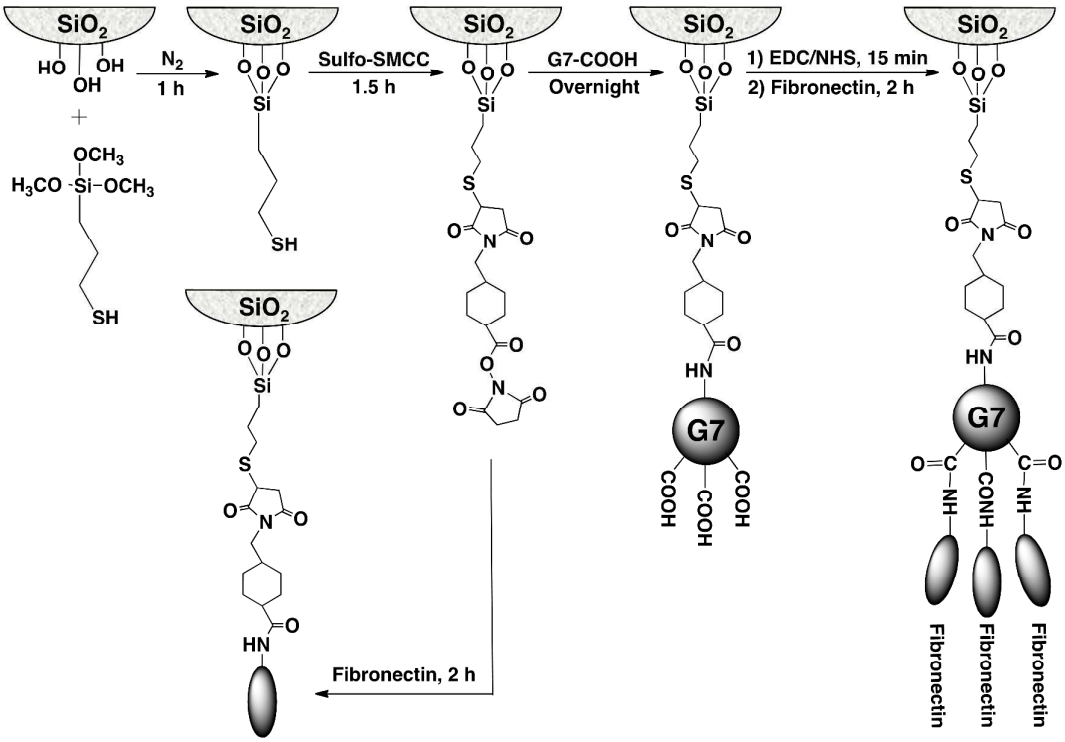

Figure S1. Schematic of AFM probe modification. Spherical silicone oxide probe cantilevers (diameter = 5 μm) were washed in absolute ethanol and treated with 3-mercaptopropyl trimethoxysilane (4% v/v in absolute ethanol) for 1 h, and rinsed with ethanol. The cantilevers were further incubated in ddH_2O containing sulfo-SMCC (2 mM) for 1.5 h. Sulfo-SMCC was replaced with G7-dendrimers overnight (0.5 mg/mL, 90% surface carboxylation), followed by incubation with EDC (100 μM) and NHS (100 μM) for 15 min to activate the surface carboxyl groups. Fibronectin (1 mg/mL) was incubated with cantilevers for 2 h. After rinsing the cantilevers with phosphate buffered saline (PBS), they were ready for imaging. AFM, atomic force microscopy.

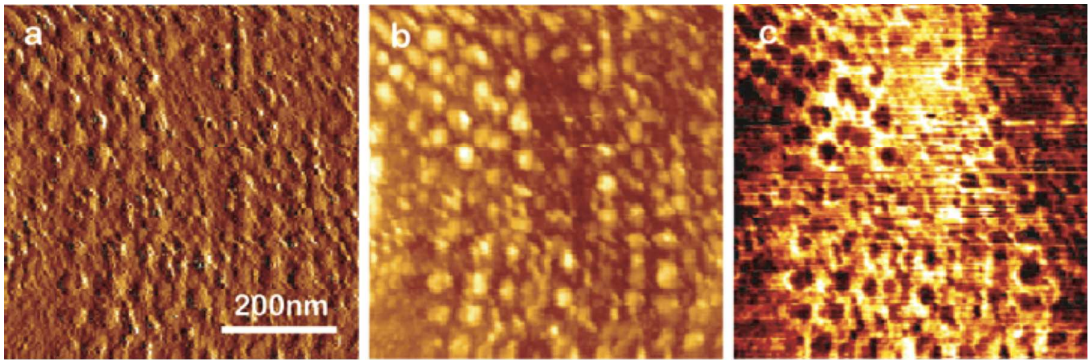

Figure S2. AFM images of fibronectin (FN) conjugated on a surface of a spherical AFM probe. An FN-conjugated spherical AFM probe surface was imaged using an anti-FN antibody-immobilized AFM tip via nano-scale dynamic recognition imaging using a method published elsewhere[1]: **(a)** An amplitude image; **(b)** A topography image; and **(c)** A recognition image. Dark spots in the recognition image represent specific interaction events between anti-FN antibody on the AFM tip and FN molecules conjugated on the probe surface. The size of image is 600 nm x 600 nm (0.36 μm^2). Scale bar: 200 nm.

Experimental Description: Direct imaging of FN conjugated on the probe surface was performed in AFM MAC mode. Anti-FN antibody was immobilized on the sharp AFM cantilever (Applied NanoStructures, Inc., Santa Clara, CA) according to literature.[2] Imaging analysis of a given probe area was performed to count the number of FN conjugated on the surface of spherical AFM surface. Dark spots in **Figure S2** counted were at least an order of magnitude darker than background threshold (n = 6).

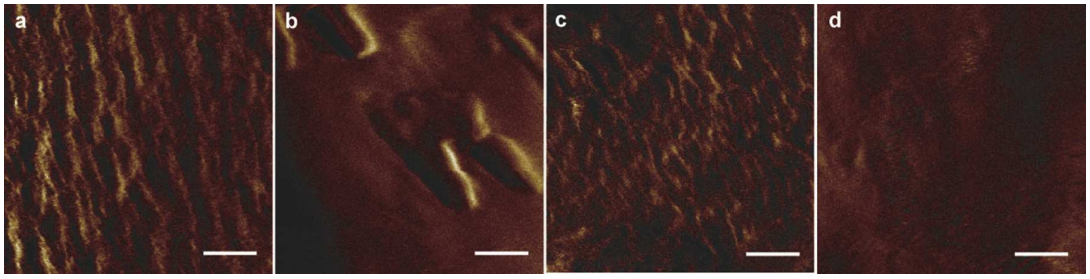

Figure S3. RMS roughness and AFM imaging of probe surfaces with ultra-sharp cantilevers in AC mode AFM. Amplitude images of a bare AFM probe surface (**a,** RMS roughness = 14.49 nm), an SMCC-FN-conjugated AFM probe surface (**b,** RMS roughness = 8.99 nm), a G7 dendrimer-conjugated AFM probe surface (**c,** RMS roughness = 11.71 nm), and G7 dendrimer-FN-conjugated AFM probe surface (**d,** RMS roughness = 5.91 nm). Scale bar = 200 nm.

Experimental Description: The AC mode AFM imaging was performed for the RMS roughness measurement of the various AFM cantilever surfaces (**Figure S3**). The unmodified, G7 dendrimer-conjugated, SMCC-FN conjugated, and G7-FN conjugated probes were placed in PBS (400 μL) and imaged in the AFM AC mode with ultra-sharp AFM cantilevers (Applied NanoStructures, Inc., Santa Clara, CA) whose resonance frequency was calibrated prior to imaging. The rate of AC mode imaging was 0.4 lines/sec (400 nm/sec) with 512 × 512 pixels.

References

1. Chtcheglova, L. A.; Waschke, J.; Wildling, L.; Drenckhahn, D.; Hinterdorfer, P. Nano-Scale Dynamic Recognition Imaging on Vascular Endothelial Cells. *Biophys. J.* **2007,** *93*, L11-L13.

2. Lee, S.; Mandic, J.; Van Vliet, K. J. Chemomechanical Mapping of Ligand-Receptor Binding Kinetics on Cells. *Proc. Natl. Acad. Sci. U.S.A.* **2007,** *104*, 9609-9614.