Embryogenesis Explained

Biochemical and mechanical regulation of extracellular matrix signaling: insights from fibronectin

Michael Smith

March 5, 2014

BOSTON UNIVERSITY

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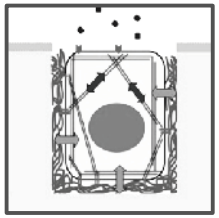
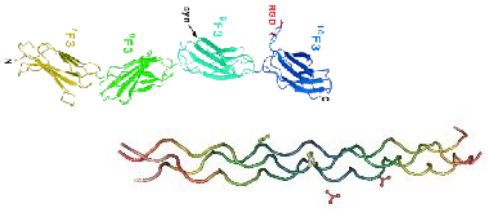
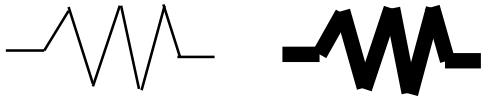


Cell sensory toolbox

biochemical



mechanical



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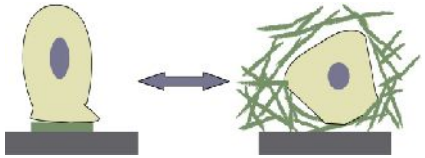

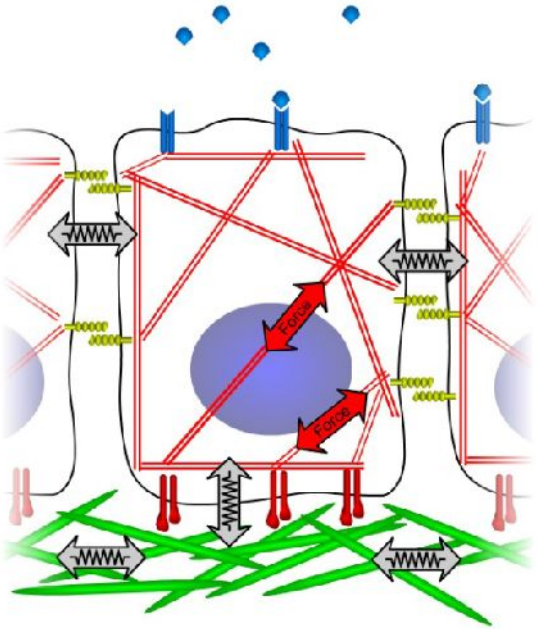

Goal: Determine how extracellular matrix fibers communicate biochemical and mechanical signals to cells

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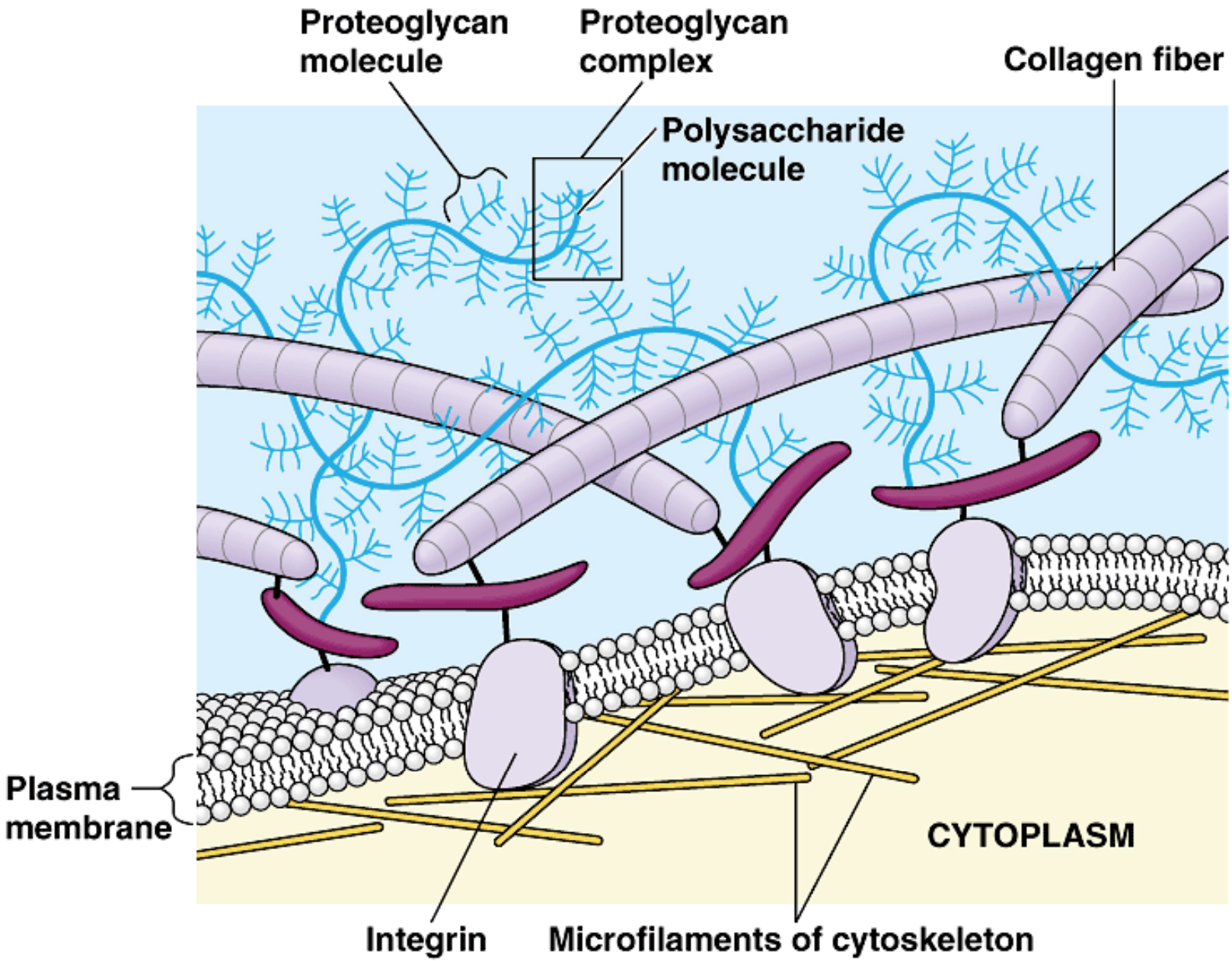

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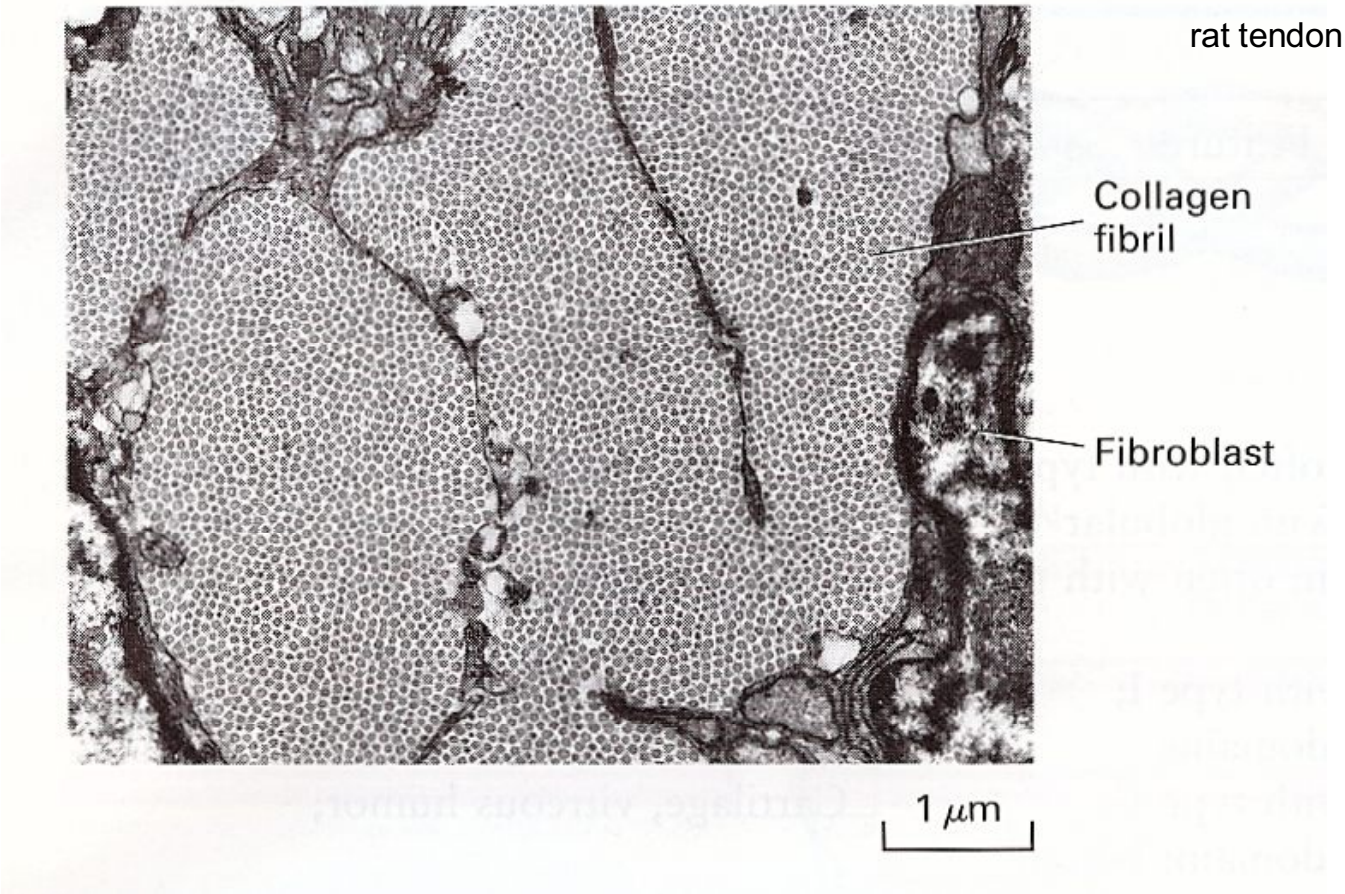

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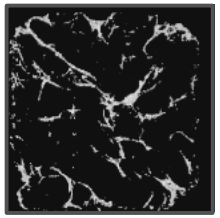
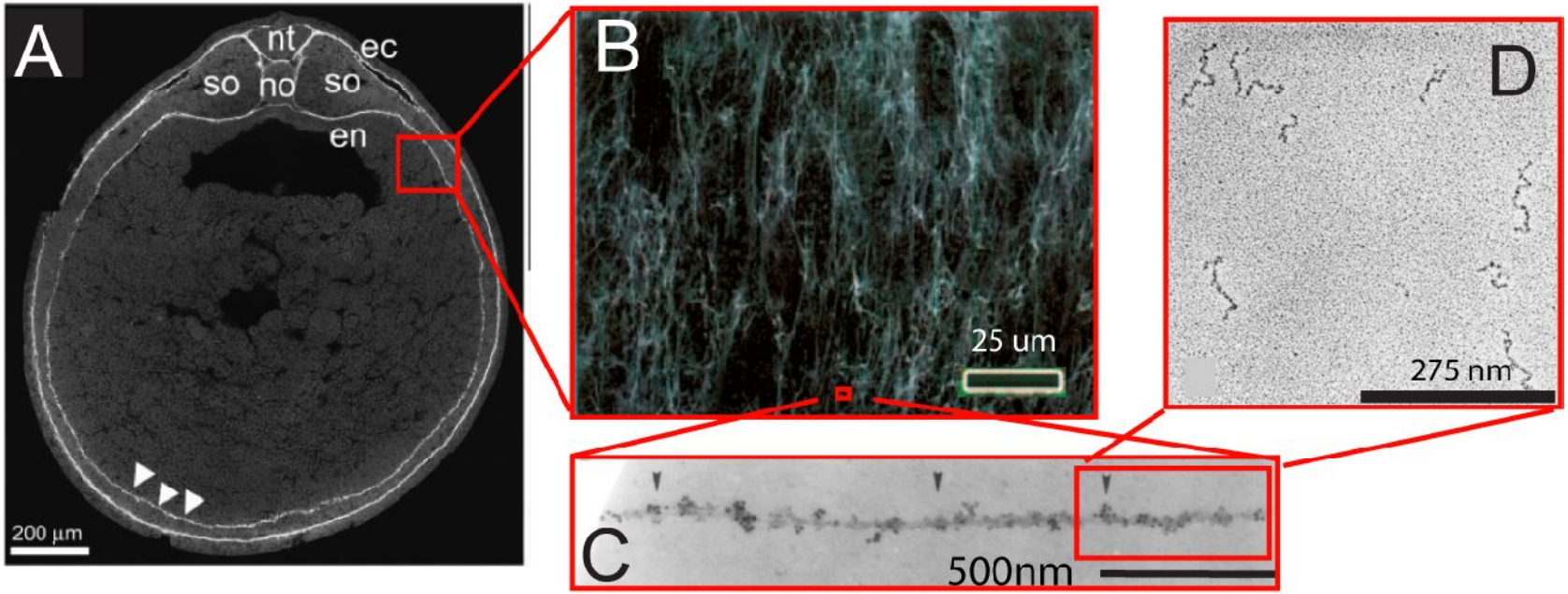


Fibronectin



Davidson, Keller, DeSimone. 2004. DevDyn. 231: 888.
Erickson, Carrell. 1983. JBiolChem. 258: 14539.
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Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin

Elizabeth L. George*, Elisabeth N. Georges-Labouesse†, Ramila S. Patel-King, Helen Rayburn and Richard O. Hynes‡

Cell and fibronectin dynamics during branching morphogenesis

Melinda Larsen, Cindy Wei and Kenneth M. Yamada*

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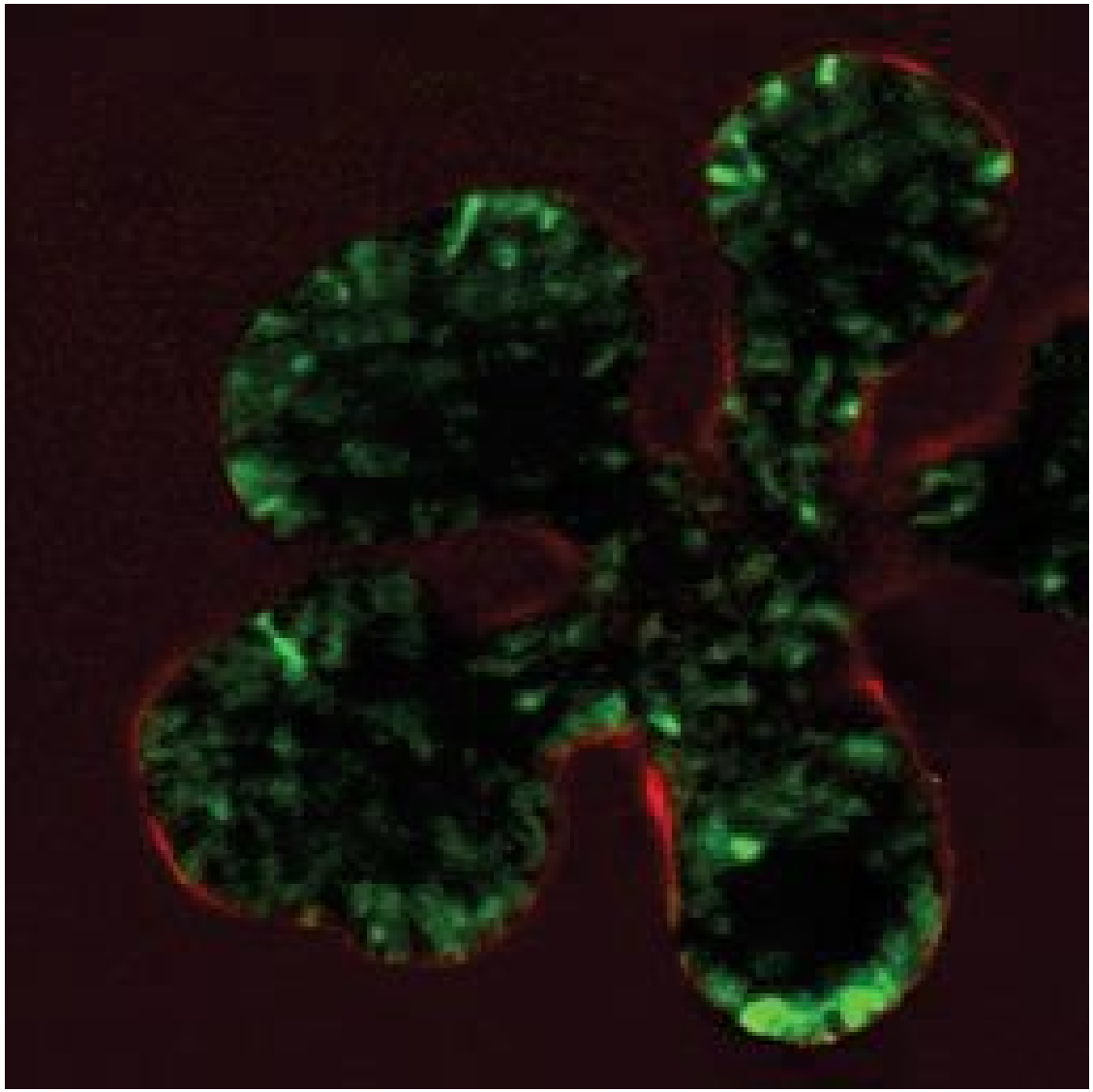

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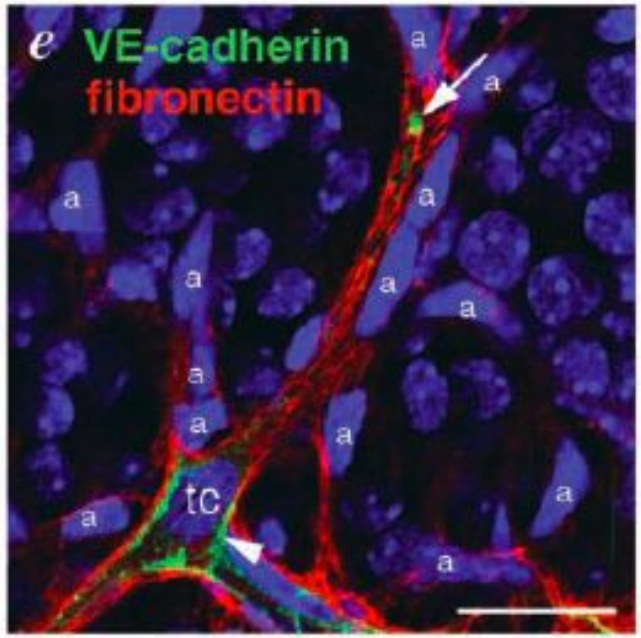
VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia

Holger Gerhardt,[1] Matthew Golding,[2] Marcus Fruttiger,[3] Christiana Ruhrberg,[2] Andrea Lundkvist,[1] Alexandra Abramsson,[1] Michael Jeltsch,[4] Christopher Mitchell,[5] Kari Alitalo,[4] David Shima,[2] and Christer Betsholtz[1]

We show here that VEGF-A controls angiogenic sprouting in the early postnatal retina by guiding filopodial extension from specialized endothelial cells situated at the tips of the vascular sprouts. The tip cells respond to VEGF-A only by guided migration; the proliferative response to VEGF-A occurs in the sprout stalks. These two cellular responses are both mediated by agonistic activity of VEGF-A on VEGF receptor 2.



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Proposed strategy to create bioartificial heart

1. Decellularize cadaveric heart

➔ create whole-heart scaffold

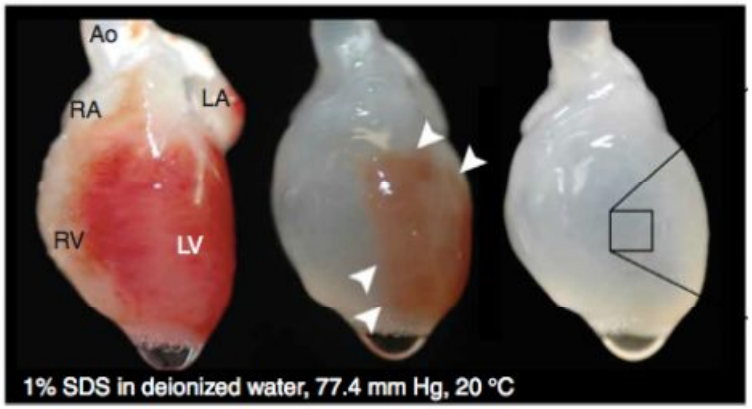
2. Repopulate decellularized heart

➔ cardiac and endothelial cells

3. Functionalize construct

➔ form contractile myocardium

1.



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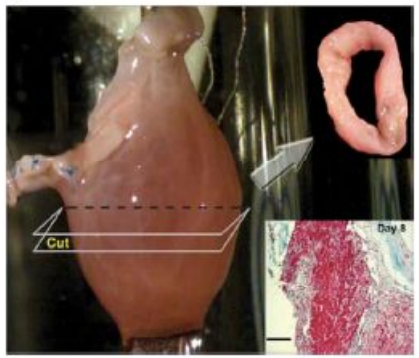

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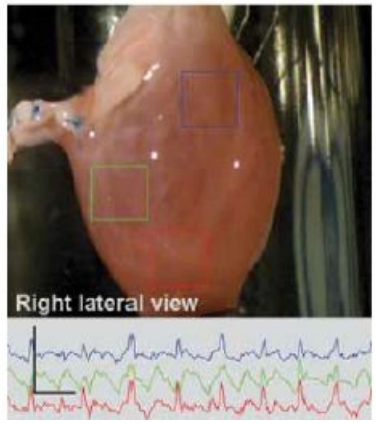

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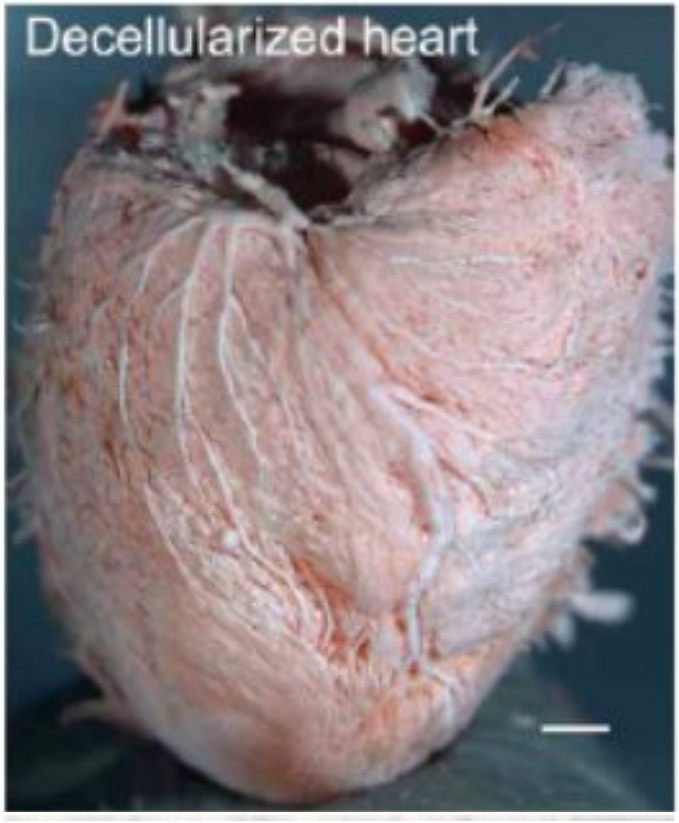

Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart

Harald C Ott[1], Thomas S Matthiesen[2], Saik-Kia Goh[2], Lauren D Black[3], Stefan M Kren[2], Theoden I Netoff[3] & Doris A Taylor[2,4]

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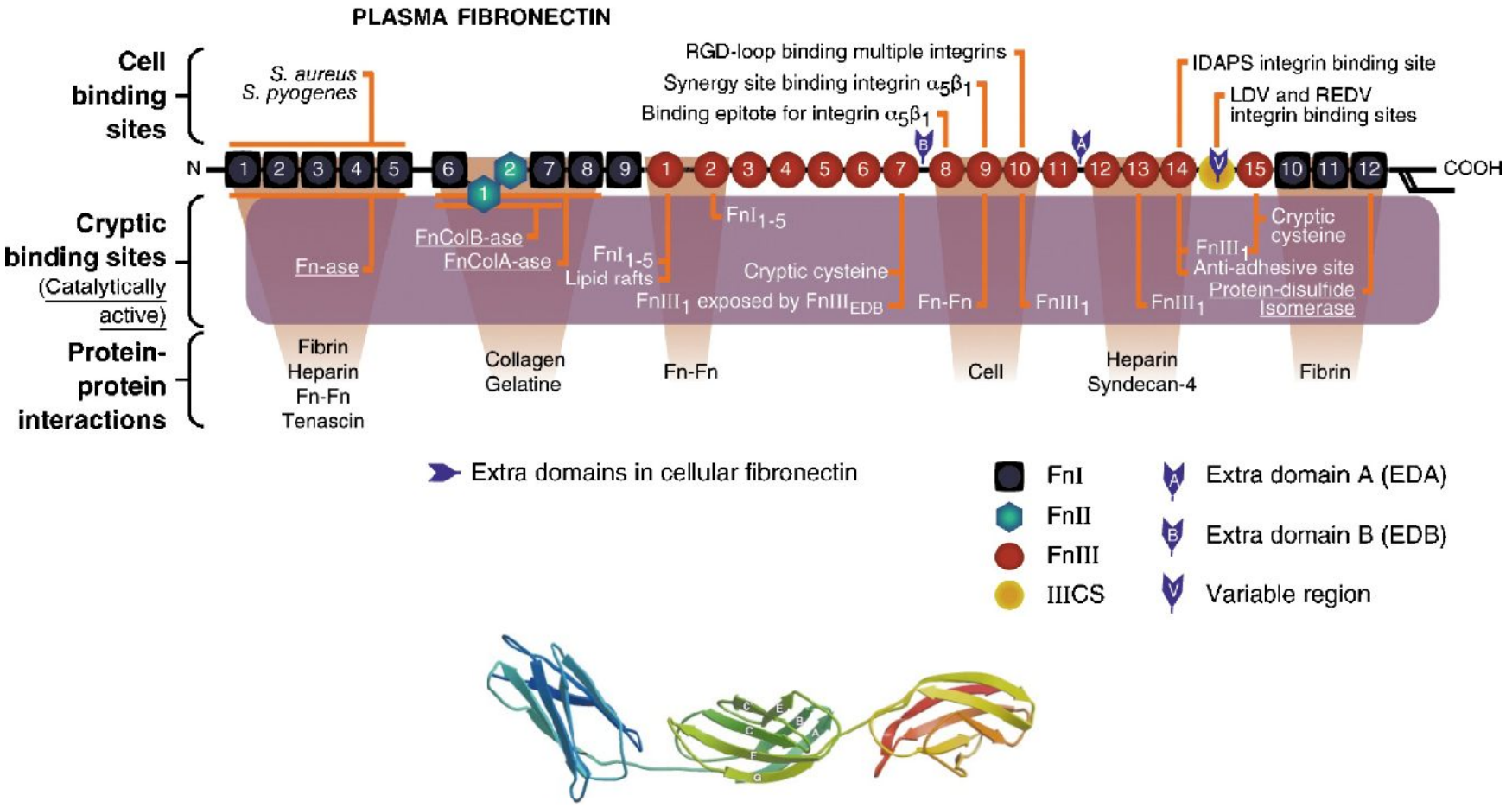

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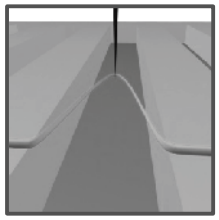

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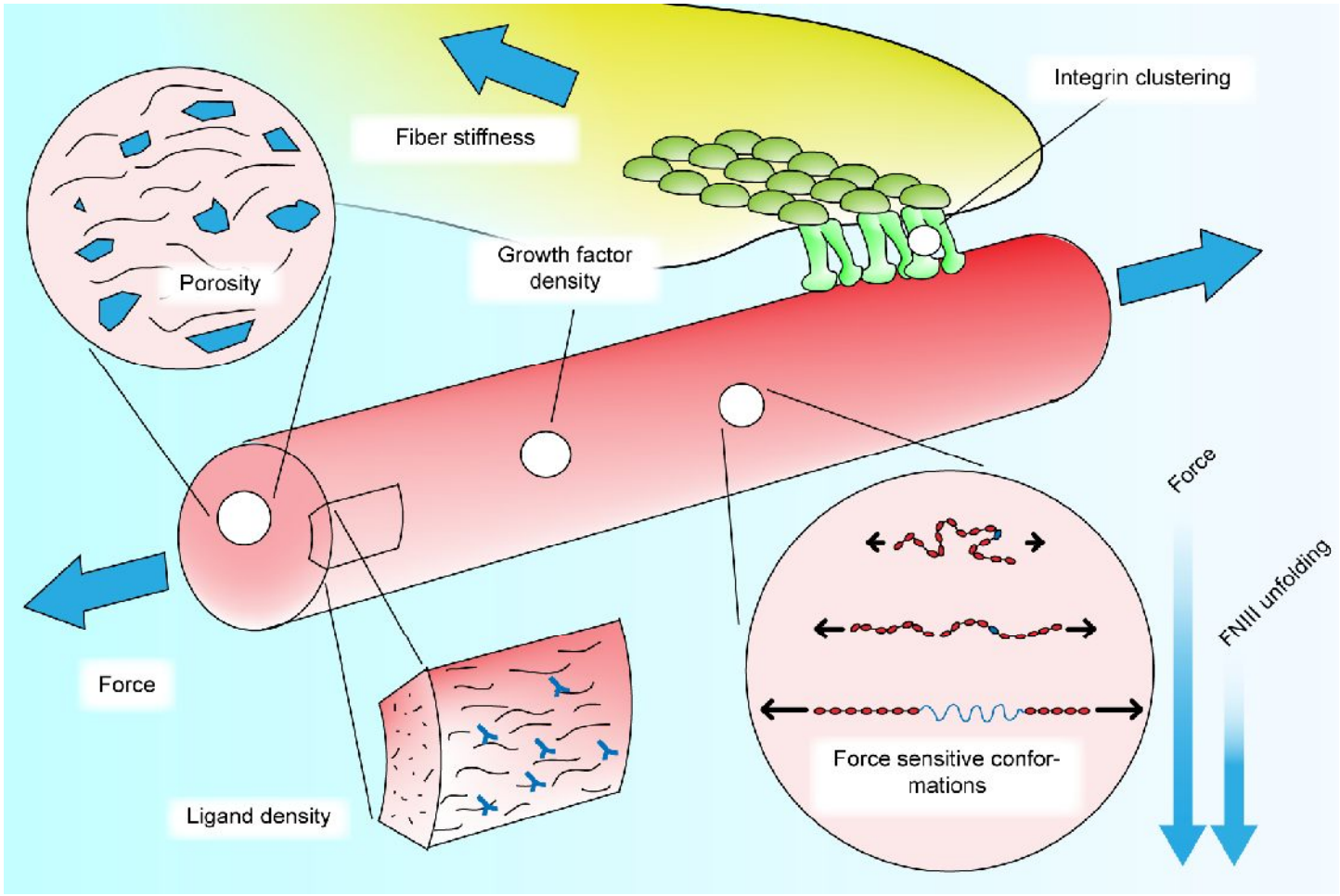

Bradshaw, Smith. 2013. ActaBiomater. In Press.

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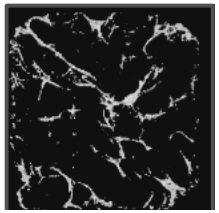

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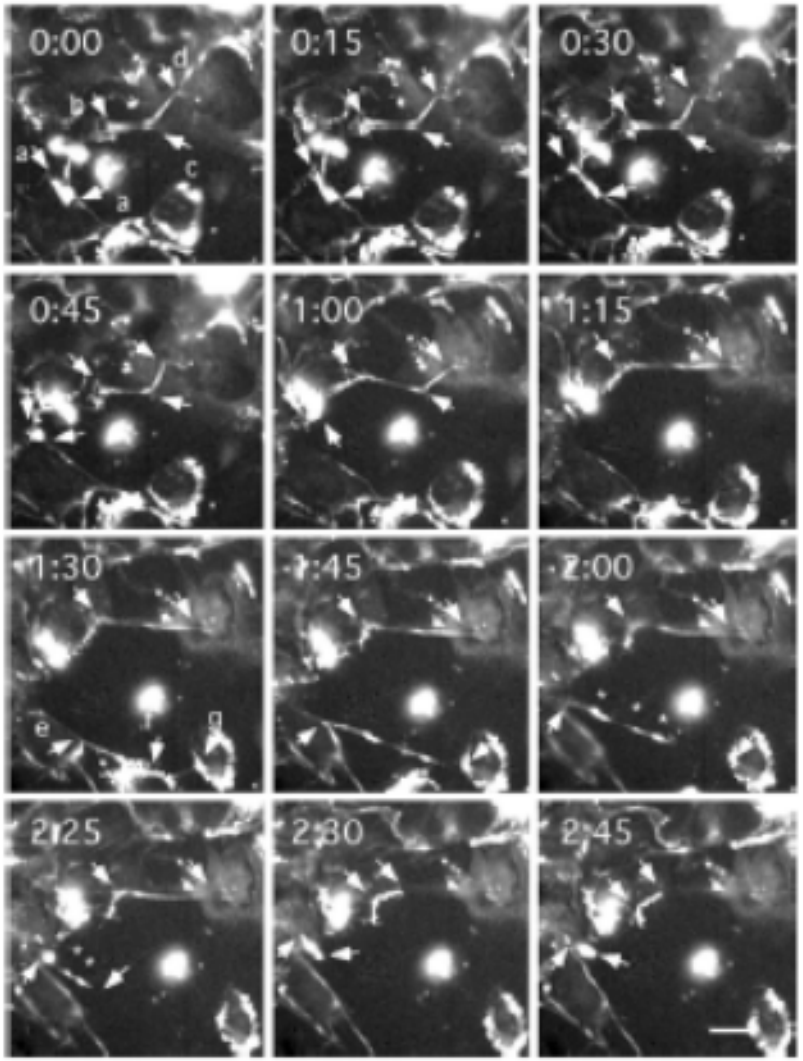

Ohashi, Kiehart, Erickson. 1999. ProcNatlAcadSci. 96: 2153.

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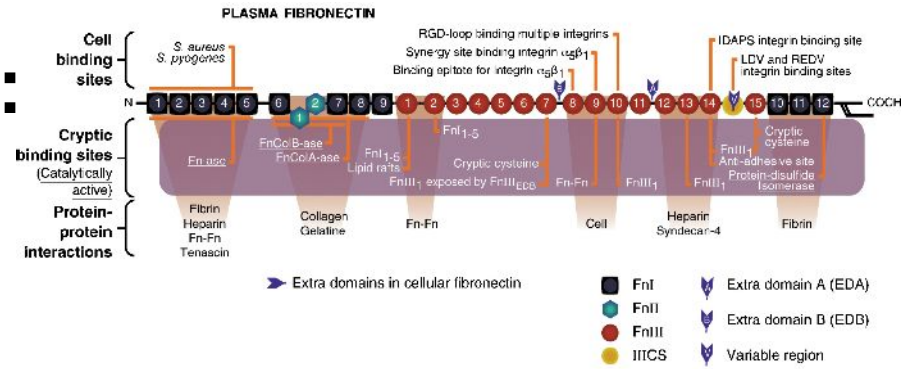

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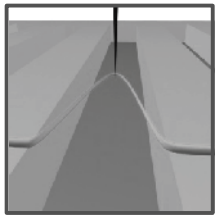
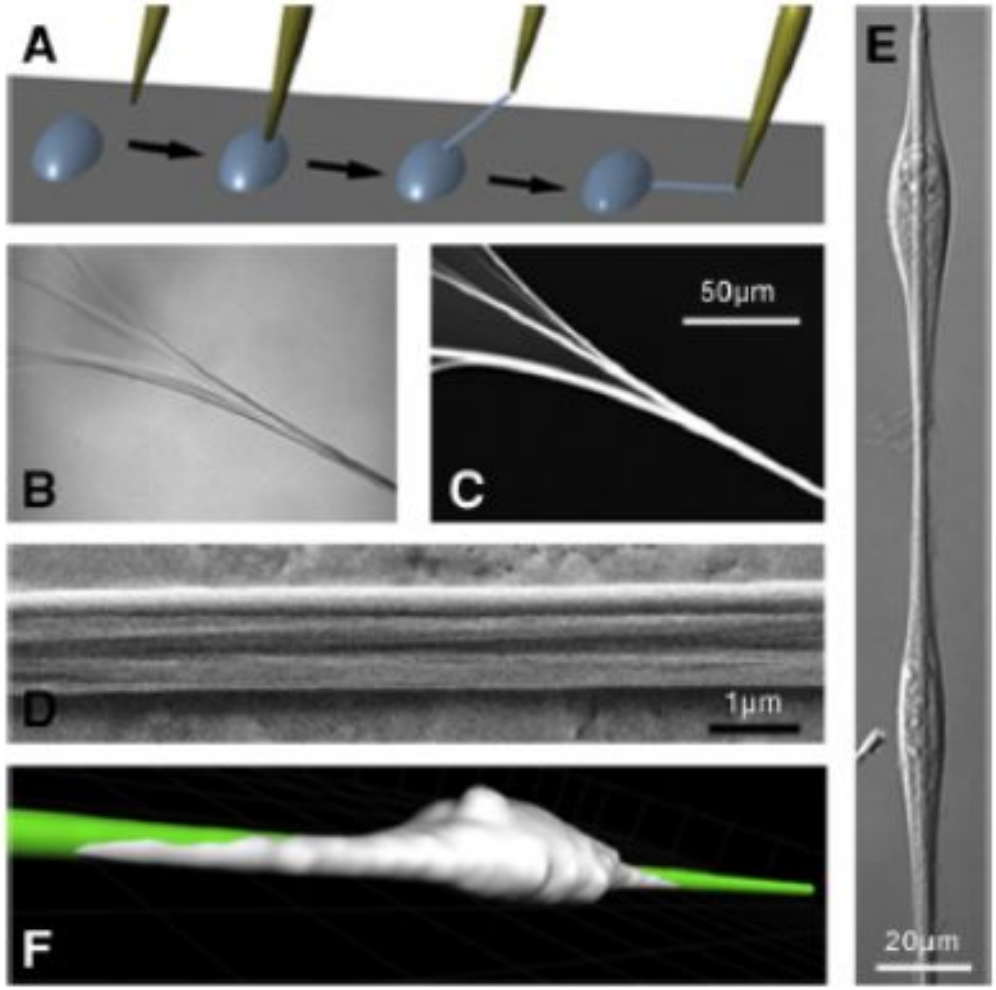


Fabricating fibronectin fibers



Ejim, Blunn, Brown. 1993. Biomaterials. 14: 743.

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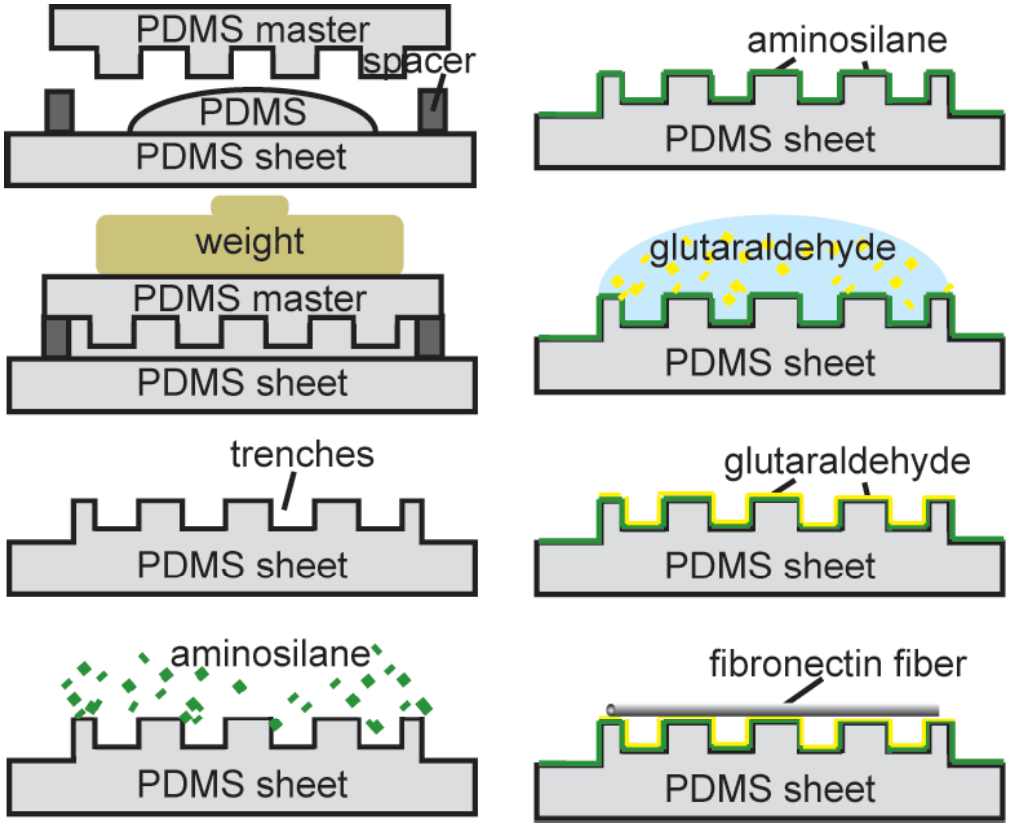
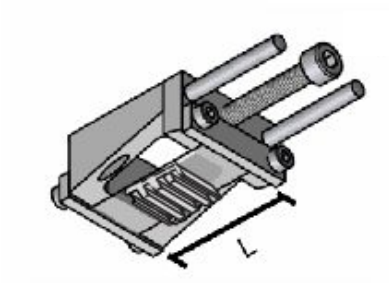
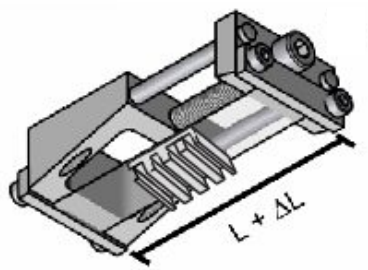
Fibronectin extensibility







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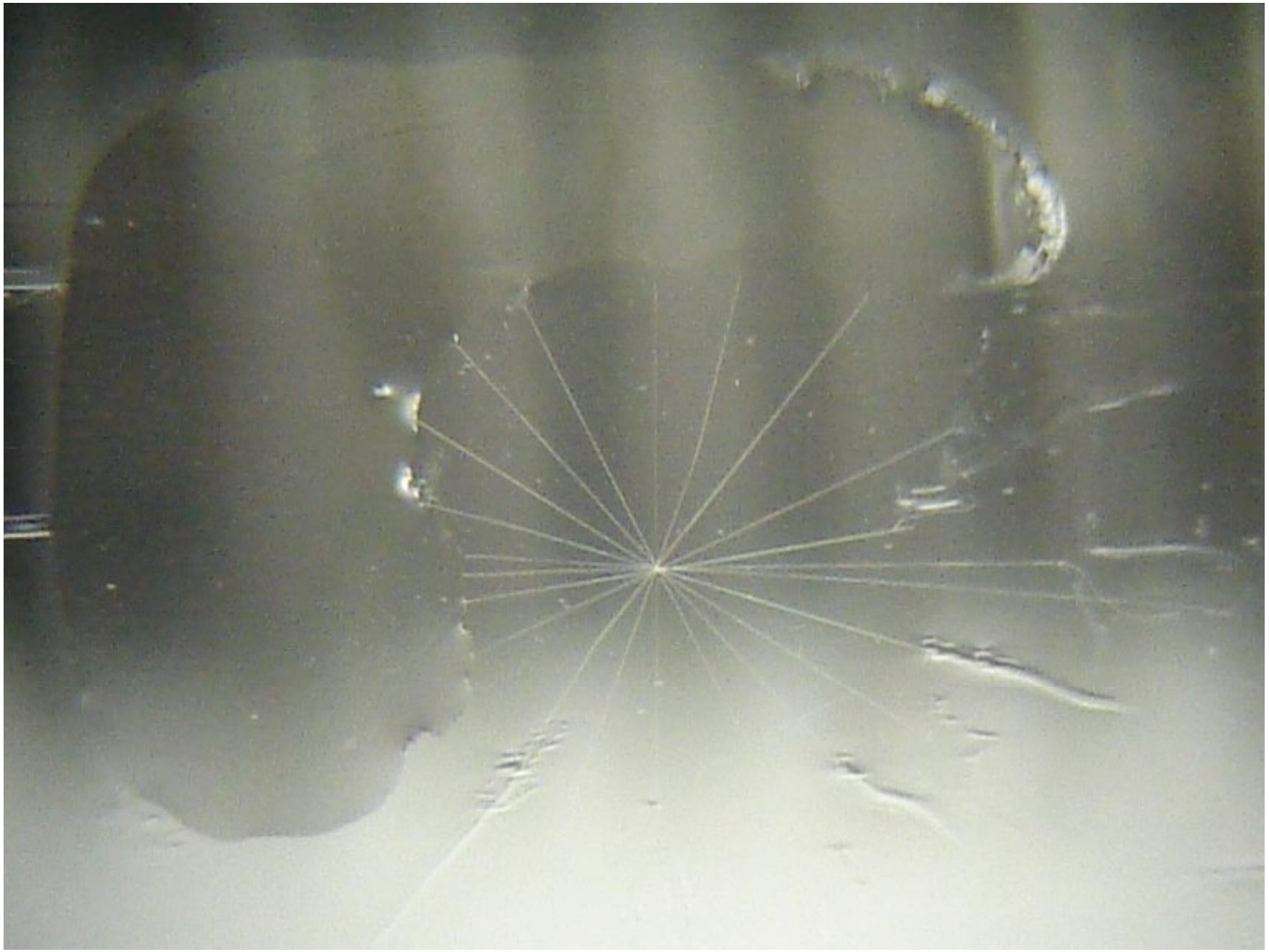

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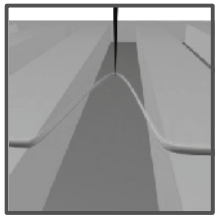

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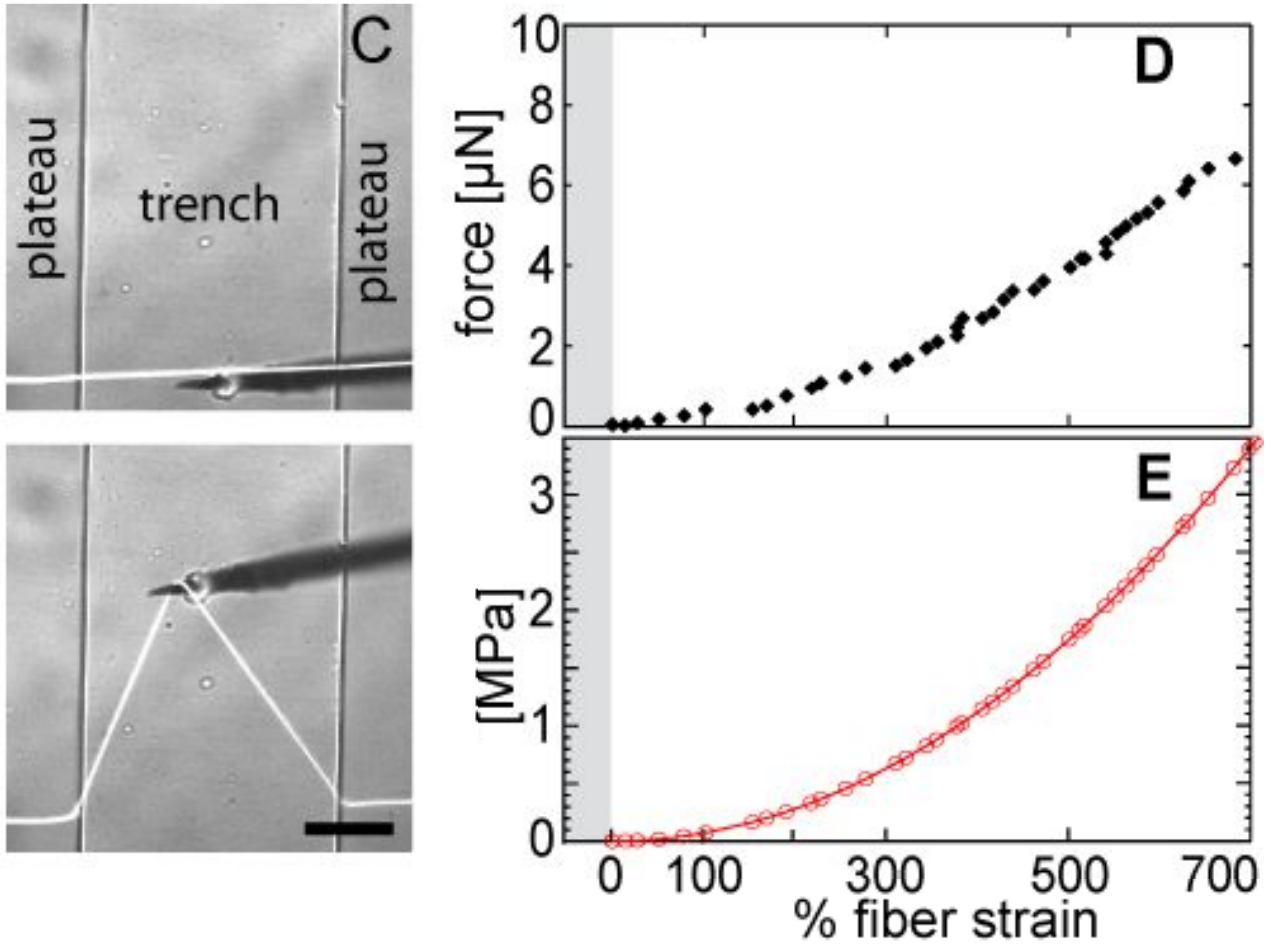

Klotzsch, Smith, Kubow, Muntwyler, Little, Beyeler, Gourdon, Nelson, Vogel. PNAS. 106: 18267.

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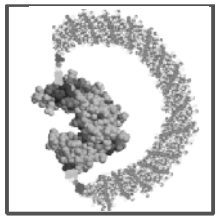
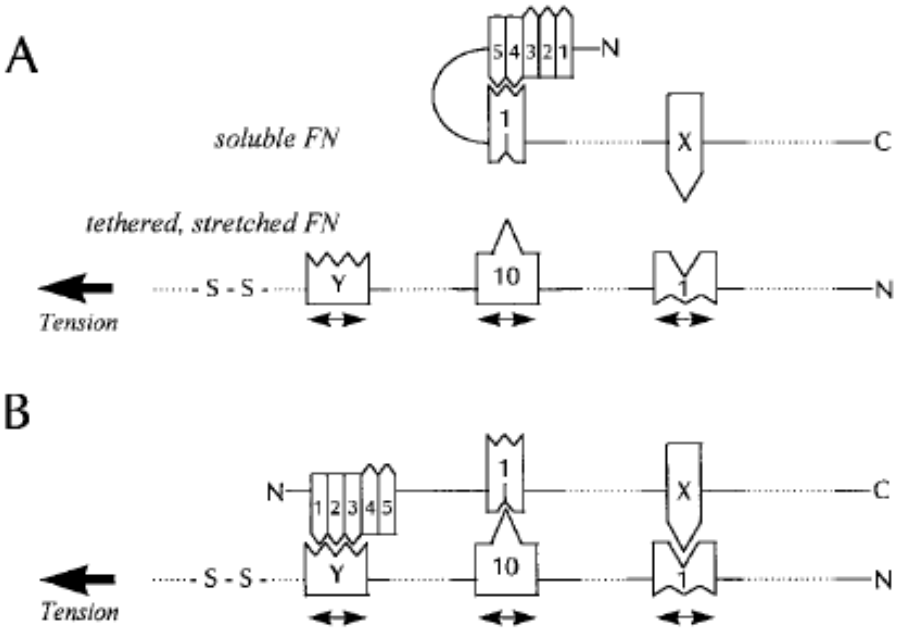


Strain-dependent biochemistry?

Rho-mediated Contractility Exposes a Cryptic Site in Fibronectin and Induces Fibronectin Matrix Assembly

Cuiling Zhong, Magdalena Chrzanowska-Wodnicka, James Brown, Amy Shaub, Alexey M. Belkin, and Keith Burridge

Department of Cell Biology and Anatomy, and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, North Carolina



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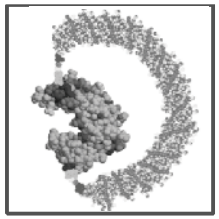
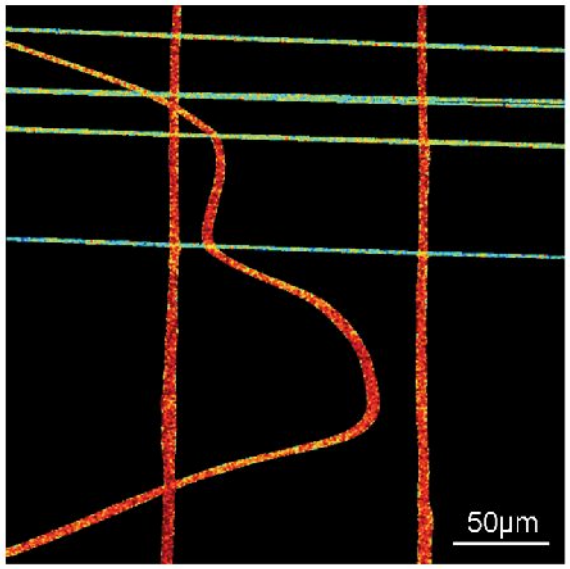


Strain-dependent biochemistry?



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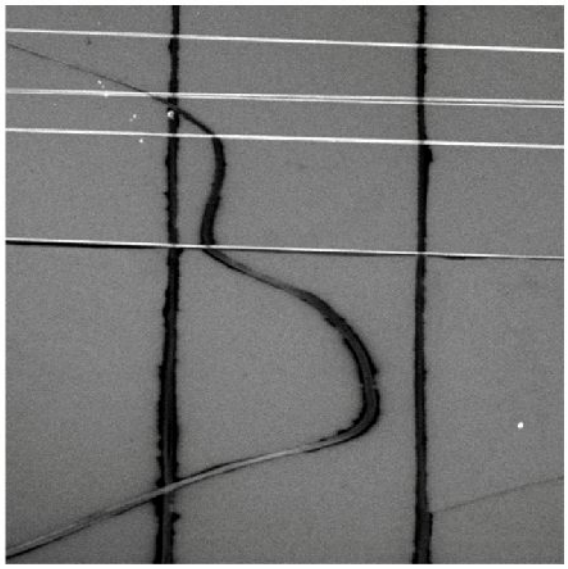

Alexa633 fibronectin adsorbed to the sample

Little, Smith, Ebneter, Vogel. 2008. MatrixBiol. 27: 451.

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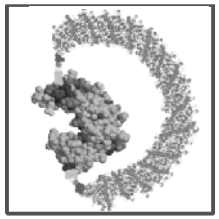
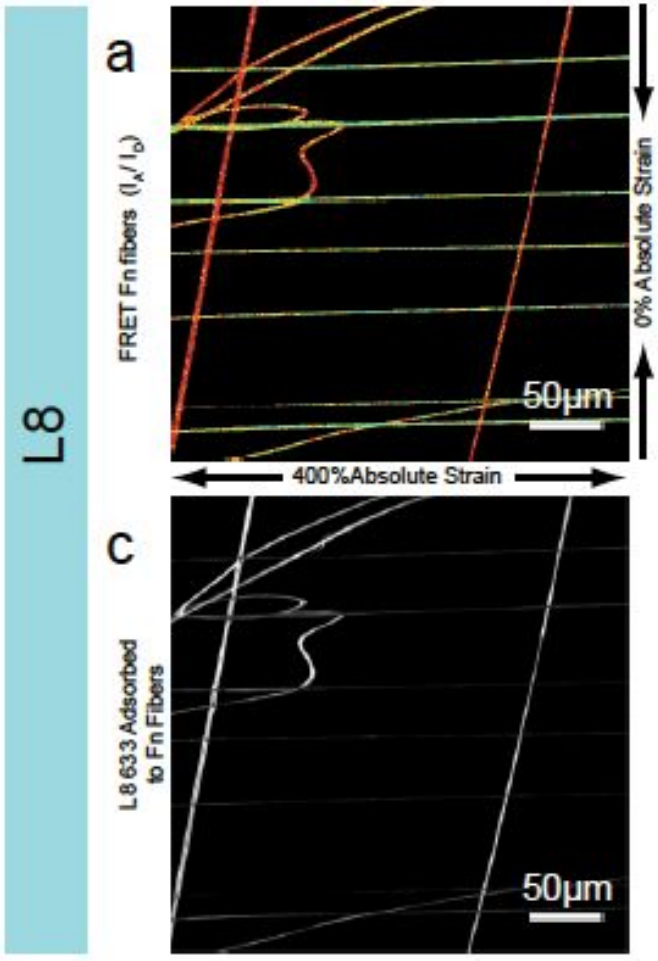


Strain-dependent biochemistry?



Little, Schwaertlander, Smith, Gourdon, Vogel. 2009. NanoLett. 9(12): 4158-4167.

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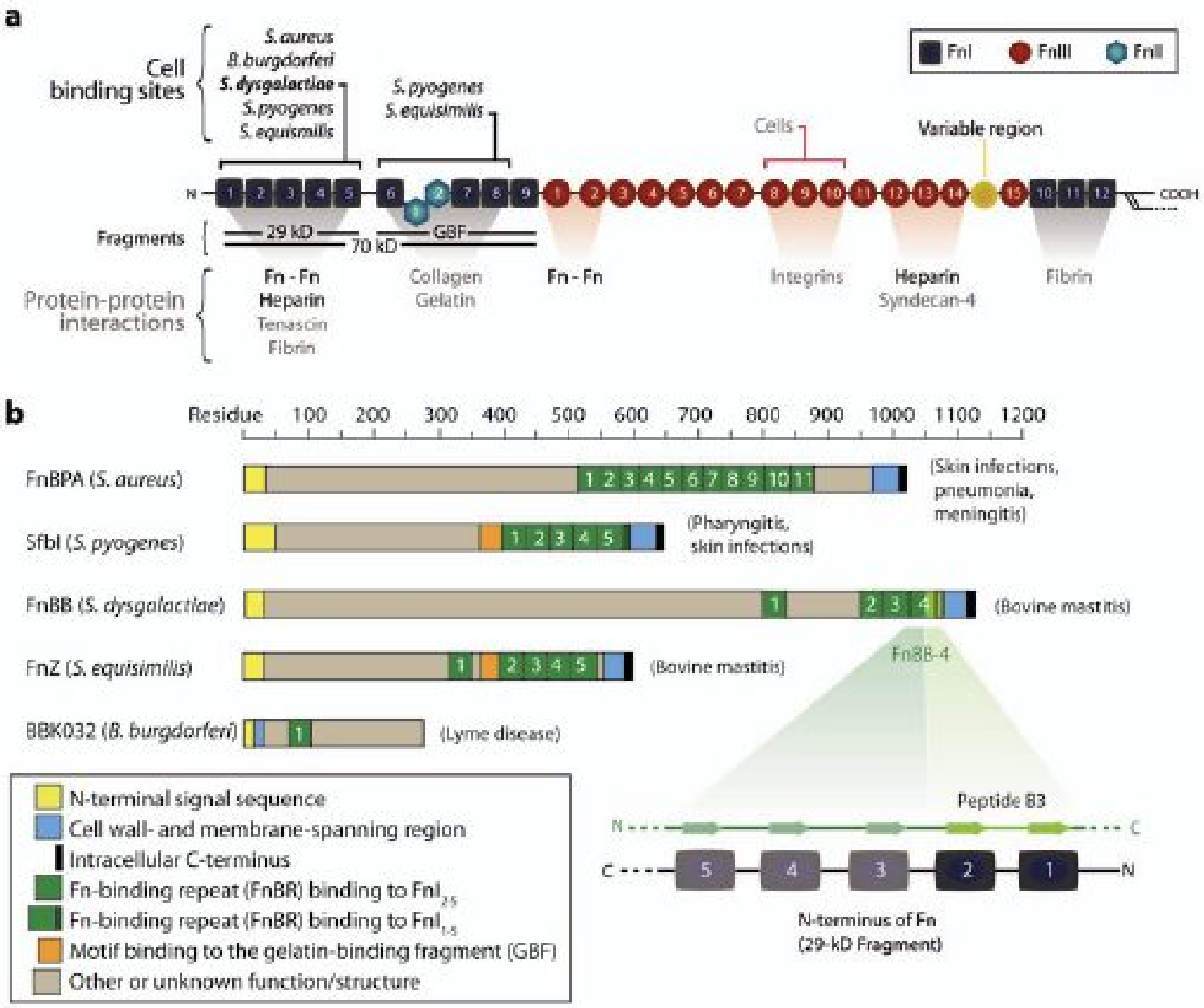

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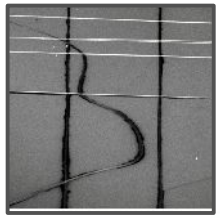
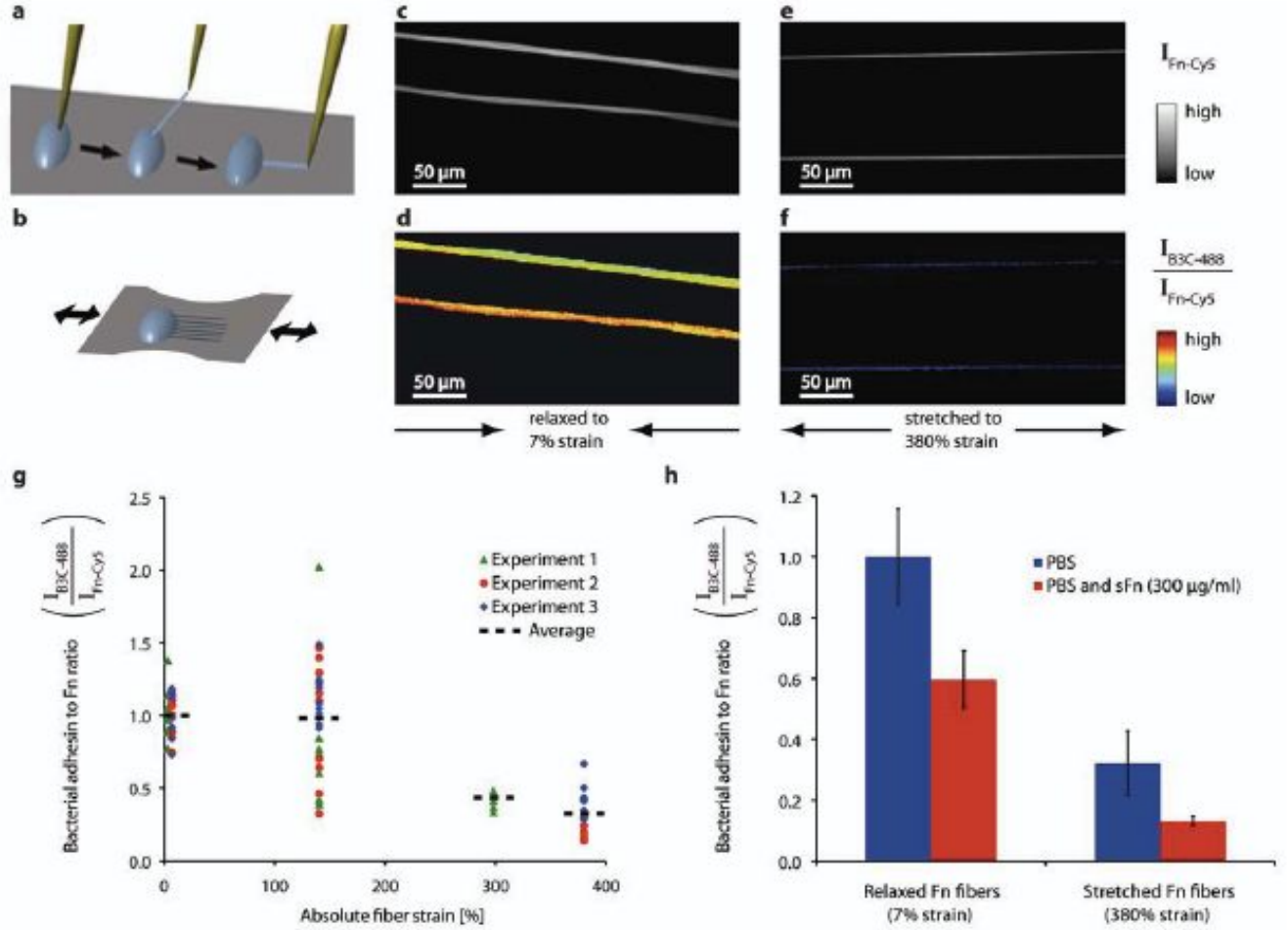


Relevance to disease



M. Chabria, S. Hertig, M.L. Smith, V. Vogel. 2010. *NatCommun.*

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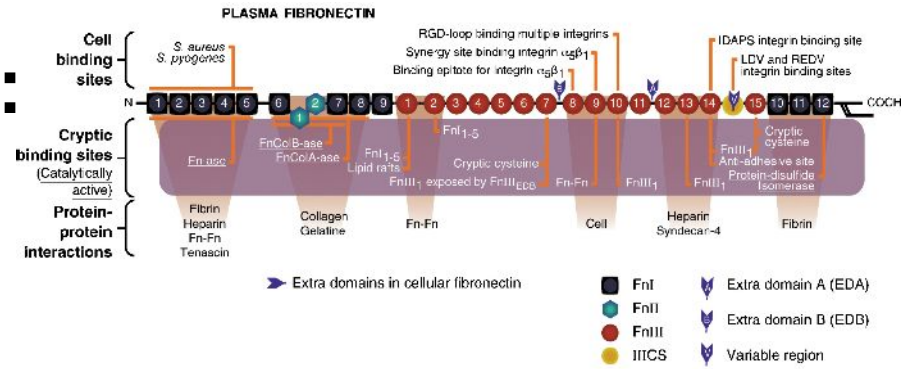

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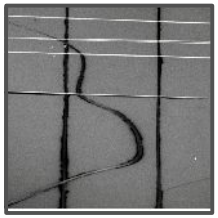
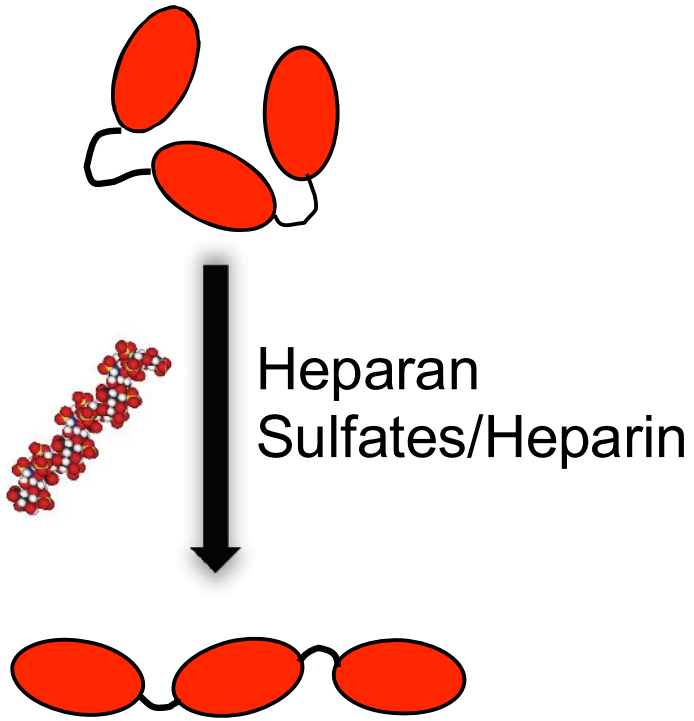
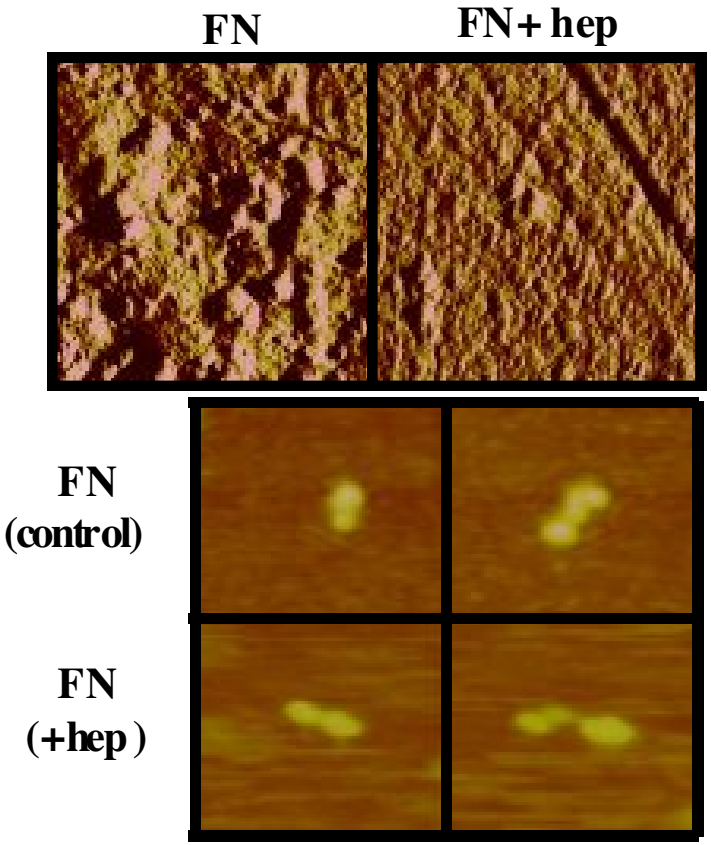


Allosteric binding?





Mitsi, Hong, Costello, Nugent. 2006. Biochemistry. 45.

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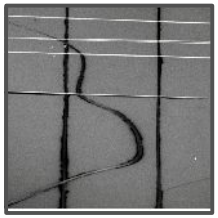

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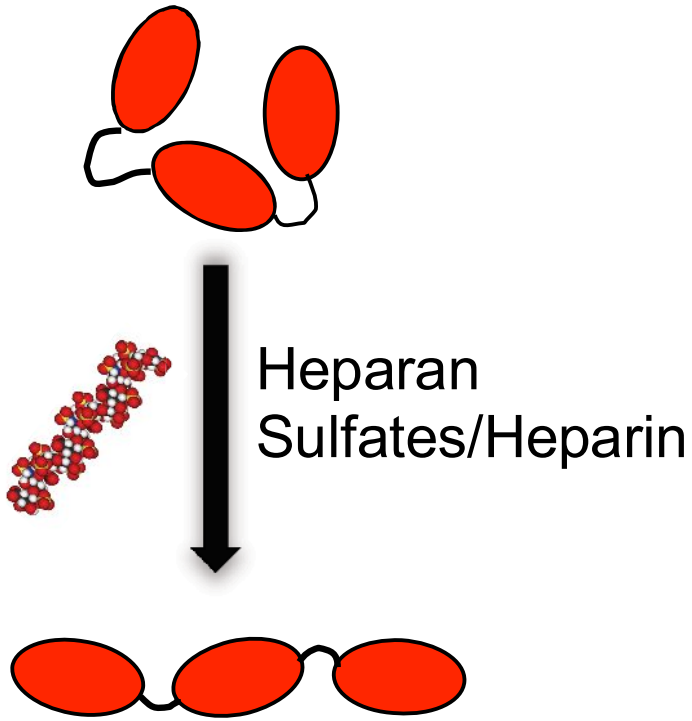

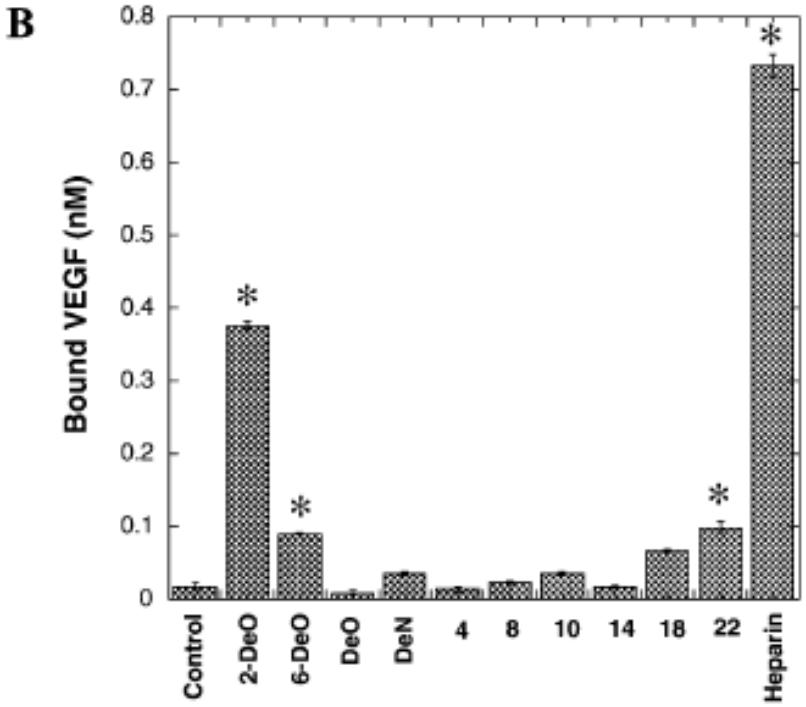

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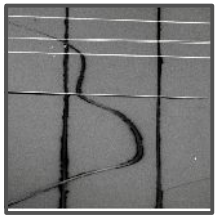
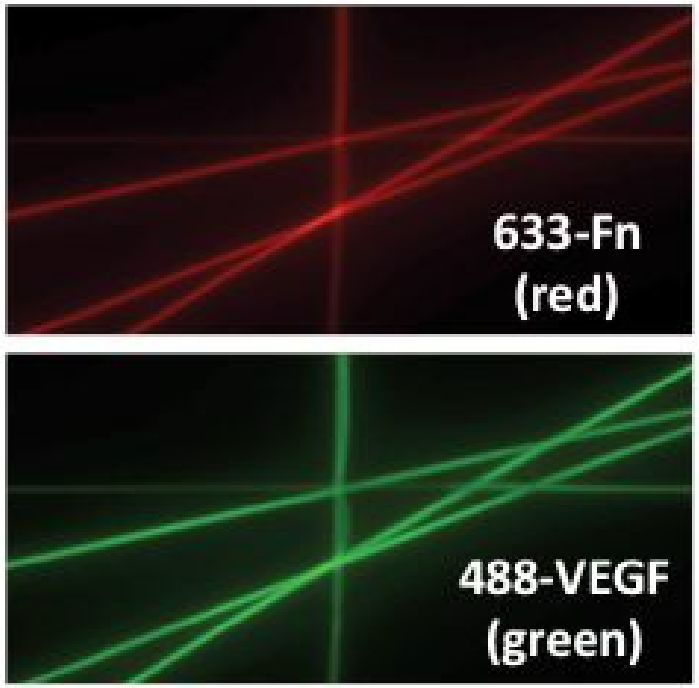
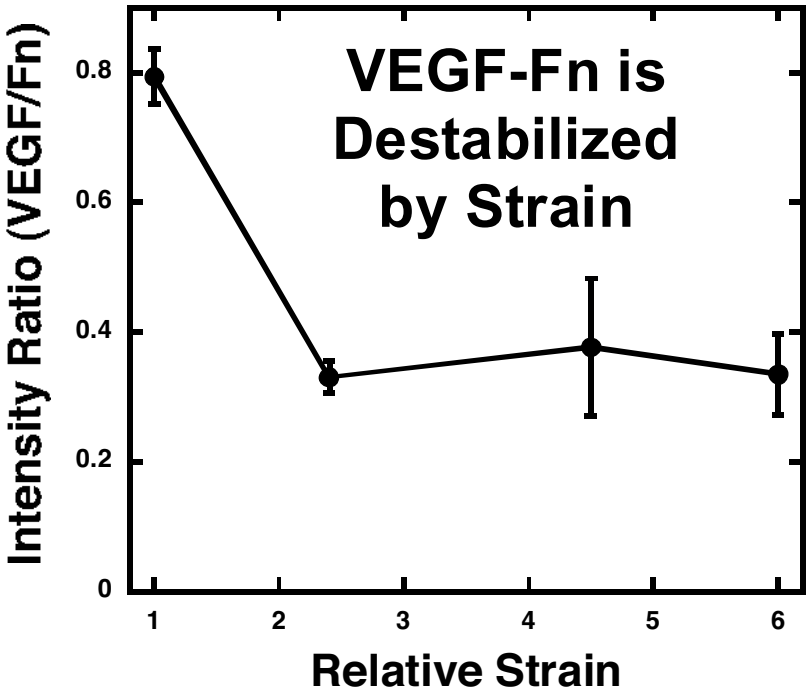


Strain-dependent biochemistry?





Hubbard, Nugent, Smith. Unpublished.

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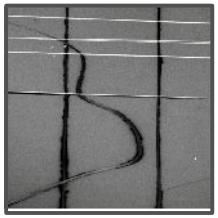
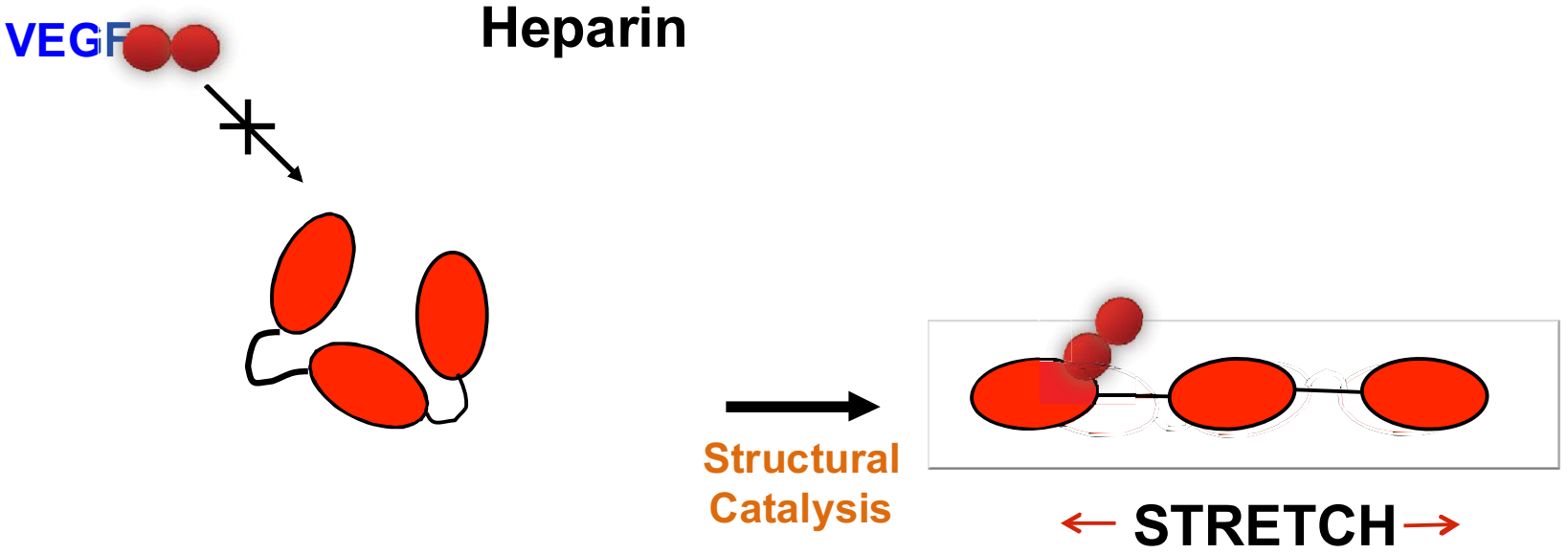


Strain-dependent biochemistry?



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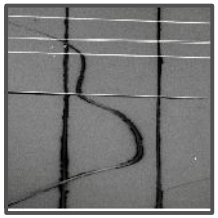
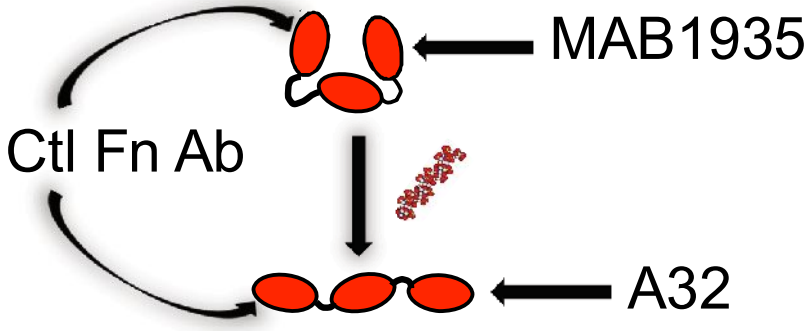
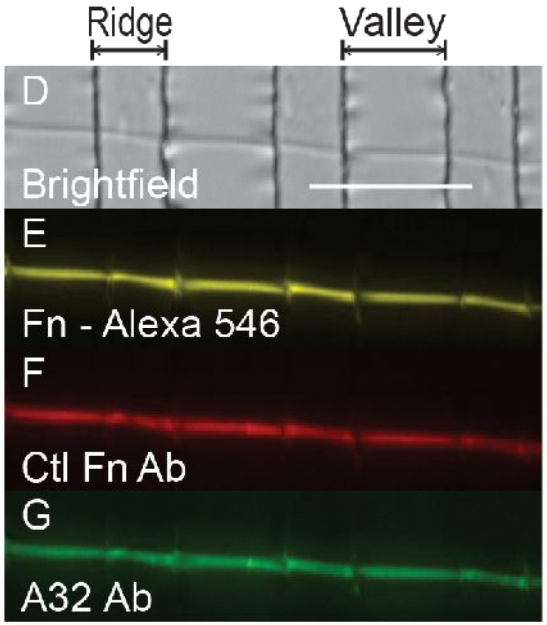
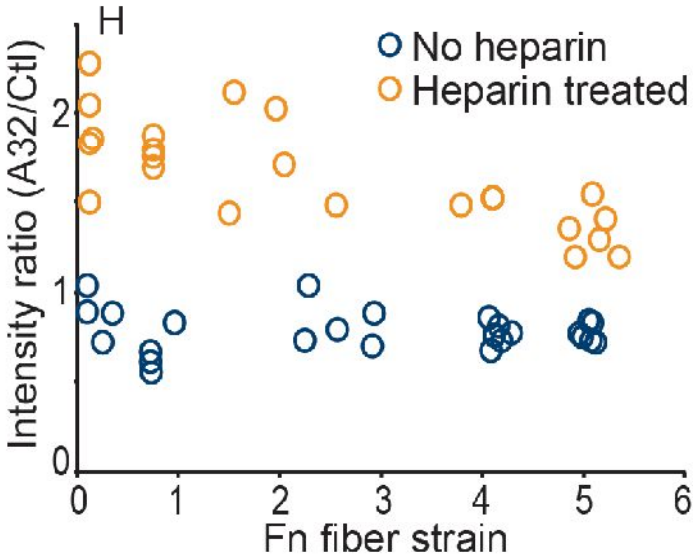


Allosteric versus mechanical regulation







Hubbard, Buczek-Thomas, Nugent, Smith. 2013. Matrix Biol. In Press.

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Enthalpic and Entropic contributions to extension:

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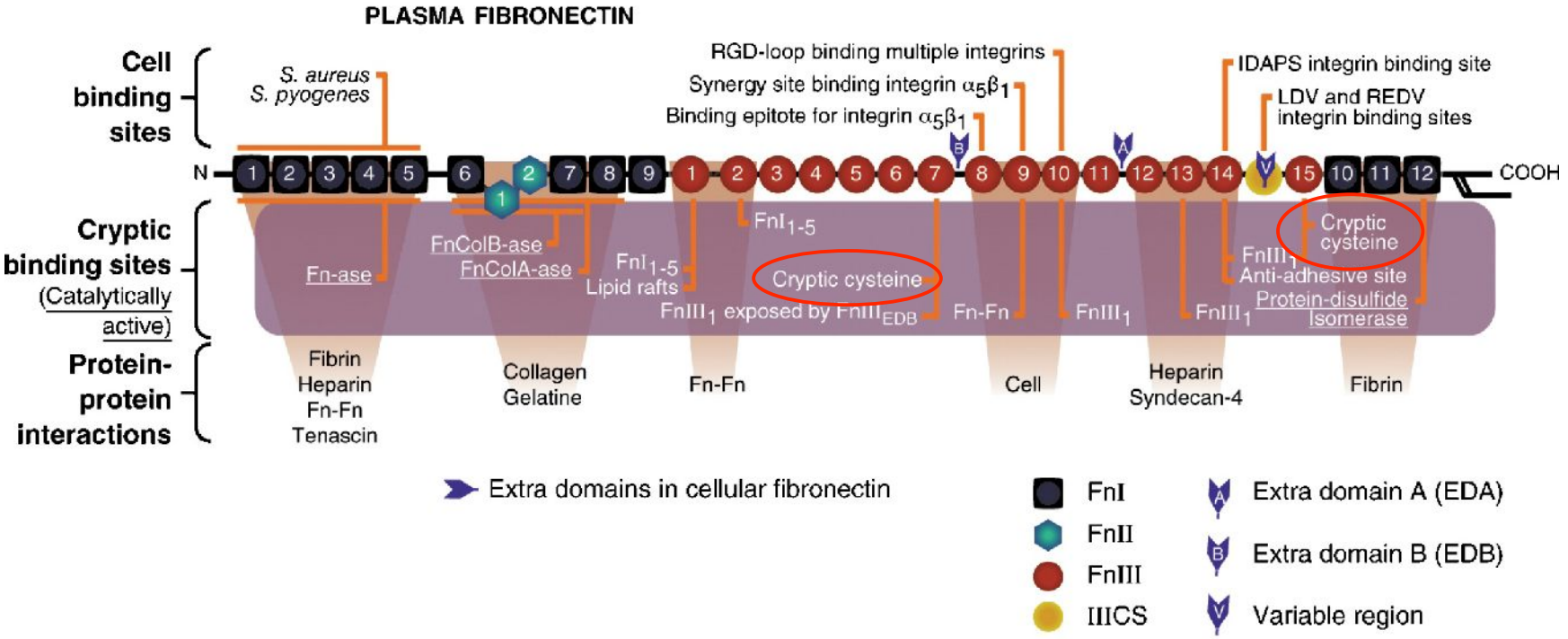
How do fibers extend?



Vogel. 2006. AnnuRevBiophysBiomolStruct. 35:459.

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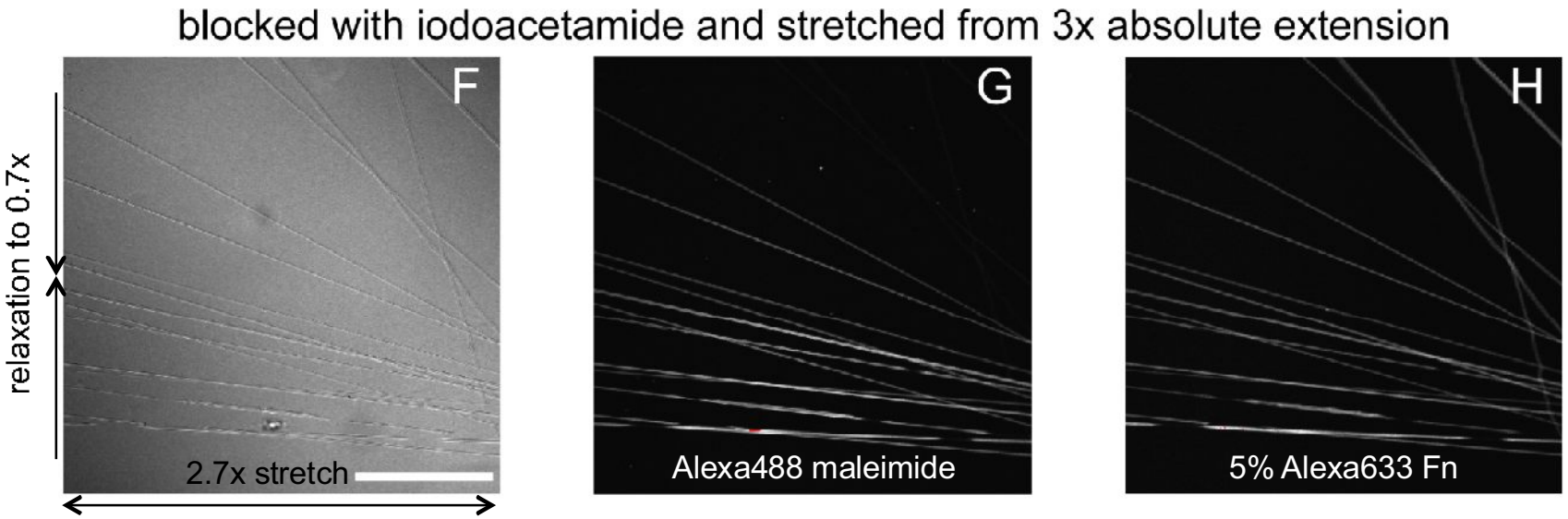
How do fibers extend?



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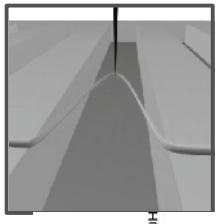
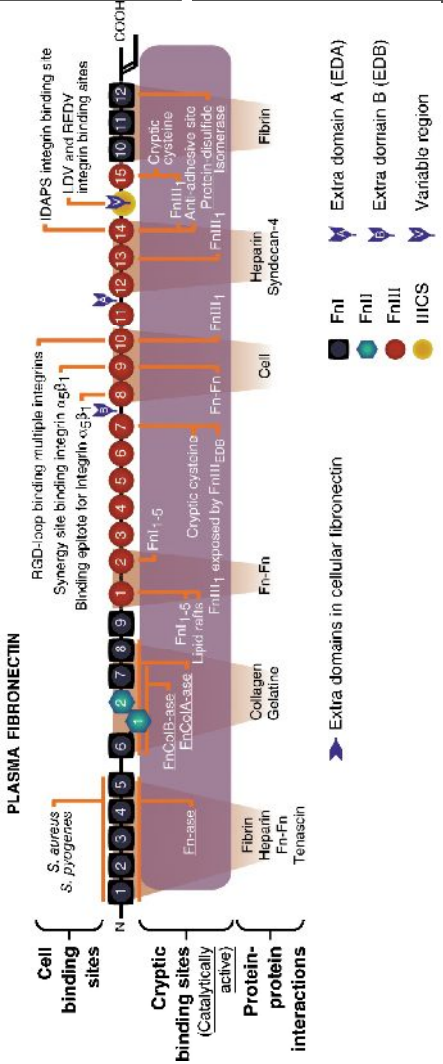
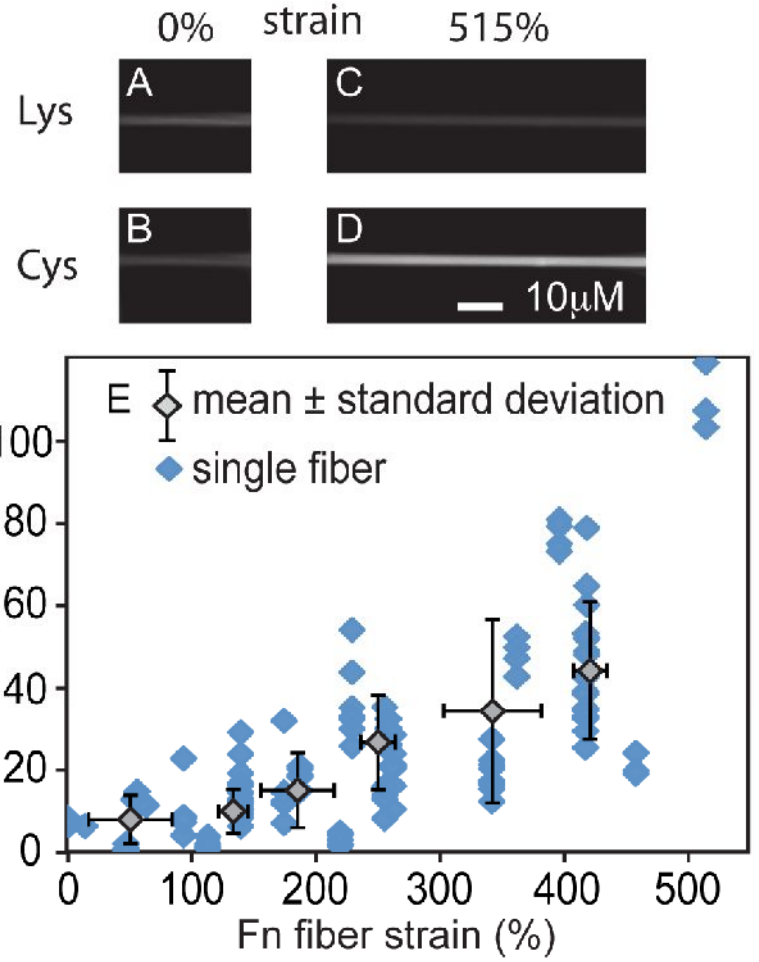


Unfolding profile





Bradshaw, Smith. 2011. BiophysJ. 101: 1740.

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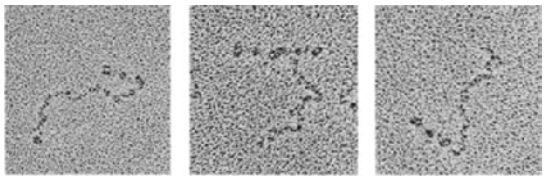
Linking nm to μm length scales

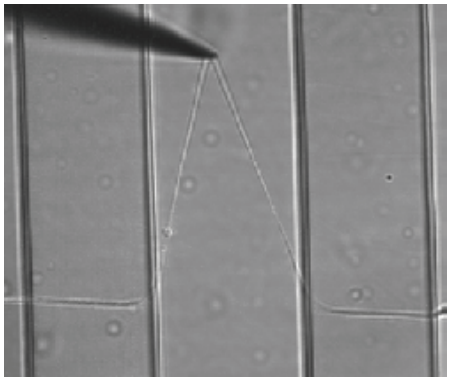
molecular properties

+

intermolecular arrangement



Molecules are probabilistic



Fibers are deterministic

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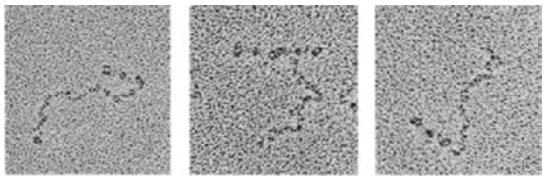
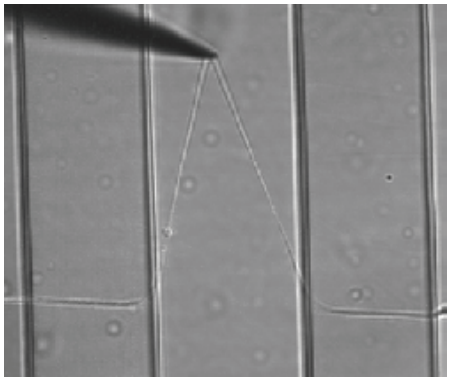
Multi-scale model

Benefits: predictions of intermolecular arrangement, molecular force, molecular conformation, ligand density, and porosity



Challenge:

Molecules are probabilistic



Fibers are deterministic

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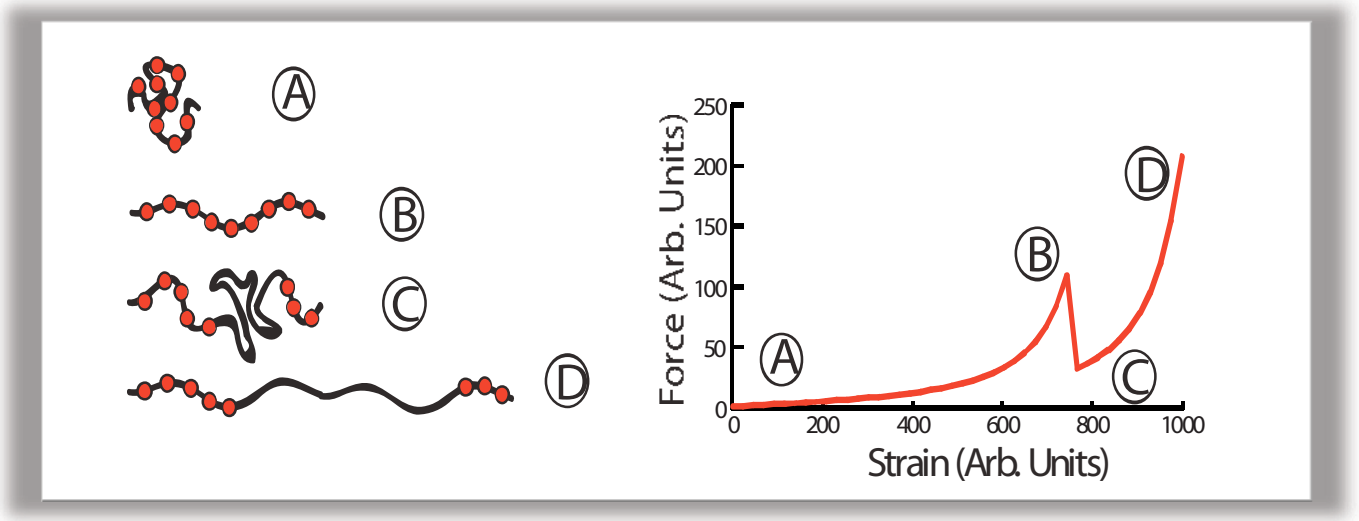
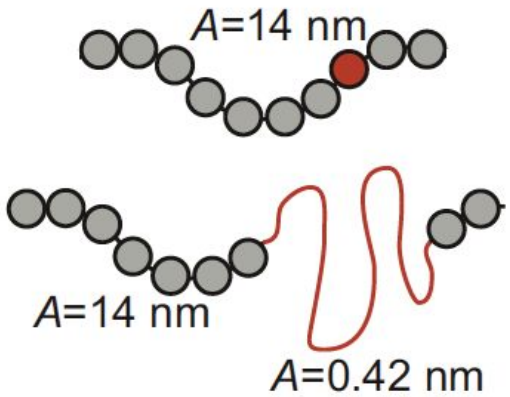
In silico: single molecule





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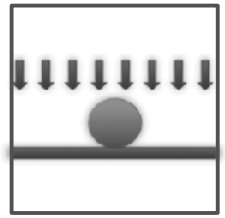
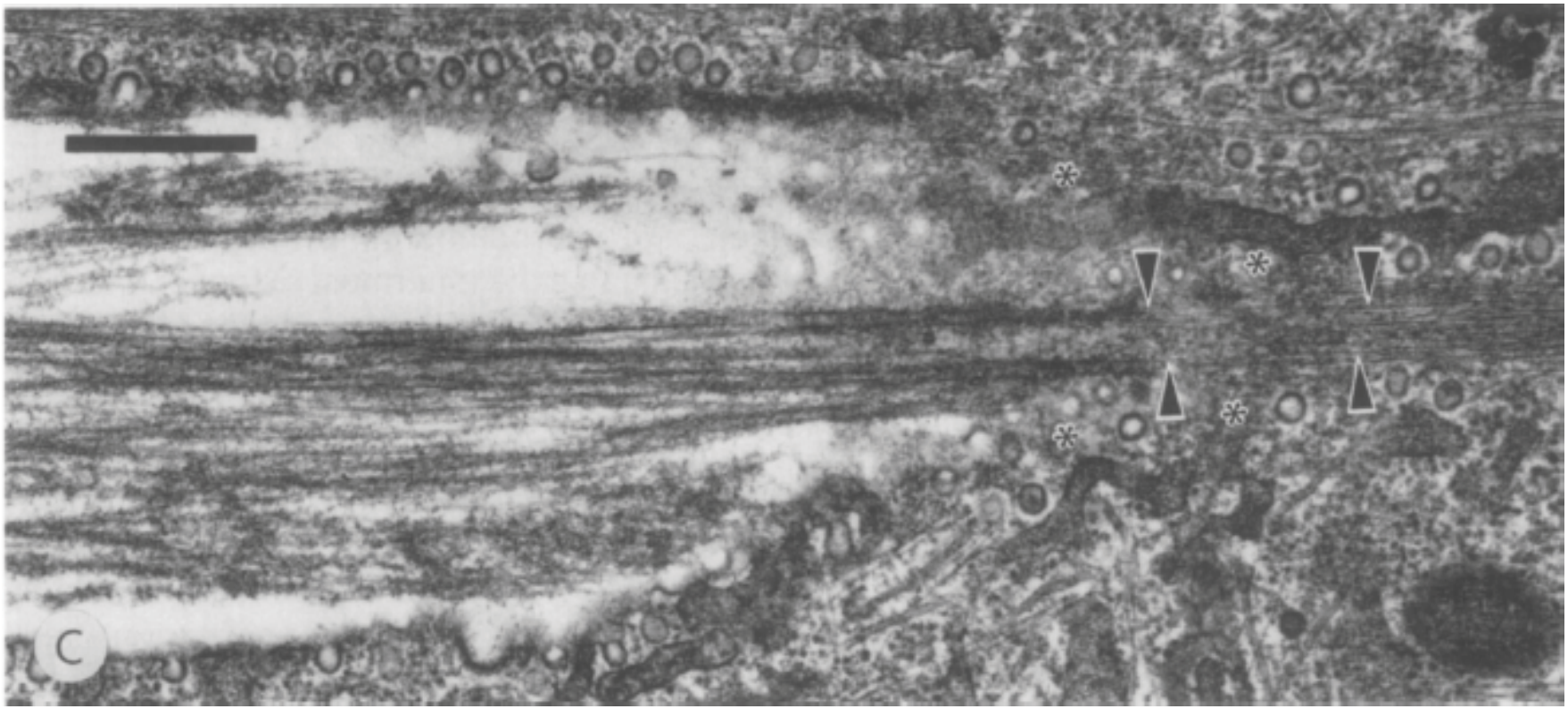


Fibronectin fiber architecture

transmission electron microscopy



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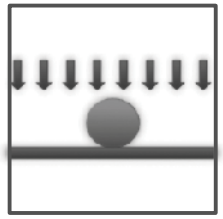
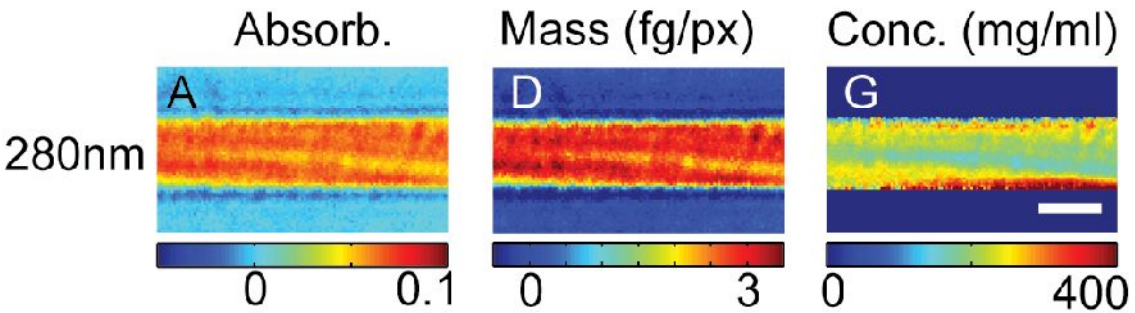
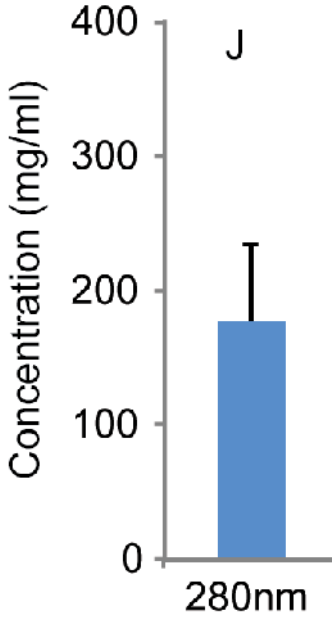


UV absorption microscopy



's Law

$$OD_{wavelength} = e_{wavelength} * c * l$$



Bradshaw, Cheung, Ehrlich, Smith. 2012. PLoSComputBiol. 8(12): e1002845.

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In silico: model fibronectin fiber

$$f = (k_B T / A)\ z/L + 1/(4(1 - z/L)^2) - 1/4$$

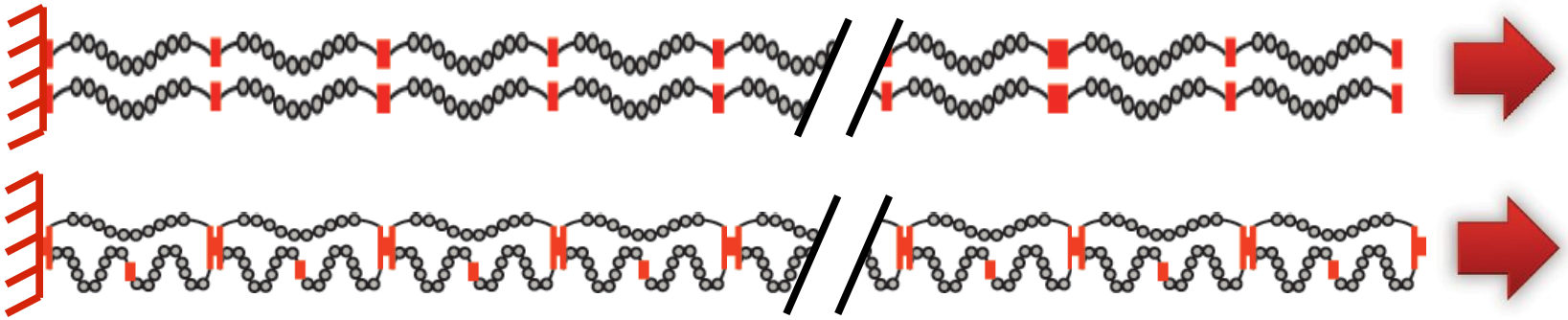
(1) Worm like chain equation.

$$U = \sum_i \left[\left(\frac{k_b T}{A_i} \right) \left(\frac{z_i^2}{2L_i} + \frac{L_i}{4} \left(1 - \frac{z_i}{L_i} \right)^{-1} - \frac{z_i}{4} \right) \right]$$

(2) Integrate (1) and sum over all molecules to find potential energy.

$$P_u = (k_u^0 * \Delta t)(\exp(f * \Delta x_u / k_B T))$$

(3) The probability of an unfolding event.



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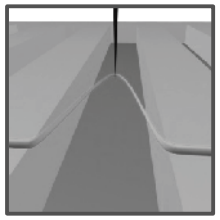
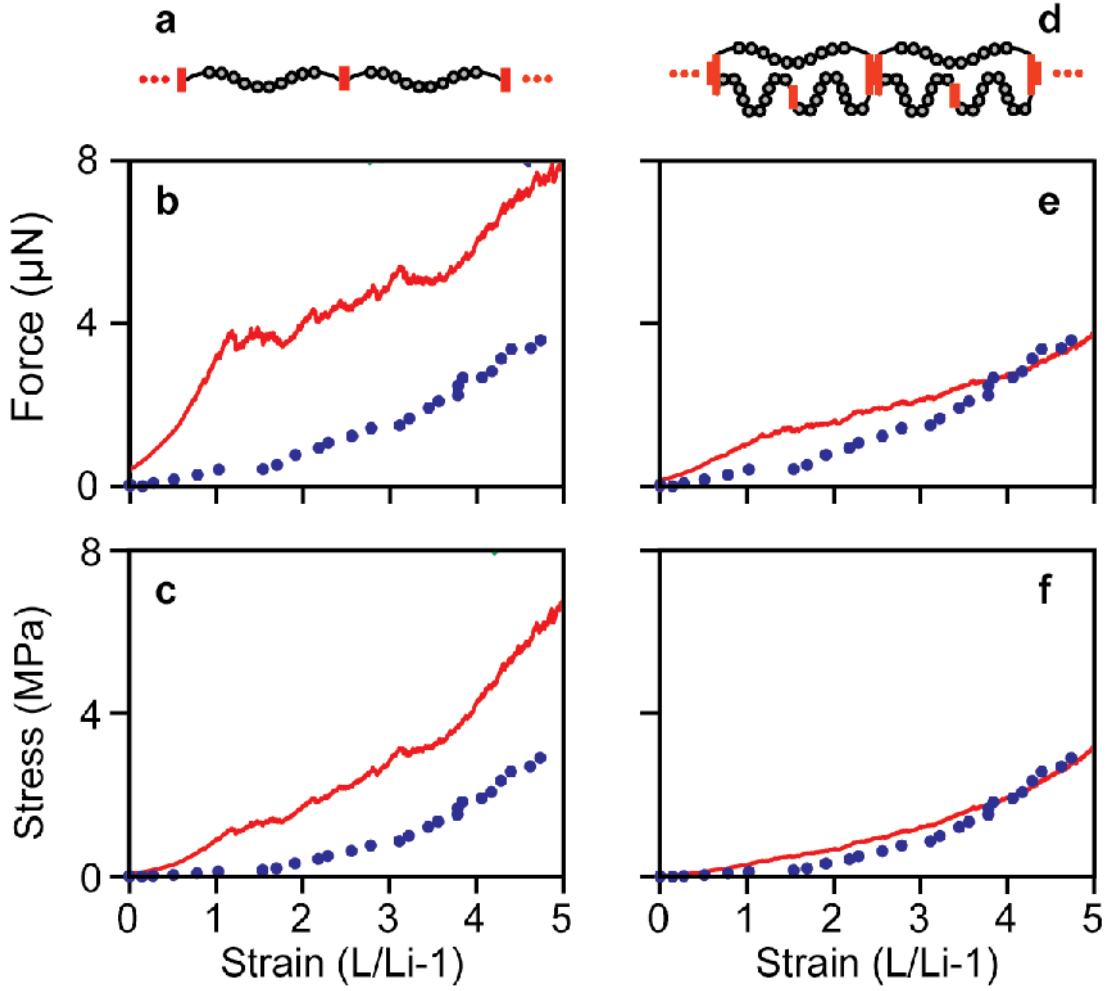


Mechanical comparison



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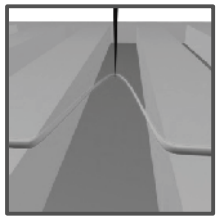
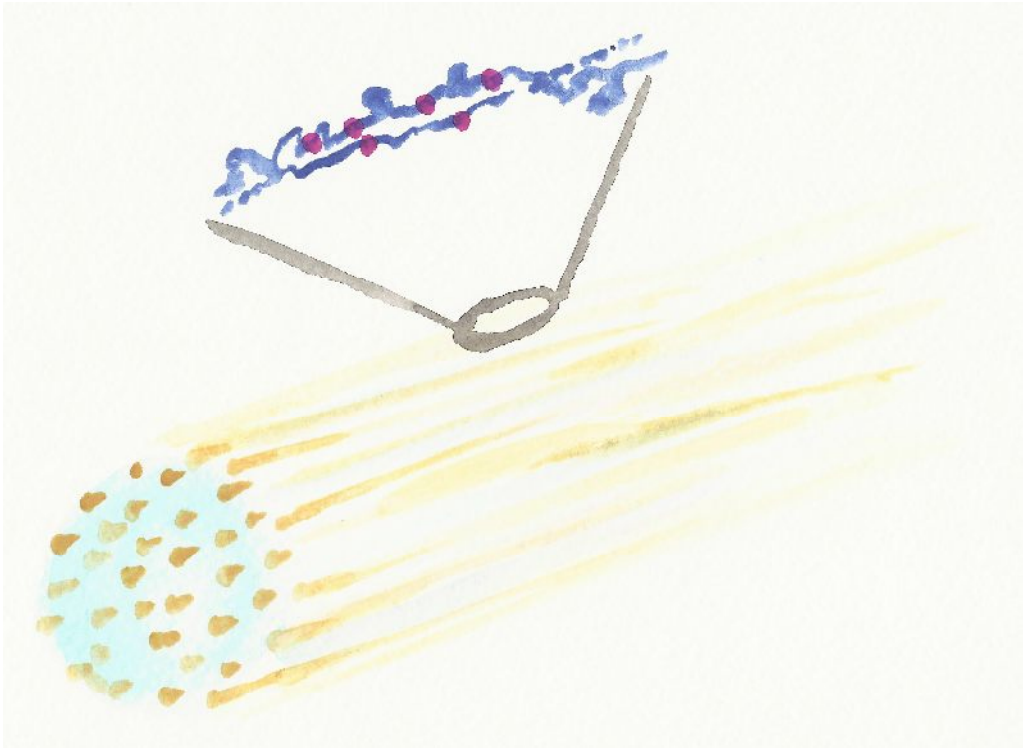


Insight: ligands and pores



Architecture predictions:

~2500 RGD/μm^2

~15 nm spacing between nanofibers

~these properties are strain dependent!

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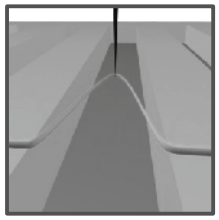
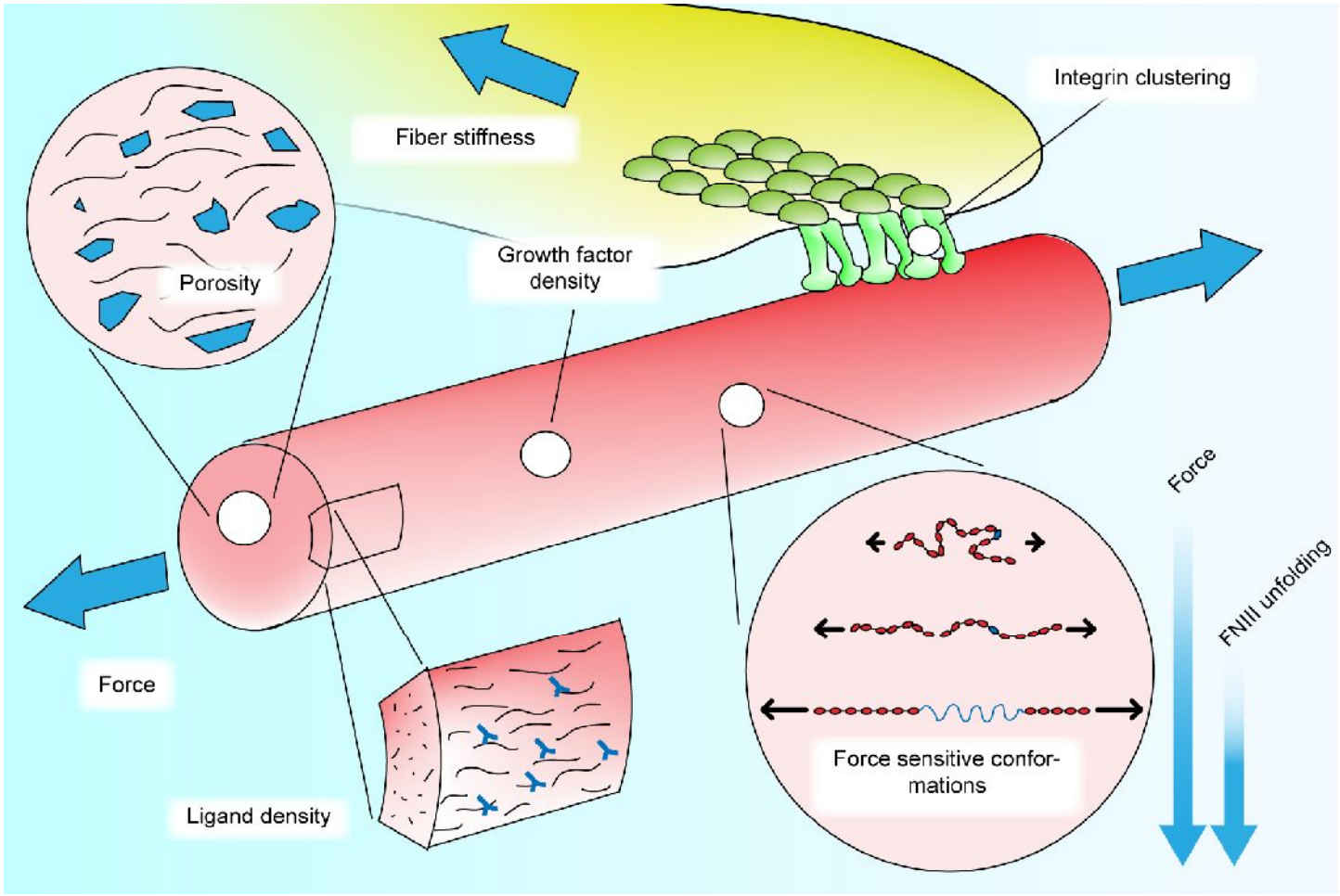


Insight:



Bradshaw, Smith. 2013. ActaBiomater. In Review.

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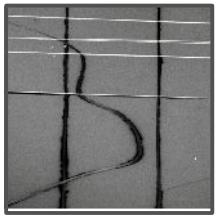
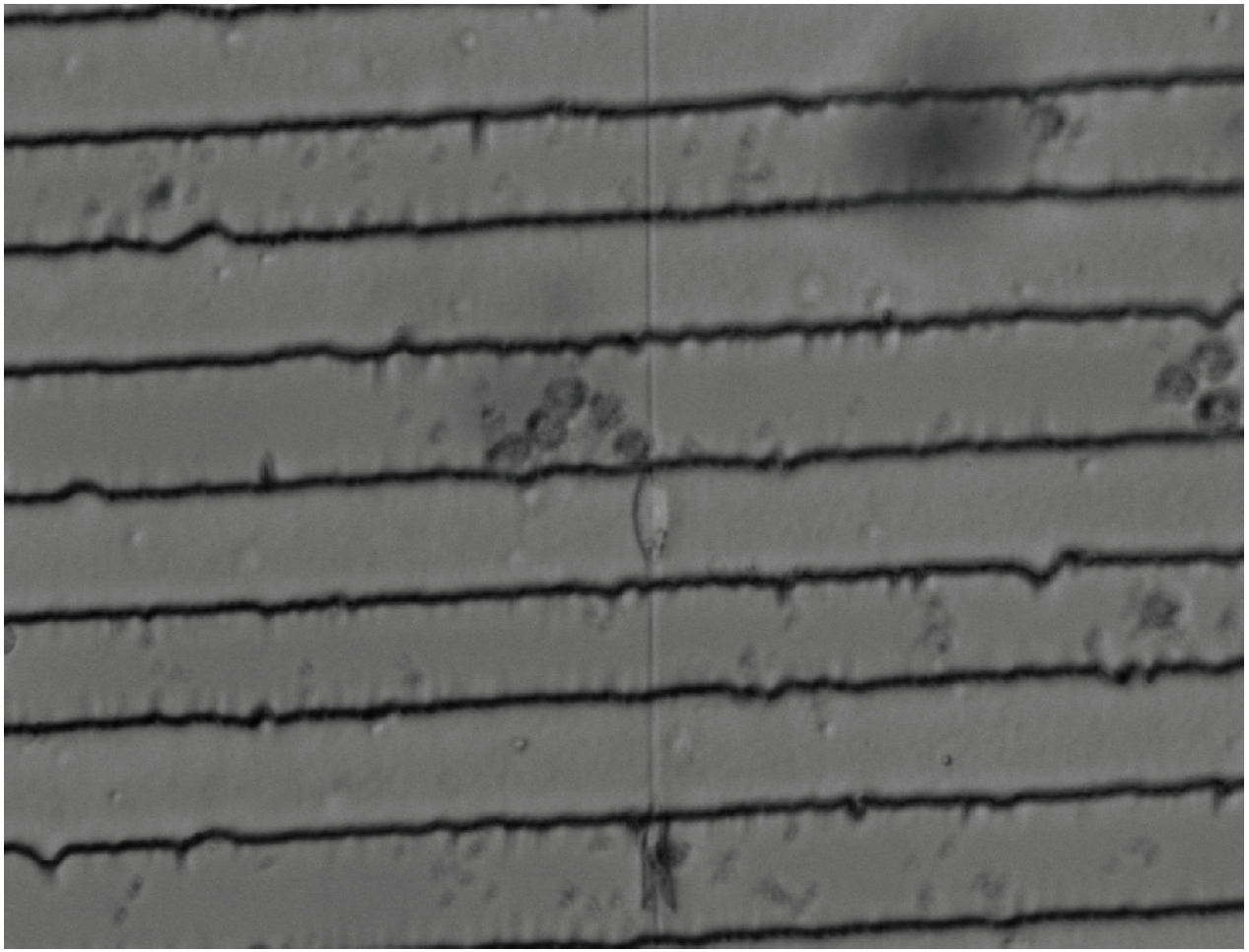


Direct regulation of cell behavior



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Dan Ehrlich, BU
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