

**BIOGRAPHICAL SKETCH**

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NAME: Dunn, Alexander Robert

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POSITION TITLE: Associate Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
California Institute of Technology, Pasadena, CA	B.S.	06/1998	Chemistry
California Institute of Technology, Pasadena, CA	Ph.D.	06/2003	Chemistry
Stanford University School of Medicine, Stanford, CA	postdoc	12/2008	Biochemistry

**A. Personal Statement**

Our laboratory studies how mechanical forces govern the structure and behavior of living cells. We used a genetically encoded, fluorescent molecular force sensor to measure forces transmitted between adjacent epithelial cells (Borghi *et al.*, PNAS 2012). These experiments, along with *in vitro*, single-molecule optical trap experiments (Buckley *et al.* Science 2014), clarified how the physical linkages between neighboring epithelial cells are established, a finding with important implications for our understanding of embryonic development and cancer biology. In complementary work, we used a battery of biophysical techniques to reveal the prominent role of the spectrin cytoskeleton in protecting neurons from mechanical damage (Krieg *et al.* Nat. Cell Biol. 2014, eLife, 2017). Recently, we found that the molecular linkages between cell adhesion complexes and the F-actin cytoskeleton are intrinsically directional (Huang *et al.* Science 2017, Owen *et al.* PNAS 2022), a finding that suggests how cellular adhesions can potentially seed long-range order in the actin cytoskeleton. The results of these and other, ongoing projects suggest deep physical commonalities in how mechanical cues act to organize cells and tissues.

**B. Positions and Honors****Positions and Employment**

Sept. 1994 – June 1998	Undergraduate, California Institute of Technology
Sept. 1998 – Jan. 1998	Graduate Student, Cornell University
Jan. 1999 – June 2003	Graduate Student, California Institute of Technology
July 2003 – Dec. 2008	Postdoctoral Scholar, Stanford University School of Medicine
Jan. 2009 – Sept. 2016	Assistant Professor, Stanford University, Dept. of Chemical Engineering
Sept. 2016 – present	Associate Professor, Stanford University, Dept. of Chemical Engineering

**Other Experience and Professional Memberships**

14. Co-organizer ASCB subgroup “How Cells Build on the Micron Scale” ASCB national meeting (2021)
13. Co-organizer ASCB subgroup “Cytoskeletal Ensembles Across Scales” ASCB national meeting (2020)
12. Co-organizer ASCB subgroup “Mechanics of Large Cellular Machines” ASCB national meeting (2019)
11. Program committee, ASCB national meeting (2018)
10. Chair, Mechanobiology Subgroup, Biophysical Society (2017)
9. Standing Member, Intercellular Interactions (ICI) NIH study section (2017-present)

8. Editorial Board Member, *Molecular Biology of the Cell* (2017 – present)
7. Editorial Board Member, *Biophysical Journal* (2016 – present)
6. Chair for American Institute of Chemical Engineers National Meeting, Areas 15d/e.
5. DARPA/Hertz Foundation Future Ideation Session, Arlington, VA Jan. 10-11, 2013.
4. NSF Proposal Review Panel, Directorate of Engineering (2013)
3. Secretary, Cellular Mechanobiology Subgroup, Biophysical Society (2013 – 2016)
2. Scientific Advisory Board, Myokardia, South San Francisco, CA.
1. Reviewer for *Nature*, *Proceedings of the National Academy of Sciences USA*; *Nature Cell Biology*; *Nano Letters*; *Nature Communications*; *Physical Review Letters*; *Journal of the American Chemical Society*; *Current Biology*; *Biomaterials*; *EMBO Journal*; *Integrative Biology*; *Arteriosclerosis and Vascular Biology*; *Journal of Cell Science*; *Optics Express*; *Interface Focus*; *Physical Review E*; *Journal of Molecular Biology*; *Biophysical Journal*; *PLoS Computational Biology*; *Scientific Reports*; *Journal of Cell Biology*; *Biomacromolecules*.

### Memberships

American Institute of Chemical Engineers  
 Biophysical Society  
 American Society for Cell Biology

### Honors

1995	Carnation Prize (Caltech)
1996	Caltech Merit Award
1996 – 1997	Barry Goldwater Scholarship
1997	George W. Green Memorial Prize (Caltech)
1997	Caltech Merit Award
1998	Richard P. Schuster Award (Caltech)
1998 – 2003	Fannie and John Hertz Fellowship
2003	Herbert Newby McCoy Award (Caltech)
2003 – 2006	Jane Coffin Childs Postdoctoral Fellowship
2007	American Heart Association Postdoctoral Fellowship
2008 – 2015	Burroughs Wellcome Career Award at the Scientific Interface
2010 – 2015	NIH Director's New Innovator Award
2016 – present	HHMI Faculty Scholar Award

## **C. Contribution to Science**

**1) Mechanosensing at cell-cell adhesions.** Until a few years ago, it was not known how, and even whether, cells might sense mechanical forces generated by their neighbors. We developed a fluorescent, genetically encoded molecular force sensor to, for the first time, directly measure the forces transmitted between neighboring cells<sup>1</sup>. This study provided the first conclusive evidence that the adhesion protein E-cadherin indeed acts as a linkage between the cytoskeletons of neighboring cells and that this cell-cell linkage experiences constitutive mechanical tension. We then used a novel single-molecule optical trap assay to show that the mechanical linkage between the E-cadherin complex and actin acts as an exquisitely sensitive force sensor<sup>2</sup>. Together, these studies provided a firm mechanistic basis for understanding how mechanotransduction can occur at cell-cell contacts, a result with important implications for our understanding of both embryonic development and cancer biology. In more recent work we have extended these studies to reveal an unexpected role for vinculin in generating long-range polarity in migrating cells<sup>3,5</sup>. We have likewise used genetically encoded force sensors to determine when and where desmosomes transmit mechanical forces between adjoining cells in simple epithelia<sup>4</sup>.

1. Borghi, N., Sorokina, M., Shcherbakova, O. G., Weis, W. I., Pruitt, B. L., Nelson, W. J. & Dunn, A. R. E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 12568-12573 (2012). PMC3411997
2. Buckley, C. D., Tan, J., Pruitt, B. L., Weis, W. I., Nelson, W. J. & Dunn, A. R. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. *Science* **346**, 1254211 (2014). PMC4364042
3. Huang, D. L., Bax, N. A., Buckley, C. D., Weis, W. I. & Dunn, A. R. Vinculin forms a directionally asymmetric catch bond with F-actin. *Science*, **357**, 703-706 (2017). PMC5821505

4. Price, A. J., Cost, A.-L., Ungewiß, H., Waschke, J., Dunn, A. R.\* & Grashoff C.\* Mechanical loading of desmosomes depends on the magnitude and orientation of external stress. *Nature Comm.* **11**, 5284 (2018).
5. Bax, N. A., Wang, A., Huang, D. L., Pokutta, S., \*Weis, W. I. & Dunn, A. R.\* Multi-level force-dependent allosteric enhancement of  $\alpha$ E-catenin binding to F-actin by vinculin. *bioRxiv*, 10.1101/2022.05.07.491039 (2022).

**2) Mechanosensing at integrin-based adhesions.** We have made important contributions to the current understanding of how cells sense mechanical forces at integrin-based adhesions to the extracellular matrix (ECM). When we joined the field, surprisingly little was known about how integrin-based mechanosensing occurs at the molecular level. Even the magnitude of forces experienced by individual integrins was uncertain, with estimates ranging from  $<1$  to  $>100$  pN. This knowledge gap made it difficult to formulate models of how cellular adhesion works at a basic, physical level. We developed FRET-based molecular tension sensors (MTSs) to directly visualize the traction forces exerted by single integrins. We found that the large majority of individual integrins produce forces  $<3$  pN, considerably less than the forces required to break integrin-ligand bonds. In complementary work, we combined the MTSs with super-resolution light microscopy to produce the first super-resolved force maps of cellular adhesions<sup>1</sup>, which revealed the physical organization of individual adhesion complexes. We found that the majority of integrins transmit forces  $<3$  pN, much less than the maximal loads supported by integrin-ligand bonds. These and other data indicate that a large pool of minimally tensioned integrins, which could not be readily characterized using other tools, are likely to play an essential role in providing cells and tissues with mechanical integrity<sup>2</sup>. In more recent work, we used single-MTS measurements, theory, and single-molecule imaging to perform a direct, molecular-scale characterization of how cells transmit mechanical cytoskeletal forces through adhesion complexes to the extracellular matrix<sup>3,5</sup>. These data motivated a reevaluation of the consensus, “molecular clutch” model for how cells transmit cytoskeletal forces to their surroundings. A revised model can account for our results, and suggests how mechanical stability can arise as a consequence of the dynamical properties of the actin cytoskeleton.

1. Morimatsu, M., Mekhdjian, A. H., Chang, A. C., Tan, S. J. & Dunn, A. R. Visualizing the interior architecture of focal adhesions with high-resolution traction maps. *Nano Letters*, **15**, 2220-2228 (2015). PMC5924765
2. Chang, A. C., Mekhdjian, A. H., Morimatsu, M., Denisin, A. K., Pruitt, B. L. & Dunn, A. R. Single molecule force measurements in living cells reveal a minimally tensioned integrin state. *ACS Nano*, **10**, 10745-10752 (2016). PMC5886374
3. Tan, S. J., Chang, A. C., Miller, C. M., Anderson, S. M., Prah, L. S., Odde, D. J. & Dunn, A. R. Regulation and dynamics of force transmission at individual cell-matrix adhesion bonds. *Science Advances* **6**, eaax0317, (2020) PMC7228748.
4. Owen, L. M., Bax, N. A., Weis, W. & Dunn, A. R. The C-terminal actin binding domain of talin forms an asymmetric catch bond with F-actin. *Proc. Natl. Acad. Sci. U. S. A.*, **8**, e2109329119 (2022) PMC8915792.
5. Miller, C. M., Korkmazhan, E. & Dunn, A. R. Extraction of accurate cytoskeletal actin velocity distributions from noisy measurements. *Nature Comm.*, *in press* (2022).

**3) Biophysical basis of physical resiliency in peripheral neurons.** In this project we examine how peripheral neurons can both withstand the large mechanical strains generated by body movement and also detect the minute physical stimuli that mediate the sense of touch. We used a combination of atomic force microscopy (AFM) measurements and laser axotomy to demonstrate that touch-responsive neurons (TRNs) in *C. elegans* are under mechanical *pre-stress* and that this prestress requires the cytoskeletal protein spectrin. To establish the molecular origin of this pre-stress, we embedded a genetically encoded Förster resonant energy transfer (FRET)-based molecular tension sensor in  $\beta$ -spectrin, and found that individual spectrin molecules are held under  $\sim 2$  pN of tension in living animals<sup>1,2</sup>. Spectrin is a ubiquitous component of the neuronal cytoskeleton from worms to humans. Our results indicate that spectrin-mediated pre-stress may protect neurons from damage<sup>3</sup>, and may additionally amplify the mechanical signals that activate stretch-activated channels in the context of touch sensation. Importantly, these conclusions are consistent with our current understanding of mechanotransduction at cell-cell and cell-matrix adhesions (see **1** and **2**, above), suggesting that a core set of biophysical commonalities may underlie mechanotransduction in a broad range of cellular contexts.

1. Krieg, M., Dunn, A. R. & Goodman, M. B. Mechanical control of the sense of touch by  $\beta$ -spectrin. *Nat. Cell Biol.* **16**, 224–33 (2014). PMC4046587

2. Krieg, M., Dunn, A. R. & Goodman, M. B. Mechanical systems biology of *C. elegans* touch sensation. *Bioessays*, **37**, 335-344 (2015). PMC4418551

3. Krieg, M., Stühmer, J., Cueva, J. G., Fetter, R., Spilker, K., Cremers, D., Shen, K., Dunn, A. R. & Goodman, M. B. Genetic defects in  $\beta$ -spectrin and tau sensitize *C. elegans* axons to movement-induced damage via torque-tension coupling. *eLife*, **6**, e20172 (2017). PMC5298879

**4) Endothelial cell response to fluid flow.** Little is presently known about how endothelial cells respond to spatial variations in fluid shear stress such as those that occur locally during embryonic development, at vascular and heart valve leaflets, and at sites of aneurysm formation. We built a novel flow device that exposes endothelial cells to gradients of shear stress. Using this device, we investigated the response of microvascular endothelial cells to shear-stress gradients that ranged from 0 to a peak shear stress of 9–210 dyn/cm<sup>2</sup>. Our observations suggest that endothelial cells are exquisitely sensitive to both magnitudes and spatial gradients in wall shear stress<sup>1,2</sup>. In subsequent work, we determined that the G protein-coupled receptor (GPCR) Sphingosine 1-phosphate receptor 1 (S1PR1), a protein known to be critical for vascular development, is required for directional cell migration in response to flow<sup>4</sup>. We are building on these results to discover the molecular mechanisms that drive vascular and lymphatic valve formation, with the long-term goal of growing transplantable valves in an *in vitro* setting.

1. Ostrowski, M. A., Huang, N. F., Walker, T., Vervijlen, T., Khoo, A. S., Poplawski, C., Cooke, J. P., Fuller, G. G. & Dunn, A. R. Microvascular endothelial cells migrate upstream and align against the shear stress field created by impinging flow. *Biophys. J.* **106**, 366-374 (2014). PMC3907231

2. Nakayama, K. H., Surya, V. N., Gole, M., Walker, T., Yang, W., Lai, E. S., Ostrowski, M. A., Fuller, G. G., Dunn, A. R. & Huang, N. F. Nanoscale patterning of extracellular matrix alters endothelial function under shear stress, *Nano Lett.* **16**, 410-419 (2016). PMC4758680

3. Surya, V. N., Michalaki, E., Huang, E. Y., Fuller, G. G. & Dunn, A. R. Sphingosine 1-phosphate receptor 1 mediates the response of lymphatic endothelial cell migration to fluid shear stress. *J. R. Soc. Interface*, **13**, 20160823 (2016). PMC5221531

4. Chang A. H., Raftrey, B. C., D'Amato, G., Surya, V. N., Poduri, A., Chen, H. I., Goldstone, A. B., Woo, J., Fuller, G. G., Dunn, A. R. & Red-Horse, K. DACH1 stimulates shear stress-guided endothelial cell migration and coronary artery growth through the CXCL12-CXCR4 signaling axis. *Genes Dev.* **31**, 1308-1324 (2017). PMC5580653

### **Complete List of Published Work in MyBibliography**

**78** total peer-reviewed publications, **25** publications in 2016-2020. A list of recent publications can be found at: <https://www.ncbi.nlm.nih.gov/sites/myncbi/alexander.dunn.1/bibliography/47417402/public/?sort=date&direction=descending>

### **D. Research Support Ongoing or Completed in the Past 3 years**

1. NIH R01GM112998 (PI: Dunn) 05/01/15 - 01/31/20

Biophysical mechanisms of mechanical tension sensing at cellular integrin complexes

The goal of this project is to understand how cells sense and transmit mechanical force at integrin-based cellular adhesion complexes.

2. NIH R01HL128779 (PI: Dunn) 07/01/15 - 06/30/19

Molecular mechanisms underlying flow sensing in lymphatic endothelial cells

The main goal of this project is to find out how during embryonic development cells in the lymphatic system spontaneously arrange to form one-way valves that prevent fluid from building up in the peripheral tissues (edema). At present we do not know how cells build these intricate structures. By finding out, we hope to learn how to regenerate or repair damaged lymphatic valves in order to treat lymphedema and other diseases of the lymphatic system.

3. NIH R01GM114462 (PI: Dunn) 09/22/15 - 04/30/20

Understanding force-dependent binding of alpha-catenin to actin

This proposal focuses on how force regulates the conformation and binding properties of the filamentous (F)-actin binding protein  $\alpha$ E-catenin, a critical part of the cadherin cell-cell adhesion complex and a regulator of cellular actin dynamics, allowing it to transduce mechanical tension into biochemical signals.

4. NIH R01GM117457 (PI: Dunn) 09/01/15 - 08/31/20  
Molecular mechanisms underlying force sensing at intercellular junctions  
The goal of this project is to understand how mechanical forces at cell-cell junctions guide the hierarchical assembly of protein complexes within adherens junctions and desmosomal adhesions.
5. HHMI Faculty Scholar Award (PI: Dunn) 10/01/16 – 09/30/21  
Biophysical Mechanisms of Mechanotransduction at Cellular Adhesions  
The goal of this project is to test the hypothesis that mechanical tension at cellular adhesion complexes drives adhesion receptor clustering, which in turn activates downstream signaling via tyrosine kinases.
6. NIH R35GM130332 (PI: Dunn) 05/01/19 – 04/30/24  
Molecular mechanisms underlying force transduction at cellular adhesion complexes  
(This award continues the projects funded by R01GM112998, R01GM114462, and R01GM117457)