# Research Interests and Plans

The theme of my research is to understand various aspects of biological motion – its physical basis and its biochemical control. Motion is one of the most defining attributes of living things and involves a series of mechanical transduction processes between the release of chemical energy and actual movement. Nature has engineered many molecular and cellular machines that induce motion at different scales – from the level of a single molecule to that of multicellular tissues. One challenge for physicists is to understand how biology exploits physical principles to cause movement. Cells also maintain exquisite spatial and temporal control over the process of force generation and use it to power directed movement in response to external signals such as chemical, adhesion or osmotic gradients. An investigation of the mechanics of moving biological objects is important in understanding the principles of biological design.

Below, I outline three questions at the interface of physics and biology that I intend to pursue. (1) How does actin polymerization generate force and motion at the microscopic sub-cellular level? (2) How is actin-based motility controlled by the cell in order to respond to internal and external directional cues? (3) At larger length scales, how do growth and interfacial forces, in addition to intrinsic forces, cause motion? In all cases, I seek quantitative information which can be used to validate existing models or design new models that best describe the data.

## 1. Force generated by actin polymerization

The movement of cells is largely powered by the formation and disassembly of actin filament networks at the boundary between the cytoskeleton and the cell membrane [1]. The polymerization of actin filaments adjacent to the cell membrane drives membrane protrusion in crawling cells. One of the challenges in understanding cell motility is to dissect the elementary biochemical and biophysical steps that link actin polymerization to mechanical force generation. Research using *in vitro* biomimetic systems has led to significant progress in understanding actin based motility [2], by reducing the enormous complexity of motile cells.

I have developed an *in vitro* system of phospholipid vesicles coated with an actin nucleating factor, ActA. Once added to a solution containing actin and other necessary proteins, they self-organize into motile vesicles propelled by polymerizing actin 'comet' tails [3]. The actin filaments in the comet tail are tightly cross-linked into an elastic gel. The polymerization forces cause significant deformation of the moving vesicles. A measure of the spatial and temporal distribution of forces can be obtained by analyzing the curvature of the vesicle contour, a purely geometric quantity. A major advantage of this *in vitro* approach is that both biochemical and physical parameters can be controlled precisely to obtain quantitative information about the mechanism of force generation. I propose to use this system to elucidate the role of the cell membrane – both in serving to localize actin filament nucleation and contributing to force generation. This research will be instrumental in understanding the mechanistic basis of force generation for motility.

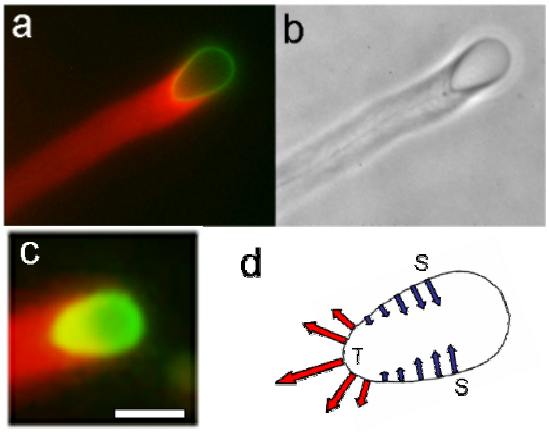


Figure 1: Phospholipid vesicles propelled by actin comet tails. a) Fluorescent image of vesicle (green) and actin (red). b) Phase image of the vesicle and comet tail. c) Magnified image of a vesicle showing that the vesicles deform into a teardrop shape. d) Vesicle contour with the calculated stress distribution due to actin polymerization. Actin exerts a squeezing force on the sides (S) and a pulling force on the trailing end (T). Scale bar is 3 μm.

One outstanding question is how the forces generated depend on the structure and mechanical properties of the actin gel. Changing the nucleating protein or the composition of the protein mixture in the motility medium, for example, the crosslinking protein will alter the mechanical properties and the structure of the actin gel. Recent experiments have shown that altering the mix of proteins results in the formation of comet tails with parallel actin bundles rather than branched structures. I shall use vesicles or oil drops to study shape deformations under different biochemical conditions in order to probe the forces generated by gels with different structures. The quantitative information obtained from these experiments can be used to validate different theoretical models of force generation by actin polymerization [4]. These studies will also provide insight into the differences in the conditions that result in the formation of filopodia (with aligned bundles of actin filaments) and lamellipodia (with branched actin networks) in crawling cells.

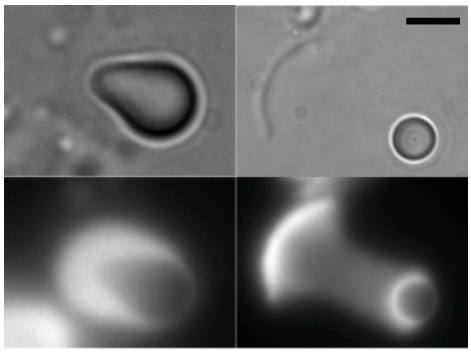

Figure 2: Changing the composition of the motility medium. Oil drops do not deform in medium with a few purified proteins (right panels), but significantly deform in cell extracts which have several additional proteins (left panels). Top images are in phase contrast and bottom images show fluorescently labeled actin. Scale bar is 3 μm.

The forces generated by actin polymerization depend critically on the geometry and physical properties of the membrane, such as curvature, composition and tension. For example, membrane tension regulates the rate of cell spreading on a substrate [5]. Our *in vitro* system allows us to manipulate membrane properties in a controlled manner to study their effects on polymerization dynamics. I propose to encapsulate actin and other proteins inside lipid vesicles using microinjection to recreate the geometry of polymerization in a cell. The curvature and tension of the membrane will be controlled using micropipette aspiration or optical trapping to investigate the role of membrane mechanics in regulating polymerization dynamics. This will be a first step towards creating a minimal model system for cell movement which can lead to a tremendous advance in our understanding of cell motility.

Vesicle properties can also be varied by changing its composition. For instance, the stiffness and fluidity of the membrane can be modulated by the amount of cholesterol. I shall use vesicles with varying composition to further elucidate the role of membrane properties on polymerization. In addition to their structural role, membrane components (such as phosphoinositides, PI) also appear to play an important role in signaling to various components within the cell such as the cytoskeleton. For example, one type of phosphoinositide, PIP2, activates actin-nucleating proteins. Further, since experiments suggest the possible curvature dependence of PI localization and clustering [6], cells may use such localization to physically regulate signaling. A longer term objective in my lab will be to use artificial vesicles containing phosphoinositides as well as other membrane proteins to study how local membrane composition can regulate actin polymerization.

I have also begun experiments using microfluidics to make lipid bilayer domains. This technique will allow me to make bilayers of varying compositions as well as encapsulate actin and other proteins. These will enable studies of actin polymerization on bilayer membranes of various controllable sizes and mimic the geometry inside a cell. These experiments will pave the way for future studies on biochemistry in small compartments as found in cells but in an *in vitro* controlled environment.

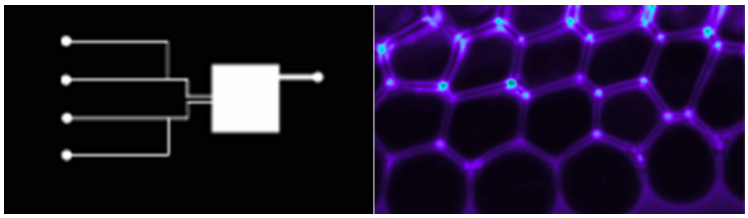

Figure 3: Left: Schematic of microfluidic device used to make lipid bilayer domains. Right: Fluorescent image of bilayer domains formed in the device.

## 2. Cell polarity and direction sensing

In order to move, cells must first decide where to go. This process of making a directional decision, whereby cellular proteins become asymmetrically distributed, is called polarization. These proteins subsequently determine where actin polymerization occurs. The establishment of polarity is important for cell motility, growth, embryogenesis and differentiation. How do cells break symmetry and polarize? An experimentally tractable system to study cell polarity is the budding yeast. Advanced imaging techniques with fluorescent proteins (such as Green Fluorescent Protein, GFP) and ease of genetic manipulation make it an attractive model system.

Yeast cells polarize to establish a cellular axis and bud at a single site on the cell membrane. In the absence of any pre-existing cues, cells can spontaneously polarize by accumulation of proteins in a spatially localized patch on the cell membrane. An outstanding problem is to understand the spontaneous generation of the polarized protein patch.

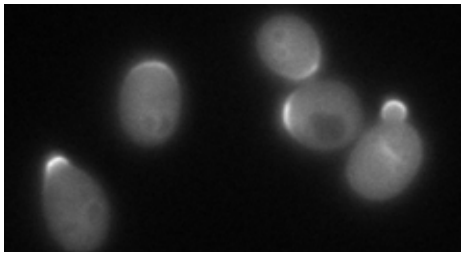

Figure 4: Polarized yeast cells showing accumulation of a fluorescently tagged protein patch on one site. The cell on the extreme right shows a bud. Cell size is ~ 5 μm.

I shall use a combination of genetic and fluorescence imaging techniques and quantitative modeling to uncover the basic mechanisms behind the initial process of polarization. This will include experiments to visualize the dynamics of key proteins involved in the polarity establishment pathway using simultaneous imaging of two or more fluorophores tagged to different proteins. Recent experiments have implicated positive and negative feedback loops that regulate the establishment of polarity [7]. Genetic manipulation will be used to perturb the postulated feedback components in order to gain a better understanding of the underlying network.

Cells also polarize in response to external signals such as chemical gradients, adhesion, heat or light. Cells' ability to orient in response to external chemical gradients is important in various biological processes. In collaboration with M. Poznansky's group at Harvard Medical School, I have developed quantitative measures to characterize the movement of human neutrophils in precise chemoattractant gradients prepared using microfluidic devices [8]. I am now developing a similar microfluidic device based system to study the orientation and polarization of yeast cells in response to controlled pheromone gradients. The response of yeast cells to external pheromone is one of the best characterized signal transduction pathways in eukaryotic cells. Though the genetic techniques have elucidated the biochemistry of the pathway, the cellular response to well defined chemical gradients has not been quantified. I shall use genetics to perturb different components of the network required for directional decisions and quantify the cellular responses to controlled pheromone gradients. Insights gained from this simple system can be used develop our understanding of how motile cells polarize and move in response to stimuli.

## 3. Surface forces and mechanics of developing tissues

At a larger length scale and an increasing level of complexity, cells in tissues need to move in a coordinated manner to create coherent structures. However, at the macroscopic scale, it is possible to ignore the molecular details of individual cells and their constituents and focus on certain generic physical properties. Tissue specific interfacial tensions arise from adhesive interactions of constituent cells. Surface forces arising from differences in adhesion drive rearrangement resulting in segregation of different cell types [9] or spreading of one cell type over another. Patterning of tissues during development, such as in the retina of *Drosophila*, is in part determined by surface mechanics [10]. Spreading of a cell aggregate on an adhesive substrate resembles the wetting of a surface by a liquid droplet. The question that I would like to ask as one aspect of my research program is – to what extent does the physics of adhesion and elasticity determine the dynamics of certain morphogenetic events?

In my previous work, I have quantified the dynamics of cell movements in some *in vitro* experiments that resemble morphogenetic phenomena [11]. I have characterized the spreading of multicellular aggregates on adhesive substrates and the engulfment of one tissue type by another. However, in all these studies the number of cells in the aggregates stayed fixed. Growth of the tissue changes the physics of the problem as it generates elastic stresses. The effect of growth on dynamics is a fundamental question in tissue biomechanics and is crucial in understanding cellular reorganization during development.

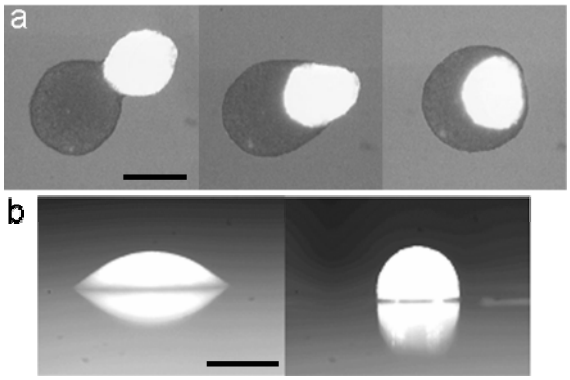


Figure 5: a) Engulfment of embryonic heart tissue (bright sphere) by neural retinal tissue (dark sphere). Scale bar is 300 μm. b) Yeast colonies with different adhesivities on an agar substrate. Left image – wild type cells; right image – cells that over express surface adhesion proteins. Note the difference in contact angles. Scale bar is 100 μm.

Genetically modified yeast strains in which the level of adhesion can be modulated are a simple model for developing tissues. In collaboration with B. Nguyen (UC Irvine), M. Brenner (Harvard University) and G. Fink (Whitehead Institute), I have studied the role of adhesion and elasticity in controlling the shapes of growing yeast colonies and compared our experimental observations to a mathematical model [12]. Using genetically modified yeast strains, I plan to study phenomena such as segregation and engulfment between cells having different levels of adhesion. Fluorescent labeling using GFP will be used to visualize the dynamics of single cells within a growing colony. A longer term objective will be to quantitatively study the dynamics of cells in the living embryo of a model developmental organism such as *Drosophila*. The large advances in genetics and visualization techniques make it an ideal time to ask the question of how physical forces regulate development.

References

1. Mitchison, T.J. and Cramer, L.P. (1996). "Actin-based cell motility and cell locomotion", *Cell*, **84**:359-369.
2. Upadhyaya, A. and van Oudenaarden, A. (2003). "Biomimetic systems for studying actin-based motility", *Curr. Biol.* **13**:R734-R744.
3. Upadhyaya, A. Chabot, J.R., Andreeva, A. and van Oudenaarden, A. (2003). "Probing polymerization forces by using actin-propelled lipid vesicles", *PNAS* **100**:6493-6948.
4. Carlsson, A.E. (2001). "Growth of branched actin networks against obstacles", *Biophys. J.* **81**:1907-1923. Mogilner, A. and Oster, G. (2003). "Force generation by actin polymerization II: The elastic ratchet and tethered filaments". *Biophys. J.* **84**:1591-1605. Gerbal, F., Chaikin, P., Rabin, Y. and Prost, J. (2000). "An elastic analysis of *Listeria monocytogenes* propulsion", *Biophys. J.* **79**:2259-2275.
5. Raucher, D. and Sheetz, M.P. (2000). "Cell spreading and lamellipodial extension rate is regulated by membrane tension", *J. Cell Biol.* **148**:127-136.
6. Hubner, S *et al.* (1998). "Enhancement of phosphoinositide 3-kinase activity by membrane curvature and inositol-phophate binding peptides", *Eur. J. Biochem.* **258**:846-853.
7. Wedlich-Soldner, R., Altschuler, S., Wu, L. and Li, R. (2003). "Spontaneous cell polarization through actomyosin based delivery of the Cdc42 GTPase", *Science*, **299**:1231-1235. Irazoqui, E.J., Gladfelter, S.A. and Lew, J.D. (2003). "Scaffold-mediated symmetry breaking by Cdc42", *Nature Cell Biol.* **5**:1062-1070.
8. Tharp, W., Upadhyaya A. *et al.* "Directional decisions and gradient perception in neutrophil migration away from a chemokine" (2004). (submitted to *Nat. Cell Biol.*).
9. Steinberg, M. (1963). "Reconstitution of tissues by dissociated cells", *Science*, **141**:401.
10. Hayashi, T. and Carthew, R.W. (2004). "Surface mechanics creates pattern formation in the developing retina", *Nature*, **431**:647-652.
11. Rieu, J-P., Upadhyaya, A., Sawada, Y., Ouchi, N.B. and Glazier, J.A. (2000). "Diffusion and deformation of single *Hydra* cells in cellular aggregates", *Biophys. J.* **79**:1903-1910.
12. Nguyen, B., Upadhyaya, A., van Oudenaarden, A. and Brenner, M.P. (2004) "Elastic instability in a growing yeast droplet", *Biophys. J.* **86**: 2720-2726.

*Arpita Upadhyaya*

# Teaching Statement

I believe that teaching students and being a good mentor is an important part of the scientific process. The future is in the hands of students and we need to teach them the fundamentals of science and instill in them a strong appreciation of research. One of the reasons I am in academia is because I enjoy interacting with students, both at the undergraduate and graduate level. I have been involved in teaching and training students from the beginning of my graduate career. I was a teaching assistant for introductory physics courses both in the laboratory and classroom as well as graduate level experimental physics, classical mechanics and quantum mechanics courses during the first three years of graduate school.

Some of the most fascinating examples of physics in action are found in the study of living organisms, for example, swimming bacteria can be used to highlight concepts in fluid dynamics, statistical physics is indispensable in the study of molecular motors, biopolymers such as DNA as well as gene transcription. My goal is to make physics more approachable to students by teaching them that physics is important to understand living systems. Because of the current revolution in biology, I think that there is a pressing need for a course in biological physics that is accessible to students from diverse backgrounds – physics, biology and engineering. I would like to develop a course in which physics is used to understand a range of biological phenomena on the microscopic and macroscopic scales. The course would include concepts of soft condensed matter physics such as membrane biophysics and biopolymers, biomechanics of cells and tissues, quantitative molecular biology including reaction kinetics and genetic networks. A possible textbook would be Philip Nelson's "Biological Physics: Energy, Information and Life". This course can be tailored to be an advanced undergraduate or a beginning graduate course.

In parallel, I would also like to develop an experimental biophysics course since I believe that a laboratory component is essential in order for students to fully appreciate the physical principles behind biological phenomena. The course would comprise of 3-4 experimental projects on topics such as membrane biophysics, molecular motors and the cytoskeleton, cell motility, bacterial chemotaxis and construction of genetic circuits. The students will learn some basic techniques for each of the modules such as the use of microscopy, imaging and quantitative image analysis and have the option of working on independent projects. The students will be able to apply the concepts learned from the lecture class to interpret and model the experimental data.

Apart from being involved in teaching at the classroom level, I have had extensive experience in supervising and mentoring students. During my Ph.D., I supervised two undergraduate seniors' theses and several summer students. At MIT, I have supervised research leading up to a seniors' thesis, four undergraduate students for the summer internship program and a high school student as part of the Research Science Institute program. I also supervised a graduate student, for his Master's thesis in Physics. This close interaction with students has made me aware of the challenges, responsibilities and rewards of good mentoring. I will make a serious effort to involve undergraduates in the research activities of my lab and to maintain a diverse environment where all students can feel comfortable working. I look forward to being a principal investigator and creating a dynamic research group where students can learn to be creative, independent and active scientists.