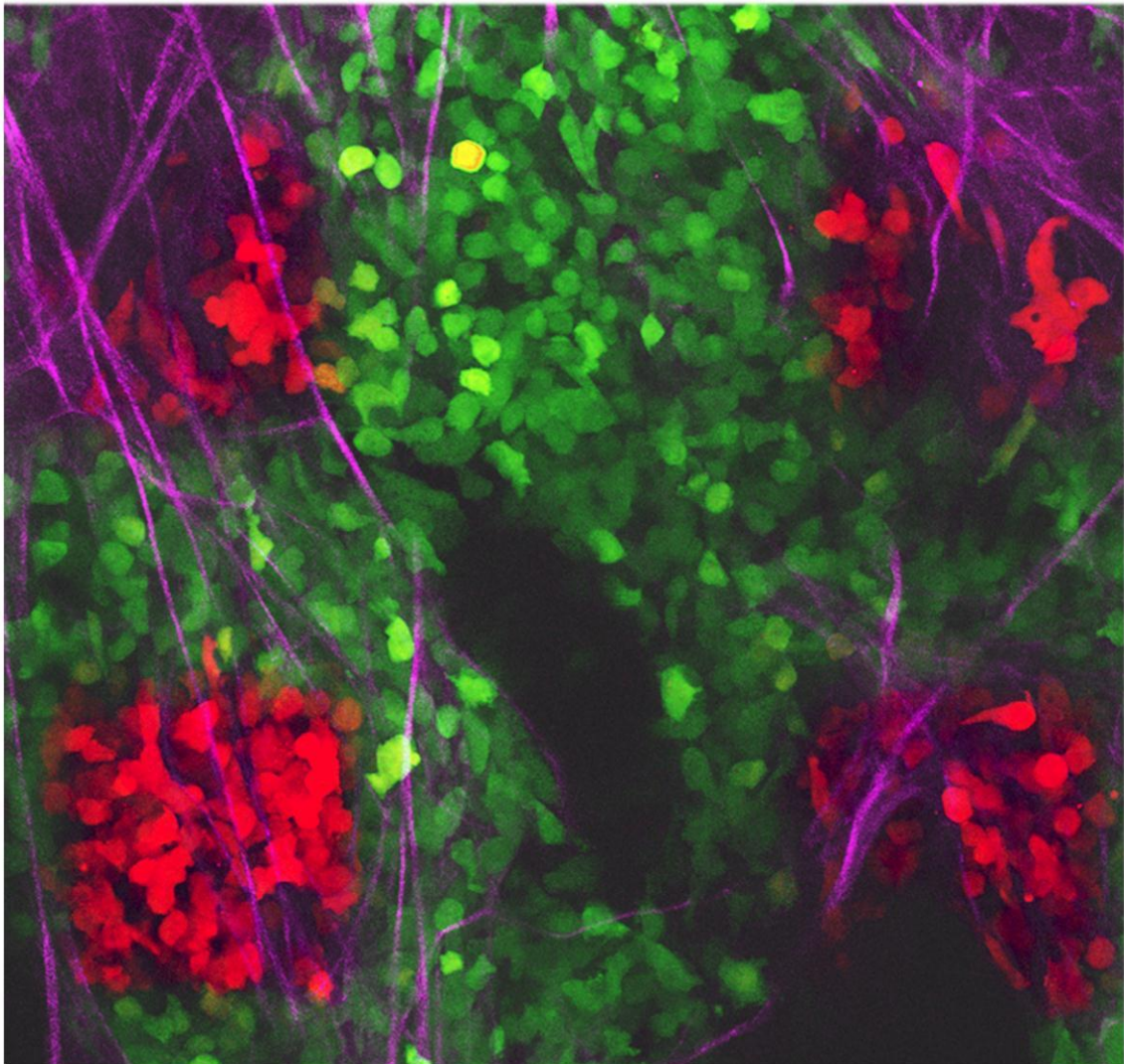


2015 - 2016

**GRADUATE TRAINING
IN CELL AND MOLECULAR BIOLOGY**



Photoconversion of tumor cells in living animal with a mammary tumor (image taken by Dr. Bojana Gligorijevic) using a mammary imaging window¹ and a custom built multiphoton microscope².
1. Kedrin and Gligorijevic et al Nature Methods 5:1019 (2008)
2. Entenberg et al, Nature Protocols 6:1500 (2011)

**DEPARTMENT OF ANATOMY
AND STRUCTURAL BIOLOGY**

ALBERT EINSTEIN COLLEGE OF MEDICINE

INTRODUCTION

The Department of Anatomy and Structural Biology is committed to graduate education. This small booklet describes the research activities and opportunities in our department for visitors and friends, but particularly for students. We have a group of outstanding faculty members who have exciting programs and world class laboratories with a good mix of postdoctoral fellows, graduate students, research associates and visitors. In the following pages, each investigator describes current projects in the laboratory and provides a list of relevant references for ongoing research. The department has many special features that make this a stimulating place in which to work, including an open door policy which encourages the members of the various laboratories to interact and share knowledge, techniques and equipment. State of the art facilities in light and electron microscopy, genomics, proteomics and cell and animal models development are available to provide investigators with the ability to perform sophisticated analyses of cell structure and function both *in vitro* and *in vivo*. Complementing a series of departmental seminars where eminent visitors present reports of their work, is a monthly internal departmental dinner, where graduate and postdoctoral students present their newest results. There are regular weekly meetings of the students and faculty in most laboratories and a graduate and postdoctoral student group that gets together frequently to discuss their mutual concerns about science, course work and instrumentation. Once every other year there is a departmental retreat in which faculty and students spend considerable time considering and planning the future direction of the program. In addition to the Department of Anatomy and Structural Biology, there are 9 additional basic science departments at Einstein, with seminars, courses, and equipment shared and available to all.

The faculty has a record of success in research and graduate and postgraduate training in cell and molecular biology, particularly relating to cell membranes, RNA trafficking, nuclear organization, the cytoskeleton and cell motility. They are interested in being mentors for new graduate students who want to develop challenging research and academic careers. If you are interested in joining us as a graduate student, you will find the details of our program in the booklet and on our Web Page <http://www.einstein.yu.edu/departments/anatomy-structural-biology/>. Our program is carefully designed, with unique features to facilitate ease of entry into the research laboratory. We believe that you will find our program exciting, different and rewarding. After looking at the descriptions here, if you have any questions, please call or drop by to talk to any member of the department.

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SINGLE MOLECULE IMAGING OF TRANSCRIPTION FACTORS INVOLVED IN CANCER AND AGEING

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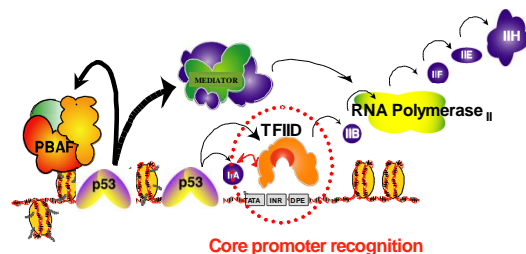
The focus of our laboratory is to develop single molecule systems to understand the molecular mechanistic basis of actions of disease driven transcription factors on their respective target genes. Specifically, we are using a powerful combination of single molecule fluorescence, biochemistry, proteomics and molecular biology to understand how these key factors dynamically discriminate their various targets in vitro and also within the complicated milieu of the cell.

Gene expression initiated by mammalian RNA polymerase II (Pol II) involves a highly coordinated assembly of over 100 different polypeptides residing within several multi-subunit complexes to form the pre-initiation complex (PIC). One critical step in gene activation involves direction of the core recognition complexes to specific target promoters. The multi-subunit TFIID complex is a principle component within the transcriptional machinery capable of

recognizing and targeting specific promoter DNA. Current models suggest that the binding of TFIID to the core promoter is followed by a sequential recruitment of other general transcription factors including TF-IIA, -IIB, -IIE, -IIH, -IIF, Mediator and Pol II that culminates with transcription initiation and promoter escape of Pol II.

As such, an ordered assembly pathway provides multiple points that can be regulated to ultimately affect gene expression. Indeed, activators, such as the tumor suppressor p53 protein and the onco-protein c-Jun, can stimulate transcription by targeting multiple factors (see Figure below), such as TFIID, TFIIB, TFIIF, Mediator, and Pol II, along with multiple steps (TFIID recruitment, Pol II promoter escape, elongation and re-initiation).

Pre-initiation complex (PIC) formation is a multi-step process stimulated by transcriptional activators

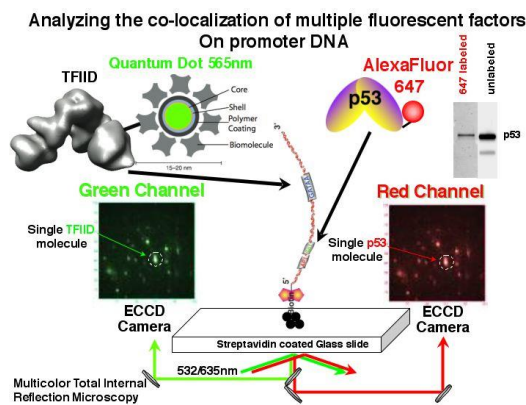


Imaging the dynamic assembly of the transcription PIC in real time

Single molecule-fluorescence has become an advanced and sensitive tool to study protein dynamics of individual molecules in a population in real time. It is therefore a technique particularly suited to study complex behavior within populations of these critical megadalton

sized endogenous macromolecular machines.

To gain a better mechanistic understanding of how the p53 and c-Jun activators dynamically regulate transcription pre-initiation complex (PIC) formation at the single molecule level, we developed a system to specifically label multisubunit human transcription complexes with Quantum dots to image their real-time assembly on promoter DNA. Total Internal Reflection Microscopy is used to examine the recruitment of single quantum dot labeled TFIID and AlexaFluor labeled p53 molecules at the promoter DNA (see Figure below).



Time resolved studies revealed that p53 helps recruit TFIID to the promoter DNA, in addition to affecting a step in the transcription cycle after TFIID has arrived at the promoter DNA. Current efforts are focused on examining the role of p53 in transcriptional elongation by Pol II.

Imaging the dynamic binding of tumor suppressors and oncogenic factors to chromatin in live cells

In order to better understand how tumor suppressors and oncogenic mutants behave in vivo, we established novel microscopes, reagents, and analytical methods to perform 2D/3D single

molecule tracking of our factors in live cells. Using these new single molecule imaging methods, we can now address how the dynamic chromatin binding activity of our factors varies in select subnuclear regions. Multi-color live cell single molecule tracking also allows us to selectively determine how our factors interact with gene targets packaged in euchromatin versus heterochromatin.

We also developed single molecule assays to determine how our factors interact with a mononucleosome in vitro. In addition, through a strong collaboration with the Liu laboratory here at Einstein, we use high-resolution cryo-EM to obtain 3D structures of our disease related factors bound to chromatin.

Screening for novel peptides that target key components of the transcription machinery and oncogenic transcription factors

To search for small peptides that target key transcription components for therapeutic development, we currently use a robust bacterial flagella display screening approach. Through several rounds of screening, we obtained numerous small peptides that can bind tightly to either p53 or TFIID. A similar approach will be used to screen for novel peptides that both bind to oncogenic mutants of our transcription factors. Peptide hits are further screened using our live cell single molecule tracking assays to determine any spatiotemporal effects on the activity of our wild type and oncogenic mutant transcription factors in vivo. We will eventually determine if these novel peptides can counteract oncogenic transformation and cancer progression in animals.

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Key Words: *Cancer, invasion, metastasis, cell motility, imaging*

Research Title: *Cell motility, the tumor microenvironment and metastasis.*

John Condeelis is The Judith and Burton P. Resnick Chair in Translational Research, Professor and Co-Chairman of the Department of Anatomy and Structural Biology at the Albert Einstein College of Medicine (AECOM). He is the director of the Cancer Center program “Tumor microenvironment and Metastasis” and co-Director of the Gruss Lipper Biophotonics Center of AECOM, a center dedicated to the development and application of optical imaging technologies. He is co-Director of the Integrated Imaging Program which is dedicated to the translation of methods using combined imaging modalities into clinically useful prognostics and treatment endpoints.

His research interests are in optical physics, cell biology and biophysics, cancer biology and mouse models of cancer. He and his

collaborators developed the multiphoton imaging technology and animal models used to identify invasion and intravasation microenvironments in mammary tumors. This led to the discovery of the paracrine interaction between tumor cells and macrophages in vivo, and the role of macrophages in the migration of tumor cells and their dissemination from primary tumors via blood vessels to distant metastatic sites. Based on these results, cell collection techniques were developed for the collection of migrating and disseminating macrophages and tumor cells. This led to the discovery of the mouse and human invasion signatures.

John Condeelis has devised uncaging, biosensor detection and multiphoton imaging technologies for these studies and has used novel caged-enzymes and biosensors to test, in vivo, the predictions of the invasion signatures regarding the mechanisms of tumor cell dissemination and metastasis. This work has supplied markers for the prediction of breast tumor metastasis in humans. Three of these markers, TMEM, MenaCalc and cofilin x p-cofilin, have been used in retrospective studies of cohorts of breast cancer patients to predict metastatic risk and are now in clinical validation trials. He has authored more than 280 scientific papers on various aspects of cell and cancer biology, biophysics and optical imaging. His current research remains on these topics.

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MACROPHAGE PHAGOCYTOSIS AND MOTILITY

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Macrophage Phagocytosis and Motility

Macrophages play an important role in host defense against invading micro-organisms and they are also key players in initiating and maintaining an immune response. However, macrophages can also play negative roles, such as in chronic inflammatory disease. Also, tumor-associated macrophages (TAMs), which are present in large numbers in many tumors, appear to play an important role in promoting the progression of solid tumors to an invasive, metastatic phenotype. Macrophages are therefore a prime target for therapies, but it is important to elucidate the mechanisms by which they are recruited to and activated in tissues.

Studying the molecular mechanisms of phagocytosis

Phagocytosis via receptors for the Fc portion of IgG (Fc γ Rs) or for complement (CR3) requires actin assembly, pseudopod extension, and phagosome closure. Actin polymerization in response to particle binding requires the activation of members of the Rho GTPases, either Rac or Cdc42 for Fc γ R-mediated phagocytosis or Rho in the case of CR3-mediated phagocytosis. Both Rac and Cdc42 regulate the cytoskeleton in part through the activation of the Wiskott Aldrich Syndrome/ WASP verprolin-homologous (WASP/WAVE) family of proteins. Several human pathogens, such as

Cryptococcus neoformans, and *Legionella pneumophila* and are internalized by macrophages, escape destruction and grow and divide inside the macrophage. The phagocytic processes by which these organisms are internalized are currently unknown. We are currently dissecting the downstream signaling pathways that mediate these processes during phagocytosis.

Studying the molecular mechanisms of chemotaxis

The directed movement of cells in response to chemoattractants involves several complex, interrelated processes, including directional or chemotactic sensing, polarity, and motility. These processes are mediated by complex, interacting signaling pathways that appear to have many similarities but yet have distinct characteristics depending on the chemoattractant and receptor. We are currently dissecting the signaling pathways required for macrophage chemotaxis towards;

1. CSF-1, a growth factor for macrophage survival and differentiation produced by many tumors and found in high concentrations in arthritic joints.
2. Chemokines that direct monocyte recruitment to different tissues

Understanding the specific signaling components required for migration towards each of these factors will allow us to test the importance of these signaling components *in vivo*. The ability of macrophages, altered in pathways identified *in vitro*, to migrate into the tissue space will be tested in animal models of peritoneal infection, atherosclerosis, and breast cancer.

The role of macrophages in the tumor microenvironment

It is now increasingly recognized that the tissue microenvironment plays a critical role in tumor progression, but most studies on

tumor metastasis involve the use of endpoint assays or in vitro studies on cell lines. It appears that macrophages and tumor cells participate in a paracrine interaction, with the tumor cells secreting CSF-1 and macrophages secreting EGF, but the precise roles of this paracrine interaction in tumor metastasis are unknown. We have developed a number of *in vitro* assays that reconstitute paracrine interaction between macrophage and carcinoma cells that mimic *in vivo* interactions of macrophages and tumor cells in the tumor microenvironment that have been observed by intravital imaging (Dovas et al., *J. Microscopy* 2012). We are currently using these assays to understand the roles of both soluble factors and direct interaction between macrophages and tumor cells through tunneling nanotubes to mediate long distance signaling to promote tumor metastasis.

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AUTOPHAGY IN AGING AND NEURODEGENERATION

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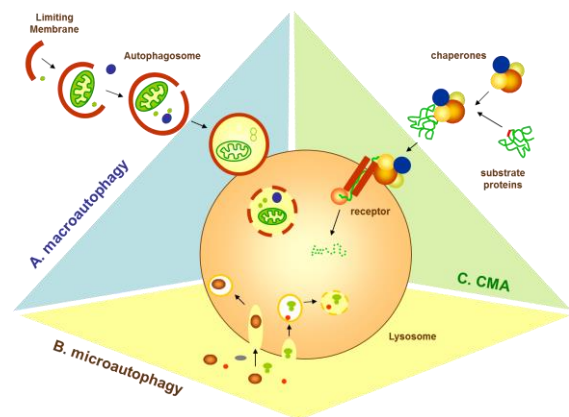
The **main focus** of our laboratory is on understanding how cytosolic proteins are transported into lysosomes for their degradation (*autophagy*) and how impaired autophagy contributes to aging and age-related diseases.

A common feature of senescent cells is the accumulation of abnormal or damaged proteins in their cytosol that, undoubtedly, impairs cellular function. Protein accumulation results, at least in part, from impaired protein degradation with age. Among the different systems that participate in the intracellular degradation of proteins, lysosomes are the most affected by age. We have previously identified in many tissues of aged animals a decrease with age in the activity of a selective pathway for the degradation of cytosolic proteins in lysosomes known as *chaperone-mediated autophagy*. The **main goal of our research** is to identify the defect(s) that lead to the decreased activity of chaperone-mediated autophagy with age and in age related pathologies, and to analyze if the correction of those defects and recovery of normal proteolytic activity in old cells leads to an improvement in cellular function.

Chaperone-mediated autophagy is responsible for the degradation of as much as 30% of cytosolic proteins, and it is mainly activated under conditions of stress, such as nutrient deprivation and oxidative stress. Substrate proteins are

selectively recognized by cytosolic chaperones (hsc70 and cochaperones) that stimulate their

binding to a glycoprotein receptor in the lysosomal membrane (LAMP-2A). The transport of the cytosolic proteins into lysosomes for their degradation requires also the presence of another chaperone in the lysosomal matrix (lys-hsc70).



Autophagic pathways in mammalian cells

Our efforts are currently directed to the:

1. Characterization of the different components involved in chaperone-mediated autophagy and identification of new players for this pathway.-

We can isolate intact lysosomes from several tissues (liver, kidney and spleen) of rodents. For the identification of new CMA components we are using different immunochemical approaches and a global proteomic and lipidomic approach. We have also developed a photoswitchable reporter which allows us to identify changes in CMA in intact cells. We are currently using this reporter to perform RNAi screenings in order to discover novel unknown regulators of this pathway.

2. Understanding the consequences of the age-related defect in chaperone-mediated autophagy.-

We have generate conditional and inducible transgenic mouse models incompetent for CMA in different tissues and have started to investigate possible tissue-dependence differences in the requirements for functional CMA. We are also analyzed the cellular response to CMA

blockage and the different compensatory mechanisms elicited. We have found that blockage of CMA leads to important alterations in cellular quality control and cellular metabolism and deficiencies in the response to different stressors.

3. Consequences of impaired autophagy in age related-disorders.- In collaboration with the groups of David Sulzer (Columbia University) and Randolph Nixon (New York University), we have been analyzing changes in autophagy in specific neurodegenerative disorders, as example of age-related diseases. By combining metabolic assays, cellular fractionation procedures and our in vitro lysosomal transport assays in different animal and cellular models of these diseases, we have found a primary defect in CMA in some familial forms of Parkinson's disease. We are currently analyzing other neurodegenerative disorders, Alzheimer's and Huntington's disease. We are interested in identifying the primary defect, the compensatory mechanisms elicited by the cell and the changes that lead to this general failure of the proteolytic systems in these and other age-related disorders.

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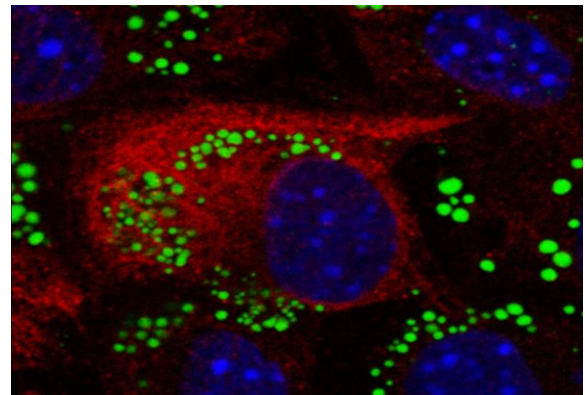
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Failure of chaperone-mediated autophagy results in lipid accumulation (green)

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MICROTUBULE-BASED TRANSPORT IN HEALTH AND DISEASE

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Key words: cell division, microtubule cytoskeleton, molecular motors, cytoplasmic dynein, bidirectional organelle transport, human pathologies, single-molecule biophysics, high-resolution fluorescence microscopy, and microscopy development.

My lab is interested in the fundamental molecular mechanisms of motor proteins and their associated biological processes. Our current research is focused on the microtubule motor cytoplasmic dynein and its role in cell division and the long-distance transport of organelles and mRNAs. We combine single-molecule biophysics with cell biology and biochemistry to interrogate the molecular mechanism of dynein and to determine the molecular basis of human diseases associated with dynein dysfunction.

By utilizing ultrasensitive single-molecule assays (high-resolution optical trapping and single-molecule fluorescence) and structure-function studies to probe dynein's molecular mechanism combined with high-throughput

screens for potent small molecules that reactivate dynein function, we are seeking to establish dynein as a therapeutic target for treating devastating human disorders including but not limited to motor neuron degeneration and lissencephaly.

Cytoplasmic dynein performs microtubule-based transport critical for mitosis, nuclear positioning, cell migration, intracellular transport of organelles and mRNAs, and the advancement of microtubules (MTs) during the outgrowth of axons. The cytoplasmic dynein complex is the largest (~2.5 MDa) and arguably the most complex cytoskeletal

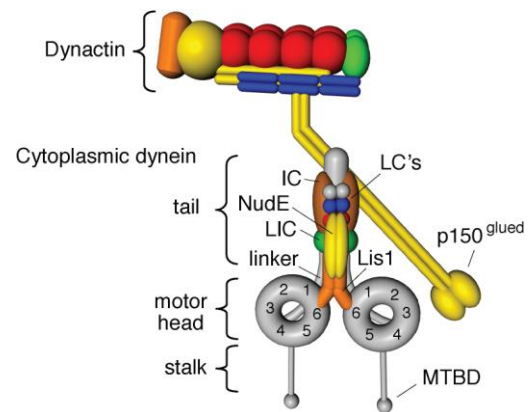


Figure 1. The cytoplasmic dynein motor complex. Illustration of the dynein dimer with associated chains and cofactors dynactin, Lis1 and NudE (LIC, light intermediate chain; IC, intermediate chain; LC, light chains).

motor protein. Dynein is composed of two identical heavy chains (HCs) and several associated subunits (Fig. 1). The dynein HC contains a MT-binding domain (MTBD) and 6 AAA domains (AAA: ATPase associated with various cellular activities) arranged in a

ring. AAA domains 1-4 can bind and hydrolyze ATP. Dynactin, dynein's largest regulatory subunit (~1.2 MDa), interacts with dynein's intermediate chain through the p150^{glued} subunit, while Lis1, in which several disease mutations reside, binds directly to the HC (Fig. 1).

Dynein harnesses the chemical energy of ATP hydrolysis to transport organelles toward the minus-ends of MTs. It has the ability to take hundreds of steps along MTs before it dissociates and diffuses away. This property allows dynein to shuttle cargoes over micrometer distances between the cell periphery and center. Such continuous movement requires coordination between the biochemical cycles of both heads of the dynein dimer so that the front head remains bound to the MT while the rear head detaches. However, it is unknown how a dynein head 'senses' and responds to the nucleotide state of its identical partner and how the nucleotide cycles of both motor domains are coordinated. Furthermore, we do not know which nucleotide states are assumed by dynein's leading and trailing heads during processive motion and how the four active AAA domains of a dynein head contribute to dynein function. My lab addresses these intriguing questions by integrating single-molecule biophysics and biochemical approaches.

Although it is known that dynein's diverse cellular functions depend on its associated regulatory subunits, Lis1 and dynactin (Fig. 1), the mechanisms by which these proteins regulate dynein function remain unclear. To determine how dynein's associated proteins regulate dynein function and to identify dynein dysfunction caused by mutations in its regulatory subunits, we are simultaneously measuring the force-generation and nanometer-scale motion of dynein while tracking the position of an attached fluorescent regulatory protein (Fig. 2) both in the wild type and mutant background. To this end, we have built a combined ultrasensitive single-molecule fluorescence and optical trapping

microscope. This instrument allows us to probe dynein's motion- and force-generating

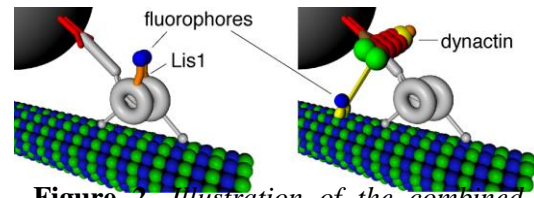


Figure 2. Illustration of the combined single-molecule fluorescence and optical trapping assay with dynein bound to fluorescently tagged Lis1 (left inset) and dynactin (right inset).

mechanism and to identify the regulatory/impairing effects of dynein's associated proteins and disease mutations. We anticipate that these studies will provide critical insights into the molecular basis of dynein function and dynein-linked human diseases.

The Gennerich laboratory is accepting rotation students, graduate students and postdoctoral fellows with interests in biochemistry, molecular and cell biology, single-molecule biophysics, and microscopy development.

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INVASIVE CANCER AND DRUG RESISTANCE

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Research Area:

- Breast Cancer
- Chemo and Radio Resistance
- Metastasis and cancer stem cells
- Biomarkers for early detection

Research Description:

Metastasis and drug resistance are major hurdles in curative cancer treatment. More attention is needed in decoding these aspects of cancer in order to develop more effective therapies. The ability of cancer cells to disseminate from primary tumors (and metastases) gives rise to a growing tumor burden that is distributed in multiple sites in the body, resulting in death for many cancer patients. Understanding the steps at the cellular level that are used by cancer cells during invasion can form the basis for new diagnostic, prognostic and therapeutic approaches that allow control of cancer metastasis. A greater understanding of the role of common signaling pathways involved in tumor invasion will lead the way to the development of more potent and selective inhibitors. The mechanism of drug resistance in cancer cells that form the secondary mets is poorly understood and more work is needed to identify these.

Recently we have discovered that the invasive breast cancer cells in two different rodent models are resistant to standard chemotherapeutic drugs. Classically, drug resistance is thought to be an aftereffect of the drug treatment, here we found that these invasive cells are *a priori* resistant to the drugs. Identification of molecular markers for both invasive and drug resistant cells from within the primary tumor would open a new vista in cancer molecular diagnosis. These would also

potentially identify new pathways for therapeutic intervention which will be a useful adjunct to conventional therapies, interfering with tumor progression at several pivotal points.

We have developed an *in vivo* invasion assay, which provides an opportunity to collect primary tumor cells that are actively in the process of invasion. The *in vivo* invasion assay has been combined with array-based gene expression analyses to investigate the gene expression patterns of carcinoma cells in primary mammary tumors during invasion. The expression of genes involved in cell division and survival, and cell motility were most dramatically changed in invasive cells indicating a population that is neither dividing nor apoptotic but intensely motile. This invasion signature provides a general resource of possible targets for future anti-invasion and drug resistance therapy. We have also been successful in isolating the cancer cells at different steps of the metastatic process and studied their gene expression pattern.

The main area of research in my laboratory is the identification and characterization of potential markers for invasion and drug resistance in cancer cells. Using various animal models and unique cell separation techniques we have been successful in separating the invasive breast cancer cells from different steps of the metastatic process which are invasive tumor cells, circulating tumor cells collected from blood and metastatic cells separated from lung metastasis. We aim to identify the molecular mechanism of the above mentioned drug resistance, and identify drugs that will be most effective in metastatic and drug resistant cancers.

Current Projects:

1. Identification and characterization of invasive and chemotherapy resistant mammary cancer cells
2. Identification of tumor initiating (cancer stem cells) amongst the invasive mammary cancer cells
3. Study of radio resistance in invasive mammary cancer cells
4. Single cell transcriptogenomics

Recent Publications:

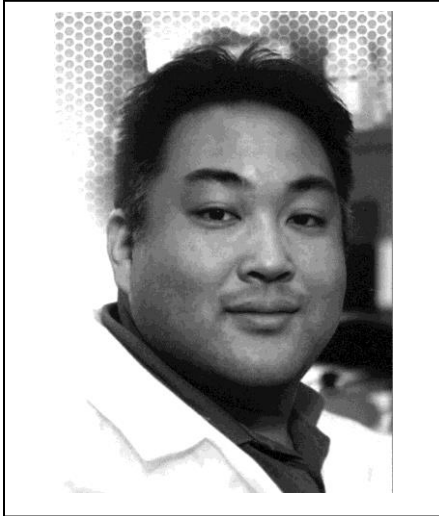
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**SPATIOTEMPORAL DYNAMICS OF PROTEIN ACTIVITIES
VISUALIZED BY FLUORESCENT BIOSENSORS
IN LIVING CELLS**

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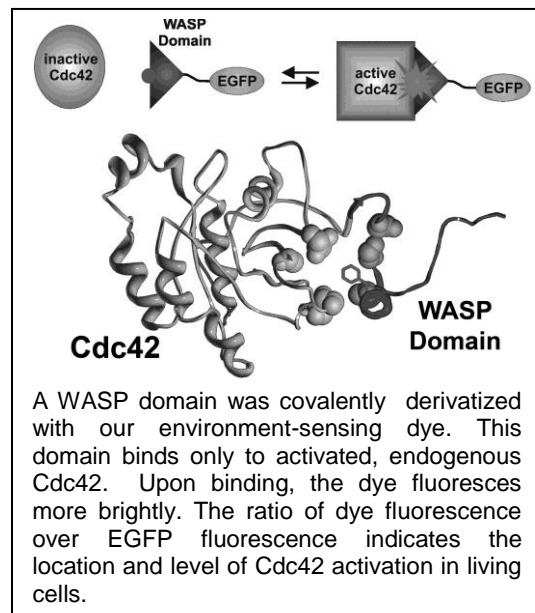


P21 Rho family small GTPases are critically important in many disease processes including malignant cancers, developmental defects, arthrosclerosis, and autoimmune dysfunction. This class of signaling molecules is critical in these diseases by impacting directly: cell polarity, motility, and migration through their actions on downstream cytoskeleton and adhesion dynamics; and proliferation by intersecting mitogenic and apoptotic signaling pathways. Rho-family GTPases regulate these processes by tightly coordinating their activities in response to various environmental cues. Only a very small fraction of GTPases turn on or off at different locations at different times to produce specific effects. Furthermore, most Rho GTPases exist in an interdependent cascade of activation/inhibition pathways resulting in a tight coordination of activation dynamics between each other. It is this coordination of multiple GTPases that is thought to regulate a variety of cellular signaling outcomes. However it has been difficult if not impossible to dissect the spatiotemporal dynamics of signal

regulation by conventional imaging or biochemical techniques.

My primary research interest is the development of fluorescent biosensors to visualize and decipher these complex spatiotemporal dynamics of protein activations in living cells in real time. These biosensors enable direct visualization of spatiotemporal dynamics of protein signaling pathways at high resolution, previously inaccessible by traditional biochemical methods. Knowledge gained from these studies will open a new window into previously unseen, coordinated mechanisms of GTPase signal regulation.

Spatiotemporal regulation of multiple GTPases: Understanding the regulatory mechanism of GTPases is very important and has potential impact in many areas including the regulation of cancer metastasis and cell migration. Regulatory and coordinating effects of multiple GTPases at the leading edge of cell migration have yet to be fully elucidated and offer an



A WASP domain was covalently derivatized with our environment-sensing dye. This domain binds only to activated, endogenous Cdc42. Upon binding, the dye fluoresces more brightly. The ratio of dye fluorescence over EGFP fluorescence indicates the location and level of Cdc42 activation in living cells.

exceptionally rich area of study in the field of cell and cancer biology. Spatiotemporal regulation of GTPase activation during cellular processes including lamellipodial protrusions and cell migration are tightly choreographed in space and time to produce specific cellular outcomes.

Protein activity and post-translational modification can be monitored in real-time in living cells by designing fluorescent biosensors. In my lab, we take multiple approaches including genetically encoded biosensors using mutants of fluorescent proteins, and solvatochromic-dyes that change the fluorescence intensity as a function of protein binding or phosphorylation. These biosensors are engineered to maximize signal-to-noise ratio (SNR) and dynamic range of response and are optimized especially for simultaneous imaging of two or more biosensors at a time in living cells using state-of-the-art high-resolution multichannel microscope system. These biosensors can be used in high-throughput screening assays (HTS) that can rapidly identify novel, small molecule compounds which can specifically target these proteins at previously unprecedented specificity, potentially providing previously inaccessible therapeutic targets for many disease processes.

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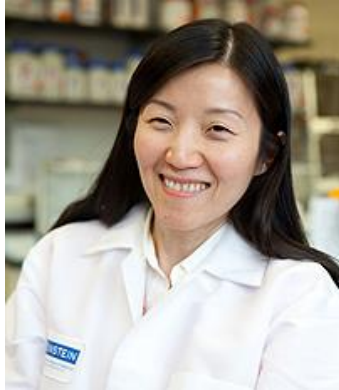
NANOSCALE PROBING OF GENE EXPRESSION

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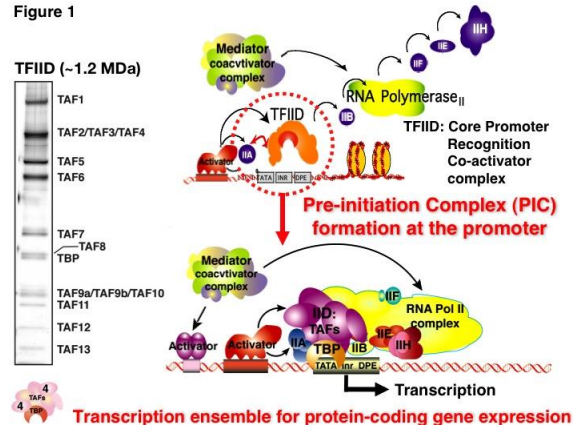
The principal focus of our work is to interrogate how protein-coding genes are transcribed in human cells by utilizing advanced single particle/molecule imaging techniques and biochemical assays. We focus on studying select human transcription initiation apparatuses responsible for directing diverse cellular processes including tumor suppression and embryonic stem cell-/cell type- specific differentiation.

Background

Expression of protein-coding genes mediated by mammalian RNA polymerase II (Pol II) is a highly coordinated and elaborate process. To accurately transcribe a protein-coding gene, a preinitiation complex is required to form at specific regions of the promoter DNA (Figure 1). One critical step during PIC assembly involves directing the core promoter recognition complex to specific target genes. The core promoter recognition complex TFIID is a principal component within the transcriptional machinery responsible for recognizing and binding

specific promoter DNA (Figure 1). Human holo-TFIID consists of the TATA-binding protein (TBP) and 10-14 evolutionarily conserved TBP-associated factors (TAFs). Once TFIID alights on core promoter DNA, it directs a sequential recruitment of other general transcription factors including TF-IIA, -IIB, -IIE, -IIH, -IIF, CRSP/Mediator, and Pol II that culminates with activated transcription at specific promoters.

Figure 1

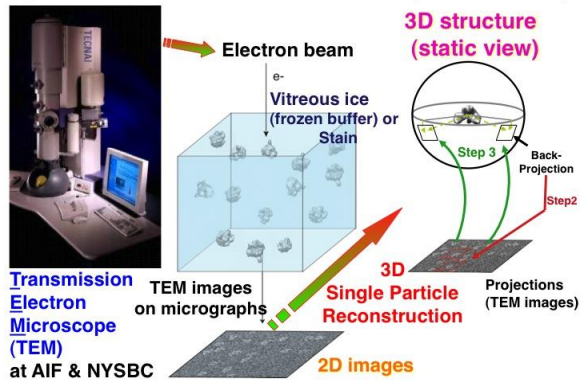


Current research projects

Although a long history of biochemical reports has documented TFIID-mediated gene expression for the last 20 years, a detailed structural and functional understanding at the single particle/molecule level of this key transcription initiation process remains elusive.

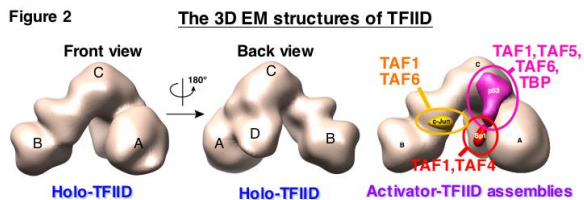
Currently we utilize high-resolution single particle cryo Electron Microscopy (cryo-EM, see Figure 1A) and a number of biochemical assays to directly visualize mega-Dalton size TFIID-mediated transcription assembly on promoter DNA.

Figure 1A Single Particle EM of Transcription Machinery



Nano-scale probing of the p53 tumor suppression transcription initiation machinery

Many tumor suppressors and disease-derived mutant proteins act as sequence-specific DNA binding activators to target TFIID and stimulate transcription directing vital cellular processes including proliferation, apoptosis and differentiation. To begin the challenging task of probing the activator/TFIID-mediated transcription initiation assembly at the single particle level, we have determined the 3D structures of holo-TFIID and TFIID bound to three distinct activators (i.e. the tumor suppressor p53 protein, Glutamine-rich Sp1 and the oncoprotein c-Jun) and compared their structures as determined by electron microscopy and single particle reconstruction (1, 2) (Figure 2).



Remarkably, each activator contacts TFIID via select TAF interfaces within TFIID (right panel). The unique and localized arrangements of these three activators contacting different surfaces of TFIID could be indicative of the wide diversity of potential activator contact

points within TFIID that would be dependent on both the specificity of activation domains as well as core promoter DNA sequences appended to target gene promoters.

To further provide nanoscale information on activator/TFIID-mediated transcription, we are presently focusing on the tumor suppressor p53 protein and three representative p53-responsive genes (i.e. *hdm2*, *bax*, and *gadd45*) that respectively express proteins regulating the activities of p53 and p53-dependent cell cycle arrest and cell death. Importantly, more than 50% of cancer patients harbor p53 mutations, highlighting the essential role of p53 in tumor suppression. Thus, it is critical to understand how p53 targets TFIID to stimulate transcription.

Armed with advanced cryo-EM, we will identify (i) if p53 and p53 target gene promoters induce specific structural changes within TFIID and (ii) if these changes generate a unique TFIID conformation responsible for transcription initiation. We aim to reveal how p53 stimulates TFIID-mediated transcription initiation that is important for maintaining cell integrity.

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RIBONUCLEOPROTEINS IN CANCER AND HUMAN REPRODUCTION

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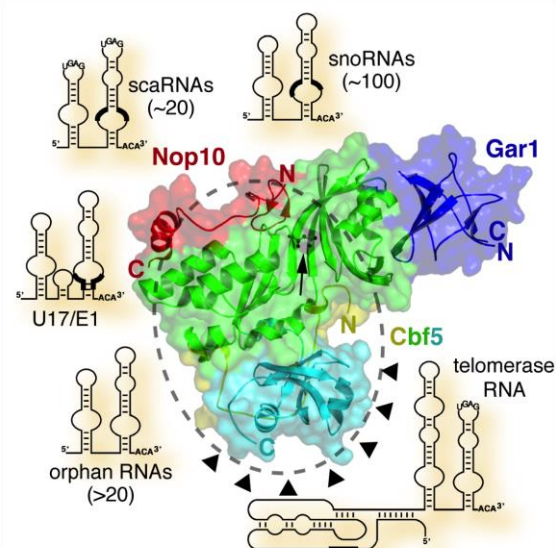
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Our group is studying the mechanism and regulation of nucleolar ribonucleoprotein biogenesis in relation to genetic disease, cancer, and human reproduction. Presently we are pursuing two main areas of research:

First, we are analyzing the biogenesis and function of small nucleolar ribonucleoproteins (snoRNPs) of the H/ACA class and how minor deviations from their natural assembly pathway can lead to cancer and bone marrow failure. Human H/ACA ribonucleoproteins are important for many basic cellular processes including protein synthesis, pre-mRNA splicing, and genome integrity. The different functional classes of H/ACA RNPs isomerize some 130 uridines to pseudouridines in ribosomal (r) and spliceosomal small nuclear (sn) RNAs,

process rRNA, stabilize telomerase RNA, yield microRNAs, and harbor yet to be determined roles. Each of these functions is specified by one of over 150 H/ACA RNAs, each of which associates with the same four core proteins to form an H/ACA RNP. The central core protein, NAP57 (aka dyskerin or in yeast Cbf5p), is mutated in the predominant X-linked form of the inherited bone marrow failure syndrome dyskeratosis congenita (DC). NAP57 was recently implicated as an oncogenic protein together with the RNP assembly chaperones pontin and reptin, whereas the H/ACA RNP-specific assembly factor SHQ1 is a tumor suppressor of prostate and other cancers. We identify how oncogenic point mutations in these proteins perturb H/ACA RNP assembly and how it can lead to an imbalance in cellular protein expression and genome instability. For this purpose, we are collaborating with structural biologists and clinical scientists.



Meier, TIBS 2006

Second, we investigate the function of nucleolar channel systems (NCSs) in the cell and in human reproduction. During the height of receptivity of each menstrual cycle, NCSs transiently develop in the nuclei of endometrial epithelial cells (EECs). They are implicated in the preparation of the endometrium for uterine attachment of the fertilized egg. Although the molecular mechanisms of embryo implantation in humans are poorly understood, NCSs remain unexplored as candidate markers or potential prerequisites for implantation. This can be attributed to the fact that, despite their discovery over 50 years ago, until recently identification of NCSs was limited to electron microscopy. We identified molecular markers of NCSs, a subset of related nuclear pore complex proteins that are recognized by the monoclonal antibody mAb414. For the first time, this allows simple and quantitative detection of these organelles at the light microscopic level. We are now exploiting our discovery to understand the cellular biology of NCSs and their regulation and function in uterine biology. The latter should have broad applicability to fertility, its regulation, and cancer.

Students are welcome to choose between the two major and the many smaller projects currently ongoing in the laboratory.

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Events that precede integrin-mediated attachment of cells to an extracellular matrix (ECM) are key in regulating the nucleation and growth of focal adhesions. Recently, my lab developed a model that provides a mechanistic understanding of the processes that govern the formation of the earliest integrin adhesions from an essentially planar plasma membrane. We call these early adhesions, "nouveau adhesions", and using a combination of single molecule tracking fluorescence microscopy and correlated confocal interference microscopy, we seek to verify the predictions of our model. In particular, the model predicts that polymerizing actin filaments are required to locally deform the membrane and translate an integrin's extracellular binding domain toward the extracellular matrix so as nucleate adhesions with radii less than a quarter of the wavelength of visible light. Without this active mechanism, cell surface glycans, which act as sensors of the ECM, inhibit the relatively short stalks of the activated integrin from reaching a ligand on the ECM; thermal fluctuations of the membrane are not large enough to form integrin adhesions. Several of the mechanisms that govern the formation of integrin adhesions are also involved in the formation of invadopodia in metastatic cells. These structures contain many of the molecular components found in adhesions and it is known that integrin signaling effects the growth of

invadopodia, however, the mechanisms that govern the formation and turnover of invadopodia remain elusive.

The novel form of interference microscopy that we have developed has enabled us to measure dynamic nanometer fluctuations in membrane topography during invadopodia formation. Furthermore, we have implemented a form of high-speed single molecule tracking using Photoactivated Localization Microscopy (PALM) and we have tracked the dynamics of actin and mRNA near focal adhesions.

To observe the dynamics of cell surface glycans, we have combined, in collaboration with the Wu lab at Einstein, bioorthogonal click chemistry with super-resolution single molecule tracking; the combination of these exceptional methodologies has enabled the imaging of the dynamics of cell surface glycans with exquisite selectivity and unprecedented spatial and temporal resolution. We have measured the diffusion of single fluorescent molecules on labeled N-linked and O-linked cell surface glycans and using statistical models to interpret our measurements of the mean square displacement and probability density functions, we have observed distinct differences in the dynamics of cell surface glycans that are correlated with the cell's metastatic potential. Although large changes in the glycome have previously been shown to be correlated with perturbations in the cell's genome, transcriptome and proteome such that altered glycosylation is considered a "universal feature" associated with many cancers, subtle variations in the dynamic behavior of glycans not yet been characterized.

Since 2008, I have taught the laboratories for "Quantitative Imaging of Cells". Based upon a "hands-on" approach, students explore the fundamentals of: (1) polarization; (2) wide-field fluorescence, DIC and Structured Illumination; (3) TIRF and single molecule imaging with EMCCD detection; (4) confocal microscopy; (5) FRAP and (6) intensity and lifetime based FRET.

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**REGULATION OF SIGNAL TRANSDUCTION IN SECRETORY VESICLE
MEMBRANE FUSION IN
EXOCYTOSIS AND CELL INVASION**

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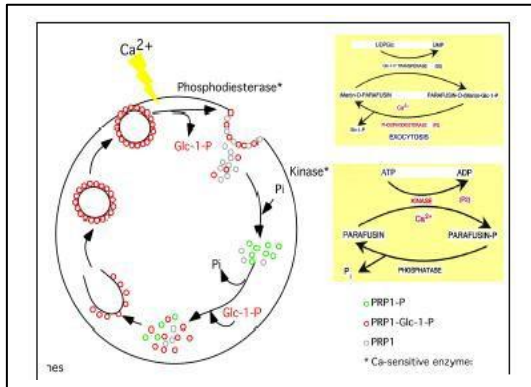
**THE EXOCYTIC NANO-MACHINE
AND ITS ASSOCIATED COMPONENTS**

The questions being addressed here concerns a basic biological process crucial to the maintenance of normal homeostasis in cells, namely regulation of trafficking and destination of secretory vesicles and release of their content via regulated exocytosis. The study takes advantage of how such basic processes and many of the main components already were in place early in evolution. It uses as a paradigm the known dynamic events found to be associated with exocytosis in the well known free-living apicomplexan ciliate *Paramecium tetraurelia*. This study will demonstrate that posttranslational modifications of protein(s) can change their known enzymatic functions and reveal new additional roles in vesicle trafficking, destination and exocytosis. In particular, we study the novel modifications involved in carbohydrate cycling of parafusin, a member of the phosphoglucomutase family. Several of the main biochemical and structural modifications of this protein, first demonstrated in *Paramecium*, have been shown to hold for mammalian systems as well. However, the exact function of parafusin and its interacting partners in the exocytic nano-machinery have not yet been demonstrated. We are trying to do this so by designing recombinant proteins of parafusin (with domain deletions or site specific mutagenesis), in combination with a novel assay that test their function in LIVING cells. In this

ortholog assay, the fluorescently labeled recombinant parafusin molecules that have been tested *in vitro* for their capability to be posttranslationally modified, will be electroporated into living *Paramecium* and their location, their transport and their location after exocytosis will be followed with different fluorescent microscopy techniques.

**THE ROLE OF THE PARAFUSIN
ORTHOLOG (PRP1) IN *T. GONDII* CELL
INVASION.**

Cryptosporidium parvum and *Toxoplasma gondii* are related Apicomplexa and have been designated as food and water borne pathogens with the highest bioterrorism potential. This is attributed to their ability to infect vast numbers of the population through contaminated water and/or food supplies. Additionally *T. gondii* can be genetically manipulated to increase virulence or resistance to anti-infectives. *T. gondii* initiates infection upon direct contact with the host cell. This is followed by a sequential release of protein from three secretory organelles, the micronemes, rhoptries and dense granules, respectively. The trigger for invasion has been linked to changes in calcium levels within the parasite. Micronemes are the only organelle with calcium dependent secretion. We have previously shown that a calcium-sensitive, modified, and dynamic molecule, called parafusin is pivotal for exocytosis in *Paramecium*. *T. gondii* possesses a homologue called Parafusin-related protein (PRP1), which we have shown: localizes to the parasites micronemes, undergoes dynamic localization changes after exocytosis and is modified in a calcium-dependent fashion. **We hypothesize that part of the Ca²⁺-dependent exocytic cascade preceding *T. gondii* invasion, is controlled by biochemical and structural changes of a vesicle-associated protein, PRP1.** Specifically, in collaboration with Dr. Kristin Hager, we want to (1) determine the kinetics and biochemical parameters for PRP1 modification during the parasite lytic/invasion cycle, (2) evaluate the importance of posttranslational



Model For PFUS Modification Cycle as it Correlates to Exocytosis. Green, red, and grey circles indicate some of the possible PFUS isoforms. Star indicates enzymes thought to be calcium sensitive, the reaction they are thought to mediate are shown

modification of PRP1 by using transgenic parasites expressing mutant PRP1 created by site-directed mutagenesis. We will then characterize localization and modification changes both in vitro, using a novel ortholog assay and in vivo in *T. gondii*, (3) identify the functional role of PRP1 in invasion by creating PRP1 knockouts and determining whether PRP1 knockout parasites are invasion competent. Our studies will help determine whether PRP1 or its modifying enzymes are potential targets for chemotherapeutic intervention to prevent *T. gondii* infection.

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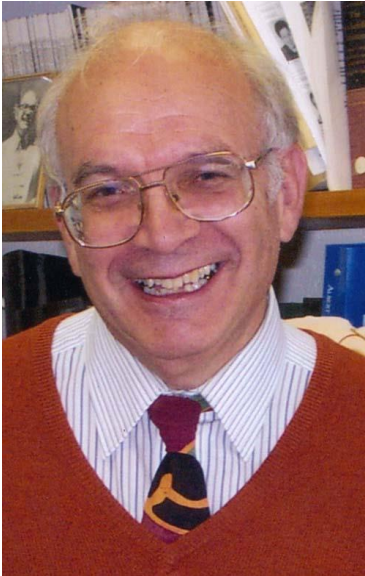
THE BIOLOGY OF CILIA AND MICROTUBULE BASED MOTILITY

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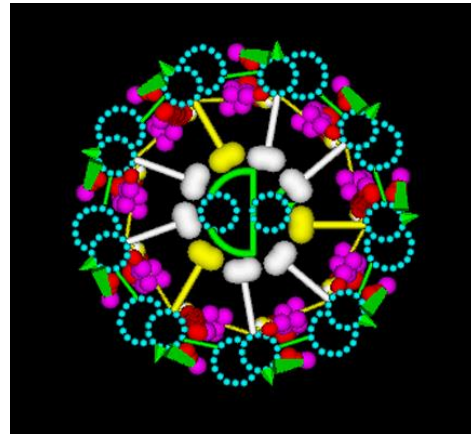
<http://www.einstein.yu.edu/faculty/805/peter-satir>



My laboratory studies the role of motile and primary cilia in human biology. Particularly interesting is the new information linking kinesin and dynein intraciliary transport (known as IFT) components to human diseases, including left-right asymmetry determination (situs inversus), primary ciliary dyskinesia (PCD), polycystic kidney disease (PKD), retinitis pigmentosa and perhaps to cancer. These are the ciliopathies. As a model of primary cilia function, my laboratory is studying the role of ciliary signaling in mouse embryo fibroblast migration by molecular genetic and imaging techniques, including high resolution immunoelectron microscopy and CLEM (correlated light and scanning electron microscopy).

Current projects on which incoming students may work are:

- Immuno light and electron microscopy of mouse embryo fibroblasts as primary cilia grow
- Control of cell migration via primary cilia signal transduction



- Signal transduction molecules as cargo in IFT.
- Role of primary cilia in stem cell differentiation and in human disease
- Evolution of the ciliary barriers: role of the ciliary necklace

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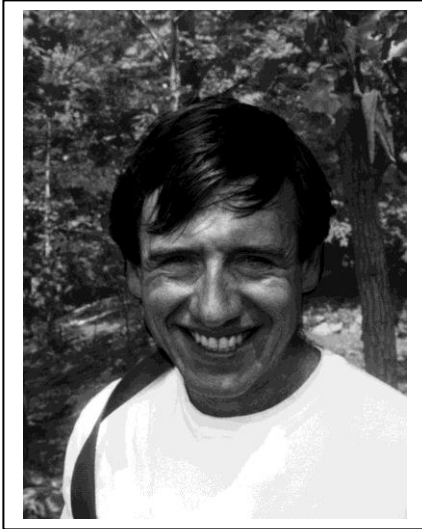
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MECHANISMS OF INVASION AND METASTASIS

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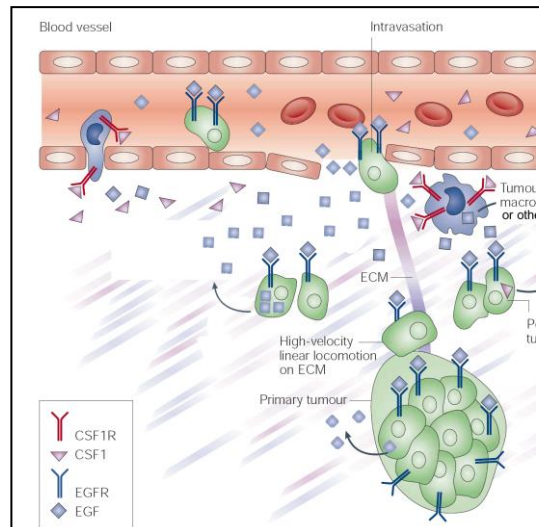


Invasion and Metastasis

For most cancers, the cause of patient death is either local invasion into critical areas or metastasis - dissemination of tumor cells from the primary tumor to many parts of the body followed by formation of new tumors at those distant sites. By understanding the mechanisms by which tumor cells invade and metastasize, we will have better chances of developing appropriate therapies. Tumor cell motility and the orientation of tumor cells by chemotaxis make important contributions to invasion and metastasis.

Three types of cancer are being studied. Glioblastoma and head and neck cancer invade locally into critical areas and the primary tumors are not fully removable by surgery. Breast cancer, although the primary tumor can be fully removed, metastasizes to distant

sites, and the resulting metastases can lead to poor prognosis. Tissue culture studies of invasion and signaling of these cancers are combined with *in vivo* analysis in mice. Two types of *in vivo* approaches being used for studying invasion and metastasis: 1) injection of tumor cells into the orthotopic site (brain, floor of mouth, or mammary fat pad for glioma, head and neck cancer, and breast cancer respectively), and 2) formation of tumors in transgenic mice using oncogenes. The injection assays allow the rapid molecular manipulation of cell lines to identify important signaling pathways that contribute to metastasis. The transgenic mouse system provides a more clinically relevant model in which tumors develop from the appropriate tissue directly.



Simplified diagram of tumor microenvironment focused on tumor cells and macrophages showing potential EGF and CSF-1 gradients.

Tumor cell motility can be visualized directly using expression of green fluorescent protein (GFP) in the tumor cells combined with in vivo imaging of

the tumor cells around the primary tumor. Web site: <http://www.aecom.yu.edu/segalllab/page.aspx>

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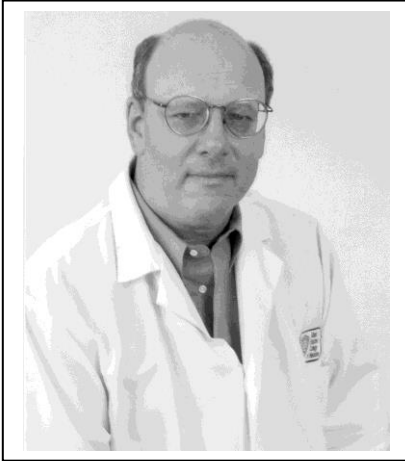
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FOLLOWING THE TRAVELS OF RNA

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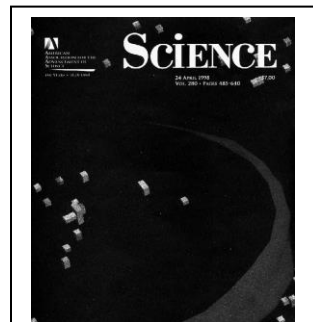
Our work is focused on the expression and travels of RNA within the cell: from the site of its birth to its ultimate biological destiny in the cytoplasm where it makes proteins in specific locations.

Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

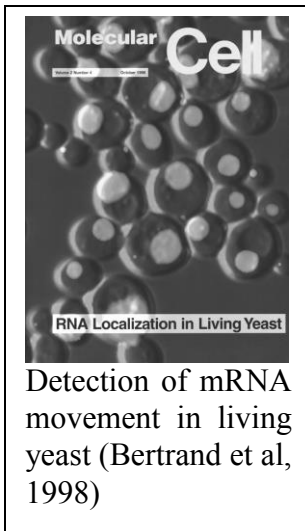
We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. This enables the detection of specific nucleic acid molecules for comparison between normal or cancer cells. This method of molecular diagnosis is the clinical application of the technology. As an additional result of this approach, we have found specific RNA sequences located in particular cellular compartments. An example is the messenger RNA for beta-actin, which is located in the periphery of the cell where actin protein is needed for cell motility.

These transcripts are not free to diffuse, and appear to be associated with a cellular matrix or skeleton from the moment of their synthesis through translation. We are investigating how this spatial information is encoded within the gene and how the RNA transcript is processed within the nucleus and then transported to its correct compartment in the cytoplasm, resulting in asymmetric protein distribution.

RNA localization also occurs in yeast. During budding, a nuclear factor represses mating type switching asymmetrically, only in the daughter cell. This is because the factor is synthesized only in the bud because the mRNA was transported there by a motor, myosin. This discovery has provided a model by which to investigate the mechanisms responsible for moving RNA within the cell. For example, we have constructed genetically altered yeast and vertebrate cells in order to elucidate the sequences responsible for mRNA localization. A reporter gene can be "delivered" to a variety of cellular compartments by using specific sequences, or "zipcodes" from the mRNAs found in those compartments. These "zipcodes" consist of short sequences in the 3' untranslated region of the mRNA.



Single molecules of mRNA being synthesized and diffusing within the nucleus (Femino et al, 1998)



Detection of mRNA movement in living yeast (Bertrand et al, 1998)

Recently we have developed technology that allows us to image RNA movement in living cells and tissues and characterize how the motors connect with and drive the RNA. Recent developments have allowed us

to visualize transcription and RNA life cycle from birth to death in transgenic mice.

Selected Publications:

Halstead, J. M., T. Lionnet, J. H. Wilbertz, F. Wippich, A. Ephrussi, R. H. Singer and J. A. Chao (2015). "Translation. An RNA biosensor for imaging the first round of translation from single cells to living animals." *Science* **347**(6228): 1367-1671

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MOLECULAR MECHANISMS OF MISFOLDED SECRETORY PROTEIN DISEASES

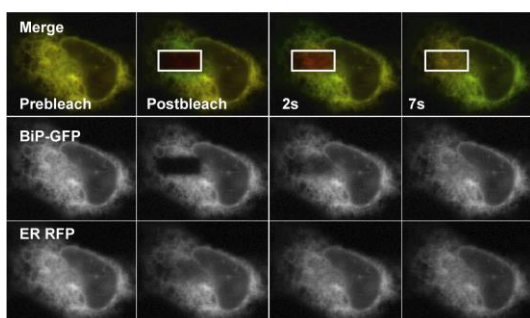
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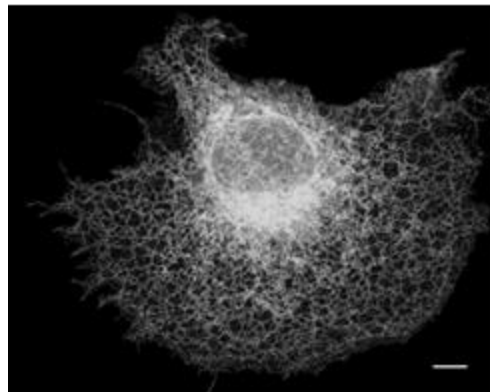
The focus of our laboratory is to understand the cell biology of secretory protein quality control. Specifically, we are using a combination of live cell imaging, biophysical fluorescence methods, biochemistry, and molecular biology to study the regulation, organization and dynamics of secretory protein folding, retention, release and destruction in cells. Our cutting edge quantitative fluorescence microscopy methods including FRAP, FLIP, photoactivation and FRET reveal information about molecules in live cells including protein mobility, environment, protein complex size, protein-protein interactions, and membrane dynamics.



FRAP and comparative recovery of the chaperone BiP, fused to GFP, versus an inert ER reporter, ER-RFP.

The endoplasmic reticulum (ER) is the largest eukaryotic organelle and carries out multiple functions including: 1) secretory protein biosynthesis, and export, 2) protein glycosylation and disulfide bond formation. To prevent protein misfolding, the ER contains a variety of proteins, termed chaperones. A functional secretory protein must be successfully translocated into the ER and then interact with the correct subset of chaperones to correctly fold. Failure of any one of these steps can lead to secretory protein-

misfolding diseases, such as α 1-antitrypsin deficiency.



Confocal micrograph of a Cos-7 cell expressing a luminal ER GFP.

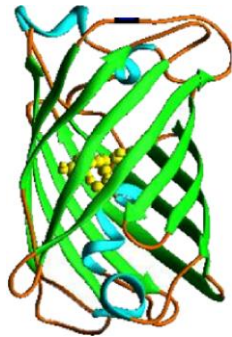
Regulation, organization, dynamics of ER quality control machinery and associated ER stress diseases

Analysis of the human genome has revealed that one fourth of all proteins are either membrane or secretory proteins. These proteins enter the ER through a large multi-protein channel termed the translocon. As the nascent peptides co-translationally insert into the translocon, the peptides encounter chaperones, which promote proper folding, disulfide bond formation, prevent protein aggregation, and form the basis of ER quality control.

Misfolded transmembrane and secretory proteins frequently accumulate in aging cells and are a feature of several ER stress-associated diseases, such as type II diabetes, heart disease, cancer, and α 1-antitrypsin deficiencies.

Our long term goal is to elucidate the physical and molecular basis of homeostasis and misfolded protein stress in the ER. Correct folding and quality control of secretory proteins by ER chaperones is essential for the viability of cells and organisms. Failure to correctly fold proteins results in loss of protein function, can activate the Unfolded Protein Response (UPR), and stimulate apoptotic death. The steps and molecular changes leading to the transition between homeostasis and UPR activation in the ER are poorly understood. The nature of the network of ER chaperones has deep implications for how the ER senses and copes with misfolded protein stress. While much is known about individual components of ER homeostasis and

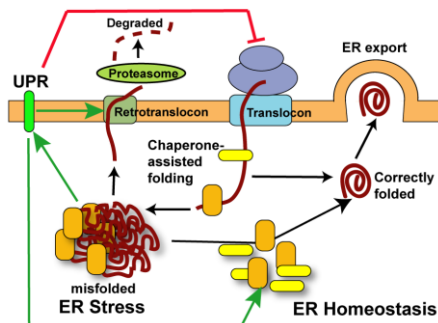
the UPR, the functional consequences of the naive, stressed, and adapted ER folding environments are underexplored. Each folding environment is distinguishable by changes in substrate burden and chaperone network complexity, which will depend on the number, dynamics, and organization of luminal ER chaperones. We have created the first live cell reporters of the unfolded protein burden and for chaperone availability leading to the transitions between ER homeostasis, stress, and UPR adaptation. These reporters and the power of yeast genetics have enabled us to begin characterizing compounds to improve the ER folding environment.



Professor Snapp is a member of the Einstein Center for AIDS Research, and the Gruss-Lipper Biophotonics Center. He is Co-Director for the Einstein Fluorescent Protein Resource Center and Director of the Marion Bessin Liver Research Center Hepatic Imaging and Cell Structure Core. He is course director for the graduate course Quantitative Imaging of Cells.

Development of Fluorescent Proteins and Biosensors for the Secretory Pathway

The environment of the ER places special constraints on fluorescent proteins (FPs). Specifically, the environment is oxidizing and is the site of protein glycosylation. Many FPs are subject to these modifications, which either complicate use of the FPs or even render the FPs unusable. We have adapted FPs to remove cysteines and glycosylation consensus sequences. In addition, the same posttranslational modifications can be exploited to turn FPs into reporters and biosensors.



Secretory protein translocation, folding, and Quality Control.

FLUORESCENT PROTEINS AND APPLICATIONS

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The **long-term goal** of our laboratory is development of a collection of chromophore containing molecular nanotools based on fluorescent proteins, which could be employed for analysis, manipulation or modification of biochemical processes in living cells, tissues and organisms with light photons. This growing field was termed as a molecular biophotonics reflecting its essence: interaction between photons and biomolecules. Cloning of homologs of a green fluorescent protein (GFP), which emit not only green but also yellow, red and far-red fluorescence, provided a powerful boost for labeling and detection technologies due to availability of colors and biochemical features never before encountered in GFP variants. Recent studies in evolution of GFP-like proteins suggest that the spectroscopic and photochemical properties of the known fluorescent proteins represent just a fraction of the naturally occurring diversity, and it is very possible to stumble upon proteins with completely new combinations of useful features.

Design and characterization of molecular biophotonic tools require highly

interdisciplinary research including molecular biology, structural biology, computer modeling, analytical and organic chemistry, and living cell microscopy. Methods from all of these fields we extensively use in our laboratory.

Photoactivatable and kindling fluorescent proteins (PAFPs and KFPs) are irreversible and reversible photoactivatable probes, respectively. They are capable of switching from a dark to a fluorescent state in response to the irradiation by a light of the specific wavelength, intensity and duration. KFPs and PAFPs are excellent tools for the precise optical labelling and tracking of proteins, organelles and cells within living systems in a spatiotemporal manner. They bring a new dimension to the kinetic microscopy of living cells, which has been traditionally associated with fluorescence recovery after photobleaching approaches.

Molecular biosensors consisted of GFP variants fused with sensitive domains, such as specific binding peptides or scaffolds, made significant progress last years. However, GFP-based fusions have a low range of fluorescence contrast. In this respect, KFPs with their capability to drastically change fluorescent intensity represent promising templates for the next generation of biosensors. Our results show that besides the light-irradiation, a partial loosening of KFP structures also results in the chromophore triggering between the dark and fluorescent states. KFPs fused with sensitive domains will result in biosensors that exhibit the fluorescence changes of two orders of magnitude and thus will allow spatiotemporal visualization of extremely low levels of intracellular signalling.

We also develop **fluorescent timers** (FTs) that change their color from blue-green to orange-red with time. FTs are biophotonic tools for visualization of up- and down-

regulation of target promoters, relative age of organelles and vesicles, and cell differentiation. For example, newly formed vesicles tagged with a FT, which changes the fluorescence from green to red, will mostly contain green FT at early stages of their maturation, older vesicles will be yellow or completely red due to presence of the mature red FT. Similarly, when FT is expressed in tissue under a target promoter, the green fluorescent areas will indicate recent promoter activation, yellow regions will correspond to continuous promoter activity and red fluorescence will denote areas in which promoter activity is stopped.

KFPs and PAFPs open new perspectives to studies of protein–protein interactions using **Forster resonance energy transfer** (FRET). FRET is a non-radiative transfer of energy from one excited chromophore, called donor, to another chromophore, called acceptor, which is in a close proximity to the donor and has appropriate excitation spectrum, overlapping with donor emission. FRET results in a fluorescence of the acceptor when the donor is excited by external light. Photoactivatable PA-FRET microscopy will make it possible to study spatiotemporal localization and interaction of fusion proteins simultaneously. PA-FRET detected between PAFP donor and acceptor in one cellular compartment while the photoactivation occurred in different cellular region could indicate that the target protein fused with PAFP moved between these two places. PA-FRET pair can be also used as a benchmark for determining intermolecular distances in a living cell.

Papers

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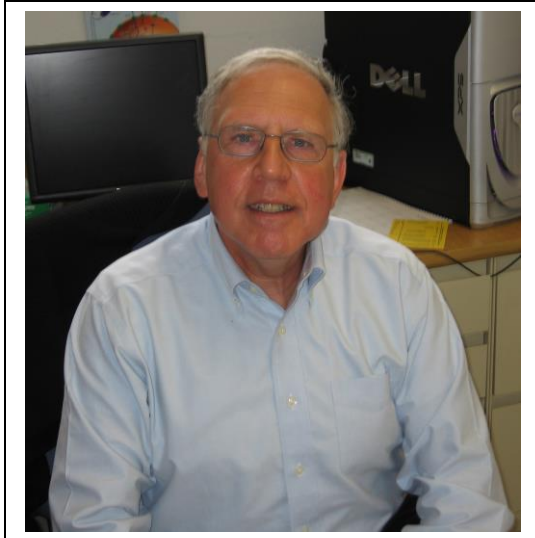
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CELL BIOLOGY OF HEPATOCYTE TRANSPORT MECHANISMS

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A major function of the hepatocyte is the removal of xenobiotic and endogenous organic anionic compounds from the circulation. Much of this transport activity resides in the hepatocyte. The focus of our research has been elucidation of two interrelated, physiologically important hepatocellular transport mechanisms, one for anionic drugs and the other for receptor-mediated endocytosis.

We have identified and cloned members of what has turned out to be a new family of organic anion transport proteins (oatps). They have 12 transmembrane domains and similar biochemical characteristics. Although evidence suggests that the oatps are important in clearance of drugs from the circulation, little is known regarding the mechanism by which they act, their oligomerization state, or mechanisms for subcellular trafficking. In recent studies, we found that many of the oatps have PDZ consensus binding domains and interaction of oatp1a1, a major oatp of the hepatocyte, with PDZK1 is required for its

expression on the cell surface. We have found that oatps and several other important drug transporters cycle on microtubules between the cytosol and cell surface, regulated by transporter-specific kinases, nanomotors, and accessory proteins such as Rabs. Elucidation of these novel mechanisms may provide an important link between trafficking of these transporters and alterations in their function that could result in drug toxicity.

These studies of mechanisms of transporter trafficking relate to our other studies of receptor-mediated endocytosis, characterized by internalization of ligand-receptor complexes into an endocytic vesicle (endosome). Subsequently, these complexes dissociate as the endosome acidifies, and ligand and receptor segregate into separate compartments. Ultimately ligand traffics to the lysosome where it is degraded, while receptor recycles to the cell surface where it is reutilized. Our previous studies have shown that this segregation process requires integrity of microtubules. We are investigating the role of microtubules in providing a directed path for these processes and the potential importance of microtubule-associated motor molecules such as kinesins and dynein in providing the force for vesicular movement. To accomplish this, we have devised a cell free *in vitro* system to dissect the functional components of these processes. In this system, endocytic vesicles on microtubules can be viewed using microscopy technologies that permit quantitation of direction and rates of movement. We have reconstituted vesicle fission and segregation, and identified regulatory proteins. Using a proteomics-based

approach on highly purified endocytic vesicles, we have discovered a number of novel vesicle-associated proteins and are pursuing studies to define their role in the endocytic process.

Recent Publications:

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Yuan, F., Snapp, E.L., Novikoff, P.M., Suadicani, S.O., Spray, D.C., Potvin, B., Wolkoff, A.W., Stanley, P. Human liver cell trafficking mutants: characterization and whole exome sequencing. *PLoS. One.* 9: e87043, 2014.

GRADUATE PROGRAM REQUIREMENTS

Dept. Anatomy & Structural Biology

Albert Einstein College of Medicine

All graduate students at Einstein are in the Sue Golding Graduate Division of Medical Sciences, and there is extensive interaction at all levels between members of different departments. Ph.D. degrees are granted by each individual department and the requirements of different departments vary slightly. The graduate program of the Department of Anatomy and Structural Biology has features that are unique among the AECOM departments. These are designed to equip the student with a broad base of fundamental knowledge in cell and molecular biology suitable for a research career in academia or industry. The department chooses students for admission who are strongly committed to research careers in cell and molecular biology and who seek special training with one of the members listed in this brochure.

As described in more detail below, a typical graduate career lasts about 5 years. In addition to taking classes, first year students can rotate through laboratories in any basic science department in order to select a thesis advisor. Students choosing an advisor in the Department of Anatomy and Structural Biology are encouraged to work in any of the laboratories of the department to learn specific techniques, as the department has an open door policy by all investigators. During the second year, students begin exploring possible thesis projects in their home laboratory as well as completing classwork. At the end of the second year, a qualifying examination determines the readiness of the student to begin thesis work. Passing the qualifying examination admits a student to candidacy for the Ph.D., leading to full time research on the thesis topic. Upon completion of research (about three years after passing the qualifying examination), the thesis work is written up and presented to a thesis examination committee. Successful completion of the thesis examination results in the student being awarded a Ph.D. in Biological Sciences.

I Admission Requirements

Students are admitted to the Sue Golding Graduate School and to the department based on the following credentials:

1. GRE scores
2. Letters of recommendation (minimum of three)
3. Transcripts
4. Essay of career goals
5. Personal interview, if possible

MSTP students / Alternate Path students are admitted with the advisor's agreement and similar rules apply for this program as for the graduate program.

II Courses

The Department requires that students take Biochemistry, Molecular Cell Biology (Parts A and B), Quantitative Skills for the Biomedical Researcher, Responsible Conduct of Research, and three electives for a minimum of 21 credits. Students are expected to participate in the ongoing Departmental Seminars, Retreats and laboratory forums. The

Department encourages students to choose Histology as one of the electives (either in the summer MSTP course or in the fall). Students who have passed Histology can then acquire teaching experience by teaching the course as a laboratory instructor. A supplementary stipend is provided for teaching the course. Graduate students are expected to pass all required coursework before proceeding to the qualifying examination.

III Advisory Committee

The role of the Advisory committee is to help in the choice of courses to be taken and to oversee that academic and research progress is satisfactory. The Advisory Committee consists of four to five faculty members, at least one from within the department (primary or secondary) in addition to the mentor. The composition of the Advisory committee can be changed as appropriate and must be approved by the thesis advisor and the Anatomy and Structural Biology Graduate Committee.

After students have declared a lab in the Anatomy and Structural Biology Department, they must assemble an Advisory Committee and have that committee approved by the Anatomy and Structural Biology Graduate Committee by November 30th of the first year in the department. The student must have an Advisory Committee meeting by the end of February of that academic year.

The Advisory Committee will meet at least once a year during the student's second and third academic years. From year four and on, the student will meet twice a year with his/her Advisory Committee. The student is required to prepare a one-page progress summary for the yearly meeting. This summary should be handed out to each committee member one week prior to the meeting. At the beginning of the meeting the student will have the opportunity to talk to the Advisory committee members in the absence of the thesis advisor. Subsequently the student will briefly leave the room. The committee must forward a report of the student's progress to the chairperson of the Anatomy and Structural Biology Graduate Committee. Continued enrollment in the program is predicated on satisfactory progress, as recommended by the Advisory committee and endorsed by the Graduate Committee of the Department on a year-by-year basis.

IV Examinations

A. The Qualifying examination follows the uniform AECOM-wide format, except that ASB students will take the exam in the Spring of the second year of the graduate program. However, ASB students will take the second year Fall exam preparation course along with all other second year graduate students. For details see the Academic Policies and Guidelines of the Sue Golding Graduate Division. For qual exam due date and dates for setting up the examination please contact the Graduate Division. If you have any questions, please contact Dr. Snapp.

B. The Thesis examination should be taken within 3 years of passing the qualifying examination. Candidacy for a Ph.D. will terminate three years after taking the qualifying examination and admittance to candidacy. In exceptional cases, with the endorsement of the advisor, a candidate may apply to the Graduate committee for an extension of this deadline.

1. The Thesis committee must contain three faculty members from the Department of Anatomy and Structural Biology, two faculty members from outside of the department, and one member from an outside university (6 minimum). The Chair of the committee should be selected by the student and mentor. It is strongly recommended that the Chair be a more senior member of the faculty (Associate or Full Professor).
2. The Thesis examination includes:
 - a) A thesis which must be delivered in final form to each member of the Thesis committee at least three weeks prior to the thesis defense.
 - b) A public departmental seminar to be presented on the thesis immediately preceding the thesis defense.
 - a) A thesis defense before the thesis committee in which the student answers questions on the thesis.

V Other Degree Granting Programs

Entrance into any other degree granting program cannot occur while enrolled in the graduate (Ph.D.) program in this department.

VI Other Requirements

There are formal and informal seminar series at which attendance is mandatory. There is a student/postdoc run work in progress seminar series in which all students participate. In addition, individual laboratories and interest groups run journal clubs and informal presentations that the students are required to attend. Once a year the students and postdocs arrange seminars by distinguished outside lecturers. Students are encouraged to participate in biannual department retreats.

VII Postdoctoral Training

The philosophy of the Department of Anatomy and Structural Biology is to actively encourage our graduate students to do their postdoctoral work in other institutions in order to broaden their experience. The department has a record of placing students in postdoctoral positions at major universities, often with prestigious Fellowships.