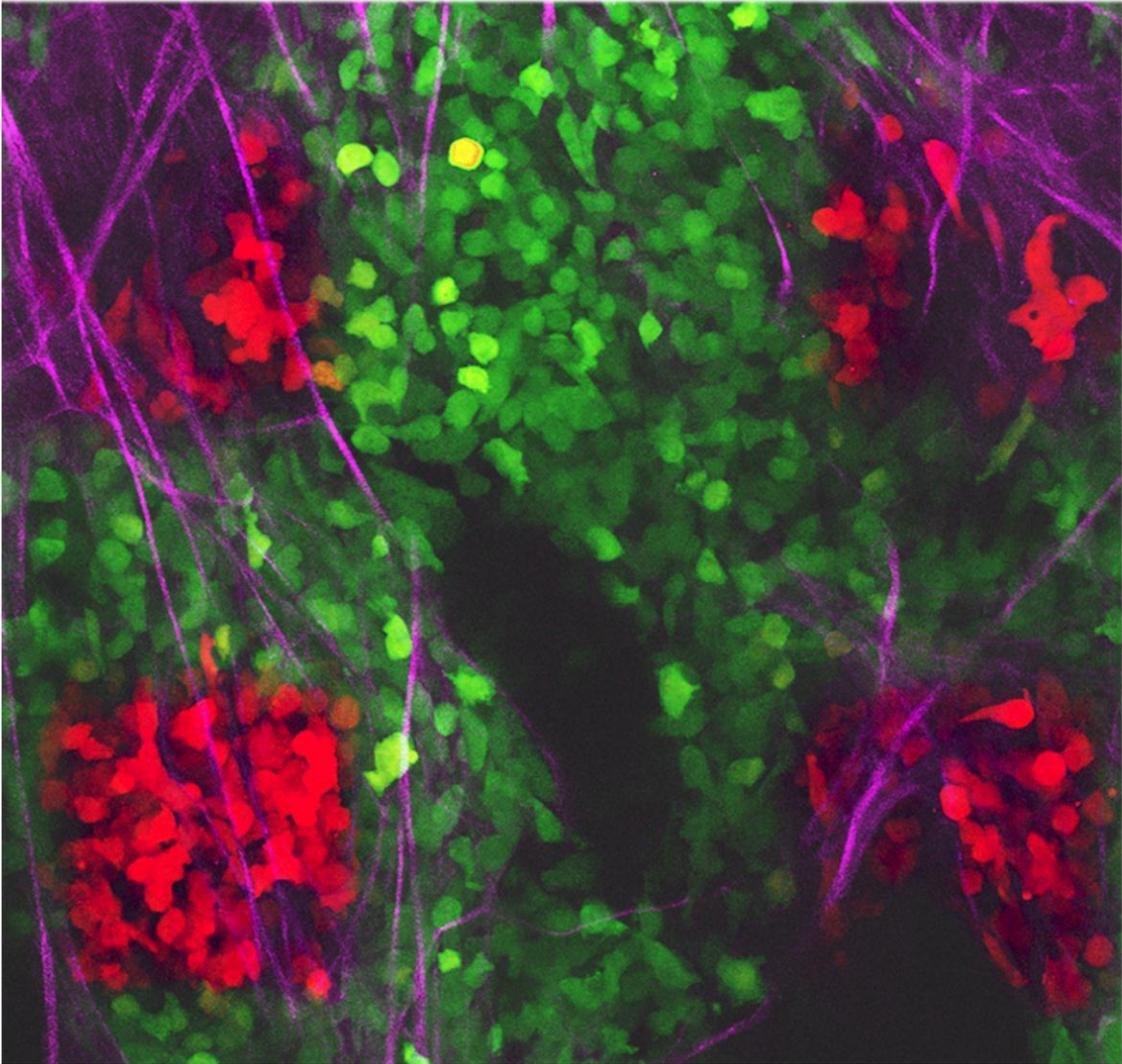


**2021 - 2022**  
**GRADUATE TRAINING**  
**IN CELL AND MOLECULAR BIOLOGY**



**Photoconversion of tumor cells in living animal with a mammary tumor (image taken by Dr. Bojana Gligorijevic) using a mammary imaging window<sup>1</sup> and a custom built multiphoton microscope<sup>2</sup>.**  
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**

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## 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 their laboratory and provide 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, are Work in Progress seminars where graduate and postdoctoral students present their newest results. There are also regular weekly meetings of the students and faculty in most laboratories and a graduate and postdoctoral student group that get together frequently to discuss their mutual concerns about science, course work, and instrumentation. In addition to the Department of Anatomy and Structural Biology, there are nine 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, molecular and cancer 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 <https://www.einsteinmed.org/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.

Best,

Dr. John S. Condeelis, Professor and Co-Chair

Dr. Robert H. Singer, Professor and Co-Chair

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**GRADUATE PROGRAM REQUIREMENTS**  
**Department of Anatomy & Structural Biology**  
**Albert Einstein College of Medicine**

All graduate students at Einstein are in the Sue Golding Graduate Division of Medical Sciences. Ph.D. degrees are granted by each department and the requirements vary. The Department chooses students for admission who are strongly committed to research in cell and molecular biology and who seek research training with faculty members listed in this brochure. Successful completion of the thesis examination results in the student being awarded a Ph.D. in Biological Sciences.

**I**      **Courses**

The following courses are required:

- 1) Biochemistry
- 2) Molecular Cell Biology
- 3) Quantitative Skills for the Biomedical Researcher
- 4) Responsible Conduct of Research

Additionally, students have to pass three (3) elective courses.

The Department encourages students to choose Histology (in the summer MSTP course) as one of the electives. With approval of their mentor, students who have passed Histology can then acquire teaching experience as a Histology laboratory instructor. A supplementary stipend is provided for teaching the course.

**II**      **Advisory Committee**

The role of the Advisory committee is to help in the choice of elective courses to be taken and to oversee that academic and research progress is satisfactory.

- 1) The committee consists of four to five faculty members,
  - a. At least one from within the department (primary or secondary) in addition to the mentor.
  - b. The composition of the Advisory committee can be changed as appropriate and must be approved by the thesis advisor and the Departmental Graduate Committee.
- 2) After a student has declared a lab in the Department, the Advisory Committee must be assembled and approved by the Departmental Graduate Committee by November 30th of the first year in the department.
- 3) The student must have an Advisory Committee meeting by the end of February of that academic year.

- 4) The Advisory Committee shall meet at least once a year during the student's second and third academic years. From the fourth year, the Advisory Committee shall meet twice a year.
  - a. A one-page progress summary must be prepared by the student and distributed to the Committee one week prior to each meeting.
  - b. At the beginning of the meeting the advisor talks to the Advisory Committee in the absence of the student. After the student has returned, the thesis advisor will briefly leave the room.
  - c. The Advisory Committee shall forward a report of the student's progress to the chairperson of the Departmental Graduate Committee.
  - d. 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.

### **III Examinations**

#### Qualifying Examination

- 1) Students shall pass all required coursework before scheduling the qualifying examination.
- 2) Graduate Division administers the examination, with the exceptions that:

ASB students shall take the exam in the Spring of the second year of the graduate program after they passed all required course work.
- 3) ASB students shall take the second year Fall exam preparation course along with all other second year graduate students and follow the schedule of the Graduate Division for writing and handing-in the proposal.

#### Publication Requirement for Graduation

- 1) Publication of at least one first-author peer reviewed research paper is required, three weeks prior to scheduling the thesis defense. The Departmental requirement supersedes that of the Graduate Program.
    - a. Published means accepted for publication, i.e. "in press" or later.
    - b. A co-first authorship publication meets the requirement.
-

### Thesis Examination

- 2) The Thesis Examination follows the uniform Einstein-wide format. The Departmental requirements supersede those of the Graduate Program.
  - a. The Examination should be taken within 3 years of passing the qualifying examination. However, with the endorsement of the advisor, a candidate may apply to the Departmental Graduate Committee for an extension of this deadline.
- 3) The Thesis committee shall consist at a minimum of 6 examiners:
  - a. 3 primary faculty members from the Department
  - b. 2 faculty members from outside of the department
  - c. 1 faculty member from outside Einstein.
- 4) The Chair of the committee must be a senior rank primary faculty member and shall be selected by the student and the mentor.
- 5) The Thesis must be approved by the advisor 4 weeks prior to the date of defense.
- 6) The Thesis must be delivered in final form to members of the committee 3 weeks prior to the date of defense.
- 7) The Chair of the committee signs off that the thesis document is defensible.
- 8) In the Thesis, all collaborative work that contributes to the Dissertation must be clearly indicated. Each Chapter should indicate which publications are represented by the described work.
- 9) A public-forum Departmental seminar shall be presented on the thesis.
- 10) A closed-forum thesis defense shall follow immediately after the public-forum.

### **IV Other Degree Granting Programs**

Entrance into any other degree granting program is not allowed while enrolled in the graduate (Ph.D.) program in this department.

### **V Other Departmental Requirements**

- 1) Attendance in Departmental Seminars.
- 2) Attendance and participation in Departmental Works in Progress.
- 3) Attendance in Departmental Retreats.

Approved by the ASB Graduate Committee

Date 4-24-20

# SINGLE MOLECULE IMAGING OF TRANSCRIPTION FACTORS INVOLVED IN CANCER AND AGEING

**Dr. Robert Coleman**

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Email: robert.coleman2@einsteinmed.org**



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.

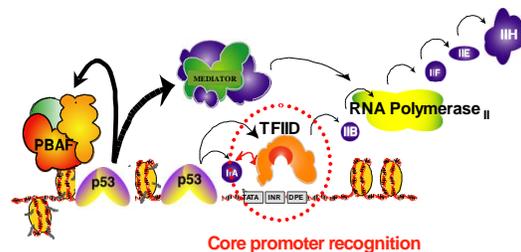
Gene expression initiated by mammalian RNA polymerase II (Pol II) involves a highly coordinated assembly of over 100 different polypeptides residing within several multi-subunit complexes to form the pre-initiation complex (PIC). One critical step in gene activation involves direction of the core recognition 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**

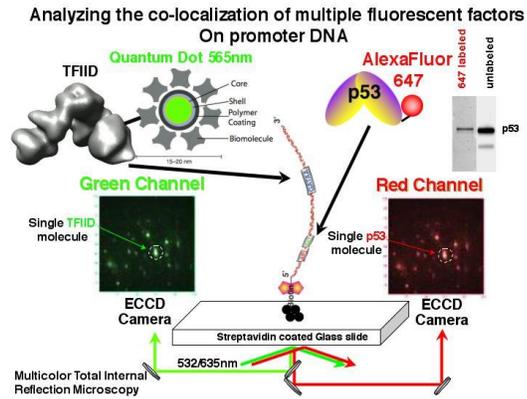


**Im**  
**aging the dynamic assembly of the transcription PIC in real time**

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.

# Tumor MicroEnvironment of Metastasis

**Dr. John S. Condeelis**

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**For more information, visit: <https://www.einsteinmed.org/faculty/6712/john-condeelis/>**



## **Areas of Research:**

Cell biology and biophysics, cancer biology, and the integration and validation of clinical imaging platforms with high resolution intravital multiphoton microscopy. Development of multiphoton imaging technology to identify tumor cell dissemination mechanisms in both primary and secondary tumor sites during metastatic progression. Development of new optical microscope technology in the Gruss Lipper Biophotonics Center.

## **Professional Interests:**

John Condeelis' 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 micro-environments in mammary tumors. Integration of intravital multiphoton imaging with computational /systems analysis of living breast tumors identified the dominant tumor cell phenotypes contributing to invasion and dissemination during metastasis. This led to the discovery and verification of the paracrine interaction between tumor cells and macrophages in vivo, the role of macrophages in the migration of tumor cells during HGF-

dependent tumor cell streaming to blood vessels and the mechanism of tumor cell dissemination from primary tumors via TMEM (Tumor MicroEnvironment of Metastasis) to distant metastatic sites. Based on these results, cell collection techniques, including the in vivo invasion assay 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, and the TMEM, MenaCalc and MenaINV markers for assessing risk of metastasis and prediction of response in breast cancer patients to both chemotherapy, and receptor tyrosine kinase and tyrosine kinase inhibitors used to suppress metastasis.

John Condeelis has devised optical microscopes for uncaging, biosensor detection and multiphoton imaging 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. He is one of the founding co-directors of the Integrated Imaging Program dedicated to the integration and validation of clinical imaging platforms, including digital pathology, with high resolution optical imaging in the Gruss Lipper Biophotonics Center. He has authored more than 300 scientific papers on various aspects of his research.

### **Selected Publications - in the last 3 years**

Entenberg, D., Oktay, M.H., D'Alfonso, T., Ginter, P.S., Robinson, B.D., Xue, X., Rohan, T.E., Sparano, J.A., Jones, J.G., and Condeelis, J.S. (2020) Validation of an Automated Quantitative Digital Pathology Approach for Scoring TMEM, a Prognostic Biomarker for Metastasis. Cancers (Basel) 124,. [PMID: 32244564].

Ginter PS, Karagiannis GS, Entenberg D, Lin Y, Condeelis J, Jones JG, Oktay MH. (2019) Tumor Microenvironment of Metastasis (TMEM) Doorways Are Restricted to the Blood Vessel Endothelium in Both Primary Breast Cancers and Their Lymph Node Metastases. Cancers (Basel). 2019 Oct 8;11(10). pii: E1507. doi: 10.3390/cancers11101507. [PMID: 31597373]

Coste-Abramson A, Oktay MH, Condeelis JS, Entenberg D. (2019) Intravital Imaging Techniques for Biomedical and Clinical Research. Cytometry Part A. [PMID: 31889408].

Stone OJ, Pankow N, Liu B, Sharma VP, Eddy RJ, Wang H, Putz AT, Teets FD, Kuhlman B, Condeelis JS, Hahn KM. (2019) Optogenetic control of cofilin and alphaTAT in living cells using Z-lock. Nature Chem Biol. (2019)15:1183-90. Epub 2019/11/20. doi:10.1038/s41589-019-0405-4. [PubMed PMID: 31740825; PMCID: PMC6873228].

DesMarais V, Eddy RJ, Sharma VP, Stone OJ, Condeelis JS. (2019) Optimizing leading edge F-actin labeling using multiple actin probes, fixation methods and imaging modalities. Biotechniques. 2019 Mar; 66(3):113-119. doi: 10.2144/btn-2018-0112. [PMID: 30869550]

Nobre AR, Entenberg D, Wang Y, Condeelis J, Aguirre-Ghiso JA. (2018) The Different Routes to Metastasis via Hypoxia-Regulated Programs. Trends Cell Biol. (18)30109-0. [PMID: 30041830]

Norwood Toro LE, Wang Y, Condeelis JS, Jones JG, Backer J, Bresnick AR. (2018) Myosin-IIA Heavy Chain Phosphorylation on S1943 Regulates

Tumor Metastasis. Exp Cell Res. (18)30369-0. [PMID: 29953877/PMCID: In Process]

Meirson T, Genna A, Lukic N, Makhnii T, Alter J, Sharma VP, Wang Y, Samson AO, Condeelis JS, Gil-Henn H. (2018) Targeting invadopodia-mediated breast cancer metastasis by using ABL kinase inhibitors. Oncotarget. 24; 9(31):22158-22183. [PMCID: PMC5955141]

Arwert, E.A, Harney, A.S., Entenberg, D., Wang, Y., Sahai, E., Pollard, J.W., Condeelis, JS (2018) A unidirectional transition from migratory to perivascular macrophage is required for tumor cell intravasation. Cell Rep. 23(5):1239-1248. PMCID: PMC5946803

Cabrera RM, Mao SPH, Surve CR, Condeelis JS, Segall JE. (2018) A novel neuregulin - jagged1 paracrine loop in breast cancer transendothelial migration. Breast Cancer Res. 2018 20(1):24. [PMCID: PMC5894135]

Karagiannis GS, Condeelis JS, Oktay MH. (2018) Chemotherapy-induced metastasis: mechanisms and translational opportunities. Clinical & Experimental Metastasis. Jan 6. [PMCID: PMC5762276]

Genna, A., Lapetina, S., Lukic, N., Twafra, S., Meirson, T., Sharma, V.P., Condeelis, J.S. and Gil-Henn, H (2018) Pyk2 and FAK differentially regulate invadopodia formation and function in breast cancer cells. Journal of Cell Biology. 217(1):375-395. [PMCID: PMC5748976]

Karagiannis GS, Condeelis JS, Oktay MH. (2017) Chemotherapy-induced metastasis in breast cancer. Oncotarget, 8(67):110733-110734 [PMCID: PMC5762276]

Linde N, Casanova-Acebes M, Sosa MS, Mortha A, Rahman A, Farias E, Harper K, Tardio E, Reyes Torres I, Jones J, Condeelis J, Merad M, Aguirre-Ghiso JA. (2018) Macrophages orchestrate breast cancer early dissemination and metastasis. Nat Commun. 9(1):21. [PMCID: PMC5750231]

Roh-Johnson M, Shah AN, Stonick JA, Poudel KR, Kargl J, Yang GH, di Martino J, Hernandez RE, Gast CE, Zarour LR, Antoku S, Houghton AM, Bravo-Cordero JJ, Wong MH, Condeelis J, Moens

CB. (2017) Macrophage-Dependent Cytoplasmic Transfer during Melanoma Invasion In Vivo. *Developmental Cell.* 4;43(5):549-562.e6. [PMCID: PMC5728704]

Entenberg D, Voiculescu S, Guo P, Borriello L, Wang Y, Karagiannis GS, Jones J, Baccay F, Oktay M, Condeelis J. (2017) A permanent window for the murine lung enables high-resolution imaging of cancer metastasis. *Nature Methods.* 15(1):73-80 [PMCID: PMC5755704]

Sparano JA, Gray R, Oktay MH, Entenberg D, Rohan T, Xue X, Donovan M, Peterson M, Shuber A, Hamilton DA, D'Alfonso T, Goldstein LJ, Gertler F, Davidson NE, Condeelis J, Jones J. (2017) A metastasis biomarker (MetaSite Breast $\alpha$ , $\beta$  Score) is associated with distant recurrence in hormone receptor-positive, HER2-negative early-stage breast cancer. *NPJ Breast Cancer.* 8;3:42.[PMCID: PMC5678158]

Donnelly, S.K., Cabrera, R., Mao, S.P.H., Christin, J.R., Wu, B., Guo, W., Bravo-Cordero, J.J., Condeelis, J.S., Segall, J.E., and Hodgson, L. (2017) Rac3 regulates breast cancer invasion and metastasis by controlling adhesion and matrix degradation. *Journal of Cell Biology* 4;216(12):4331-4349. [PMCID: PMC5716284]

Entenberg D, Pastoriza JM, Oktay MH, Voiculescu S, Wang Y, Sosa MS, Aguirre-Ghiso J, Condeelis J.(2017) Time-lapsed, large-volume, high-resolution intravital imaging for tissue-wide analysis of single cell dynamics. *Methods.* 1;128:65-77. [PMCID:PMC5659295]

Harney, A.S., Karagiannis, G.S., Pignatelli, J., Smith, W.C., Kadioglu, E., Wise, S.C., Hood, M.M., Kaufman, M.D., Leary, C.B., Lu, W-P, Al-Ani, G., Chen, M., Entenberg, D., Oktay, M.O., Wang, Y., Chun, L., De Palma, M., Jones, J.G., Flynn, D.L. and Condeelis, J.S. (2017) The selective Tie inhibitor rebastinib blocks recruitment and function of Tie2<sup>Hi</sup> macrophages in breast cancer and pancreatic neuroendocrine tumors. *Molecular Cancer Therapeutics.* 16(11):2486-2501. [PMCID: PMC5669998]

Jiang C, Ding Z., Joy M., Chakraborty S., Kim S., Bottcher R., Condeelis J., Singh S., Roy P. (2017)

A balanced level of profilin-1 promotes stemness and tumor-initiating potential of breast cancer cells *Cell Cycle* [PMCID: PMC5788419]

Karagiannis GS, Pastoriza JM, Wang Y, Harney AS, Entenberg D, Pignatelli J, Sharma VP, Xue EA, Cheng E, D'Alfonso TM, Jones JG, Anampa J, Rohan TE, Sparano JA, Condeelis JS, Oktay MH. (2017) Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism. *Science Translational Medicine* 5;9(397). [PMCID: PMC5592784]

Brian J. Rosenberg, B.J., Gil-Henn, H., Mader, C.C., Halo, T., Yin, T., Condeelis, J.C., Machida, K., Yu, Y.I. and Koleske, A.J. (2017) Phosphorylated cortactin recruits Vav2 guanine nucleotide exchange factor to activate Rac3 and promote invadopodial function in invasive breast cancer cells. *Molecular Biology of the Cell.* 15;28(10). [PMCID: PMC5426849]

Eddy, RJ, Weidmann, M and Condeelis, J.S. (2017) Tumor cell invadopodia: Invasive protrusions that orchestrate metastasis. *Trends in Cell Biology.* [PMCID: PMC5524604]

# MACROPHAGE PHAGOCYTOSIS AND MOTILITY

Dr. Dianne Cox

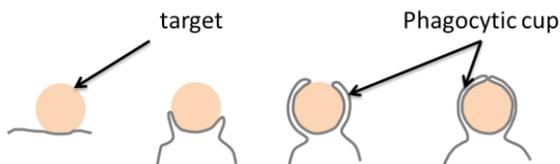
Laboratory: Price 207 Phone: 718-678-1066  
E-mail: Dianne.cox@einsteinmed.org



## Macrophage Phagocytosis and Motility

*Macrophages play important roles 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.*

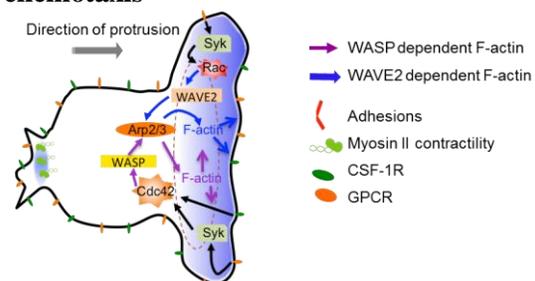
## Studying the molecular mechanisms of phagocytosis



Among their many roles, macrophages are best known for their striking ability to engulf a large number of big (>0.5 $\mu$ m) particles that are very diverse in size and shape in a process called phagocytosis. Phagocytosis is important in many situations such as the clearance of pathogen and particles (bacteria, yeast, pollen and pollutants). Crucially, phagocytosis may also be a major player of

cancer immunity by mediating the engulfment and killing of cancer cells. Phagocytosis requires actin assembly, pseudopod extension, and phagosome closure (Figure 1). Actin polymerization in response to particle binding requires the activation of members of the Rho GTPases, either Rac or Cdc42 for Fc gamma Receptor-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. RhoG, another family member, is also important for phagocytosis but the precise role of RhoG is currently unknown. We are exploring the roles of these signaling pathways as well as those regulating the myosin family of molecular motors in phagocytosis. We are employing a novel technique called traction force microscopy to understand the roles of these factors in the protrusive forces needed to engulf the diverse particles of various sizes and stiffnesses found in nature.

## Studying the molecular mechanisms of chemotaxis



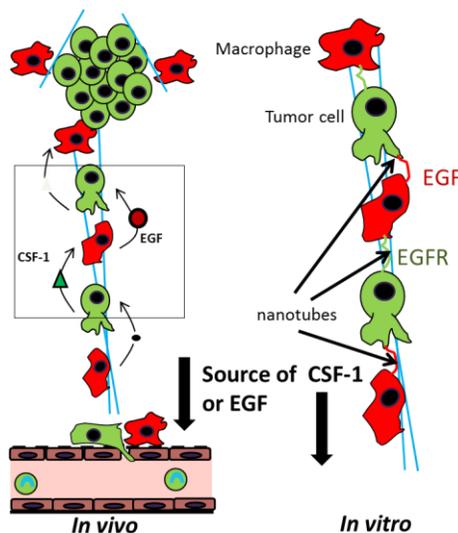
The directed movement of cells in response to chemoattractants involves several complex, interrelated processes, including directional or chemotactic sensing, polarity, and motility. These processes are mediated by complex, interacting signaling pathways that appear to have many similarities but yet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

result in the appearance of different structures (Figure 2). 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.

### Determining 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 using multiphoton microscopy (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 (Figure 3).

### Development of tools for live cell imaging

In order to determine the role of individual molecules in macrophage functions it is essential to understand the timing and localization of specific protein involved. We have been actively involved in the generation of new probes for single live cell imaging including photoconvertible actin probes that label various structures in live cells. We have also developed and employed biosensors to monitor localized protein activity in live cells, including the recent work in collaboration with Dr. Louis Hodgson on the development of isoform specific single chain RhoGTPase biosensors.

### Selected Publications (Students in bold)

**Abou-Kheir, W.**, Gevrey, J-C., Issac, B.M., Yamaguchi, H., and Cox, D. (2005) A WAVE2/Abi1 complex mediates CSF-1-induced F-actin rich membrane protrusions and migration in macrophages. *J. Cell Science* 118:5369-5379.

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# AUTOPHAGY IN AGING AND AGE-RELATED DISEASES

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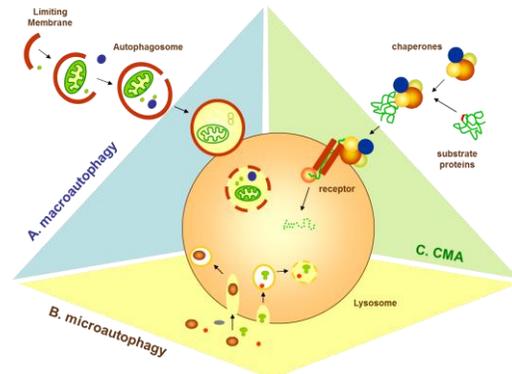


The **main focus** of our laboratory is on understanding how cytosolic proteins are transported into lysosomes for their degradation (*autophagy*) and how impaired autophagy contributes to aging and age-related diseases.

A common feature of most cells in aging organisms is the accumulation of abnormal or damaged proteins in their cytosol that impair cellular function. Protein accumulation results, at least in part, from impaired protein degradation with age. We have previously identified the activity of a selective pathway for the degradation of cytosolic proteins in lysosomes known as *chaperone-mediated autophagy (CMA)*. The **main goal of our research** is to identify the defect(s) that lead to the decreased activity of CMA and other forms of selective autophagy with age and in age related pathologies, and to determine if the correction of these defects in old cells leads to an improvement in cellular function.

**Chaperone-mediated autophagy (CMA)** and **endosomal-microautophagy (eMI)** (co-discovered by our group) are two forms of autophagy that degrade proteins bearing a specific targeting motif present in about 75% of cytosolic proteins. Both pathways are activated in response to different stressors but they also play important regulatory roles by degrading fully functional

proteins to terminate their function. Substrate proteins are selectively recognized by cytosolic chaperones (hsc70 and cochaperones) that stimulate their binding to a receptor in the lysosomal membrane (LAMP-2A) in the case of CMA, or directly to lipids in the membrane of late endosomes in the case of eMI. The transport of substrates into lysosomes in CMA 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 CMA and eMI.** - We can isolate intact lysosomes and late endosomes from several tissues (liver, kidney and spleen) of rodents and use comparative proteomics to identify new CMA and eMI components. We are also using full-genome CRISPRi screens to identify novel regulators of these pathways under normal physiological conditions and in diseases related with aging. To learn more about the physiology of these pathways, we have also developed fluorescent functional reporters that allow us to identify changes in CMA and eMI in intact cells, and in the case of CMA a reporter mouse model where we can study spatial and temporal properties of CMA activation using two photon microscopy.

**2. Understanding the consequences of the age-related defect in CMA.** We have generate conditional and inducible transgenic mouse models incompetent for CMA in different tissues and are investigating 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 phenocopies many aspects of aging such as degenerative traits in tissues, metabolic alterations, loss of stemness and increase cancer propensity.

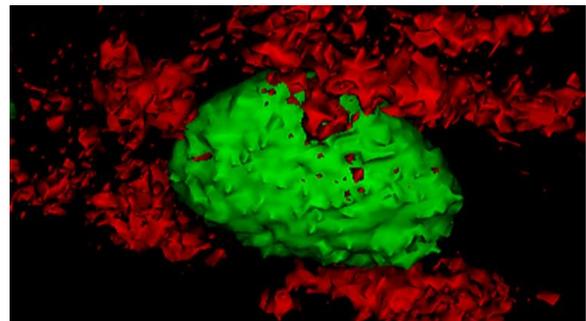
**3. Consequences of impaired autophagy in age related-disorders.** We have been analyzing changes in autophagy in three main groups of age-related diseases: **neurodegeneration, metabolic disorders** and **cancer**. By combining metabolic assays, cellular fractionation procedures, fluorescence reporters and our in vitro lysosomal transport assays in different animal and cellular models of these diseases, we have found that autophagy malfunctions in conditions such as diabetes, obesity and also that the age-related decline in autophagy could be an early step in oncogenic transformation. We have identified a primary defect in CMA in some familial forms of Parkinson's disease and of taupathies such as Alzheimer's disease. We are interested in identifying the primary defect, the compensatory mechanisms elicited by the cell and developing approaches to modulate autophagy with therapeutic purposes in age-related disorders.

For more information go to our web page [here](#)

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*CMA in lipid droplets metabolism*

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## Tumor MicroEnvironment of Metastasis

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### **Areas of Research:**

Investigating mechanisms of metastasis. Design and development of novel instrumentation and imaging technologies for biological research. Use of surgical engineering for expanding mouse models of cancer and metastasis. Development of and validation of clinical biomarkers for cancer progression and metastasis. Development of new technologies within the Gruss-Lipper Biophotonics Center.

### **Professional Interests:**

David Entenberg's expertise lies in the design and development of novel instrumentation and imaging technologies for biological research. With a background in laser-based experimental quantum physics, he brings skills in optical, mechanical, electrical, software, and instrument design.

His previous work has included the design and development of several robotics-based high-throughput automated biological assays utilizing real time PCR and MALDI-TOF protocols; novel microscopes including a fast switching, multi-channel TIRF microscope, a video rate multiphoton microscope; and a two-laser OPO-based multiphoton microscope.

Current and future projects are focused on expanding the utility of mouse models for intravital imaging by utilizing surgical engineering to develop and optimize novel surgical protocols and implantable imaging windows. This work has included mammary, abdominal, brain, lymphatic, and lung imaging windows for studying cancer metastasis to secondary sites in vivo, as well surgically implantable photolithographic microdevices designed to directly alter the local (<300  $\mu\text{m}$ ) microenvironment in vivo and during intravital imaging.

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## DNA- AND MICROTUBULE-BASED TRANSPORT IN HEALTH AND DISEASE

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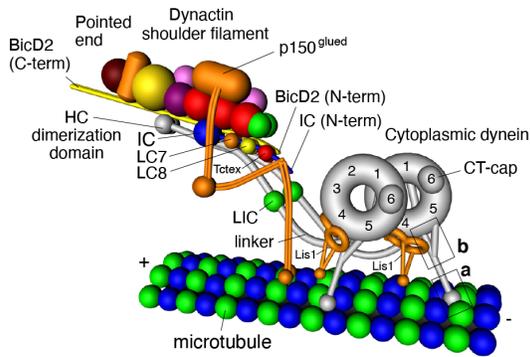


**Key words:** *transcription elongation, Pol II, nucleosomes, microtubule cytoskeleton, molecular motors, cytoplasmic dynein, bidirectional organelle transport, human pathologies, single-molecule biophysics, high-resolution fluorescence microscopy, and microscopy development.*

**My lab is interested in the fundamental molecular mechanisms of motor proteins and their associated biological processes.** Our current research is focused on the application of advanced high-resolution and single-molecule microscopy techniques to study how biomolecular motors work and generate biological motion and force. In particular, we combine single-molecule biophysics with molecular biology and biochemistry to study the molecular mechanisms underlying cell division, intracellular organelle and mRNA transport, as well as the molecular mechanisms underlying the regulation of transcription elongation by RNA polymerases. Current research is focused on the molecular

functions of the microtubule motors kinesins and cytoplasmic dynein (molecular machines that harness the chemical energy of ATP hydrolysis to perform mechanical work in eukaryotic cells), the regulation of RNA polymerase II-based transcription elongation through nucleosomes, and the molecular mechanism of transcription elongation by RNA polymerase III. We use a multidisciplinary approach integrating ultrasensitive single-molecule assays (high-resolution optical trapping and single-molecule fluorescence microscopy) and genetic approaches such as homologous recombination to dissect the mechanisms of microtubule- and DNA-based motor proteins. Our long-term goal is to understand the fundamental design principles of biomolecular motors and the molecular basis of human diseases with underlying defects in motor function.

**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 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. Only AAA domains 1, 3 and 4 can bind and hydrolyze ATP. Dynactin, dynein's largest regulatory subunit (~1.2 MDa), interacts with dynein's intermediate chain through the p150<sup>glued</sup> subunit, while Lis1, in



**Figure 1.** *The cytoplasmic dynein motor complex. Illustration of the dynein dimer with associated chains, cofactors dynactin, Lis1 and NudE (LIC, light intermediate chain; IC, intermediate chain; LC, light chains), and cargo-adaptor BicD2.*

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.

**Transcription elongation** through a nucleosome and its regulation by oncogenic transcription factors is a new research area in my lab. To answer paradigm-shifting

questions in the chromatin field, we are combining the recombinant expression of human histones, RNA polymerases and transcription factors with the correlative measurements of transcription elongation at base-pair resolution and the imaging of fluorescently tagged regulatory proteins (such as p53 and other oncogenic transcription factors) at the single-molecule level.

The Gennerich laboratory is accepting rotation students, graduate students and postdoctoral fellows with interests in biochemistry, molecular biology, structure-function studies, and single-molecule biophysics.

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# Rho GTPases in Breast Cancer Metastasis, FRET Biosensors and Optogenetics, High-resolution Optical Imaging of Protein Functions in Living Cells

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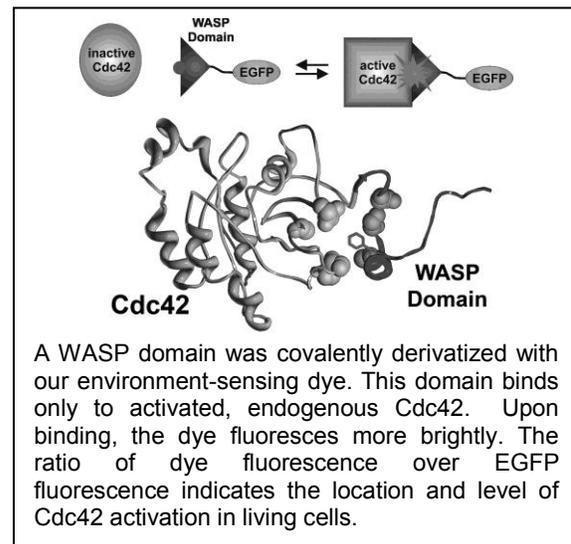
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P21 Rho family small GTPases are critically important in many disease processes including malignant cancers, developmental defects, atherosclerosis, and autoimmune dysfunction. This class of signaling molecules is critical in these diseases by impacting 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 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.

### **Original Papers:**

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# RIBONUCLEOPROTEINS IN CANCER AND HUMAN REPRODUCTION

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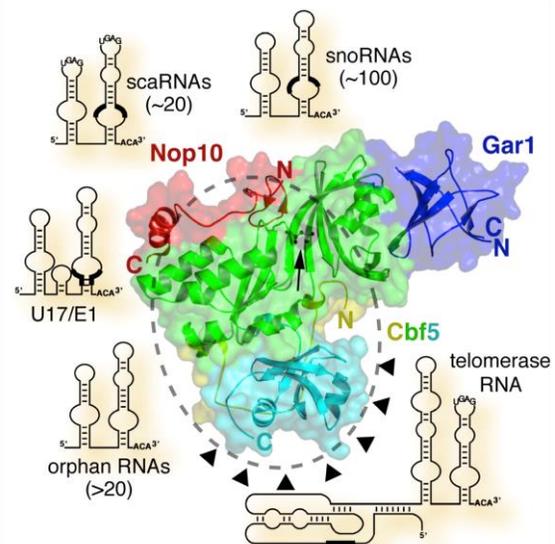
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Our group is studying the mechanism and regulation of nucleolar ribonucleoprotein biogenesis in relation to genetic disease, cancer, and human reproduction. Presently we are pursuing two main areas of research:

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 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 500 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 is an oncogenic protein, whereas the H/ACA RNP-specific assembly factor SHQ1 is a tumor suppressor of prostate and other cancers. We identify how disease causing and 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. Among other cell biological approaches, we are employing CRISPR/Cas9 and RNAseq technology to get to the bottom of this. We are further 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, which finally affords 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. We are now exploiting our technology for timing endometrial receptivity in embryo transfer cycles by NCS detection in uterine secretions.

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## Tumor MicroEnvironment of Metastasis

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### Areas of Research:

Translational and basic research (bench to bedside/ bedside to bench) in tumor microenvironment related to pro-metastatic changes including induction of invasive and stem cell phenotype in cancer cells. The effect of chemotherapy on tumor microenvironment in pre-clinical mouse models, and patient-derived samples. The analyses of disease outcome data from clinical trials comparing pre- and post-operative chemotherapy relative to patient ethnicity and race.

### Professional Interests:

Maja Oktay is a physician scientist. She is board certified anatomical pathologist and cytopathologist with a Ph.D. and post-doctoral training in cancer cell biology and cell signaling pathways. Her major interests are in cancer cell biology, the biology of breast cancer progression and metastasis, development of prognostic and predictive molecular biomarkers as well as identification of therapeutic targets for cancer cell dissemination. In addition, she is interested in the effect of commonly used chemotherapy on tumor microenvironment in patients of all racial backgrounds. Her work is based on the analysis of the cancer microenvironment using patient-derived material as well as mouse models of cancer in

combination with digital pathology and intravital multiphoton imaging. Dr. Oktay's team is now focusing on elucidating the cellular and molecular mechanisms involved in the emergence of disseminating cancer stem cells, cancer cell dissemination from metastatic foci and chemotherapy-mediated induction of invasive and stem program in breast cancer cells with the overarching goal to improve the outcome of patients treated with chemotherapy.

### Selected Publications - in the last 3 years

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# MECHANISMS OF INVASION AND METASTASIS

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## Invasion and Metastasis

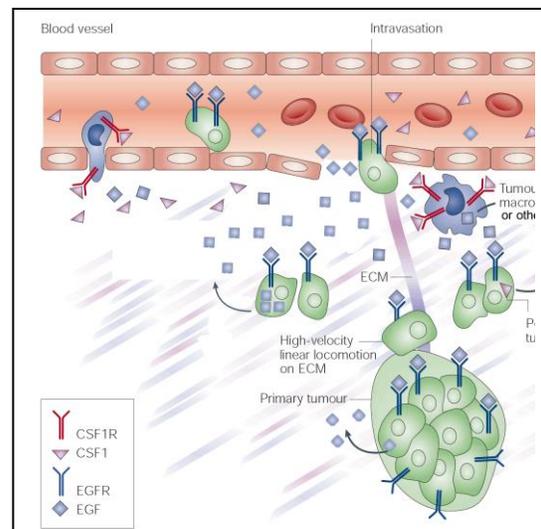
For most cancers, the cause of patient death is either local invasion into critical areas or metastasis - dissemination of tumor cells from the primary tumor to many parts of the body followed by formation of new tumors at those distant sites. By understanding the mechanisms by which tumor cells invade 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.

Two types of cancer are being studied. Head and neck cancer invades 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

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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 (floor of mouth or mammary fat pad for 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. 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.



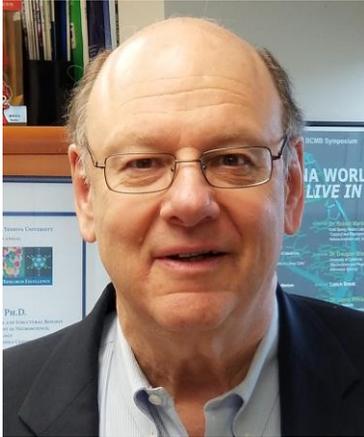
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## FOLLOWING THE TRAVELS OF RNA

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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.

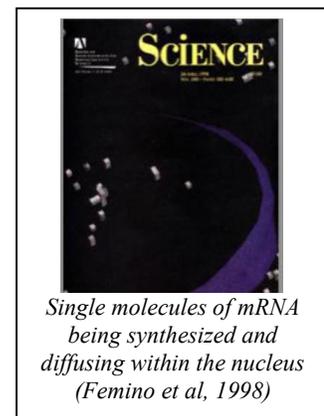
Our new technology, based on in situ hybridization allows us to visualize specific nucleic acid sequences within individual cells. Synthetic nucleic acid probes are labeled with fluorochromes. Subsequently these molecules are hybridized to the cell and detected using high resolution digital imaging microscopy.

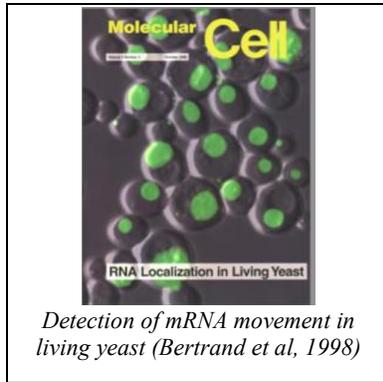
We have developed imaging methodologies and algorithms capable of detecting a single RNA molecule within a cell. This enables the detection of specific nucleic acid molecules for comparison between normal or cancer cells. This method of molecular diagnosis is the clinical application of the technology. 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.

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, including translation of single mRNAs.





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## FLUORESCENT PROTEINS, BIOSENSORS AND OPTOGENETIC TOOLS

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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 PAFP 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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## 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.

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