Computer vision-based analysis of tumor cell invasion and adhesion

James E. Bear, Ph.D. *Principal investigator*

Shawn Gomez, Eng.Sci.D *Co-principal investigator*

Abstract:

The formation of metastases in distant organs requires that cancer cells escape from the primary tumor by degrading the basement membrane that separates epithelial from stromal compartments. This degradation is carried out by matrix metalloproteinases that have been shown to accumulate in small, finger-like membrane protrusions termed invadopodia. While their role in metastasis has been recognized for some time, significant gaps remain in our understanding of invadopodia architecture and regulation. To address these shortcomings in our knowledge of these key metastasis components, we propose to implement novel image analysis techniques which, in combination with manipulation of specific structural and/or signaling proteins, will enable highly detailed, quantitative characterization of invadopodia dynamics. Specifically, in Aim 1 of this effort, we will apply novel computer vision approaches to the study of invadopodia dynamics in WM2664 cells. This Aim will provide quantitative spatiotemporal measurements of invadopodia behavior and their response to perturbation through targeted shRNA gene knockdowns. In Aim 2, we will quantify the spatiotemporal coordination between invadopodia and focal adhesions through an analysis that leverages newly developed focal adhesion image analysis methodologies with the invadopodia approaches developed in Aim 1. As metastatic cells must balance the processes of degradation with those of adhesion, the behaviors of invadopodial and focal adhesion structures are expected to be coordinately regulated. However, to date, no such study has been undertaken. Together, the computational and biological work proposed here will lead to significant advancements in our understanding of these cellular structures and their role in metastasis.

Innovation and Impact Statement:

This is a new collaborative project between the Bear and Gomez groups that brings together expertise in the cell biology of tumor cell behavior and computer vision-based analysis of massive image data sets. With the advent of multicolor live-cell imaging and gene manipulation, there is an increasing need to find new methods to extract quantitative information from images and movies. The cellular structures that are the focus of this project, invadopodia and focal adhesions, are critical for tumor cell metastasis. Yet the standard in the field for analyzing these structures is to hand select a small number (<10) and perform limited measurements of area and some dynamic properties. The method under development here will allow unbiased analysis of *all* the invadopodia and/or focal adhesions (~50-1000 per cell) over 12-16 hours. This level of data analysis is required to identify subtle or combinatorial effects of genetic and/or pharmacological manipulation. Beyond method development, this approach will allow us to understand the effects of genes known to be involved in tumor metastasis such as PTEN and LKB1 that have never been studied at this level. Furthermore, we will evaluate the effects of B-Raf inhibition on these structures using the new Plexxikon compound in a B-Raf mutant melanoma cell line. Thus, this project will have a significant impact on the field of cancer metastasis by allowing the inter-related dynamics of extracellular matrix degradation and cellular adhesion to be studied at an unprecedented level of detail.

Specific Aims:

In this proposal, we seek to develop methods to analyze tumor cell behavior using computer vision-based techniques. We will use these methods to understand the role of known pro-invasive genes and test the role of metastasis-implicated genes such as PTEN and LKB1 whose role in matrix degradation and adhesion control is unknown. To accomplish this, we propose two specific aims:

Aim 1. Computer vision-based analysis of invadopodia: In this aim, we will develop an automated, computer vision-based assay to quantify invadopodia population statistics and dynamics from live-cell movies. Using this assay, we will evaluate gene knockdowns of candidate metastasis genes such as PTEN and LKB1. In addition, we will evaluate the use of this tool with pharmacological inhibitors such as the B-Raf inhibitor.

Aim 2. Multiplex analysis of invadopodia and focal adhesions: In this aim, we will combine or multiplex the established method for quantification of focal adhesion dynamics with the new method of invadopodia quantification from Aim 1 using 3-color live-cell imaging. By simultaneously and quantitatively examining these structures, we can begin to ask questions about how cells manage to dynamically balance adhesion and matrix degradation to achieve invasive motility.

Background:

Advanced melanoma is one of the most feared human cancers [1, 2]. Although curable through surgery when diagnosed at early stage, melanoma is characterized by its therapeutic resistance, aggressive clinical behavior, and proclivity for early metastasis. Metastasis is the process by which tumor cells leave the primary lesion and move to other parts of the body, thereby initiating tumor formation at multiple sites. During this process, cells detach from their cell-cell and cell-matrix adhesive contacts, degrade barriers such as the basement membrane, migrate to other locations within the body, invade new organs, and regain adherence to re-initiate growth [3]. It is therefore not surprising that metastatic tumor cells have high *in vitro* cell motility and invasive capabilities.

Through the combined work of many labs, a conceptual framework for understanding cell migration has emerged [4]. Motile cells display a characteristic cycle of steps leading to translocation. First, cells protrude a structure such as lamellipodia in the direction of migration. Second, after the protrusion has extended forward, it must become stabilized by attachment to the underlying substratum. These attachment points are known as focal contacts or focal adhesions and contain clustered integrin receptors that create a bridge between the extracellular matrix and the actin cytoskeleton. Third, the cell body is then squeezed forward from the rear in an actomyosin-based contraction event. Finally, cells must lose substratum attachment at the rear or trailing edge. In the case of tumor cells, these general steps of migration must be preceded by the local degradation of extracellular matrix using short finger-like structures called invadopodia that are heavily enriched with matrix metaloproteases (MMPs) [5]. The coordination of matrix degradation by invadopodia and adhesion to matrix required for movement is poorly understood.

Computer vision approaches have been applied in cellular biology settings to analyze large collections of both still and time lapse microscopy to quantitatively assess cellular phenotypes [6]. The applications of computer vision approaches to analyzing microscopy images have ranged from large siRNA-based screens designed to discover proteins involved in mitosis [7] to tracking the effect of camptothecin on protein localization in individual cells [8]. In the field of cellular motility, studies have demonstrated, using computer vision techniques, the subtle connection between cell edge movement and the Rho GTPases [9]. These applications of computer vision techniques demonstrate the power of combining computational image processing techniques to determine the connection between perturbations of specific pathways and phenotype. By applying computer vision methods to examine invadopodia and the interplay between invadopodia and focal adhesions, this proposal seeks to determine the effects of several candidate genes on metastasis and develop sets of image processing tools that can be widely applied.

Preliminary Studies:

Recent work in the Gomez lab has led to the development of a set of computational tools that automates the analysis of time-lapse movies of fluorescently tagged focal adhesions. While not only automating the data collection process, these tools greatly extend both the number and the types of properties that can be

quantified. This work is in revision for *PLoS Biology* after a positive first round of reviews (manuscript available upon request). Integrated into a single software suite, these methods automatically identify all focal adhesions in each frame of a time-lapse movie, track them through time and then collect various sets of properties that describe their birth, growth, maturation, disassembly and death (Fig. 1). These properties fall into three general categories: static, dynamic and spatial. The static properties of the adhesions include such aspects as area, intensity and eccentricity averaged or at a given point in time. The dynamic properties, such as the assembly and disassembly rates, are determined by automatically analyzing the properties of the adhesions through time (Fig. 2). Spatial properties include such aspects as where within the cell (and relative to structures such as the cell edge) an adhesion is born or dies. These automated methods are comparable to prior methods used to assess focal adhesion properties [10], but the developed computer vision approaches allow all the detectable adhesions to be analyzed in an unbiased manner, and with greater fidelity. Thus, while manual methods typically quantified a small number of adhesions (10-25) per experimental condition, the automated methods we have developed can scale to collect thousands of data points (Fig. 2). We note that the software suite was designed as a series of modules to make expansion of capabilities easier. Commonalities between focal adhesion and invadopodia imaging allow relatively straightforward application of these methodologies to invadopodia analysis. In fact, we expect that several of the key software components developed to analyze focal adhesions will be able to be reused with little or no modification in the development of the invadopodia analysis.

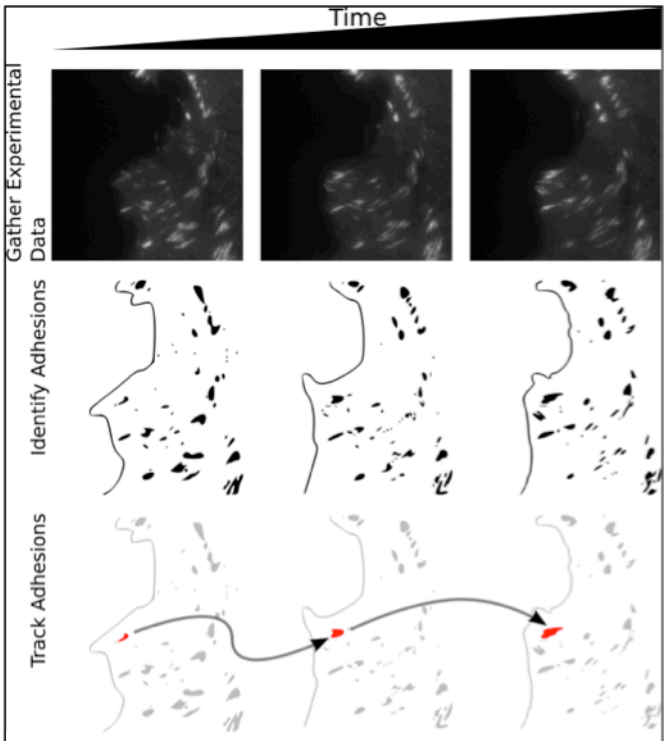

Figure 1: Summary of focal adhesion analysis methods showing a small region of a migrating fibroblast, the adhesions identified in this region and how a single adhesion is tracked.

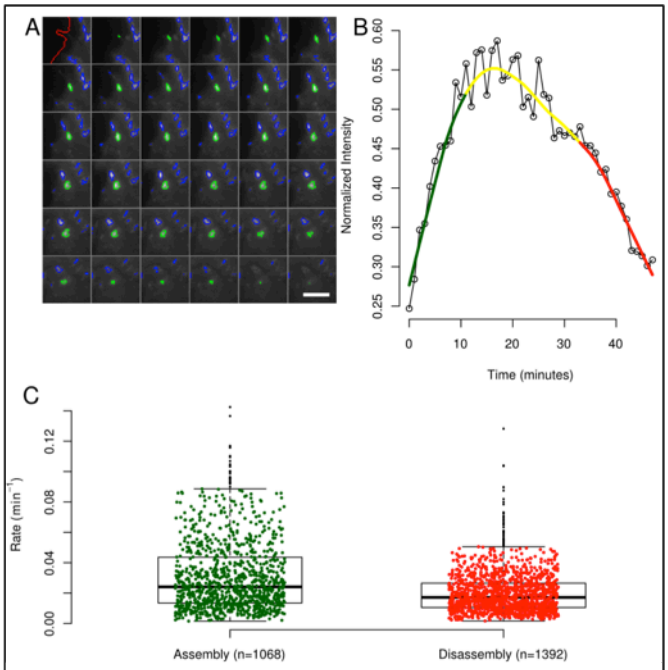

Figure 2: (A) Sample tracking of single adhesion (green), surrounding adhesions (blue) and the cell edge (red) (B) The intensity of the adhesion in A over time with the assembly phase (green), the stability phase (yellow) and the disassmbly phase (red) indicated. (C) Summaries of the assembly and disassembly rates.

Research Plan:

Aim 1. Quantitative analysis of invadopodial dynamics:

Rationale: Invadopodia are highly dynamic structures, which form and disassemble as cancer cells degrade the extracellular matrix [5]. Their role in cancer cell invasion has been known for many years, but the methods used to quantify them have been limited to relatively qualitative measurements. We propose to develop computer vision software designed to quantitatively assess the properties currently collected by hand and develop new measures of invadopodial properties to evaluate genetic and pharmacological manipulations.

Experimental plan: This aim focuses on using the well-characterized human melanoma cell line WM2664 to assess the properties of invadopodia. We have extensive expertise manipulating and imaging this cell line both *in vitro* and *in vivo* [11]. The basic experimental protocol is to plate cells onto a thin layer of fluorescently labeled gelatin (red channel) and image for 12-16 hrs. As the cells attach to the gelatin, many begin forming invadopodia, resulting in regions where the fluorescent signal from the gelatin gradually disappears due to degradation. In addition, a fluorescent tag for F-actin (Lifeact-GFP) that accumulates as a bright puncta at invadopodia is also imaged. This results in two sets of movies: one of the gelatin and the other of the actin tag. To determine the role of various proteins in influencing the properties of invadopodia, a set of candidate genes have been selected for gene knockdown using lentiviral shRNAs. These genes have either been implicated in invadopodia function or have known effects on tumor cell metastasis, but have never been tested for a role in invadopodia function (Table 1). Knockdown constructs for most of these genes already exist in the Bear lab or published studies. These shRNAs will be combined with an expression cassette for LifeAct-GFP in our knockdown-marker lentivirus published previously [12]. At least 50 cells will be analyzed per knockdown construct with a nonspecific (NS) shRNA used as a control. In cases where an shRNA has not been tested for off-target effects using RNAi-resistant rescue, we will use at least two independent targeting sequences. As WM2664 cells harbor the activating V600E B-Raf mutation common in melanoma [13], we will also use this approach to test the role of B-Raf kinase in invadopodia formation using the newly available B-Raf inhibitor from Plexxikon (PLX4032) [14]. We will use the drug at 1μM, a dose that has been shown to inhibit Erk1, a downstream target of B-Raf [15]. In future studies, this approach can be expanded to test other drugs that may have anti-invasive or anti-metastatic activity.

Table 1: Candidate Genes	Reference
Pten	[16]
LKB1	[17]
Cortactin	[18]
Coronin 1C/1B	[19]
FAK	[20]
Fascin	[21]
Exo70	[22]

The first stage of the image processing pipeline is to identify the regions of high F-actin concentration (actin puncta) in each frame of the movie. In conjunction with appropriate image registration, this is accomplished using a thresholding algorithm based on the focal adhesion identification methods described above. With each of the puncta identified, they are then tracked through the entire movie and various properties, such as size and longevity are extracted from the raw image data. With the puncta identified and tracked, the next stage of the analysis involves determining which of the long-lived puncta are actual invadopodia. Our method uses the intensity of the gelatin in the region near the puncta as a filter for identifying invadopodia (Fig. 3). Specifically, we use the difference in average value of the region immediately beneath the actin puncta and the region in a five-pixel border around the puncta as a filtering method. If this local difference value is consistently below zero, then we know that the region of gel immediately underneath the puncta has lost fluorescence relative to the surrounding region, indicating degradation of the gelatin. We also look to the frame immediately before the actin puncta was born to determine if the gelatin appeared degraded, perhaps due to a previous invadopodia or uneven coating. If there are significant differences in the degradation before and during the puncta's life, we classify the puncta as an invadopodia. In addition, we are developing a population method to scan an entire field of cells to quantify the percentage of cells that form invadopodia meeting the defined criteria.

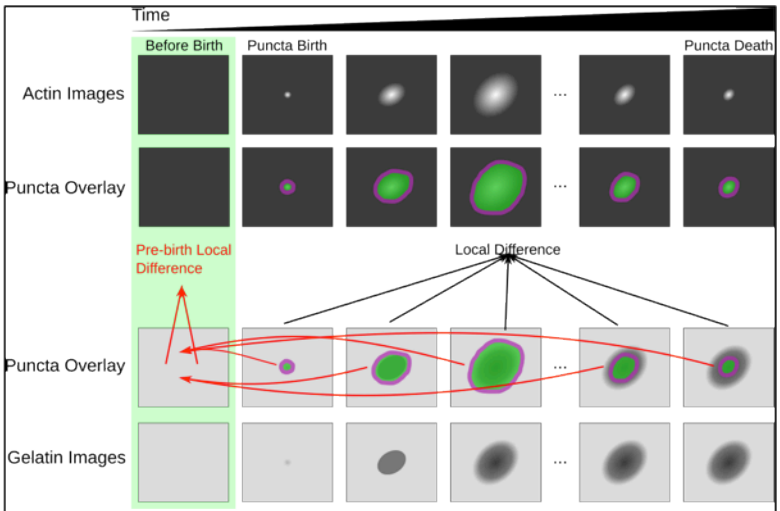

Figure 3: Overall filtering strategy for identifying invadopodia is based around the mean values of the gelatin intensity underneath and adjacent to the actin puncta. The punta overlay rows indicate the puncta extent in green and the adjacent region in purple.

Potential pitfalls: Although the focal adhesion methods are quite robust, they may not be perfectly applicable to invadopodia and could require optimization such as changes in the local difference approach. Furthermore, false positive detection of invadopodia by the algorithm may be a problem. To test for this, we will run the experiment in the presence of a broad-spectrum MMP inhibitor (BB94) that is known to block invadopodia formation [23] and check for computer-detected invadopodia vs. observer-confirmed events.

Expected outcomes: Results from this Aim will provide the most comprehensive and quantitative characterization of invadopodial dynamics known to date, Through the knockdown of implicated genes, we expect to quantify changes in associated invadopodia dynamics (e.g. number formed, lifetime, etc.) as well as potentially identify key proteins necessary for "normal" invadopodia behavior.

Aim 2. Combined imaging of invadopodia and focal adhesions:

Rationale: Tumor cells can simultaneously contain both focal adhesions that anchor the cells to the extracellular matrix and invadopodia that are degrading it (Fig. 4), although these two events have never been simultaneously monitored in live cells. How these two processes are coordinated and integrated is poorly understood, and by combining the existing focal adhesion analysis method with the newly developed invadopodia analysis method, we can begin to understand this coordinate regulation.

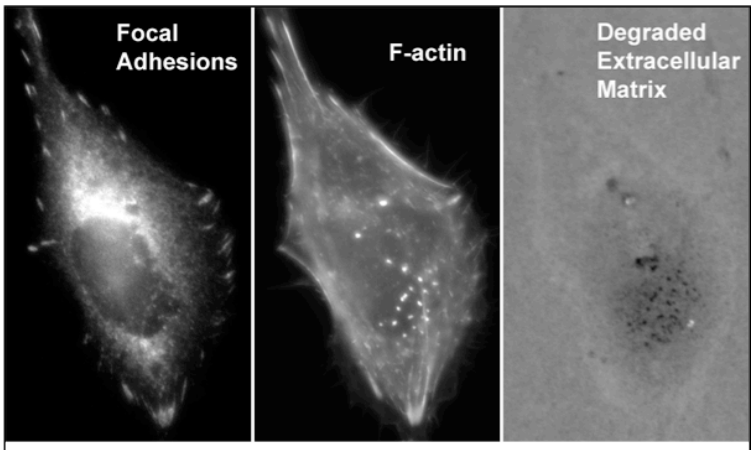

Figure 4: WM2664 cell plated on fluorescent gelantin and stained for focal adhesions (Vinculin) and F-actin (note bright puncta)

Experimental Plan: We propose to develop a 3-color imaging method that will allow us to quantify invadopodia and focal adhesions simultaneously using GFP-Paxillin to mark focal adhesions, LifeAct-mCherry (red fluorescent protein) and Cy5-gelatin (far red dye) to monitor invadopodia. Using three sets of movies (focal adhesions, actin puncta and gelatin degradation), we will determine the dynamic properties of focal adhesions and invadopodia in single cells over time. From these data sets, additional software will be written that correlates the properties of focal adhesion and invadopodia. While difficult to predict all the types of relationships we will observe in this combined data, we expect several areas to be of particular interest. Comparisons between the rate of focal adhesion turnover and the formation of successful invadopodia will indicate whether the relative timing between these events is crucial for a successful invasion. For example, we may observe that adhesion turn over rates increase, a necessary condition for rapid cell motility, following a burst of invadopodia formation events. We will also develop tools needed to compare the spatial characteristics of the invadopodia and focal adhesion populations. These tools will make it possible to quantify how formation of focal adhesion and invadopodia affect one another in local regions of the cell, suggesting how these two structures interact and negotiate sharing similar components. This combination of a novel imaging setup and sophisticated computer vision algorithms will provide the first analysis of how focal adhesions and invadopodia interact in the same cells.

Although careful observation of focal adhesions and invadopodia in control cells will undoubtedly yield important information about tumor cell invasion, the real payoff is the ability to use this approach to evaluate genetic and/or pharmacological manipulations of the cells. Our test case for a combined effect on both structures will be knockdown of FAK which has been shown recently to increase the number of invadopodia per cell while also increasing the lifetime of focal adhesions (observed in different cell types) [24, 25]. Knockdowns or drug treatments that show an effect on invadopodia as described in Aim 1 will be re-tested in the combined assay to better understand the functional relationship between invadopodia and focal adhesions.

Potential pitfalls: One potential concern with the 3-color imaging experiments is that expression of these multiple probes may adversely affect the physiology of the cells and alter the properties we are studying. To control for this possibility, we will carefully compare the dynamics of focal adhesions and invadopodia in this context vs. the single analysis of each structure separately. If there is an effect, we can either reduce expression levels by alternate promoters (eg. Tet-inducible systems) or change the probe to a more well-tolerated one (ie. swap GFP-Vinculin for GFP-Paxillin).

Expected outcomes: Results from this aim will provide the first known dataset on the coordinate regulation of matrix degradation by invadopodia and matrix adhesion by focal adhesions in the same cells. This is critical information for understanding the behavior of invasive tumor cells and may provide new therapeutic targets for anti-metastasis strategies.

References

1. Sharpless, N.E. and L. Chin, *The INK4a/ARF locus and melanoma.* Oncogene, 2003. **22**(20): p. 3092-8.
2. Chin, L., G. Merlino, and R.A. DePinho, *Malignant melanoma: modern black plague and genetic black box.* Genes Dev, 1998. **12**(22): p. 3467-81.
3. Kopfstein, L. and G. Christofori, *Metastasis: cell-autonomous mechanisms versus contributions by the tumor microenvironment.* Cell Mol Life Sci, 2006. **63**(4): p. 449-68.
4. Lauffenburger, D.A. and A.F. Horwitz, *Cell migration: a physically integrated molecular process.* Cell, 1996. **84**(3): p. 359-69.
5. Weaver, A.M., *Invadopodia: Specialized Cell Structures for Cancer Invasion.* Clin Exp Metastasis, 2006.
6. Peng, H., *Bioimage informatics: a new area of engineering biology.* Bioinformatics, 2008. **24**(17): p. 1827-36.
7. Neumann, B., et al., *Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes.* Nature, 2010. **464**(7289): p. 721-7.
8. Cohen, A.A., et al., *Dynamic proteomics of individual cancer cells in response to a drug.* Science, 2008. **322**(5907): p. 1511-6.
9. Machacek, M., et al., *Coordination of Rho GTPase activities during cell protrusion.* Nature, 2009. **461**(7260): p. 99-103.
10. Webb, D., et al., *FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly.* Nat Cell Biol, 2004. **6**(2): p. 154-61.
11. Rozenberg, G.I., et al., *Metastasis in an Orthotopic Murine Model of Melanoma is Independent of RAS/RAF Mutation.* Melanoma Res, 2010. **In Press**.
12. Cai, L., et al., *Coronin 1B coordinates Arp2/3 complex and cofilin activities at the leading edge.* Cell, 2007. **128**(5): p. 915-29.
13. Shields, J.M., et al., *Lack of extracellular signal-regulated kinase mitogen-activated protein kinase signaling shows a new type of melanoma.* Cancer Res, 2007. **67**(4): p. 1502-12.
14. Hersey, P., et al., *Small molecules and targeted therapies in distant metastatic disease.* Ann Oncol, 2009. **20 Suppl 6**: p. vi35-40.
15. Sondergaard, J.N., et al., *Differential sensitivity of melanoma cell lines with BRAFV600E mutation to the specific raf inhibitor PLX4032.* J Transl Med, 2010. **8**(1): p. 39.
16. Brader, S. and S.A. Eccles, *Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis.* Tumori, 2004. **90**(1): p. 2-8.
17. Fan, D., C. Ma, and H. Zhang, *The molecular mechanisms that underlie the tumor suppressor function of LKB1.* Acta Biochim Biophys Sin (Shanghai), 2009. **41**(2): p. 97-107.
18. Weaver, A.M., *Cortactin in tumor invasiveness.* Cancer Lett, 2008. **265**(2): p. 157-66.
19. Wu, L., et al., *Coronin-1C is a novel biomarker for hepatocellular carcinoma invasive progression identified by proteomics analysis and clinical validation.* J Exp Clin Cancer Res, 2010. **29**: p. 17.
20. Provenzano, P.P. and P.J. Keely, *The role of focal adhesion kinase in tumor initiation and progression.* Cell Adh Migr, 2009. **3**(4): p. 347-50.
21. Chen, L., et al., *Migrastatin analogues target fascin to block tumour metastasis.* Nature, 2010. **464**(7291): p. 1062-6.
22. Liu, J., et al., *The role of the exocyst in matrix metalloproteinase secretion and actin dynamics during tumor cell invadopodia formation.* Mol Biol Cell, 2009. **20**(16): p. 3763-71.
23. Ayala, I., et al., *Multiple regulatory inputs converge on cortactin to control invadopodia biogenesis and extracellular matrix degradation.* J Cell Sci, 2008. **121**(Pt 3): p. 369-78.
24. Chan, K.T., C.L. Cortesio, and A. Huttenlocher, *FAK alters invadopodia and focal adhesion composition and dynamics to regulate breast cancer invasion.* J Cell Biol, 2009. **185**(2): p. 357-70.
25. Ilic, D., et al., *Impairment of mobility in endodermal cells by FAK deficiency.* Exp Cell Res, 1996. **222**(2): p. 298-303.

BUDGET JUSTIFICATION

Personnel:

James E. Bear, Ph.D. *Associate Professor.* Dr. Bear will serve as Principal investigator for this project and will oversee experimental design, data analysis and interpretation. His major areas of responsibility will be aspects of the project related to the cell biology of cancer cell invasion and motility. 5% effort on this project is allocated with no requested salary support.

Shawn Gomez, Eng.Sci.D. *Assistant professor.* Dr. Gomez will serve as Co-principal investigator for this project and will also oversee experimental design, data analysis and interpretation. His major areas of responsibility will be aspects of the project related to computer vision image analysis. 5% effort on this project is allocated with no requested salary support.

Matt Berginski, BS. *Graduate assistant*. Mr. Berginski is a biomedical engineering graduate student who will by mainly responsible for image analysis and method development. In addition, he will receive cell biology training in the Bear lab. 100% effort on this project is allocated with appropriate salary and benefit support.

David Roadcap, Ph.D. *Postdoctoral associate*. Dr. Roadcap has developed the WM26664/LifeAct-GFP system for analyzing invadopodia and will mainly be responsible for generating knockdown cell lines for analysis and live-cell imaging. 50% effort on this project is allocated with appropriate salary and benefit support.

Supplies: (these numbers will change depending on other budget sections)

1. **Molecular biology supplies**: $6000 has bee requested for molecular biology supplies such as restriction enzymes, ligase, TAQ, PfuUltra, bacterial growth media, agarose, DNA size markers, antibiotics, tubes, plates, etc.
2. **General lab chemicals**: $2000 has been requested for general lab chemicals such as the components of buffer solutions, SDS-PAGE reagents, and other chemicals.
3. **Transfection reagents**: $1500 has bee requested for commercial transfection reagents (eg. Fugene6) that are critical do the experiments outlined in the proposal.
4. **DNA/RNA purifcation**: $3000 has been requested for materials needed to purify DNA and RNA such as Qiagen maxi-preps kits.
5. **Oligonucleotide synthesis**: $1500 has been requested for oligonucleotide synthesis. Cloning of shRNA constructs requires special purification of these particularly long (and expensive) oligos.
6. **Microscopy supplies**: $3000 has bee requested for microscopy supplies such as disposable live cell dishes from Bioptechs, anti-fade mounting buffer, fluorescent probes for the cytoskeleton (eg. Phalloidin), etc.
7. **Glassware**: $1000 has been requested for lab glassware.
8. **Tissue culture media/serum/trypsin**: $5000 has been requested for tissue culture media, serum and trypsin solution.
9. **Tissue culture consumables**: $3000 has been requested for tissue culture consumables such as plates and serological pipettes.

Sequencing: $2000 has bee requested for DNA sequencing costs. In the 96-well plate format, the cost per sequencing reaction (600-800 bp per reaction on plasmid templates) is $13. This will allow for approximately 150 reactions to verify the sequence of the plasmid constructs described in the research plan. This is necessary to ensure that mutations are not introduced during PCR-based cloning.

Computer storage & Research Computing cluster time:

Additional Computer Storage: $900 has been requested to purchase additional hard drives (4x 2Tb) for local image data storage. This is needed to maintain data flow through the proposed project as large data sets need to be stored between the time of collection and analysis on Research Computing cluster.

Reserved Research Computing cluster time: $10,400 has been requested to reserve priority time on the UNC Research Computing cluster “Kure”. The image-processing pipeline used to analyze the microscopy images requires high power computing resources to quantitatively analyze the movies and test out the results of changes to the analysis methods.

Program Director/Principal Investigator (Last, First, Middle): Bear, James

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 07/01/2010	THROUGH 06/30/2011

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
James Bear	PD/PI	.60				0	0	0
Shawn Gomez	Co-Investigator	.60				0	0	0
David Roadcap*	Postdoctoral Res. Assoc.	6.00			45,048	22,524	3,150	25,674
Matt Berginski@	Graduate Res. Assist	12.00			28,000	28,000	1,970	29,970
*SS only at 8.36% plus $2,534 for Health Insurance Prorated of effort								
@Health Insurance only at $1,970								
SUBTOTALS →						50,524	5,120	55,644

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Molecular Biology Supplies $6,000 General lab Chemicals $2,000 Transfection Reagents $1,500 DNA/RNA Purification $3,000 Oligonucleotide Synthesis $1,500 Microscopy Supplies $3,000 Glassware $1,000 Tissue Culture Media/Serum/Trypsin $5,000	26,000
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Sequencing $2,000 Computer Storage $900 Computing Cluster Time $10,400 Tuition $3,356	16,656

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 98,300
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 98,300

Program Director/Principal Investigator (Last, First, Middle): Bear, James

DETAILED BUDGET FOR YEAR 2 BUDGET PERIOD DIRECT COSTS ONLY	FROM 17/01/2011	THROUGH 06/30/2012

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
James Bear	PD/PI	.60				0	0	0
Shawn Gomez	Co-Investigator	.60				0	0	0
David Roadcap*	Postdoctoral Res. Assoc.	6.00			46,399	23,200	3,245	26,445
Matt Berginski@	Graduate Res. Assist	12.00			28,840	28,840	2,029	30,869
*SS only at 8.36% plus $2,534 for Health Insurance Prorated of effort								
@Health Insurance only at $1,970								
SUBTOTALS →						52,040	5,274	57,314

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Molecular Biology Supplies $6,000 General lab Chemicals $2,000 Transfection Reagents $1,500 DNA/RNA Purification $3,000 Oligonucleotide Synthesis $1,500 Microscopy Supplies $3,000 Glassware $1,000 Tissue Culture Media/Serum/Trypsin $5,000	26,000
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* Sequencing $2,000 Computer Storage $900 Computing Cluster Time $10,400 Tuition $3,356	16,656

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 99,970
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR YEAR 2 BUDGET PERIOD		**$** 99,970

Program Director/Principal Investigator (Last, First, Middle): Bear, James

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	55,644	57,314			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	26,000	26,000			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	16,656	16,656			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	98,300	99,970			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	98,300	99,970			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$** 198,270

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
James E. Bear	**Associate Professor**
eRA COMMONS USER NAME **James_Bear**	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Davidson College; Davidson, NC	B.S.	1993	Biology
Emory University; Atlanta, GA	Ph.D.	1998	Cell & Developmental Biology
Massachusetts Institute of Technology; Cambridge, MA	Post-doc	1998-2003	Cell Motility

A. Personal Statement I have been focused for over a decade on the regulation of actin dynamics and cell motility. As a graduate student, I discovered the founding member of the SCAR/WAVE family of Arp2/3 complex activators through a genetic screen that I designed and executed. This protein family is critical for the proper regulation of actin dynamics in such diverse contexts as morphogenesis and dendric spine function. As a postdoc, I elucidated the mechanism of Ena/VASP function through a series of cell biological and biochemical experiments. These proteins were the first known anti-capping factors that allow actin polymerization in the face of high levels of capping protein. This function is required for the formation of many cellular structures such as filopodia. In my own lab, we have begun studying the Coronins, a highly conserved family of proteins that are poorly understood. We have discovered that these proteins serve as coordinators of Arp2/3 and Cofilin activity, two major pathways that control the birth and death of actin filaments. Coronins inhibit Arp2/3 branching and target Slingshot-1L, Cofilin's activating phosphatase, to the leading edge. Recently, we discovered that Coronins remodel Arp2/3 actin branches in a manner that is antagonistic with Cortactin. Surprisingly, Coronin can actually replace Arp2/3 at branch junctions, a process that leads to the generation of a second branched actin network. We have extended our studies of the basic mechanisms of cell motility to studying melanoma metastasis. We have several strong collaborations within the LCCC on this topic (eg. Norman Sharpless) that have led to several recent publications.

B. Positions and Employment

1993-1998	Graduate student, Lab of Dr. Charles Saxe, Emory University
1998-2003	Postdoctoral Fellow, Lab of Dr. Frank Gertler, Massachusetts Institute of Technology
2003-2009	Assistant Professor, Lineberger Comprehensive Cancer Center and Dept. of Cell & Developmental Biology, University of North Carolina at Chapel Hill
2009-present	Associate Professor, University of North Carolina at Chapel Hill

Other Experience and Professional Memberships

1989-1993	Samuel H. Bell Memorial Scholar, Davidson College
1993	Phi Beta Kappa, Davidson College chapter
1993-1995	NIH Biochemistry, Cellular and Molecular Biology Training Grant recipient
1997	ASCB Predoctoral Travel Award
1999-2000	Anna Fuller Molecular Oncology Fellow
2000	NIH NRSA Award
2001-04	Leukemia and Lymphoma Society Special Fellow
2004-06	V Scholar Award
2005-07	Melanoma Research Foundation Junior Faculty Award
2006-2009	Sontag Foundation, Distinguished Scientist Award

2008-present	Research Scholar Award, American Cancer Society
2008-present	Jefferson-Pilot Award
2009-present	Faculty of 1000
2009-present	HHMI Early Career Scientist selection
2010	Ruth and Phillip Hettleman Prize for Artistic and Scholarly achievement

C. Selected Peer-Reviewed Publications (in chronological order)

1. **James E. Bear**, John Rawls and Charles L. Saxe III. 1998. SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late *Dictyostelium* development. (*Journal of Cell Biology*, 142(5): 1325-1335) PMID: 9732292
2. **James E. Bear**, Joseph J. Loureiro, Irina Libova, Reinhard Fässler, Jürgen Wehland and Frank B. Gertler. 2000. Negative regulation of Fibroblast Motility by Ena/VASP Proteins. (*Cell*, 101: 717-728) PMID: 10892743
3. **James E. Bear***, Tatyana M. Svitkina*, Matthias Krause, Dorothy A. Schafer, Joseph J. Loureiro, Geraldine A. Strasser, Ivan V. Maly, Oleg Chaga, John A. Cooper, Gary G. Borisy and Frank B. Gertler. 2002. Antagonism between Ena/VASP Proteins and Actin Filament Capping regulates Fibroblast Motility. (*Cell*, 109: 509-521) *equal contributors PMID: 12086607
4. **James E. Bear**. 2002. Formins: Taking a Ride on the Barbed End. (*Developmental Cell*, 3: 149-156) PMID: 12194842
5. Matthias Krause, Erik W. Dent, **James E. Bear**, Joseph J. Loureiro, Frank B. Gertler. 2003. ENA/VASP PROTEINS: Regulators of the Actin Cytoskeleton and Cell Migration. (*Annual Reviews of Cell & Developmental Biology*, 19: 541-64) PMID: 14570581
6. Liang Cai, Nicholas Holoweckyj, Michael D. Schaller and **James E. Bear**. 2005. Phosphorylation of Coronin 1B by PKC regulates interactions with Arp2/3 and Cell Motility. (*Journal of Biological Chemistry*, 280(36): 31913-23) PMID: 16027158
7. Janiel M. Shields, Nancy E. Thomas, Melissa Cregger, Aaron J. Berger, Michael Leslie, Chad Torrice, Honglin Hao, Shannon Penland, Jack Arbiser, Glynis Scott, Tong Zhou, Menashe Bar-Eli, **James E. Bear**, Channing J. Der, William Kaufmann, David L. Rimm and Norman E. Sharpless. 2007. Lack of ERK mitogen-activated protein kinase signaling demonstrates a new type of melanoma. (*Cancer Research,* 67(4):1502-12). PMID: 17308088
8. Liang Cai, Thomas W. Marshall, Andrea C. Uetrecht, Dorothy A. Schafer and **James E. Bear**. 2007. Coronin 1B coordinates Arp2/3 and Cofilin activity at the leading edge. (*Cell,* 128(5): 915-29) [featured as a research highlight in *Nature*] PMID: 17350576
9. Eric A. Vitrol, Andrea C. Uetrecht, Feimo Shen, Ken Jacobson and **James E. Bear**. 2007. Enhanced EGFP-CALI using deficient cells rescued with functional EGFP-fusion proteins. (*Proceedings of the National Academy of Sciences U S A,* 104: 6702-07) PMID: 17420475
10. Kimberly B. Petermann, Gabriela I. Rozenberg, Daniel Zedek, Pamela Groben, Karen McKinnon, Christin Buehler, William Kim, Janiel M. Shields, **James E. Bear**, Nancy E. Thomas, Jonathan Serody and Norman E. Sharpless. 2007. CD200 is induced by ERK and is a potential therapeutic target in melanoma. (*Journal of Clinical Investigation*, 117(12): 3922-9) PMID: 18008004
11. **James E. Bear**. 2008. Follow the Monomer. (*Cell*, 133(5):765-7) PMID: 18510919
12. Liang Cai, Alexander M. Makhov, Dorothy A. Schafer and **James E. Bear**. 2008. Coronin 1B antagonizes Cortactin and remodels Arp2/3-containing actin branches in lamellipodia. (*Cell,* 134: 828-42) [Cover] PMID: 18775315
13. Lawrence R. Shiow, David W. Roadcap, Kenneth Paris, Susan R. Watson, Irina L. Grigorova, Tonya Lebet, Jinping An, Ying Xu, Craig N. Jenne, Niko Föger, Ricardo U. Sorensen, Christopher C. Goodnow, **James E. Bear**, Jennifer M. Puck and Jason G. Cyster. 2008. The actin regulator coronin 1A is mutant in a thymic egress-deficient mouse strain and in a patient with severe combined immunodeficiency (*Nat Immunol.*, 9(11):1307-15) PMID: 18836449
14. **James E. Bear** and Frank B. Gertler. 2009. Ena/VASP: towards resolving a pointed controversy at the barbed end. (*J Cell Sci.* 122(Pt 12):1947-53) PMID: 19494122
15. Thomas W. Marshall, Heather L. Aloor and **James E. Bear**. Coronin 2A regulates a subset of focal adhesion turnover events through the Cofilin pathway. J Cell Sci. 2009 Sep 1;122(Pt 17):3061-9. Epub 2009 Aug 4.PMID: 19654210

OTHER SUPPORT

James E. Bear

ACTIVE

5-U54-CA119343-04 (Juliano) 9/1/05 8/31/10 0.60 Calendar (5.00%)
National Cancer Institute 242,287.00

Carolina Center of Cancer Nanotechnology Excellence: Project 2- Smart Nanoparticles for Cancer Therapy and Imaging

The major thrust of this proposal is to design and fabricate several types of novel nanodevices and to evaluate them in the context of powerful and informative biological models, with the emphasis on mouse tumor models.

5-U54-CA119343-04 (Juliano) 9/1/05 8/31/10 0.60 Calendar (5.00%)
National Cancer Institute 282,480.00

Carolina Center of Cancer Nanotechnology Excellence: Project 6- Nanopatterned Surfaces for the Analysis of Tumor Cell Signaling and Migration

The major thrust of this proposal is to design and fabricate several types of novel nanodevices and to evaluate them in the context of powerful and informative biological models, with the emphasis on mouse tumor models.

Not Assigned (Bear) 10/1/06 9/30/09 2.40 Calendar (20.00%)
Sontag Foundation 130,435.00

The Role of PTEN and Coronin 1C in Astrocyte Motility and Metastasis

This proposal aims to study genetically engineered mice to analyze the role of two genes in the spread of tumor cells.

1-P01-ES014635-03 (Kaufmann) 5/1/07 4/30/12 0.60 Calendar (5.00%)
National Inst. of Health 234,010.00

The System of Response to DNA Damage Suppresses Environmental Melanomagenesis - Project 3: Murine and Human In Vivo Models of Melanoma Formation

This program will use a systems biology approoach to investigate the mechanisn(s) whereby human melaocytes undergo neoplastic transformation, clonal expansion and malignant progression to cutanious melanomas.

5-RO1-GM083035-02 (Bear) 9/1/07 8/31/12 3.00 Calendar (25.00%)
National Inst. of Health 185,000.00

The Coordination of Cytoskeletal Dynamics by Coronins

This proposal seeks to elicudate the mechanisms of this coodination and determine how this activity contributes to cell migration and cancer metastasis.

RSG-08-154-01-CSM (Bear) 7/1/08 6/30/12 3.00 Calendar (25.00%)
American Cancer Society 153,509.00

The Role of Coronin 1C and Coactosin in Melanoma Metastasis

The proposed research will test the if proteins control actin dynamics and act synergistically to promote tumor cell motility and metastasis in melanoma.

PENDING

OTHER SUPPORT

Not Assigned (Bear) 7/1/09 6/30/11
American Heart Association 23,000.00

The Development of Long Circulating Nanoparticles for Future Use of Nanoparticles in Cardiovascular Medicine

We will test how our markers of self proteins and our best shape and size parameters of particles affect circulation time. All of these experiments will be conducted in mice. At the end of this project we will have developed a nanoparticle with sufficient circulation time to be used effectively for the delivery of therapeutic and imaging molecules in vivo.

1-U54-CA119343-06 (DeSimone) 9/1/10 8/31/15 1.20 Calendar (10.00%)
National Cancer Institute 396,316.00

Carolina Center of Cancer Nanotechnology Excellence- Project 1

In this project, we will exploit the advantages of PRINT (Particle Replication in Non-wetting Templates) to generate "calibration quality" nano-tools to define the geometric (size, shape) surface (zeta potential, stealthing ligands) and deformability limitations associated with the delivery of drugs using different dosage forms (IV and IP).

CA016086-36 (Earp) 12/1/10 11/30/15 0.00 Calendar (0.00%)
National Cancer Institute 212,590.00

Cancer Center Core Support Grant- Microscopy Core

To provide LCCC members access to state of the art service in ligh microscopy together with research-based EM and intravital imaging at little or no cost to members.

OVERLAP NONE

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Gomez, Shawn Michael	Assistant Professor, Joint Department of Biomedical Engineering, UNC-Chapel Hill
eRA COMMONS USER NAME (credential, e.g., agency login) SHAWN_M_GOMEZ	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Colorado, Boulder, USA	BS	06/90	Aerospace Engineering
University of Colorado, Boulder, USA	MS	06/93	Aerospace Engineering
Columbia University, New York City, USA	Eng.Sc.D	12/99	Biomedical Engineering
Columbia University, New York City, USA	PostDoc	1999-2002	Computational Biology
Institut Pasteur, Paris, FRANCE	PostDoc	2002-2005	Computational Biology

A. Personal Statement

My lab is focused on issues in computational systems biology, with particular focus on understanding the architecture and dynamics of signaling networks in human disease. We have recently initiated efforts to develop quantitative, high-content methodologies in the area of live-cell image analysis and microscopy. Most recently, we have developed computational tools for the analysis of fluorescently tagged proteins in focal adhesions and have used these tools to detect changes in adhesion dynamics as a result of blocking JNK phosphorylation of paxillin. These tools provide a novel basis for the invadopodia work given in this proposal.

B. Positions and Honors

Positions and Employment

1987 – 1990	Intern, Martin Marietta, Astronautics Group, Denver, CO
1990 – 1994	Research Assistant, BioServe Space Technologies, University of Colorado, Boulder, CO
1991	Intern, Lockheed Missiles & Space Company, Bioastronautics Division, Sunnyvale, CA
1995 – 1999	Research Assistant and Eng.Sc.D. Candidate, Biomedical Engineering, Columbia University, New York, NY
1999 – 2002	Postdoctoral Fellow, Columbia Genome Center, Columbia University, New York, NY
2002 – 2005	Postdoctoral Fellow, Institut Pasteur, Paris, France
2005 – present	Assistant Professor, Joint Department of Biomedical Engineering, UNC-Chapel Hill and NC State University
2009 – present	Adjunct Assistant Professor, Department of Computer Science, UNC-Chapel Hill

Other Experience and Professional Memberships

International Society for Computational Biology
Biomedical Engineering Society
New York Academy of Sciences

Honors

1986	National Hispanic Scholar
1986	University of Colorado Engineering Scholarship
1989	Martin Marietta/Inroads Distinguished Intern
1990	NASA/USRA Summer Conference Leader
1991-1994	NASA Fellow, Graduate Student Researchers Program
1999-2001	Research Foundation for Mental Hygiene Fellow

2002-2005 Florence Gould Scholar, Pasteur Foundation Fellow
2006 UNC Junior Faculty Development Award
2008 Carl Storm URM Fellowship

C. Selected Peer-reviewed Publications

Rzhetsky A and Gomez SM. (2001) Birth of scale-free molecular networks and the number of distinct DNA and protein domains per genome. *Bioinformatics*. Oct;17(10):988-96.

Gomez SM, Lo SH and Rzhetsky A. (2001) Probabilistic prediction of unknown metabolic and signal-transduction networks. *Genetics*. Nov;159(3):1291-8.

Gomez SM and Rzhetsky A. (2002) Towards the prediction of complete protein-protein interaction networks. *Proceedings of the Pacific Symposium on Biocomputing* 7. pp 474-85.

Krauthammer M, Kra P, Iossifov I, Gomez SM, Hripcsak G, Hatzivassiloglou V, Friedman C, Rzhetsky A. (2002) Of truth and pathways: chasing bits of information through myriads of articles. *Bioinformatics*. ISMB 2002. Jul;18 Suppl 1:S249-S257.

Gomez SM, Noble WS and Rzhetsky A. (2003) Learning to predict protein-protein interactions from protein sequences. *Bioinformatics.* Oct 12;19(15):1875-81.

Chien, M., Morozova, I., Shi, S., Sheng, H., Chen, J., Gomez, S.M., Asamani, G., Hill, K., Nuara, J., Feder, M., Rineer, J., Greenberg, J.J., Steshenko, V., Park, S.H., Zhao, B., Teplitskaya, E., Edwards, J.R., Pampou, S., Georghiou, A., Chou, I.C., Iannuccilli, W., Ulz, M.E., Kim, D.H., Geringer-Sameth, A., Goldsberry, C., Morozov, P., Fischer, S.G., Segal, G., Qu, X., Rzhetsky, A., Zhang, P., Cayanis, E., De Jong, P.J., Ju, J., Kalachikov, S., Shuman, H.A., and Russo, J.J. (2004) The genomic sequence of the accidental pathogen *Legionella pneumophila*. *Science.* 305:1966-1968.

Gomez SM*, Eiglmeier K*, Segurens B, Dehoux P, Couloux A, Scarpelli C, Wincker P, Weissenbach J, Brey PT, and Roth CW. (2005) Pilot *Anopheles gambiae* full – length cDNA study: Sequencing and initial characterization of 35,575 clones. *Genome Biology.* 6:R39. (* - contributed equally to this work)

Li J, Riehle MM, Zhang Y, Xu J, Odul F, Gomez, SM, Eiglmeier K, Ueberheide BM, Shabanowitz J, Hunt DF, Ribeiro JMC, and Vernick KD. (2006) *Anopheles gambiae* genome reannotation through synthesis of *ab initio* and comparative gene prediction algorithms. *Genome Biology*. 7:R24.

Rosinski-Chupin I, Briolay J, Brouilly P, Perrot S, Gomez SM, Chertemps T, Roth CW, Keime C, Gandrillon O, Couble P. and Brey PT. (2007) SAGE analysis of mosquito salivary gland transcriptomes during *plasmodium* invasion. *Cellular Microbiology.* 9(3):708-724.

Wu Y, Choi K and Gomez SM. (2008) Prediction of protein-protein interaction networks. Current Protocols in Bioinformatics. Unit 8.2.

Yang W, Johnson GL and Gomez SM. (2008) Data-driven modeling of cellular stimulation, signaling and output response in RAW 263.7 cells. *J Mol Sig.* 3:11.

Johnson GL and Gomez SM. (2009) Sequence patches on MAPK surfaces define protein-protein interactions. *Genome Biology*. 10:222.

Choi K and Gomez SM. (2009) Comparison of phylogenetic trees through alignment of embedded evolutionary distances. *BMC Bioinformatics.* 10:423.

Staab JM, O'Connell TM and Gomez SM. (2010) Enhancing metabolomic data analysis with Progressive Alignment of NMR Spectra (PCANS). *BMC Bioinformatics*. 11(1):123.

Doolittle JM and Gomez SM. (2010) Structural similarity-based predictions of protein interactions between HIV-1 and Homo sapiens. *Virology Journal*. 7(1):82.

D. Research Support

Ongoing Research Support

R01-DK037871 Johnson (PI) 1/15/2007 – 12/31/2011 NIH/NIDDK
Regulation of Sequential Protein Kinase Pathways
This work focuses on determining how MAPK pathways are regulated via members of the MAPKKK family and involves experimental efforts along with the development of quantitative, systems-level models for describing the spatiotemporal behavior of pathway members. Specific focus is on the role of MKKKs in the spatiotemporal activation of ERK1/2, JNK, p38 and ERK5.
Role: Co-Investigator

The Carolina Center for Computational Toxicology Russyn (PI) 4/1/2008 – 3/31/2012 EPA RD-83382501
The objective of this proposal is to create The Carolina Center for Computational Toxicology. My group's project is focused on the inference and predictive modeling of chemically-perturbed regulatory networks.
Role: Co-PI

R01-GM084071 1/01/2009 – 12/31/2012 NIH/NIGMS
MAP kinase regulation of cell-fate transitions in yeast
The major goals of this project are to learn how differences MAPK activation profiles translate into alternative transcription programs and to uncover how the two programs differentially affect the physiological and phenotypic potential of the cell.
Role: Co-PI (Errede and Elston, Co-PIs)

Completed Research Support

W911NF0910049 US Army Short-Term Innovative Research 2/6/2009 – 10/1/2009 USARO
Prediction of host-pathogen protein-protein interactions.
This proposal focuses on the development of computational approaches for the prediction of host-pathogen protein interactions. Major outputs of this work include: 1) Development of a novel method for the comparison of phylogenetic trees with potential application to detection of coevolutionary processes between a host and pathogen, 2) Development of a method for the prediction of protein interactions between a host and pathogen based on protein structural similarity and 3) Application of domain and structure based predictions to prediction of host-pathogen interactions.
Role: PI

R21-GM075941-01, MacDonald (PI) 07/01/2006 – 06/30/2009 NIH/NIGMS
Human Enterohepatic Cell Model for Predictive Toxicology
The major goals of this project are to create the first 3-D human bioartificial entero-hepatic organ-system and establish the feasibility of using this model system to evaluate the toxicity of chemicals and drugs. The proposed technology will be based on incorporating a defined population of human liver cells in an extracellular matrix thus creating a microenvironment composed of a precise composition of insoluble factors to interact with the cells.
Role: Co-Investigator

UNC University Cancer Research Fund Innovators Award Gary Johnson (PI) 1/1/08 – 6/30/09
Defining Signaling networks Controlling Tumorigenesis
The objective of this proposal is to perform a comprehensive analysis to define the specific Ste20 kinases and MAP3Ks required for tumor growth and/or metastasis.
Role: Co-Investigator

UNC Joint Department of Biomedical Engineering Research Initiation Project 9/1/2007 – 6/30/2008
A systems-biology examination of antibiotic tolerance in Pseudomonas aeruginosa for cystic fibrosis
Role: Co-PI (Giddings, Co-PI)