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RPPR Final Report
as of 10-Mar-2020

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STEM Degrees: 0 **STEM Participants:** 3

Major Goals: Our goal is to combine innovative computational approaches with novel molecular tools for imaging and manipulating intracellular signaling networks in living cells to elucidate the molecular mechanisms that drive cell migration. The ability to both manipulate and visualize signaling pathways in vivo combined with computational methods for image analysis will generate the quantitative data needed to develop predictive mathematical models. Together these tools enable unprecedented resolution for deciphering the complex control mechanisms that coordinate Rho GTPase signaling during cell migration. In particular, we will elucidate mechanisms of feedback, feed forward and cross regulation that tightly control the spatiotemporal activity of the canonical Rho GTPases, RhoA, Rac1 and Cdc42. We hypothesize that different regulatory mechanisms operate in different regions of the cell, and that this differential regulation is critical for proper cell migration.

Accomplishments: This project resulted in 6 research articles. Four have been published in peer review journals, and two are currently under review.

In a paper that appeared in PLoS Computational Biology, we use particle-based stochastic simulations to investigate the role of molecular-level fluctuations in polarity establishment (1). In particular, we developed a computational platform for performing particle-based simulations of the biochemical steps involved in polarization. Our method explicitly tracks signaling molecules in the membrane and in a thin layer adjacent to membrane. Molecules located in the interior of cell are treated implicitly. We performed extensive tests to verify our method produces results consistent with reaction-diffusion equations in the macroscopic limit. Our simulations revealed that molecular-level noise can decrease the time required for polarization and make the polarity circuit more robust to changes in molecular abundance.

We extended our platform for particle-based simulations to simulate actomyosin dynamics. Our models account for biophysical interactions between filamentous actin and non-muscle myosin II. Our simulations reveal the spontaneous formation of actin asters that may be related to the actin puncta, we observe during frustrated phagocytosis. A systematic analysis of model parameters was used to identify biochemical steps in myosin activity that are good candidates for regulatory control. Finally, we have investigated how the model responds to spatial gradients in parameter values. Interestingly, spatial regulation of motor stiffness led to a time-dependent behavior

of the actomyosin network, in which actin asters continue to spontaneously form and dissociate. A manuscript describing these results was published in Integrative Biology (2).

Cell migration requires precise and complex spatiotemporal regulation of the actin cytoskeleton. Therefore, perturbing this regulation can lead to subtle differences in cell migration. To assess these differences, it is necessary to accurately quantify cell migration in a biologically relevant manner. We therefore developed computational tools, based on stochastic modeling, to analyze time series data for the position of randomly migrating cells. Our approach allows parameters that quantitatively characterize cell movement to be efficiently estimated from experimental data. We applied our methods to two different cell types (Mouse Embryonic Fibroblasts (MEFS) and HeLa cells). Our analysis revealed that randomly migrating cells stochastically transition between distinct states of migration characterized by differences in cell speed and persistence. Using a biosensor for Rac1 activity developed in the Hahn lab, we demonstrate these two states of migration are characterized by differing number of foci of Rac1 activity. A paper describing these results has been posted on bioRxiv (doi: https://doi.org/10.1101/249656) and is under consideration for publication in Frontiers in Systems Biology.

We developed several novel computational tools for analyzing live-cell images. Using deep learning to perform structured illumination microscopy we obtained super-resolution images with 1/3 the number of raw images, and generate images under extreme low light condition (100X fewer photons). We validated the performance of deep learning networks on different cellular structures and achieved multi-color, live cell super-resolution imaging with greatly reduced photobleaching. A manuscript describing these results was positively reviewed by Nature Communications (4), and we recently submitted a revised version of the paper that addressed all the reviewers' comments.

Lattice light-sheet microscopy (LLSM) is valuable for its combination of reduced photobleaching and outstanding spatiotemporal resolution in 3D. Using LLSM to image biosensors in living cells could provide unprecedented visualization of rapid, localized changes in protein conformation or posttranslational modification. However, computational manipulations required for biosensor imaging with LLSM are challenging for many software packages. The calculations require processing large amounts of data even for simple changes such as reorientation of cell renderings or testing the effects of user-selectable settings, and lattice imaging poses unique challenges in thresholding and ratio imaging. We developed a new software package, named ImageTank, that is specifically designed for practical imaging of biosensors using LLSM. To demonstrate its capabilities, we use a new biosensor to study the rapid 3D dynamics of the small GTPase Rap1 in vesicles and cell protrusions. A manuscript describing these results was recently published in the Journal of Cell Biology (5).

Developing molecular tools to visualize and control Rho GTPase signaling in living cells has been instrumental in elucidating the mechanisms of cytoskeletal reorganization and causal relationships between activation events in cell function. An indispensable part of such studies is the quantitative characterization of the spatiotemporal GTPase activity. We developed a computational pipeline, EdgeProps, designed for comparative/correlative analysis of cell dynamics (edge velocity) and near-edge protein activity (intensity of a fluorescent signal). The tool offers a user-friendly interface with three functional modules for processing, visualization, and statistical characterization of single-cell imaging data. A paper describing EdgeProps was published in Methods in Molecular Biology (6).

1. Pablo, M, S. Ramirez, and T. C. Elston. ¬¬¬¬¬¬2018. Particle-based Simulations of Polarity Establishment Reveal Stochastic Promotion of Turing Pattern Formation. PLoS Comp Bio 14(3): e1006016.
2. Miller, C., S. Asokan, J. Haugh, J. Bear and T.C. Elston. 2019. A Computational study of emergent structures in actomyosin dynamics during chemotaxis. Integrative Biology 11(6):208-292.
3. Allen, R., C. Welch, N. Pankow, K. Hahn and T.C. Elston. 2020. Stochastic methods for inferring states of cell migration. Frontiers in Systems Biology (submitted, bioRxiv: doi: https://doi.org/10.1101/249656).
4. Jin, L, B. Liu, F. Zhao, S. Hahn, B. Dong, R. Song, T. C. Elston, Y. Xu and K. Hahn. 2019. Deep learning enables structured illumination microscopy with low light levels and enhanced speed. Nat. Comm. (submitted).
5. O'Shaughnessy, E., O. Stone, P. LaFosse, M. Azoitei, D. Tsygankov, J. Heddleston, W. Legant, E. Wittchen, K. Burridge, T. C. Elston, E. Betzig, T. Chew, D. Adalsteinsson, K. Hahn. 2019. FRET biosensor imaging with lattice light-sheet microscopy, exemplified by Rap1. J. of Cell Biol. 218(9):3153-3160.
6. Zhurikhina, A., T. Qi, K. Hahn, T. C. Elston, and D. Tsygankov. 2018. EdgeProps: a Computational Platform for Correlative Analysis of Cell Dynamics and Near-edge Protein Activity. Method in Molecular Biology (Clifton, N.J.) 1821:47-56.

Training Opportunities: This project provided partial support for three graduate students and five postdoctoral researchers.

Results Dissemination: All research results have been or will be reported in peer review journals.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

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Person Months Worked: 1.00 **Funding Support:**
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Other Collaborators:

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International Collaboration:
International Travel:
National Academy Member: N
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Project Contribution:
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
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International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:

International Collaboration:
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National Academy Member: N
Other Collaborators:

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Authors: Luhong Jin, Bei Liu, Fenqiang Zhao, Stephen Hahn, Bowei Dong, Ruiyan Song, Tim Elston, Yingke Xu,
Keywords: Structured illumination microscopy, deep learning, live-cell imaging
Abstract: Using deep learning to augment structured illumination microscopy (SIM), we obtained a fivefold reduction in the number of raw images required for super-resolution SIM, and generated images under extreme low light conditions (100X fewer photons). We validated the performance of deep neural networks on different cellular structures and achieved multi-color, live-cell super-resolution imaging with greatly reduced photobleaching.
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Nothing to report in the uploaded pdf (see accomplishments).