Biophysical tools to quantify cell adhesion and migration in normal and pathological conditions.

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Cell migration is fundamental to normal biological processes such as development, the immune response and wound healing [1]. When cell migration is not regulated properly it can lead to pathological conditions such as cancer metastasis, immune disorders and developmental disorders. Cell adhesions form between the extracellular matrix (ECM) and the internal cellular cytoskeleton creating structures required for productive cell movement. Adhesions are tightly regulated in space and time across the moving cell. They form anchor points at the front of the cell so it can pull forward and adhesions at the rear of the cell rear that need to disassemble allowing for forward motion [1]. Our research is focused on developing quantitative biophysical tools to gain an understanding of the complex molecular mechanisms that regulate adhesion dynamics (formation, stability, disassembly), protein interactions within adhesions and the ability the cell to sense the biomechanics of its surrounding environment.

Paxillin is an adaptor protein that plays a key role bringing structural and signaling proteins to sites of adhesion [2]. We have been interested in a key paxillin phosphorylation site, serine 273 (S273) that is known to regulate adhesion dynamics [3]. Image correlation microscopy (ICM) data will be presented showing variability of protein binding dynamics across individual adhesions (Figure 1) [4]. Image cross correlation microscopy (ICCM) data will be presented to show that adhesion subdomains correspond to areas where paxillin is co-binding with kinases important for S273 phosphorylation [4].

We have implicated the Lipoma Preferred Partner (LPP) protein as an important mechanosensitive integrator of TGFβ-mediated breast cancer cell migration and invasion [5,6]. Data will be presented showing that LPP plays a role in regulating cell migration and adhesion dynamics in response to TGFβ stimulation. This regulation requires LPP binding to the actin cytoskeleton. Data will be presented showing how a FRET based tension sensor has been used to show that LPP regulates tension across individual adhesions. Results will also be presented that show LPP functions as a mechanosensor to distinguish between substrates of different stiffness. Results from 3D single molecule localization microscopy data (iPALM) will demonstrate the localization of LPP relative to other adhesion proteins with up to high precision in individual adhesions. The biomechanics of the cellular environment has emerged as a key determinant of cellular behavior and disease progression including cancer metastasis. Results will be presented showing how unsupervised clustering approaches can be used to reveal
subtle phenotypes based on quantitative measurements of adhesions in cells grown on different 2D and 3D substrates (Figure 2).

Overall, this work will show how biophysical tools can take us to the next level to make sense of the tremendous amount of data acquired from single images and timelapse image series to gain a clear understanding of fundamental biological processes and processes of disease.

**Figure 1:** Image correlation microscopy (ICM) results from CHO-K1 cells expressing paxillin-EGFP localized to cell-matrix adhesions. Temporal binding times are measured for each pixel in the image and displayed as short (cyan), intermediate (yellow) or long (magenta) binding times. Adhesion subdomains of variable paxillin binding dynamics are see across adhesions. Scale bar is 10 µm.
**Figure 2:** Unsupervised Hierarchical Clustering Analysis (HCA) of adhesion properties in LPP-EGFP expressing NMuMG cells. Primary clusters (A, B, C, D) exhibit distinct phenotypes with large long adhesions in Cluster A and small round adhesions in Cluster D. HCA was conducted on data from cells expressing wt-LPP or an actin binding mutant (ΔABD) in the presence or absence of TGFβ stimulation. Orange scale is high, white is average and blue is low.

References


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