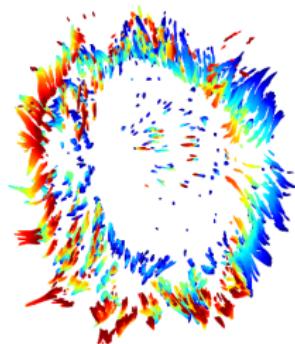


Quantitative Analysis of Focal Adhesions in TIRF Microscopy Images

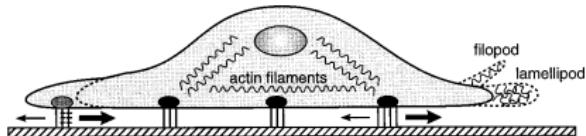
Matthew E. Berginski

Hahn Lab Meeting 8/17/2009

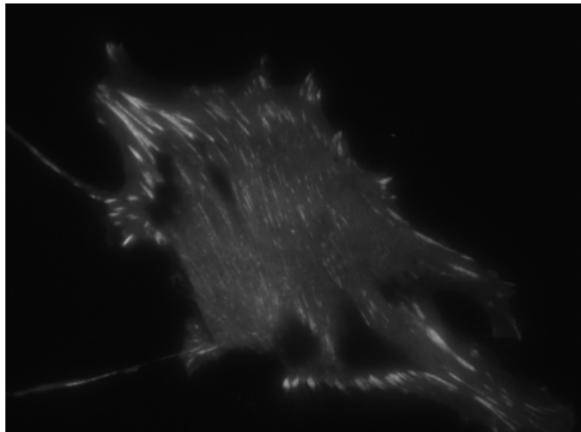


Focal What?

- ▶ Points of contact between the substrate and cells
- ▶ Consist of dozens of dynamically recruited proteins
- ▶ Important in understanding how cells navigate and sample the environment

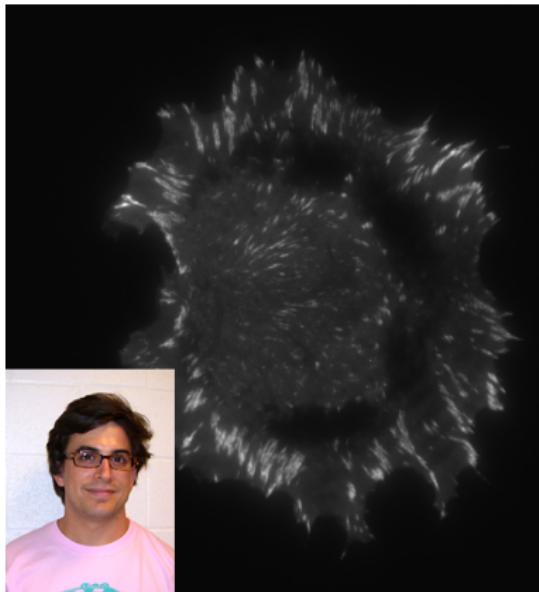


Lauffenburger, DA and Horwitz, AF. Cell, 84 359–69

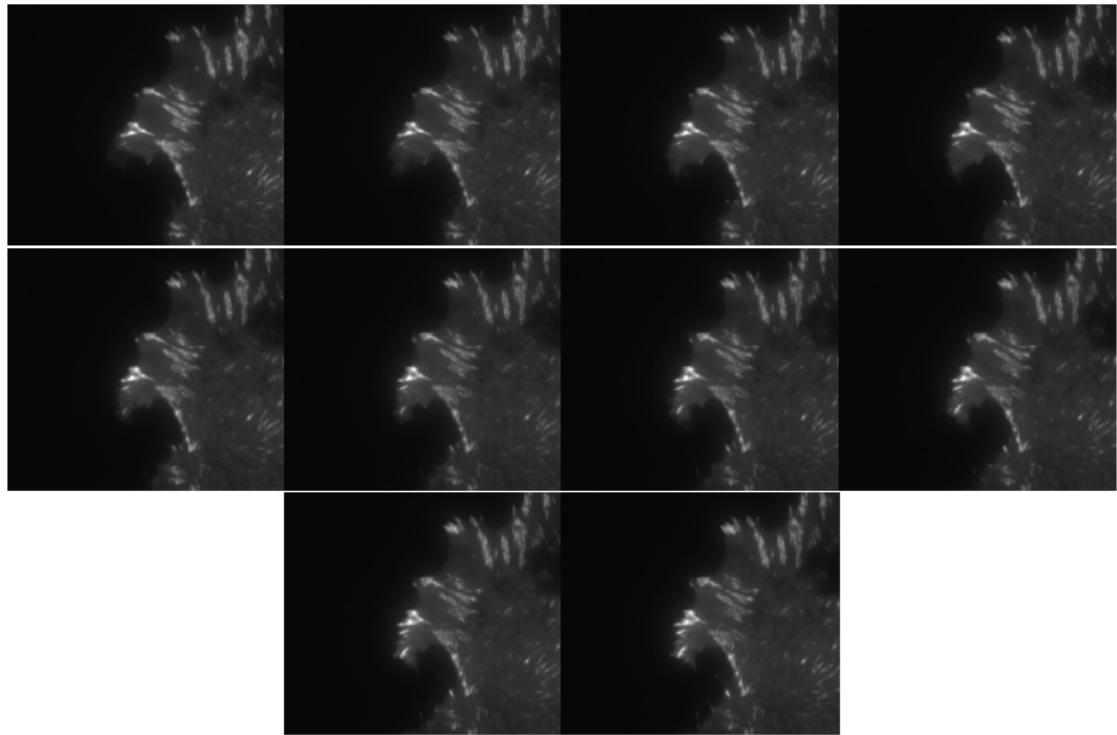


Paxillin Info/Example Movie

- ▶ Paxillin - adhesion scaffolding protein
- ▶ Recruited to adhesions early, remains associated
- ▶ Data consists of time lapse images of GFP-labeled Paxillin in 3T3 fibroblasts

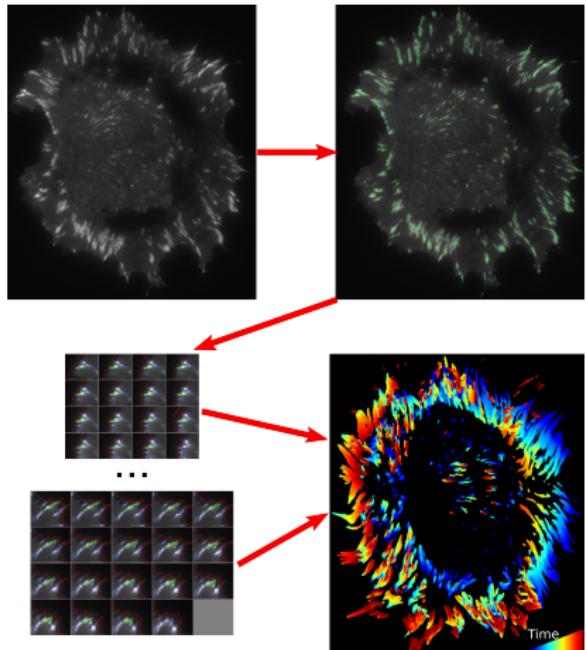


Paxillin Movie Backup

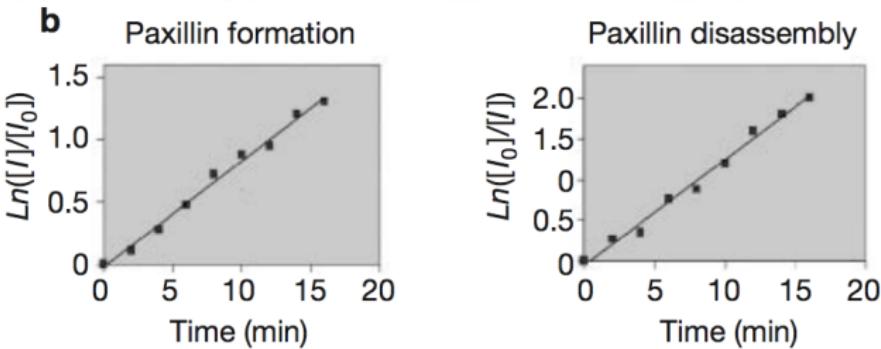
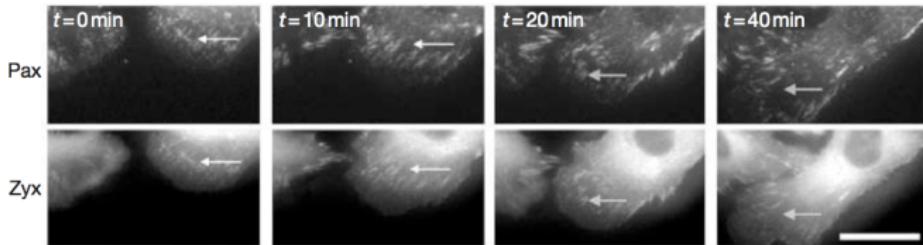


Methods Overview

- ▶ Multistage Process
 - ▶ Finding Adhesions
 - ▶ Tracking Adhesions
 - ▶ Collect Adhesion Properties
 - ▶ Visualization



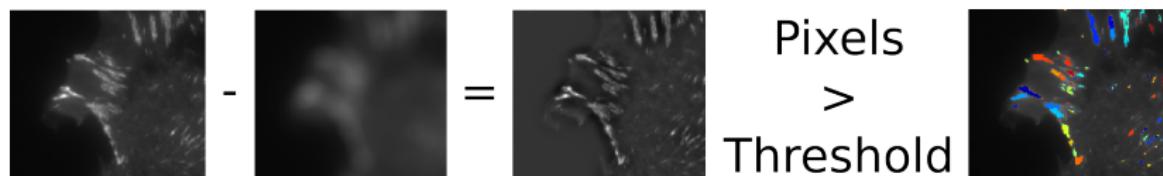
Prior FA Analysis Methods



D. Webb, et al. NCB, 6(2):154-161, 2004.

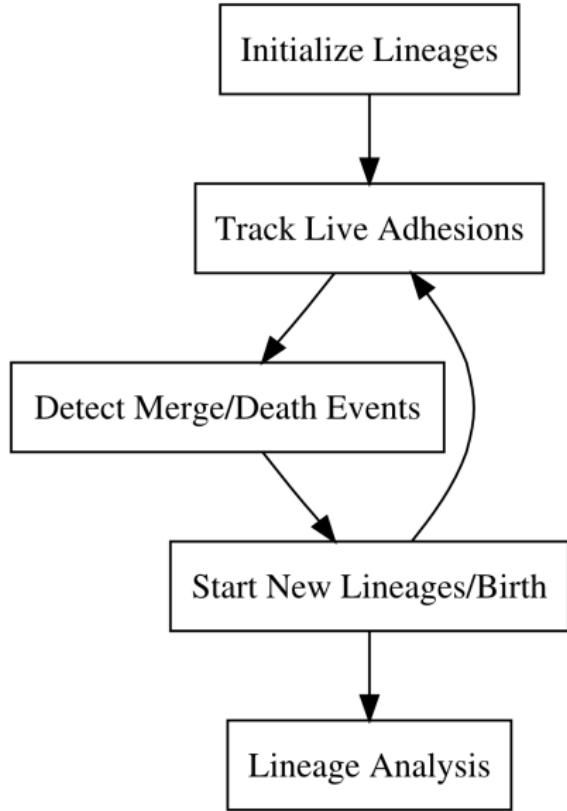
Finding Adhesions

- ▶ Technique adapted from E. Zamir, et al. JCS, 112(11):1655-1669, 1999.
- ▶ High-pass image filter applied
- ▶ Threshold used to select pixels



Tracking Adhesions

- ▶ Uses properties extracted from each of the images
- ▶ Criteria for matching adhesions between images include the percentage overlap and the distance between the adhesions



Extracted Properties Summary

Static

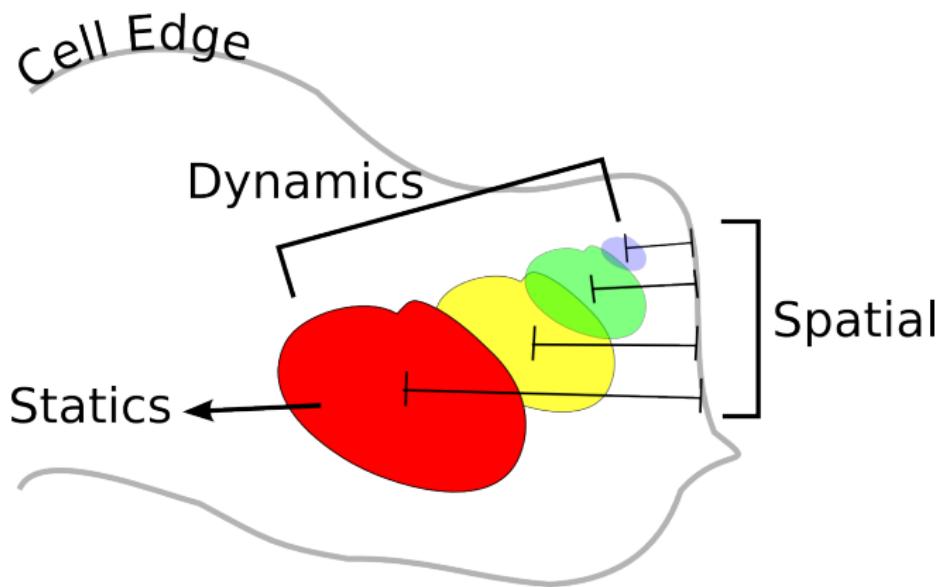
- ▶ Area, Paxillin Intensity, Axial Ratio, ...

Dynamics

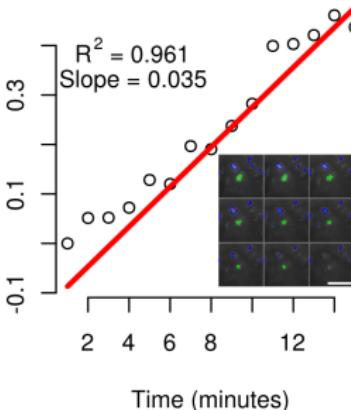
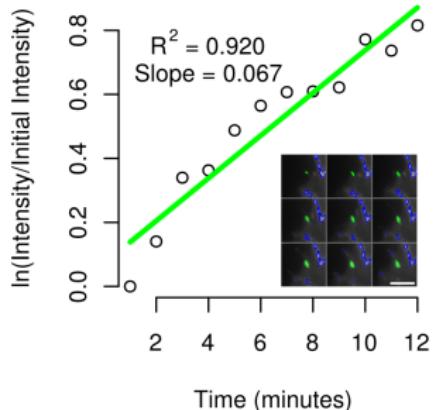
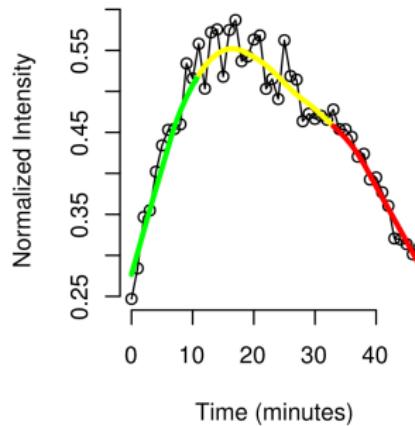
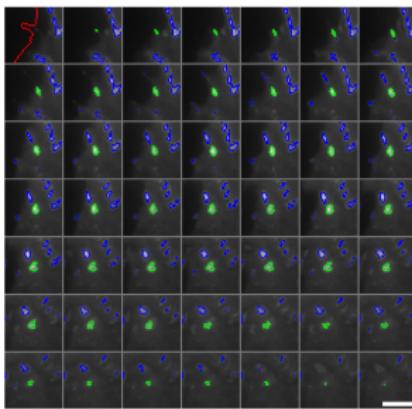
- ▶ Assembly and Disassembly Rates

Spatial

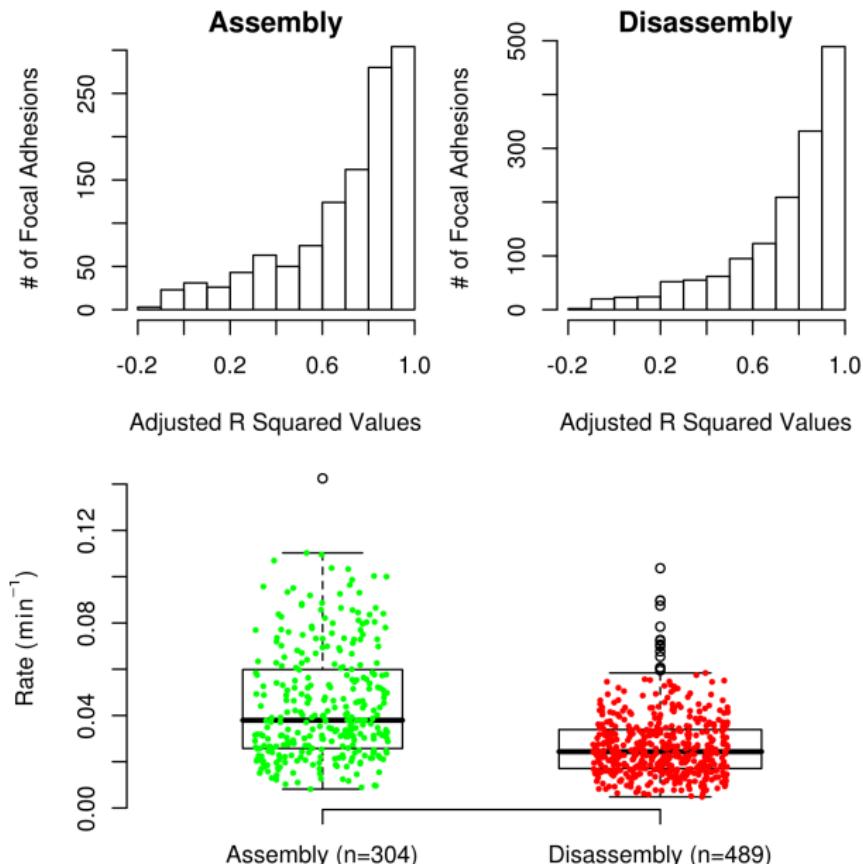
- ▶ Distance from Cell Edge



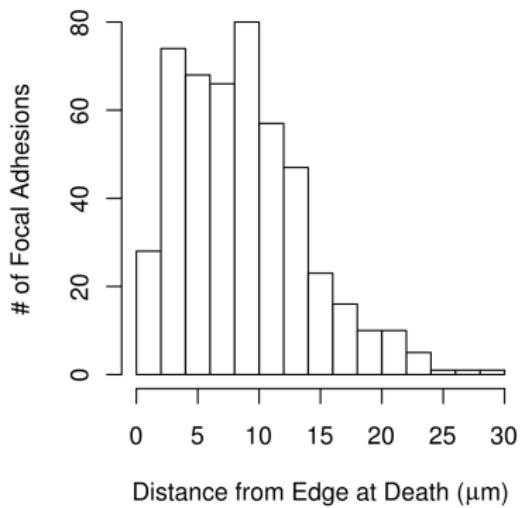
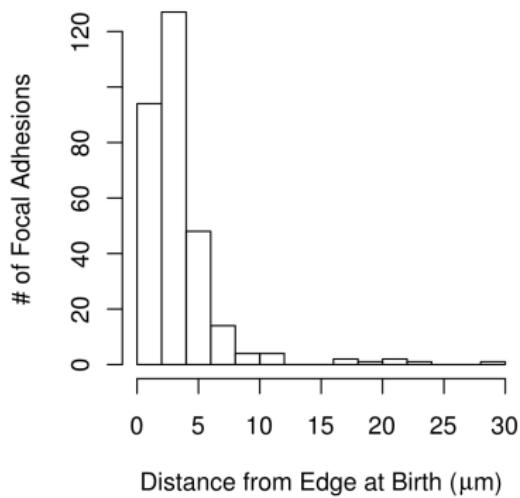
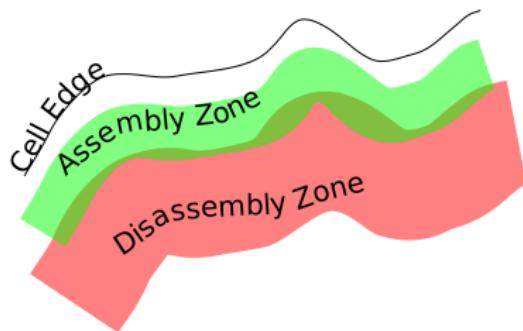
Finding Assembly and Disassembly Rates



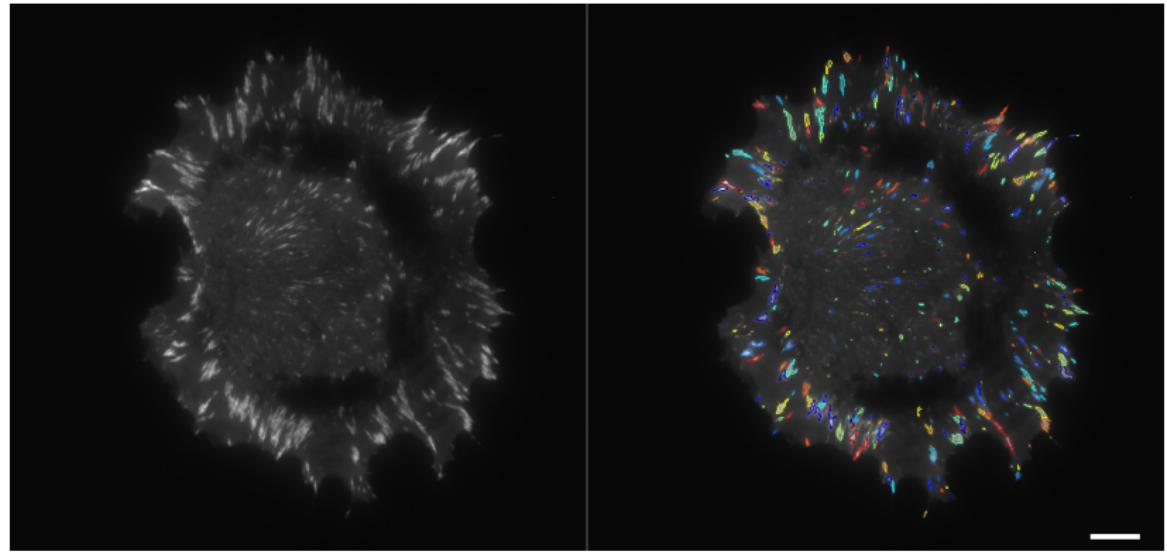
Overall Assembly and Disassembly Rates



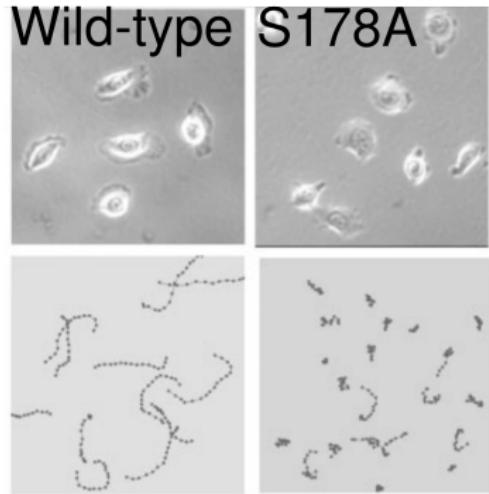
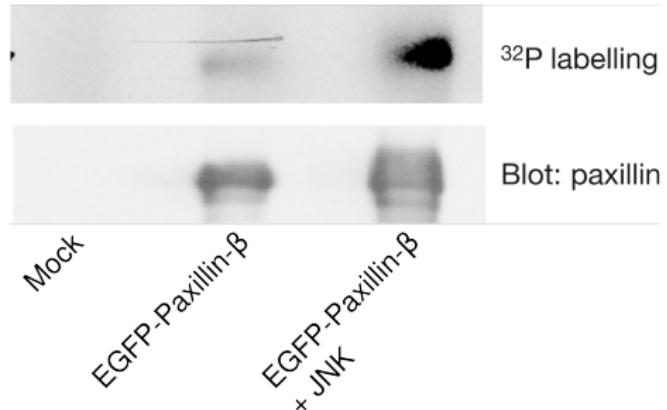
Spatial Properties



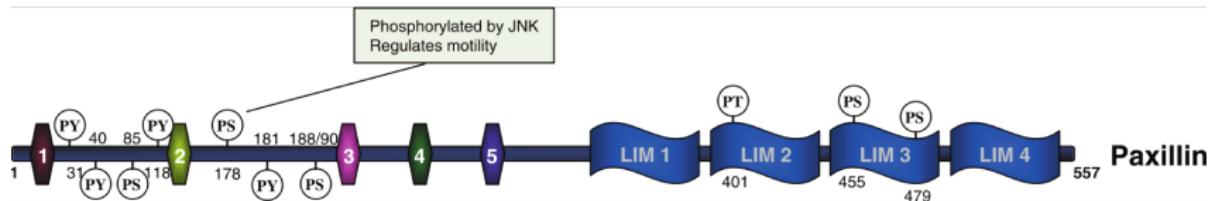
Visualizing the Results



JNK phosphorylates Paxillin on Serine 178

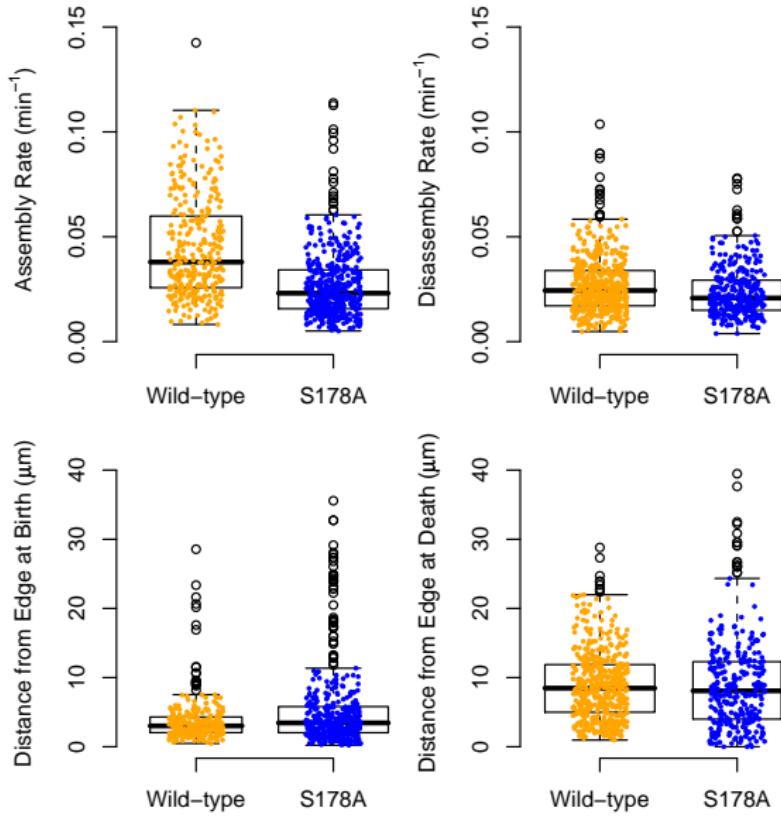


Huang, C. et al. Nature 424, 219-223

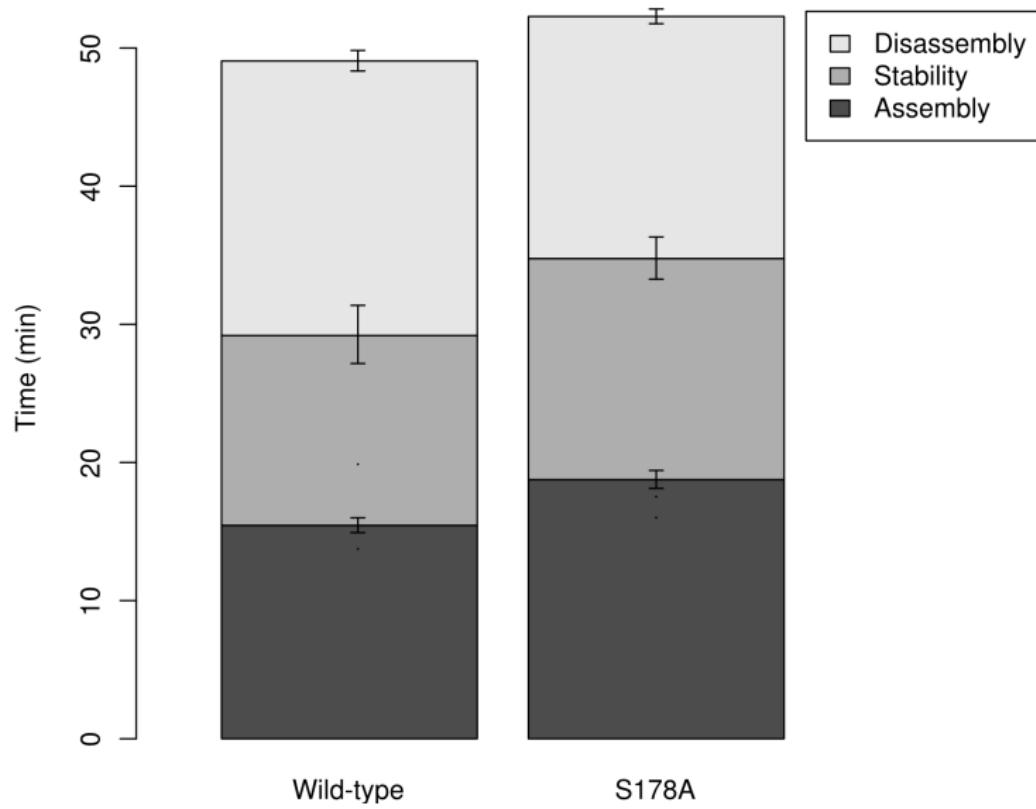


Brown, M.C. and Turner, C.E. Physiological Reviews 84, 1315--1339

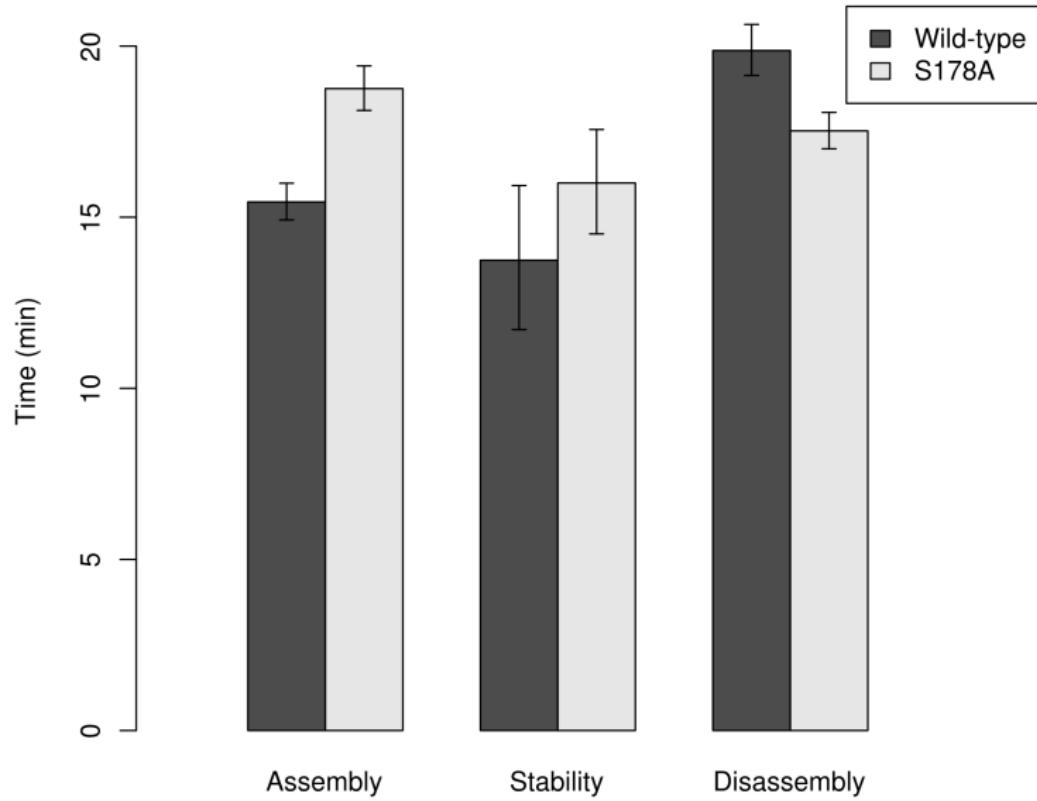
The S178A Mutant Perturbs Adhesion Development



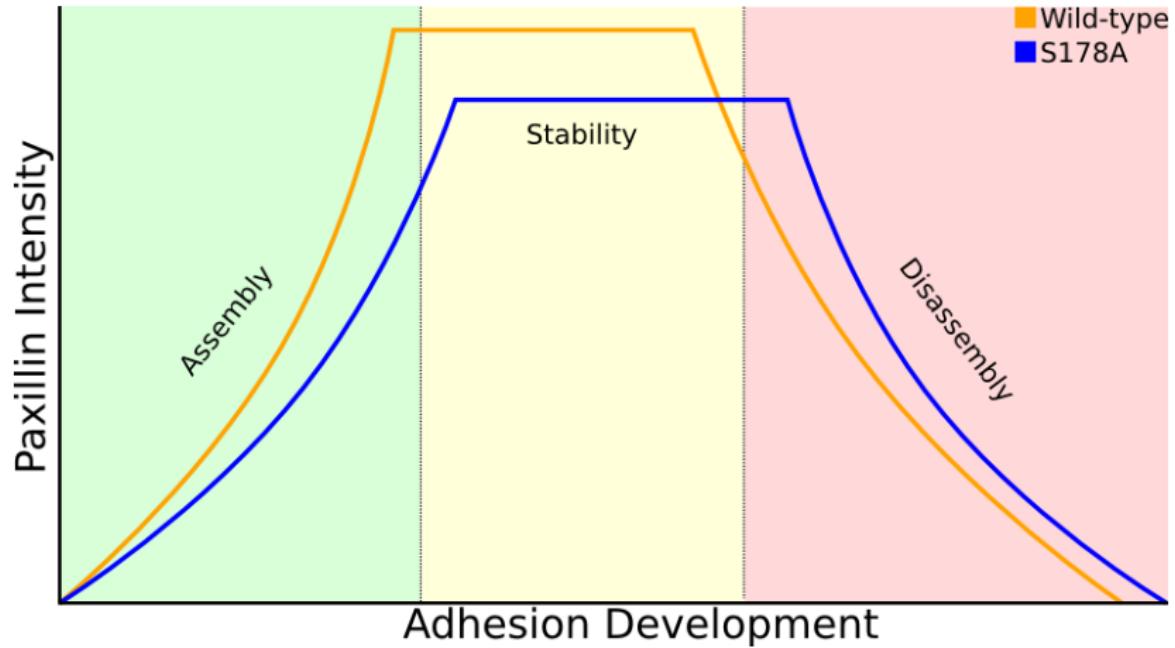
The S178A Mutation Affects Phase Length Differences



The S178A Mutation Affects Phase Length Differences



S178A Differences Summary



Future Work

- ▶ Addition of more software features
 - ▶ Integration of the cell edge velocity
- ▶ Examining other signaling network perturbations