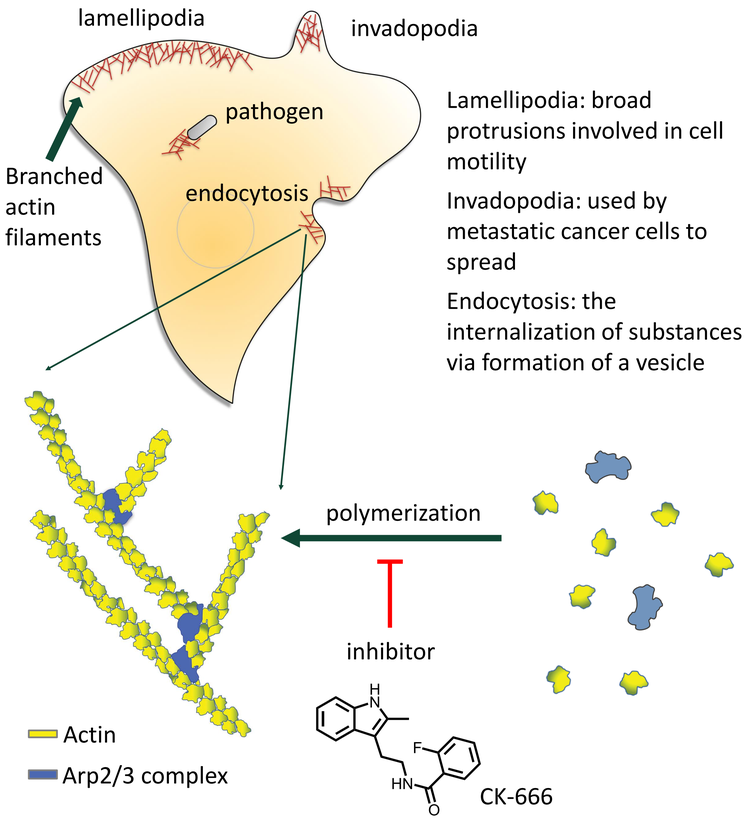
Developing an *In Vivo* Assay for Quantitative Analysis of Arp2/3 Complex Inhibitors

Maisie Topping, Heidy Narvaez Ortiz, Brad Nolen

University of Oregon, Department of Chemistry and Biochemistry, Institute of Molecule Biology

Branched actin networks are critical for diverse cellular processes



Lamellipodia: broad protrusions involved in cell motility

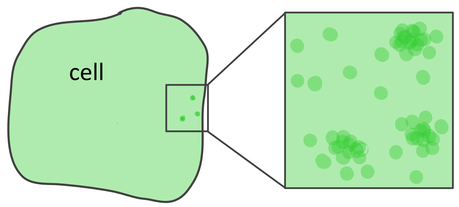
Invadopodia: used by metastatic cancer cells to spread

Endocytosis: the internalization of substances via formation of a vesicle

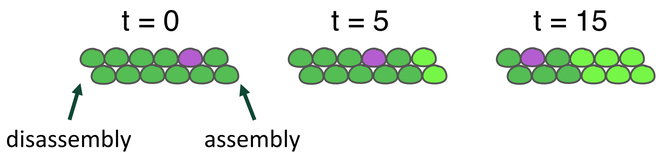
How can we quantitatively measure the effects of Arp2/3 complex inhibitors on actin cytoskeleton dynamics in live cells?

Speckle total internal reflection fluorescence microscopy (TIRF)

- Small percentage of actin monomers labelled with GFP
- Speckles represent a higher local density of GFP relative to the surroundings



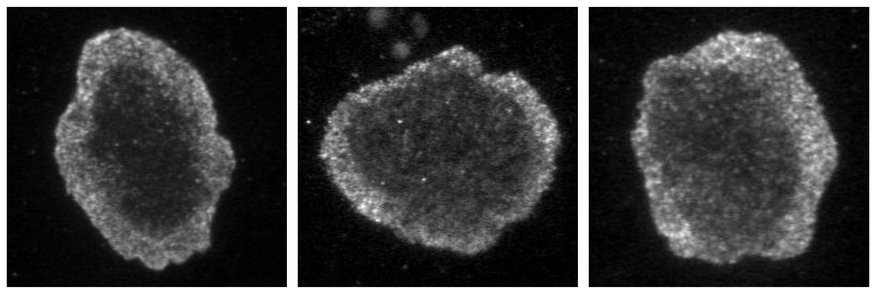
Actin filaments are assembled close to the membrane and disassembled further from the membrane and speckles move inward



Speckle velocity reflects the activity of Arp2/3 complex

Movies

Movies taken for 2.5 minutes, with 100 msec exposures every 2 seconds at 3 mW laser power

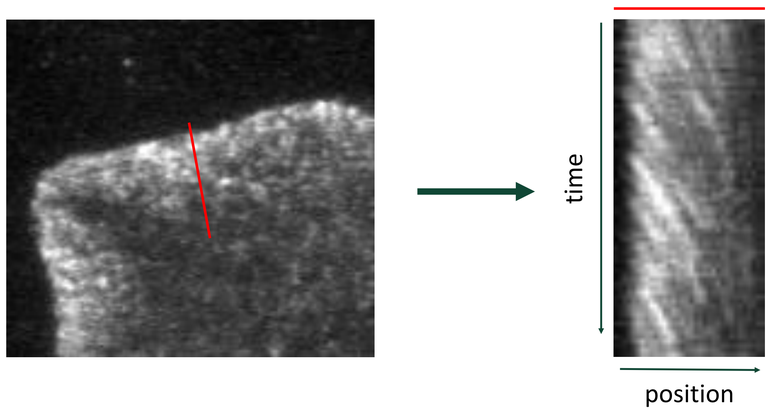


No inhibitor | With inhibitor (CK-666) | DMSO control

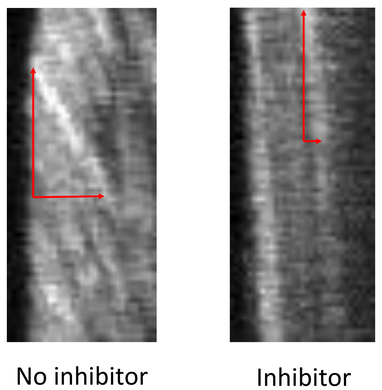
Inhibitor stocks are made in dimethyl sulfoxide (DMSO). DMSO control contains the same final concentration of DMSO as the cells with inhibitor.

Kymographs

- Graphical representations of position over time
- A line is drawn over the image along the plane of movement
- A kymograph is the compilation of that line over a series of time points (in this case the entire movie)



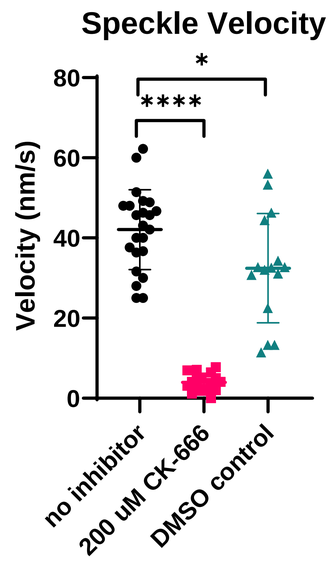
- Movement along the line appears as diagonal lines in the kymograph
- Calculate the change in x and y for the movement of individual speckles
- Convert to nm and seconds to get velocity



No inhibitor | Inhibitor

Results & Conclusions

- Average velocity without inhibitor is 42 nm/s
- With 200 uM CK-666, average velocity is 4 nm/s
  - This concentration is typically used to eliminate Arp2/3 complex activity
- Large difference before and after inhibition indicates the assay is well-designed for measuring this metric
- In DMSO control cells, average velocity is 32 nm/s
  - Would expect no difference between no inhibitor and control
- Significant difference for control cells indicates the assay needs to be improved



Future Directions

- Much more data collection
  - Expand to using a range of inhibitor concentrations
  - Potential application for characterizing Arp2/3 complex inhibitors that are discovered in the future
- Try to reduce the final concentration of DMSO to avoid any potential non-inhibitor induced velocity changes
- Automate the quantification process with programs that automatically track speckles
  - Allows more data per video
  - Reduces human error / biases

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