A Model for Cellular Blebbing

M. Lougher

THE UNIVERSITY OF
WARWICK

Aims of the Model

- Replicate observed bleb behaviour during formation.
- Investigate relationship between curvature and peak bleb speed.
- Investigate whether bleb formation rate depends on local curvature.

Model Components

- Active Contours
  - Main basis of model.
  - Internal energy from tension and curvature, plus elastic energy and pressure.
  - External energy from deformation force.

$$E_{snake} = \int_0^1 (E_{internal} + E_{external})\,\mathrm{d}s$$

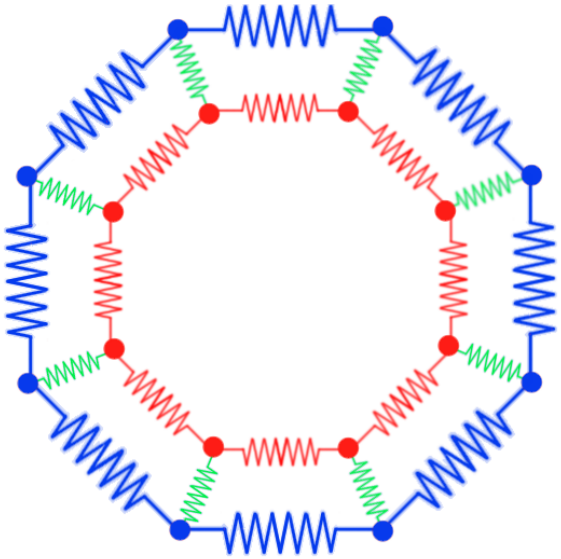
  - Tension and curvature defined by derivatives of position.

$$\nabla E_{internal} = \alpha \frac{\partial^2 v}{\partial s^2} + \beta \frac{\partial^4 v}{\partial s^4}$$

  - Approximated these using Finite Differences.

Model Components

- Elastic Energy
  - Plasma Membrane (PM), actin cortex and linkers (FREM proteins) modelled as Hookean springs.
  - Spring coefficient for PM 10x less than linkers & cortex.
  - Rest length of PM & cortex the same, so no inherent curvature.
  - Linker rest length smaller.



Model Components

- Pressure
  - Isotropic around entire contour => no location dependence.
  - Assumed force behind pressure due to cytoplasm and other cell components is constant => no flow in or out of cell.
  - Pressure varies as surface area changes, so blebs decrease pressure by increasing surface area.

Model Components

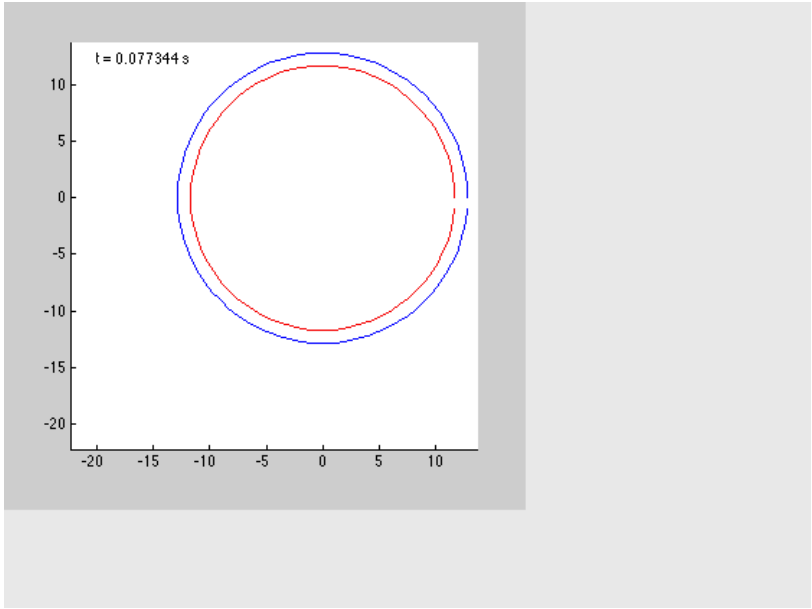
- Linker Interactions
  - Modelled as acting only on the PM, assumed cortex ‘fixed’ by other structures within the cell.
  - Linkers modelled as fragile; they break when exceed maximum extension (proportional to breaking force).
  - Finite linker width optional in model to prevent over crowding (relevant when linker density not constant).

Model Components

- Stochastic Considerations
  - Later experiments have 1% probability of each linker breaking at each time step.
  - Also 1% probability of broken linkers reforming if PM node hasn't exceeded maximum distance.
  - Implemented using XOR operation on random numbers > 0.99, plus the current linker condition for each node.

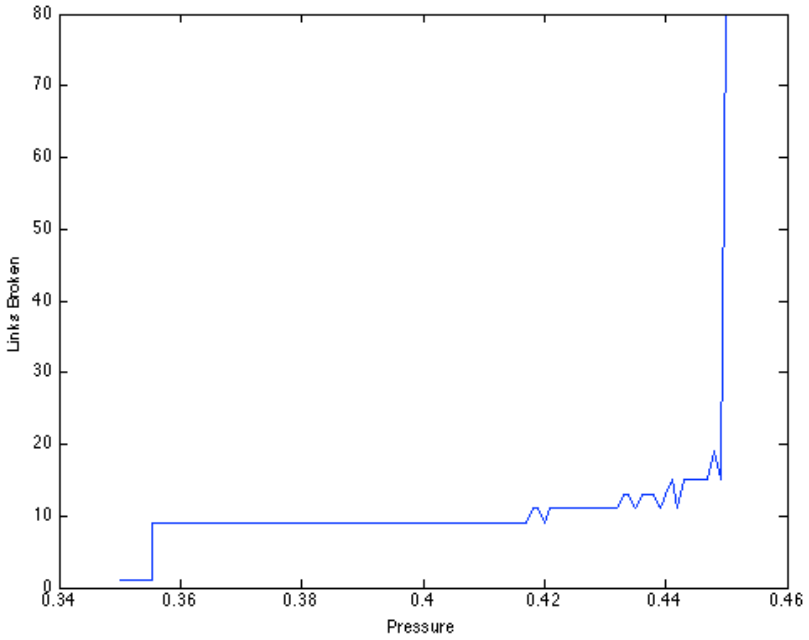
Preliminary Results

- Initial Bleb Simulation
  - Cut linker manually.
  - Linkers break until pressure decreased.
  - Bleb then rearranges into hemisphere.



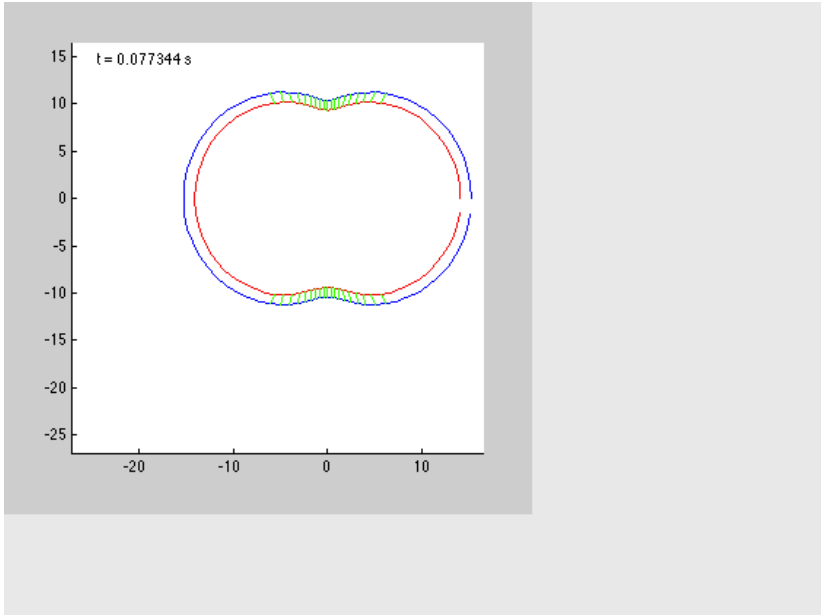
Preliminary Results

- Pressure Dependence
  - Recorded number of linkers broken as pressure increases.
  - Found mainly increased in jumps.
  - Went down to pressure steps of 10^{-15} to see only slightly smaller jump.



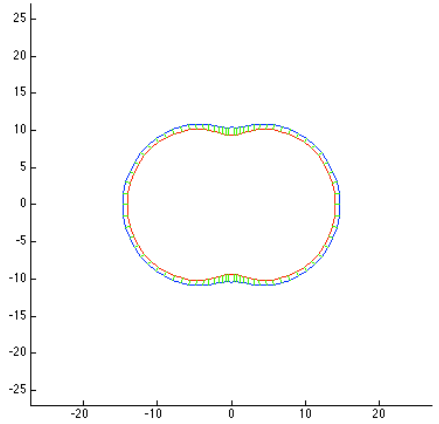
Preliminary Results

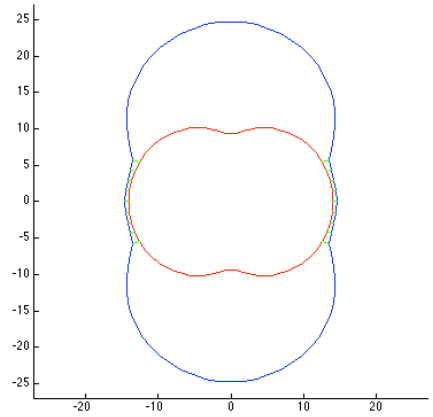
- Varying Curvature & Linker Density
  - Hard to quantify relationship.
  - Complex dependence on curvature and linker density.
  - But, if initial hypothesis proven, can see that increased linker density dominates effect of negative curvature.



Preliminary Results

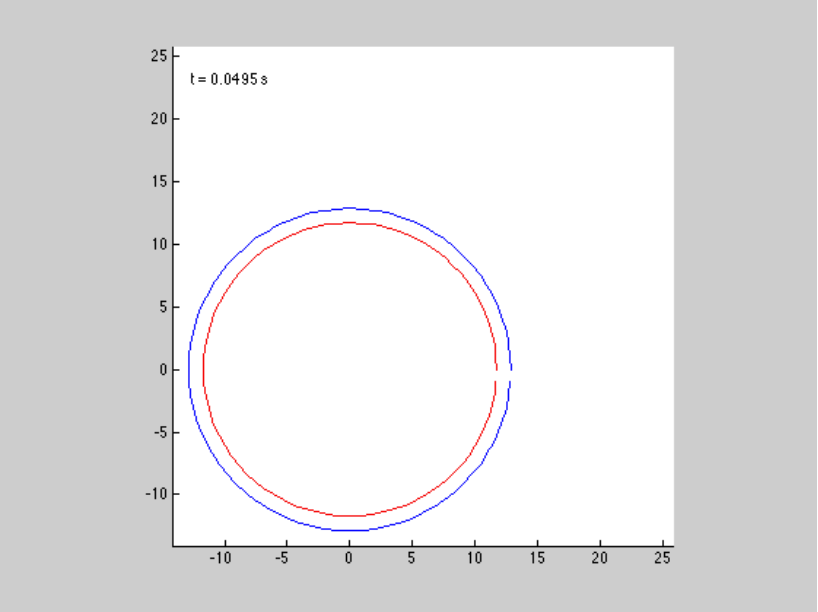
- Finite Linker Width
  - Attempt to overcome high linker density by introducing finite width.
  - Led to nice blebs if all linkers closer than minimum separation broken.
  - Added in NAND operation so only surrounded linkers would break.
  - Random chance of odd or even analysed first. Led to two very different outcomes.
  - Not included in final model.





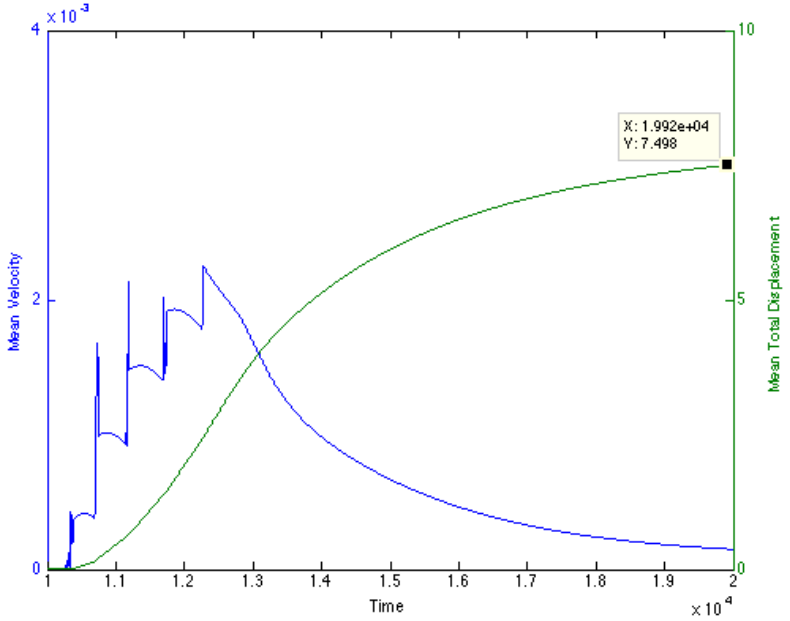
Preliminary Results

- Curvature with Fixed Linker Density
  - Started with circular vesicle and pushed inwards at a point.
  - Linkers maintained for 5 seconds to allow cortex to equilibrate.
  - Still takes another 4 seconds for linker to break.
  - Forms natural bleb in area of negative curvature.
  - Model ready for more in depth analysis.



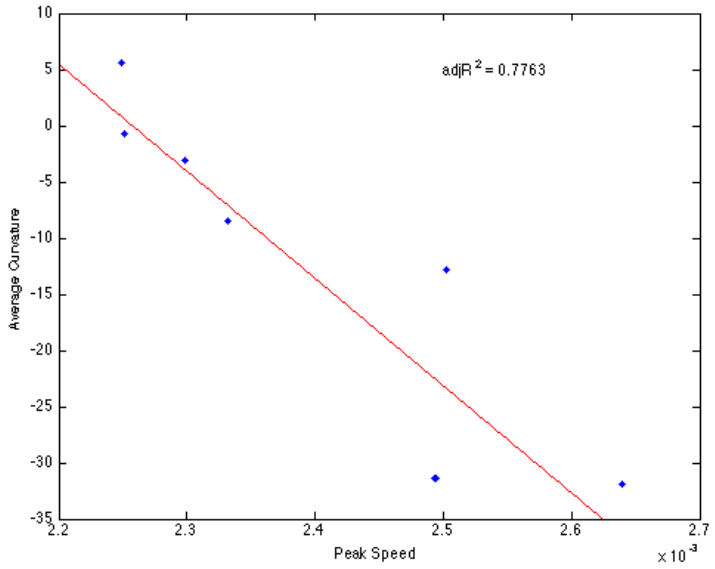
Results

- Velocity and Displacement Profiles
  - Compared to profiles in R. Tyson's thesis.
  - Maximum displacement sigmoidal.
  - Peak speed slightly different.
  - Get jumps due to instant acceleration when linker breaks.
  - Indicates linker behaviour not quite modelled right.
  - Exponential decay not seen in experiment as cortex reformation not modelled here.



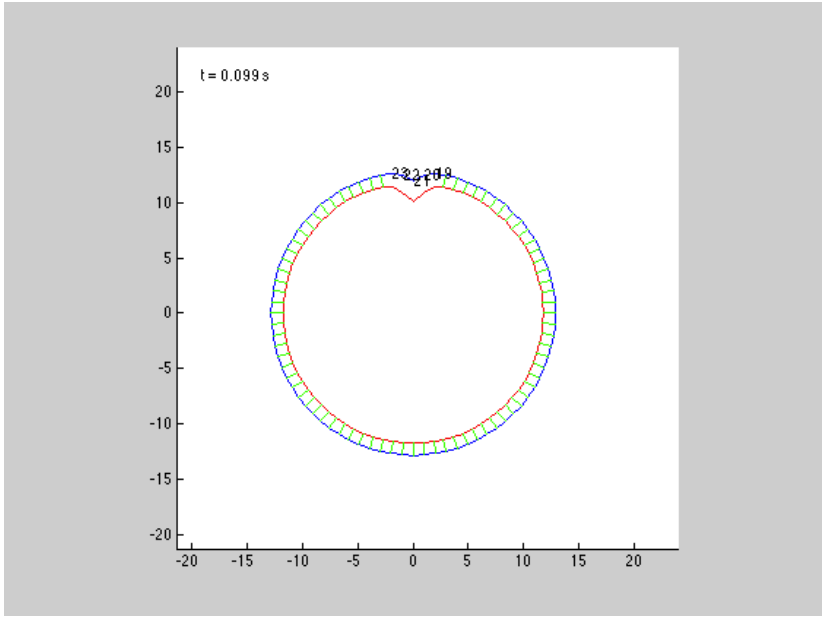
Results

- Curvature Dependence
  - Obtained maximum mean speed across bleb.
  - Plotted against average curvature in bleb formation region.
  - Hints at possible linear relationship.
  - More data needed.
  - Model limited by size.



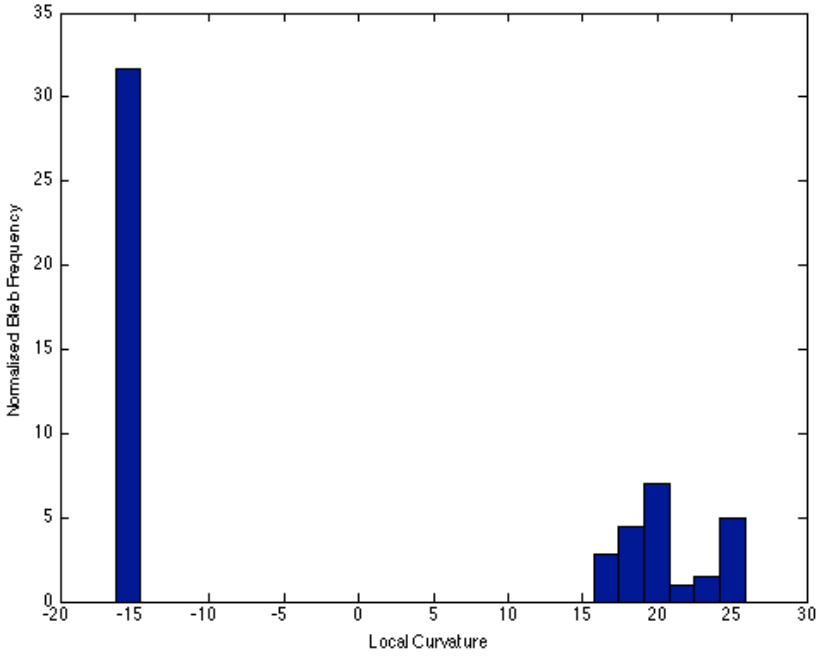
Results

- Stochastic Linkers
  - Added in 1% breaking/ fixing probability mentioned earlier.
  - Now negative region blebs almost instantly.
  - If linker breaks, acceleration too great to recover.
  - Blebs form randomly around the vesicle.
  - Sometimes get two blebs separated by just 1 linker.



Results

- Bleb Formation Rates
  - Recorded origin of 300 bleb events.
  - Histogram of blebs formed at different curvatures around vesicle.
  - Normalised against the number of nodes that share that curvature value.
  - Clear preference for negative curvature.



Further Work

- Model could be improved so lines don't cross.
- Look at wider range of curvature values.
- Look closer at interaction with inker-linker angle/distance.
- Deformation with outward force.
- Why sometimes multiple blebs prevented from fusing by single linker.
- Add realistic dimensions so results become quantitative.

Conclusions

- Model has been built that imitates behaviour of bleb during formation.
- There is evidence of a strong relationship between curvature and peak bleb speed.
- Demonstrated that there is a much higher bleb formation rate in areas of negative curvature.