

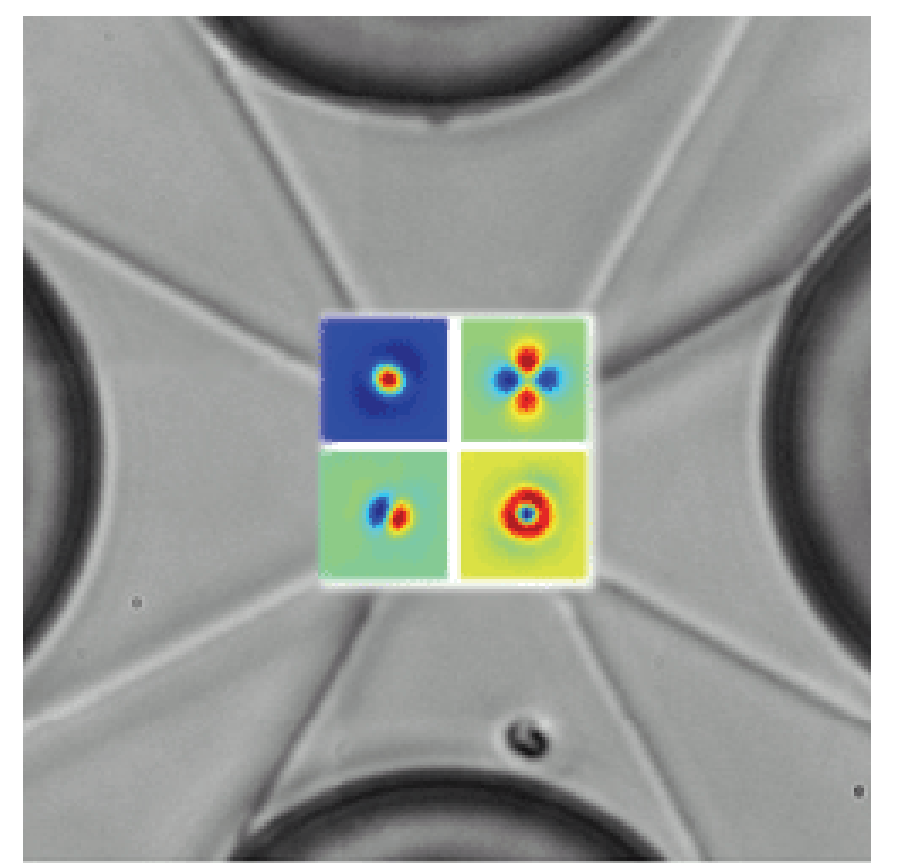
Slithering through Slime: Mechanisms of Bacterial Motility in Mucus

Jennifer H. Hou¹, Katharina Ribbeck², Adam E. Cohen^{1,3}

¹ Department of Physics, Harvard University, Cambridge, MA 02138

² FAS Center for Systems Biology, Bauer Laboratory, Cambridge, MA 02138

³ Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138

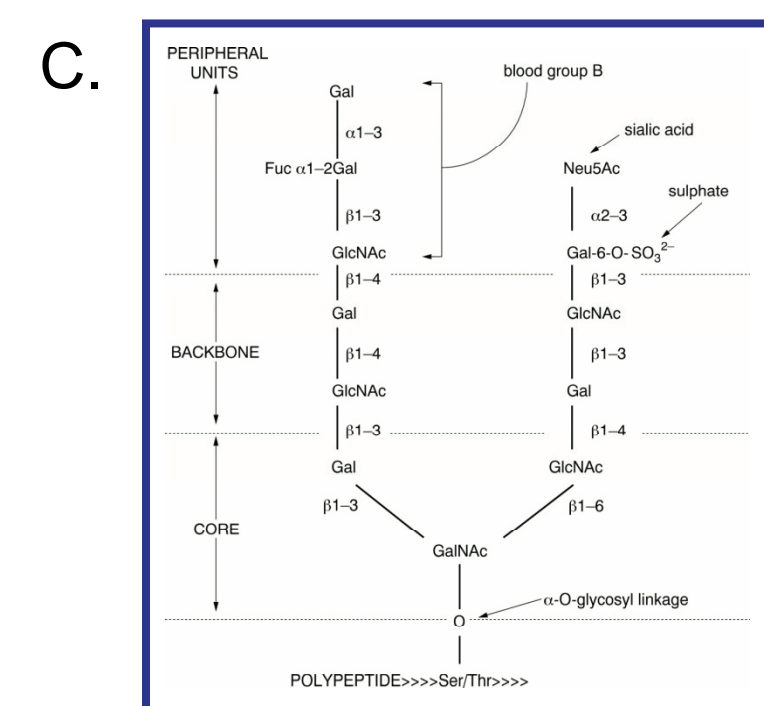
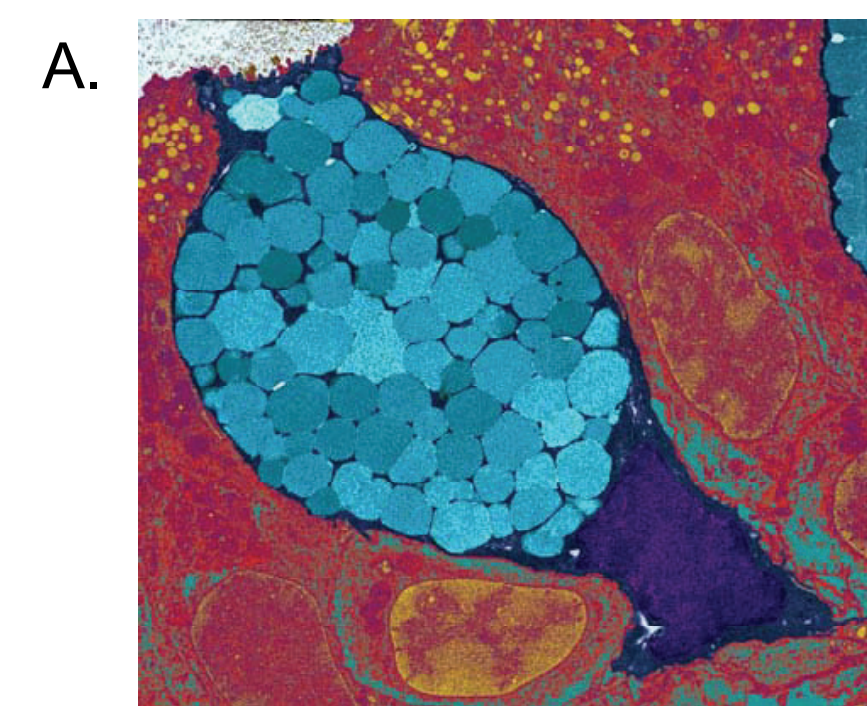


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Abstract

Mucus is a hydrogel secretion that lines epithelial cells in the respiratory, gastrointestinal, and urogenital tracts. The mucosal lining serves as a physical barrier against invading pathogens. We want to understand the mechanisms by which pathogenic bacteria break through this line of defense. Our hypothesis is that asymmetric secretion of proteases leads to asymmetric degradation of the surrounding gel. This asymmetry causes an imbalance of forces from the gel on the bacteria, which leads to motility in the direction of protease secretion. We present a series of experiments on protease-powered propulsion in mucus.

Background and Motivation

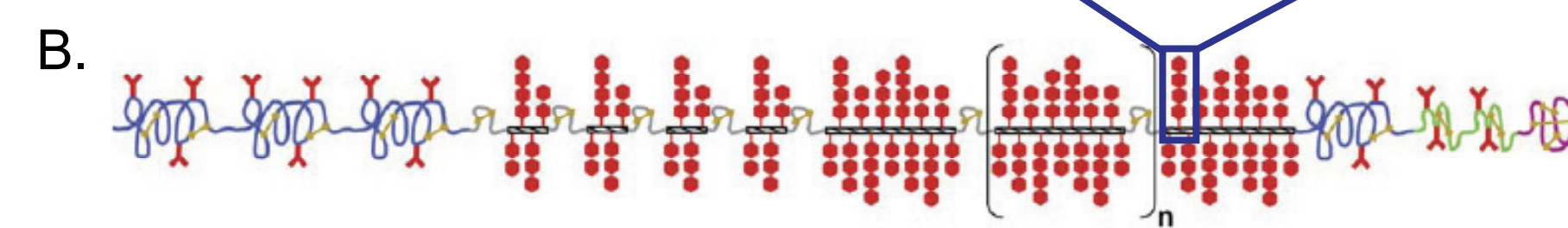


Mucin, the mucus protein

(A) Goblet cell packed with mucus globules (blue). Mucus is rapidly released to expand and form the viscoelastic mucosal gel.¹

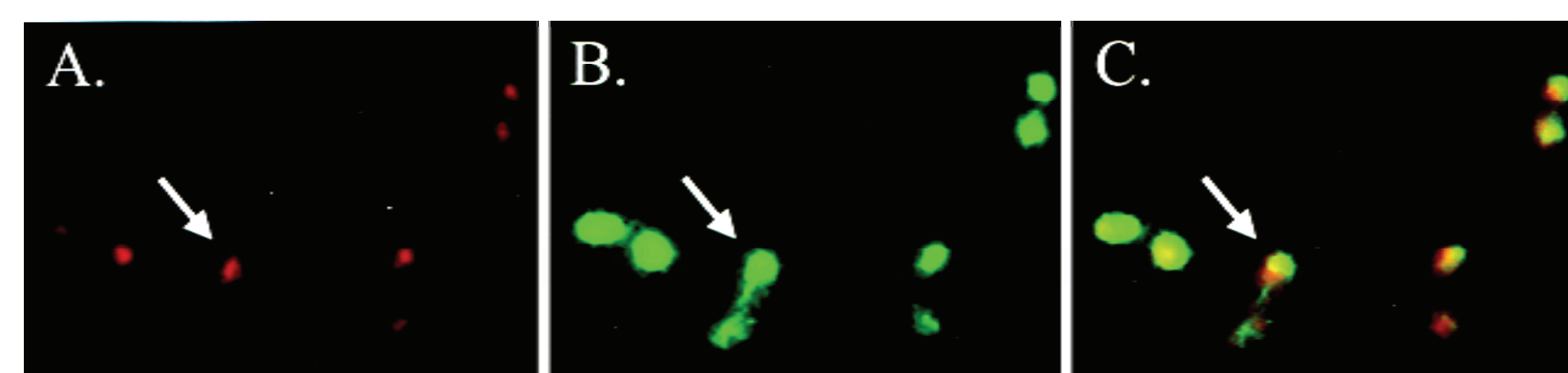
(B) Depiction of mucin molecule with regions of heavy glycosylation as well as naked regions.²

(C) Model of oligosaccharide structure attached by O-glycosylation to serine or threonine residues of the amino acid backbone.³



Hypothesized mechanism of motility:

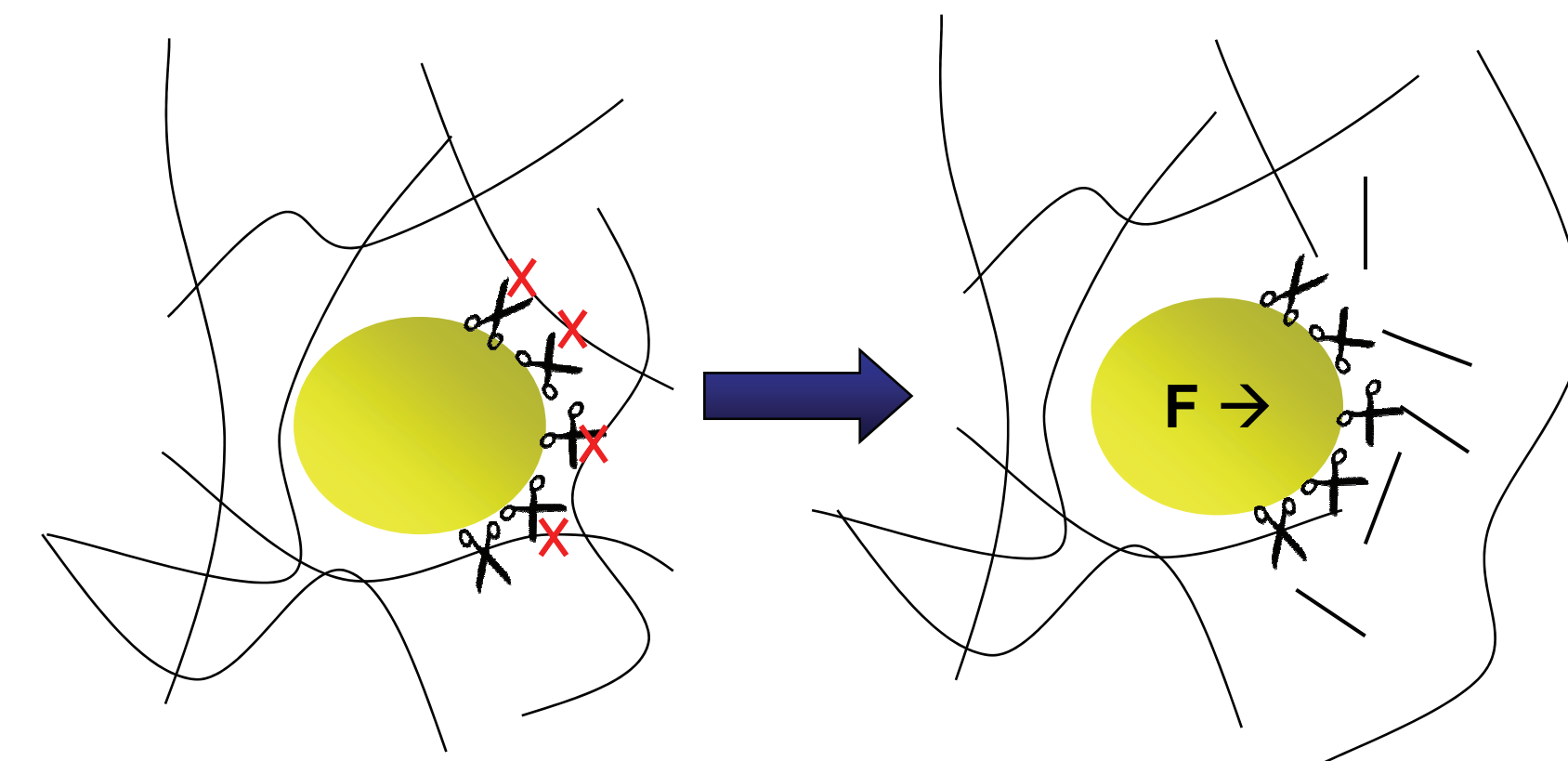
Bacteria asymmetrically secrete proteases, enzymes that degrade mucin molecules. Forces derived from asymmetric degradation of the mucin gel enhance motion towards the direction of protease secretion.



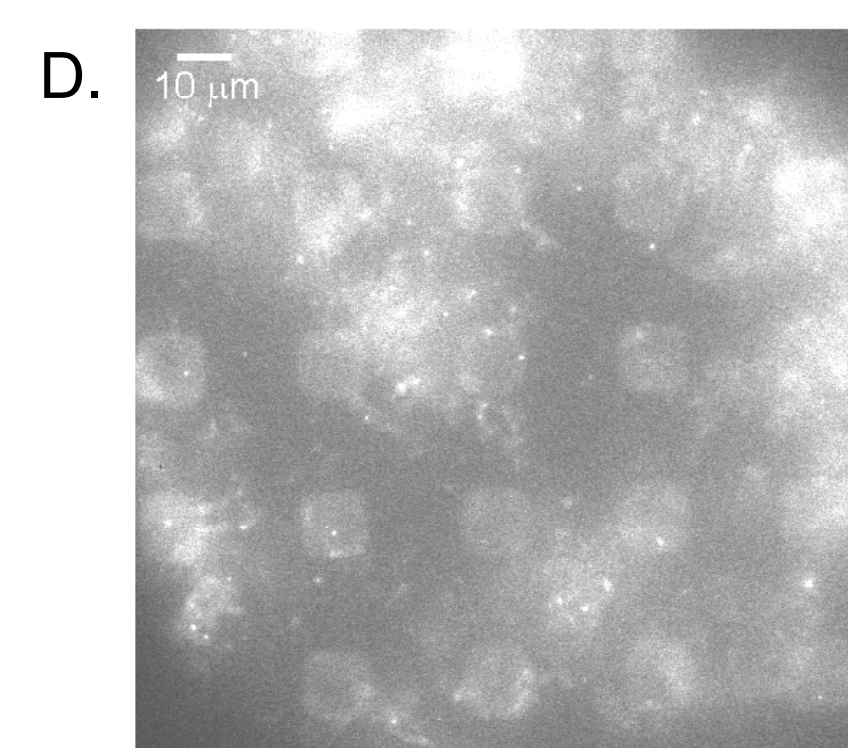
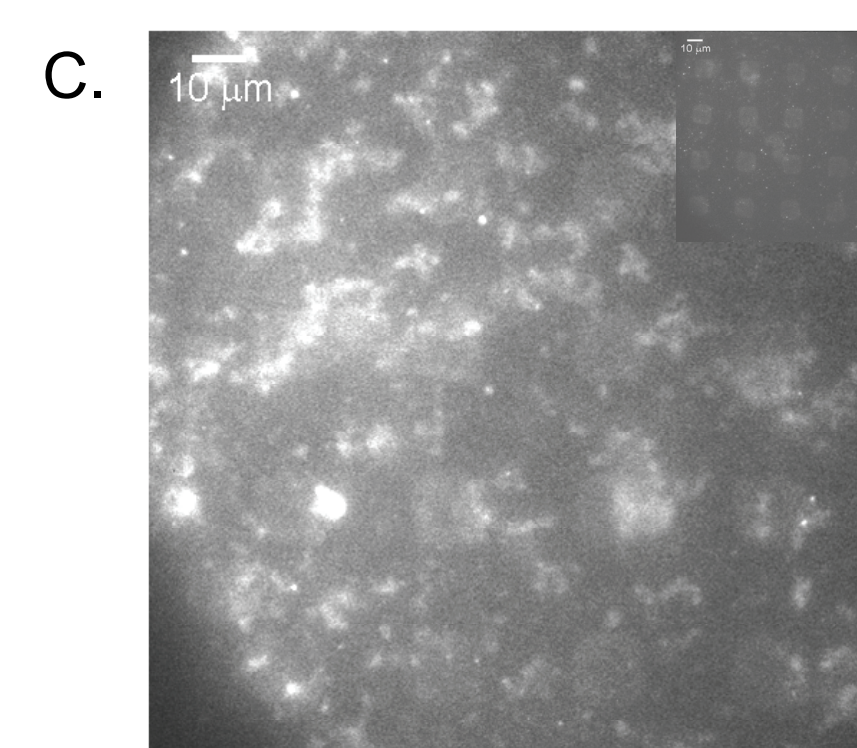
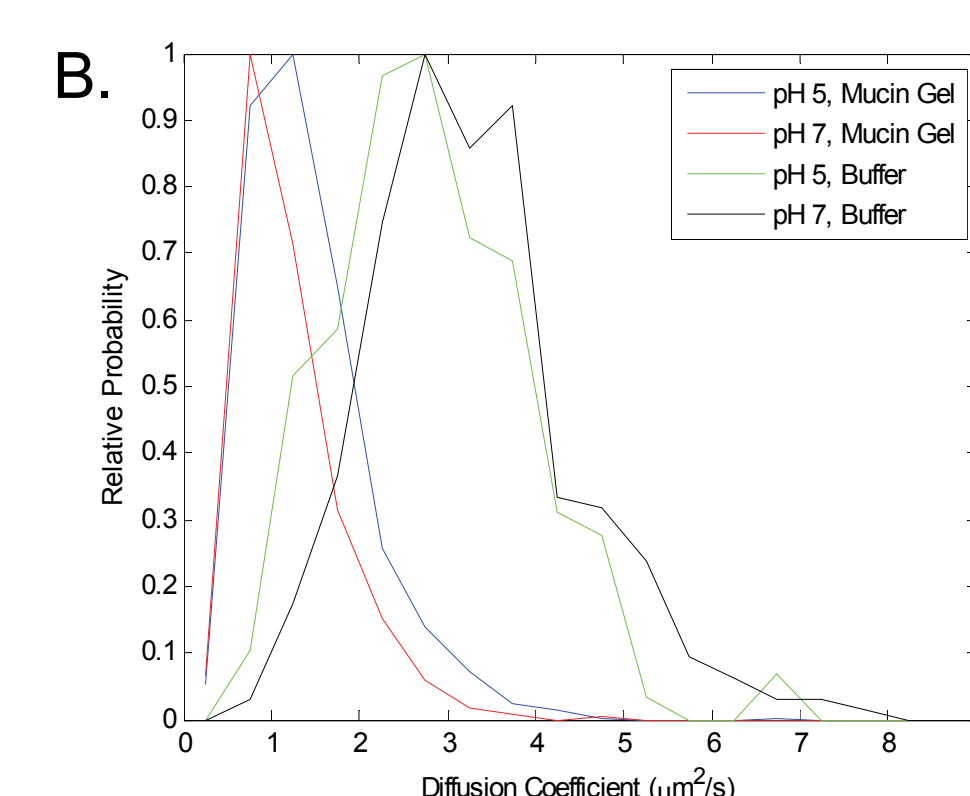
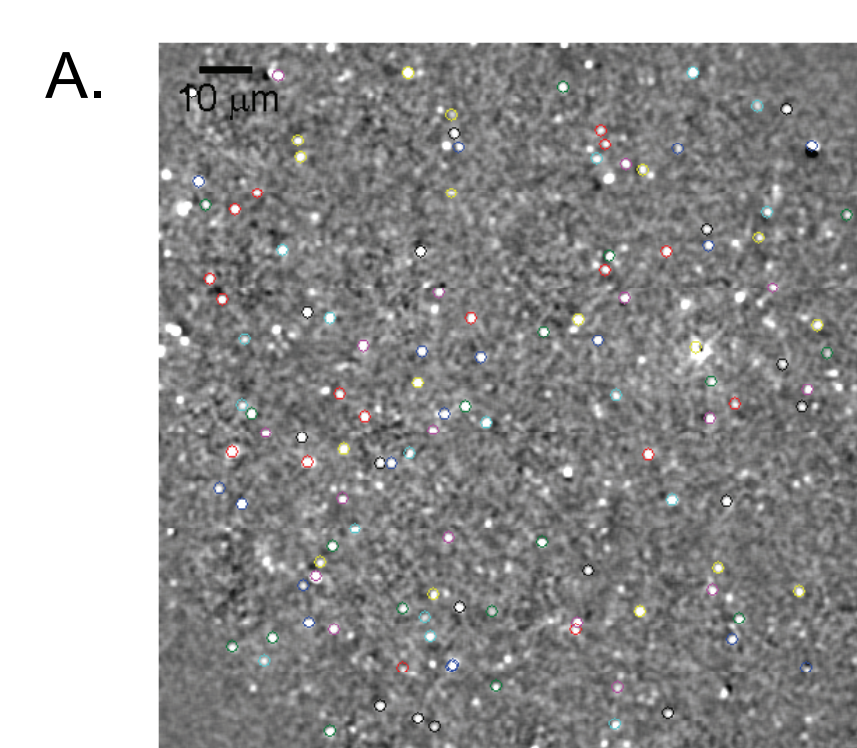
Vibrio cholera secretes proteases asymmetrically.

- (A) Fluorescent visualization of protease secretion by *V. cholera*.
- (B) Localization of the GFP-extracellular protease secretion (eps) complex at one pole of the bacterium.
- (C) Overlay of images A and B to show colocalization of EpsM and site of protease secretion.

From M.E. Scott et al. (2001). *PNAS* 98: 13978-13983.



Tracking Single Molecules of Mucus



(A) Single molecules of mucin, labeled with Alexa 647 succinimide ester, in native 1% pH 5 mucin gel. Colored circles indicate detection by particle tracking software.

(B) Histogram of diffusion coefficients for mucin molecules in various environments.

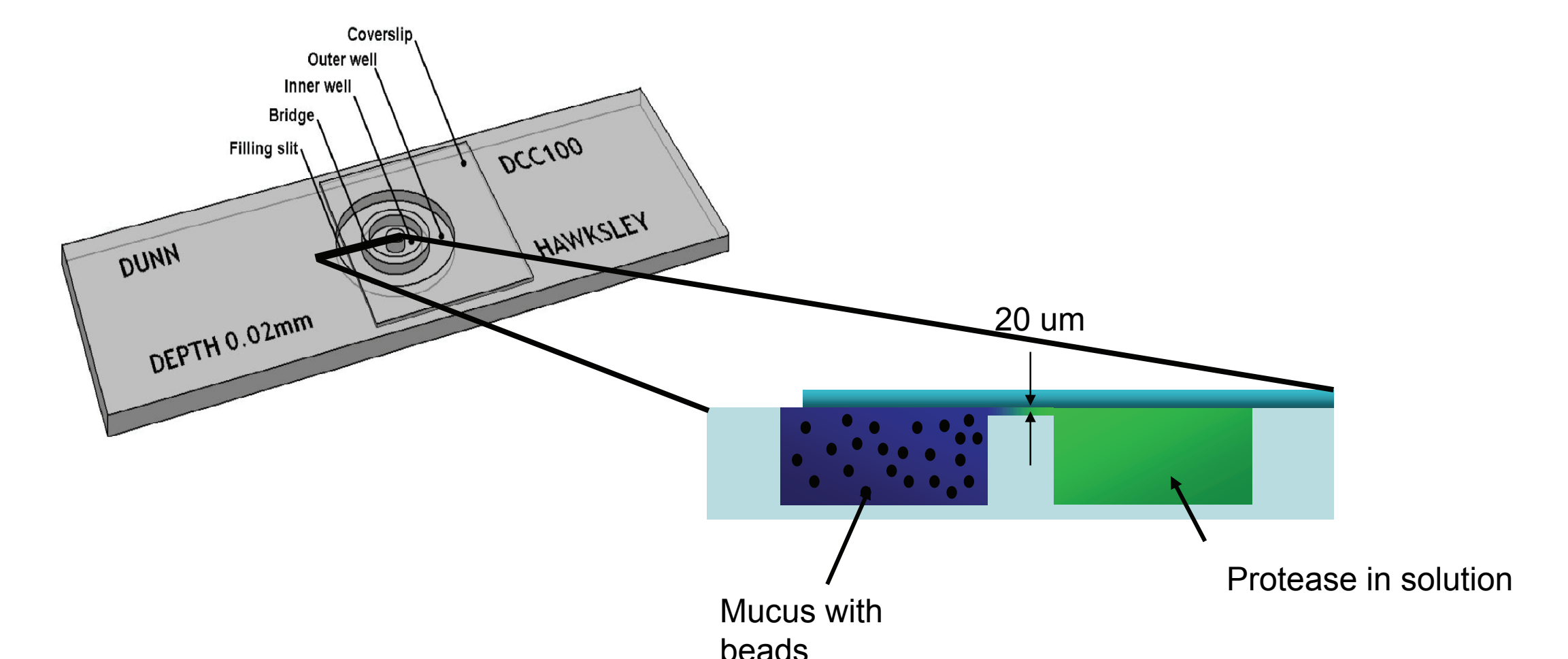
(C) Fluorescently labeled mucin remnants after 50 minutes of degradation by trypsin protease. (Inset) Mucin in native 1% pH 7 mucus before digestion.

(D) Fluorescently labeled mucin in native 1% pH 3 mucus.

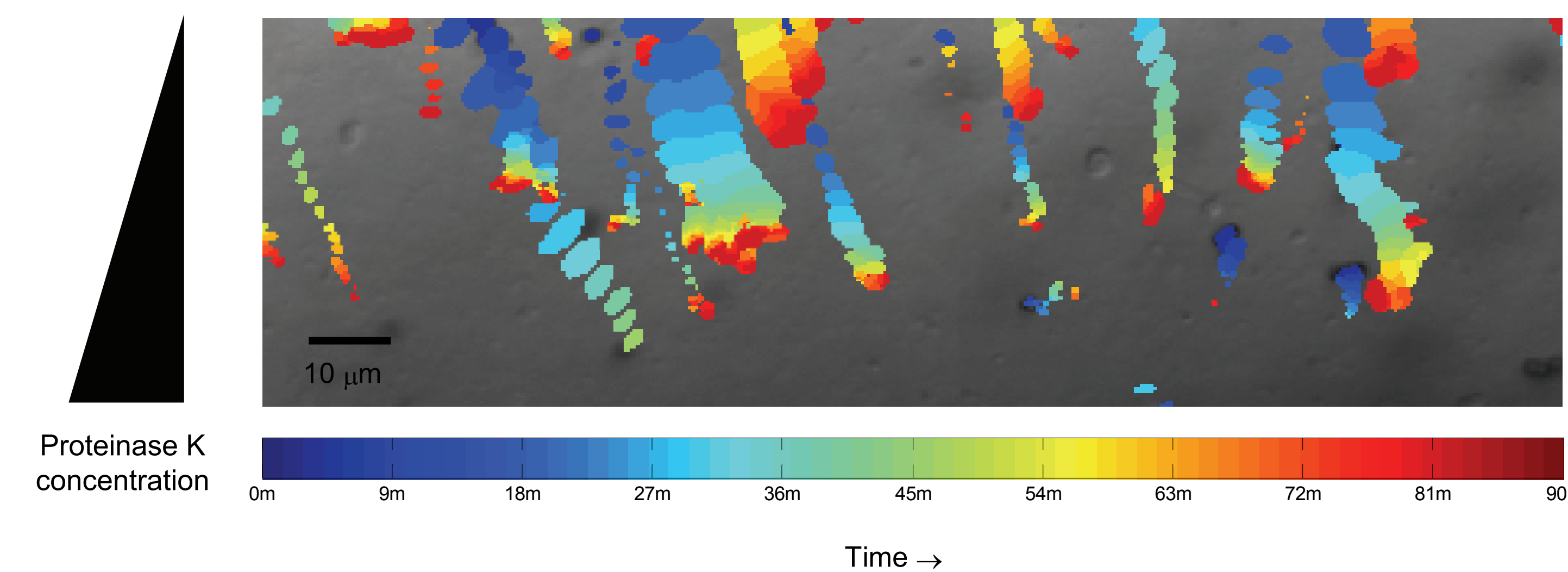
Motion in Mucus Induced by Protease Gradients

Sample chamber

Dunn gradient contraction. One chamber is filled with mucus and beads; the other with protease solution. The thin region connecting the two chambers maintains a steep protease concentration gradient. We watched the beads move in this gradient.



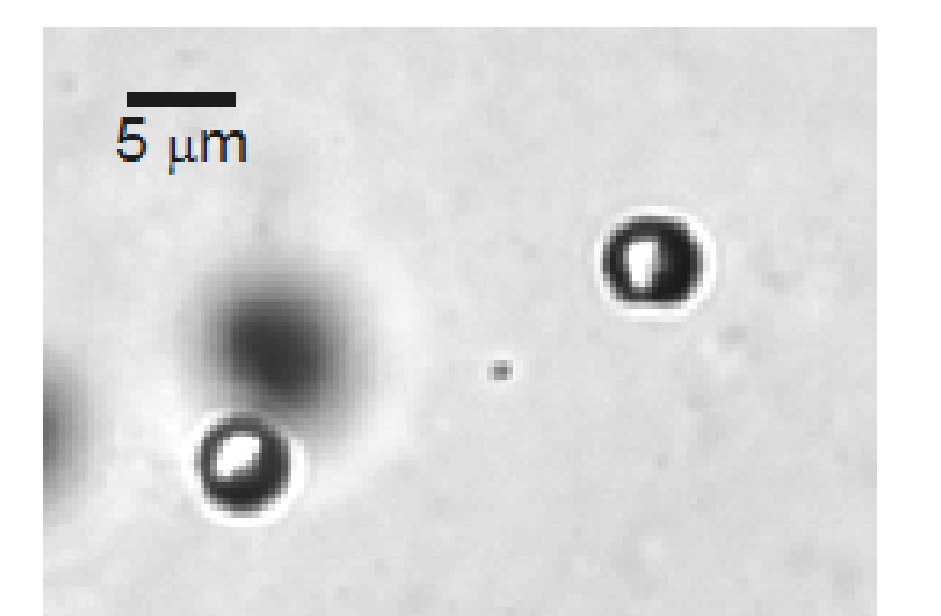
Beads moving in a protease gradient



Stroboscopic trajectories of 4 micron beads moving in 1% pH 7 mucin gel over 90 minutes. Motion is induced by asymmetric degradation of the mucin environment by the proteinase K gradient.

Modeling Bacteria with Protease-Coated Janus Particles

- Deposit beads on glass slide.
- Coat one hemisphere with aluminum by thermal evaporation.
- Release beads from slide by sonication.
- Couple proteins to remaining functional groups on bead surface.
- Watch motion of asymmetrically coated protease beads in mucus gel as proteases degrade the mucin polymers.

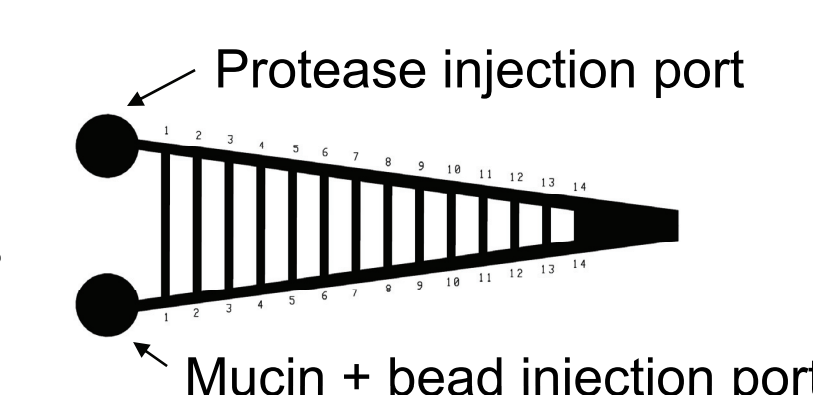


4 micron polystyrene Janus particles with one hemisphere coated in aluminum (dark side), and the other coupled to proteinase K (light side).

Outlook

- Motion in mucus in microfluidic channels

Schematic for microfluidic PDMS gradient chambers of varying lengths for parallelized data acquisition.



- In vivo* studies of *V. cholera* in mucin gel to detect correlations between direction of protease secretion and motion

Figure References:

- R. Bansil et al. (2006). *Current Opinion in Colloid & Interface Science* 11: 164-170.
- Wellcome Trust Medical Photographic Library, Biomedical Image Awards 2006. <http://www.wellcome.ac.uk/en/bia/gallery.html?image=9>
- R. Wiggins et al. (2001). *Sexually Transmitted Infections* 77: 402-408.

Acknowledgments: This work was funded by the Harvard MRSEC. JHH thanks NDSEG for supporting her graduate studies.