Electron cryomicroscopy and force spectroscopy data :

a direct role for anti-pili antibodies in inhibiting diarrheal diseases

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Acknowledgments

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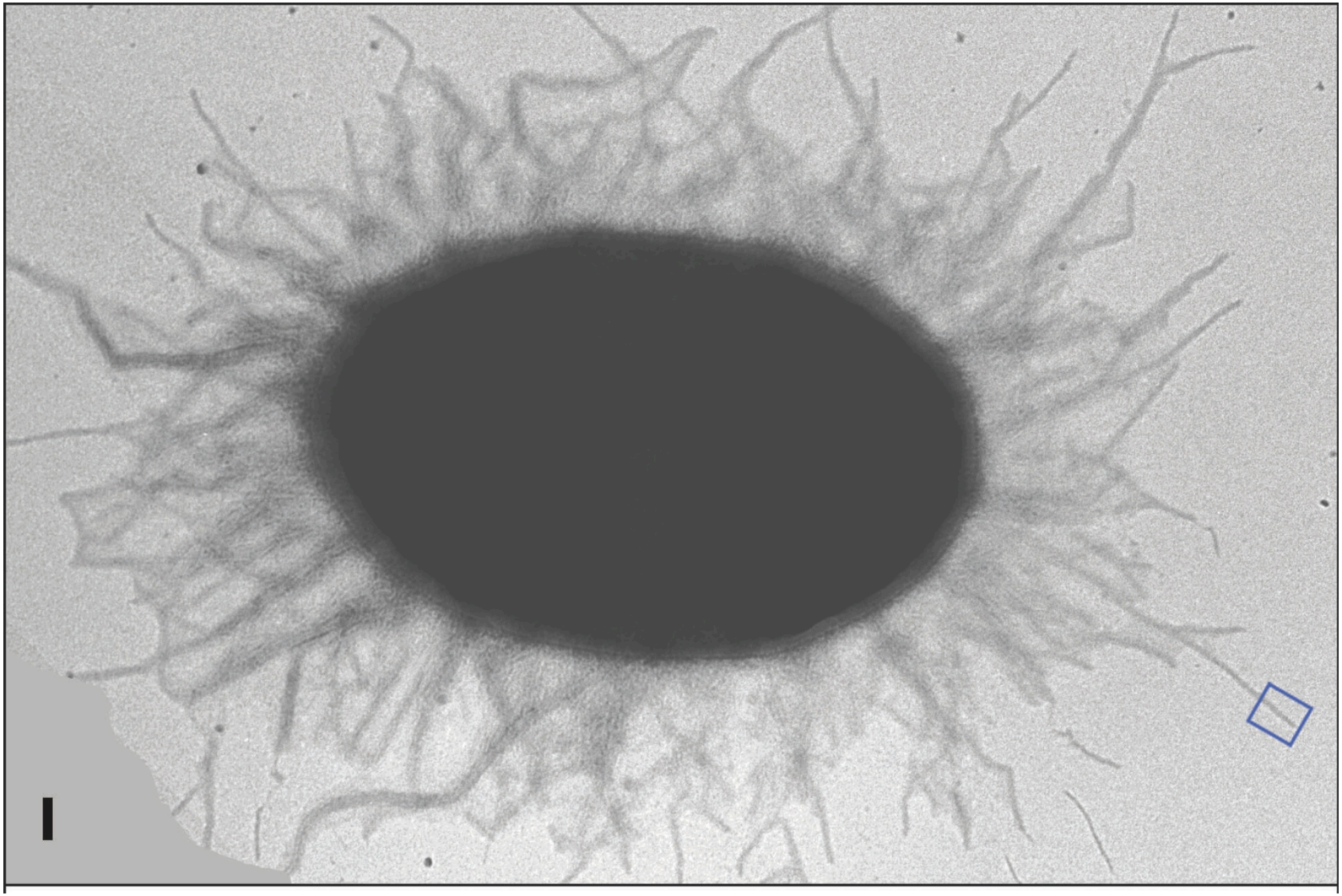
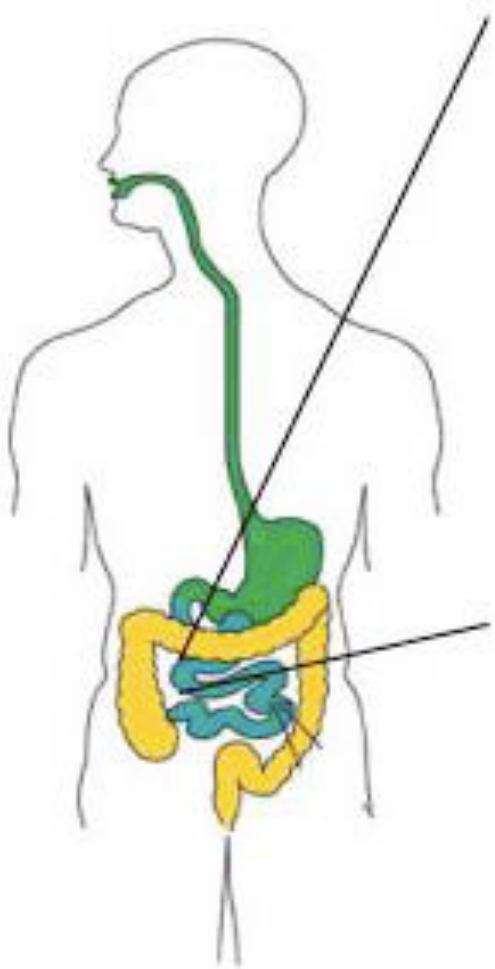
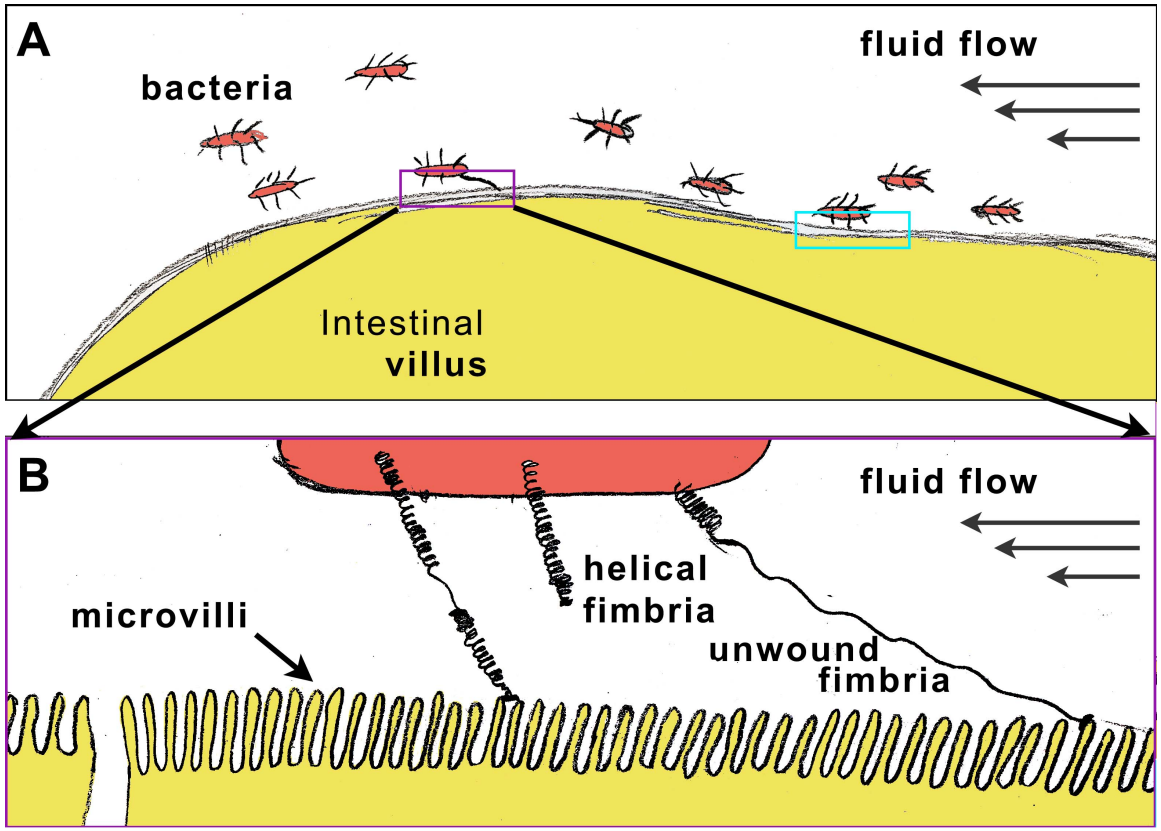
Bacteria express adhesion pili (fimbriae)



For binding to target cells

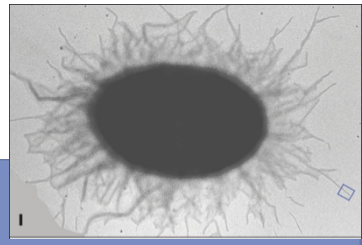


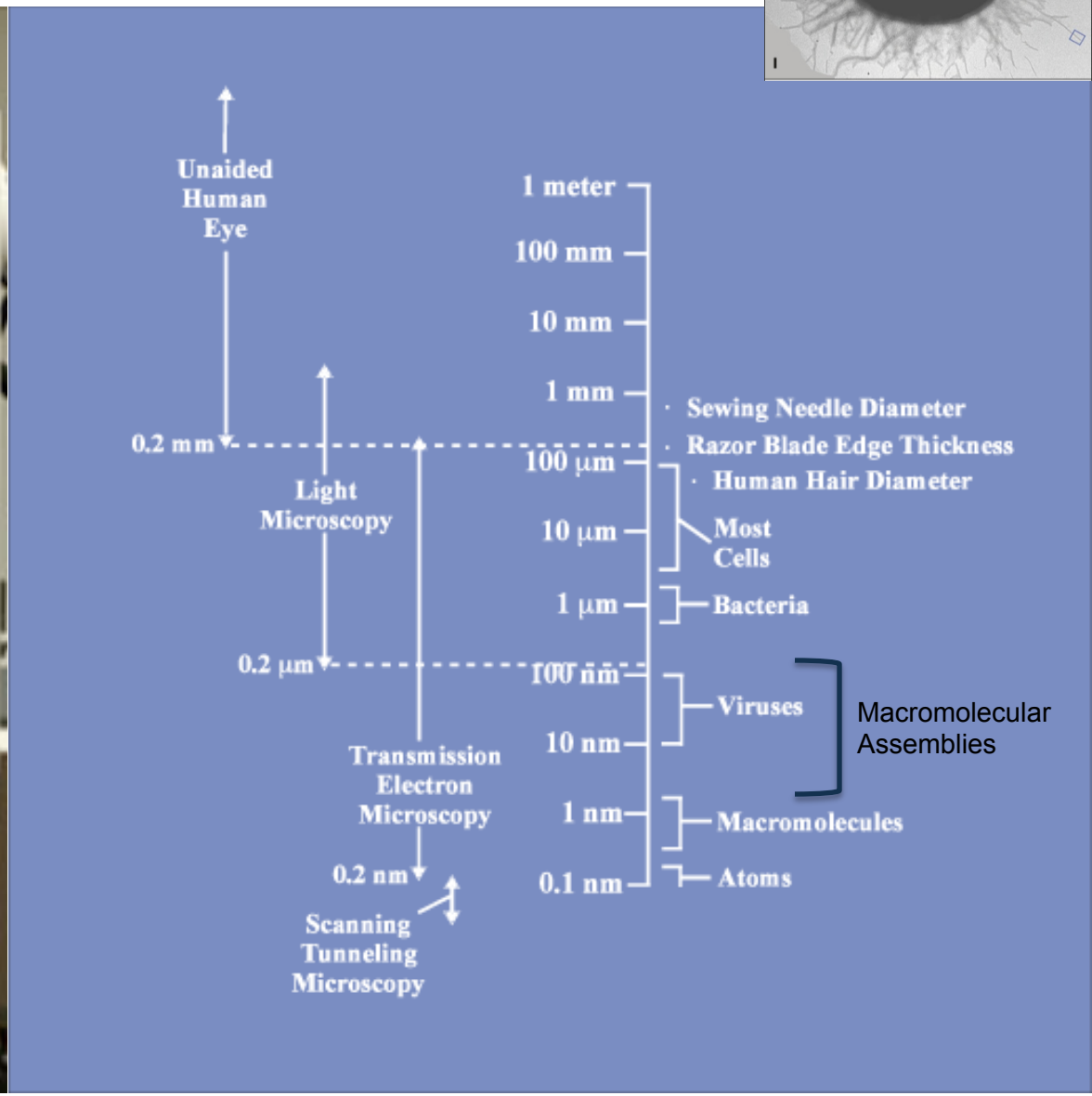


Bacteria: ~0.5 μm by 2μm
Epithelial cell: ~24μm across

Pili: 7-8 nm dia by 1μm in length

Electron Microscopy has the resolution





Electron Microscopy Principles & Techniques for Biologists (1992)
Bozzola & Russell; Jones & Bartlett Publishers

To Combat Diarrheal Diseases

- 1.87 million deaths of children aged less than 5 were caused by diarrheal disease (~ 19% of total child deaths). W.H.O. 2008
  - Fluid loss from acute infectious diarrhea is the second most common cause of death in children in the developing world (pneumonia is number one) [1]

- Diarrheal disease kills more young children than AIDS, malaria, and measles, combined. W.H.O. 2009

- ETEC (enterotoxigenic E. coli) is responsible for 30-60% of all cases of diarrhea in travelers to endemic areas [2]

Photograph courtesy of Drs. Firdausi Qadri and Alejandro Cravioto, ICDDRB, Dhaka

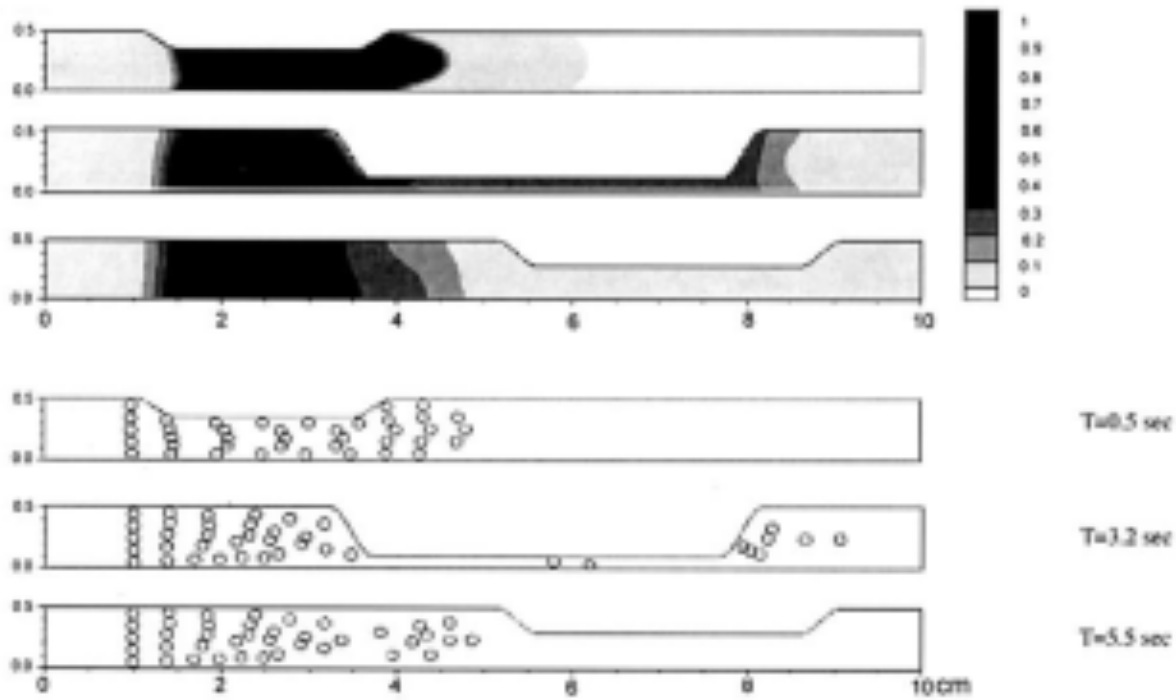
1. Kosek M, Bern C, Guerrant RL. Bull World Health Organiz. 2003; 81:197

2. Jiang ZD, Greenberg D, Nataro JP, Steffen R, DuPont HL. J Clin Microbiol. 2002; 40:4185

Bidirectional fluid flow in the intestines
[pig ileum, with ink]

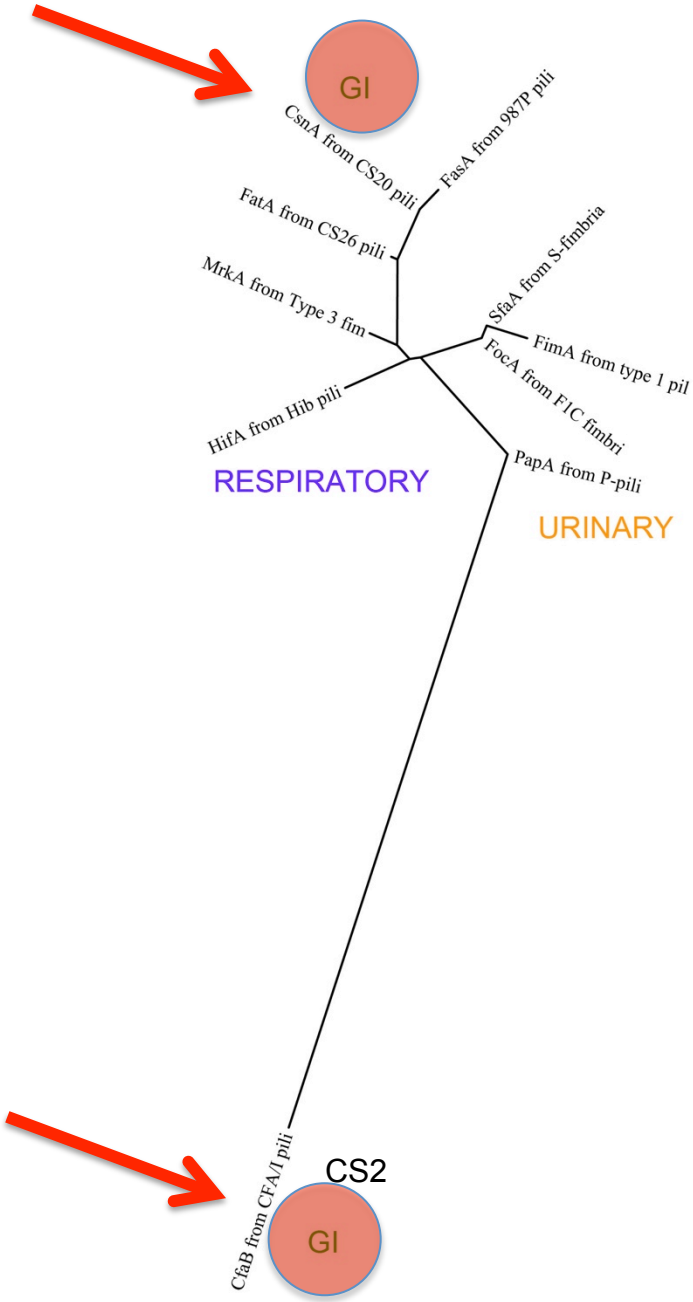
Mixing of ink

Particle redistribution



Jeffrey et al *Am. J. Physiol. Gastrointest. Liver Physiol.* **285**, G907-18 (2003)

Genetic (dis)similarity

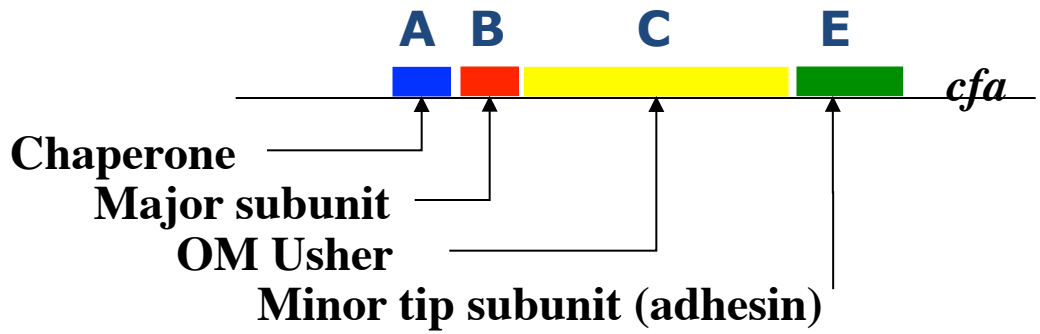


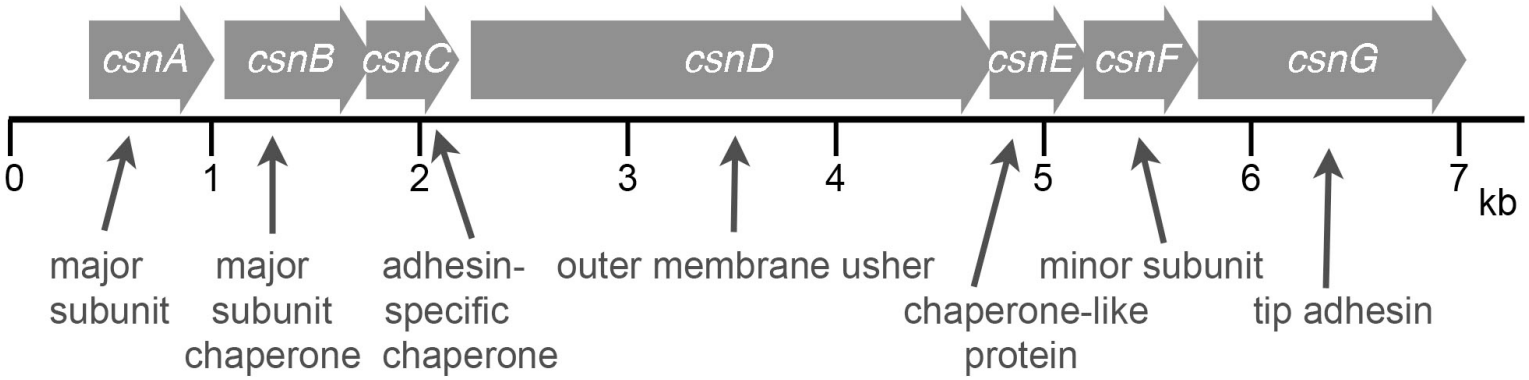
Chaperone-Usher Pathway

vs

Alternate Chaperone Pathway

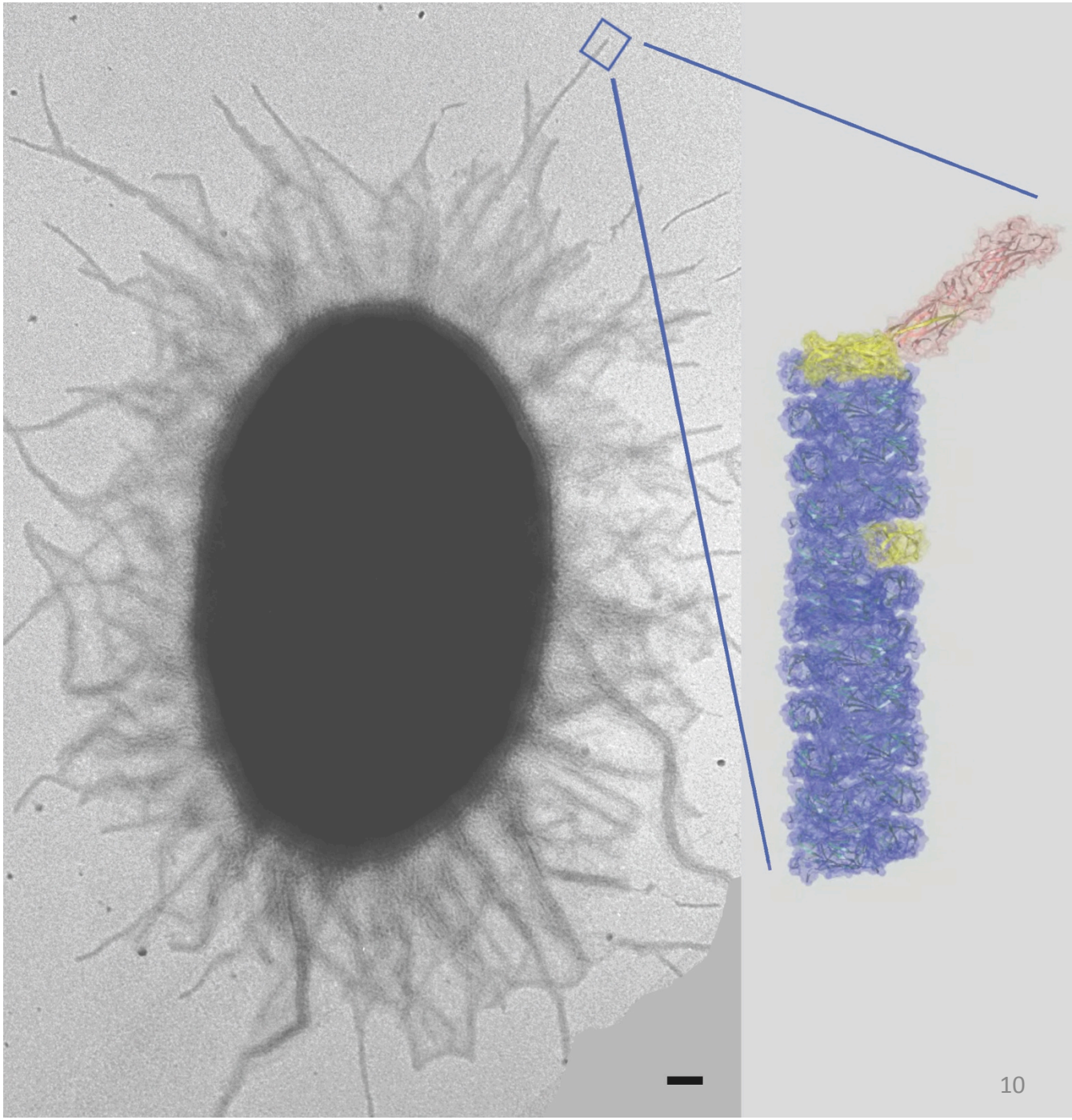
CFA/I and CS20 operons





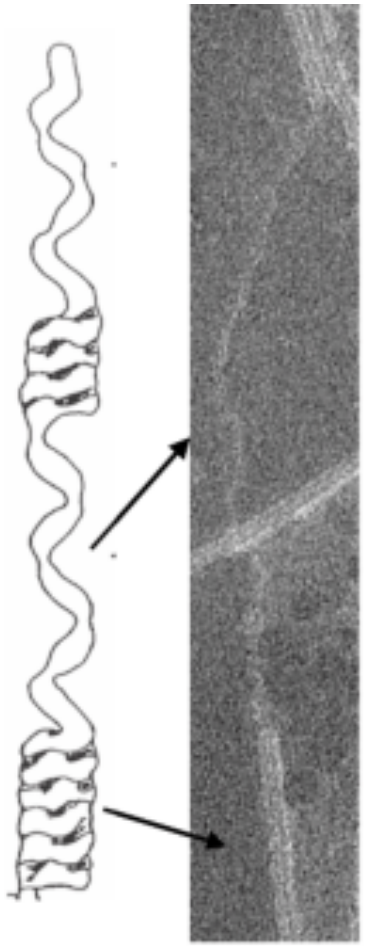
CS20 Operon

Pilus structure
(CFA/I pili)

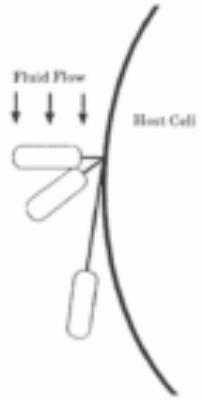


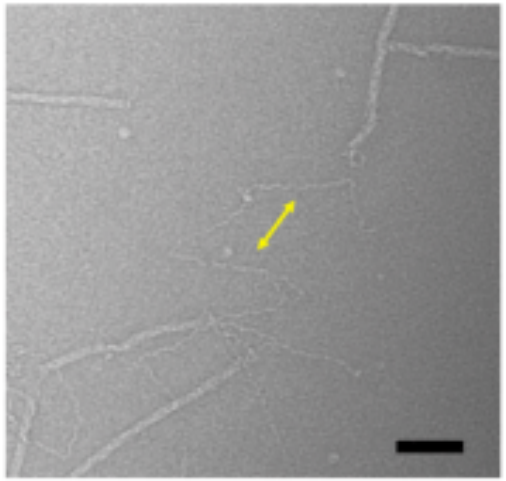
ETEC:
Enterotoxigenic E. coli

Pili can unwind

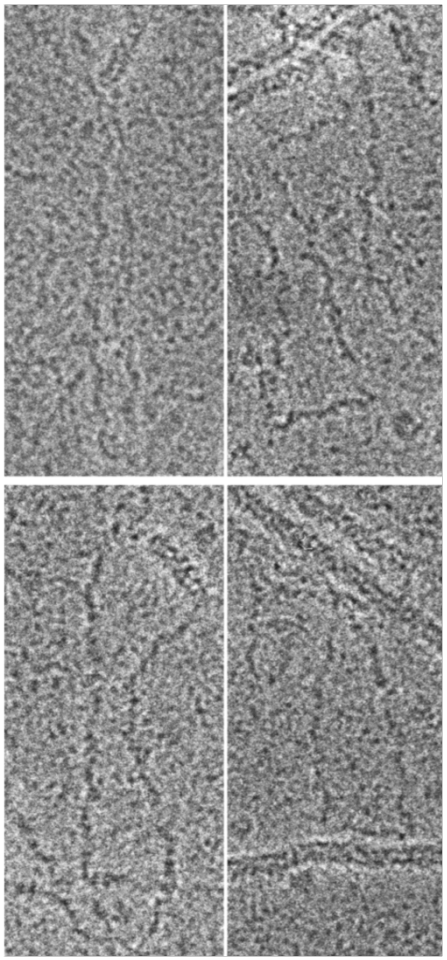


P-pili

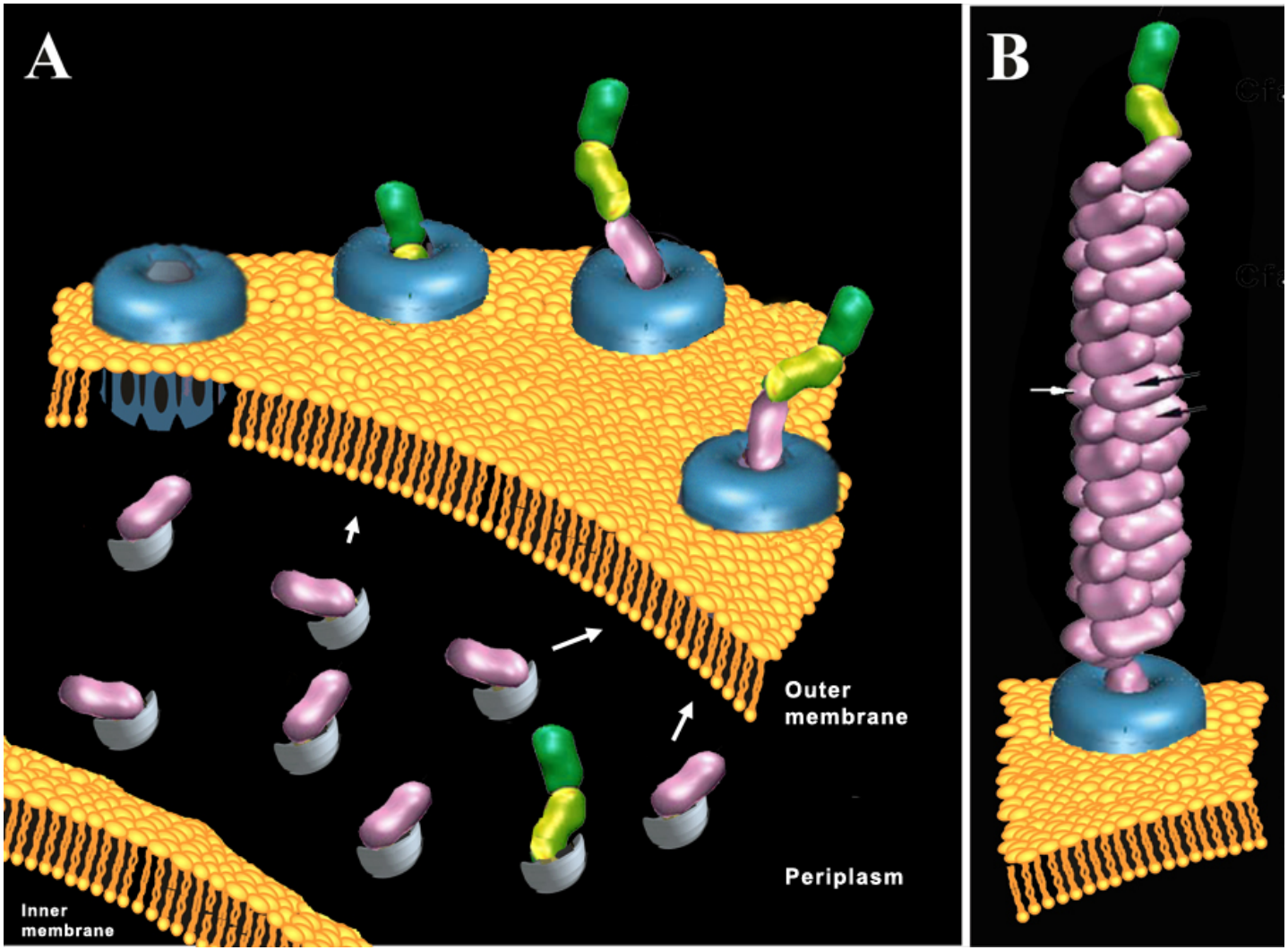




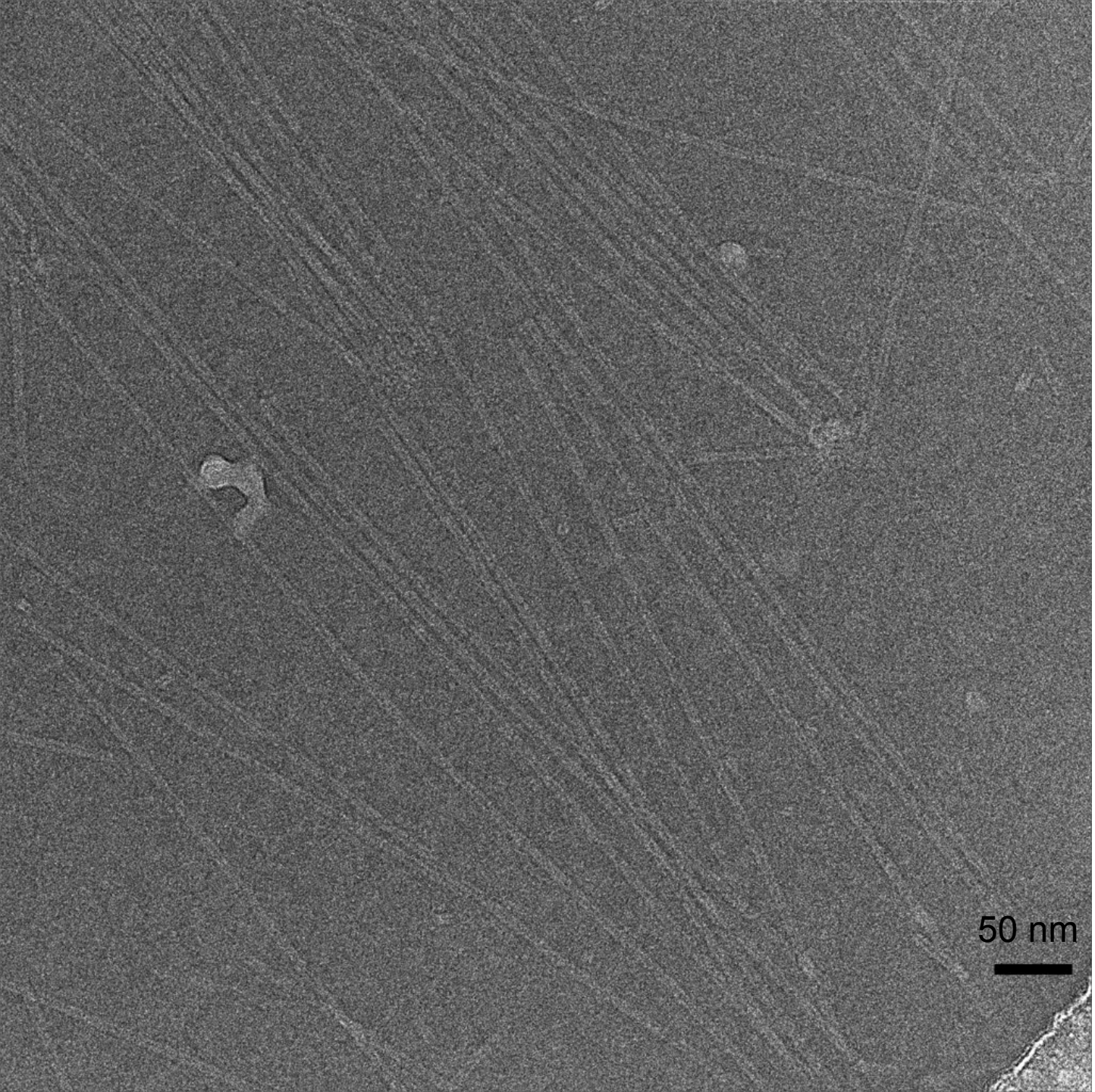
CFA/I pili



Unwinding is similar to assembly



Plunge the sample into liquid ethane

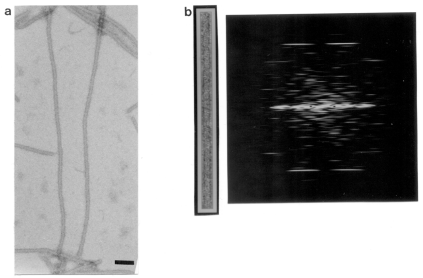


ETEC
CS20 pili
(fimbriae)

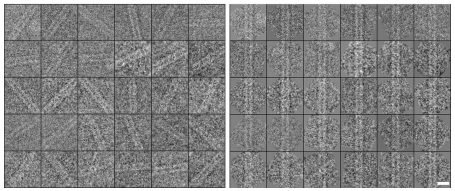
by
cryoEM

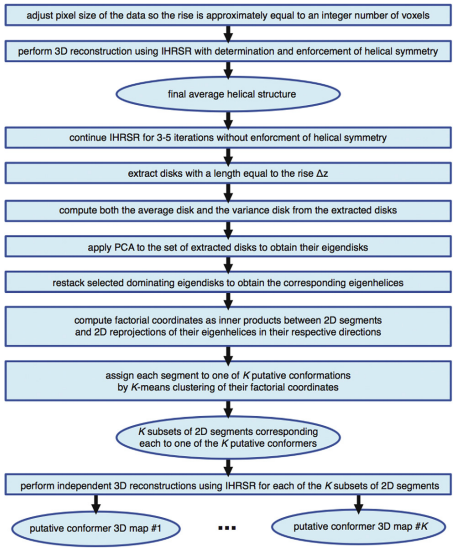
To determine the 3-dimensional structure:

1970: Fourier-Bessel reconstruction



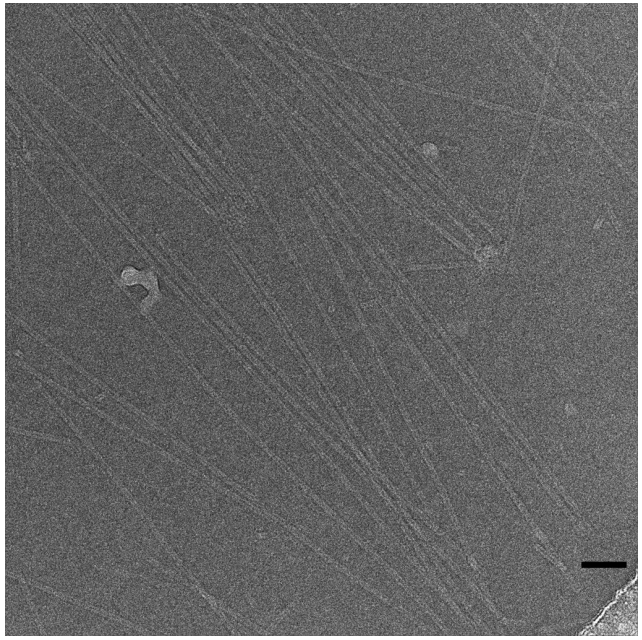
2000: IHRSR
Iterative Helical Real Space Reconstruction



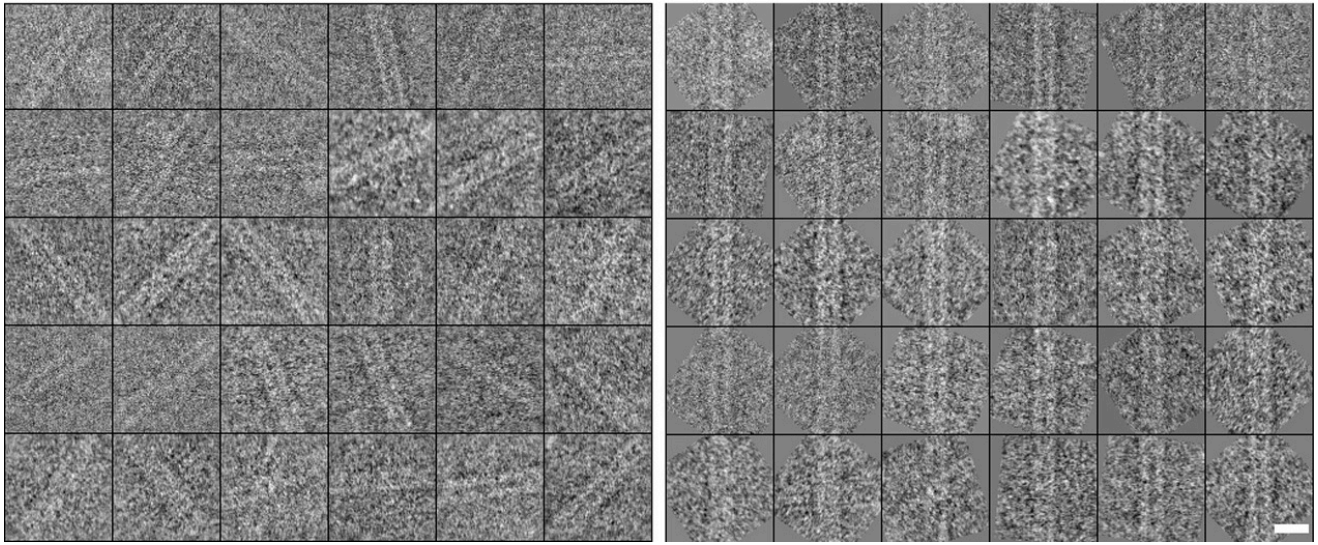


2012: Sparx Helicon

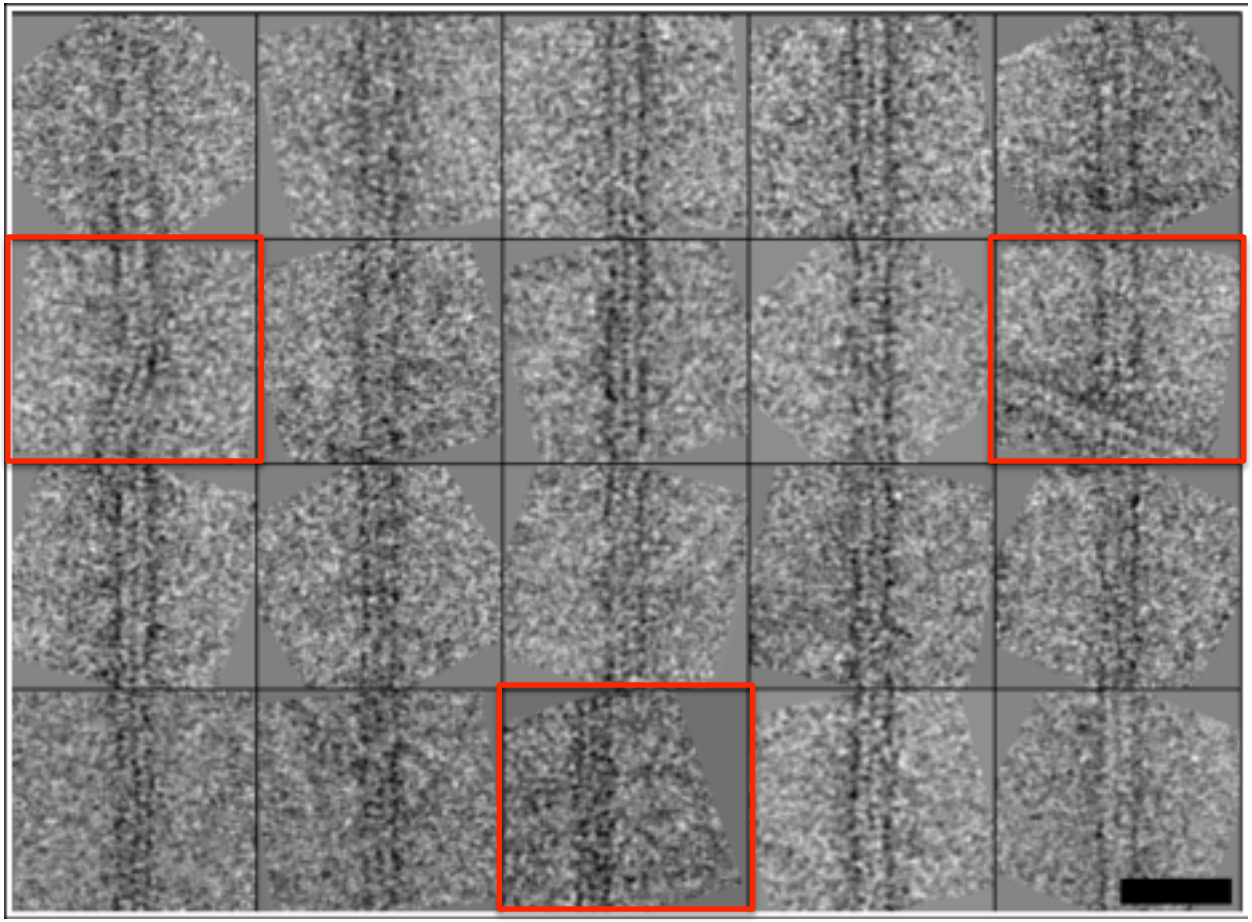
methods by
DeRosier & Moore (1970) *J Mol Biol* 52:355-369
Egelman (2000) *Ultramicroscopy* 85:225-234
Behrmann E … Penczek (2012) *J Struct Biol* 177:.302-313



Real Space Reconstructions



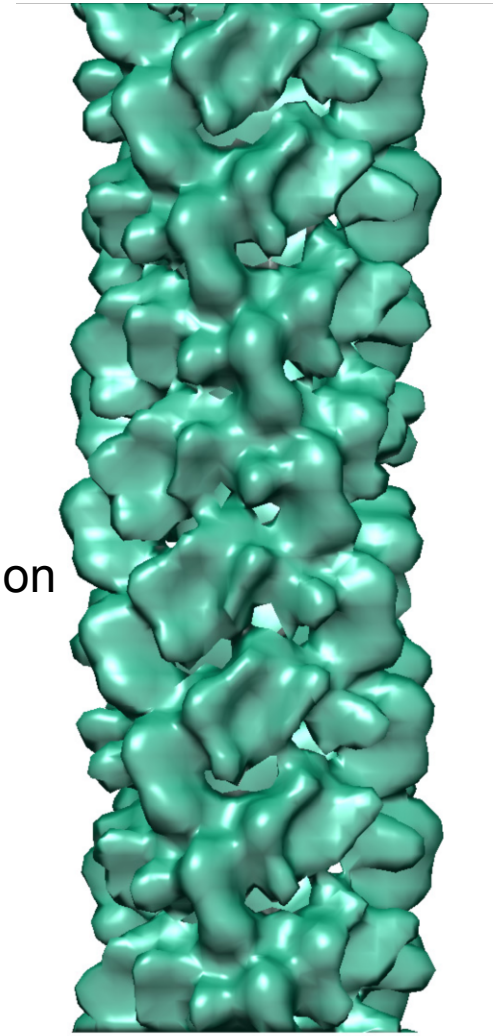
Sample ‘particles’

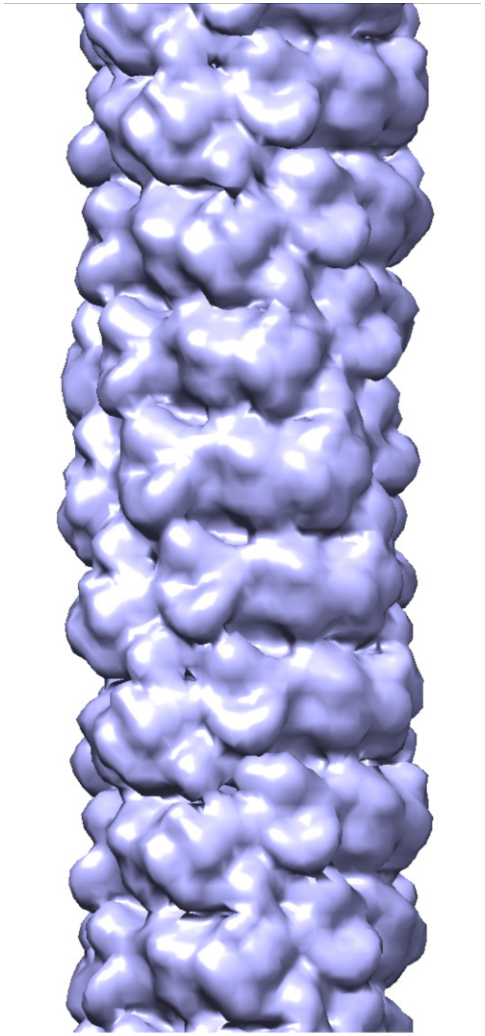


Genetically distinct E coli, both diarrhea-causing

Structure
by cryoEM

1 nm resolution





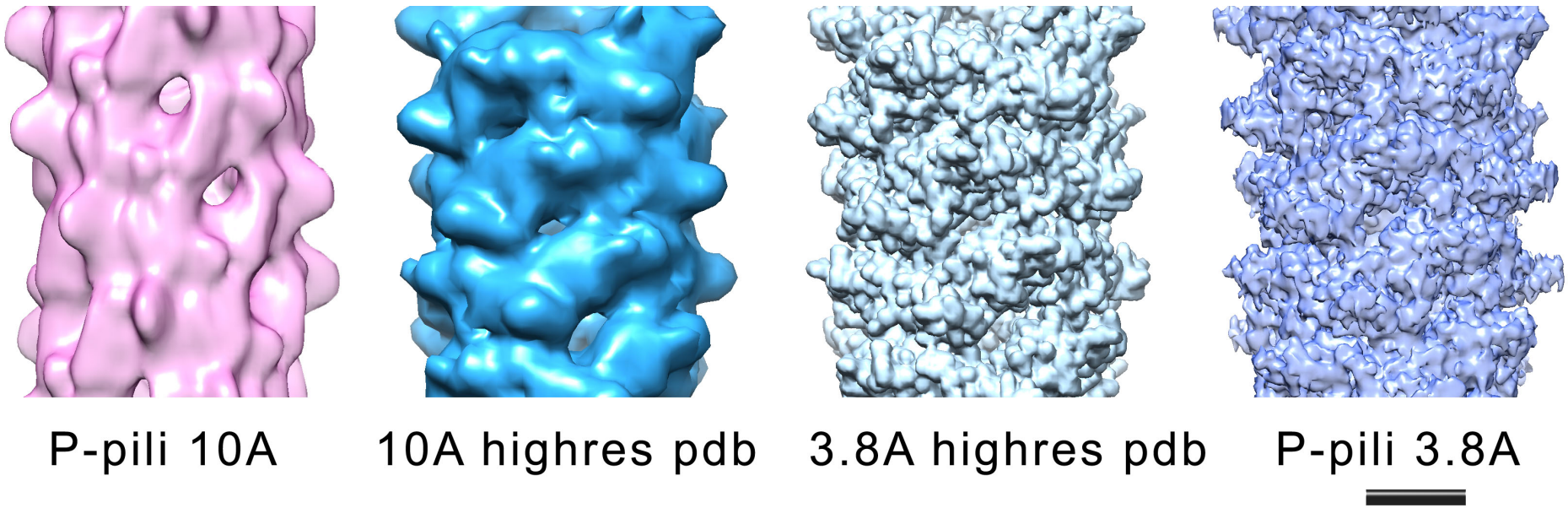
Structure by
negative stain
EM & x-ray
crystallography

50Å

CS20
GI

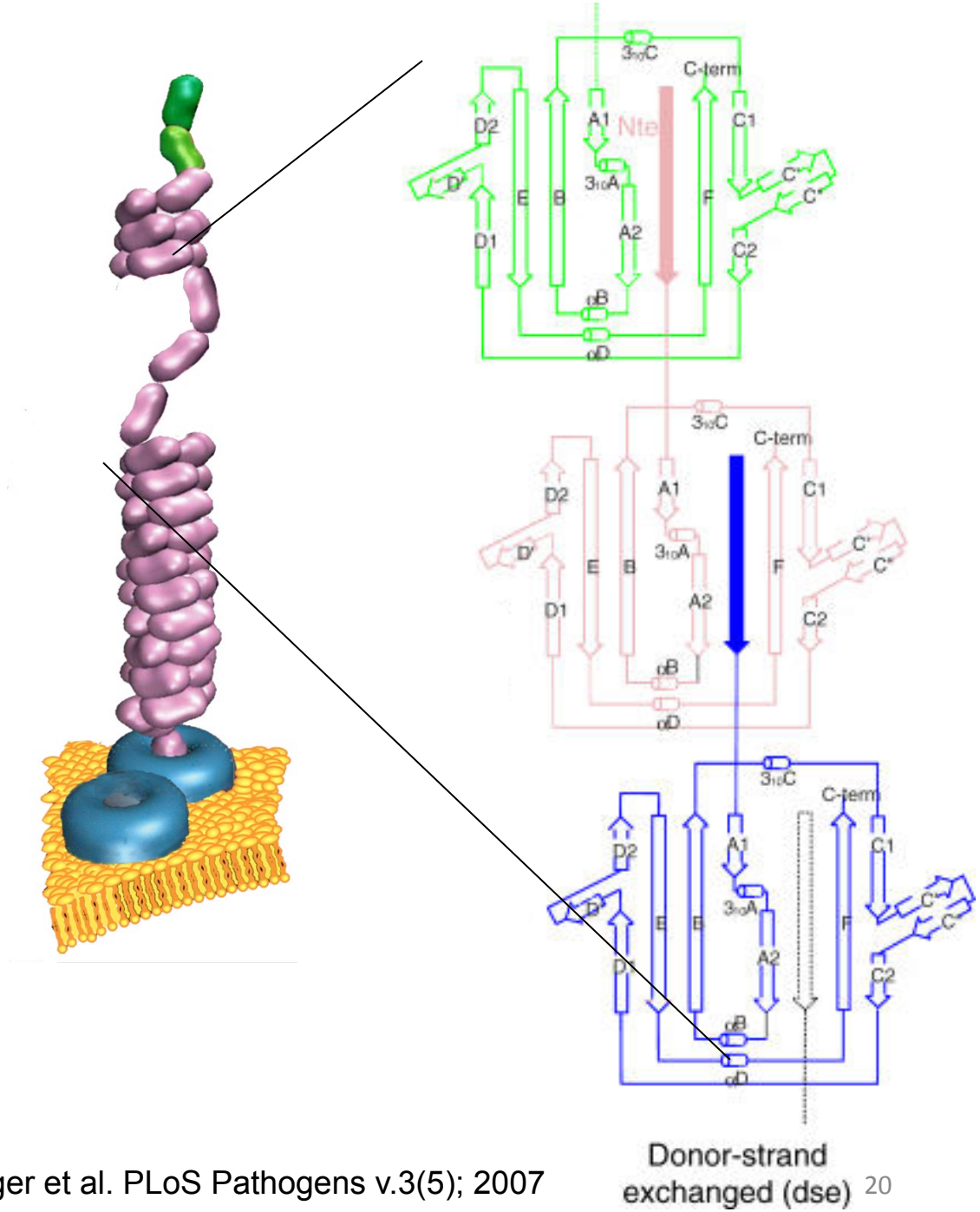
CFA/I
GI

What direct detectors can do



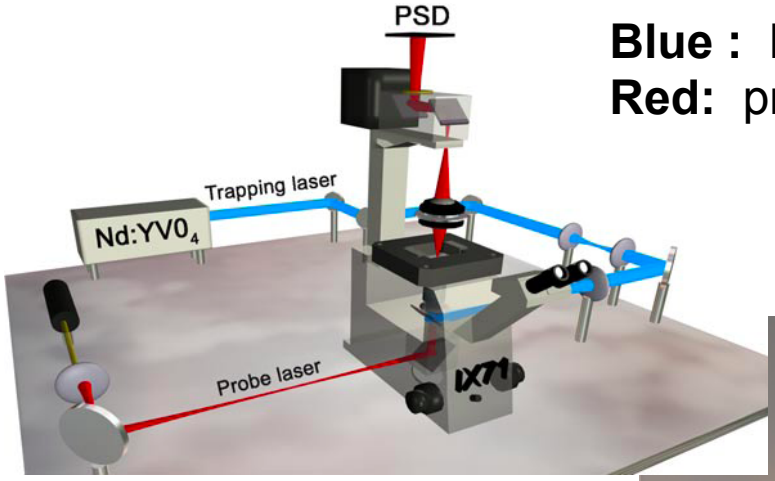
10A resolution: Li et al 2006 PNAS 103:9861
3.8A resolution: Hospenthal et al 2016 Cell 164:269

Molecular architecture
that promotes unwinding

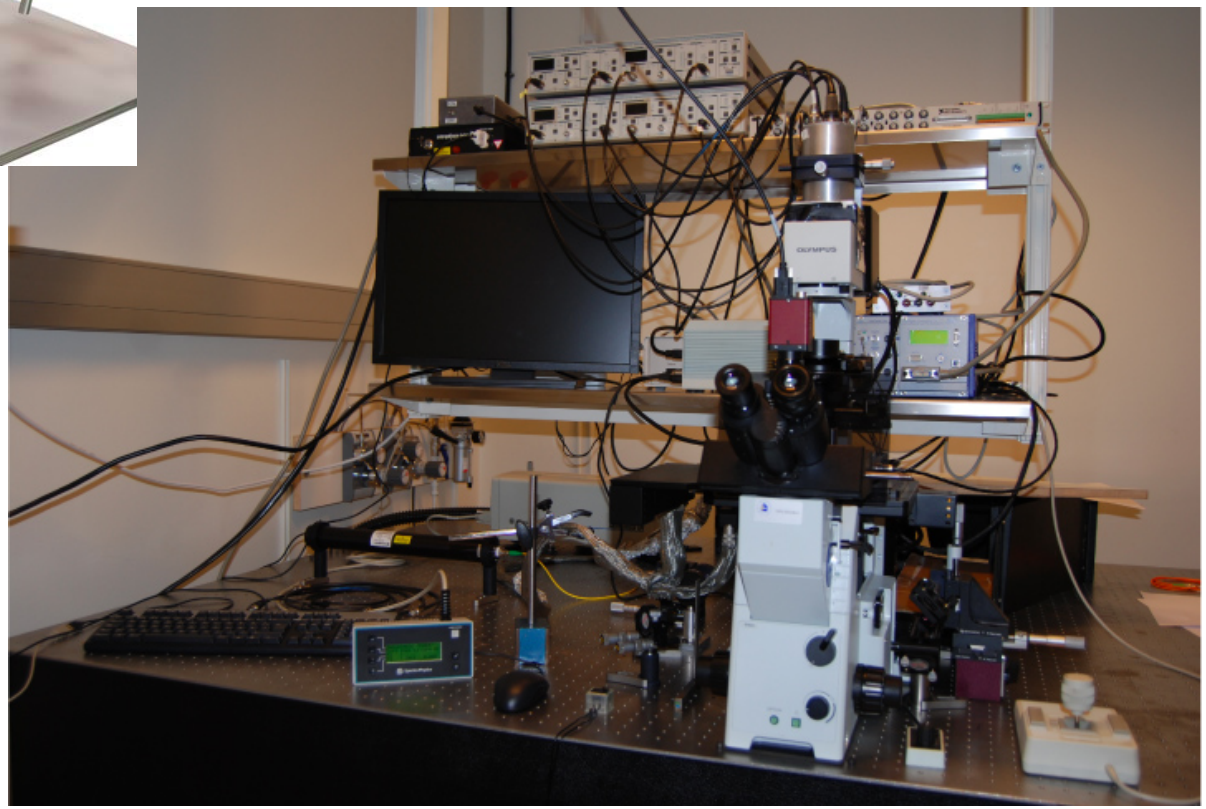


Verger et al. PLoS Pathogens v.3(5); 2007

Optical Tweezers : Pulling on Pili

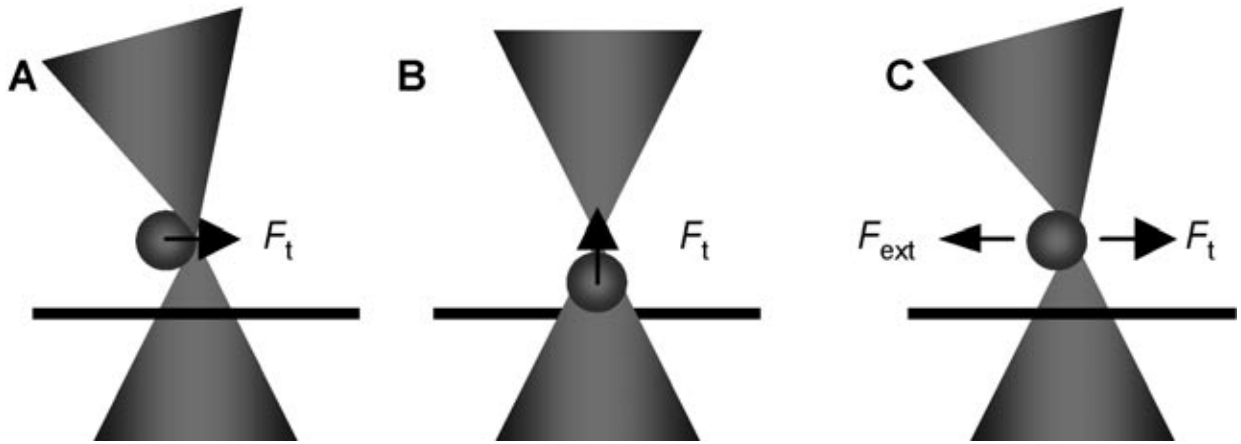


Blue : light used for trapping a particle
Red: probes the position of the trapped object



Magnus Andersson et al
ChemPhysChem 2008, 9, 221 – 235

Optical Tweezers : Pulling on Pili



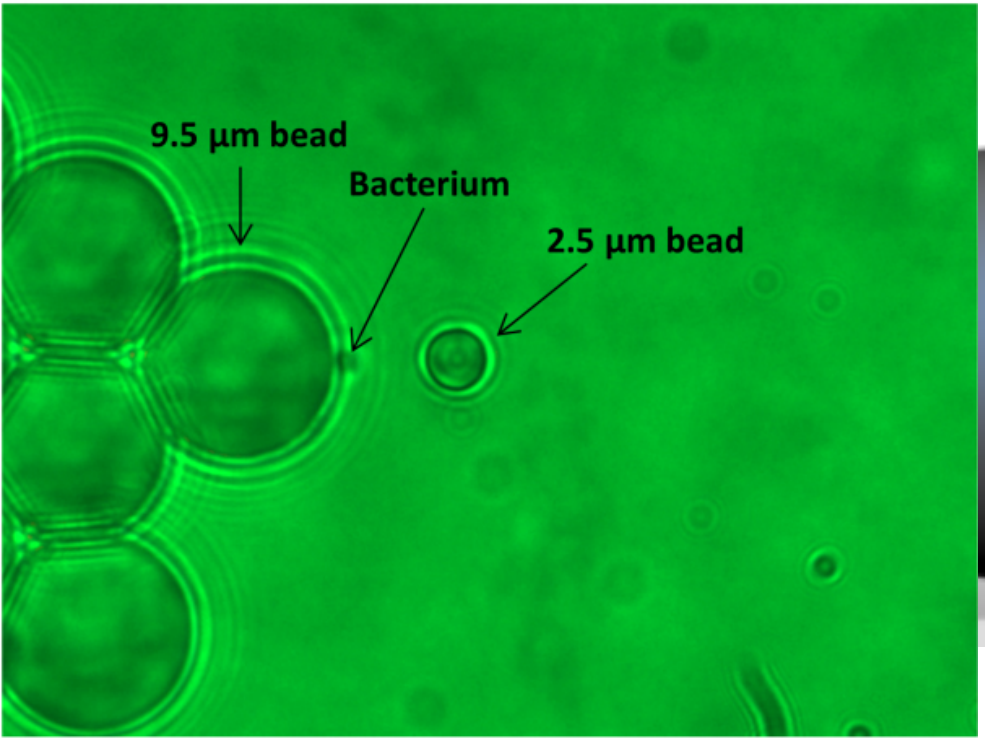
F_t – force of the trap
F_{ext} – external force

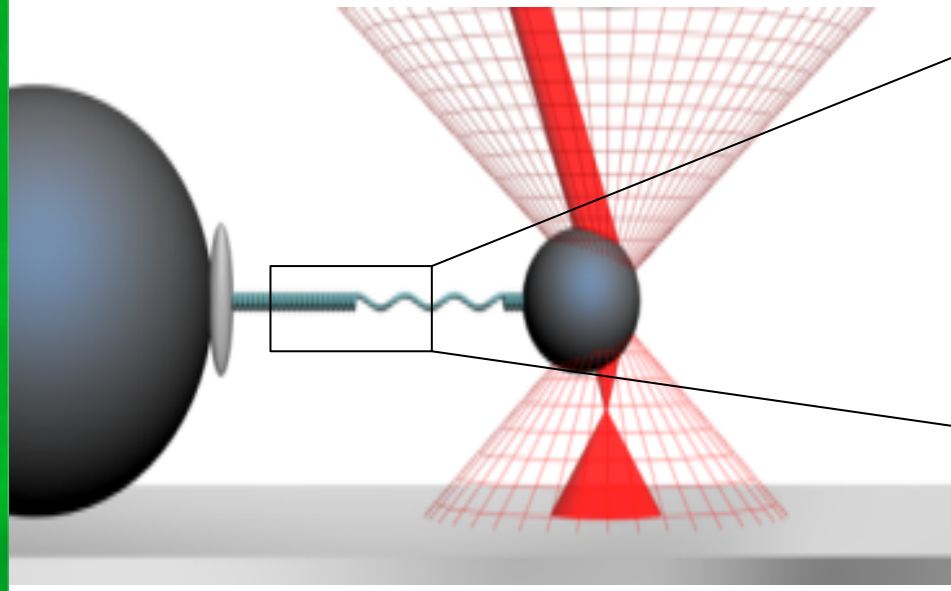
The force of the trap is related to the shift of the position of the bead in the trap through $F_t = \kappa \Delta x$.

Trap stiffness ~140pN/μm

Magnus Andersson et al
ChemPhysChem 2008, 9, 221 – 235

Force Spectroscopy : Pulling on Pili

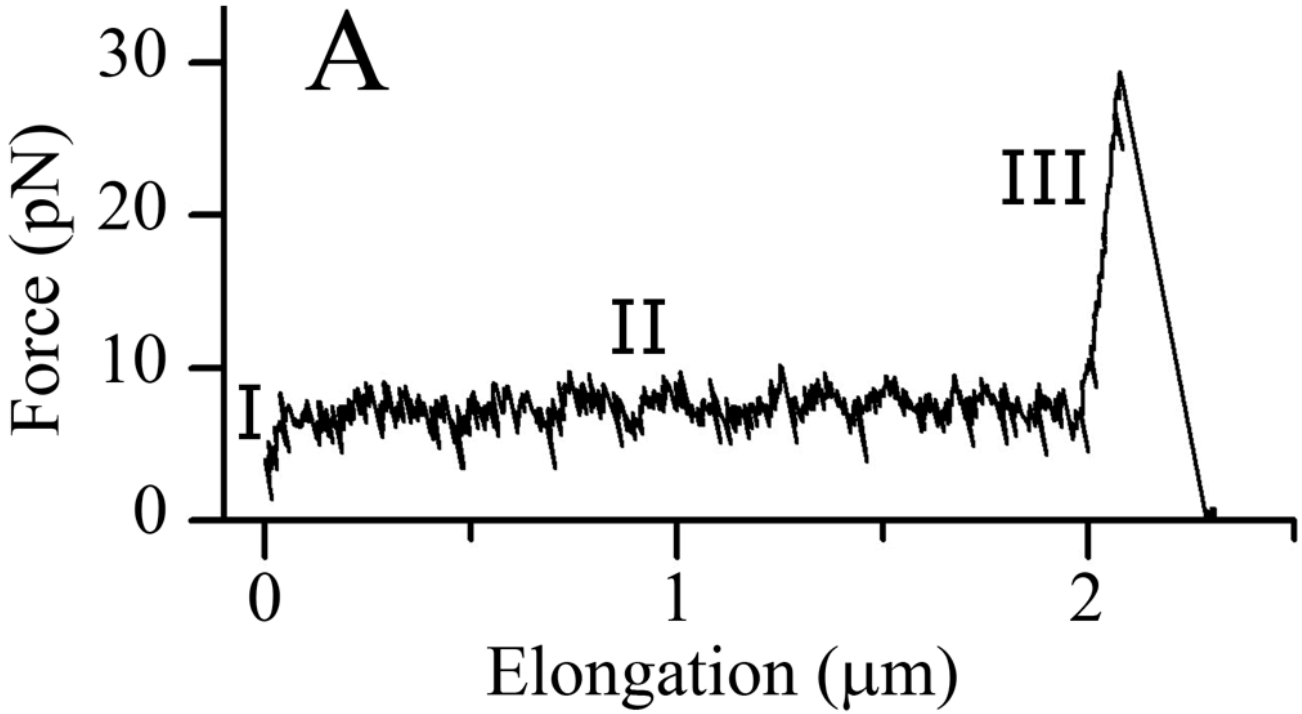


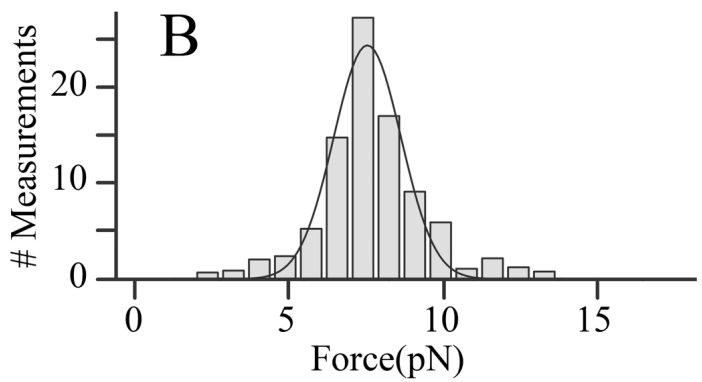


Mariam Shirdel, Master's thesis, Umeå Univ.

Mortezaei et al., (2015) Mol Microbiol. 95:116-2

CFA/I pili unwind at 8 pN force



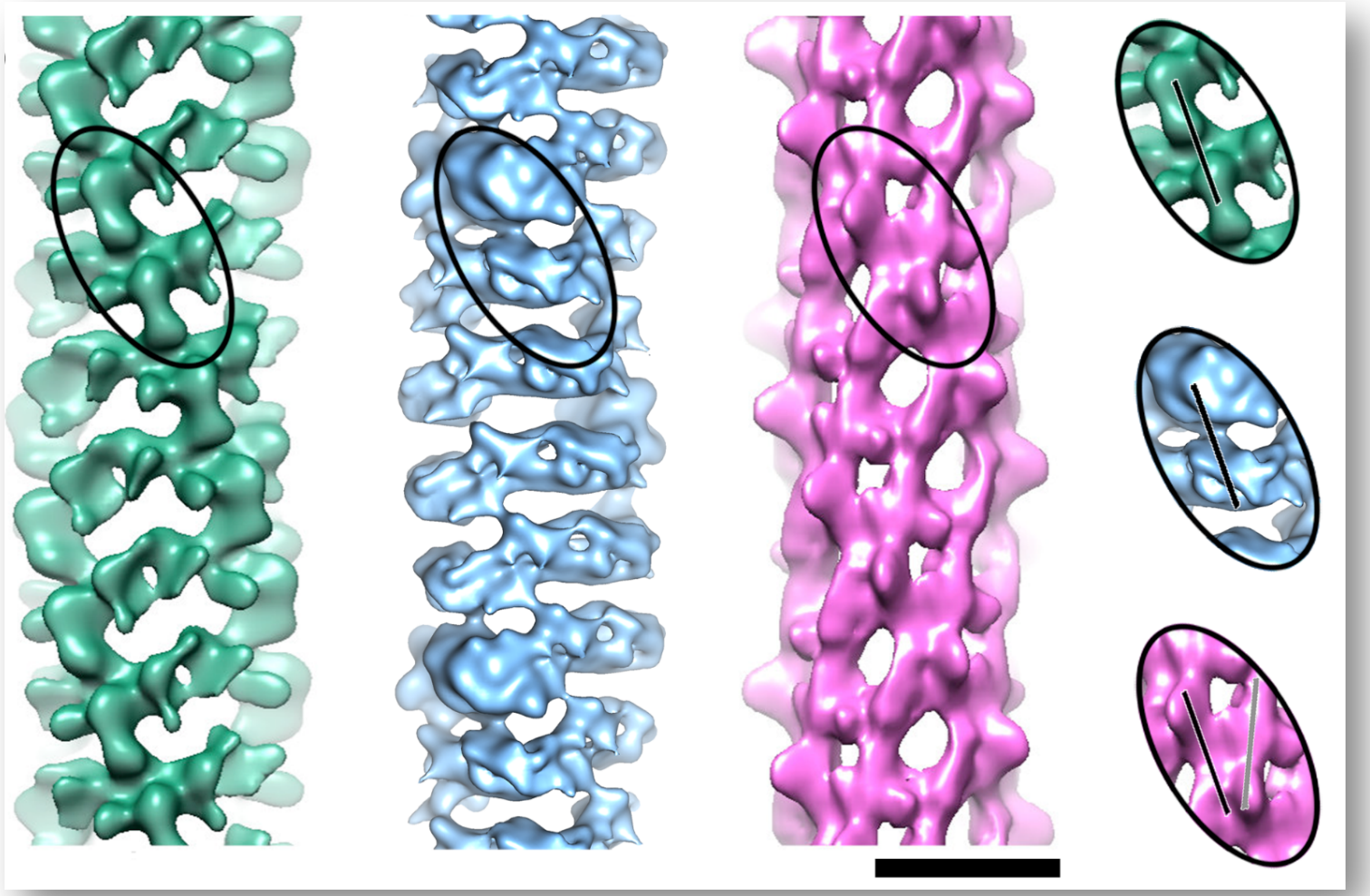


Weak connections between layers provide ‘bounce’ in the gut

GI CS20, 15pN

GI CFA/I, 8pN

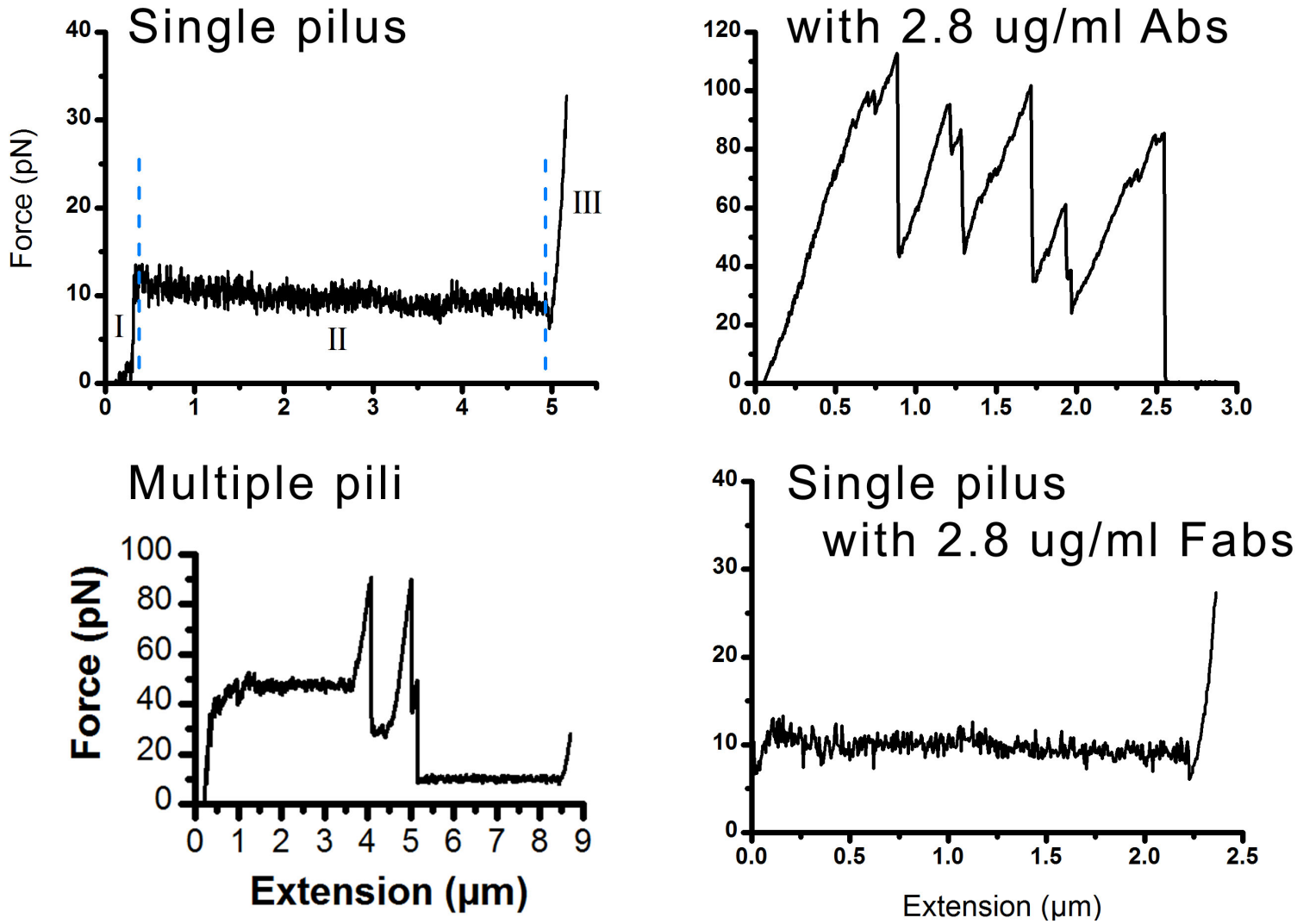
UTI P-pili, 28pN



New therapeutics against pathogenic bacteria?
We have a defense system:

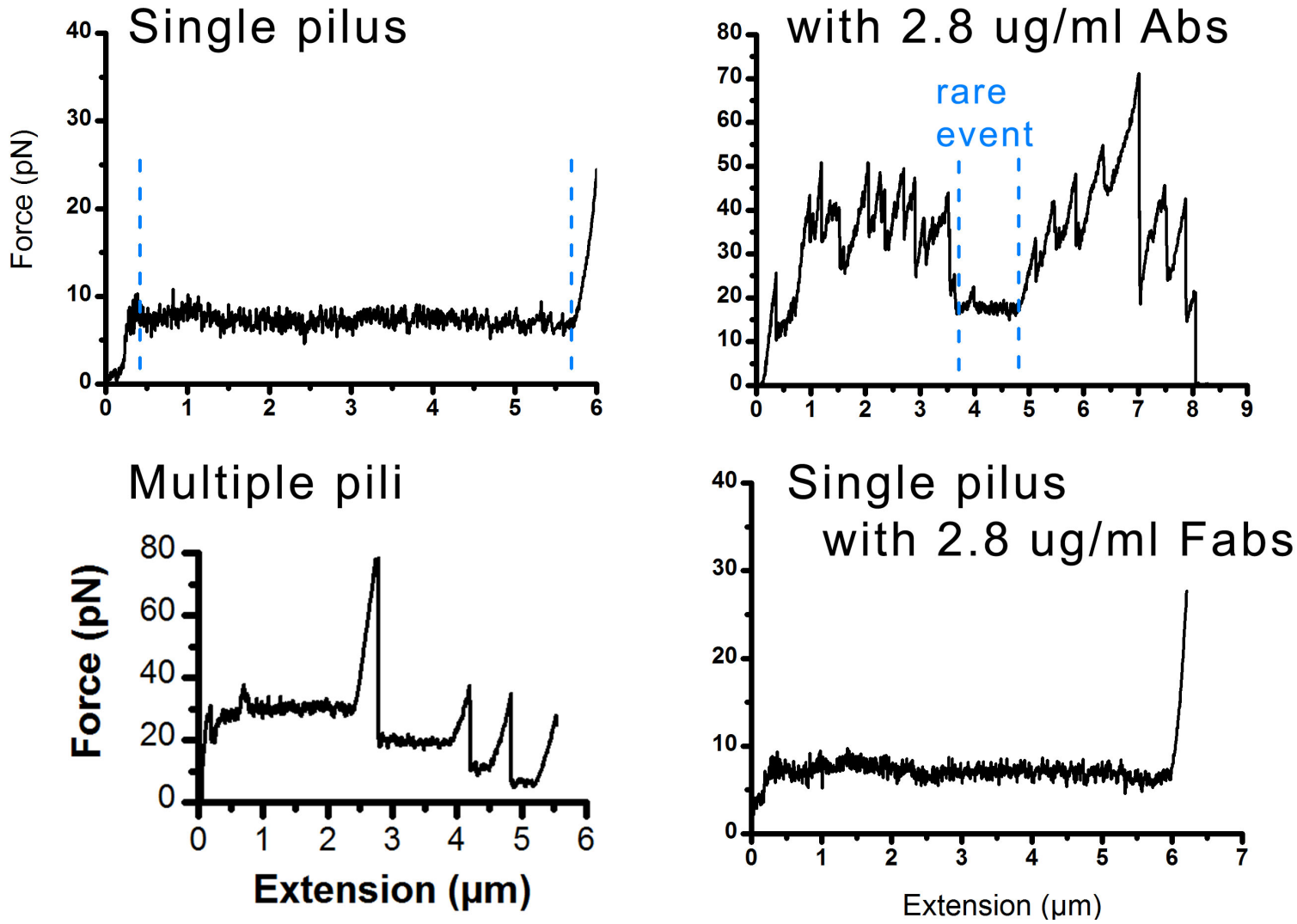
Pili + Abs

CS2 pili

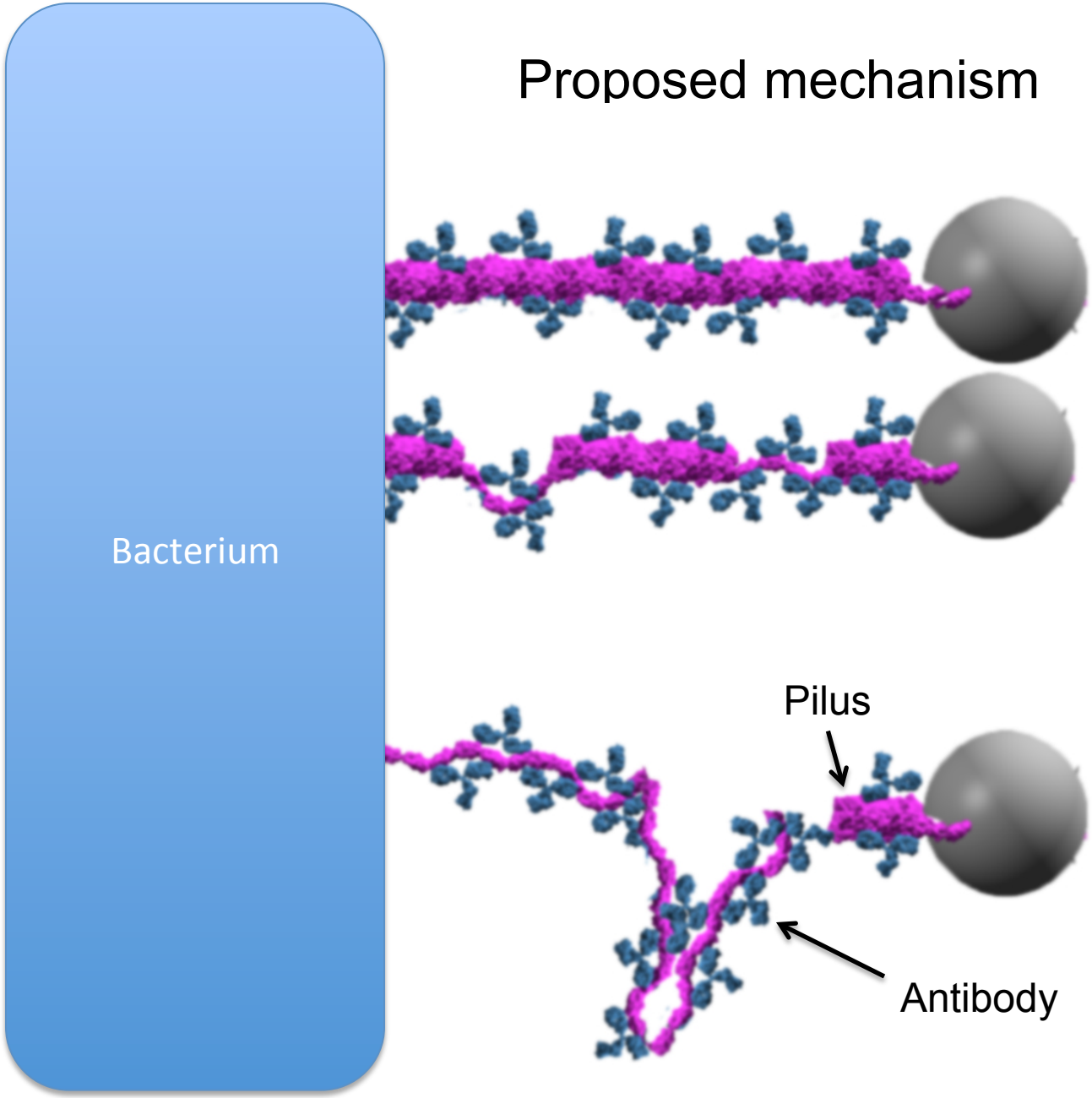


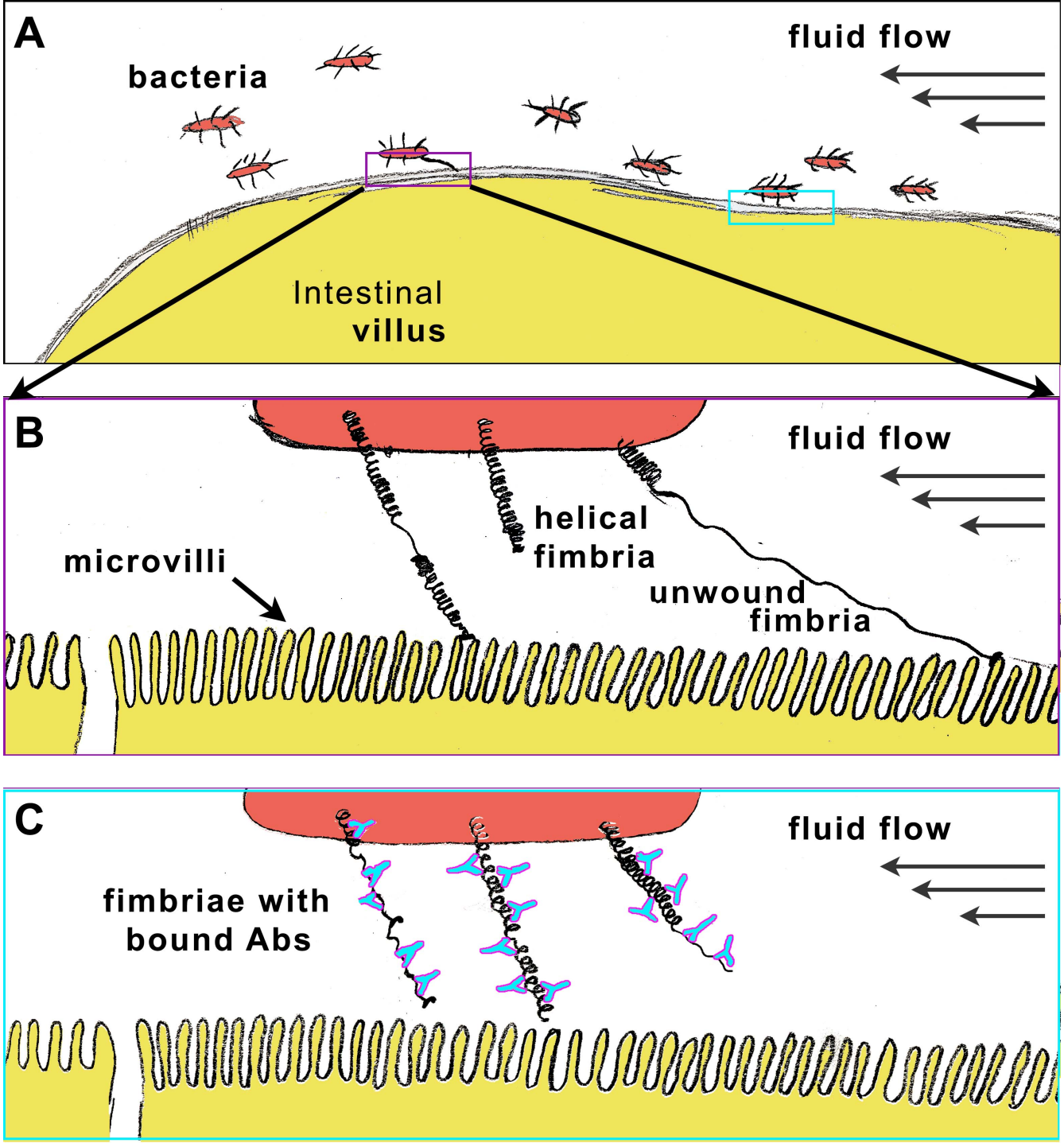
Works for different types of pili

CFA/I pili



Proposed mechanism




A
bacteria
fluid flow
Intestinal
villus
B
fluid flow
helical
fimbria
microvilli
unwound
fimbria
C
fluid flow
fimbriae with
bound Abs

Summary

Pili are specialized for their preferred environmental niche

Genetic similarity matters, and Environment matters more

Pilus structures can now be determined at high resolution with direct electron detectors and cryoEM

Force spectroscopy can be used to analyze pilus mechanics

Antibodies may play a direct role in limiting bacterial infections

Acknowledgments

Umeå University

Magnus Andersson, PhD

Bernt Eric Uhlin, PhD

Narges Mortezaei

Bhupender Singh, PhD

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Lt Commander Mike Prouty, PhD

Annette McVeigh

Boston University

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