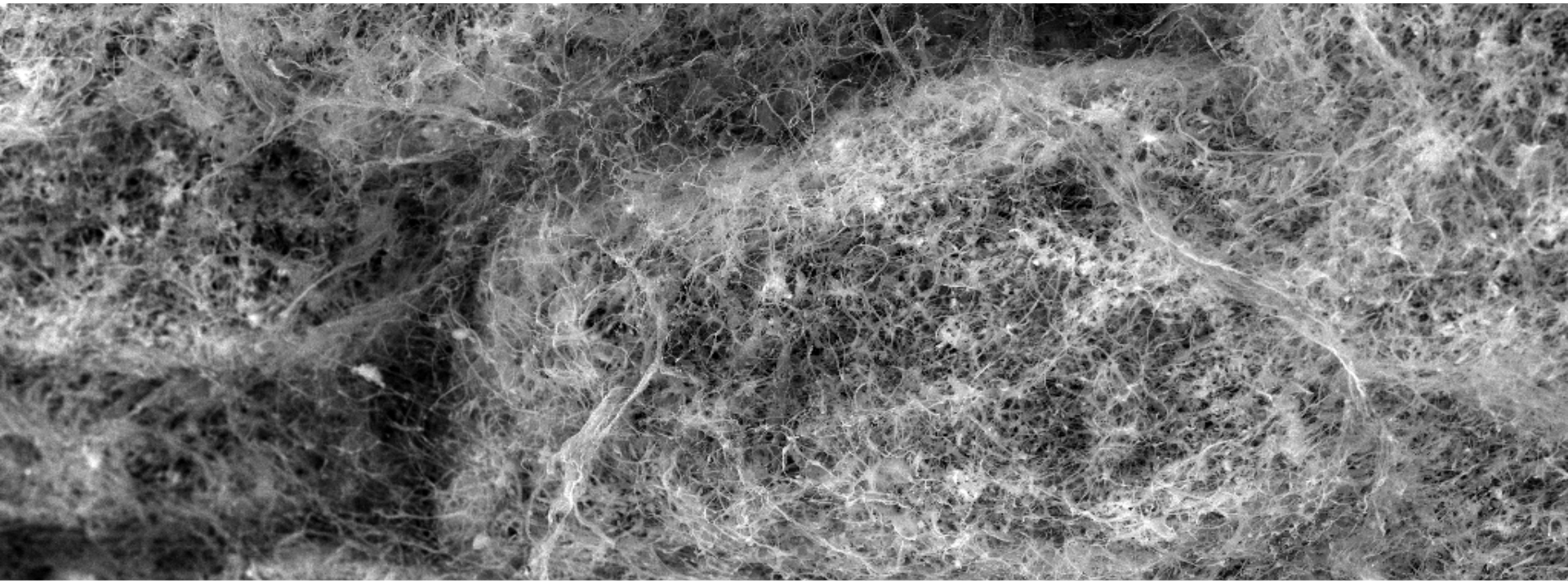
Mechanistic Insights and Therapeutic Applications from Mucosal Barrier Functions



2017 MIT Japan Conference
19th Annual Symposium for
Japanese Industry

Katharina Ribbeck

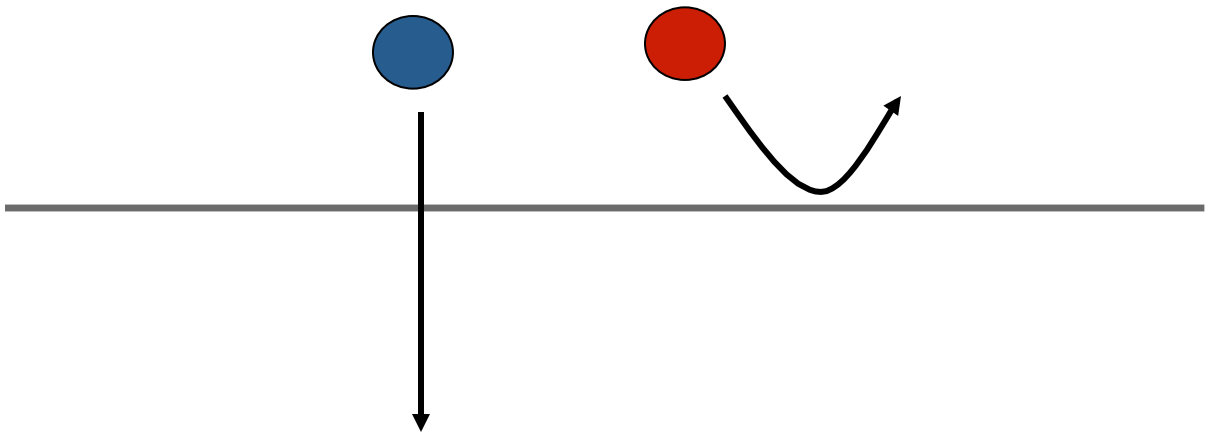
January 27, 2017

MIT BE
BIOLOGICAL ENGINEERING

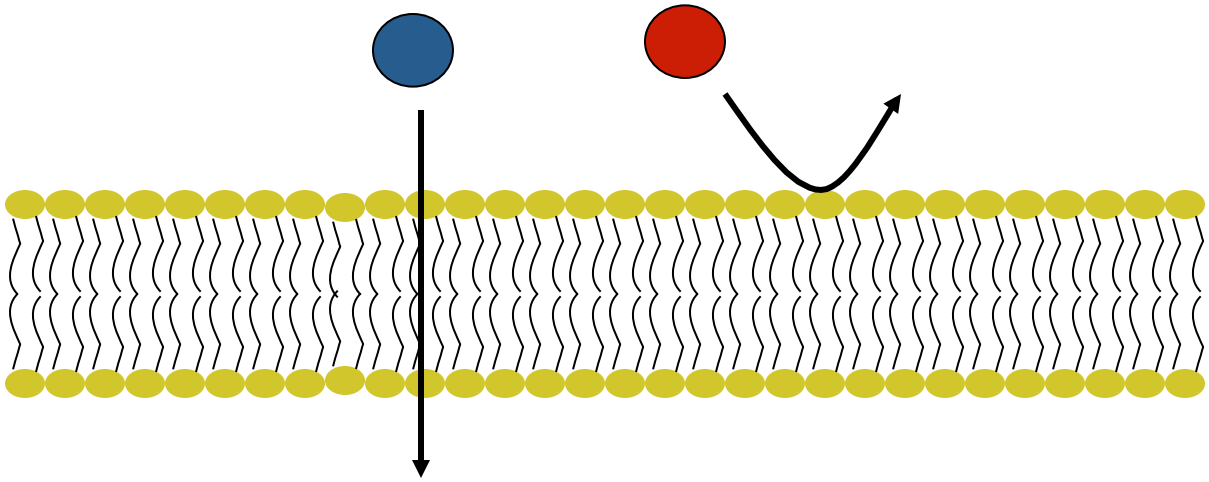
MIT CEHS

rle
AT MIT

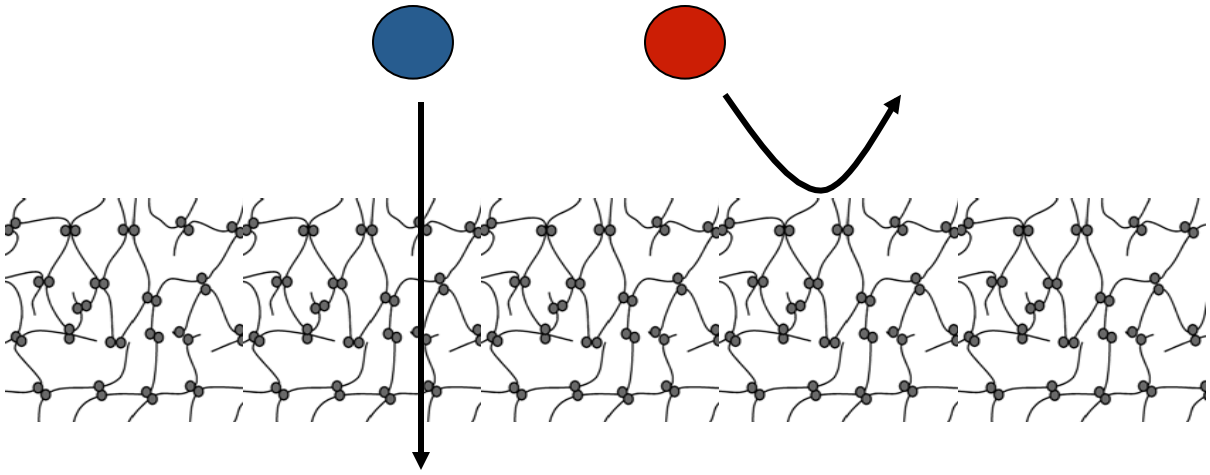
Biology depends on selective barriers

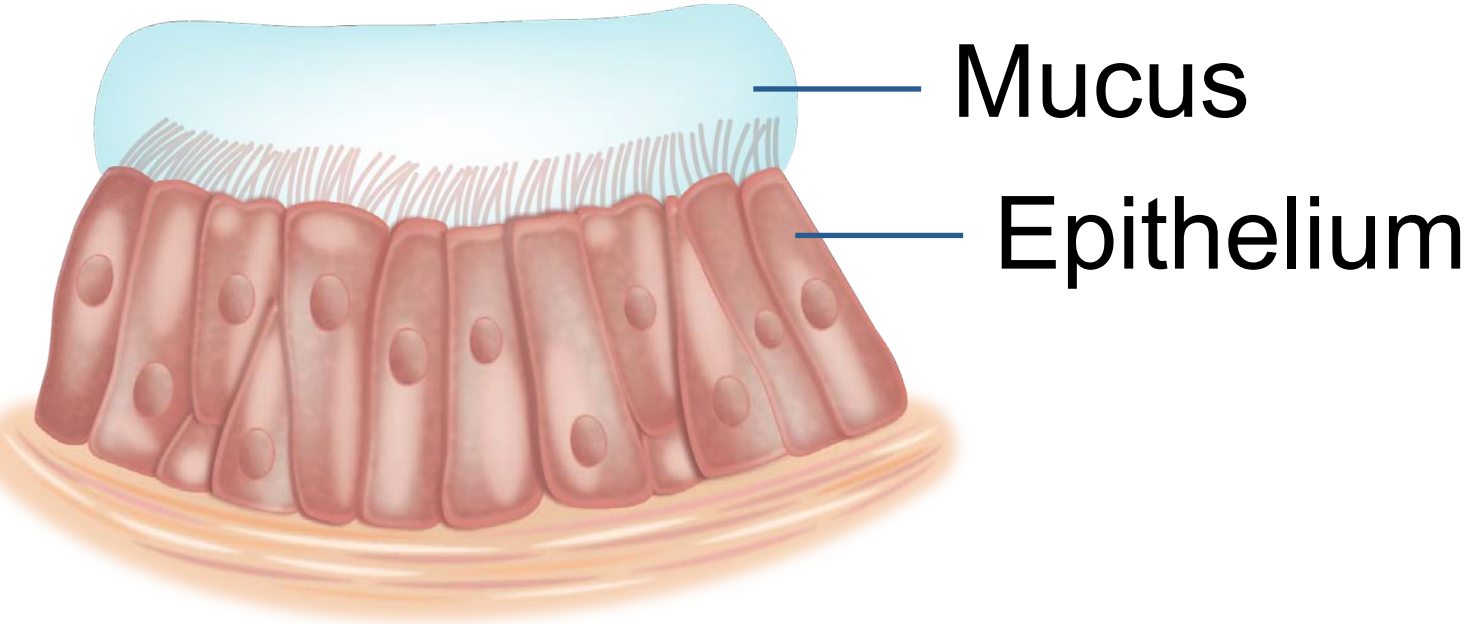


Membranes as biological filters

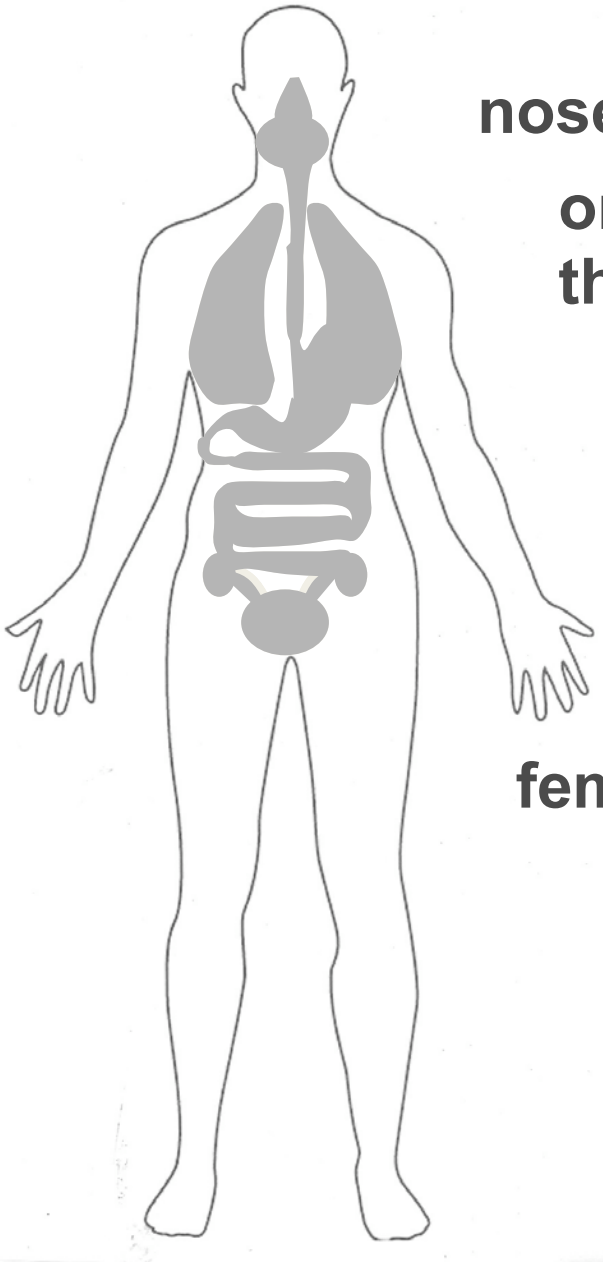


Hydrogels as biological filters




Mucus
Epithelium

Mucus has many different functions



nose

oral cavity/ throat

lungs

stomach

intestine

female genital tract

Mucus has many different functions

Viral Infections

Respiratory Allergies

Cystic Fibrosis

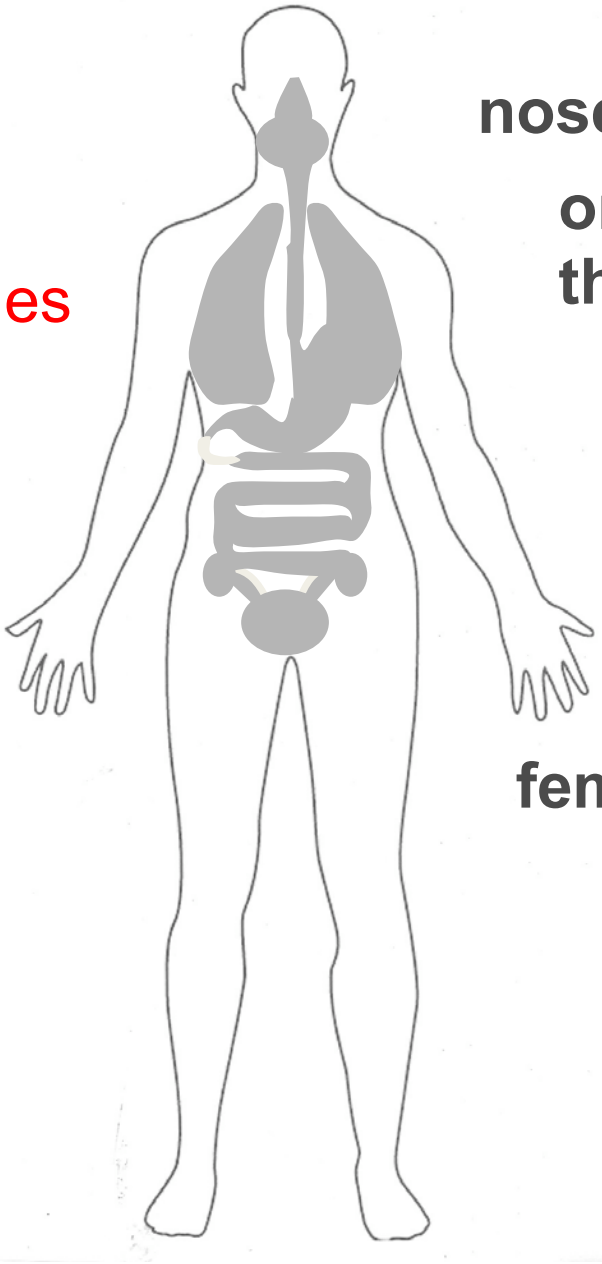
Gastric Ulcers

IDS, IBD

Diabetes

Obesity

Infertility



nose

oral cavity/ throat

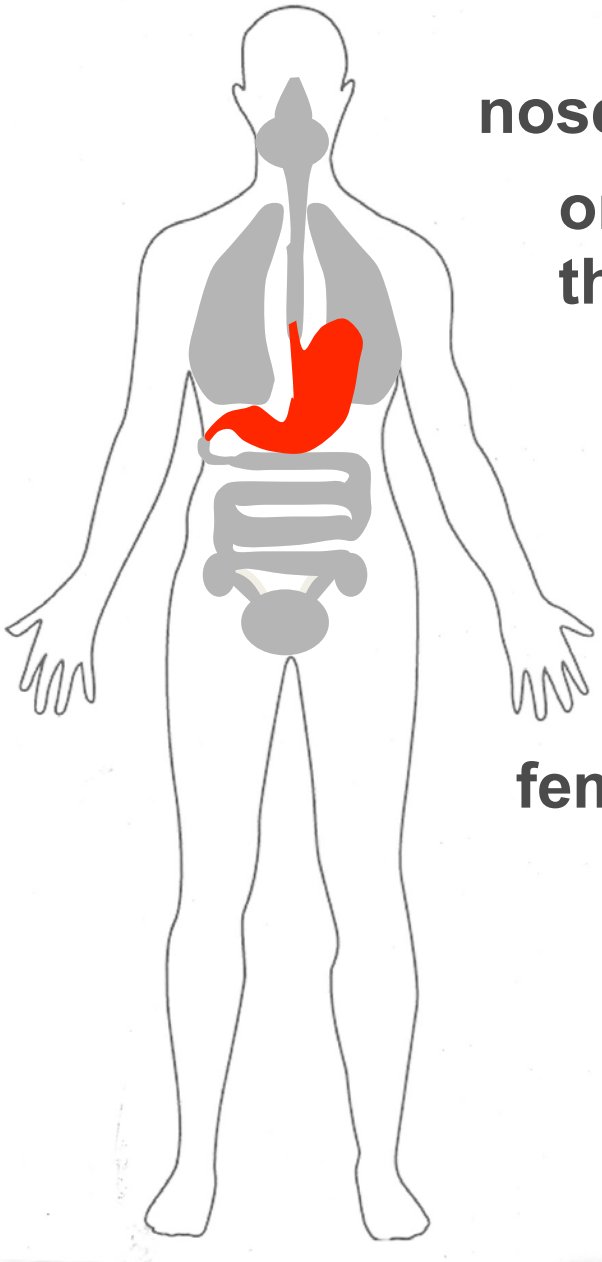
lungs

stomach

intestine

female genital tract

Mucus has many different functions



nose

oral cavity/
throat

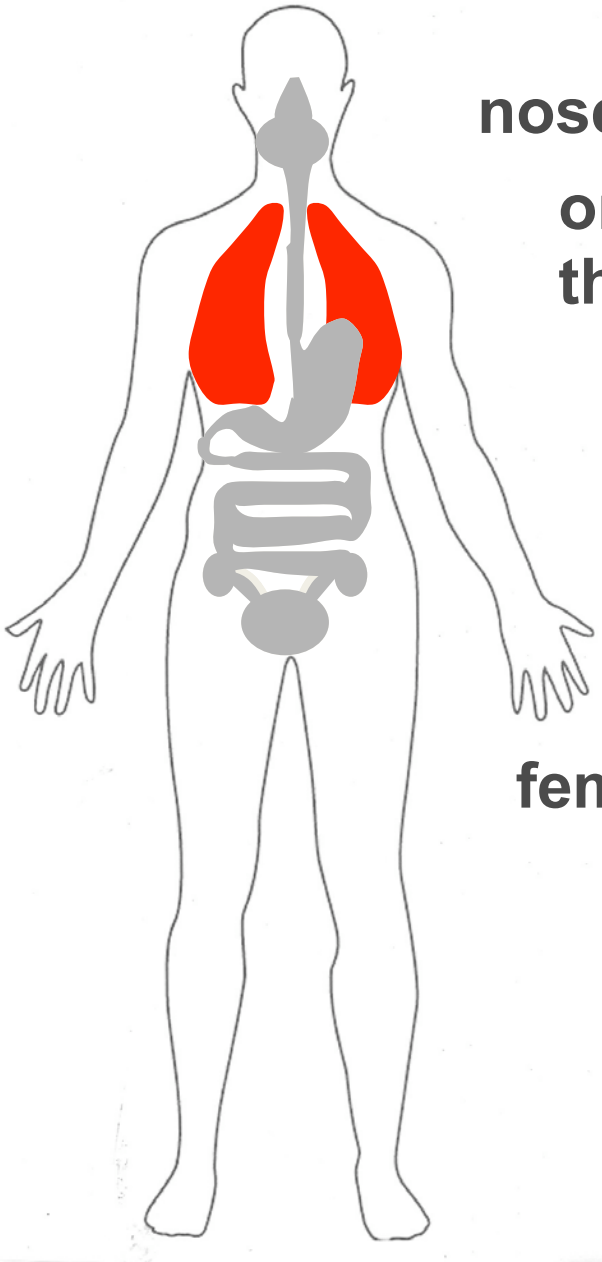
lungs

stomach

intestine

female genital tract

Mucus has many different functions



nose

oral cavity/
throat

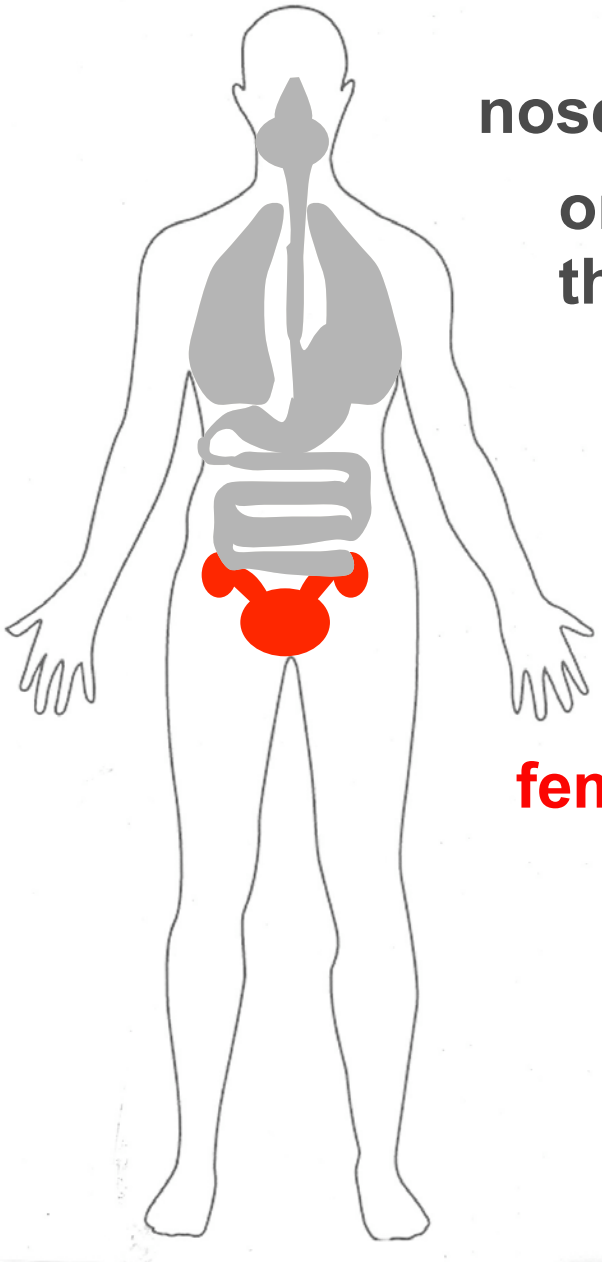
lungs

stomach

intestine

female genital tract

Mucus has many different functions



nose

oral cavity/ throat

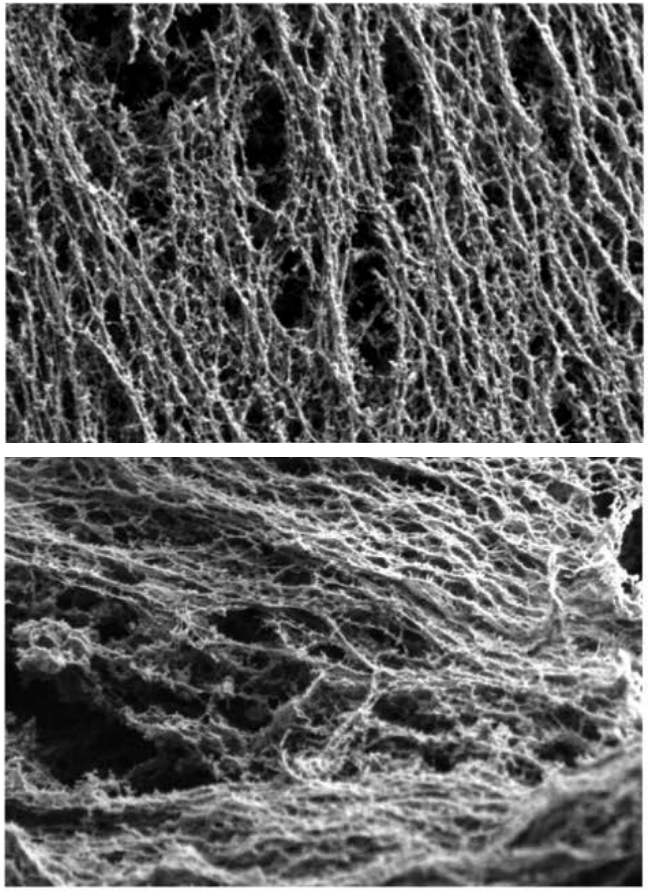
lungs

stomach

intestine

female genital tract

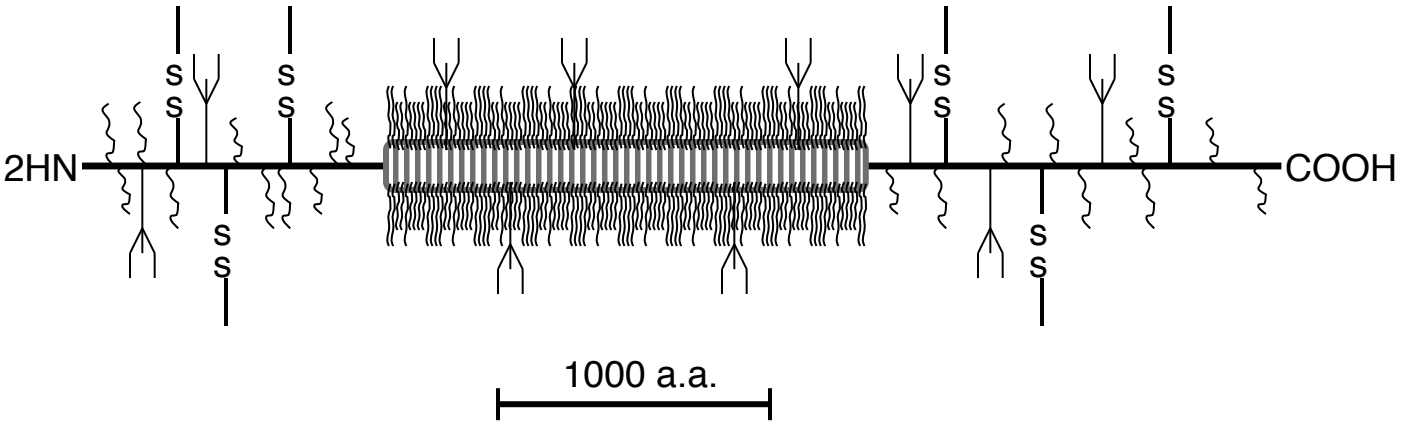
Secreted mucins form complex meshworks

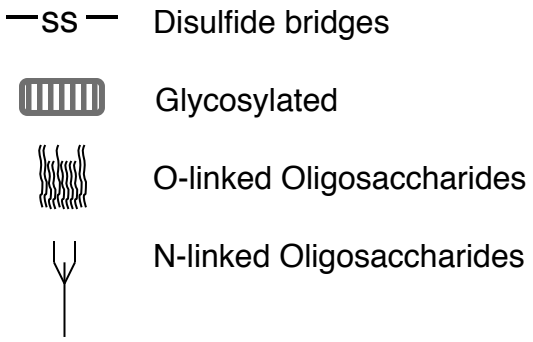


Ultrastructure of human cervical mucus

Critchfield 2013

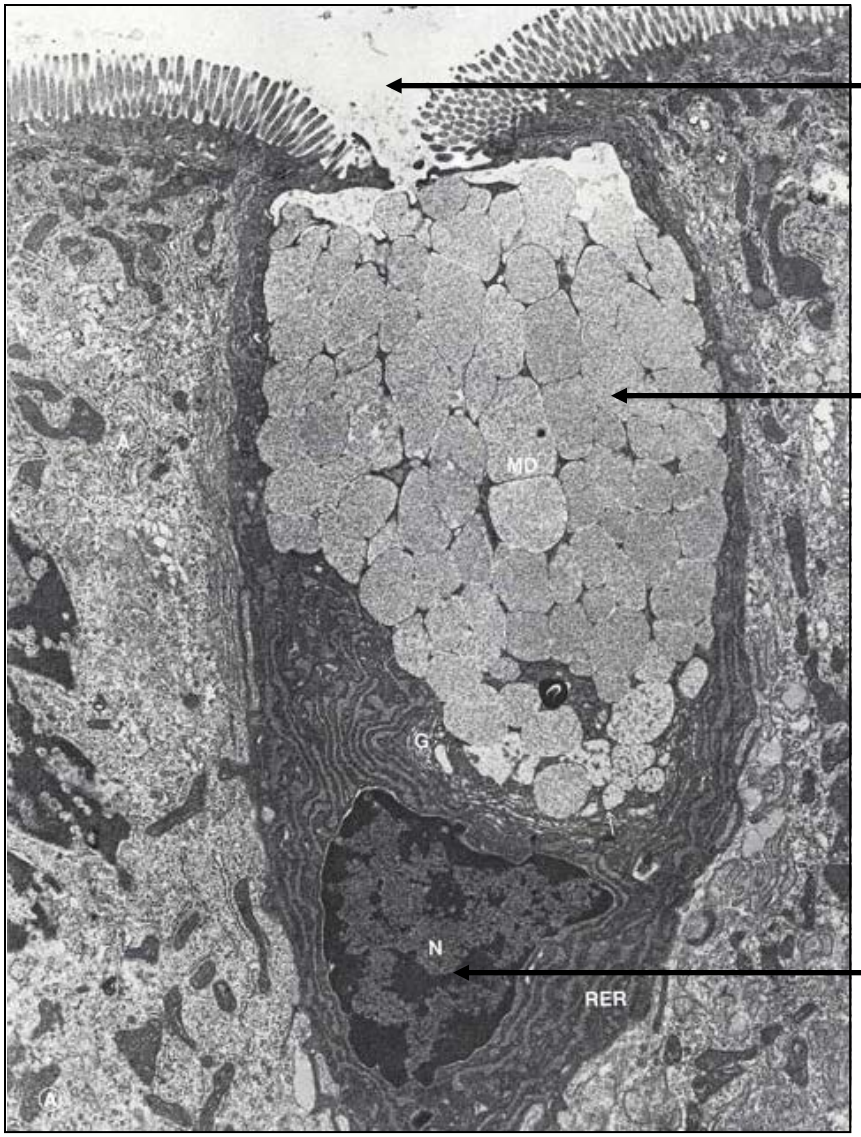
Mucin polymer





MUC2
MUC5AC
MUC5B
MUC6

Histology of a Goblet cell

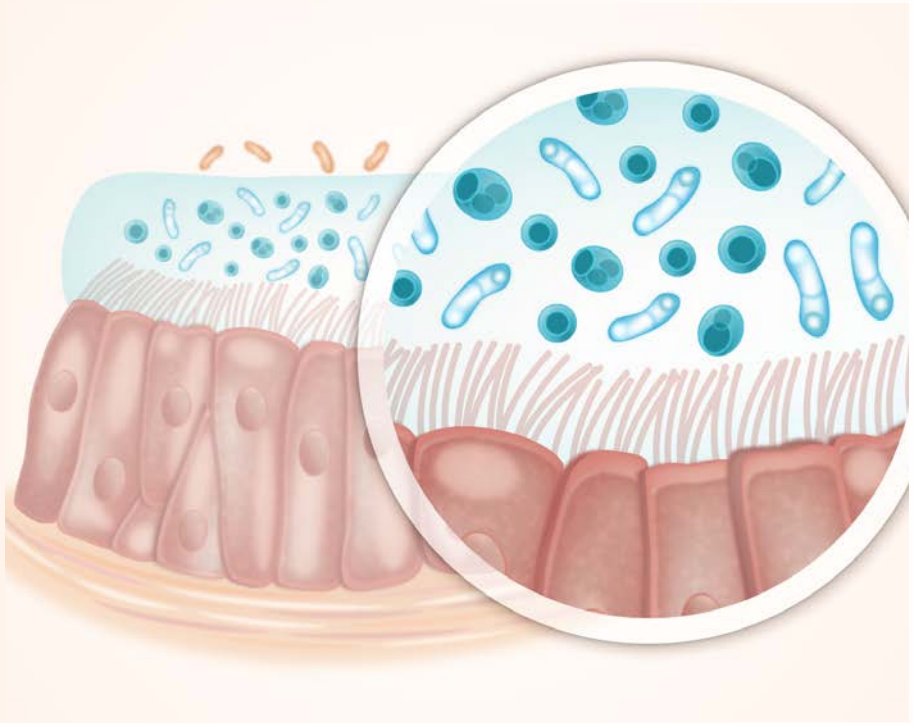


Mucus gel

Secretory granules

Nucleus

Mucus forms a major ecological niche for the human microbiota

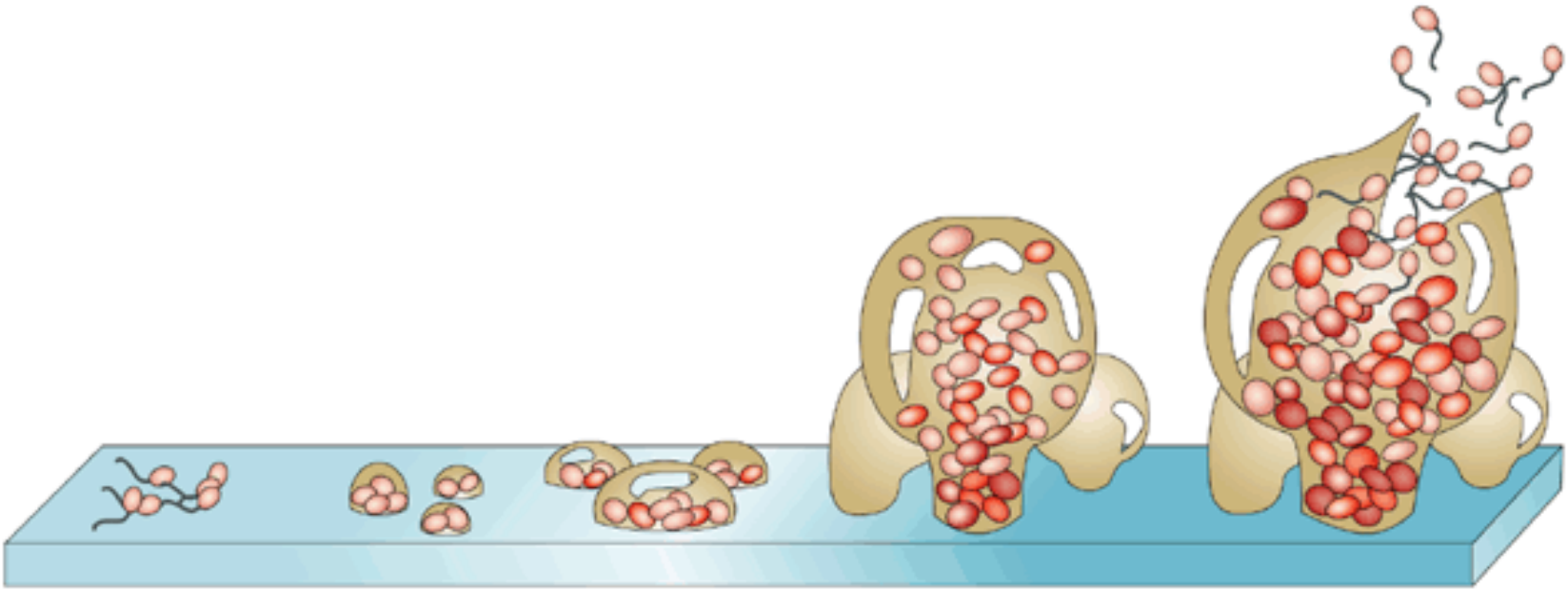


A principle arena for microbe-host interactions

Mucus is a barrier, but harbors many bacteria

Except in disease, pathogenic biofilms do not form in mucus

Biofilms - a beneficial lifestyle for microbes

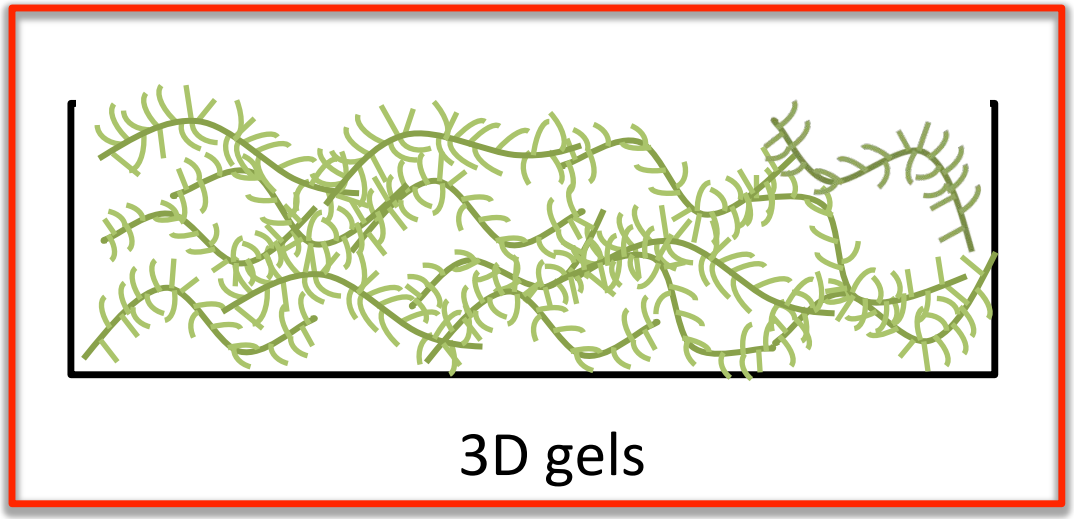


- Resistance to antibiotics/biocides
- Resistance to clearance (phagocytosis/mechanical stress)
- Synchronized toxin production
- Adapt to harsh environments through division of labor

Davies, D. 2003. Understanding biofilm resistance to antibacterial agents. Nature reviews Drug Discovery. 2(114-122)

Assaying microbial behavior in 3D mucin gels

2D coating

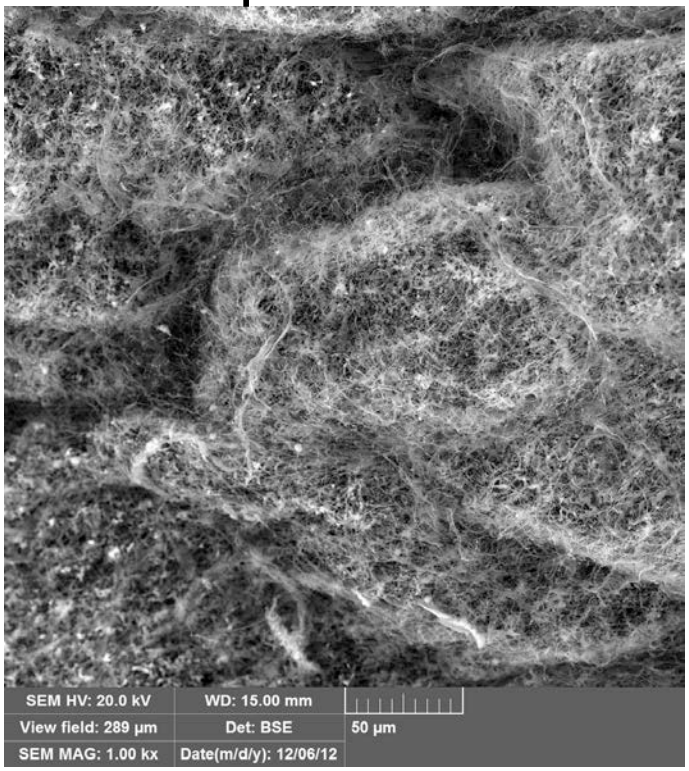


3D gels

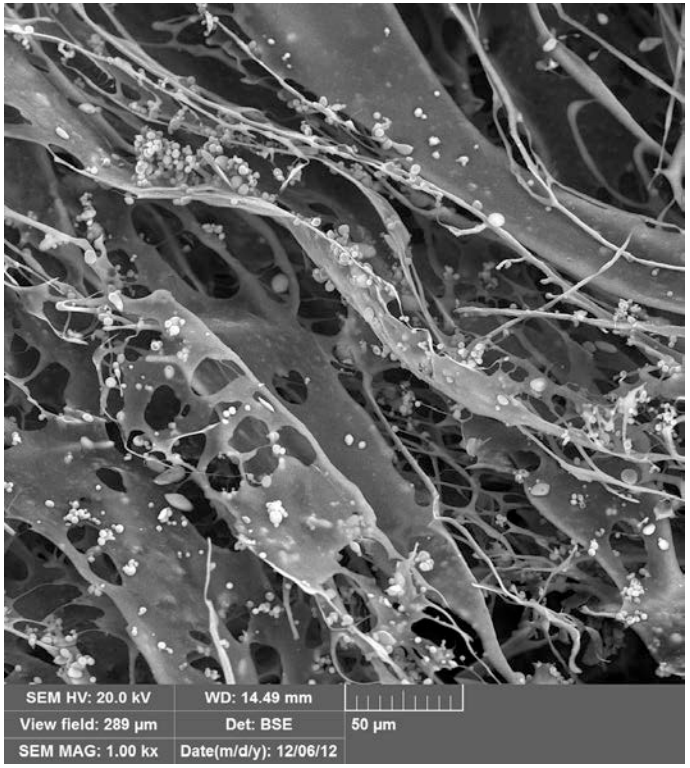
MUC5AC

Native purification of mucins is crucial for their functionality

Native purified mucins



Industrial mucins



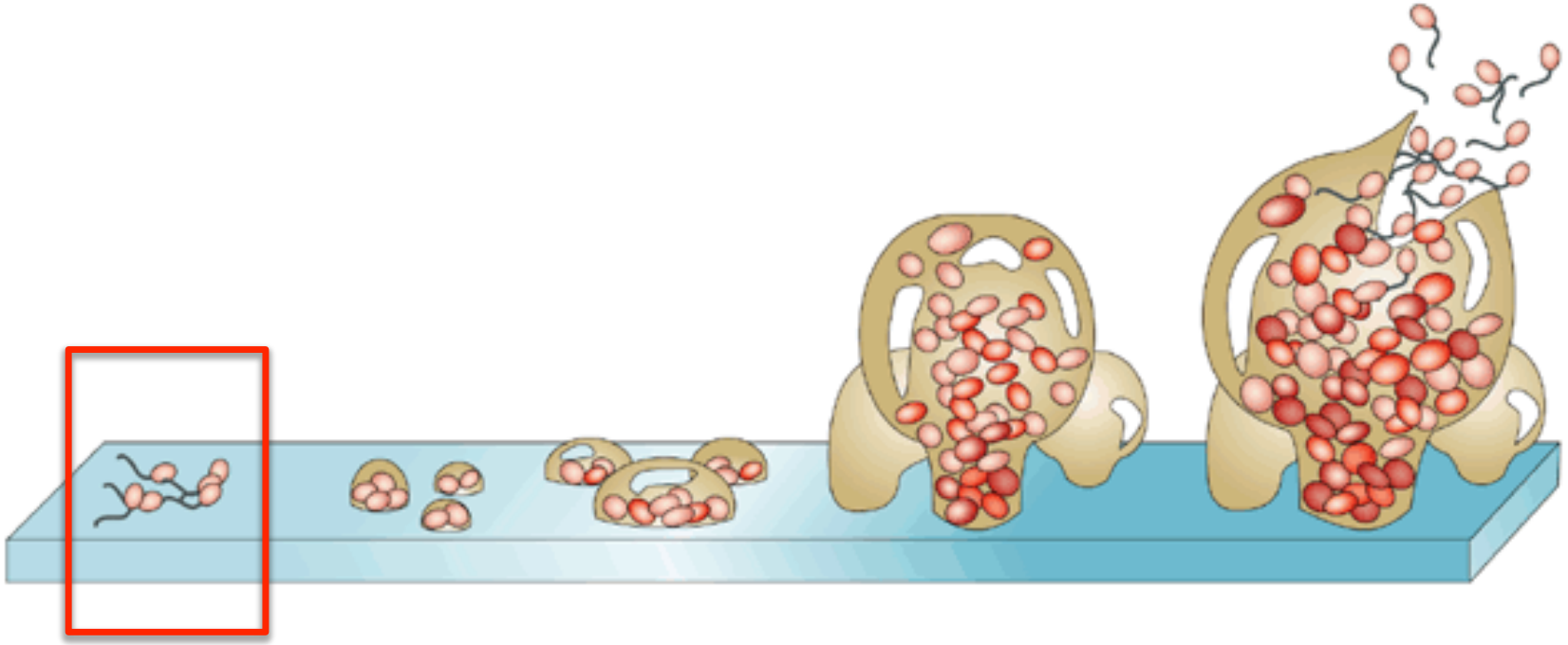
Nicole Kavanaugh

Model organism

***Pseudomonas aeruginosa* PAO1**

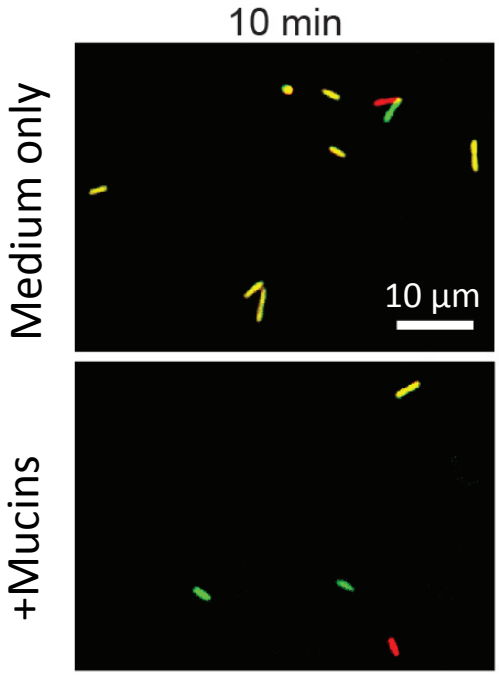
- Gram-negative, rod-shaped, motile
  - Polar flagella
- Opportunistic pathogen
  - Important in hospital acquired infections and immuno-compromised individuals, especially cystic fibrosis
- Forms biofilms implicated in chronic infections (CF)

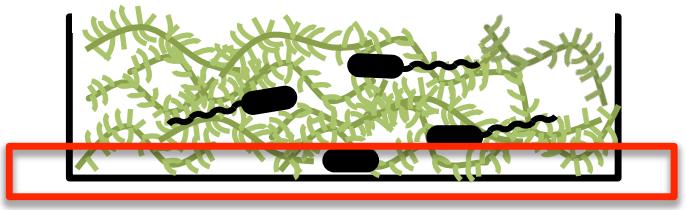
Pseudomonas aeruginosa

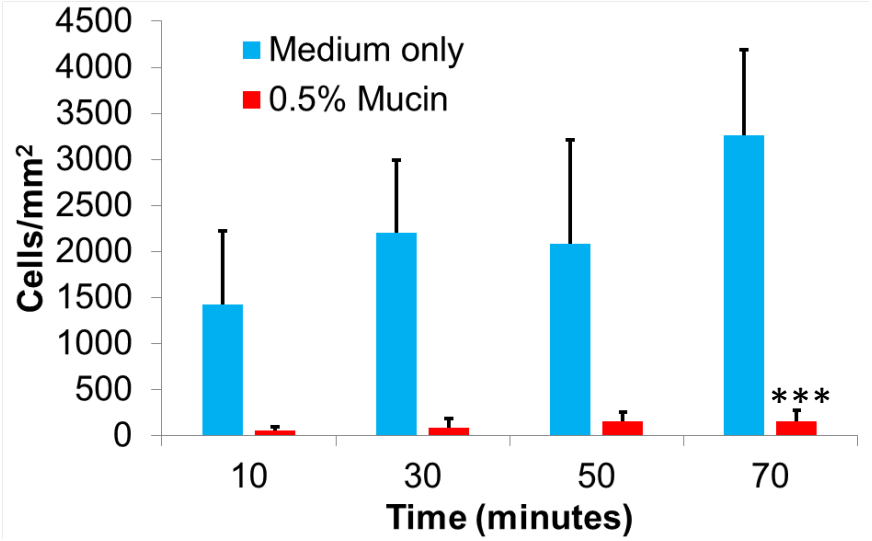


Davies, D. 2003. Understanding biofilm resistance to antibacterial agents. Nature reviews Drug Discovery. 2(114-122)

Mucins reduce bacterial adhesion



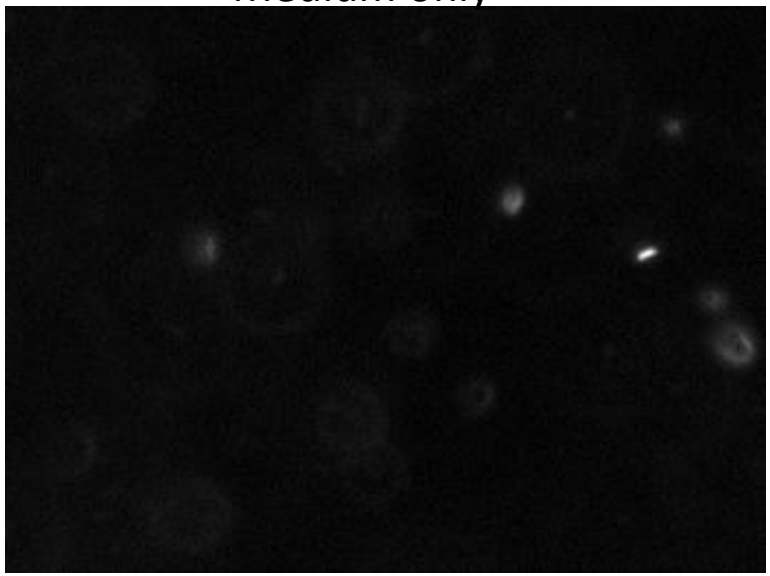




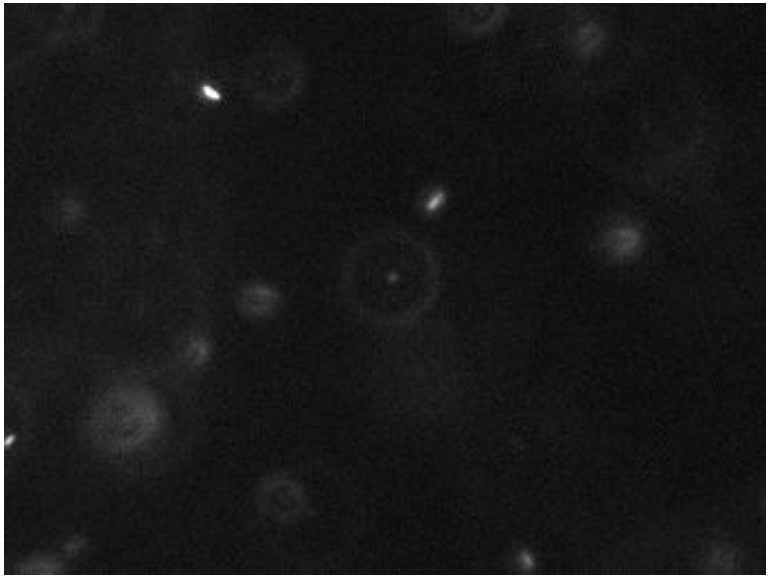
Caldara 2012

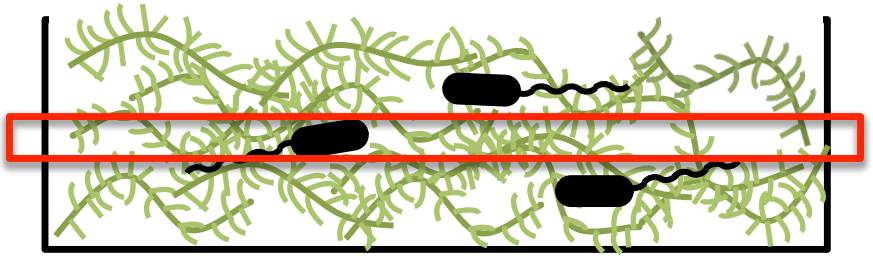
Are cells trapped in mucins?

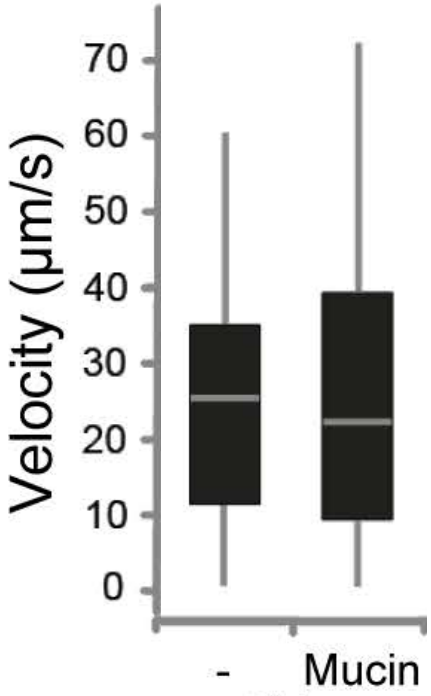
Medium only



Medium + mucin



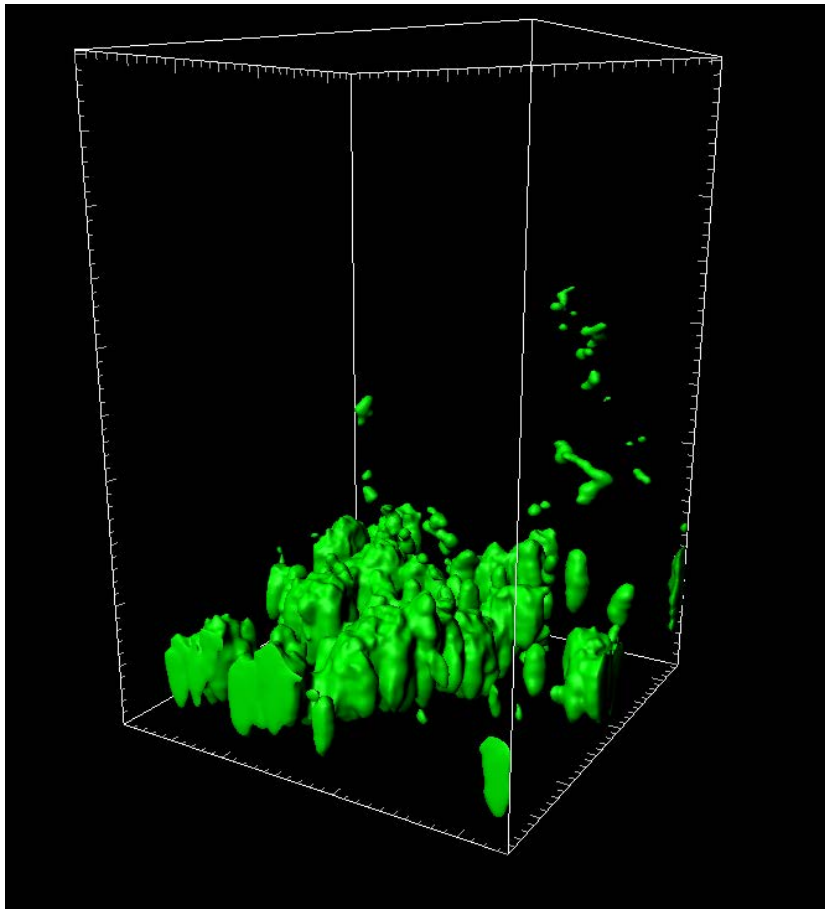




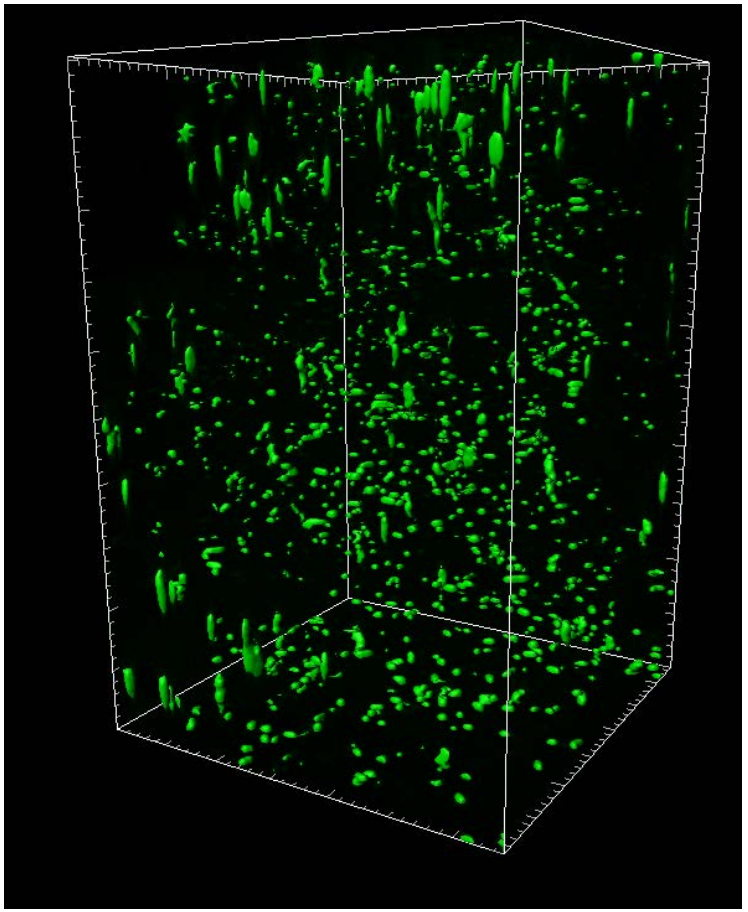
Caldara 2012

Mucins prevent biofilm formation by *P. aeruginosa*

Medium

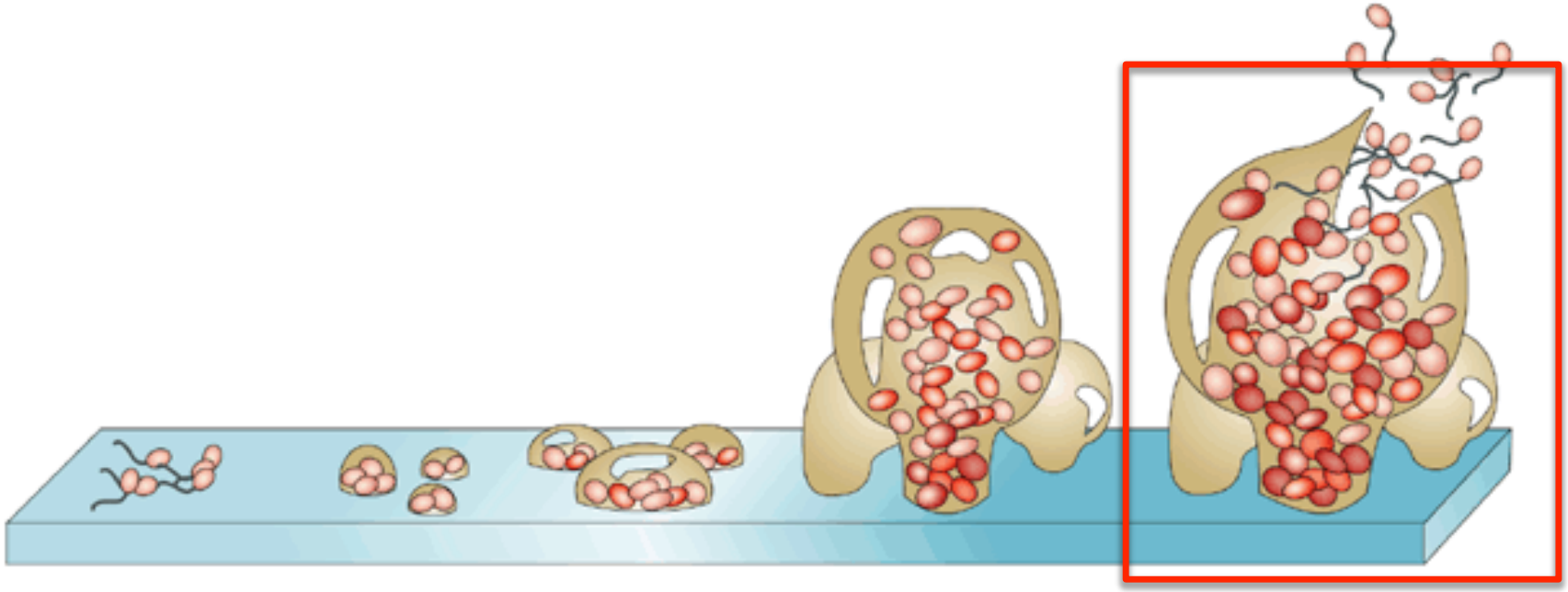


+ 0.5% Mucins (MUC5AC)



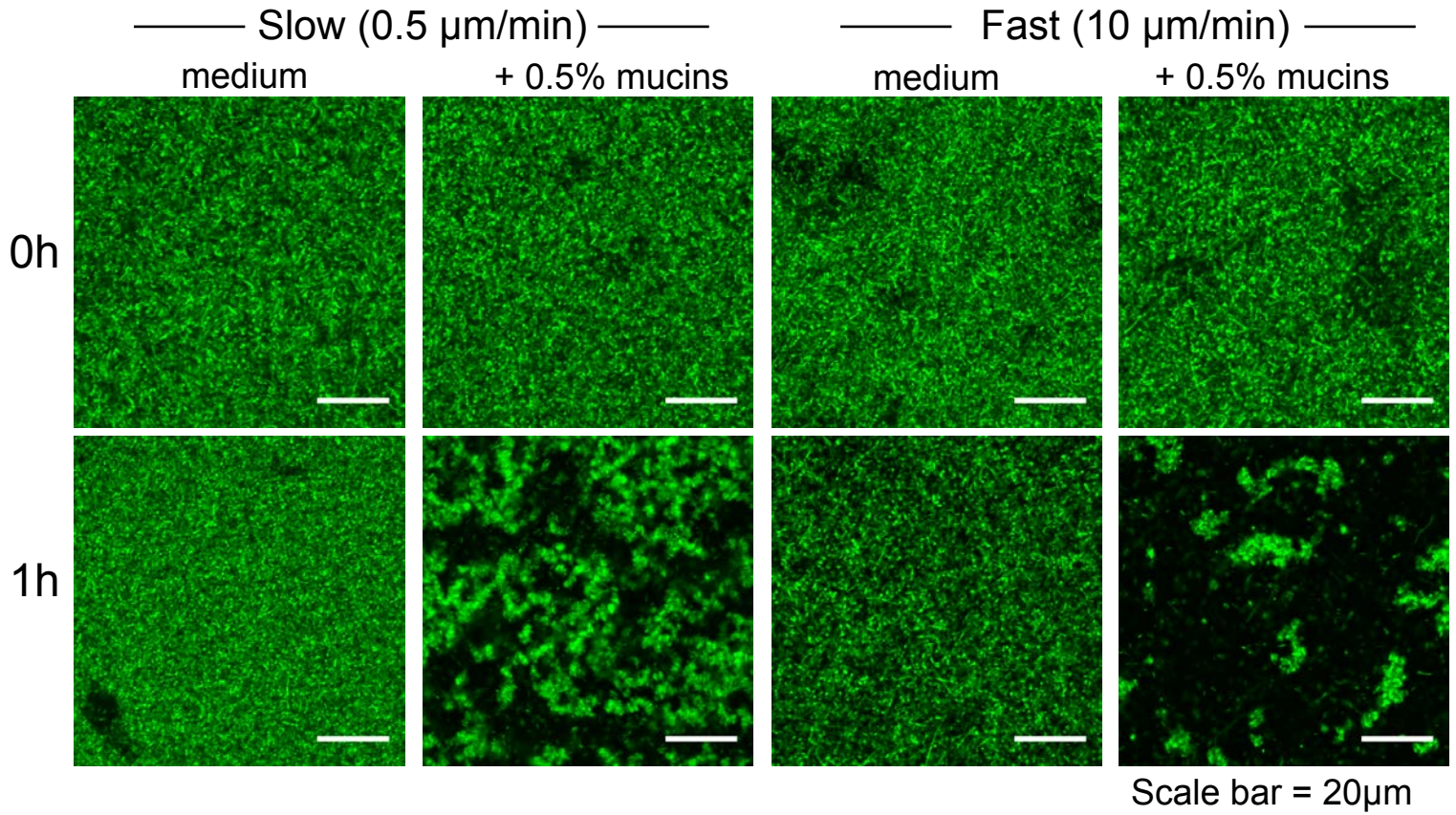
36 h, 37°C

Gerardo Carcamo Oyarce

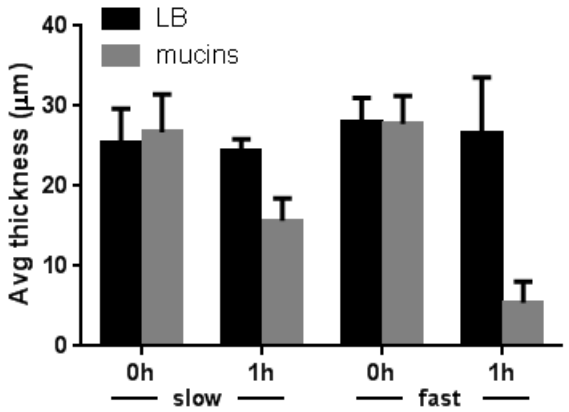


Davies, D. 2003. Understanding biofilm resistance to antibacterial agents. Nature reviews Drug Discovery. 2(114-122)

Mucins can disassemble mature biofilms

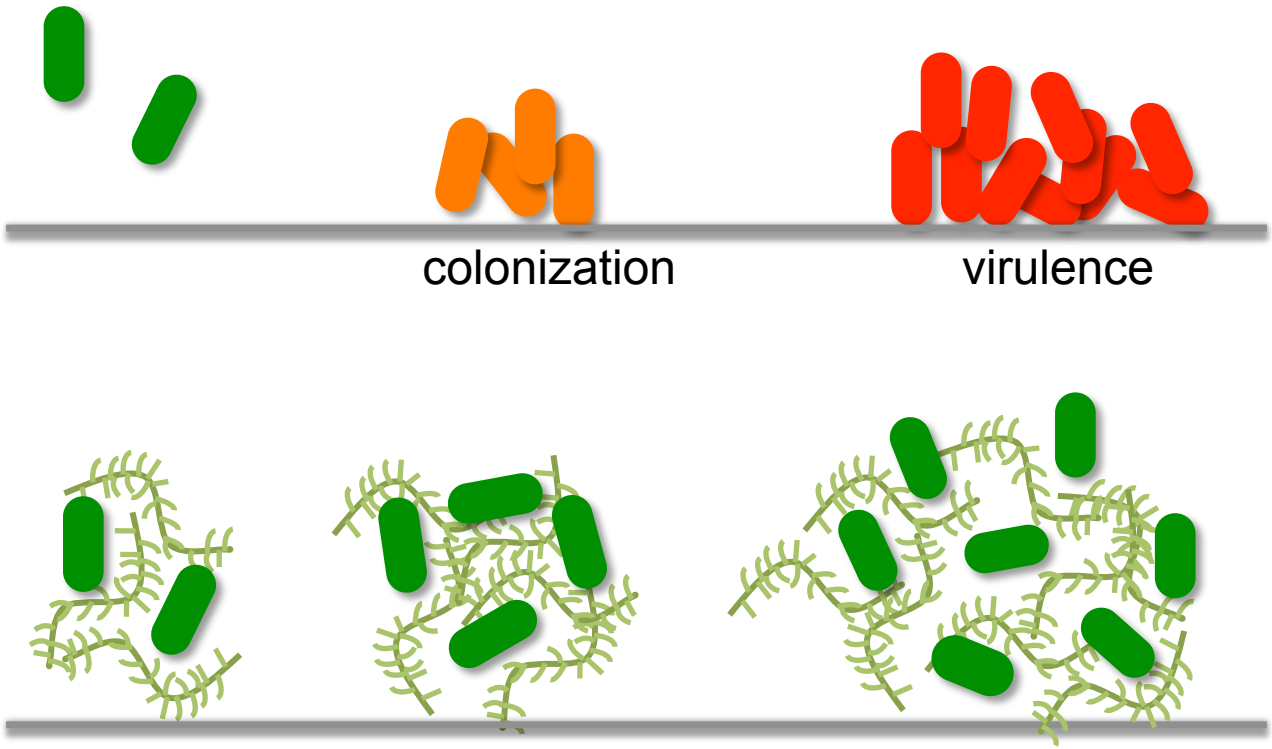


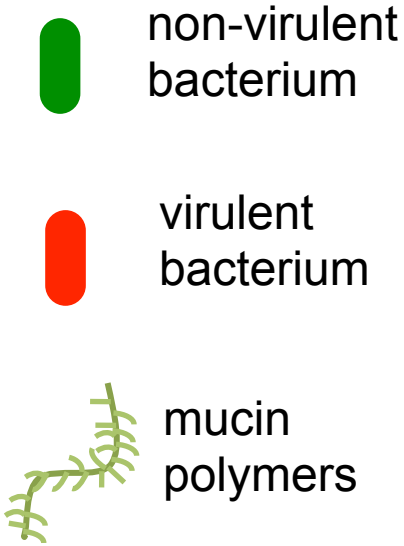
Scale bar = 20μm



Julia Co
Nicole Billings

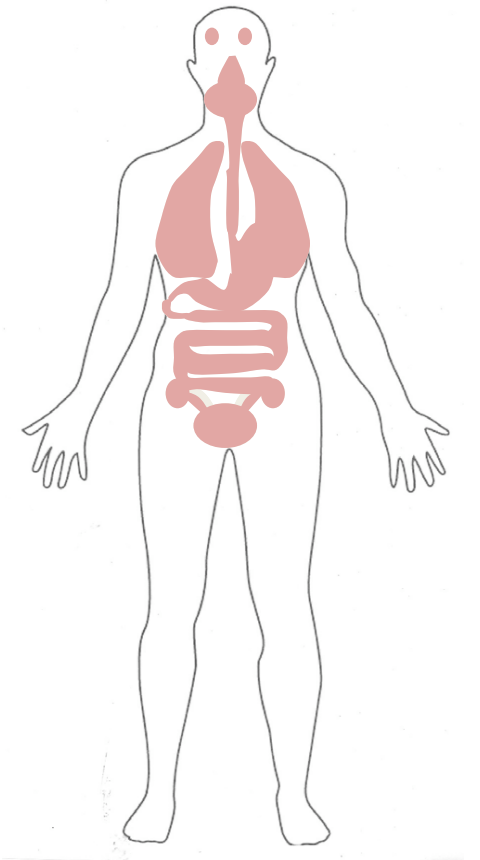
Mucins promote dispersion of microbes





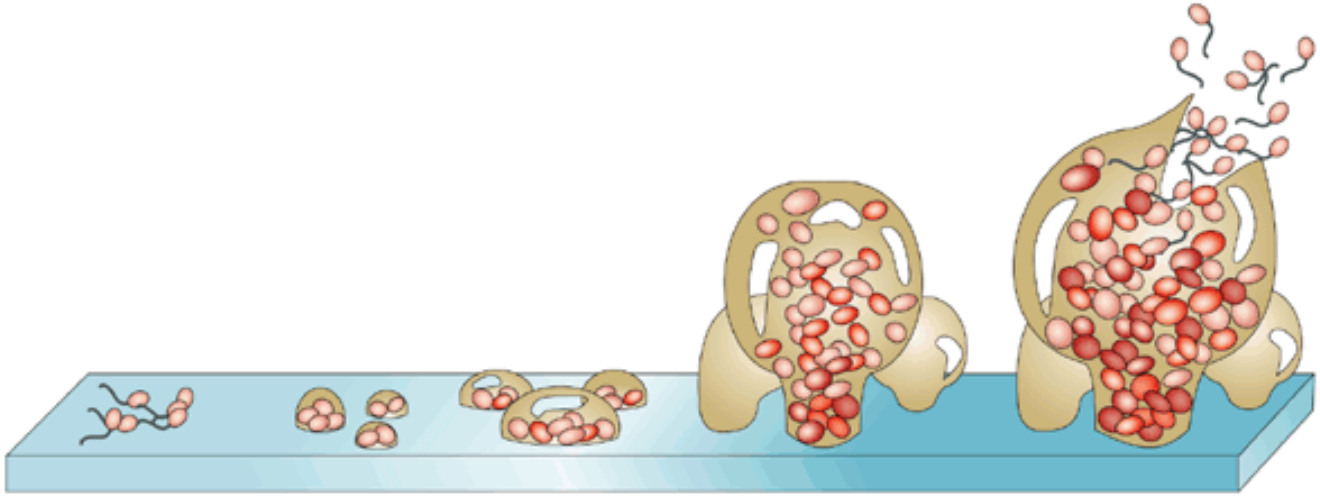
- Block surface adhesion
- Promote cellular dispersion

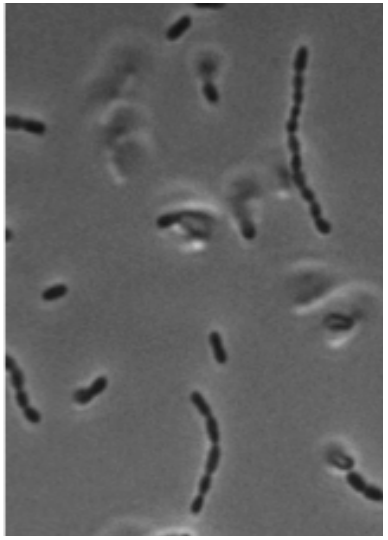
MUCUS BARRIER



Cavity Formation

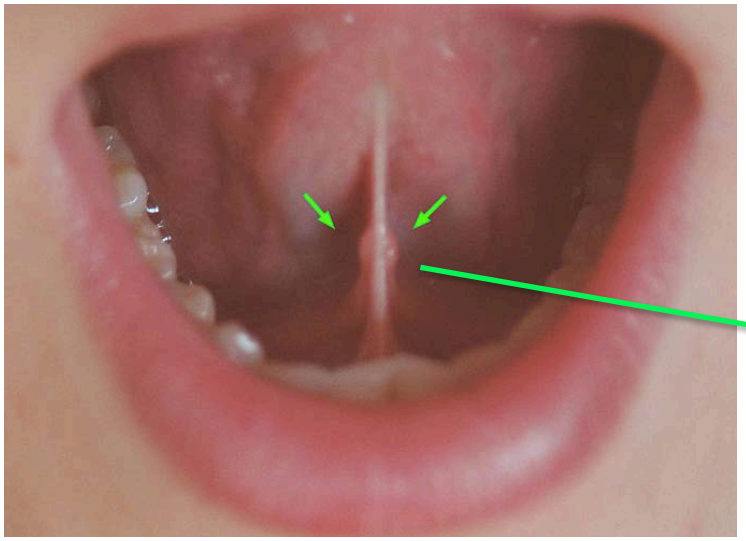
- Main culprit: *Streptococcus mutans*
  - Sugar metabolism → glucans → biofilm
  - Lactic acid production
- Biofilm formation = Plaque



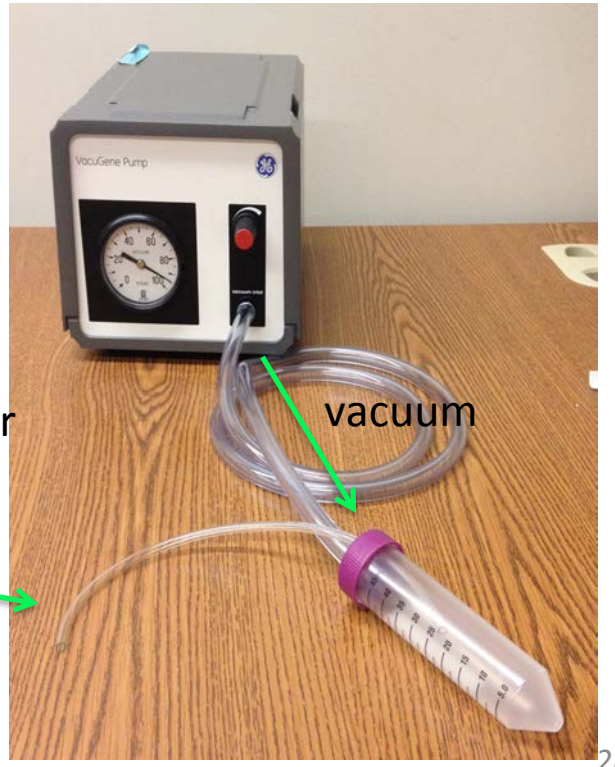


Saliva Collection

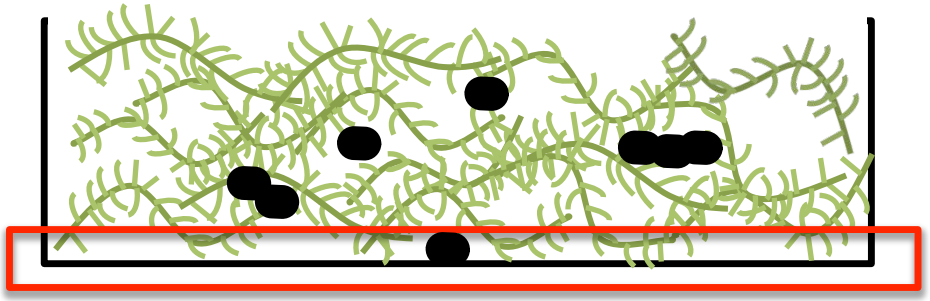
- MUC5B: Found in the mouth and respiratory tract
- Produced in the submandibular and sublingual glands
- Volunteers



Submandibular saliva

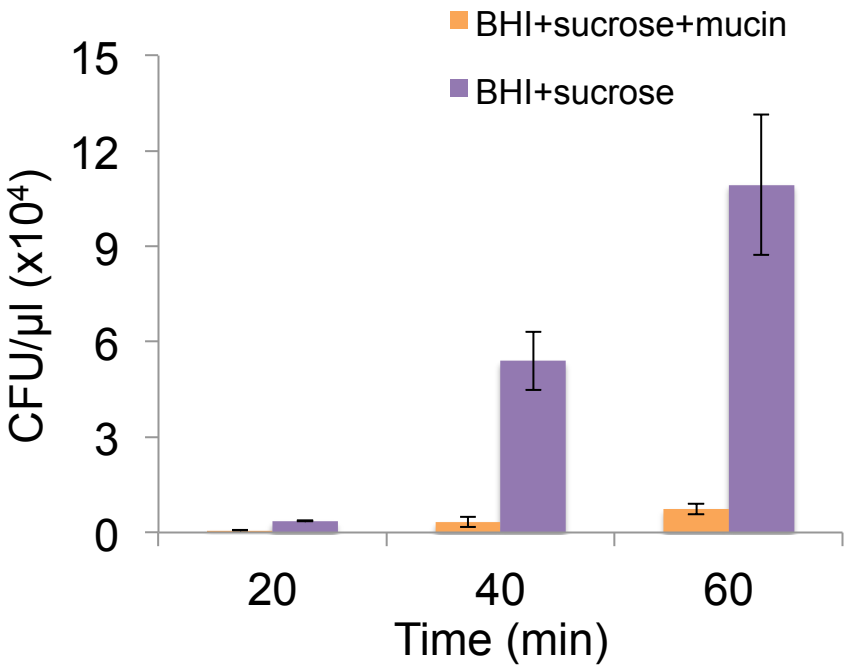


Erica Shapiro

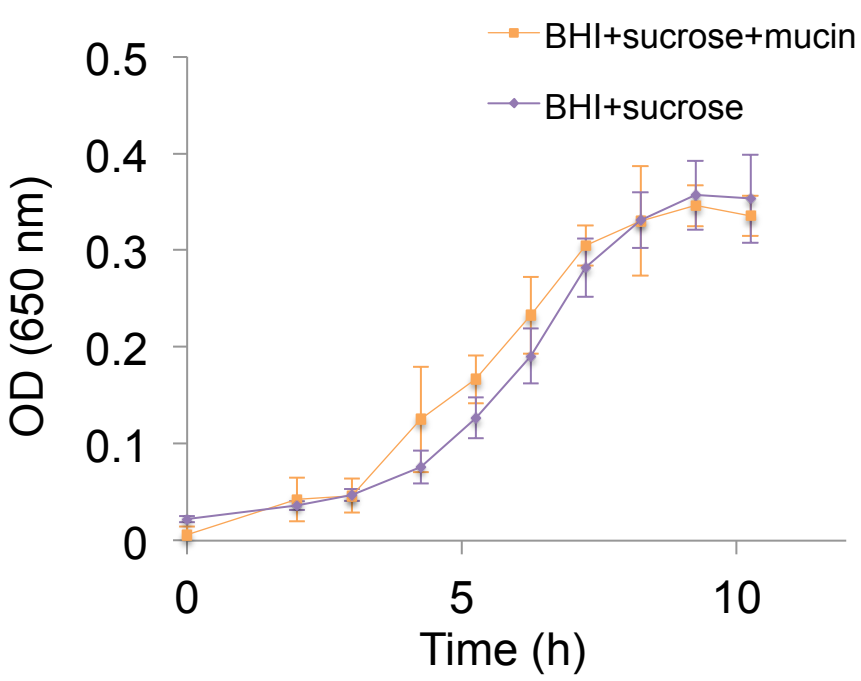


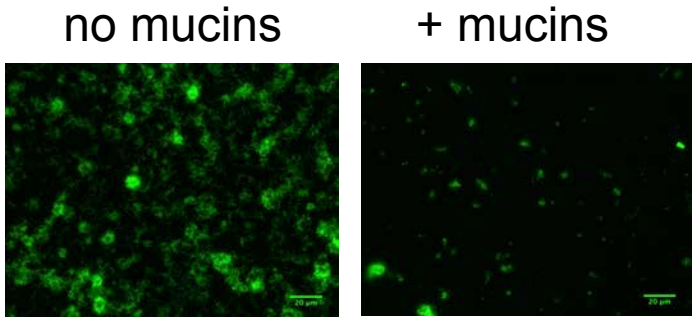
Mucins reduce surface adhesion of *S. mutans*

Surface Attachment

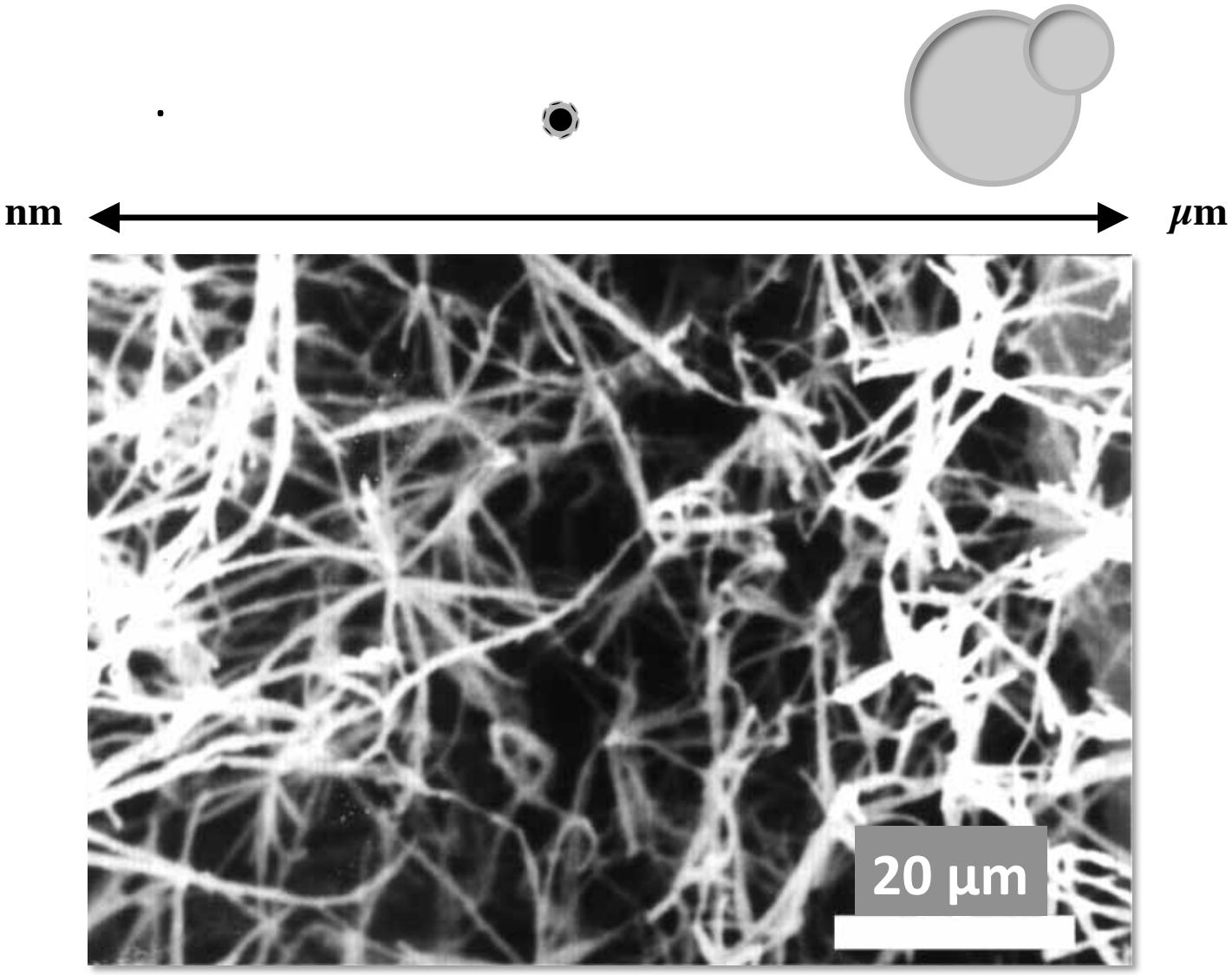


Growth





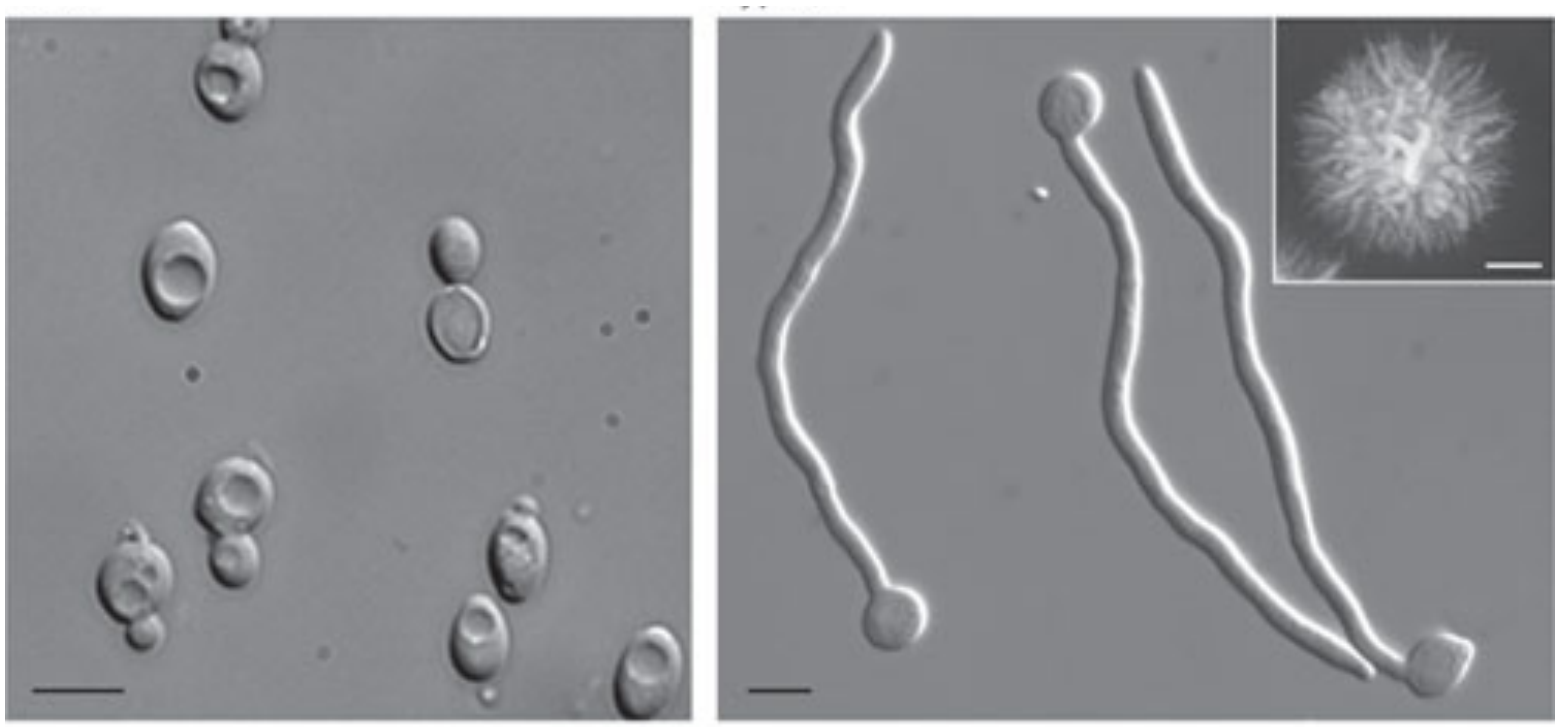
Frenkel 2015


nm
μm
20 μm

Candida albicans Morphology

Yeast

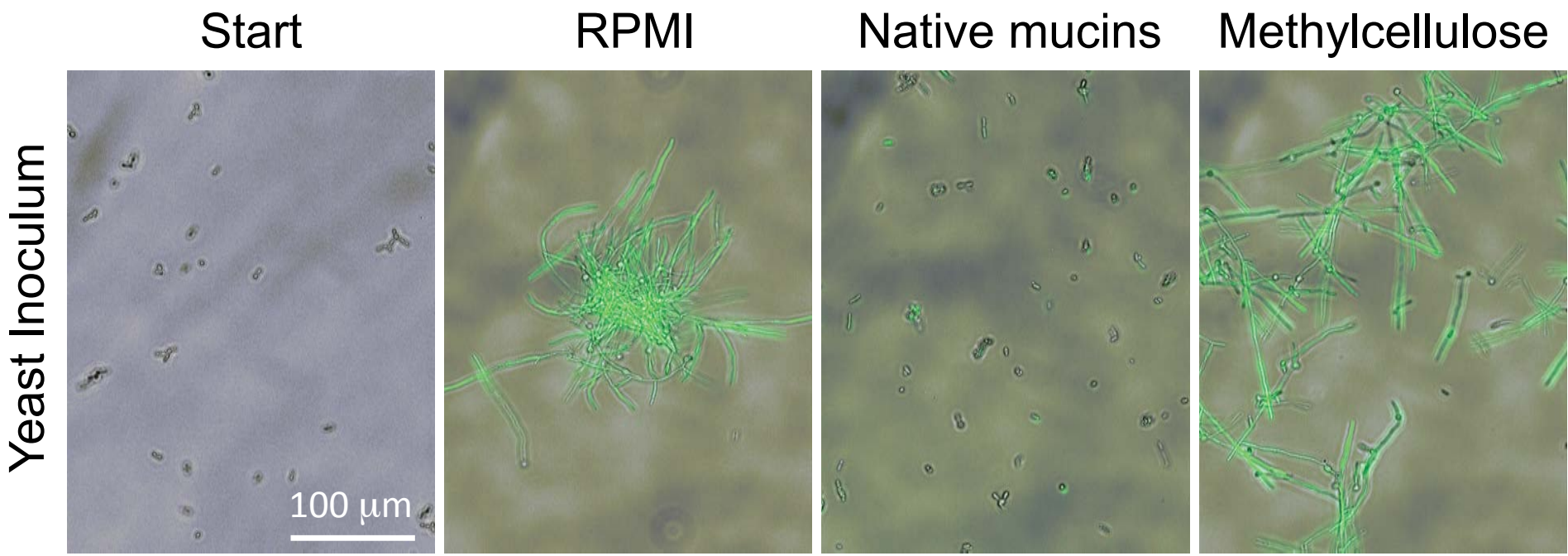
Hyphae

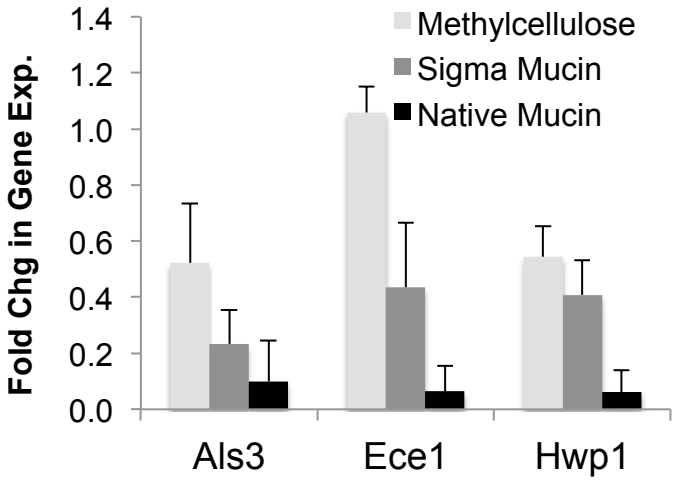


Nature Reviews | Microbiology

Adapted from: Sudbery 2011

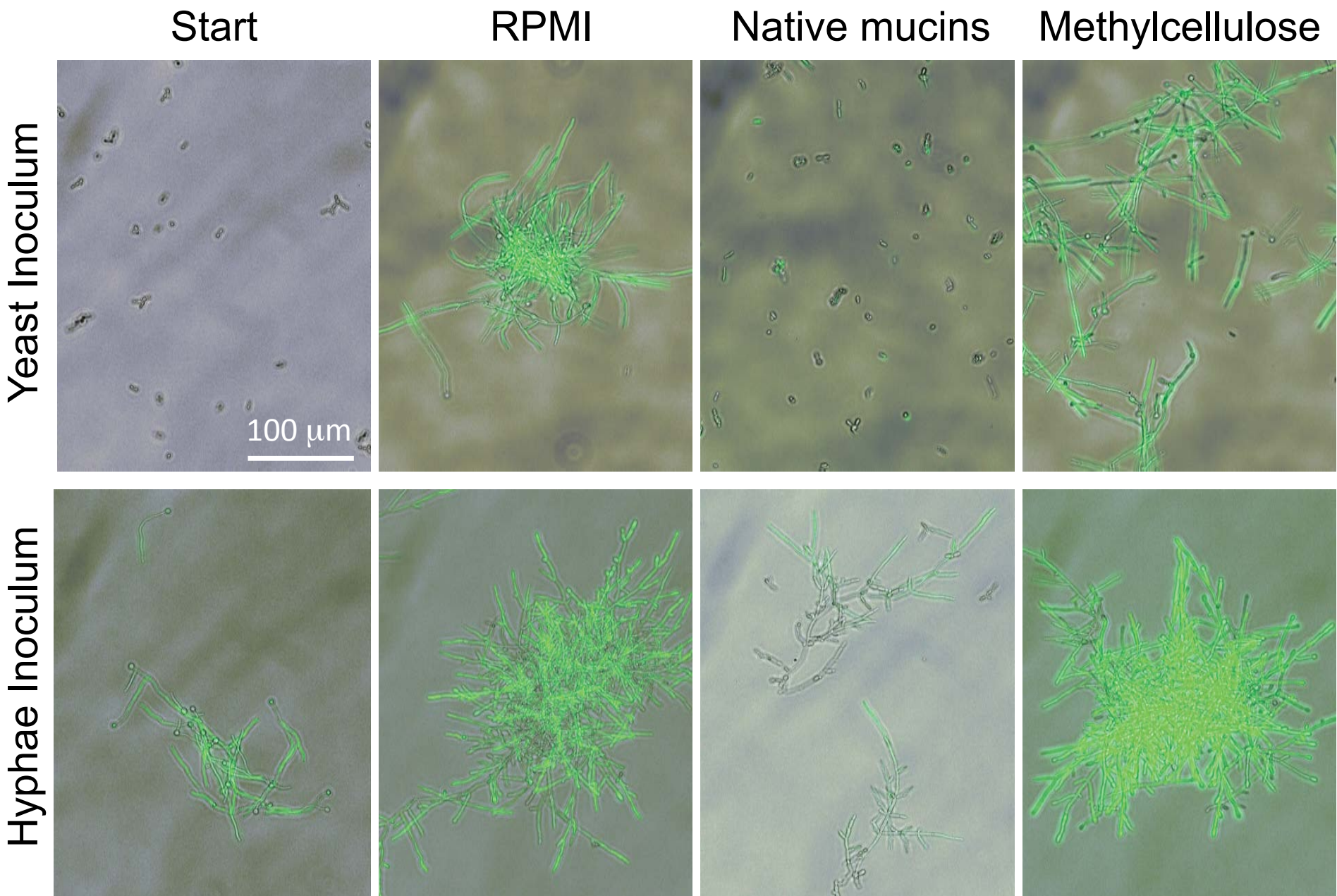
Mucins suppress the yeast-to-hyphae transition:





Kavanaugh et al., 2014

Mucins suppress the yeast-to-hyphae transition:

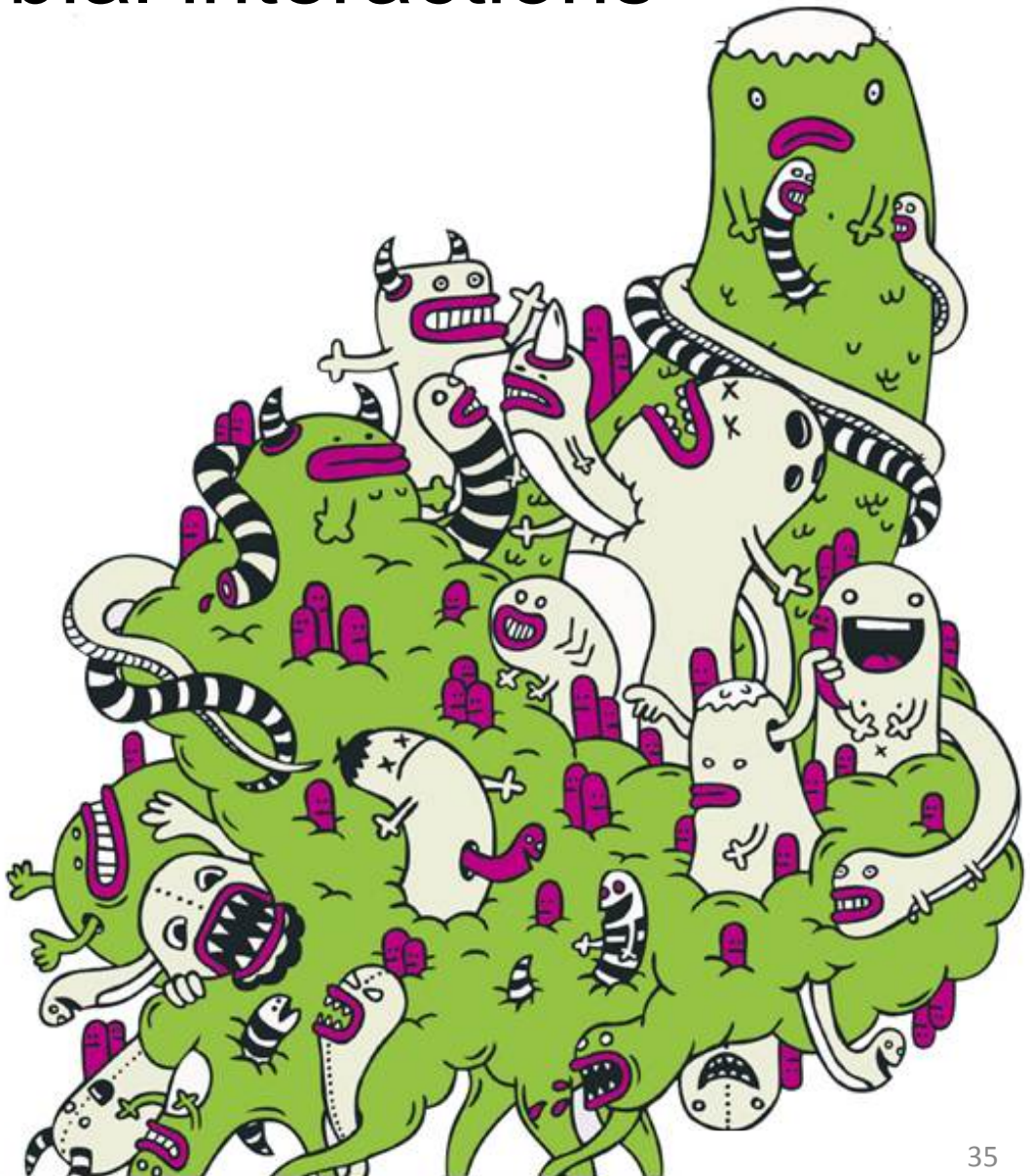


Mucus is location, and battleground, of our microbial interactions

Mucins suppress a range of virulence traits in microbes

Microbes are “disarmed”, not killed

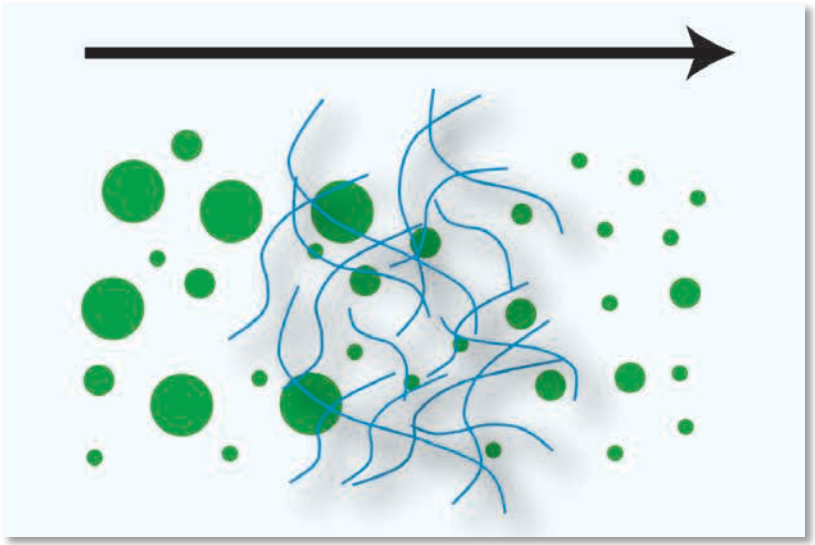
Drives microbial diversity by decreasing interspecies competition



Frankfurter Allgemeine Sonntagszeitung

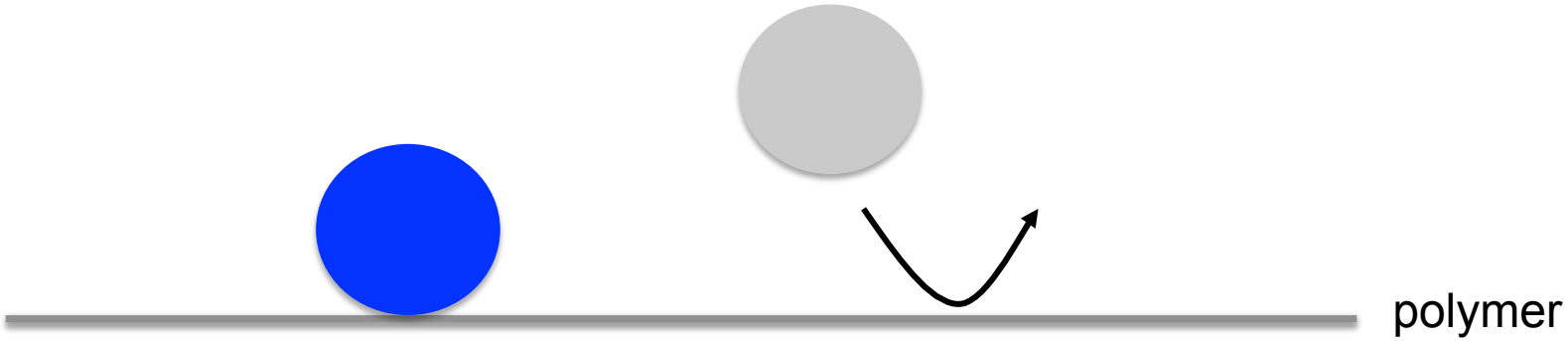
What makes a hydrogel selective?

Pore size

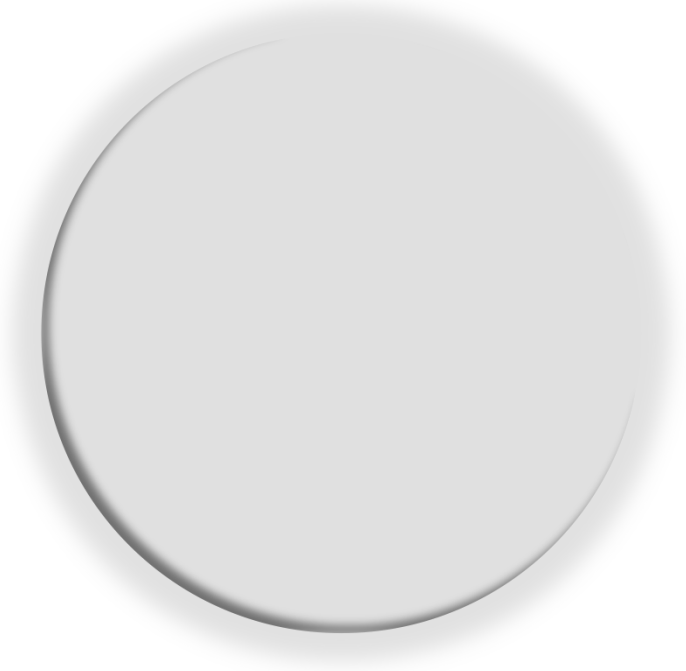


Lieleg 2011

What makes a hydrogel selective?



Nanoparticles with uniform surface chemistry



Cone 2009

Which mechanisms determine particle interactions with mucin polymers?

Which mechanisms determine particle interactions with mucin polymers?

Problems:

Substrates with defined spatial biochemical heterogeneity

Need to quantitate transport of nanoscale probes through mucus

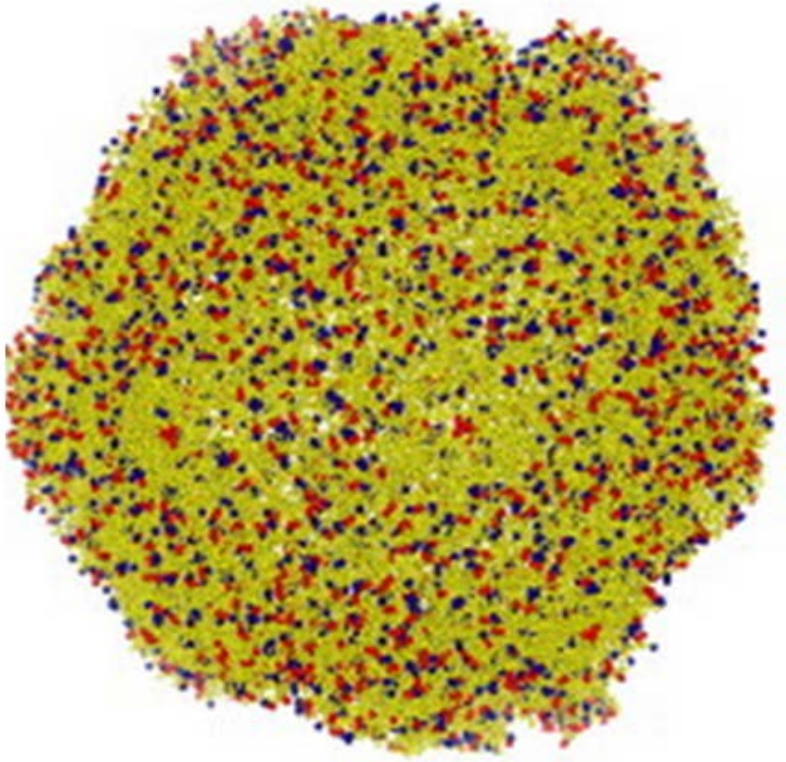
Competition of various substrates in mucus for mucin-binding sites

Peptide nanoprobes to identify mucus selectivity

Cationic peptide. Net charge = +8

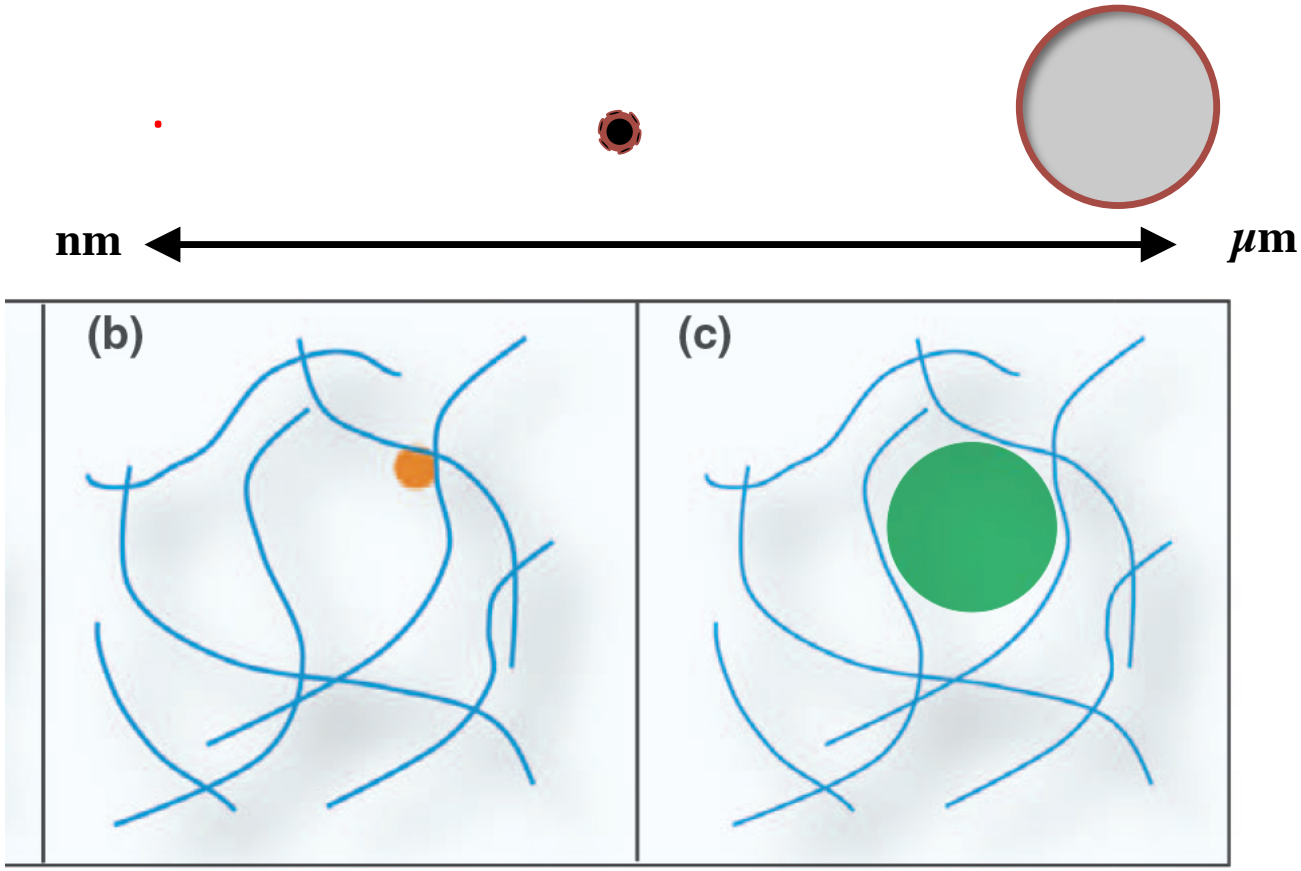
Anionic peptide. Net charge = -12

3 nm



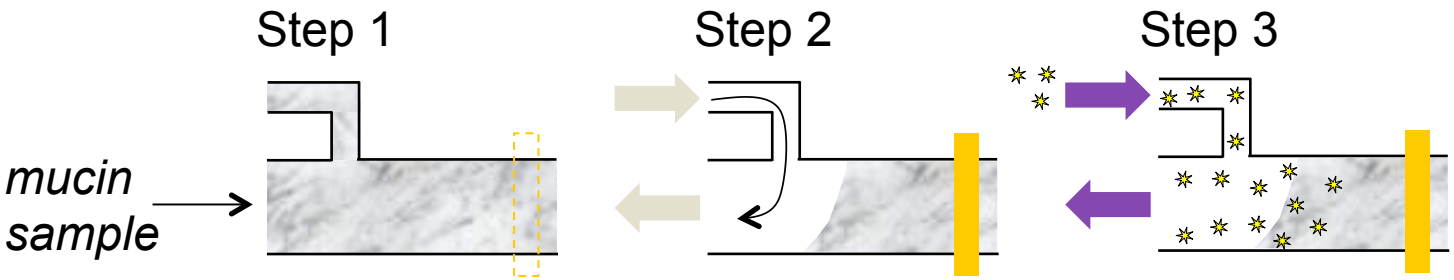
10 nm

Cone 2009



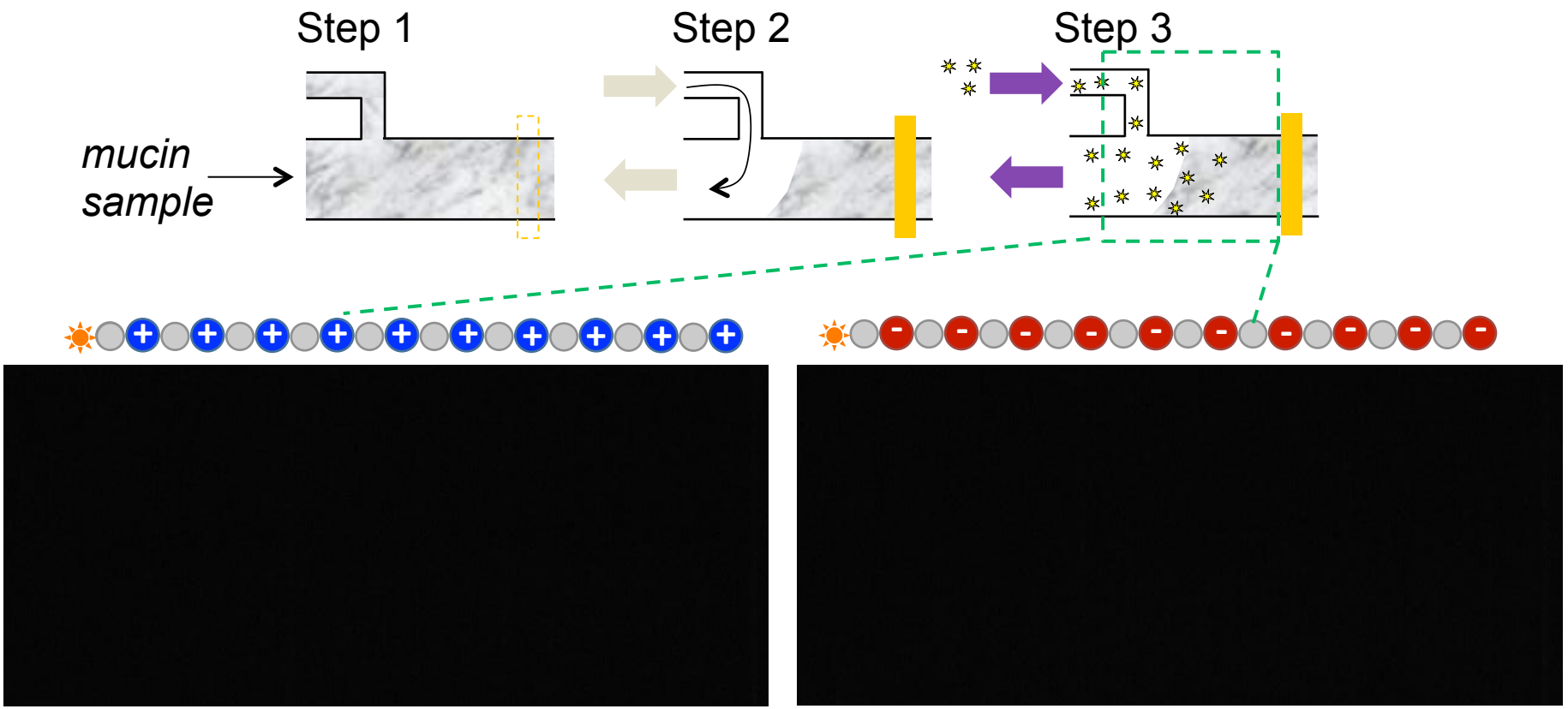
Lieleg 2011

A microfluidic system to measure transport through mucus



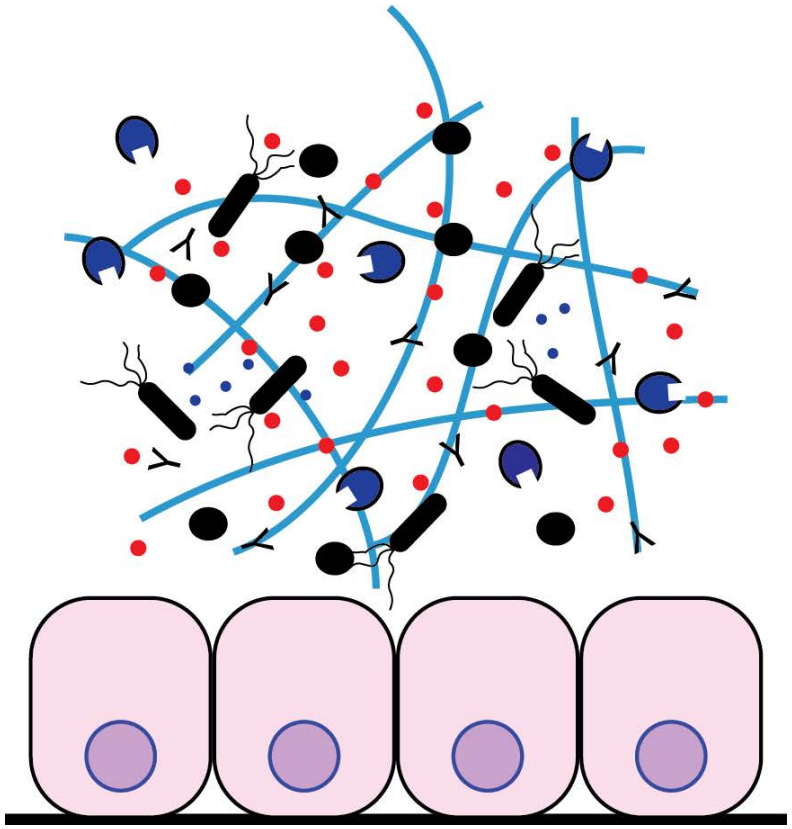
Li 2012, 2013

A microfluidic system to measure transport through mucus



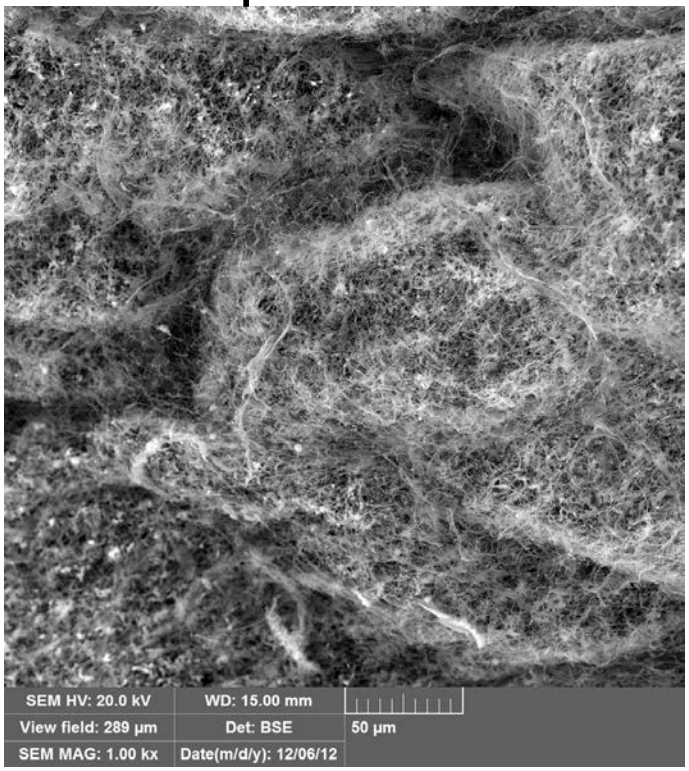
Li 2012, 2013
Kate Dupont

Native mucus

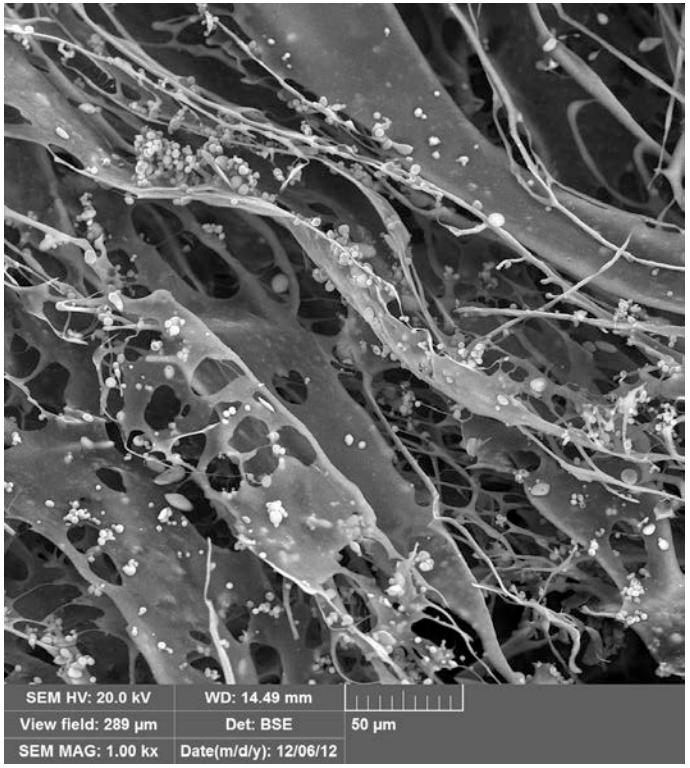


Native purification of mucins is crucial for their functionality

Native purified mucins



Industrial mucins

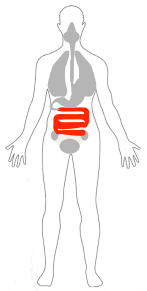


Nicole Kavanaugh

Mucin polymers govern the permeability properties of native mucus

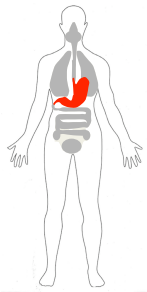
Analyte: ⊕

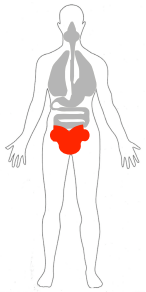
Analyte: ⊖



Native Mucus

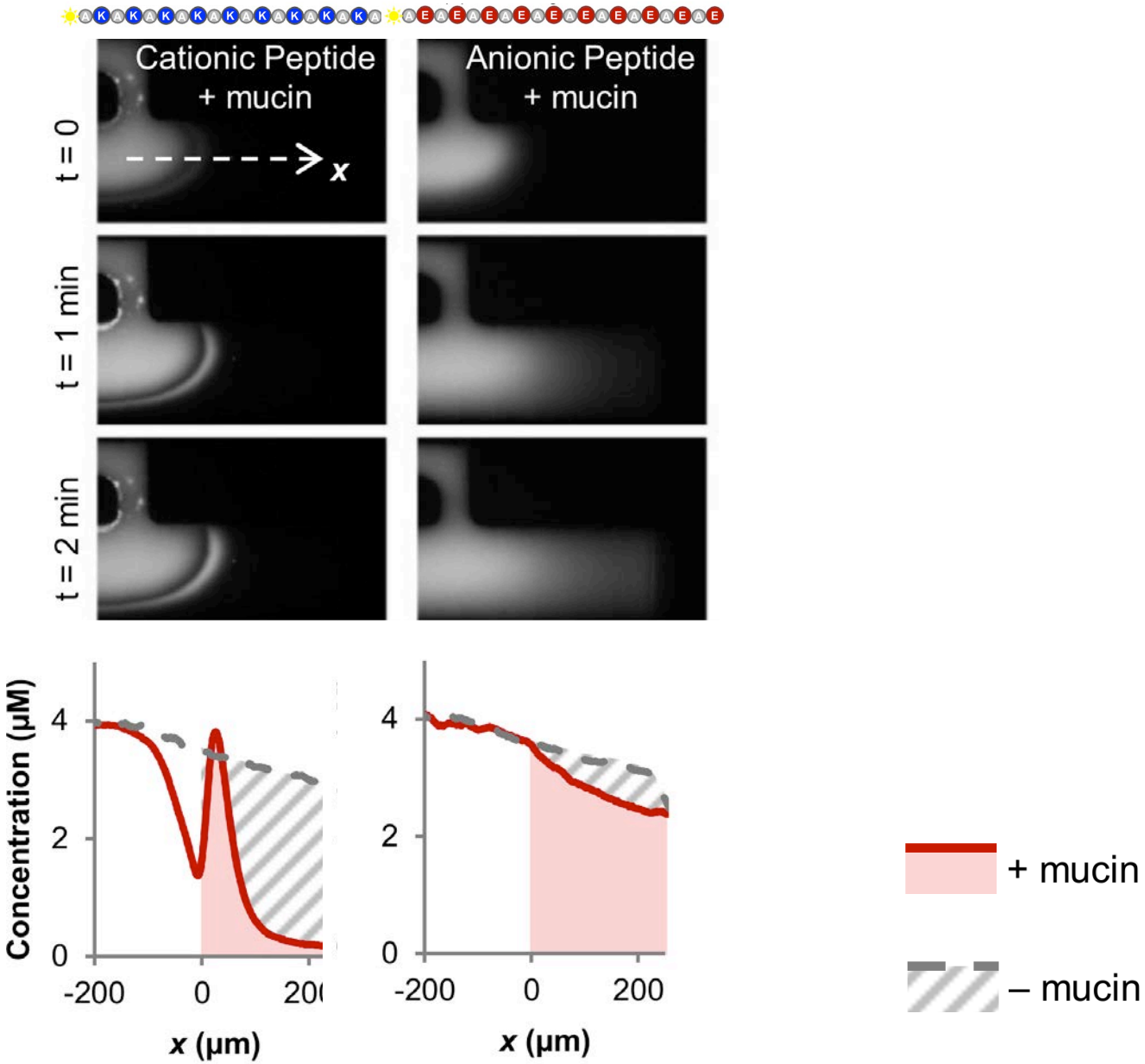
Intestine





Kate Dupont

Mucins form a charge-selective permeability barrier even to very small peptides



Li 2013

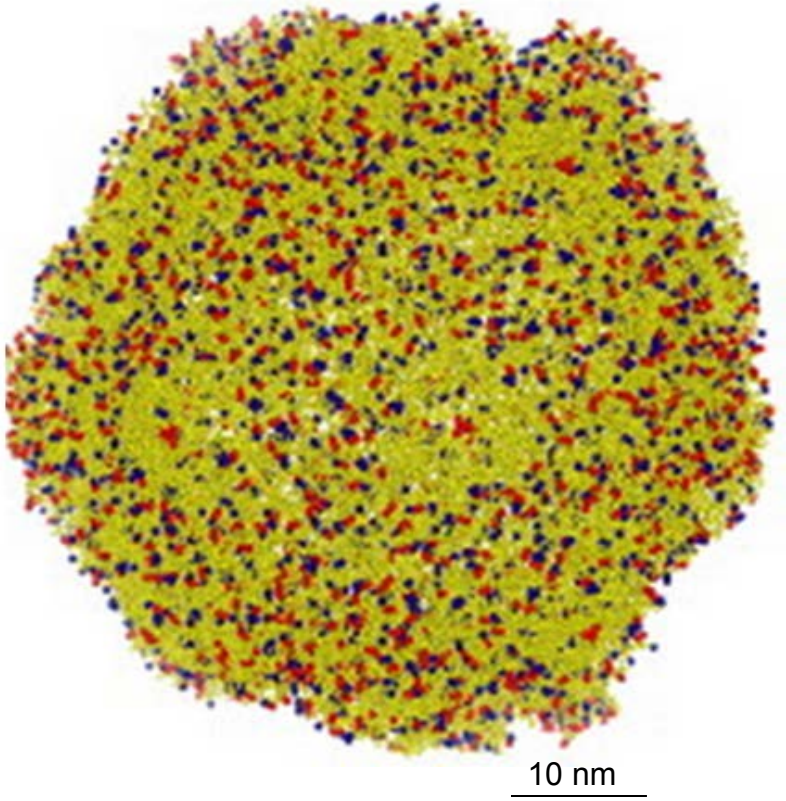
Peptide nanoprobes to identify selection criteria for transport through mucus

Cationic peptide. Net charge = +8

Anionic peptide. Net charge = -12

Same net charge, different spatial distribution:

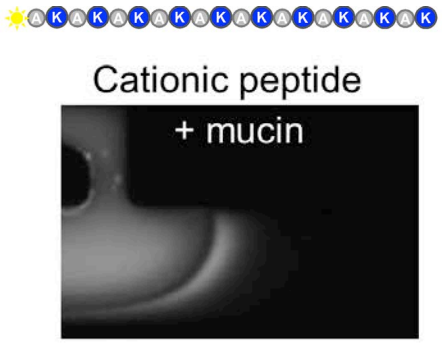
Block peptide. Net charge = -2



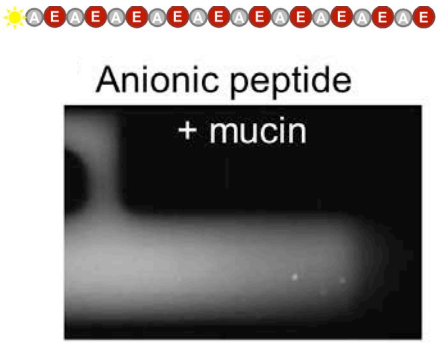
3 nm

Cone 2009

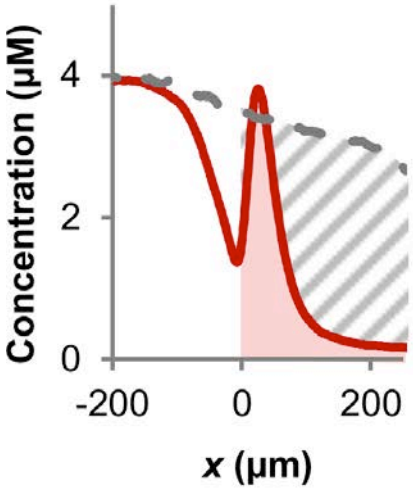
?

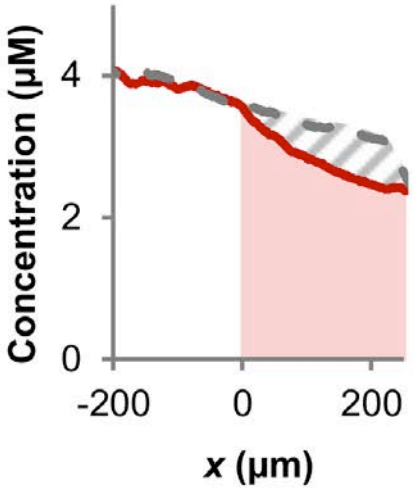


Cationic peptide



Anionic peptide



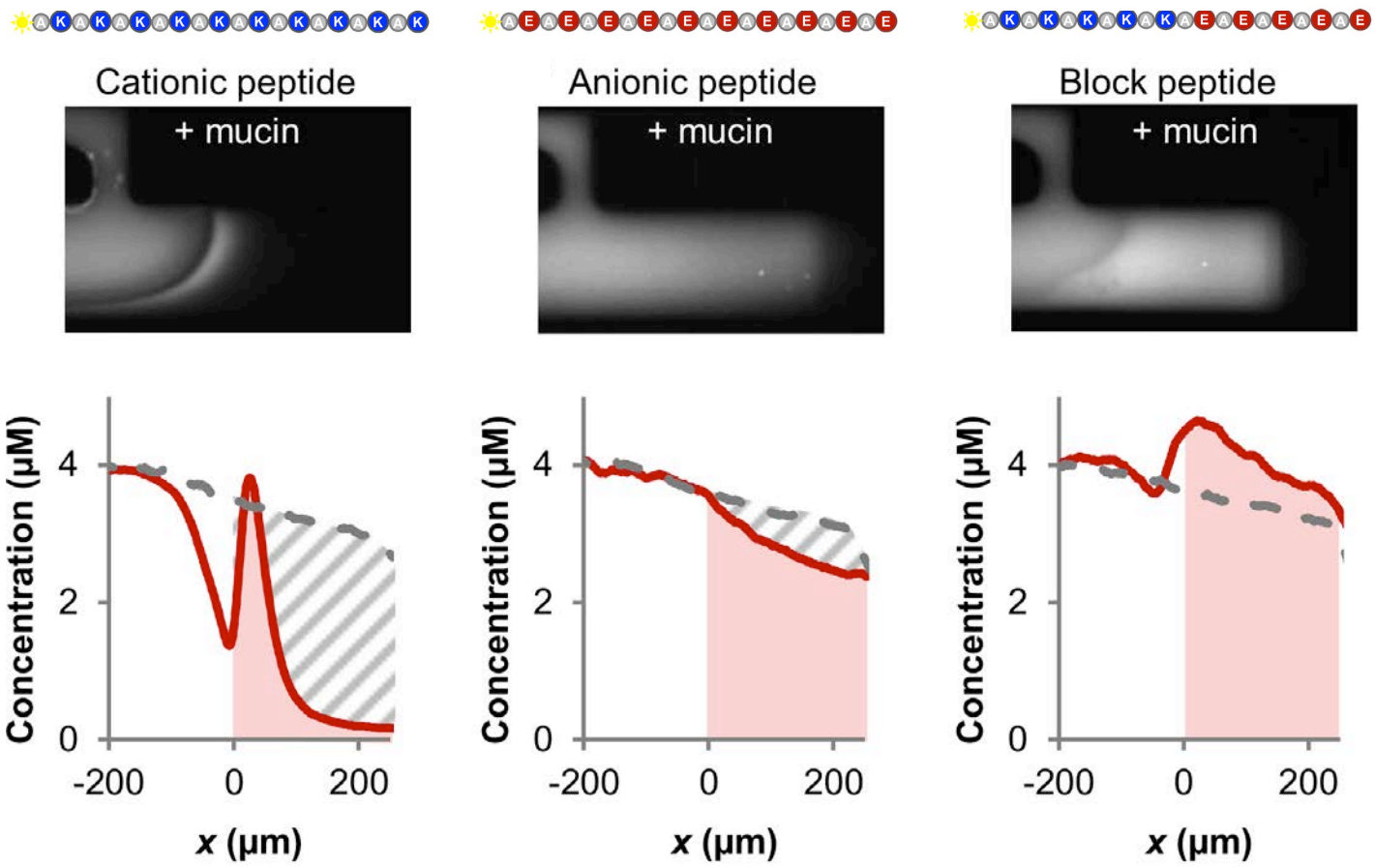


+ mucin

– mucin

Li 2013

A combination of negative and positive surface charge results in enhanced peptide transport



Li 2013

Peptide nanoprobes to identify selection criteria for transport through mucus

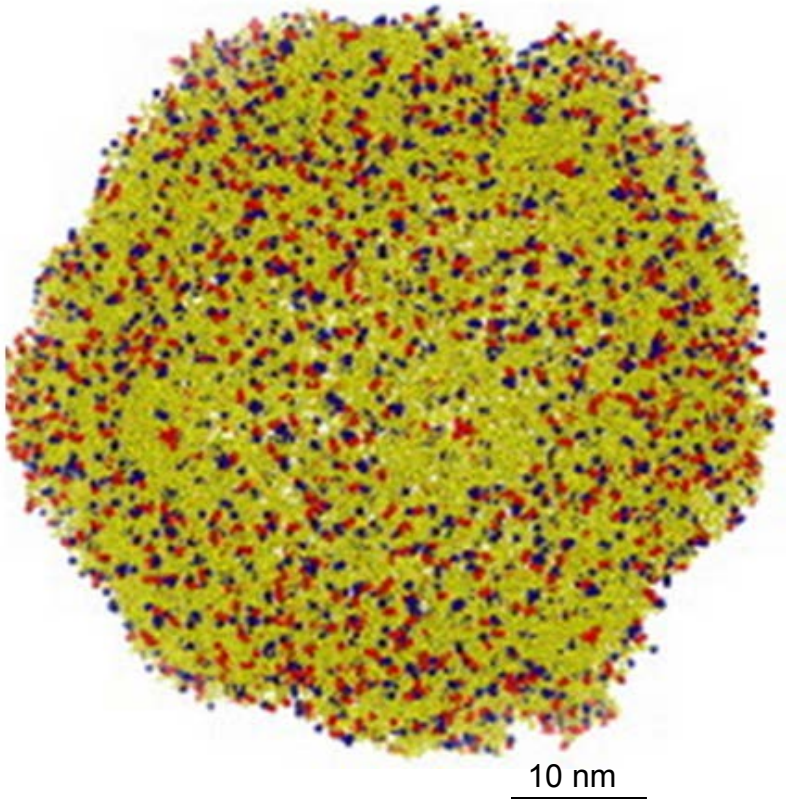
Cationic peptide. Net charge = +8

Anionic peptide. Net charge = -12

Same net charge, different spatial distribution:

Block peptide. Net charge = -2

Alternate peptide. Net charge = -2

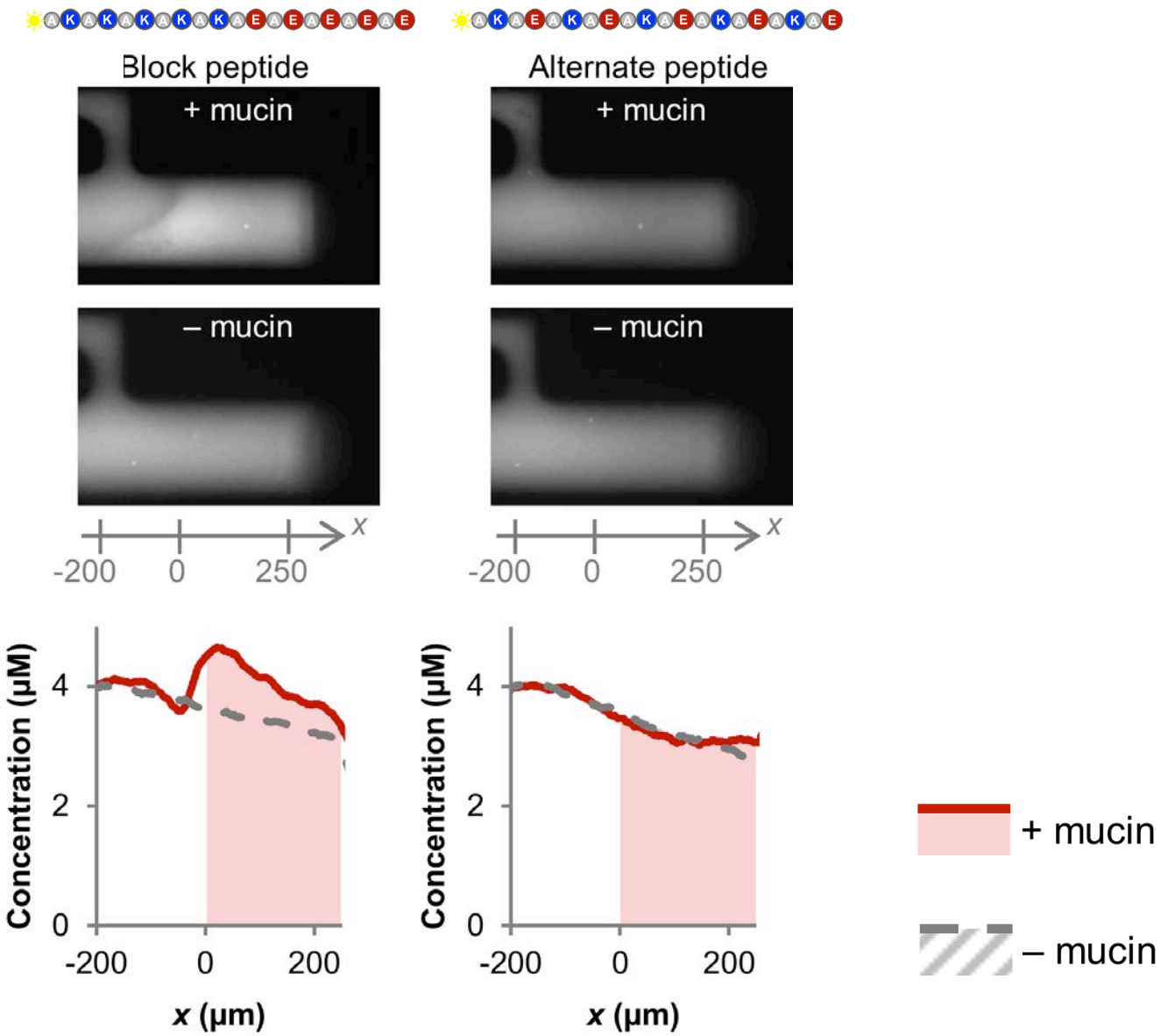


Li 2013

3 nm

Cone 2009

Spatial charge distribution influences peptide transport



Li 2013

Preterm birth

nature
International weekly journal of science

NATURE | NEWS

Pre-term births on the rise

But simple measures could cut the mortality rate of premature babies in poorer countries.

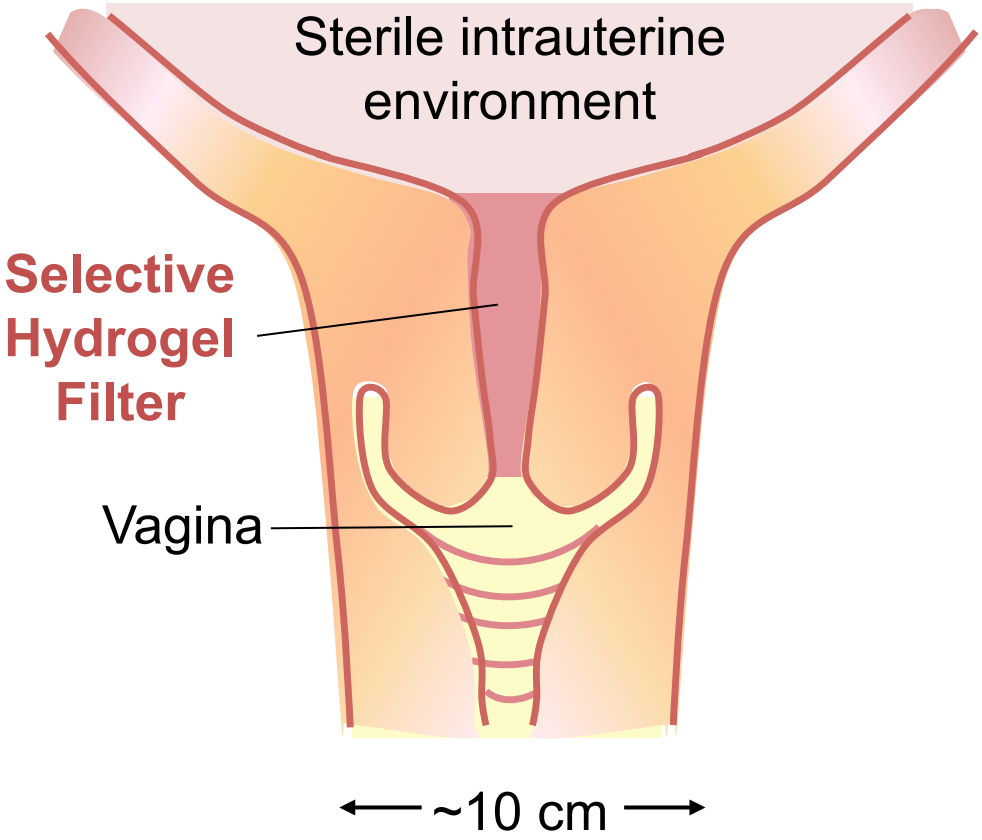
Eugenie Samuel Reich

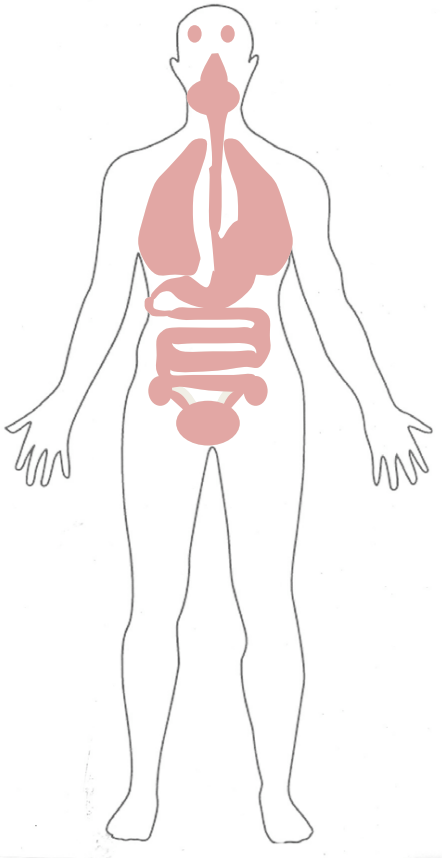
02 May 2012

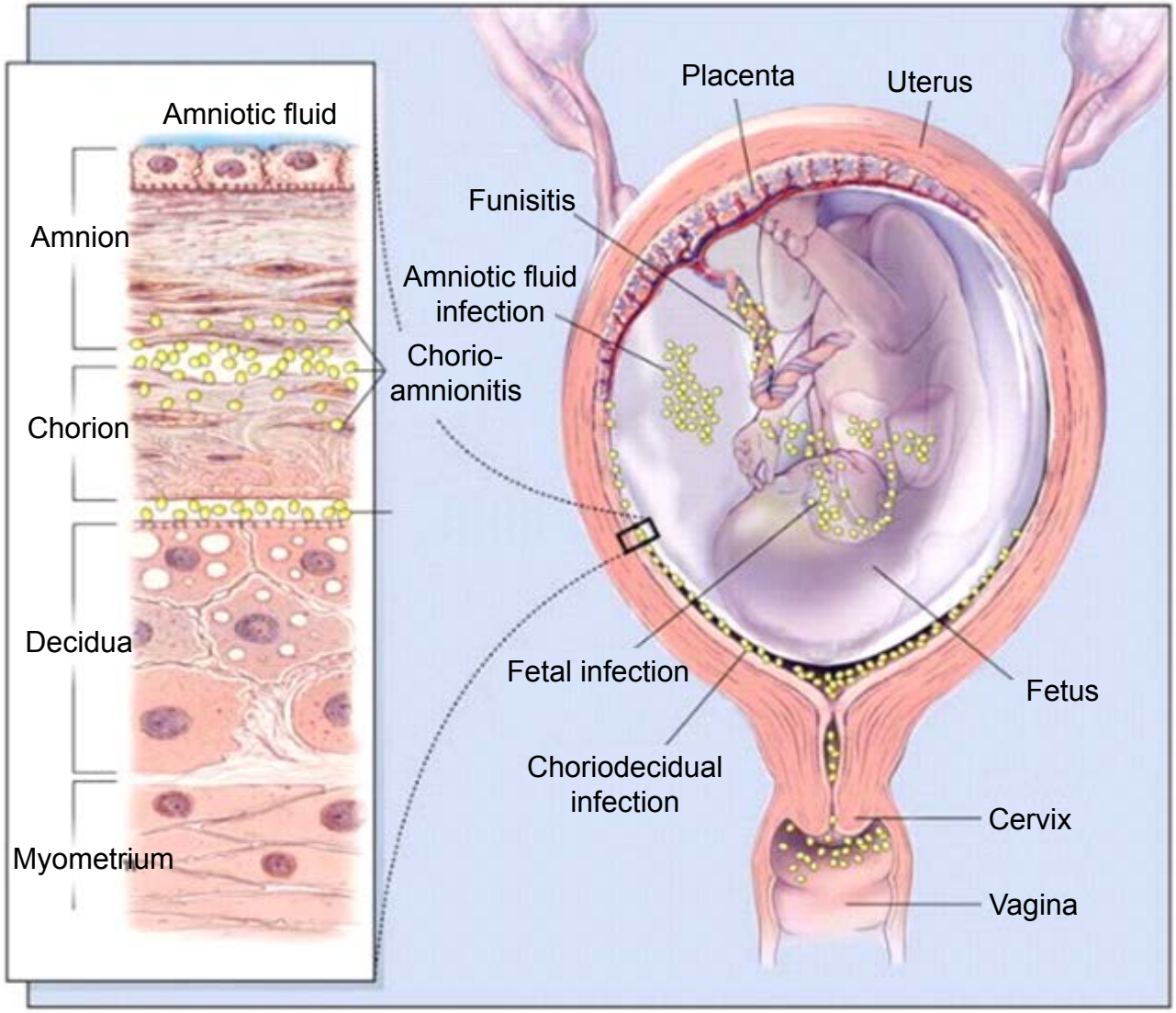
Little Shemeririwe is just one of an increasing number of babies being born prematurely.

Affects ~12% of pregnancies, often leading to infant death and illness

Theory:
uterine infection caused by ascension of vaginal bacteria



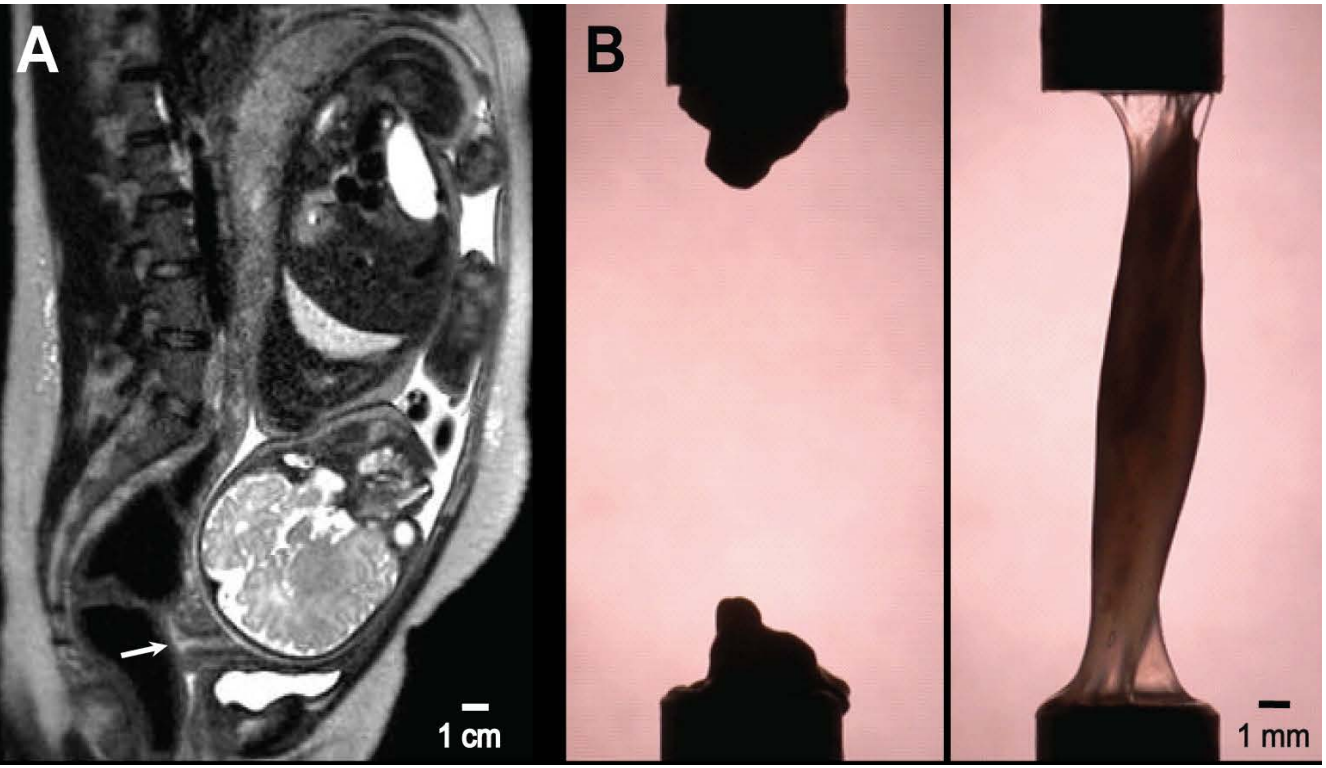



Amniotic fluid
Amnion
Chorion
Decidua
Myometrium
Placenta
Uterus
Funisitis
Amniotic fluid infection
Chorio-amnionitis
Fetal infection
Choriodecidual infection
Fetus
Cervix
Vagina

Measure mechanical properties of cervical mucus: Capillary Breakup Extensional Rheometer (CaBER)

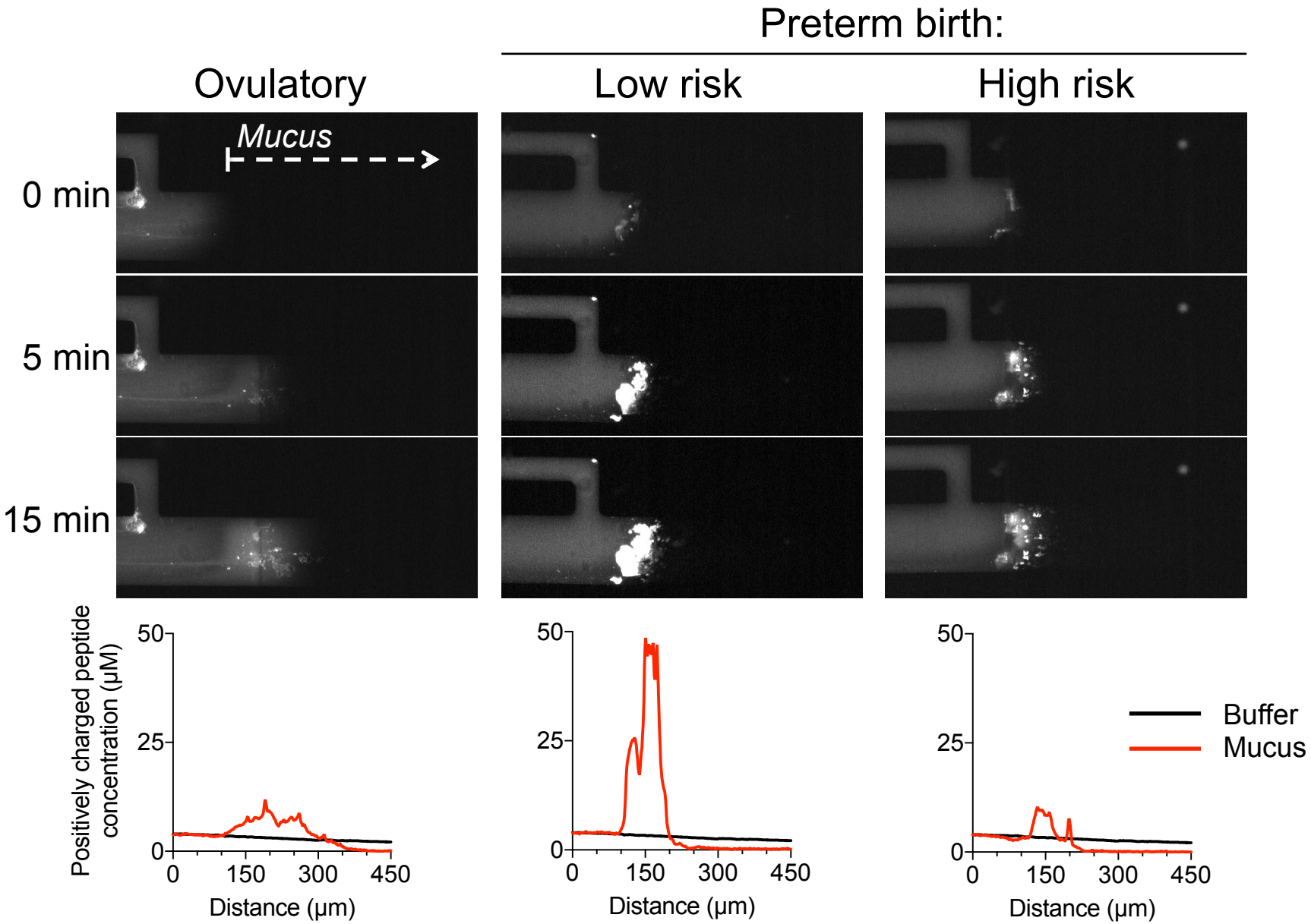
Preterm birth:

low risk high risk



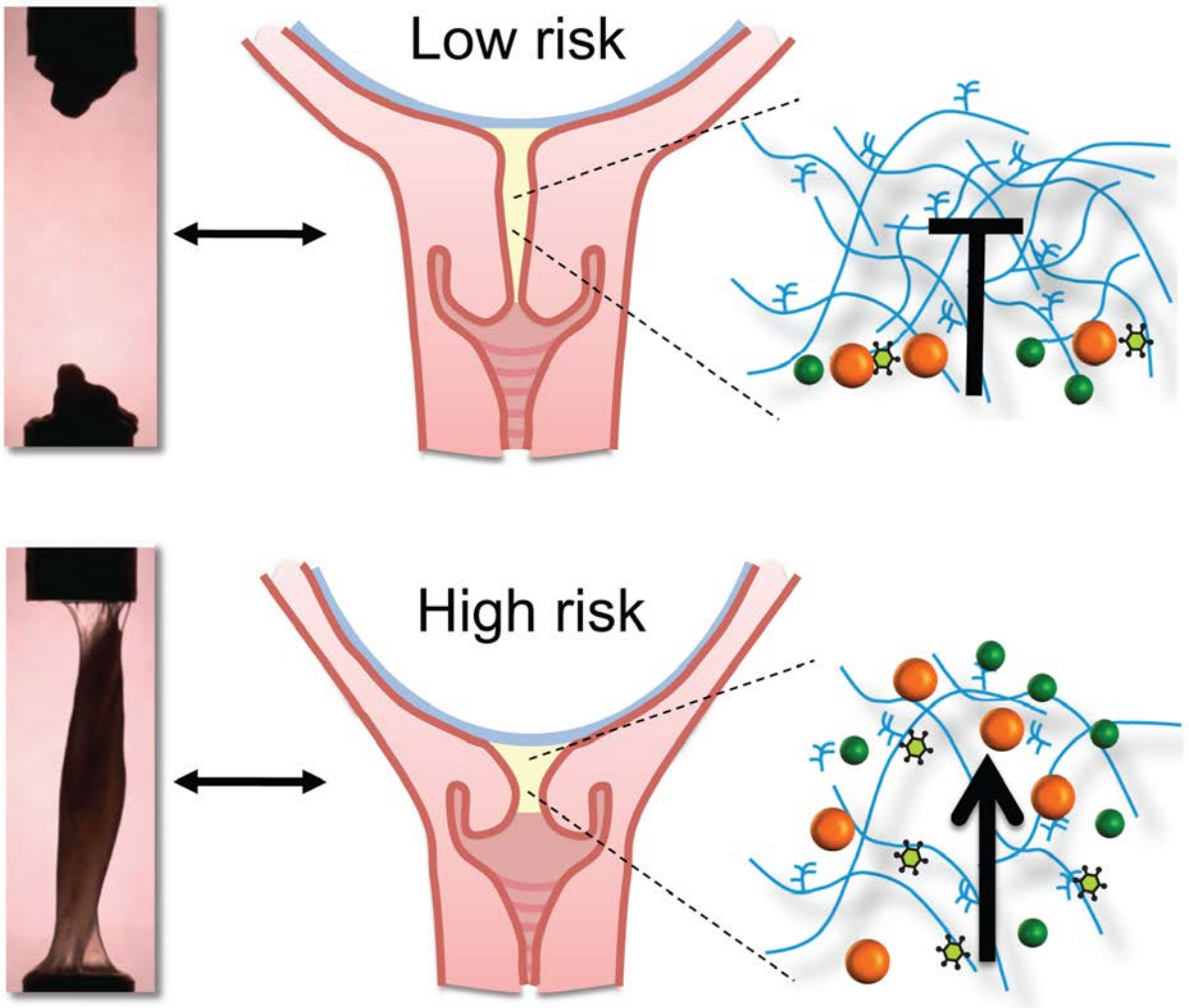
Critchfield 2013

Cervical mucus permeability stratifies risk for preterm birth



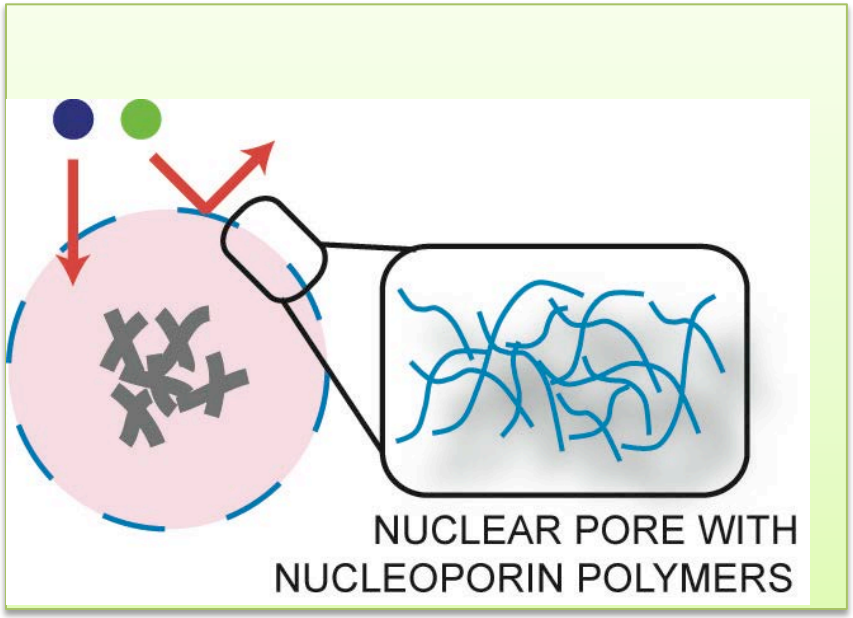
Kathryn Blakey Dupont

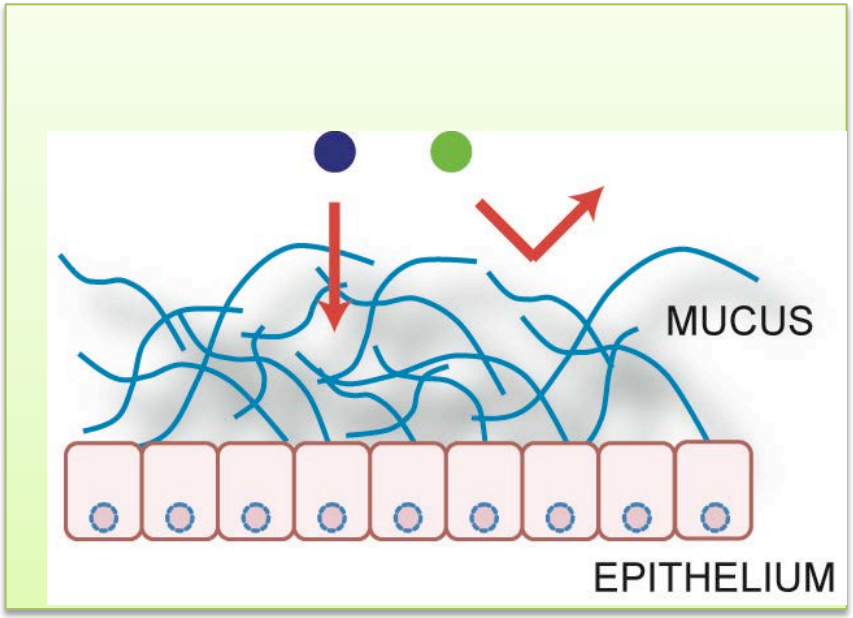
Biophysical properties of cervical mucus stratify risk for preterm birth

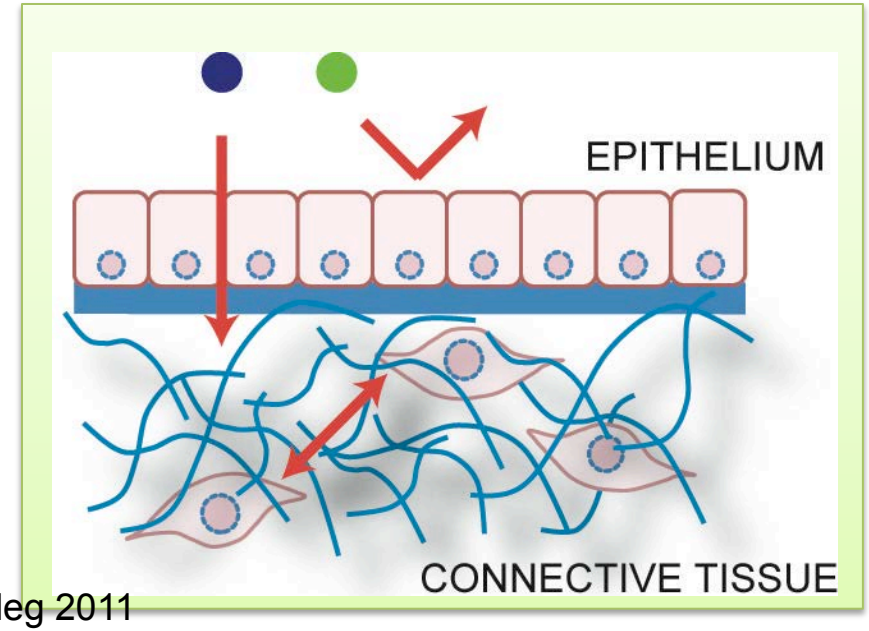


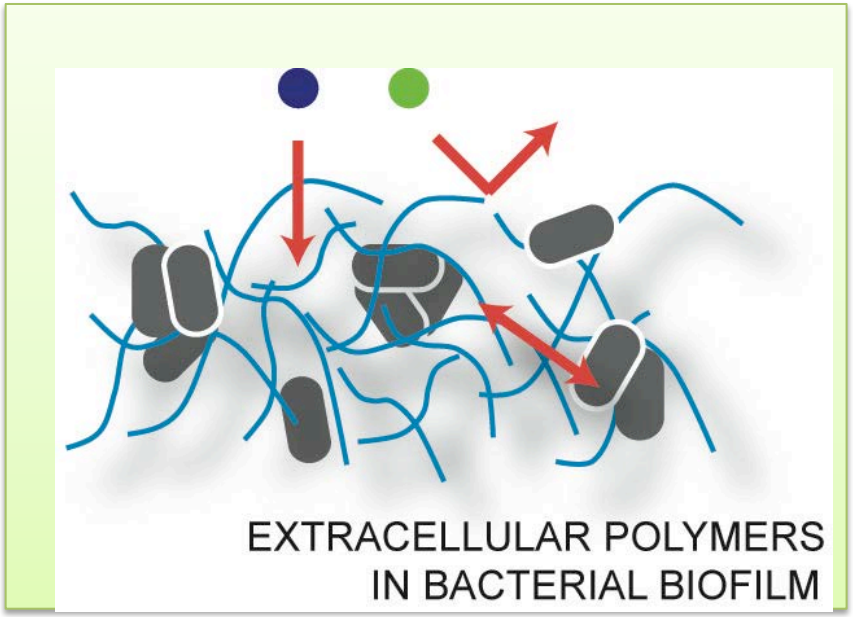
Critchfield 2013

Hydrogels are an integral part of biology









Lieleg 2011

Funding

BURROUGHS WELLCOME FUND
Investigator Award Preterm Birth Initiative

CAREER Award

R01EB017755

DTRA 1-13-1-0038

NIGMS

NATIONAL SCIENCE FOUNDATION MRSEC MATERIALS RESEARCH SCIENCE & ENGINEERING CENTERS

MIT CEHS

The Charles E. Reed Fund

DESHPANDE CENTER FOR TECHNOLOGICAL INNOVATION

Collaborators

MIT:

Doug Lauffenburger
Alan Grodzinsky
Jay Han
Darrell Irvine
Forest White
Linda Griffith
John Essigmann
Leona Samson
Scott Manalis
Nancy Hopkins
Michael Rubner
Robert Cohen
Gareth McKinley
Roman Stocker
Brad Olsen
Pat Doyle
Tim Lu
Paula Hammond
Niels Holten-Andersen
Alfredo Alexander-Katz
Yoel Fink

Joanna Aizenberg (Harvard University)
Kevin Foster (Oxford University)
Alexander Johnson (UCSF)
Clarissa Nobile (UC Merced)
Justin Hanes (Johns Hopkins Medicine)
Michael Rubinstein (UNC Chapel Hill)
Kelly Ten Hagen (NIH Bethesda)
Lucy Colwell (Cambridge University, UK)
Michael House, Agatha Critchfield, Kelley Conroy (Tufts Medical Center)
K. Pagidas and Victoria Snegovskikh (Brown University)
Xiana Teimoy (Artist, Spain)

The team (2010 - present)

Biogel lab:

Nicole Kavanaugh
Julia Co
Marina Caldara
Oliver Lieleg
Corinna Lieleg
Regina Baumgaertel
Grace Yao
Ronn Friedlander
Ioana Vladescu
Leon Li
Irena Jevtov
Nicole Billings
Caroline Wagner
Brad Turner
Erica Shapiro
Tahoura Samad
Jacob Witten
Ben Wang
Kelsey Wheeler
Gerardo Carcamo Oyarce
Anna Burgos
Kowa Chen
Maria Ramirez
Alona Birjiniuk
Wesley Chen
Kathryn Dupont

Undergraduates:

Colin Beckwitt
Connor Duffy
Hannah Rudoltz
Sae Yang
Kristina Kim
Emily Bloch
Lauren Miller
Angela Zhang
Claudio Macias
Irena Cich
Edgar Matias
Denise Neibloom
Coleen Mehl