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STINT

The Swedish Foundation for International
Cooperation in Research and Higher Education



7th and Final Meeting

The interface between cells and biomaterials

1st July – 4th July 2018

Program

Travel to Vindeln on Sunday afternoon the 1st July 2018,

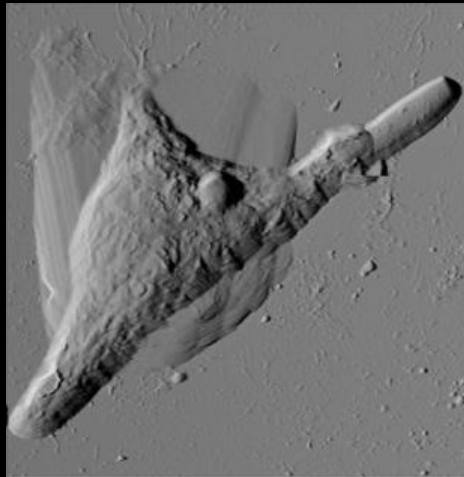
Arrival and check in

Time	Monday 2 nd July
7.30	Breakfast
9.00	Madeleine Ramstedt; Introduction + Bacterial interactions with surfaces
9.45	Cesar Rodriguez Emmenegger; adaptive Synthetic Macrophages
10.00	Discussions and coffee break
10:45	Kaline Rabelo Coutinho; Molecules interacting with phospholipids bilayers and solution
11:15	Discussions
11:45	Alexandre Macedo; Anti-infective molecules from natural sources
12:15	Discussions
12:30	Lunch
14:00	Sun Nyunt Wa,, Bacterial membrane vesicles and host interactions
14:30	Discussions
15:00	Bernt Eric Uhlin; Studies of <i>E. coli</i> adhesion: from molecular genetics to biomechanical properties
15:30	Discussions and coffee break
16:00	Discussion of joint projects and project ideas
19:00	Dinner
20:00	Evening activity

Time	Tuesday 3 rd July
7.30	Breakfast
9.00	Stefan Salentinig; Molecular Engineering for Antimicrobial Nanomaterials
9.30	Discussions
10.00	Fabienne Quilès; On the interaction of peptides with biological membranes: a physico-chemical study from model membranes to bacterial membranes
10:30	Discussions and coffee break
11:00	Sofiane El Kirat Chatel; Atomic force microscopy to decipher microbial adhesion
11:30	Discussions
12:00	Lunch
13:30	Thereza Soares; Molecular models of bacterial membranes and polymer brushes
14:00	Discussions and coffee
14:30	Julien Gautrot; Design of Biointerface Chemistry and Mechanics for Gene Delivery and Stem Cell expansion
15:00	Discussions Followed by discussions of possible joint projects, and ideas for future collaborations over coffee (15:30)
17:00	Dinner
19:00	Networking activity connected to the river
20:00	Discussions

Departure Wednesday the 4th July 2018 and travel to Umeå, visit to the university during Wednesday

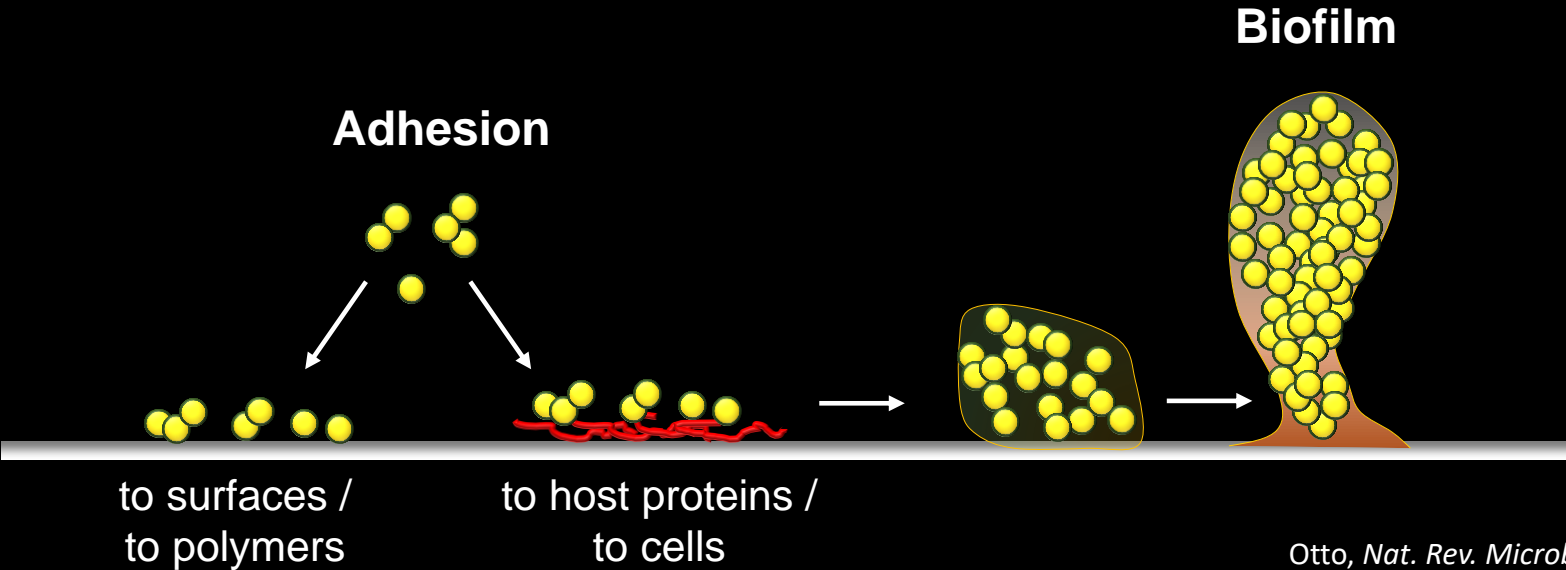
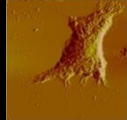
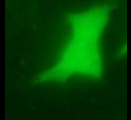
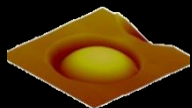
Atomic Force Microscopy to decipher microbial adhesion



Sofiane El-Kirat-Chatel

STINT “The interface between cells and biomaterials”
Vindeln, July 2018

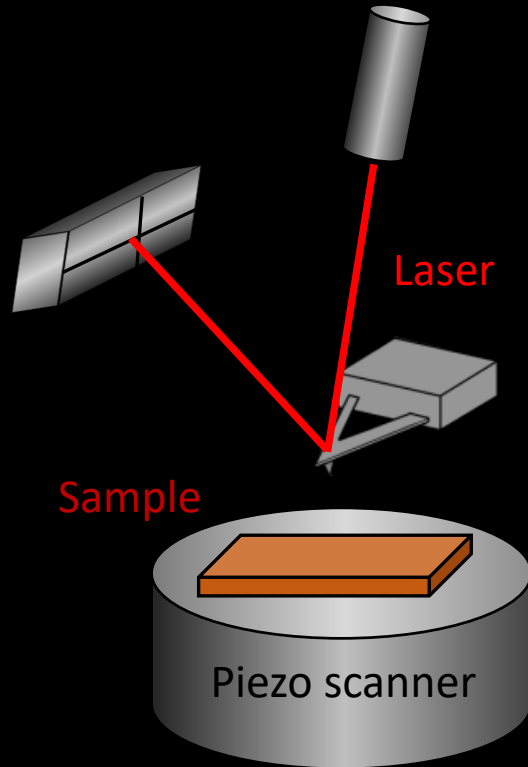
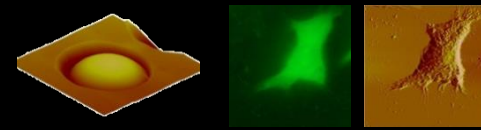
Biofilm and microbial adhesion



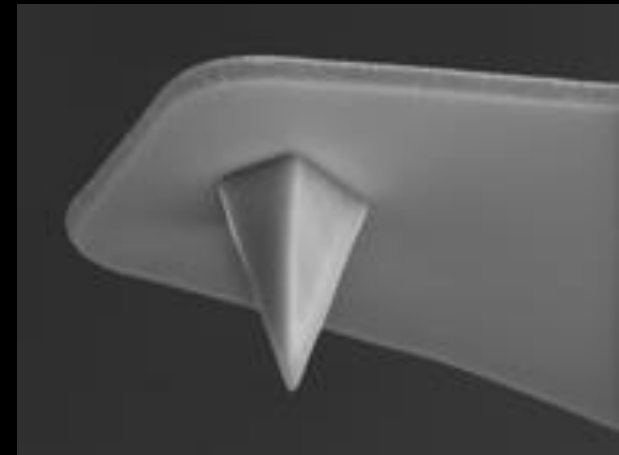
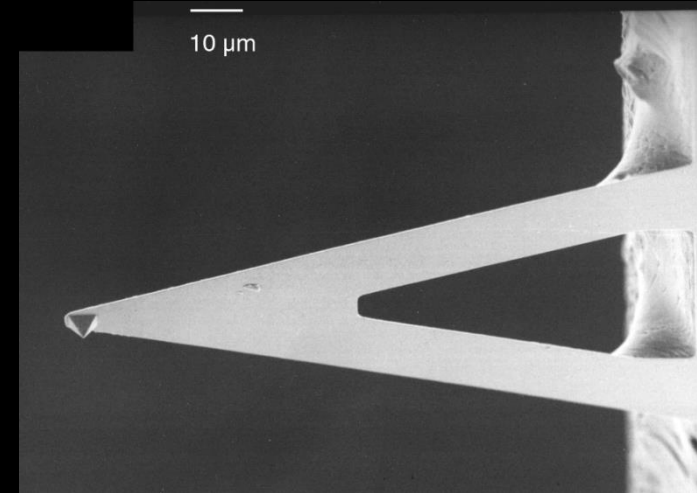
Otto, *Nat. Rev. Microbiol.* (2009)
Costerton, *Science* (1999)

- Adhesion:**
- Initial step of biofilm formation
 - Governed by cell surface

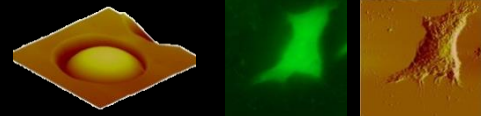
Atomic force microscopy (AFM)



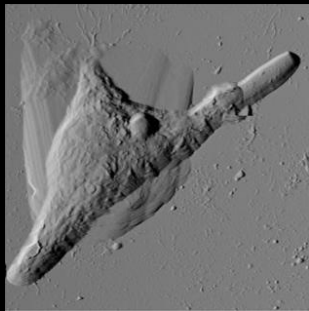
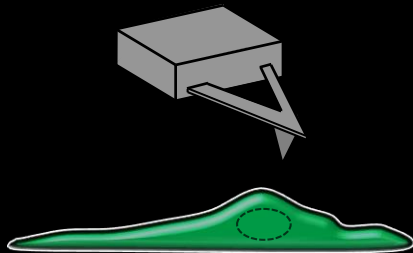
Binnig and Rohrer in the early 1980s to image the topography of surfaces.
(Nobel prize in 1986)



AFM: versatile platform for cell surface analysis

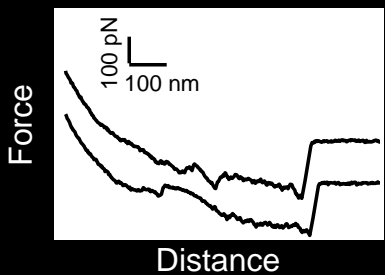
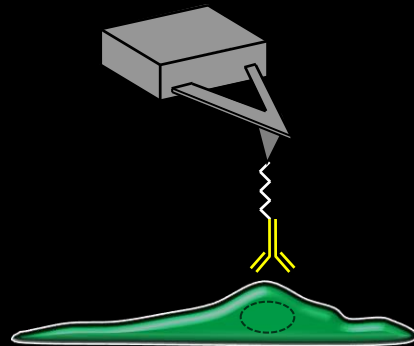


1. Surface imaging and mechanics



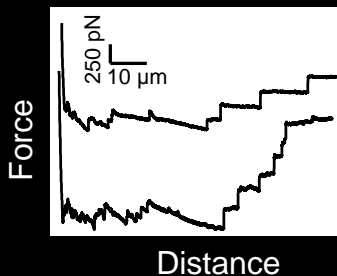
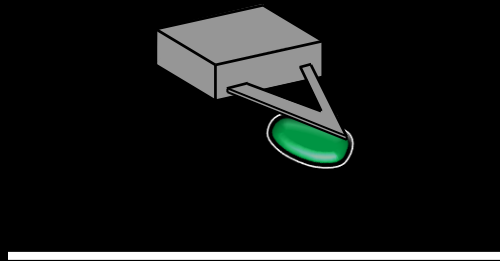
*Topography,
Elasticity*

2. Single molecule force spectroscopy



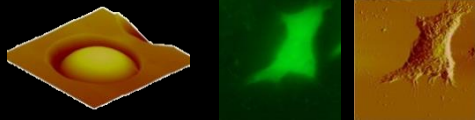
*Distribution,
Molecular mechanics*

3. Single cell force spectroscopy

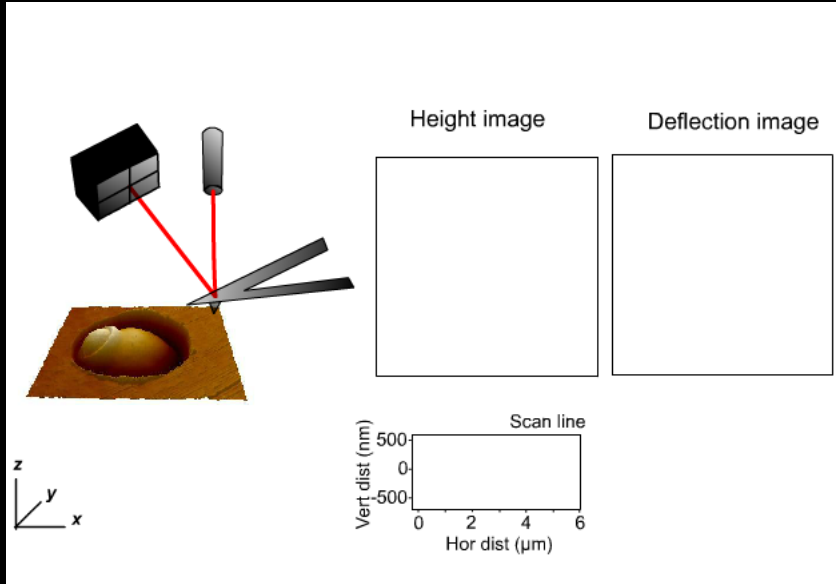


Cell adhesion

AFM: Imaging cell topography

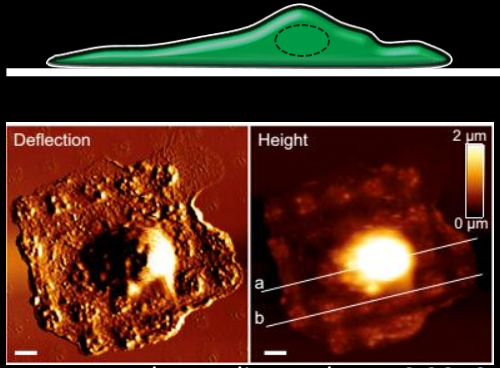


Contact mode under small force (100 pN)
 In physiological conditions (liquid)



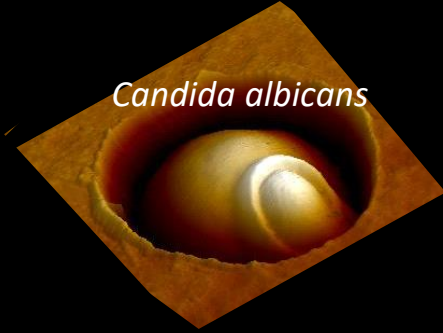
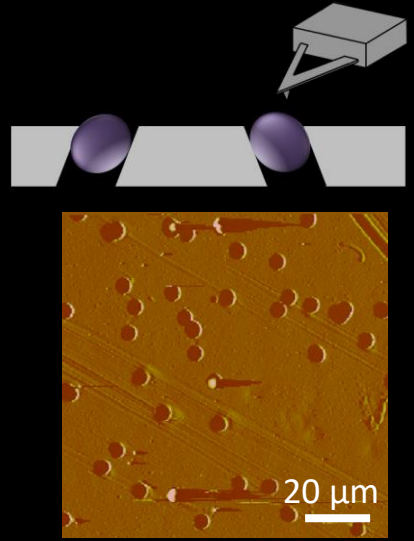
Alsteens

Spread cells

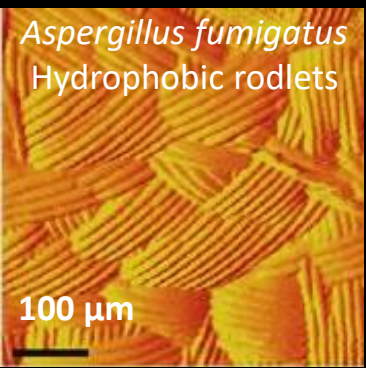


Labernadie *et al.* PNAS 2010

Mechanically trapped microbes

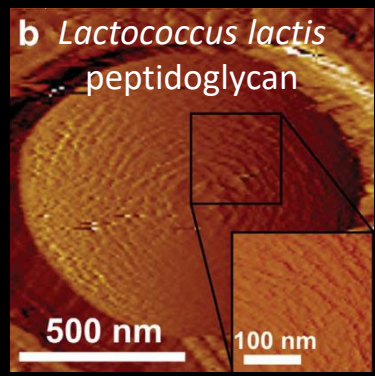


Candida albicans



Aspergillus fumigatus
 Hydrophobic rodlets

100 μm

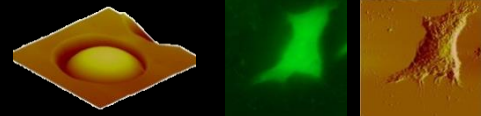


Lactococcus lactis
 peptidoglycan

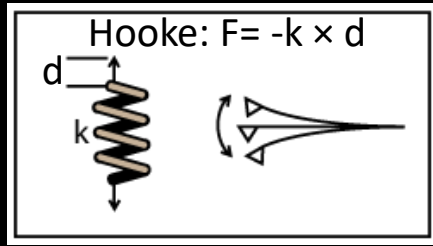
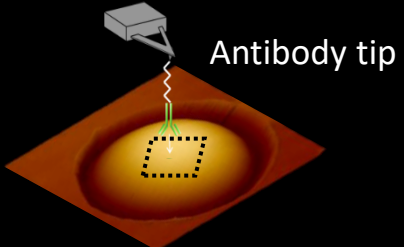
500 nm

100 nm

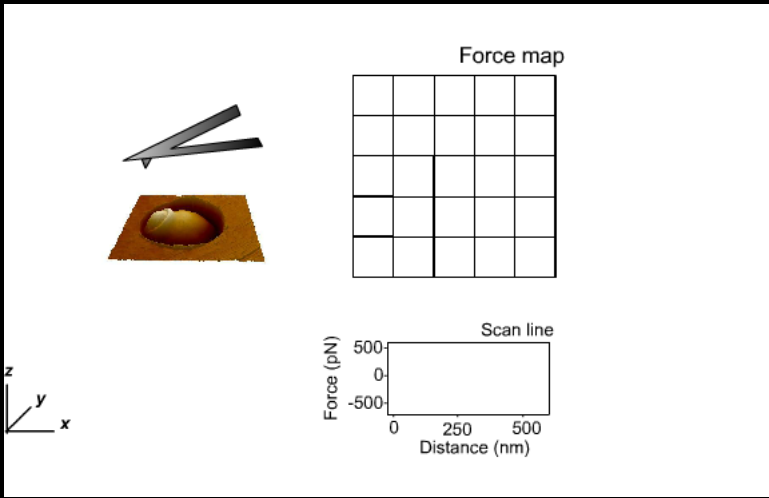
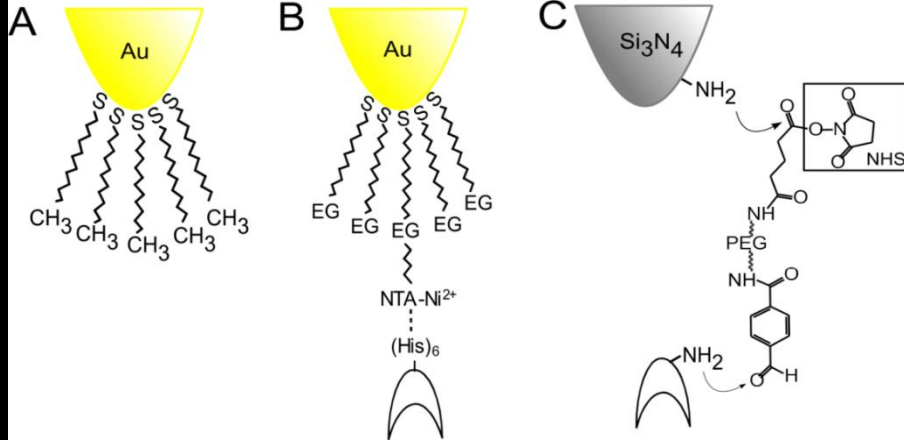
AFM: Single molecule mapping



Molecular mapping, single molecule mechanics

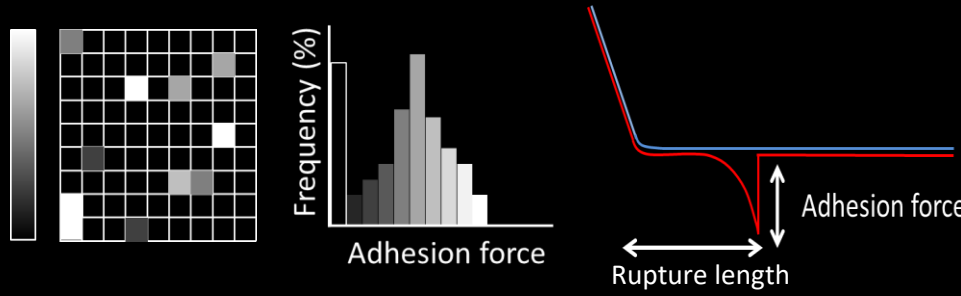


Tip functionalization

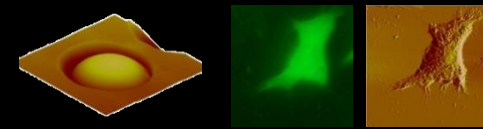


Chemical Force
Microscopy

Single Molecule
Force Spectroscopy



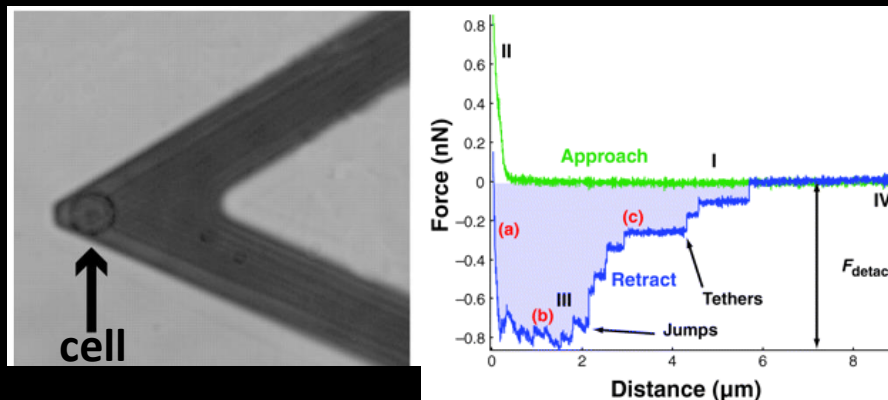
AFM: Single cell adhesion



How molecules work together at the whole cell scale?

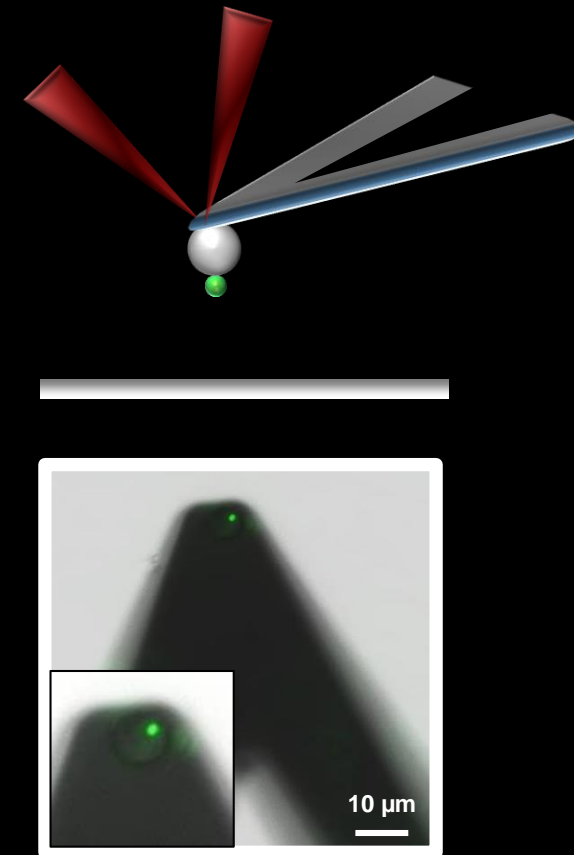
Mechanisms of cell adhesion/interaction, screening of inhibitors

Adhesion of mammalian cells



Helenius *et al.* J. Cell Sci. 2008

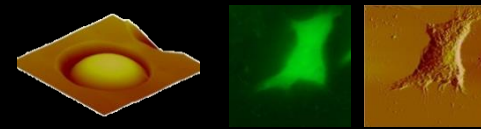
Adhesion of single bacteria



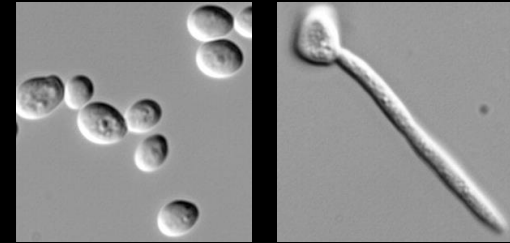
Beaussart, El-Kirat-Chatel *et al.* Nat. Protoc. 2014

Example 1:
AFM to study pathogenic yeast

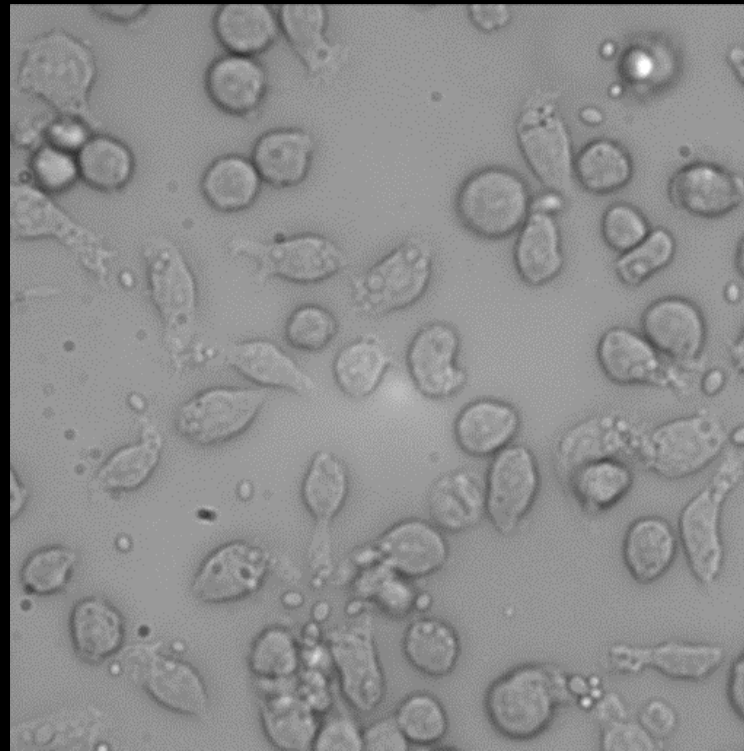
Candida albicans



- Human commensal yeast
- Opportunistic pathogen
- Few efficient antifungal drugs
- Several virulence factors



Wightman, *J. Cell. Biol.* (2004)

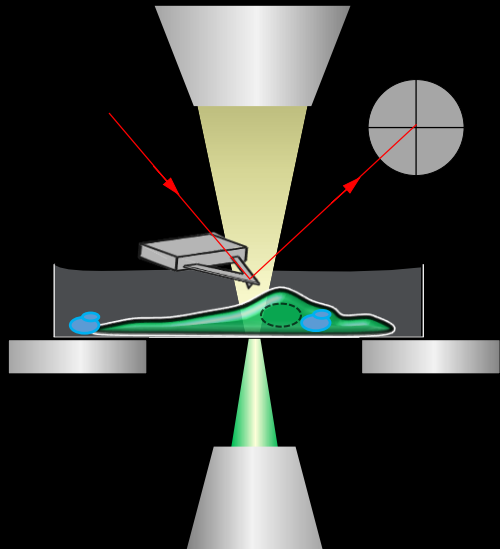
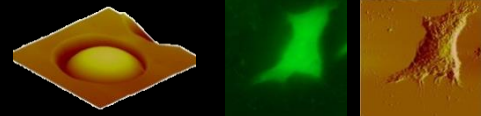


Cell wall composition?

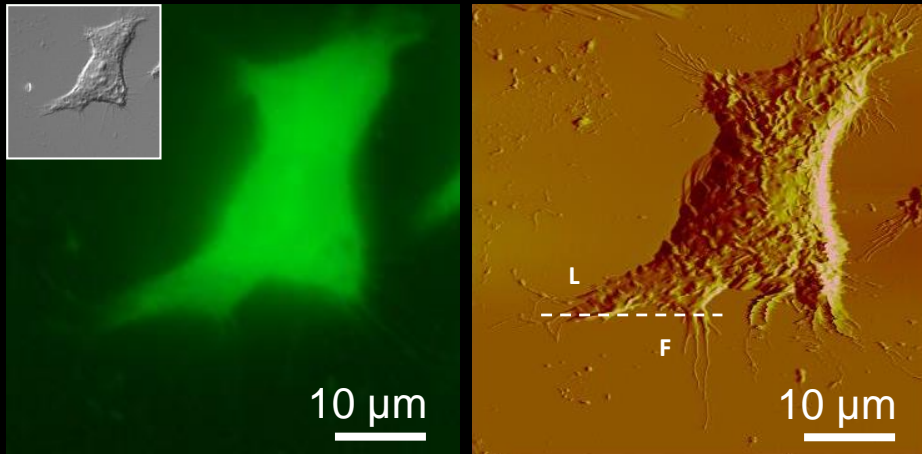
Resistance to phagolysis?

Effect of antifungal drugs?

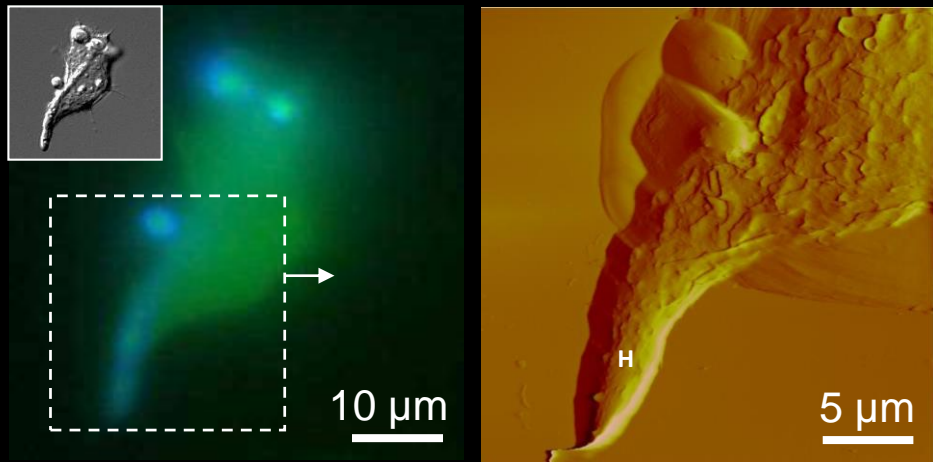
Correlative imaging of the interaction



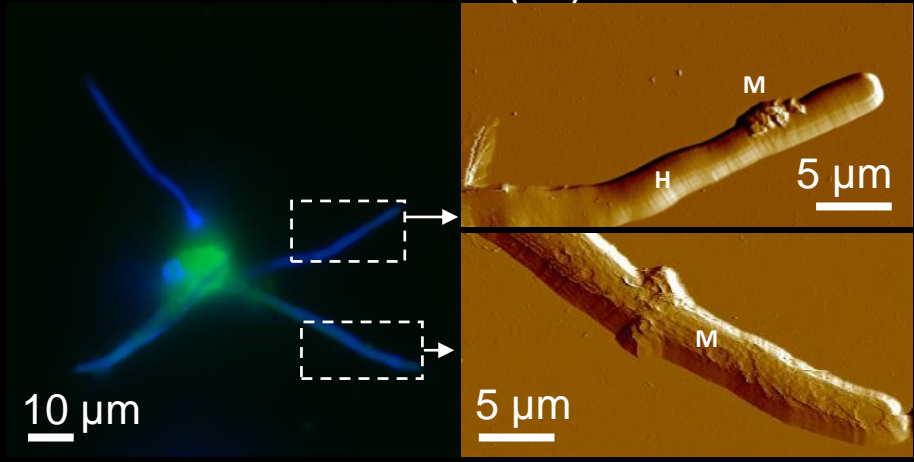
Uninfected



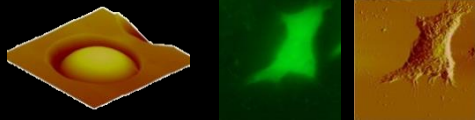
Infected (1h30)



Infected (3h)

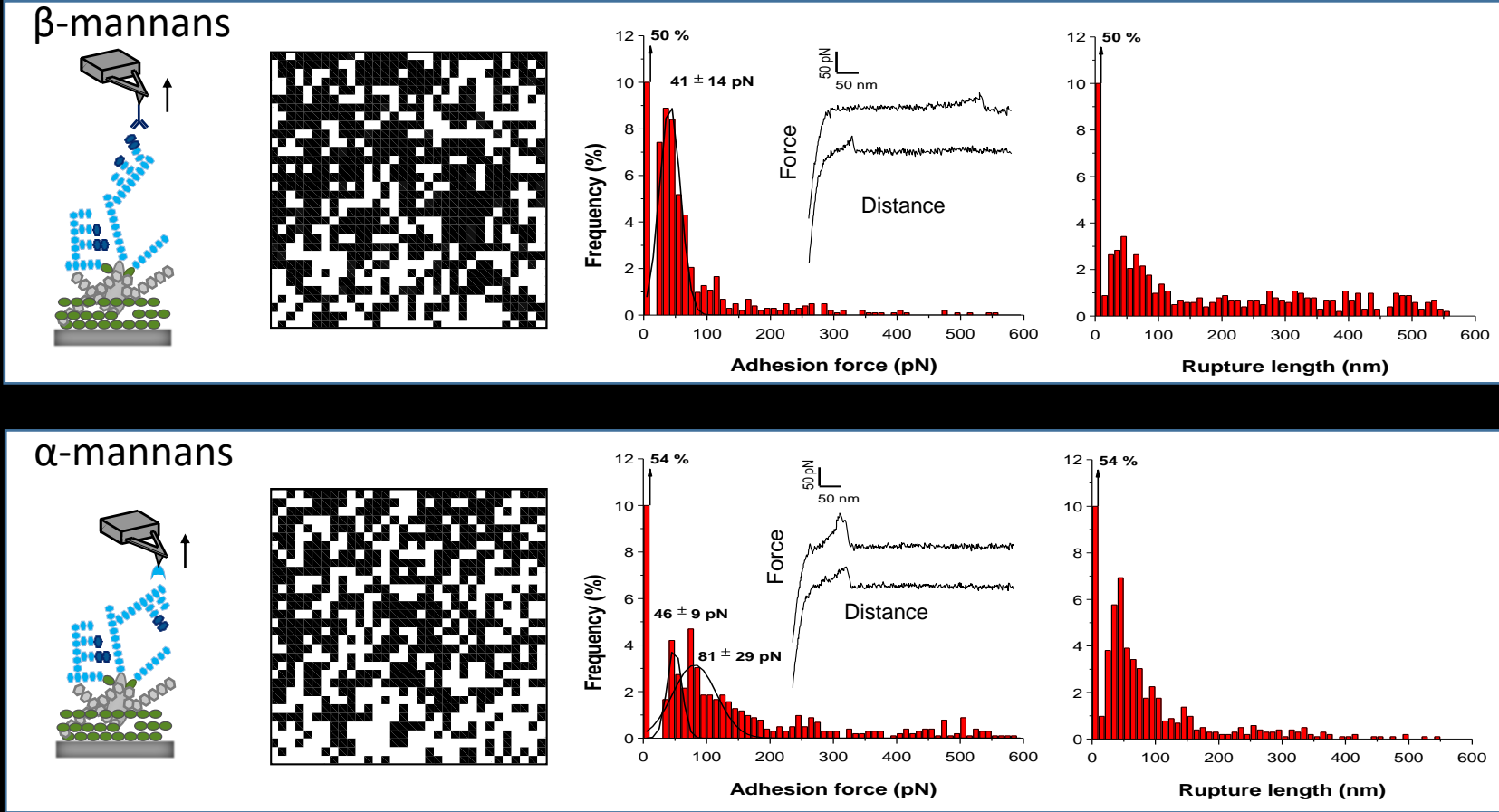


Mannose distribution on yeast surface



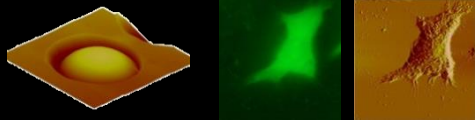
Mannosides are supposed to play pivotal role for immune recognition

Single molecule

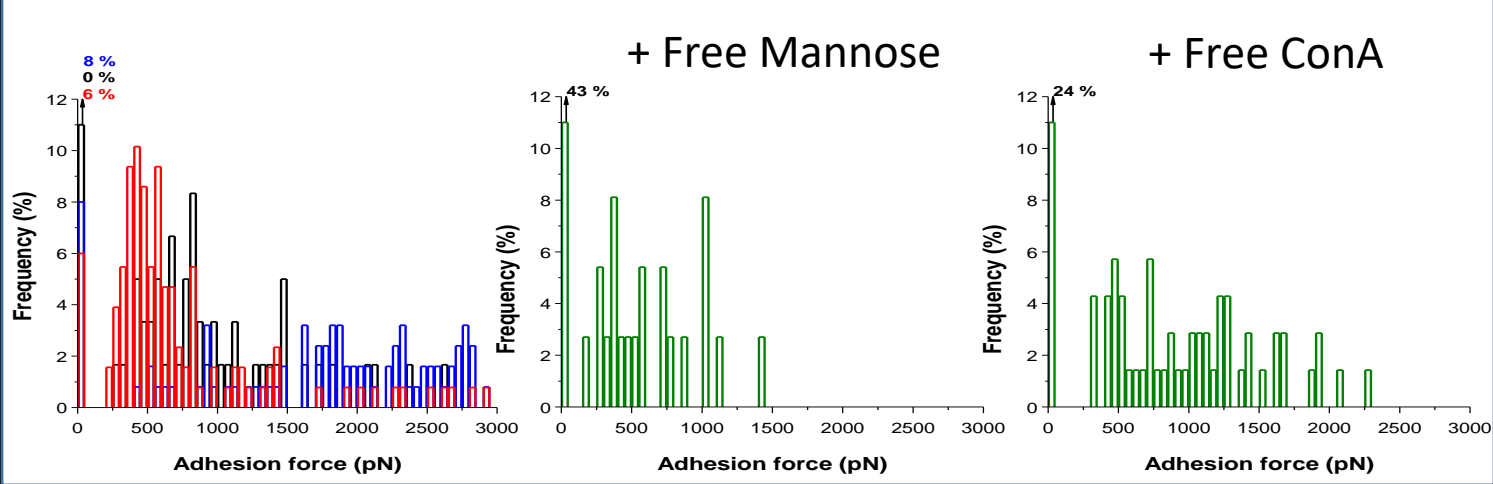
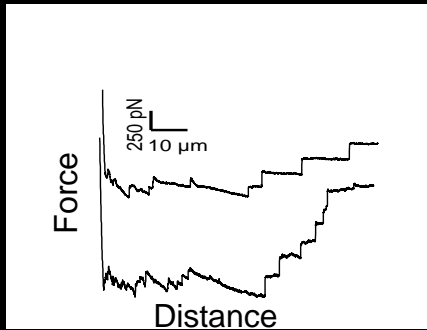
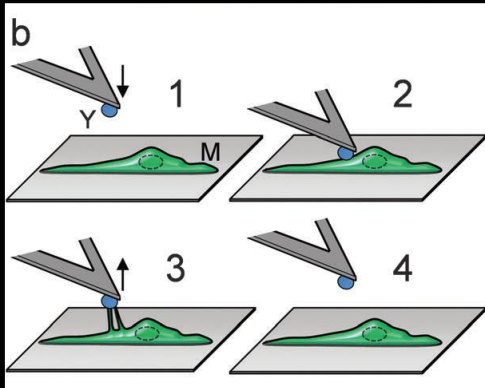
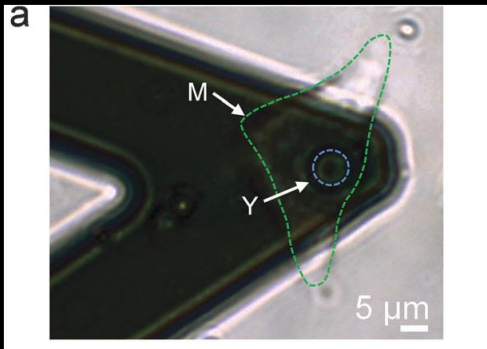


SMFS shows large and homogeneous distribution of mannans on yeast surface

Forces involved in the interaction

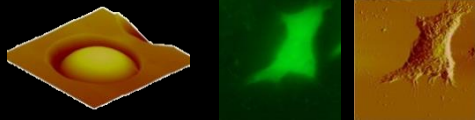


Single cell

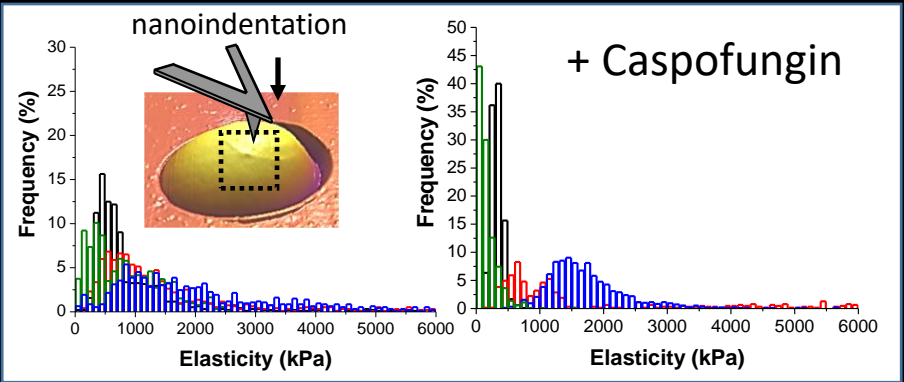
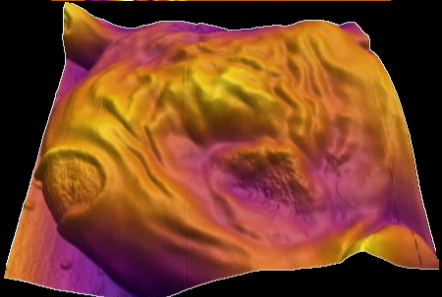
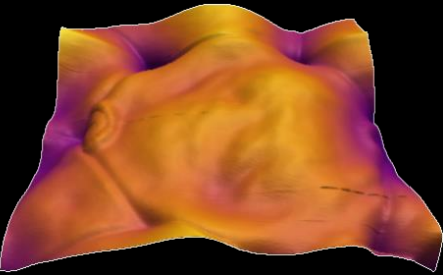
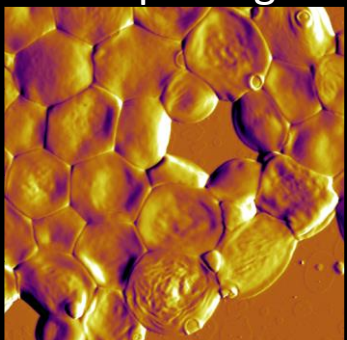
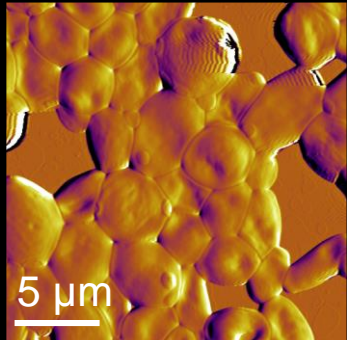


Recognition of yeast cell wall mannosides by macrophage mannose receptors governs the initial steps of phagocytosis

Effect of antifungal drugs



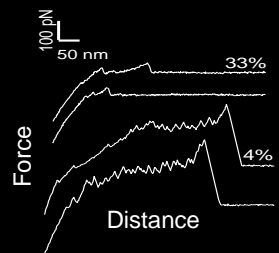
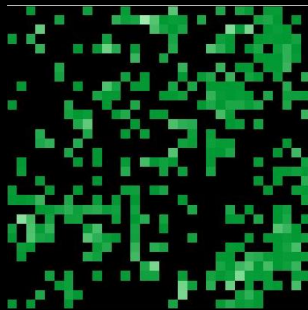
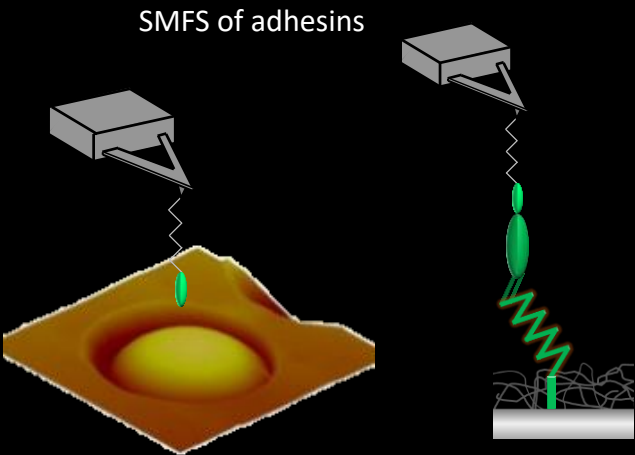
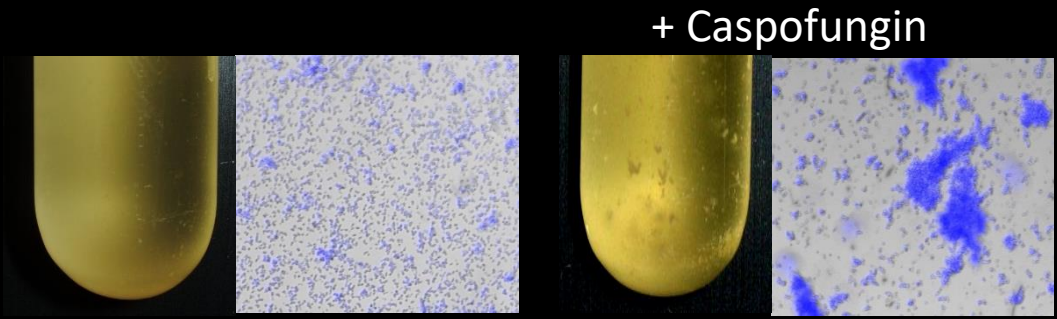
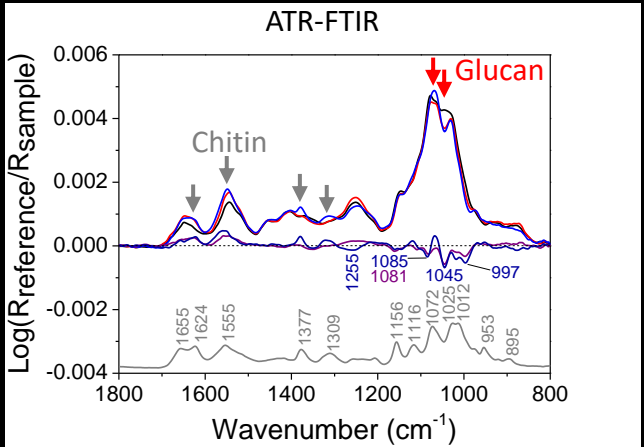
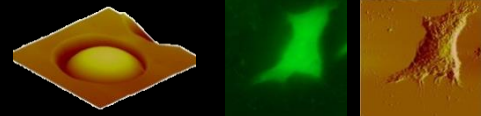
+ Caspofungin



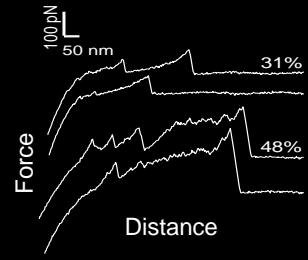
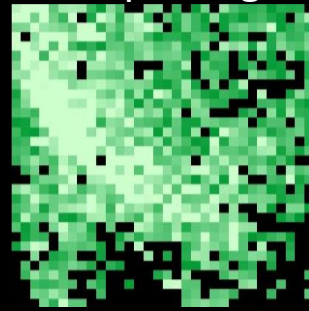
+Caspofungin:

- increased roughness and cell swelling
- decreased elasticity

Effect of antifungal drugs: stress response



+ Caspofungin

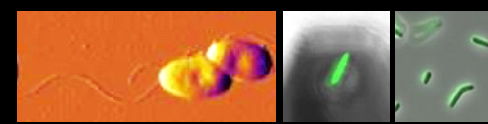


+Caspofungin:

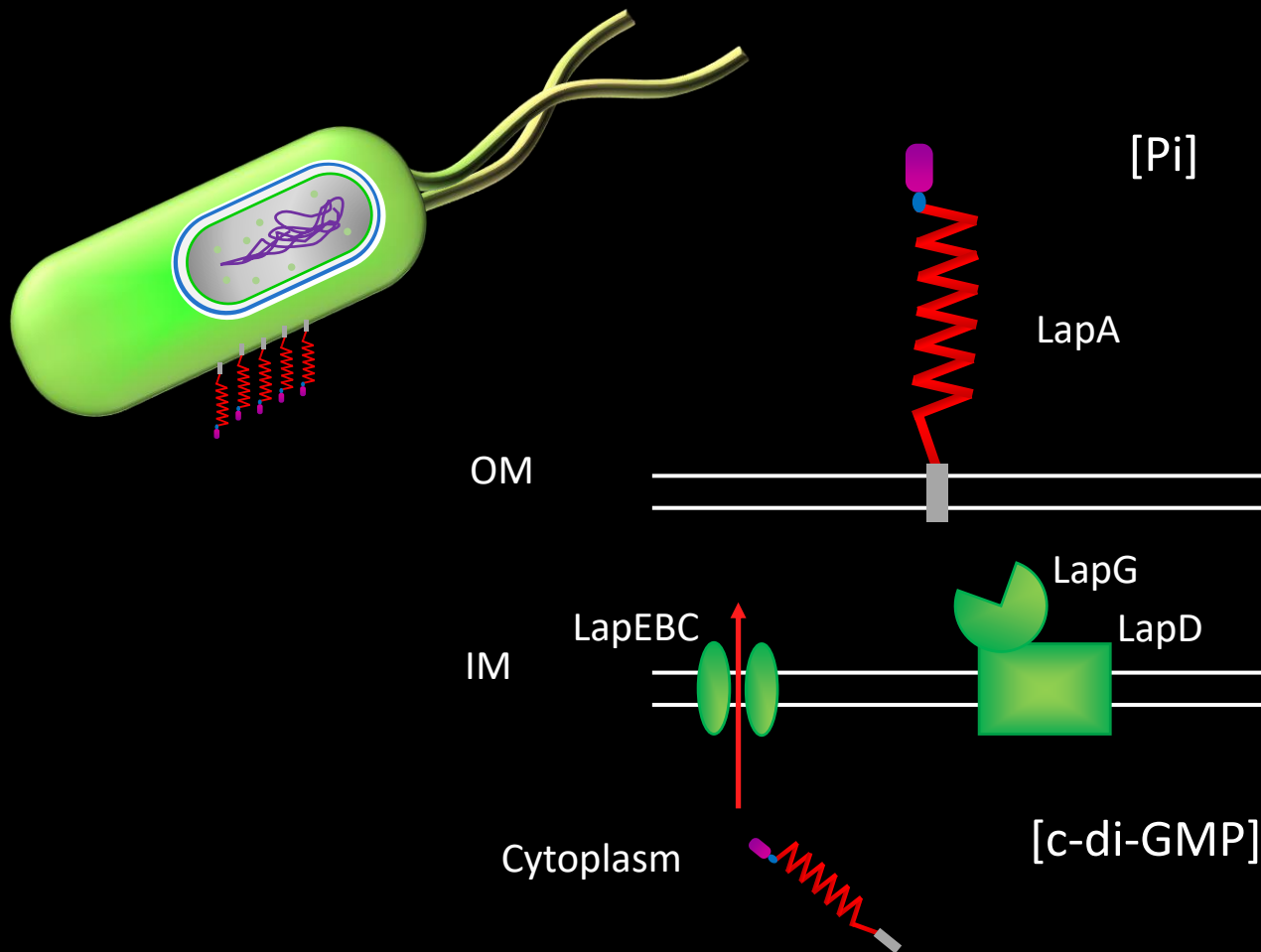
- decreased glucans compensated by increased chitin content
- overexpression of Als1 for increased adhesion

Example 2:
AFM to study bacteria

Adhesion and biofilm formation



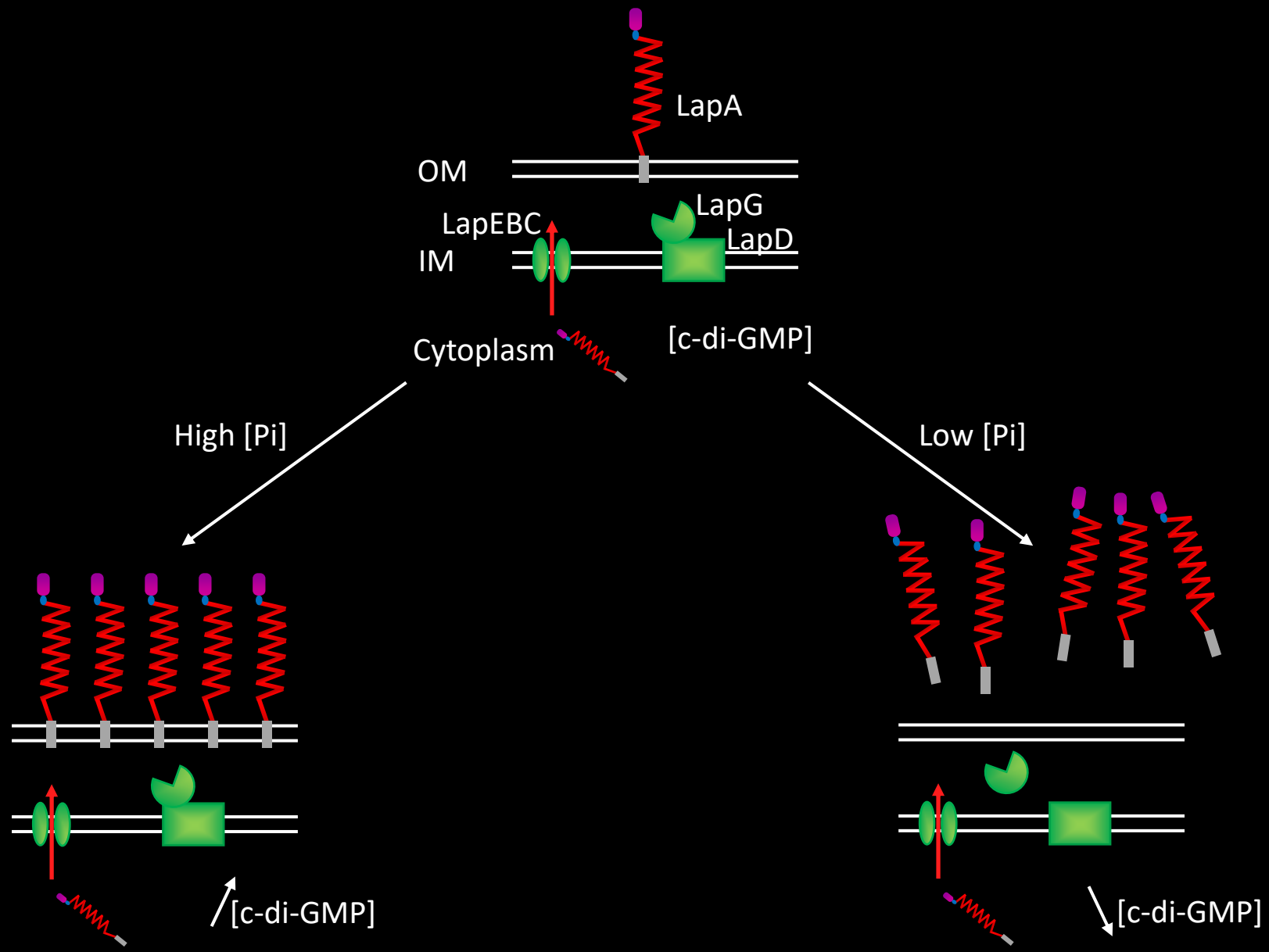
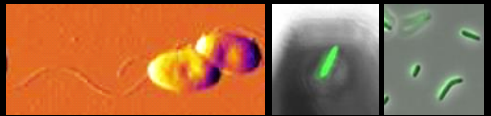
Pseudomonas fluorescens: soil bacteria, model for biofilm formation and regulation.



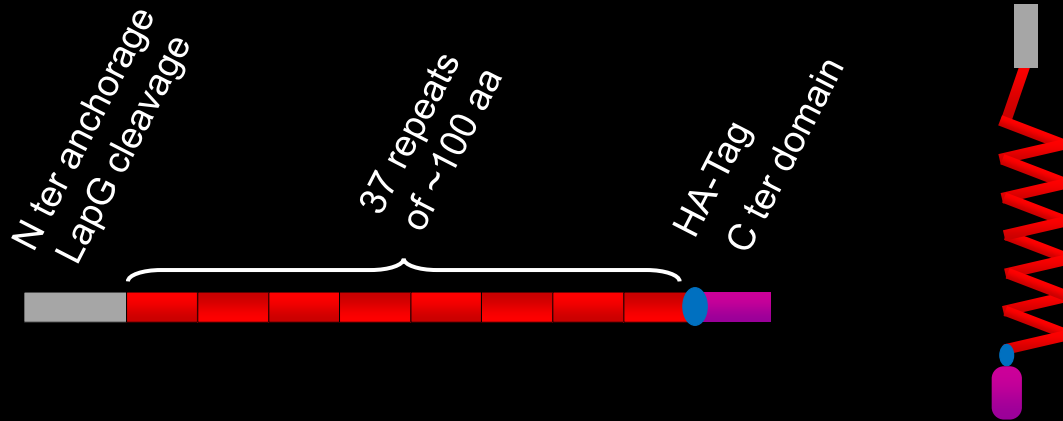
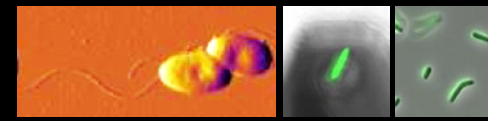
Newell, *PNAS* (2009)

Newell, *PLoS Biol.* (2011)

Adhesion and biofilm formation

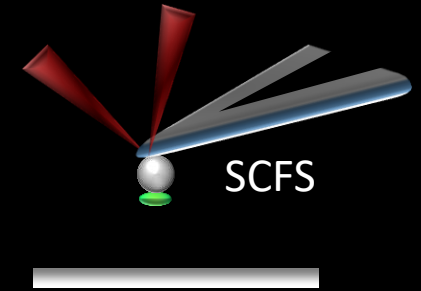
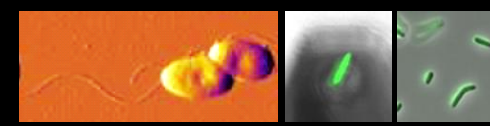


Large adhesin protein A: LapA



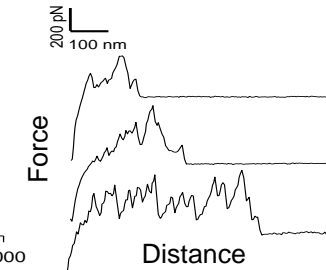
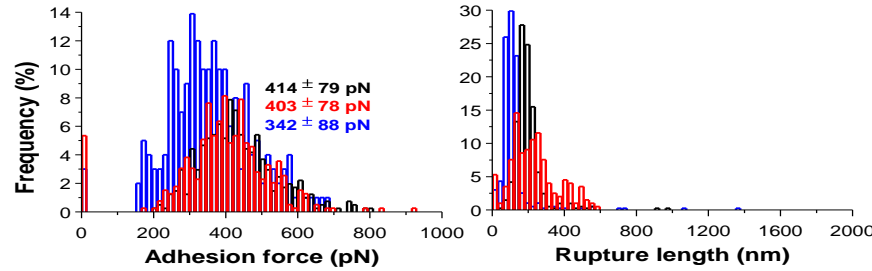
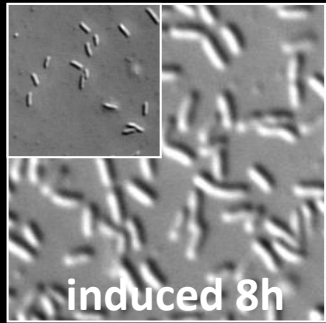
- How does it promote adhesion, *i.e.* what are the functional domains?
- What are its biophysical properties?
- How is its distributed on the cell surface?

Single-cell force spectroscopy

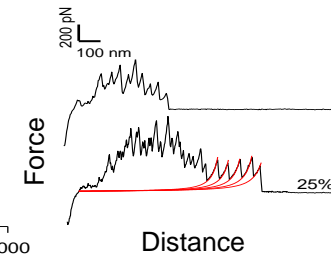
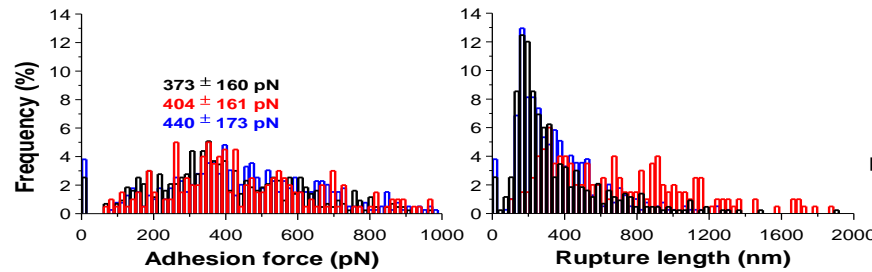
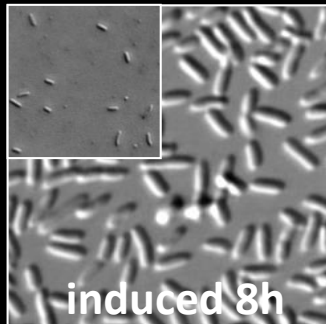


WT

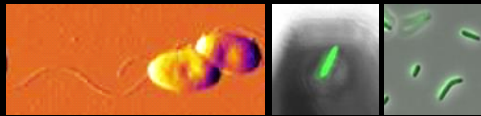
On hydrophobic surfaces



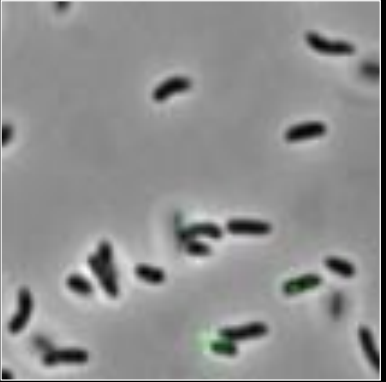
On hydrophilic surfaces



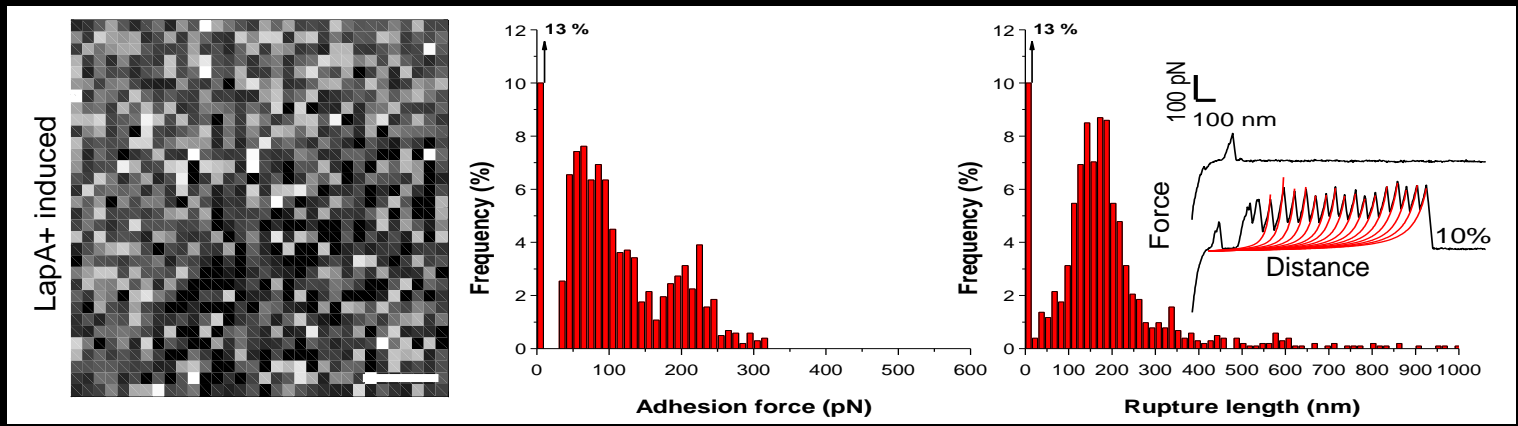
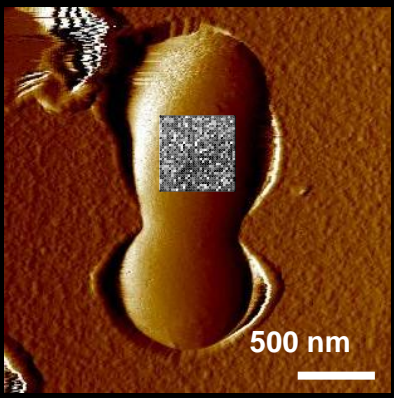
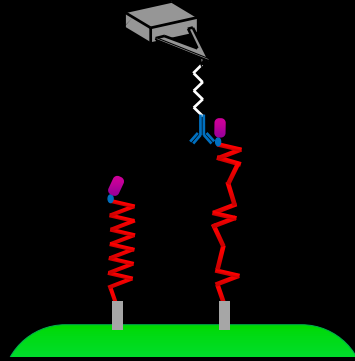
LapA cell-surface distribution



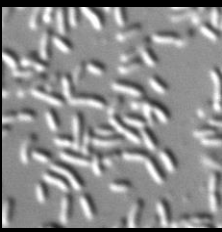
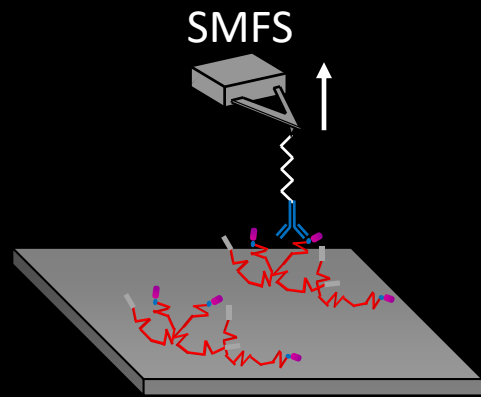
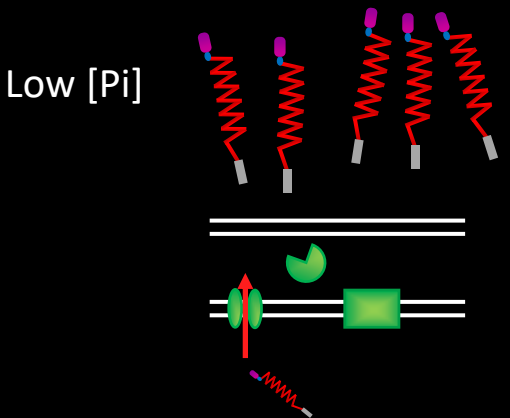
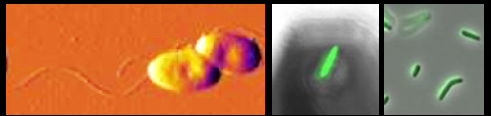
WT



SMFS



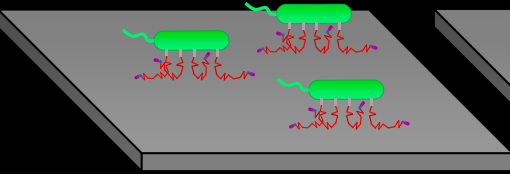
Bacterial footprints



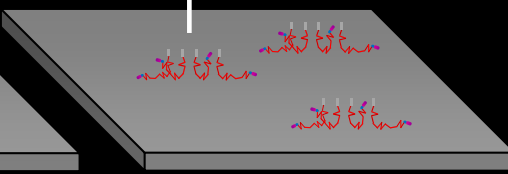
High Pi



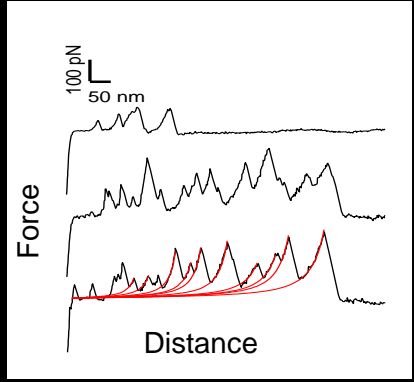
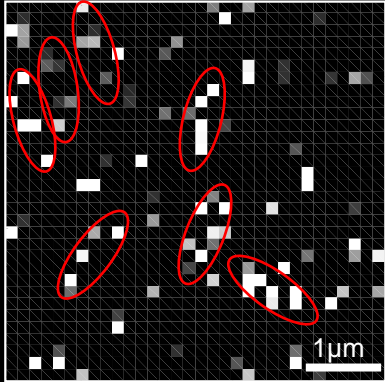
Low Pi



Cell adhesion

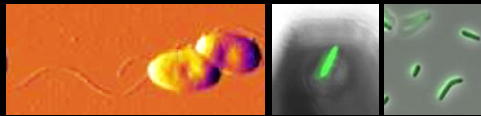


Cell detachment

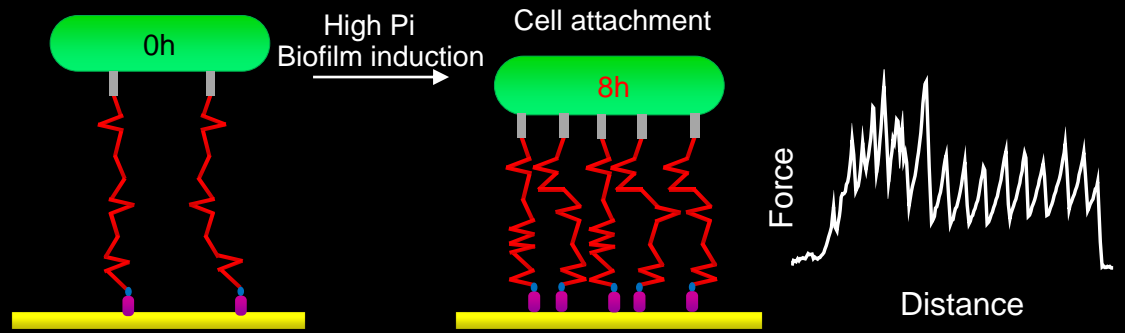


After cell detachment, LapA detected on the surface as bacterial footprint

Model for LapA mechanism: Conclusions

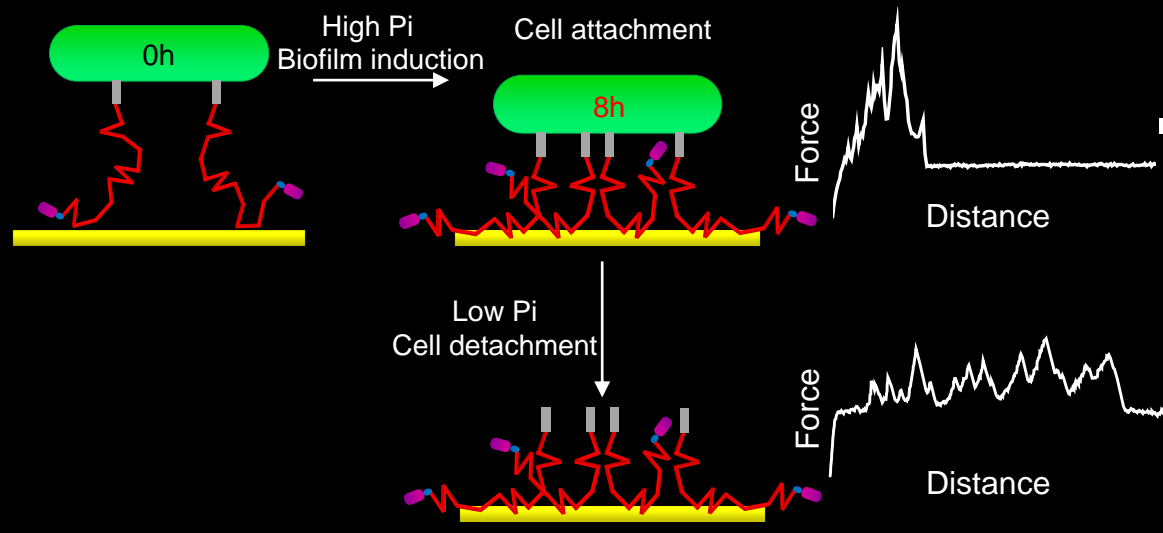


Hydrophilic



■ Adhesion by C term: unfolding

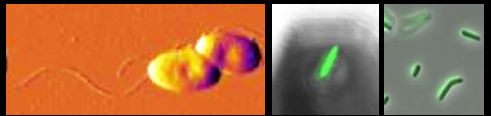
Hydrophobic



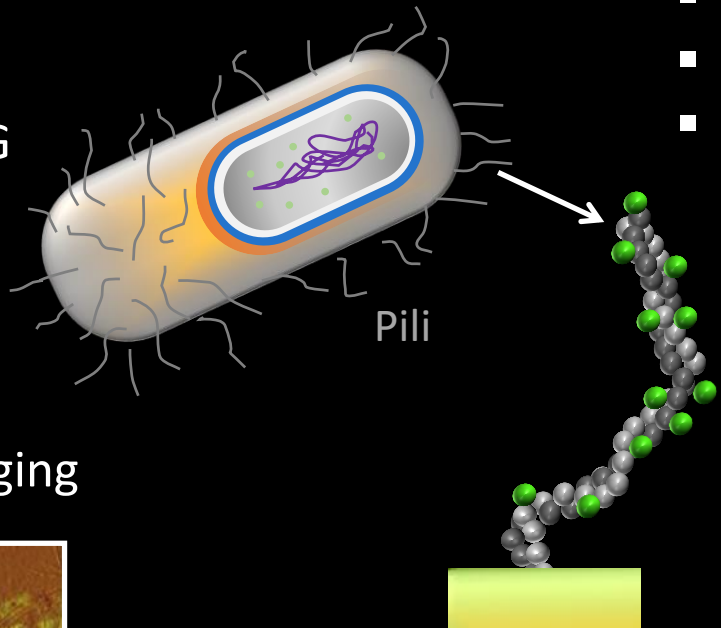
■ Adhesion by repeats region

■ LapA is a finely regulated multifunctional adhesin for alternative bacterial adhesion or detachment.

Probing appendages mechanical properties



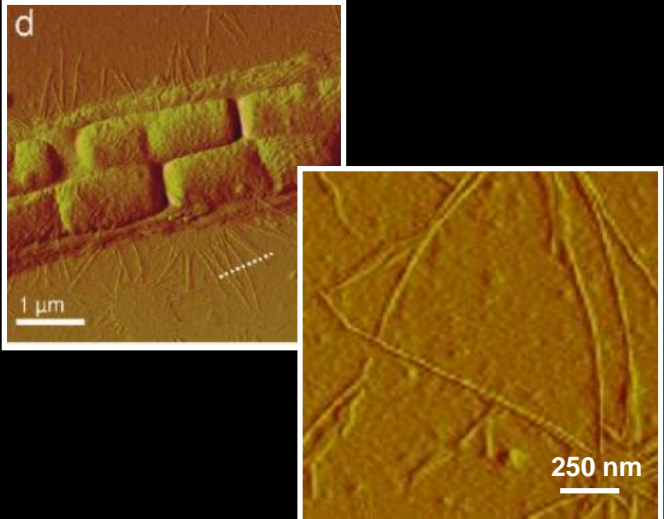
Lactobacillus rhamnosus GG



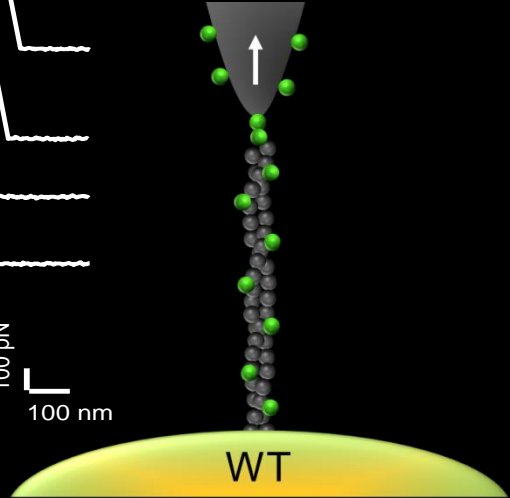
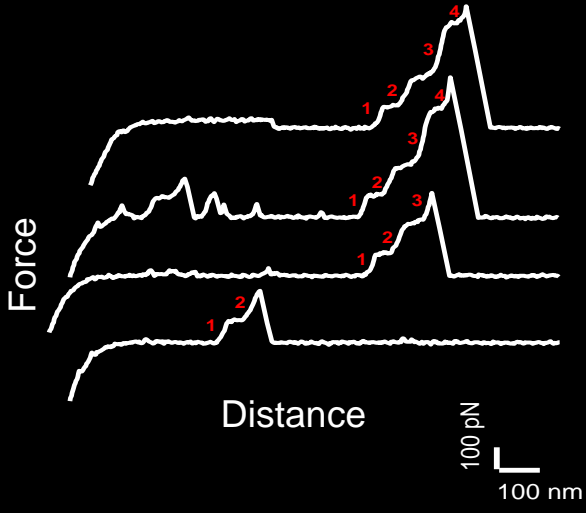
- Probiotic bacteria
- Found in gastro-intestinal track
- Pili: homotypic adhesion, interaction with mucus and epithelial cells

SpaC: Pilin subunit responsible of adhesion

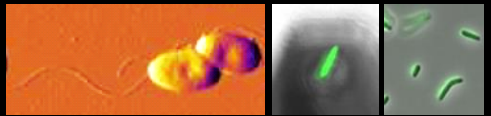
Imaging



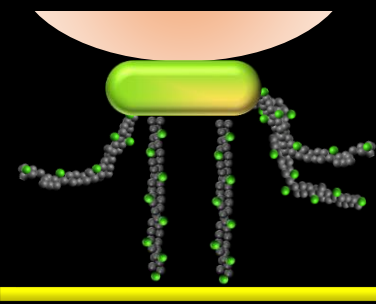
SMFS



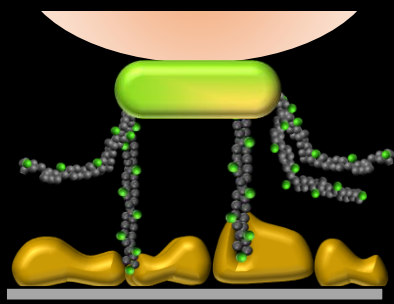
Probing appendages mechanical properties



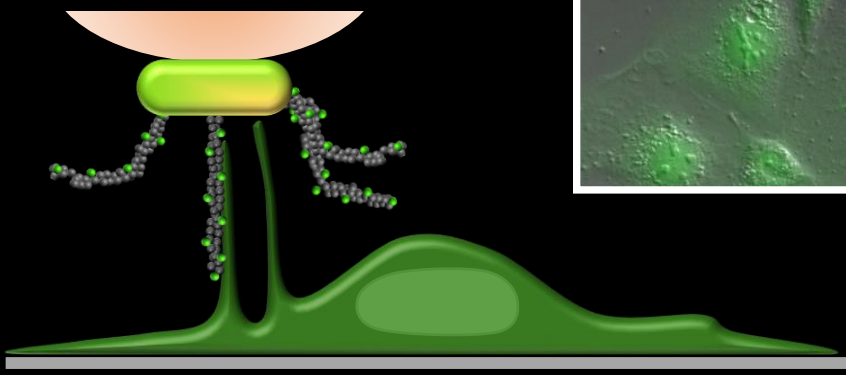
Single-cell force spectroscopy



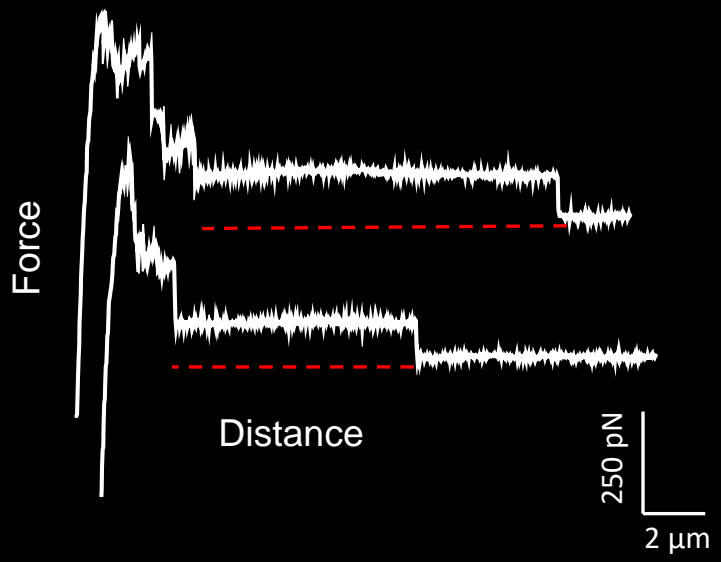
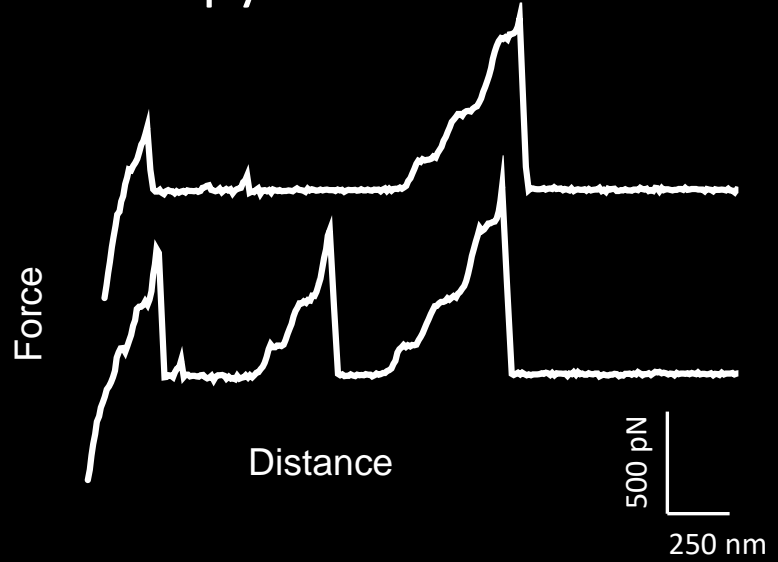
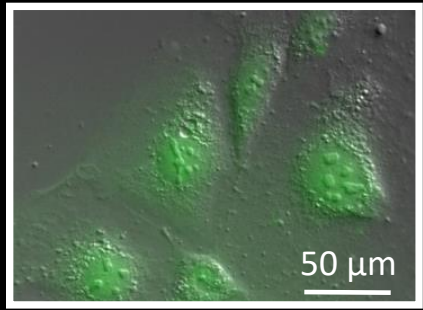
Hydrophobic

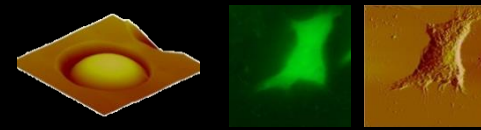


Mucin



Caco2





George O'Toole



Peter Lipke



Yves Dufrene

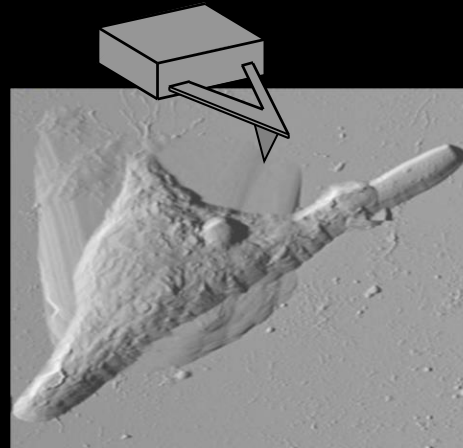


Fabienne Quilès
Gregory Francius

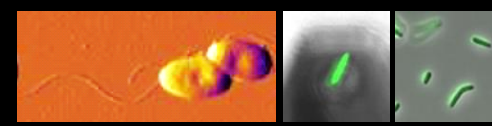


Thierry Noël

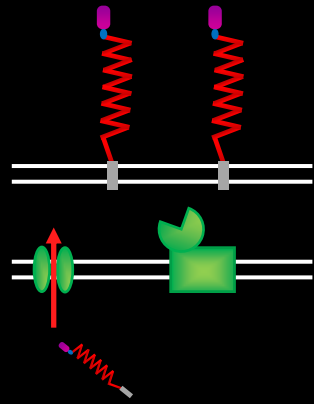
Thank you for your attention



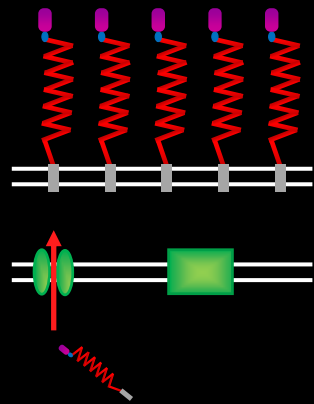
Adhesion and biofilm formation



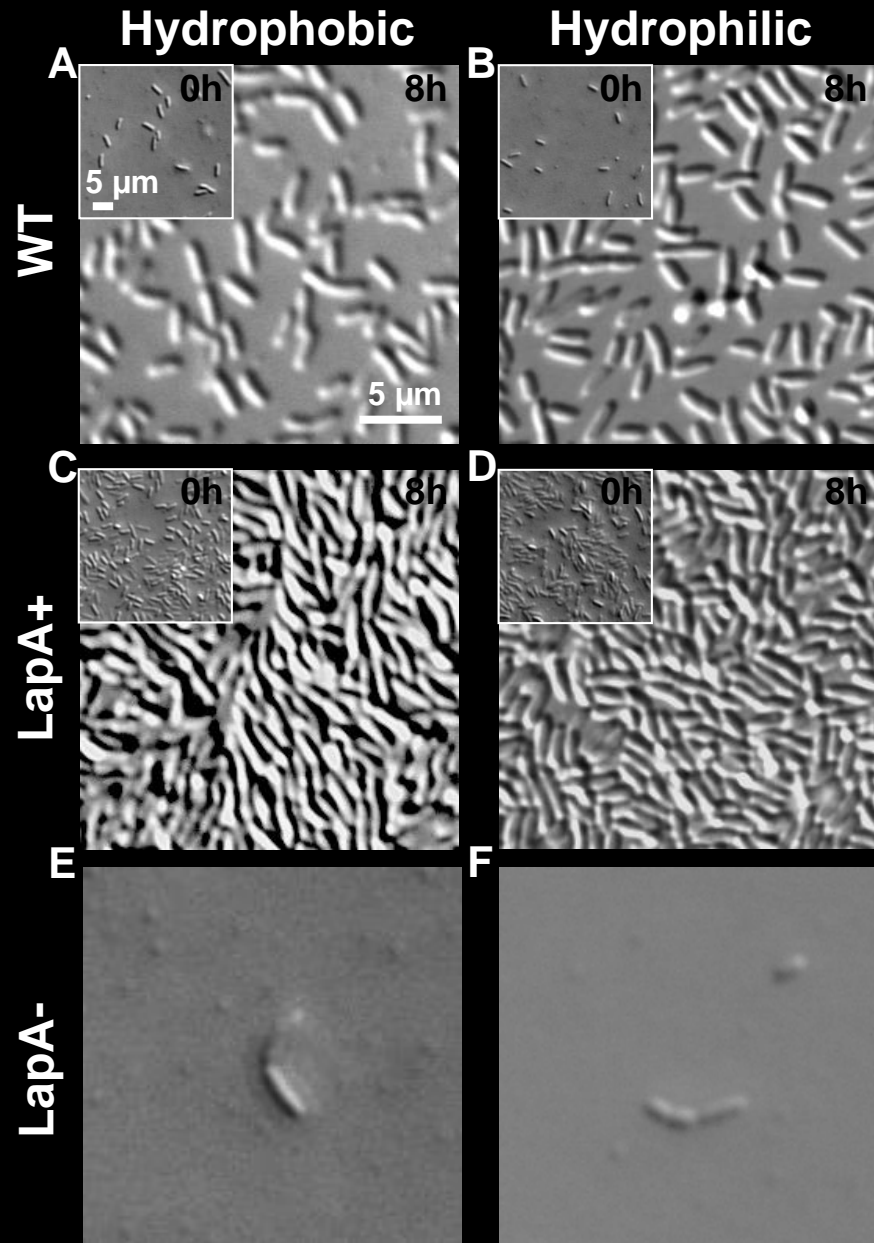
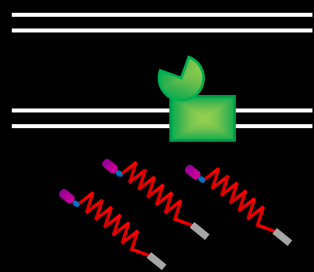
WT



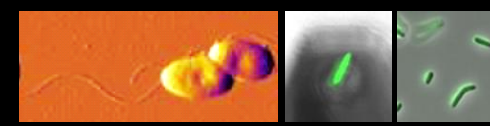
LapA+



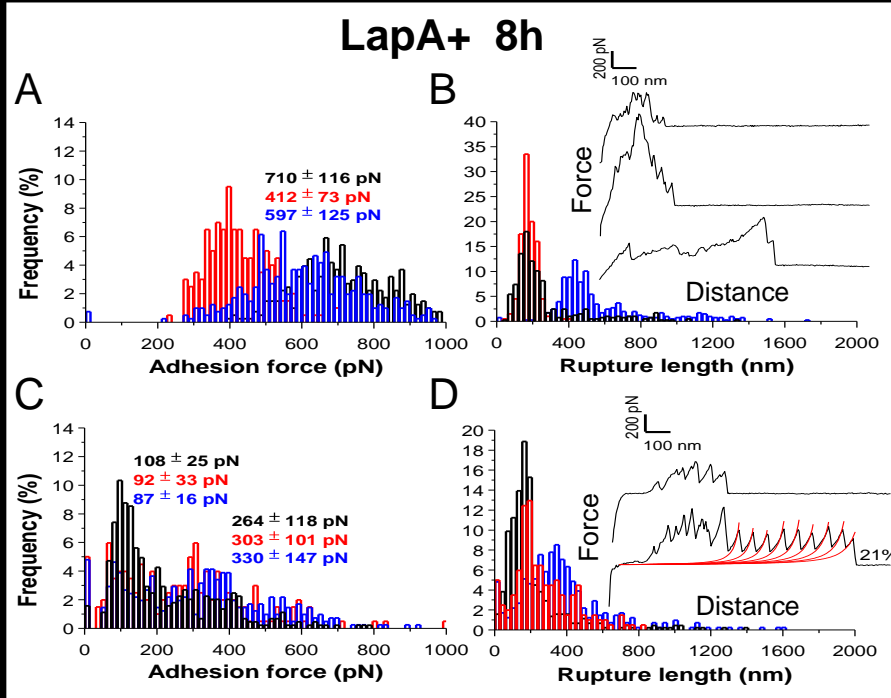
LapA-



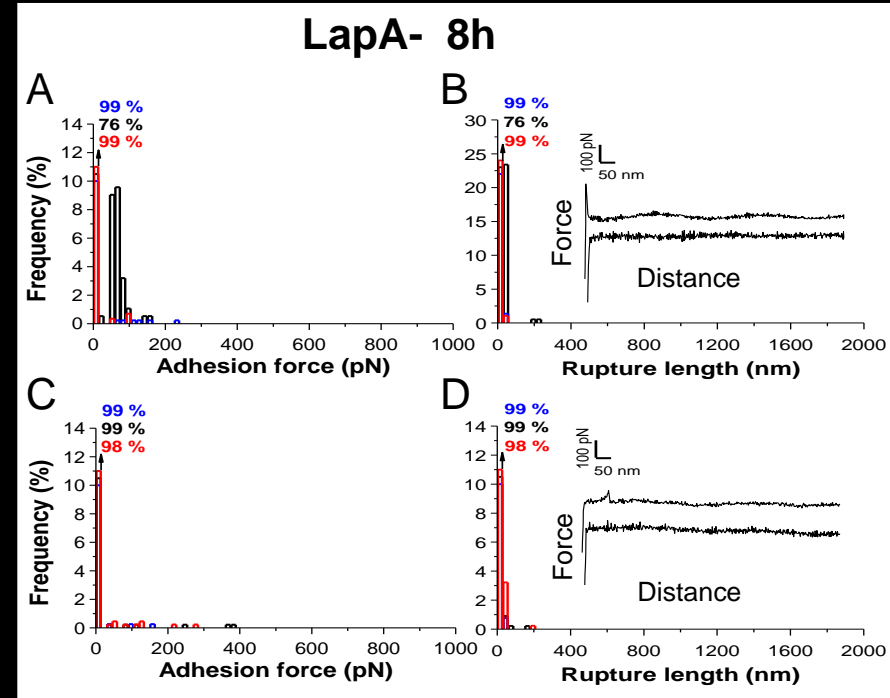
Single-cell force spectroscopy



CH₃

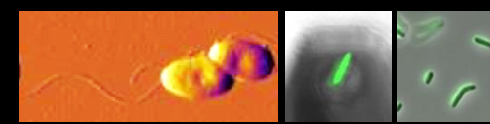


OH

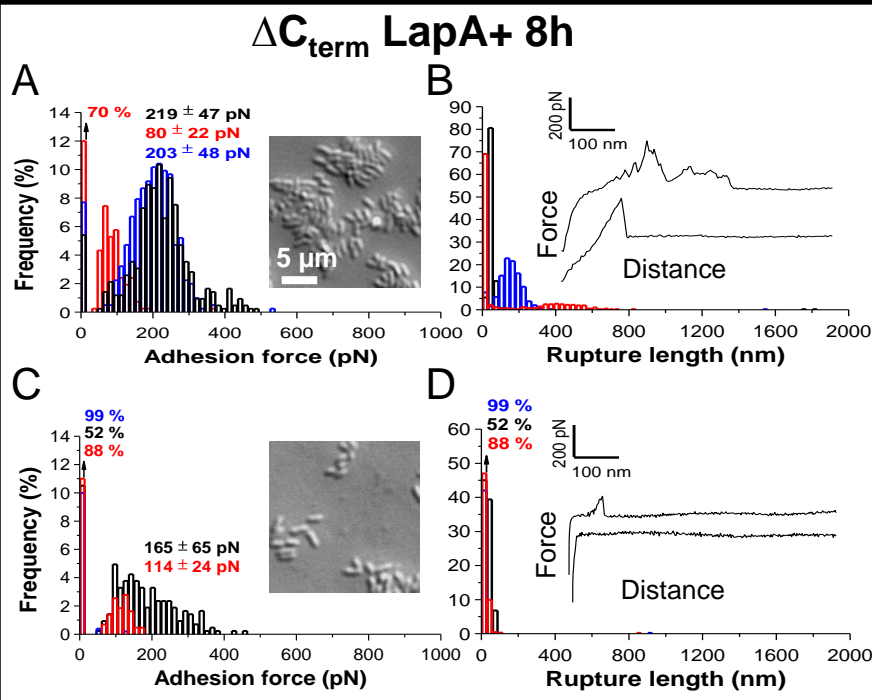


- Mutants analysis confirms LapA role in bacterial adhesion

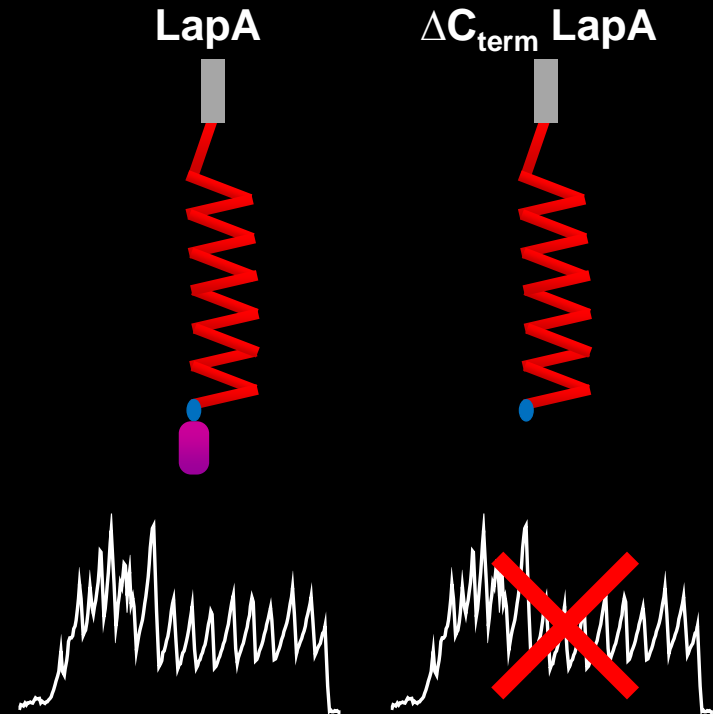
Single-cell force spectroscopy



CH₃

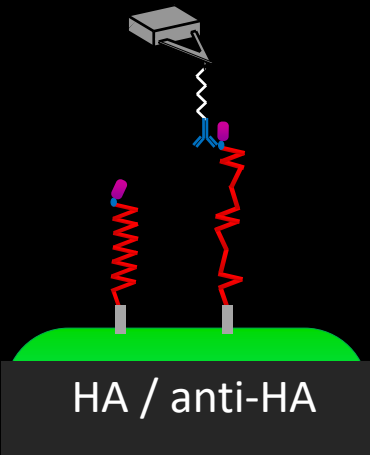
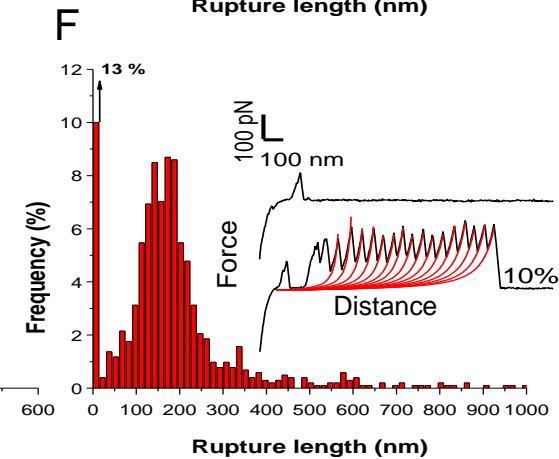
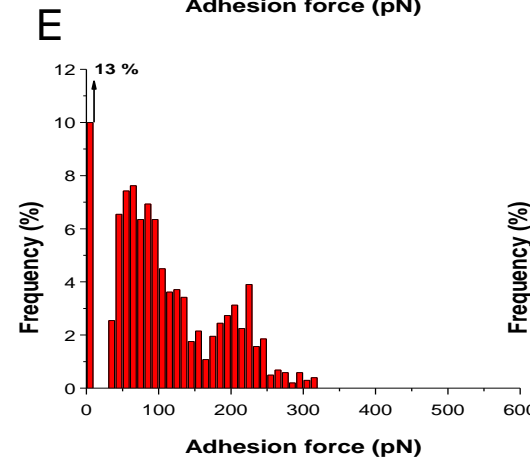
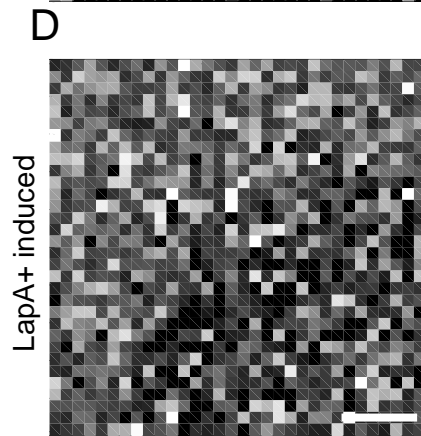
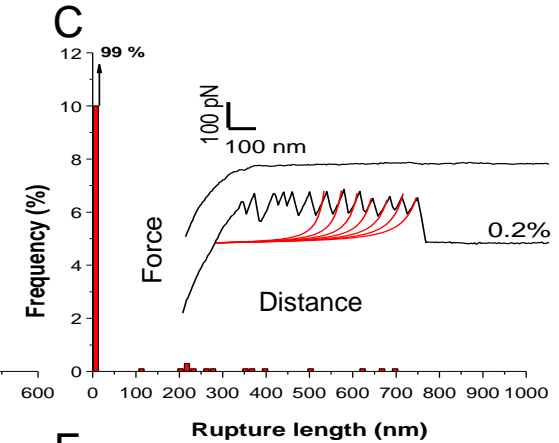
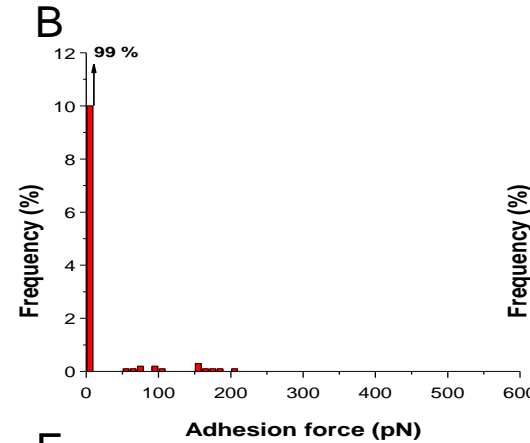
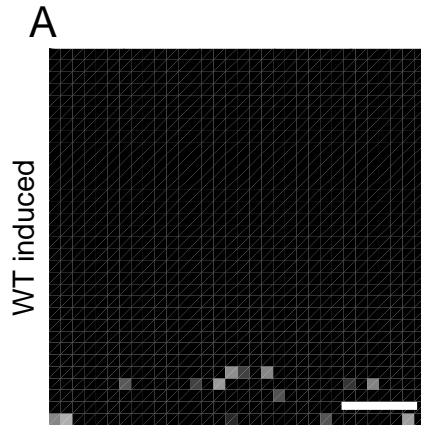
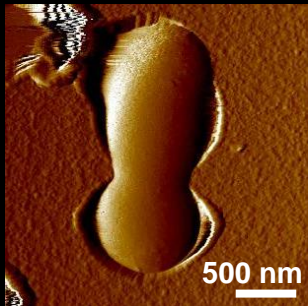
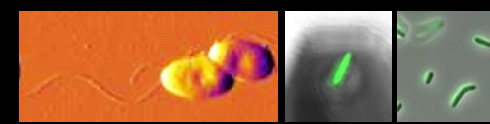


OH



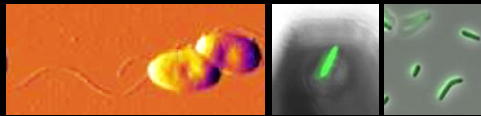
- LapA is a multipurpose adhesin
- Repeats are involved in adhesion to hydrophobic surfaces
- C terminal domain is involved in adhesion to hydrophilic surfaces, leading to protein repeats unfolding

Single-molecule force spectroscopy of LapA

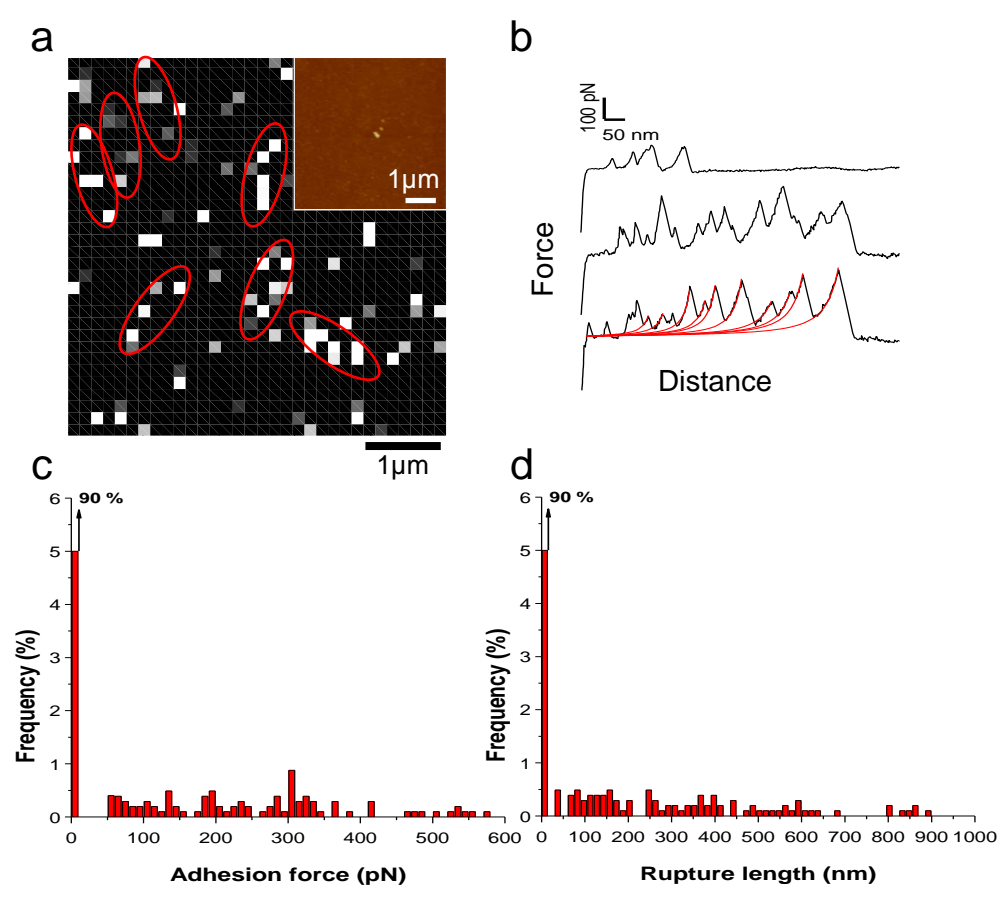
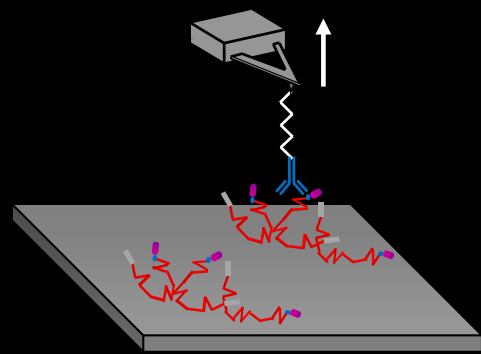


- LapA is released from cell surface depending on P_i concentration
- Unfolding in SMFS confirms LapA adhesion on hydrophilic surfaces
- Mutants confirmed these conclusions. LapA+ accumulates LapA and no detection on LapA-

Bacterial footprints



SMFS using anti-HA mAbs



- Biofilm formation in high Pi medium and subsequent cell detachment in low Pi.
- Detection of single LapA on CH₃ surfaces.
- Clustered LapA forming bacterial footprints

Bacterial footprints: controls

