INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

AFM for biological application – Theory 2

Pieter De Beule

31 October 2022

Pieter De Beule **Adelaide Miranda**

Nanodevices and Nanoelectronics

- Mathematics of signal processing for scientific instrumentation
  - Theory 1 – 13:30-15:30h – 10 October 2022 – UMinho Ed.1-0.18
  - Theory 2 – 14-16h – 10 October 2022 – UMinho Ed.2-1.10

Topics in Biophysics and Bionanosystems

- Bio-AFM
  - Theory 1 – 15:30-17:30h – 24 October 2022 – UMinho Ed.1-1.18
  - Theory 2 – 15:30-17:30h – 31 October 2022 – UMinho Ed.1-1.18
  - Laboratory 1 – 16:30-19:00h – 2 November 2022 – INL Lab
  - Laboratory 2 – 16:30-19:00h – 2 November 2022 – INL Lab

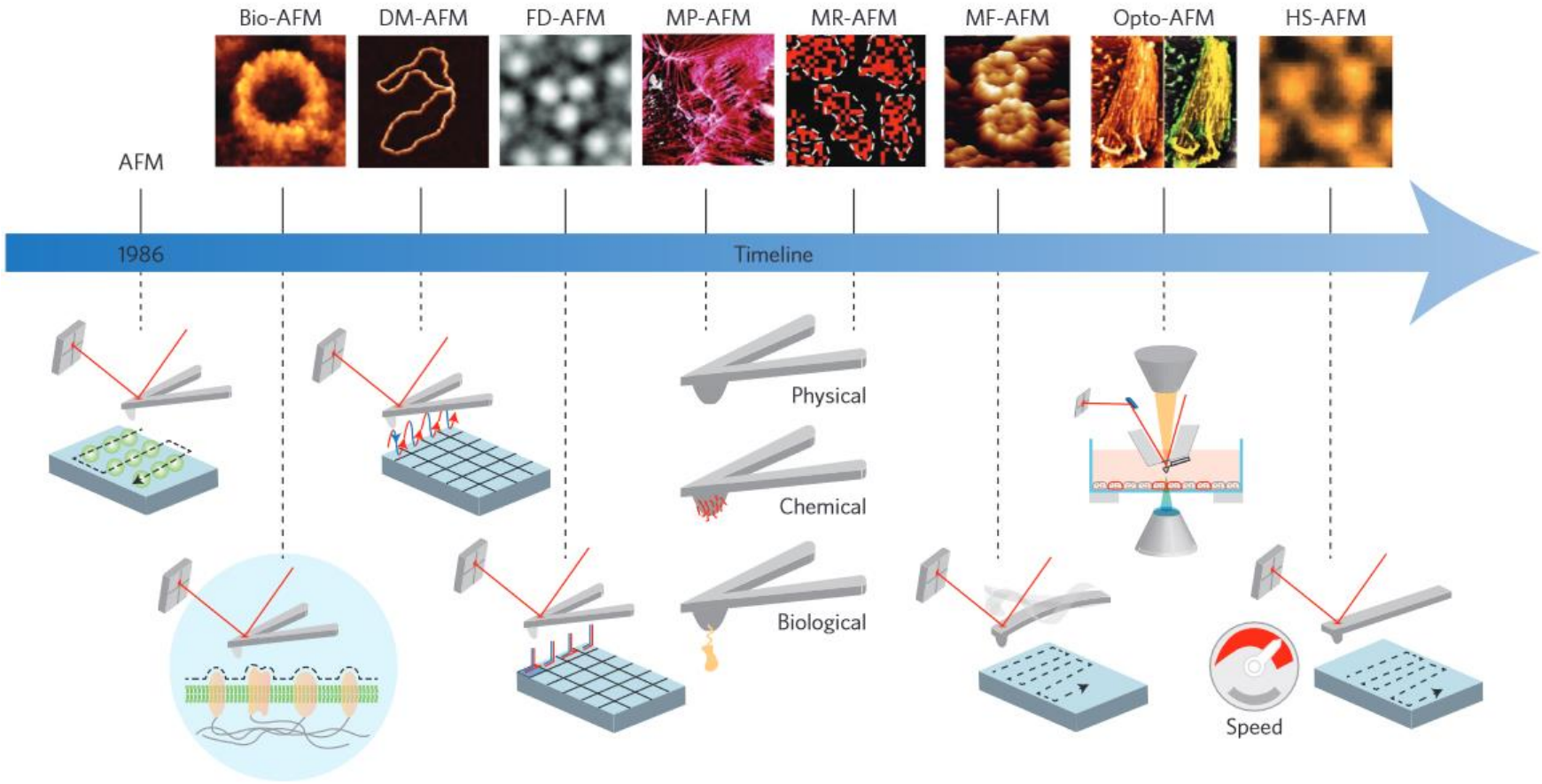
Student Feedback

Mathematics for signal processing of scientific instrumentation

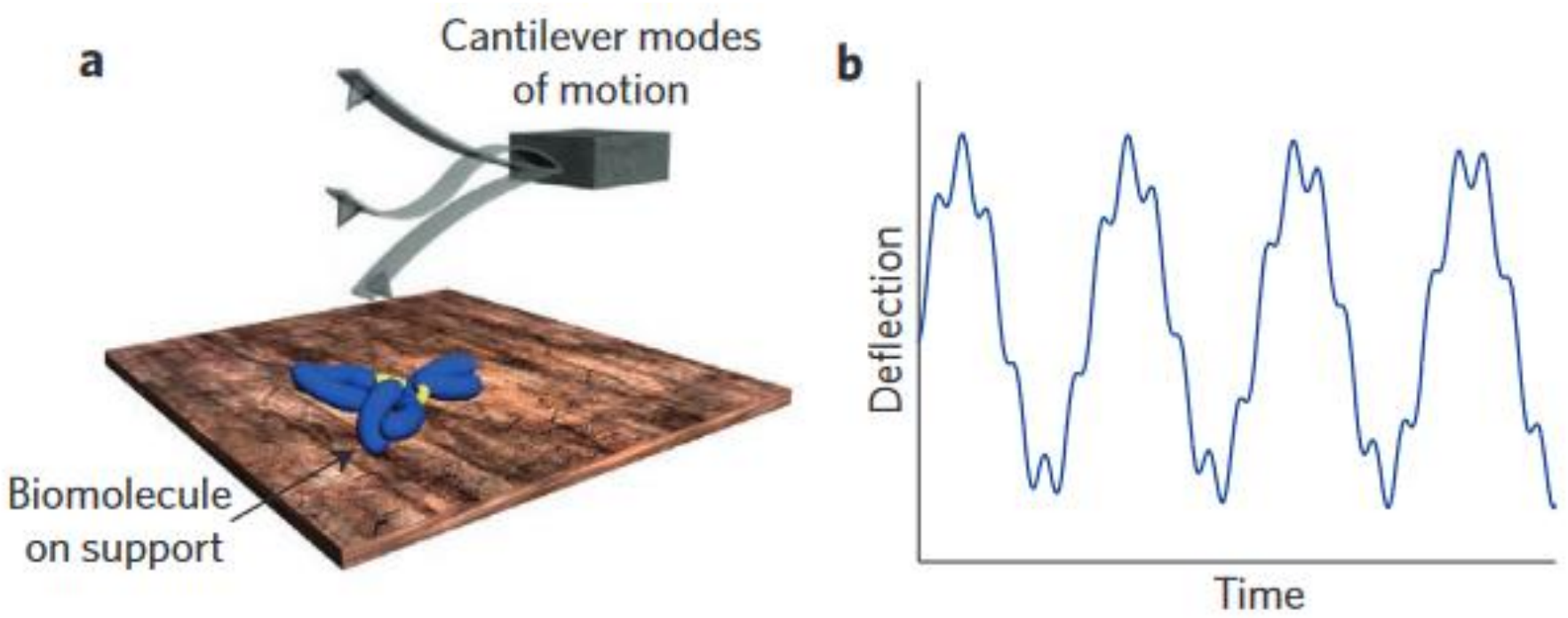
Difficulty level of class?					
	Too easy	Just about right	Too difficult		**Main comments**
	0	15	5		More exercise
					Unfamiliar with topics, need more introduction
Interesting?					
	Yes	Somewhat	No		
Too difficult	2	3	0		
Just about right	8	7	0		
	10	10	0		
Do you want lab class?					
	Yes	No			
	20	0			

Bio-AFM Imaging Modes

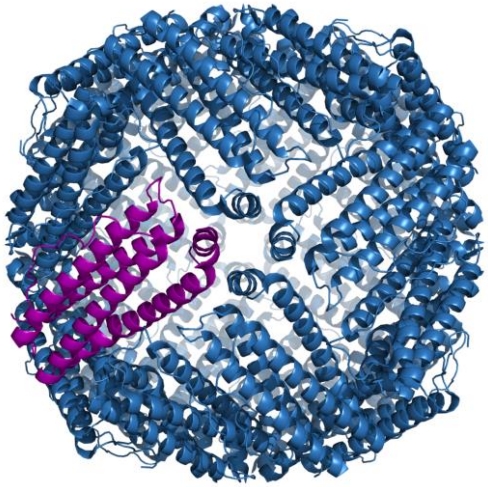
A novel approach to study biological structures



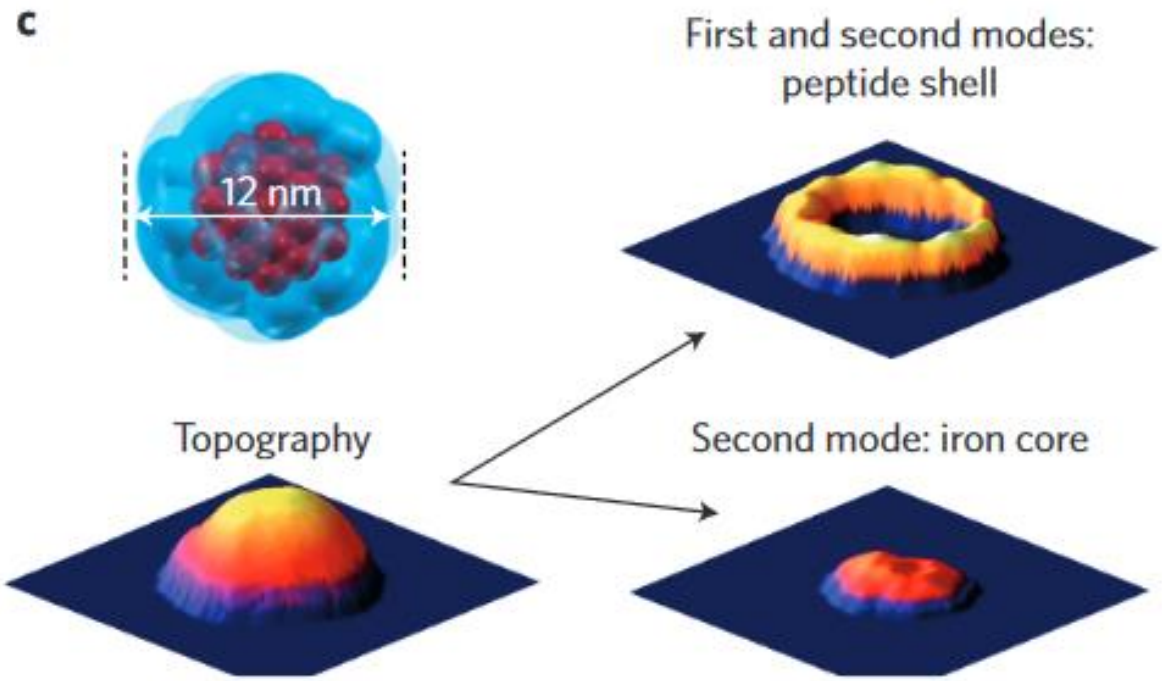
Source: Dufrêne, Y., Ando, T., Garcia, R. *et al.* Imaging modes of atomic force microscopy for application in molecular and cell biology. *Nature Nanotech* **12,** 295–307 (2017).



Scheme of the deflection of the cantilever in bimodal AFM. b, Two eigenmodes of the cantilever are excited and detected. Observables associated with both eigenmodes are recorded to determine sample properties such as flexibility, deformation and viscosity.

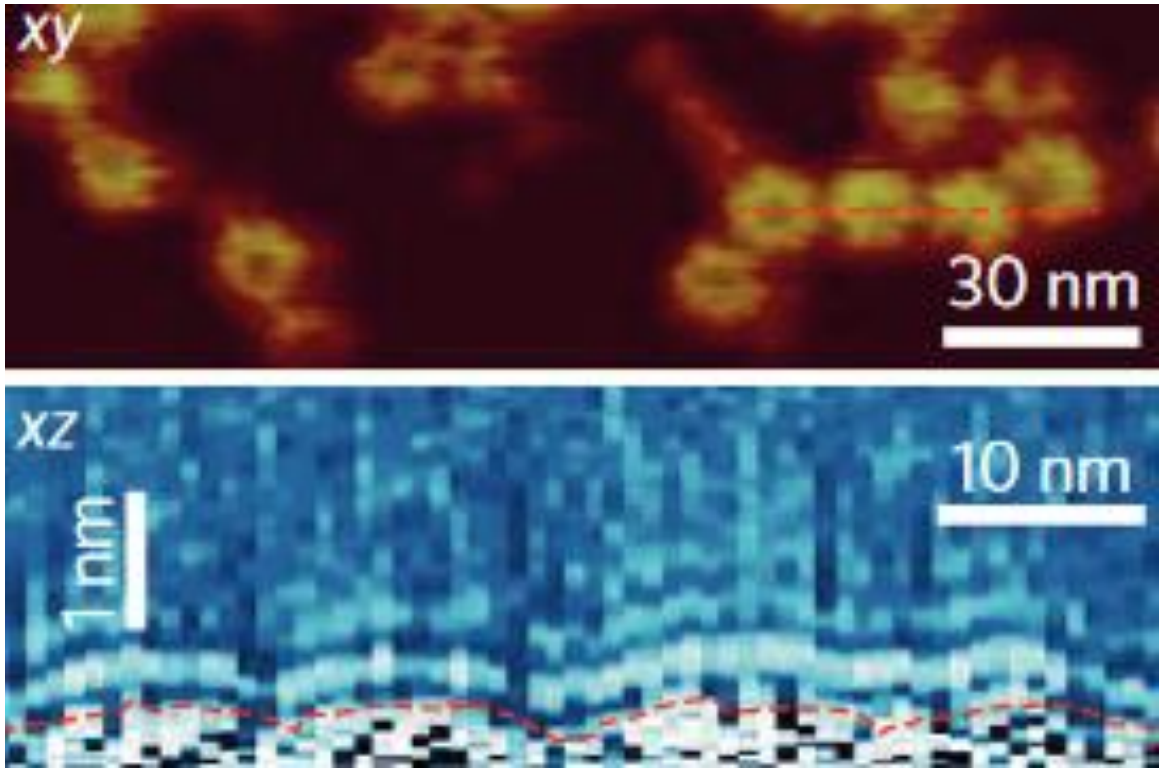


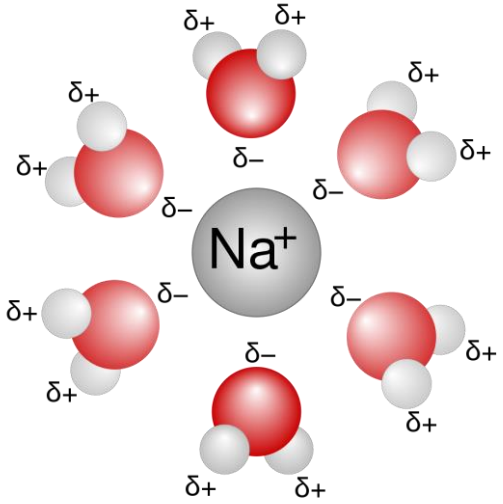
Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms, including archaea, bacteria, algae, higher plants, and animals. It is the primary *intracellular iron-storage protein* in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. In humans, it acts as a buffer against iron deficiency and iron overload.



GroEL is a protein which belongs to the chaperonin family of molecular chaperones, and is found in many bacteria. It is required for the proper folding of many proteins

Multifrequency AFM is able to resolve multiple hydration layers of the GrOEL proteins (red dashed line = protein surface)

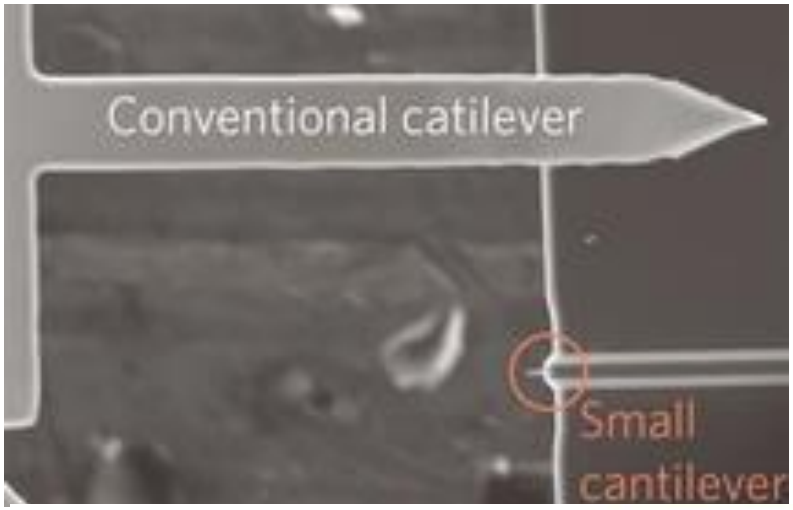


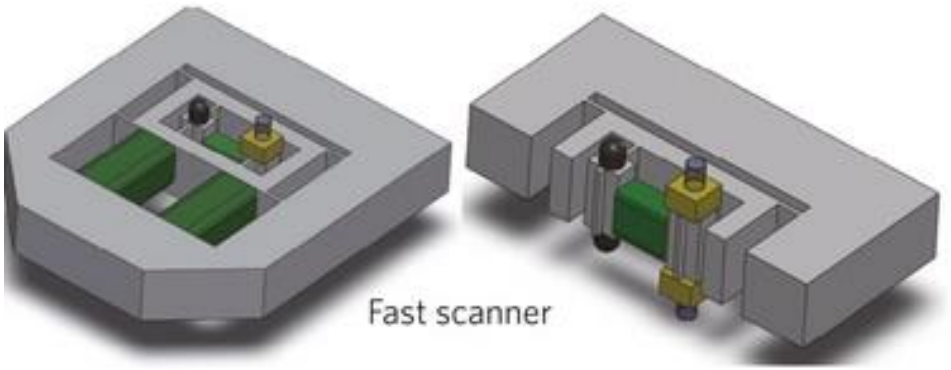


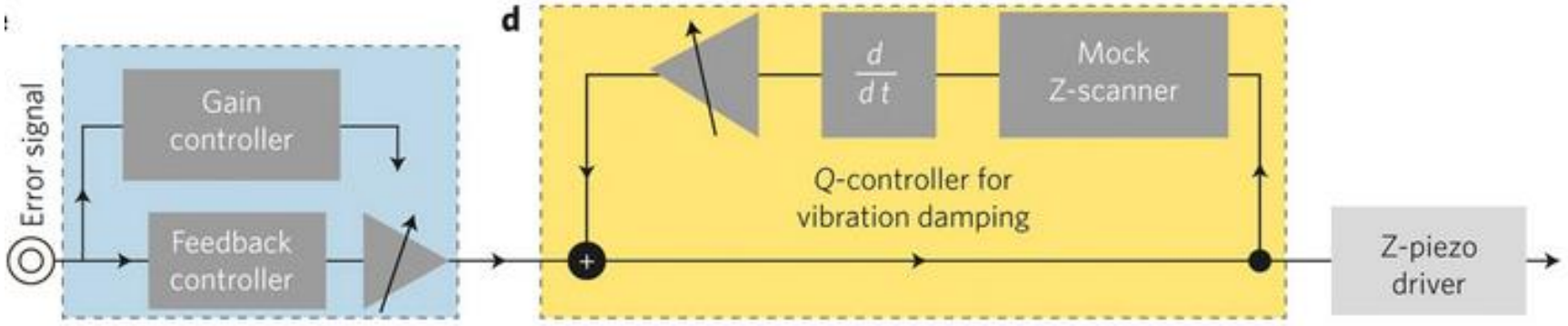
The first solvation/hydration shell of a sodium ion dissolved in water

High-Speed (HS) AFM

Imaging 1000x faster than before!





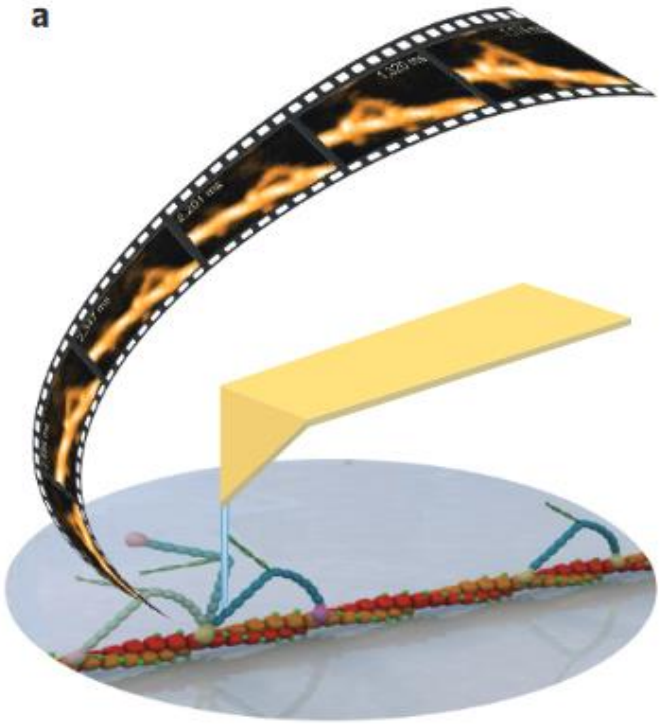


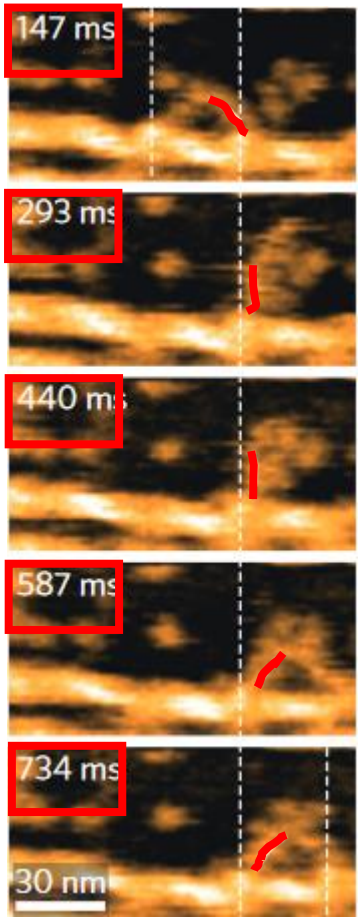
906 IEEE TRANSACTIONS ON CONTROL SYSTEMS TECHNOLOGY, VOL. 15, NO. 5, SEPTEMBER 2007

Design and Modeling of a High-Speed AFM-Scanner

Georg Schitter, *Member, IEEE*, Karl J. Åström, *Fellow, IEEE*, Barry E. DeMartini, *Member, IEEE*, Philipp J. Thurner, Kimberly L. Turner, and Paul K. Hansma

Myosins are a superfamily of motor proteins best known for their roles in muscle contraction and in a wide range of other motility processes in eukaryotes. They are ATP-dependent and responsible for actin-based motility



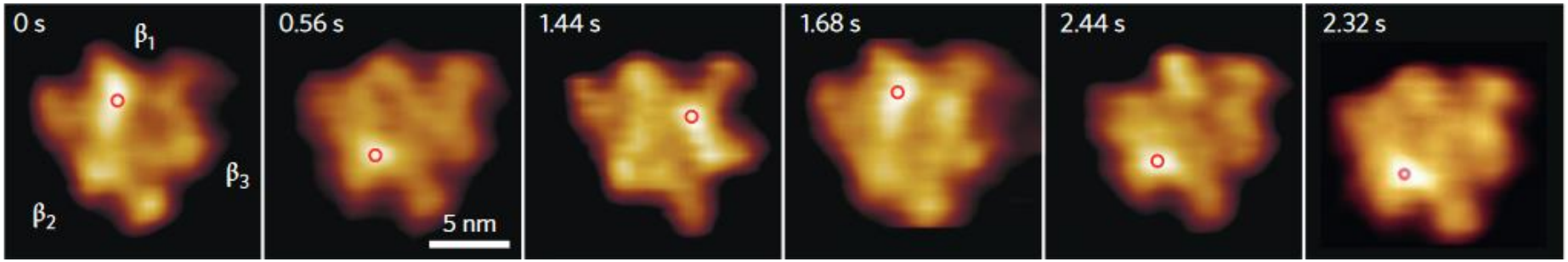


Myosin V walking unidirectionally along an actin filament, showing forward rotation of the leading lever-arm on trailing head detachment from actin

High-speed AFM Imaging example

Motorless F1-ATPase undergoing conformational changes

F-ATPase, also known as **F-Type ATPase**, is an ATPase/synthase found in bacterial plasma membranes, in mitochondrial inner membranes (in oxidative phosphorylation, where it is known as Complex V), and in chloroplast thylakoid membranes. It uses a proton gradient to drive ATP synthesis by allowing the passive flux of protons across the membrane down their electrochemical gradient and using the energy released by the transport reaction to release newly formed ATP from the active site of F-ATPase



Red circles indicate the highest positions of the topographs. Since a nucleotide-free β-subunit protrudes higher than ADP- and ATP-bound ones, it is observed that the unbound state rotates anticlockwise.

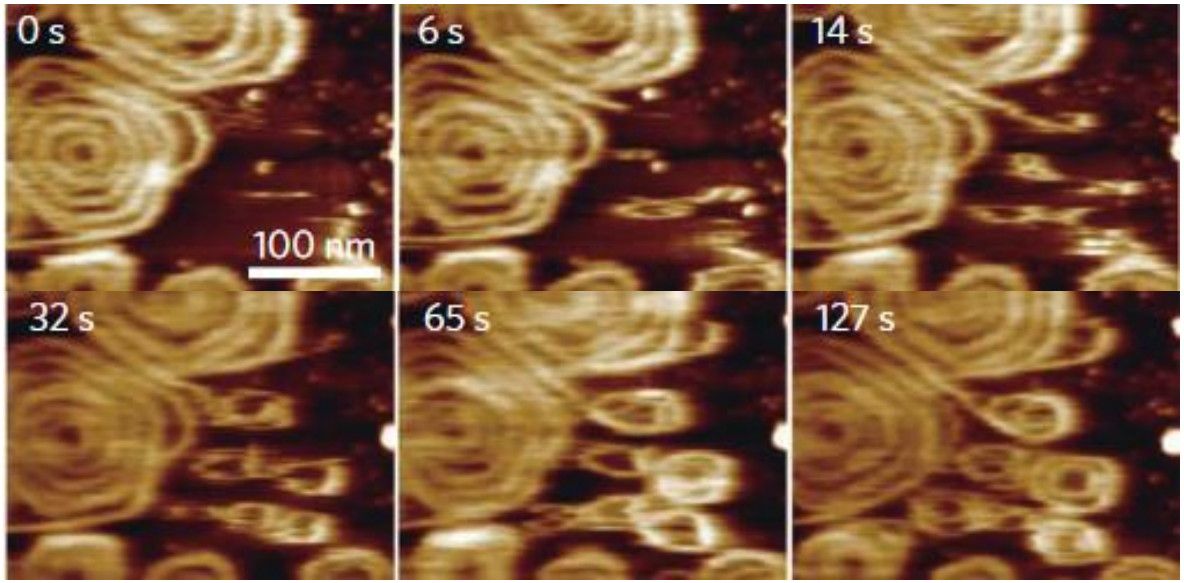
High-speed AFM Imaging example

ESCRT-III protein Snf7 polymerization into spirals on a SLB

The endosomal sorting complexes required for transport (ESCRT) machinery is made up of cytosolic protein complexes, known as ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III.
The ESCRT machinery plays a vital role in a number of cellular processes including

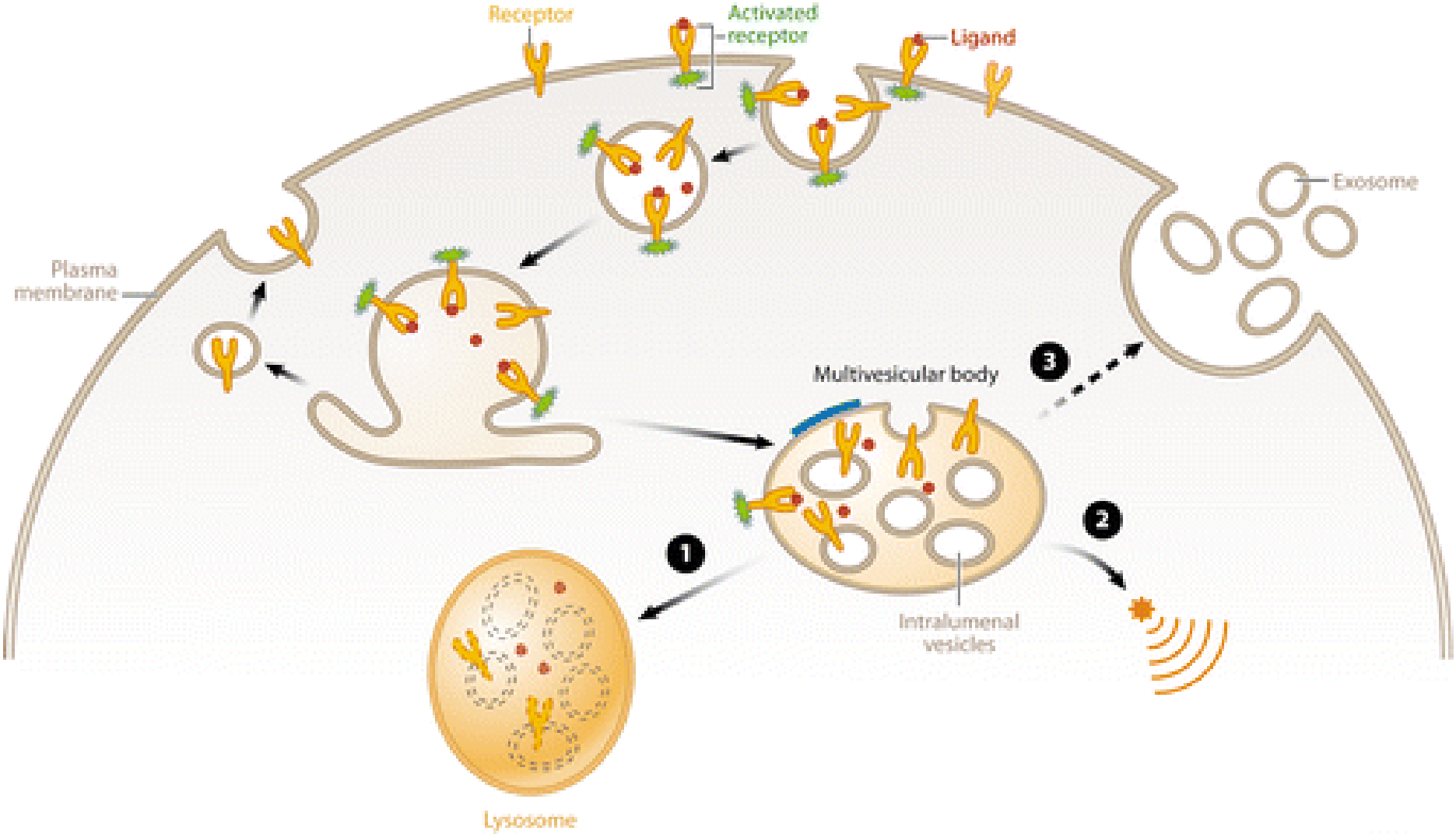
- multivesicular body (MVB) biogenesis
- cellular abscission
- viral budding

Snf7 acts a component of the ESCRT-III complex required for the sorting and concentration of proteins resulting in the entry of these proteins into the invaginating vesicles of the multivesicular body (MVB)



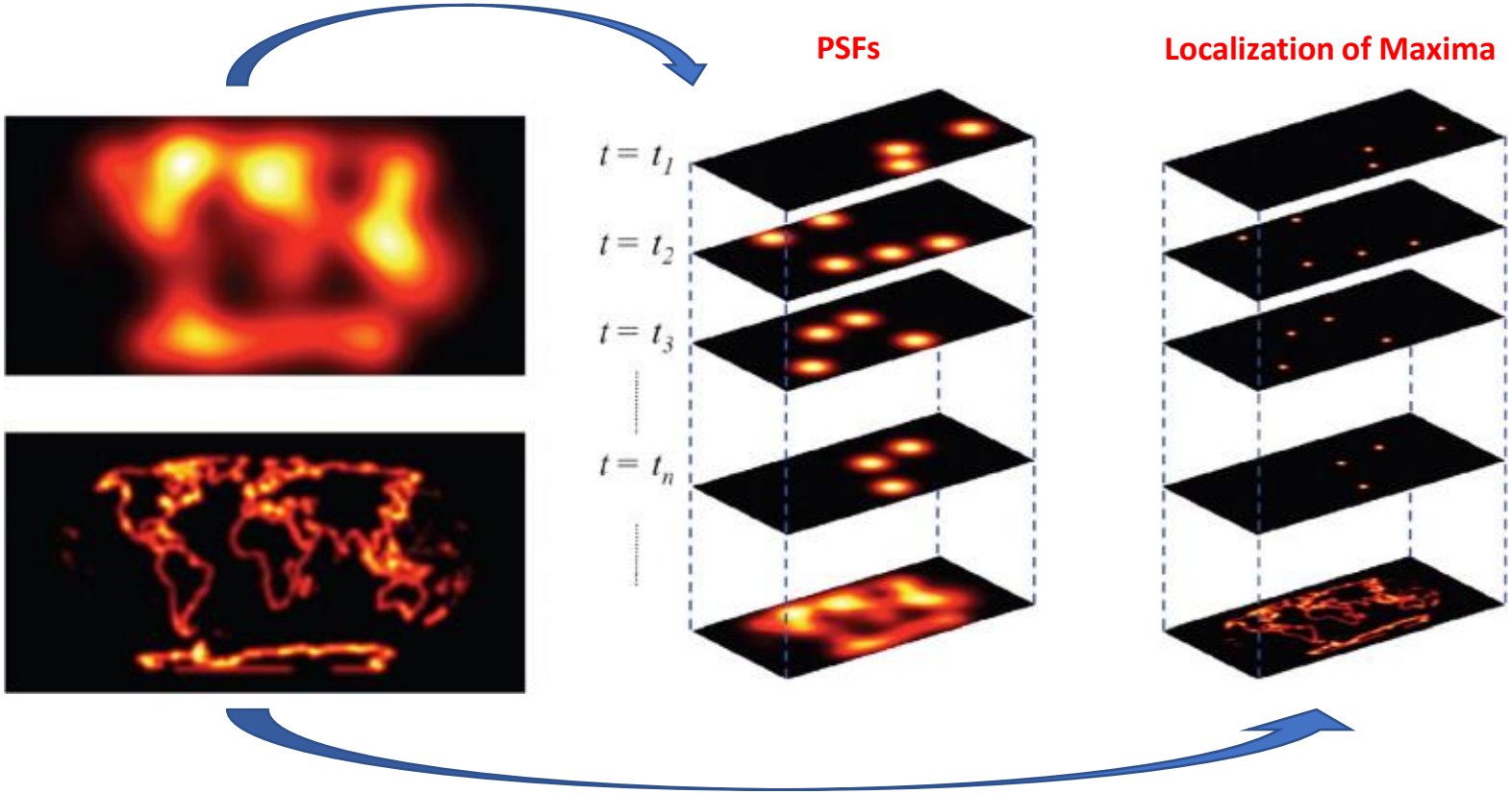
High-speed AFM Imaging example

MVB biogenesis



PALM/STORM

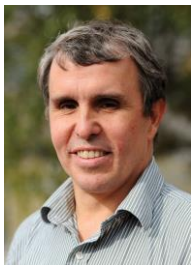
Maxima localization microscopy



Proposed method for molecular optical imaging

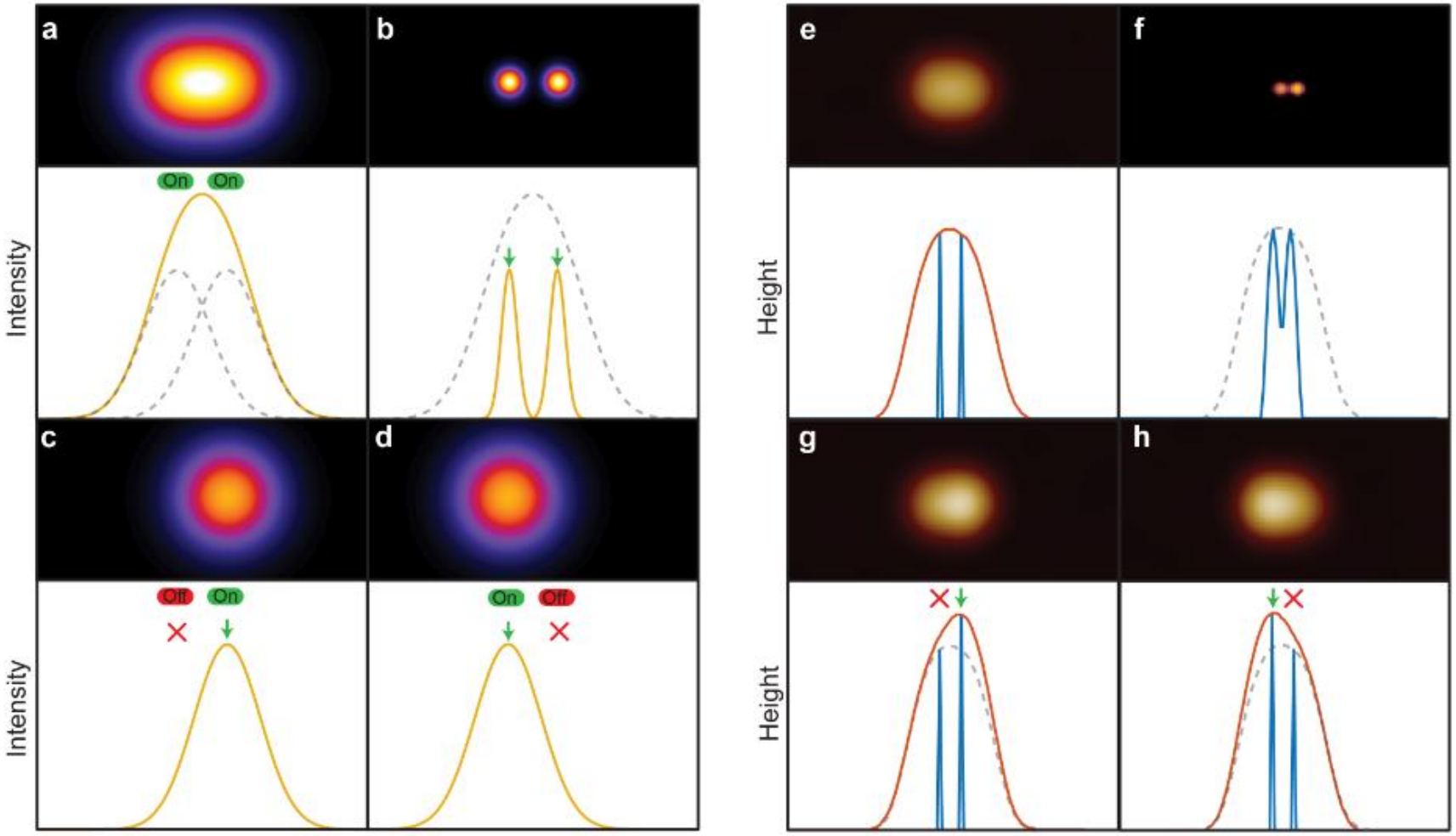
February 1, 1995 / Vol. 20, No. 3 / OPTICS LETTERS 237

E. Betzig



Localization AFM

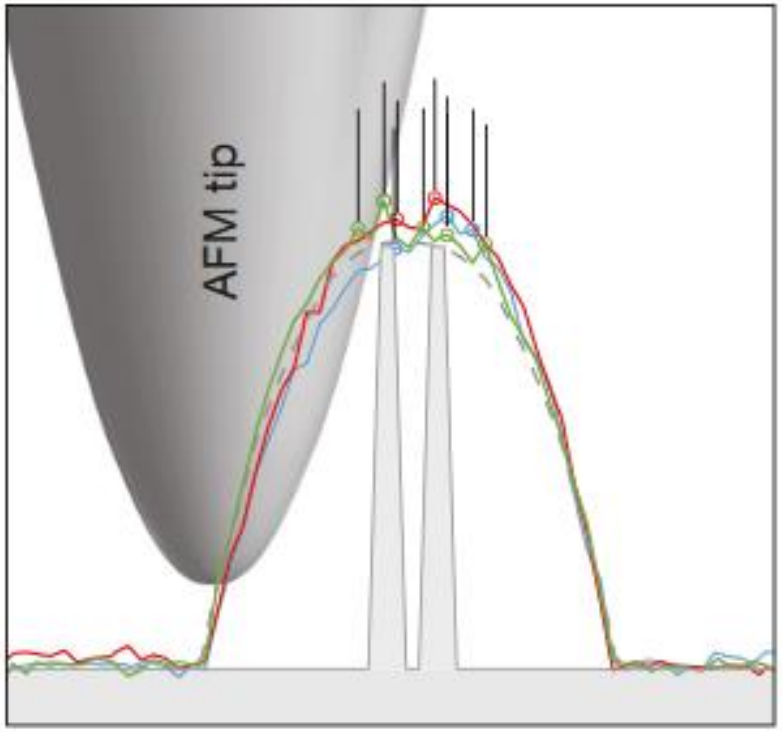
Translation of PALM/STORM concept to the AFM world

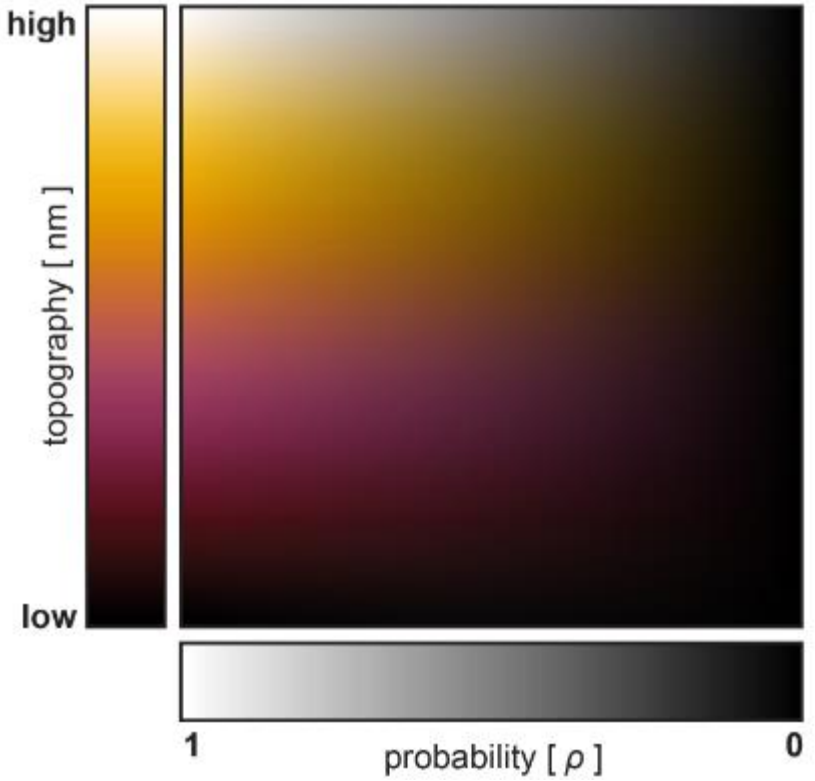


Localization atomic force microscopy

George R. Heath, Ekaterina Kots, Janice L. Robertson, Shifra Lansky, George Khelashvili, Harel Weinstein & Simon Scheuring

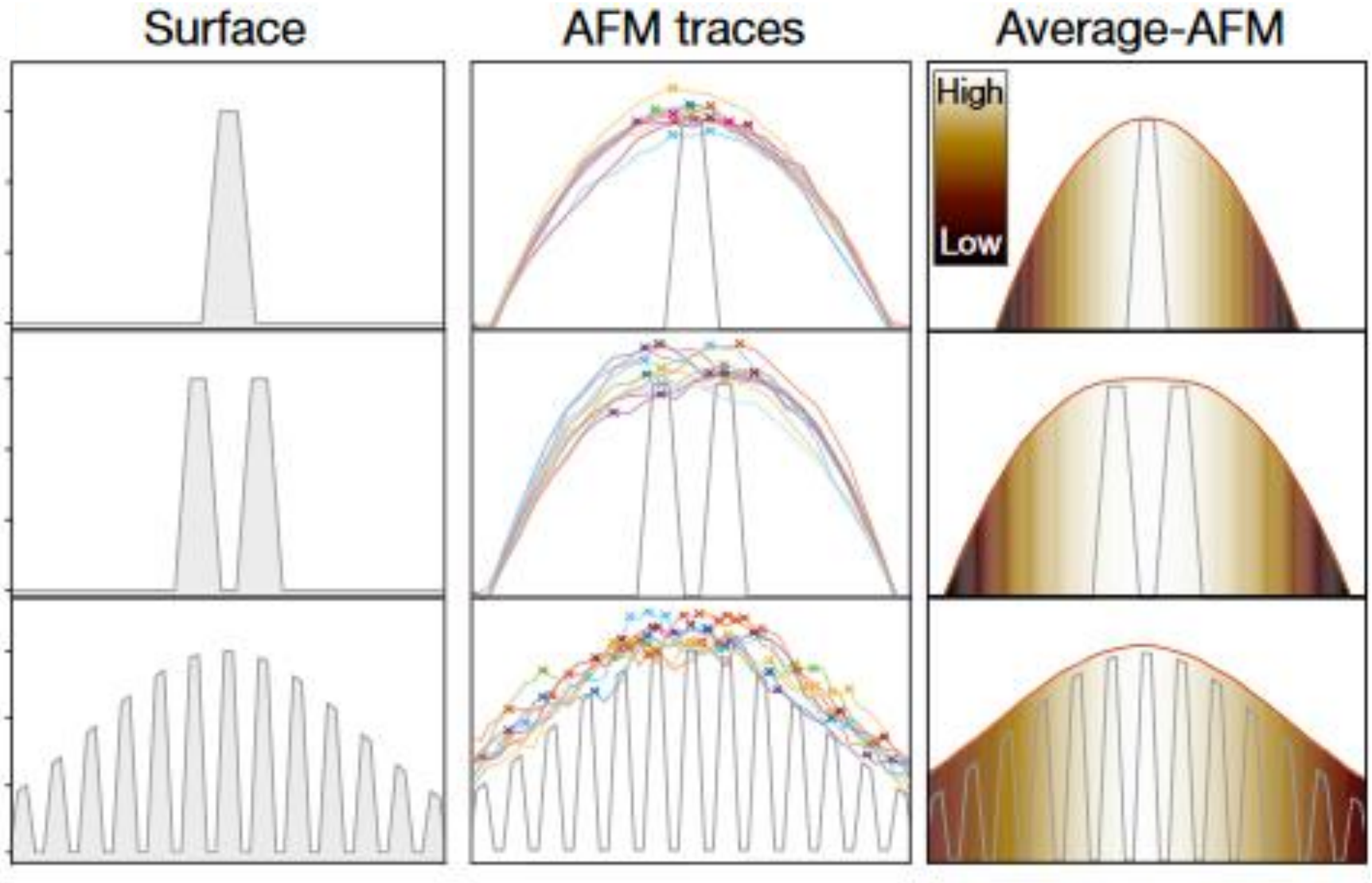
Nature **594**, 385–390 (2021) | Cite this article


AFM tip


high
topography [nm]
low
1
probability [ρ]
0

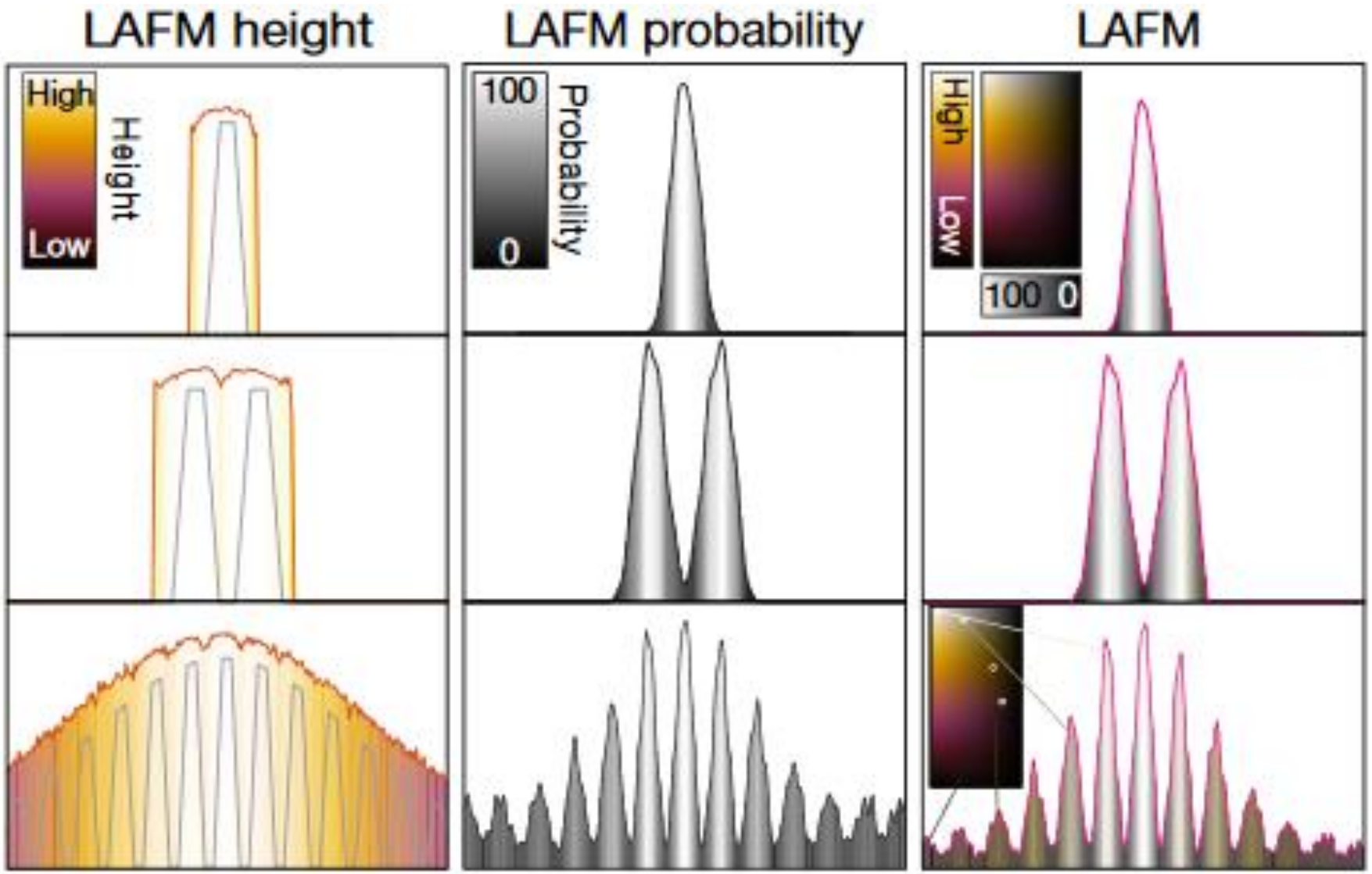
Localization AFM

Surface structure, 9 AFM traces & Average-AFM trace

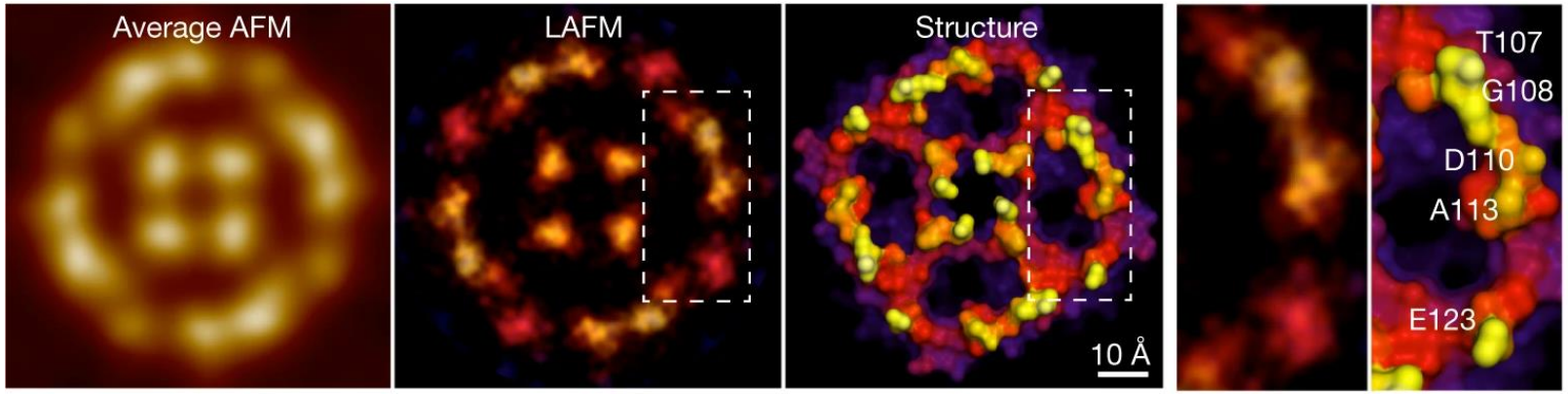


Localization AFM

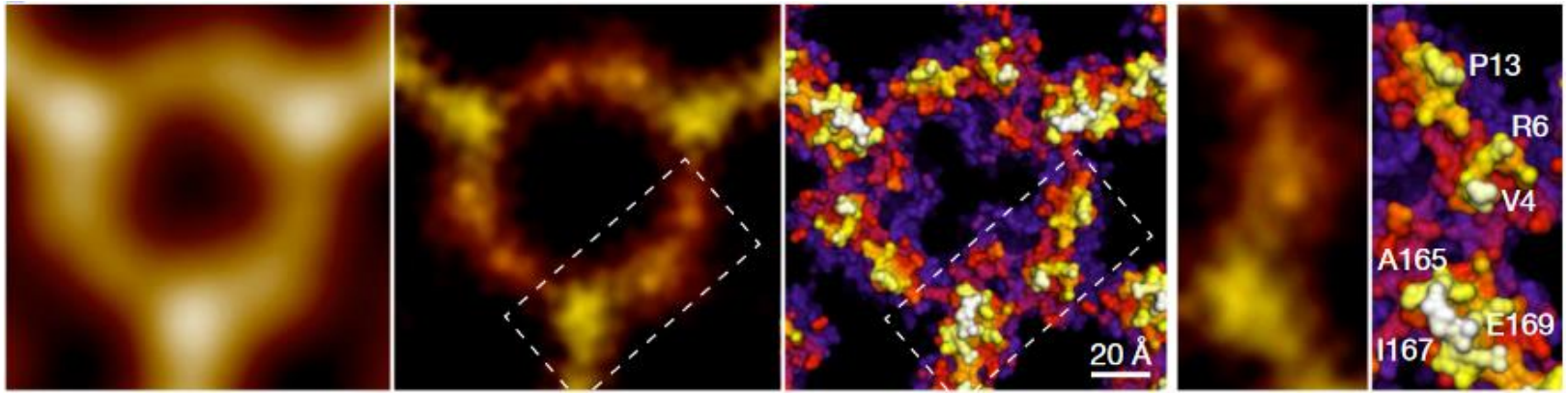
LAFM height, probability and LAFM mapping

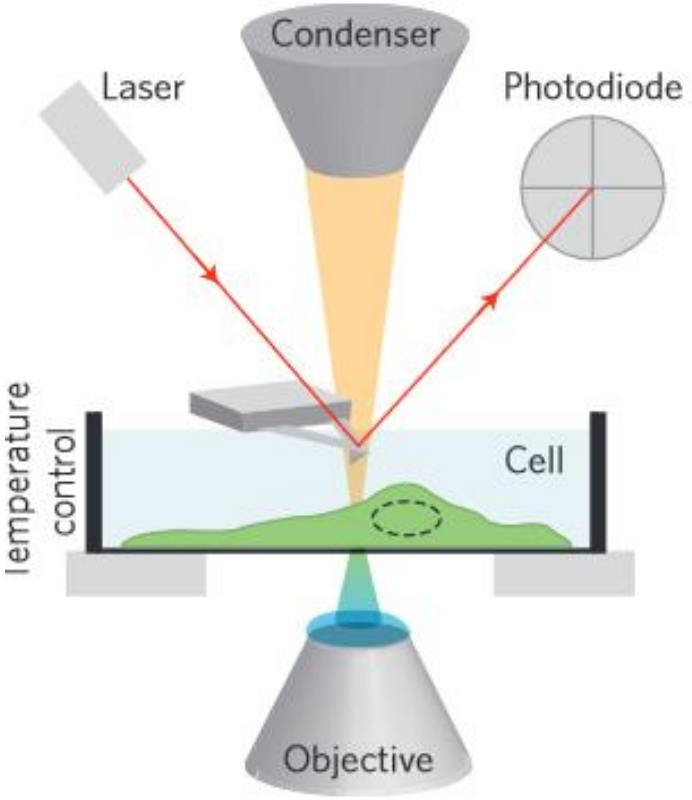


Aquaporin Z (AqpZ) is an integral membrane protein that facilitates transport of water across E. coli cells with a high rate



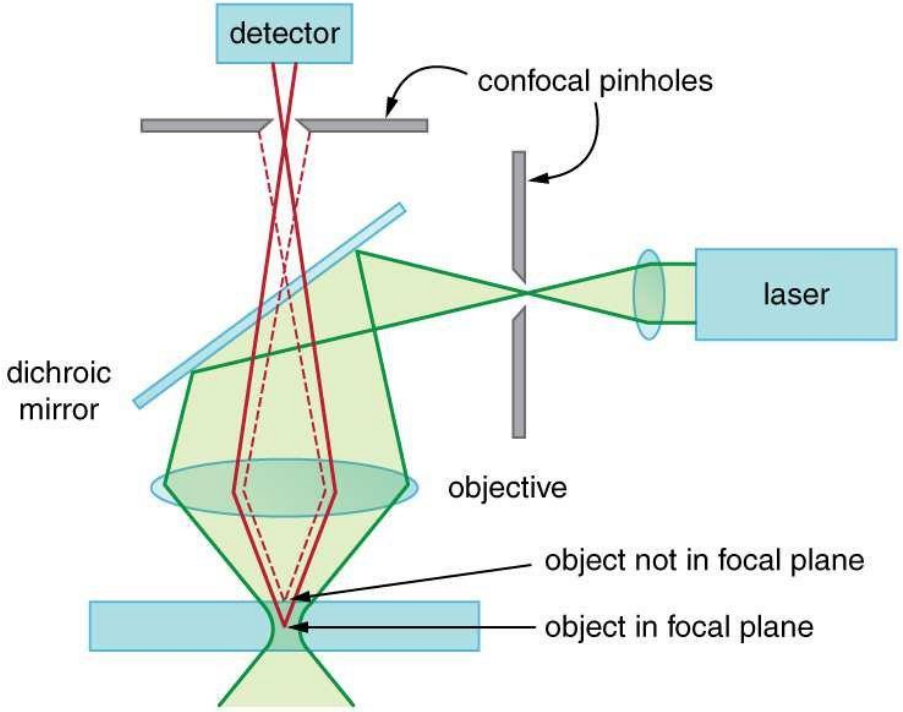
Annexin A5 (or annexin V) is a cellular protein in the annexin group. Annexin V is commonly used to detect apoptotic cells by its ability to bind to phosphatidylserine, a marker of apoptosis when it is on the outer leaflet of the plasma membrane.





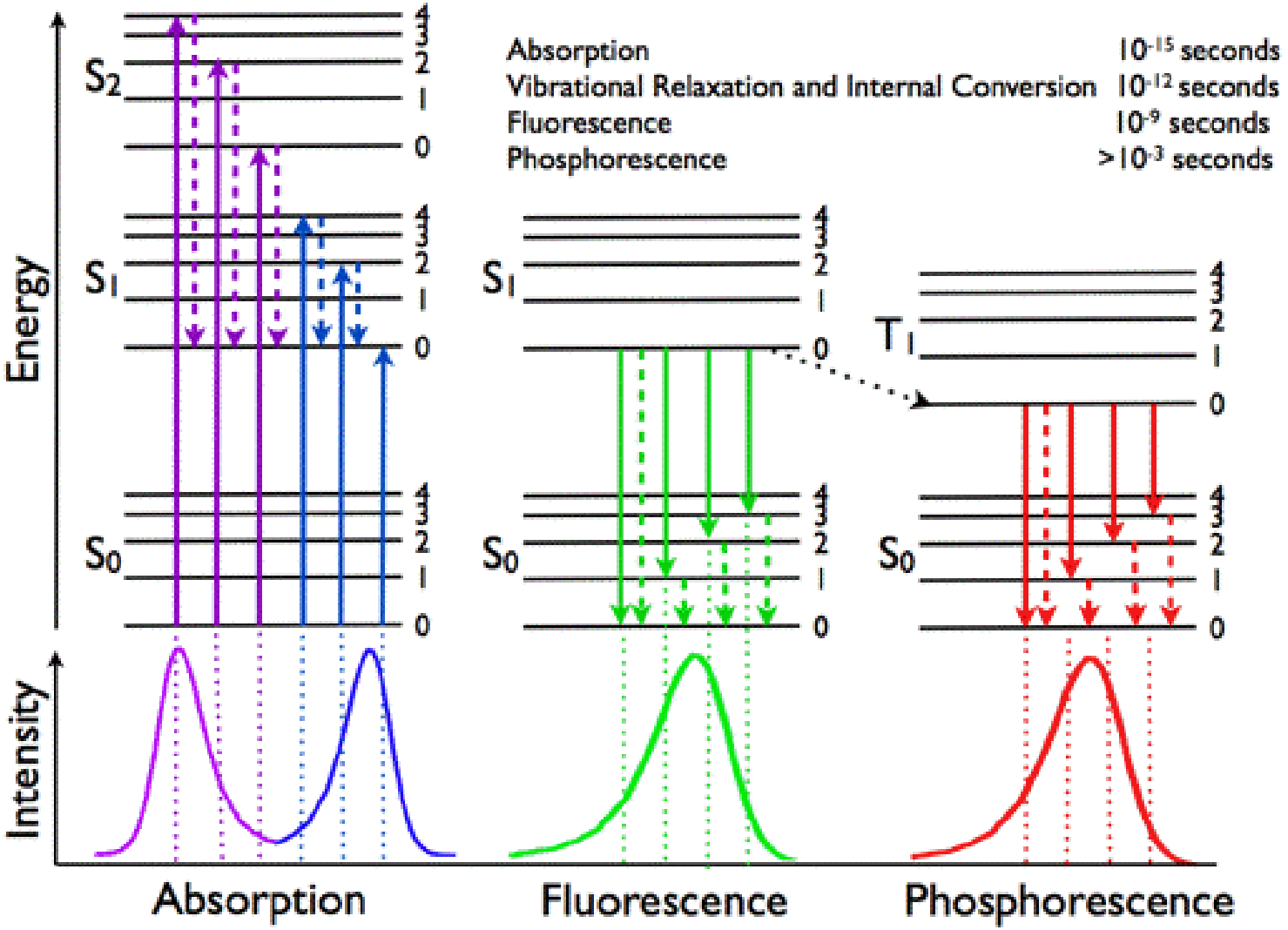
Various Flavours of Opto-AFM

Laser Scanning Confocal microscopy (LSCM) is most widely applied Opto-AFM system integration:

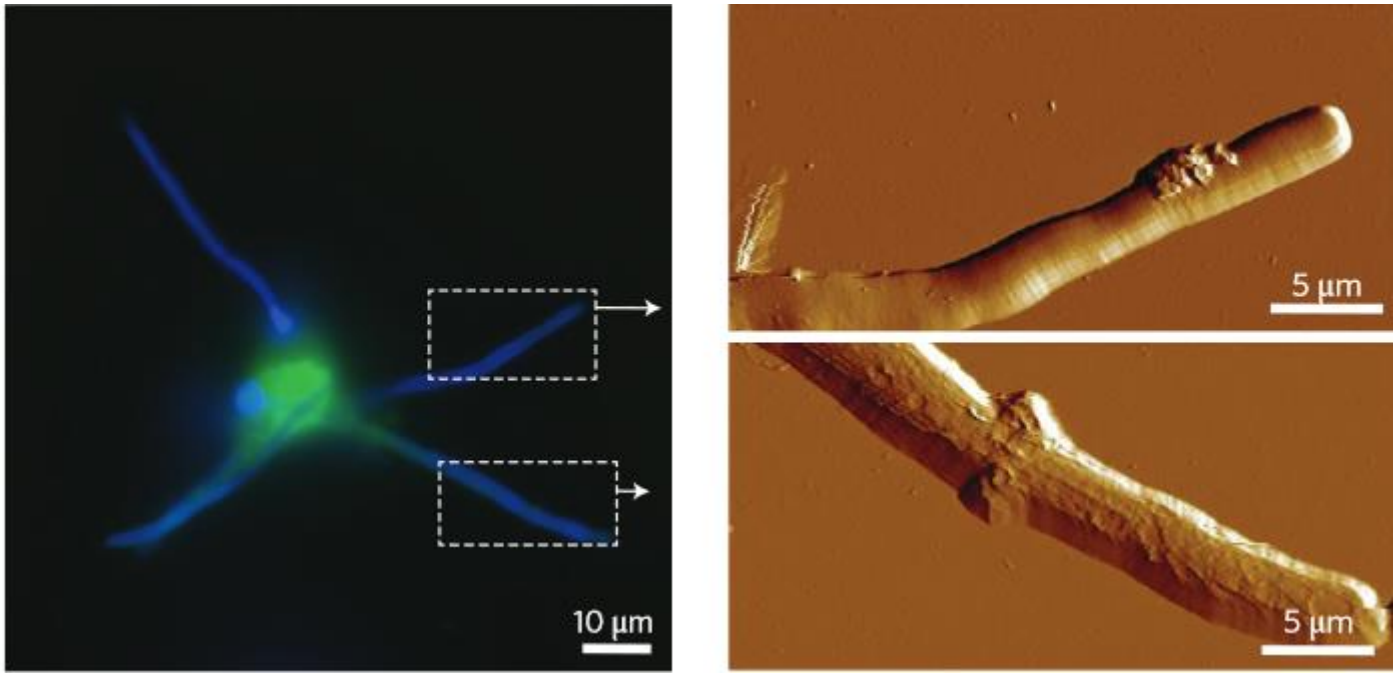


Jabłoński diagram

Transition between molecular electronic states



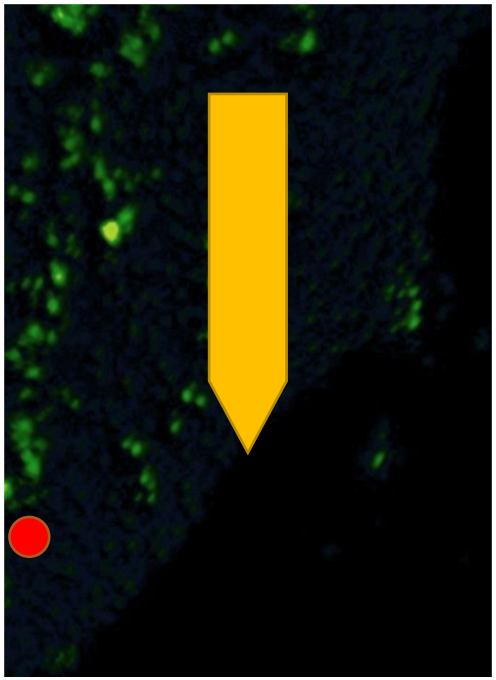
Fluorescence image (left) and correlative AFM images (right) of a macrophage (green) incubated for 3 h with cells from Candida Albicans (blue). AFM images are enlarged views of the dashed areas shown in the fluorescence image. Internalized (bottom) and externalized (top) hyphae featuring major structural differences



CLSM / AFM limitations

Artefacts restricting simultaneous and colocalized data acquistion

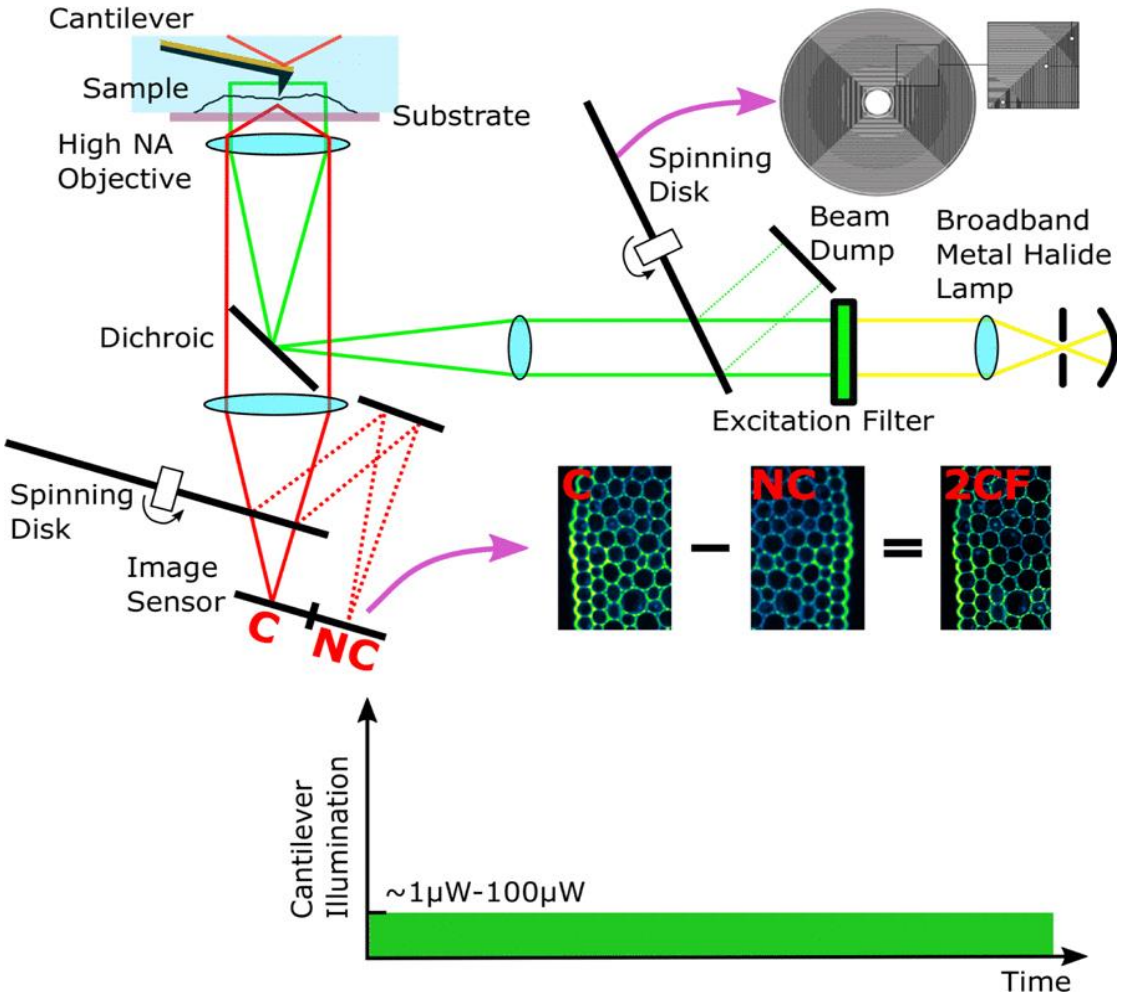
Typical scanning for live cells	Field of View	Image Rep Rate
CLSM	~220µm	1Hz
AFM	100µm	0.05Hz



- Fluorescence excitation light interacting with the cantilever causes uncontrolled cantilever bending and localized heating due to absorption, mainly of the Au cantilever surface coating
- Mechanical noise transfer from optical microscopy system to AFM camera fans & other moving parts

Simultaneous combined imaging

Differential Spinning Disk (DSD) microscopy



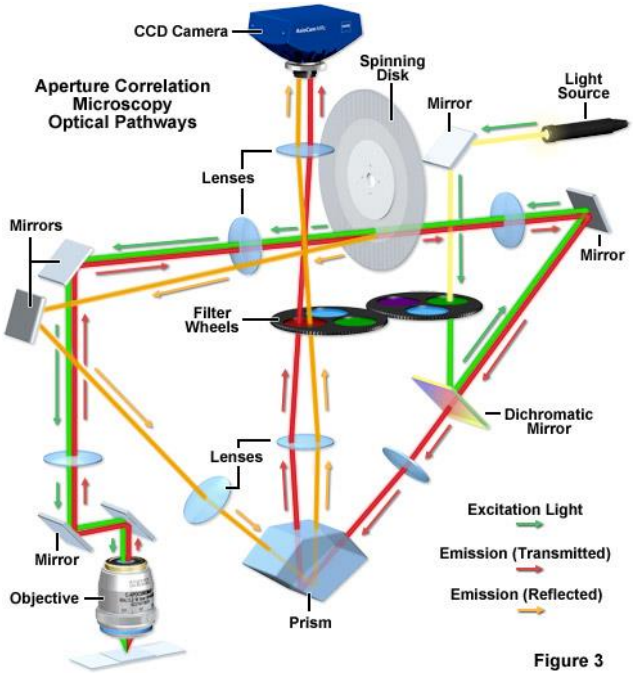
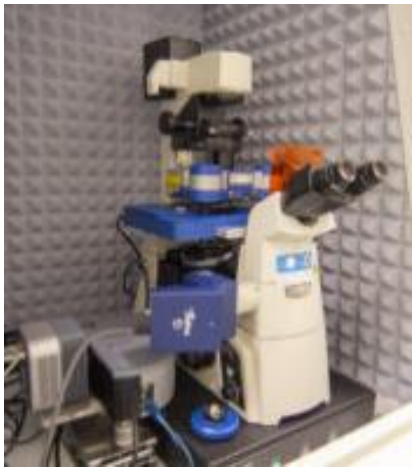


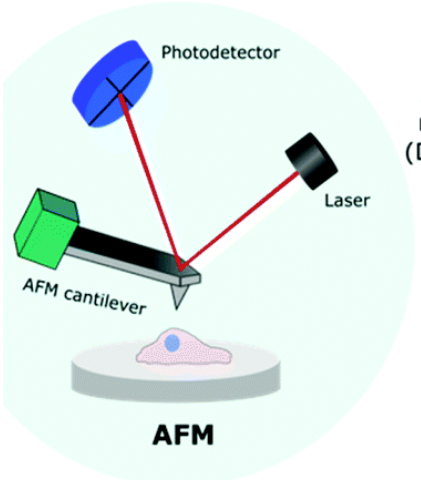
Figure 3

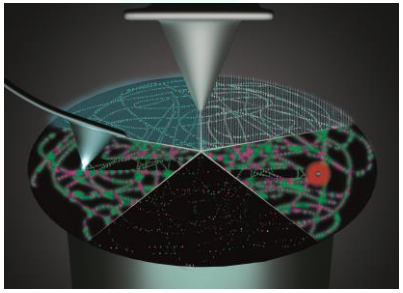


Opto-AFM with super-resolution fluorescence microscopy

Historical timeline

Some key developments in combined AFM and Super-Resolution optical schemes





Far-field techniques

- 2011 — AFM/Blink microscopy (Duim *et al.*)
- 2012 — AFM/STED integration (Chacko *et al.*)
- 2013 — AFM/dSTORM combination (Turner *et al.*)
- 2014 — *in situ* AFM & SLML (Monserrate *et al.*)
- 2015 — AFM/PALM integration (Odermatt *et al.*)
- 2017 — Correlative AFM/STED on live cells (Curry *et al.*)
- 2018 — New dye for buffer compatible in AFM/STORM (Hirvonen *et al.*)
- 2020 — Simultaneous co-localized AFM/SR-SIM (Gómez *et al.*)

Near-field techniques

- 2000 — AFM/TIRFM study of force transmission in endothelial cells (Mathur *et al.*)
- 2002 — AFM/TIRFM single cell nanomanipulation (Nishida *et al.*)
- 2006 — Spatially and temporally synchronised AFM/TIRFM (Kellermayer *et al.*)
- 2013 — HS-AFM/TIRFM simultaneous imaging (Fukuda *et al.*)
- 2020 — HS-AFM/HS-SNOM HS-AFM/tip-enhanced TIRFM (Umakoshi *et al.*)

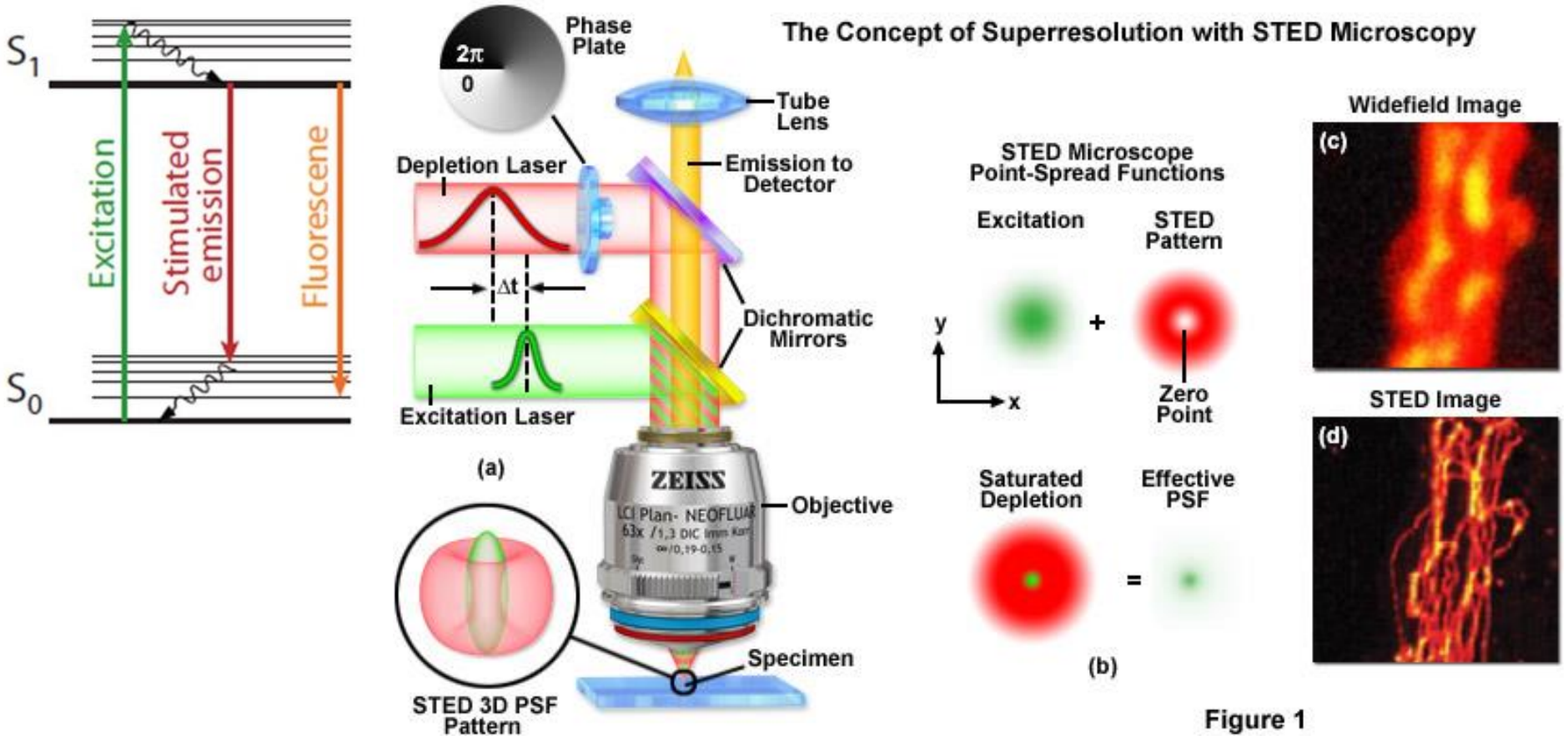
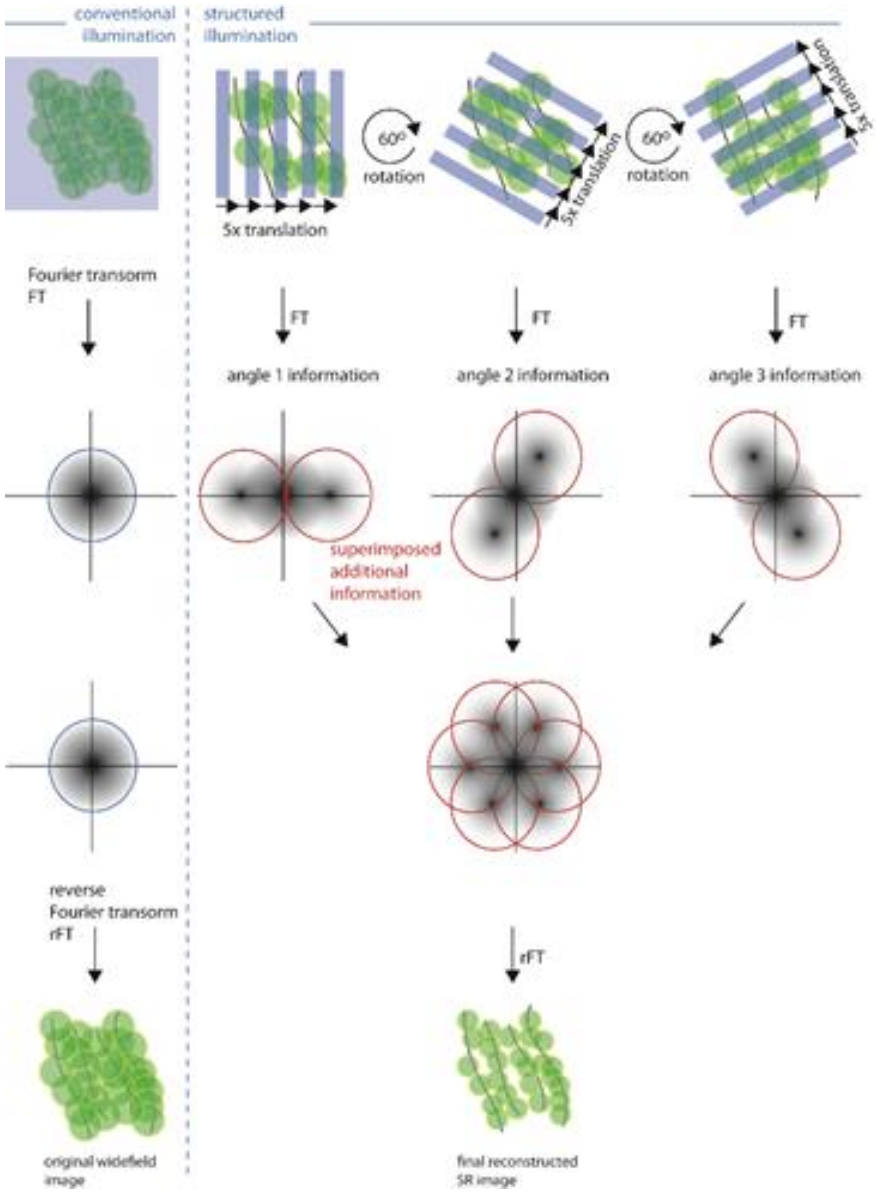

S1
S0
Excitation
Stimulated emission
Fluorescene
Phase Plate
2π
0
Tube Lens
Depletion Laser
Emission to Detector
Δt
Dichromatic Mirrors
Excitation Laser
(a)
ZEISS
Objective
Specimen
STED 3D PSF Pattern
The Concept of Superresolution with STED Microscopy
STED Microscope Point-Spread Functions
Excitation
STED Pattern
y
x
Zero Point
Saturated Depletion
Effective PSF
(b)
Widefield Image
(c)
STED Image
(d)

Figure 1

Super-resolution Structured Illumination Microscopy

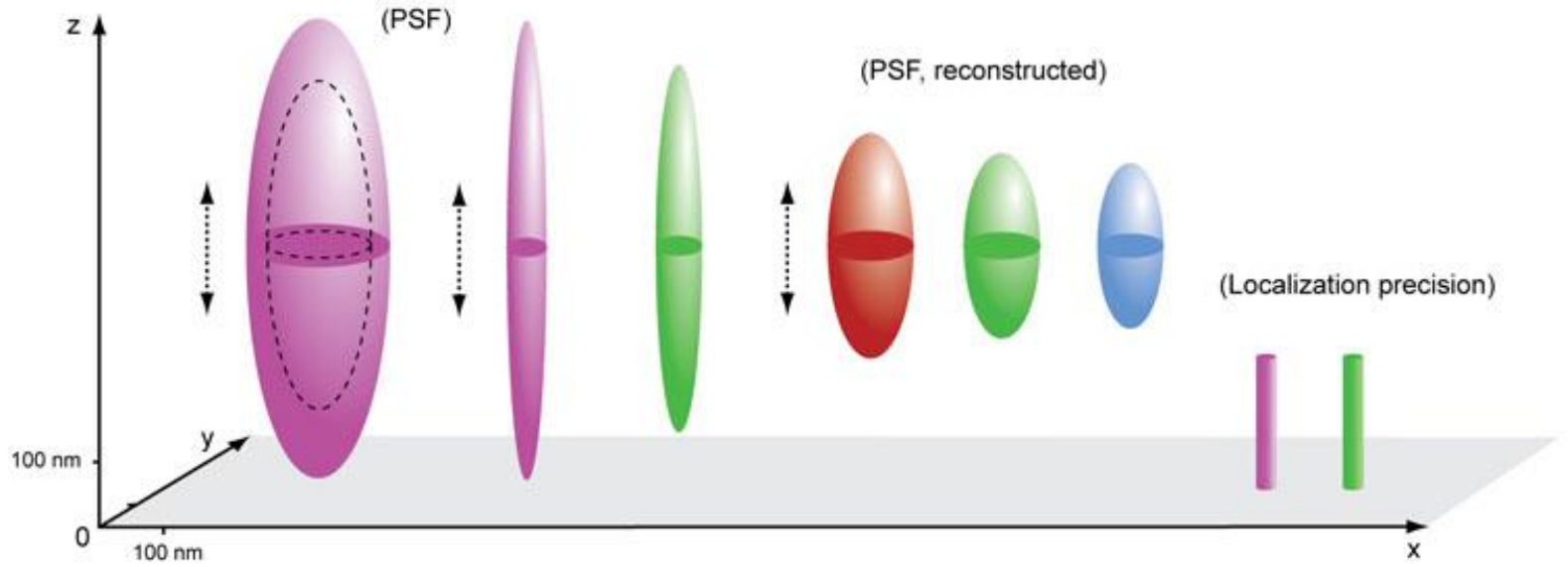
SR-SIM concept



Fluorescence super-resolution

Comparison of resolution limits

	Deconvolution / CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λem [nm]	460-670	670	520	620	520	460	670	520

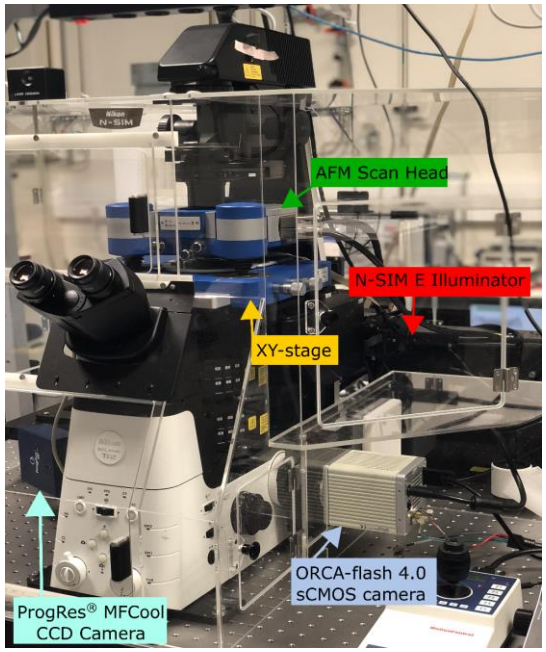


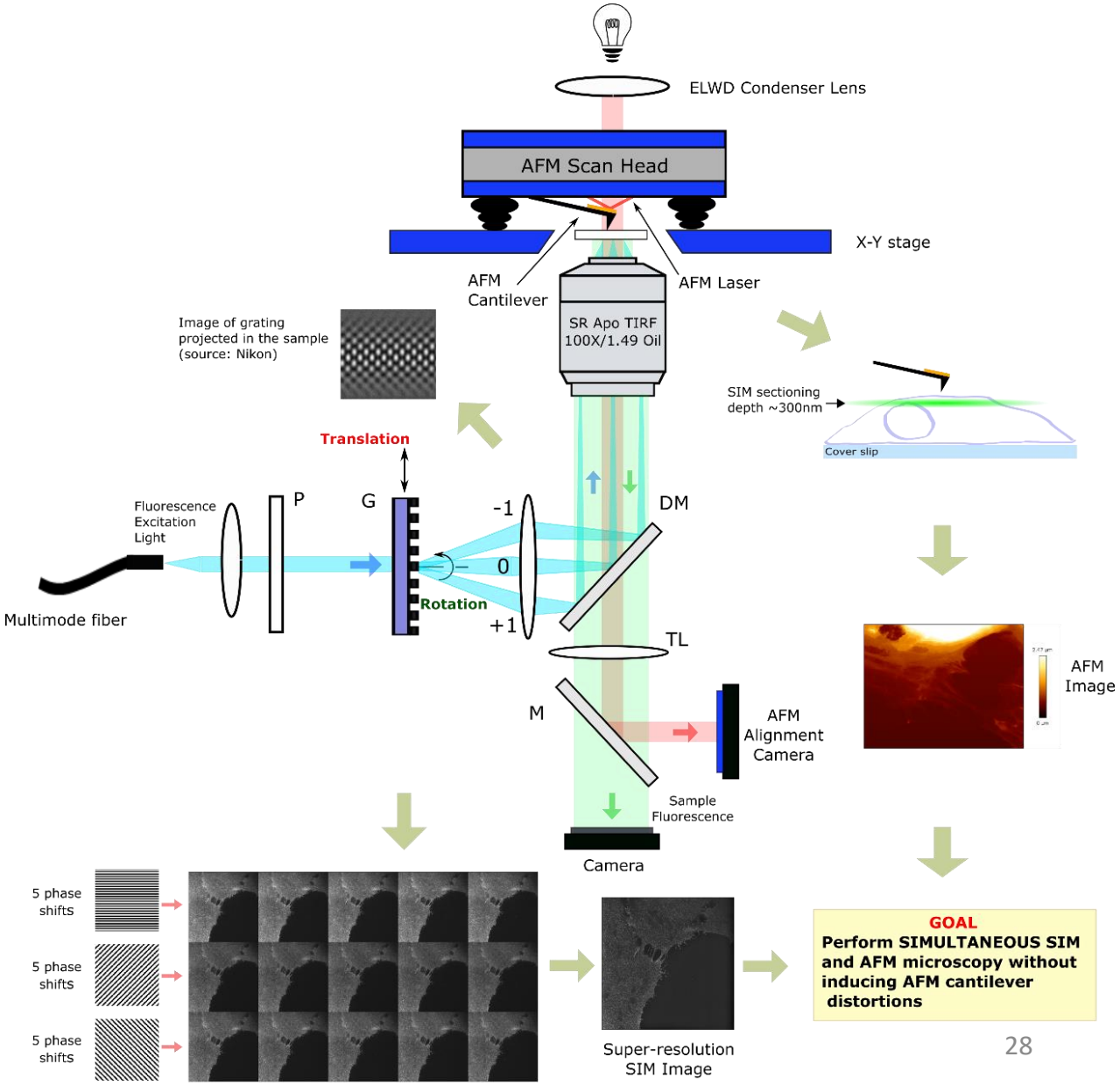
$\sim D_{x,y}$ [nm]	180-250	60	70	130	110	100	20 (precision)
$\sim D_z$ [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
$\sim V_{x,y,z}$ [$*10^{-2}$ µm^3]	7-18	1.3	1.5	3.0	1.8	1.3	< 0.1

Comparison of spatial resolutions achievable with current commercial super-resolution imaging systems (adapted from Schermelleh et al, 2010).

Super-Resolved Structured Illumination (SR-SIM) and AFM

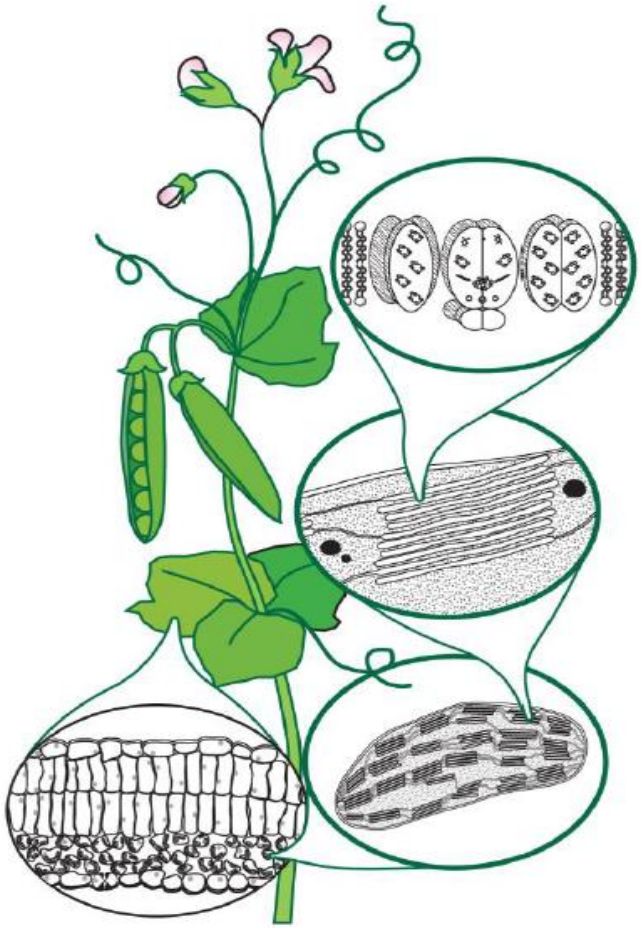
SR-SIM/AFM platform



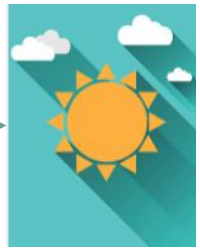


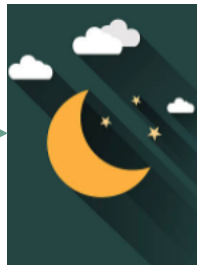
Photosynthesis adaptation to light environment

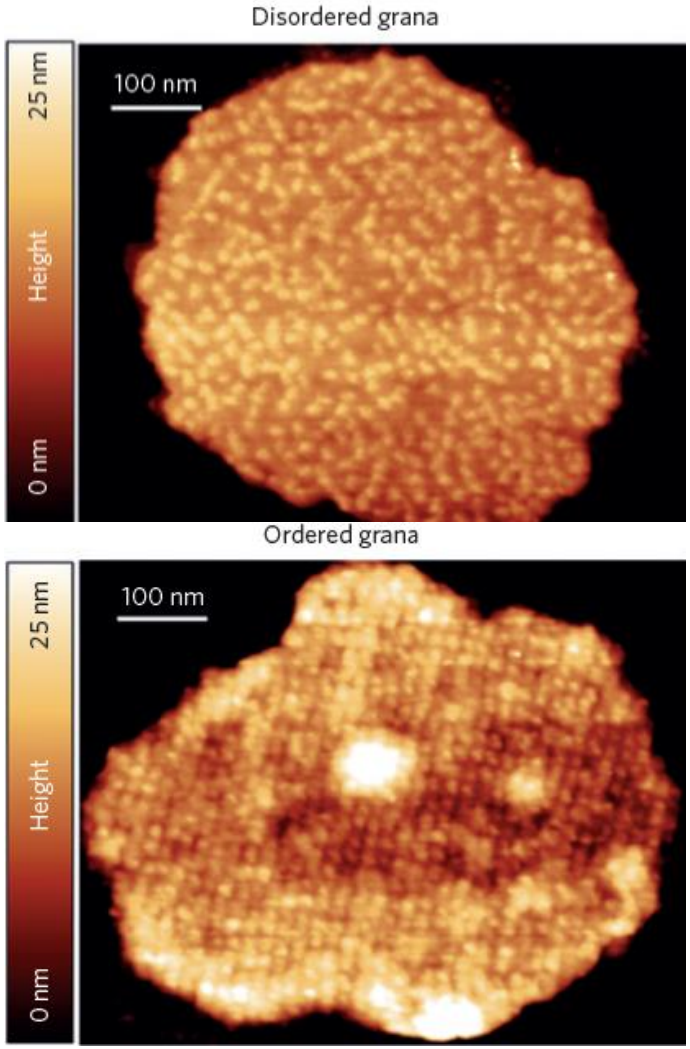
Molecular level adaption of proteins to ambient light by plants



“Real-time”
Light induced
Protein
Conformal
Adaptation

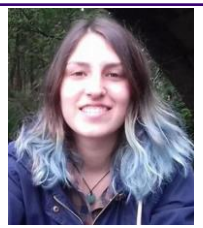


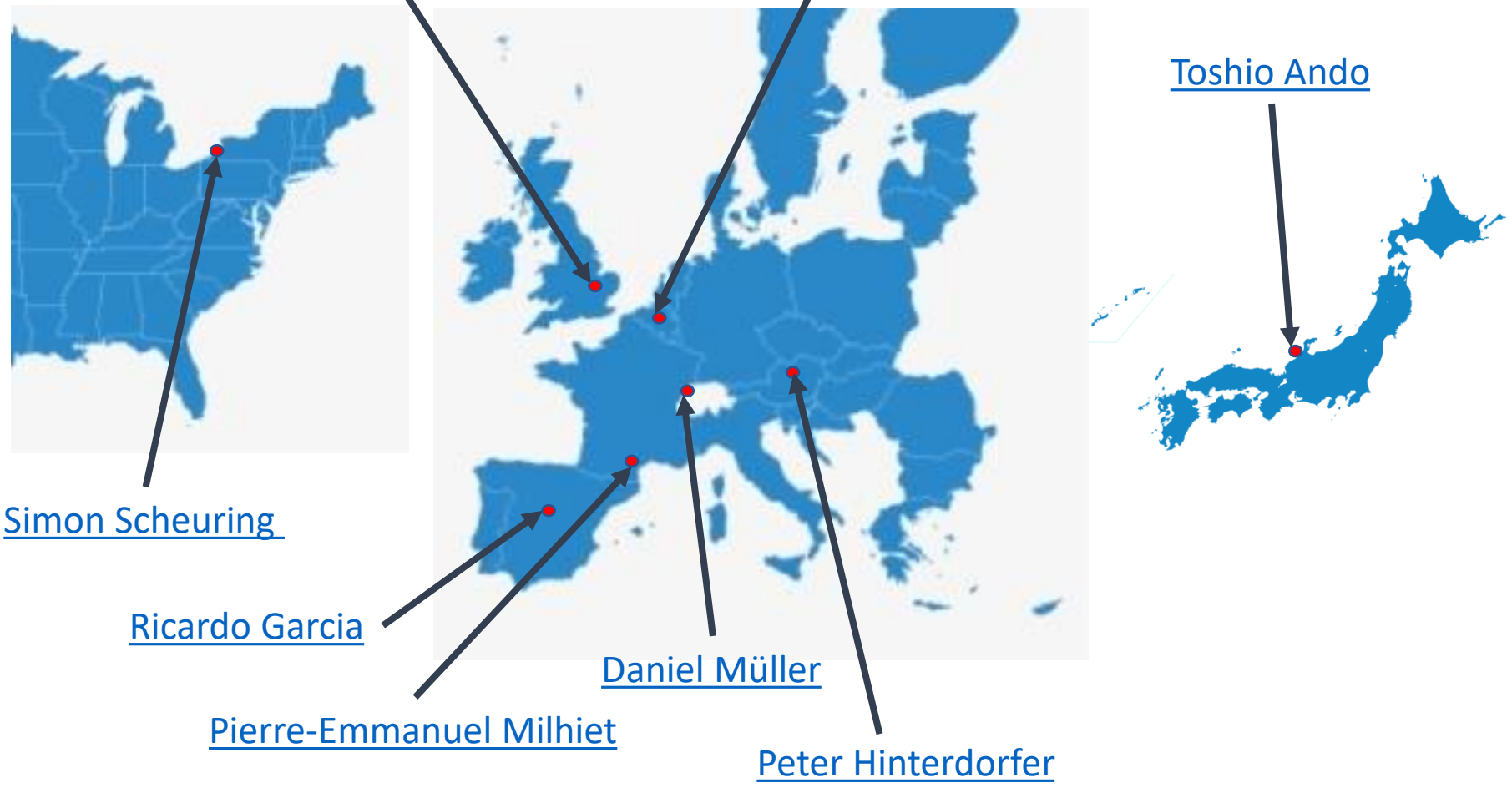


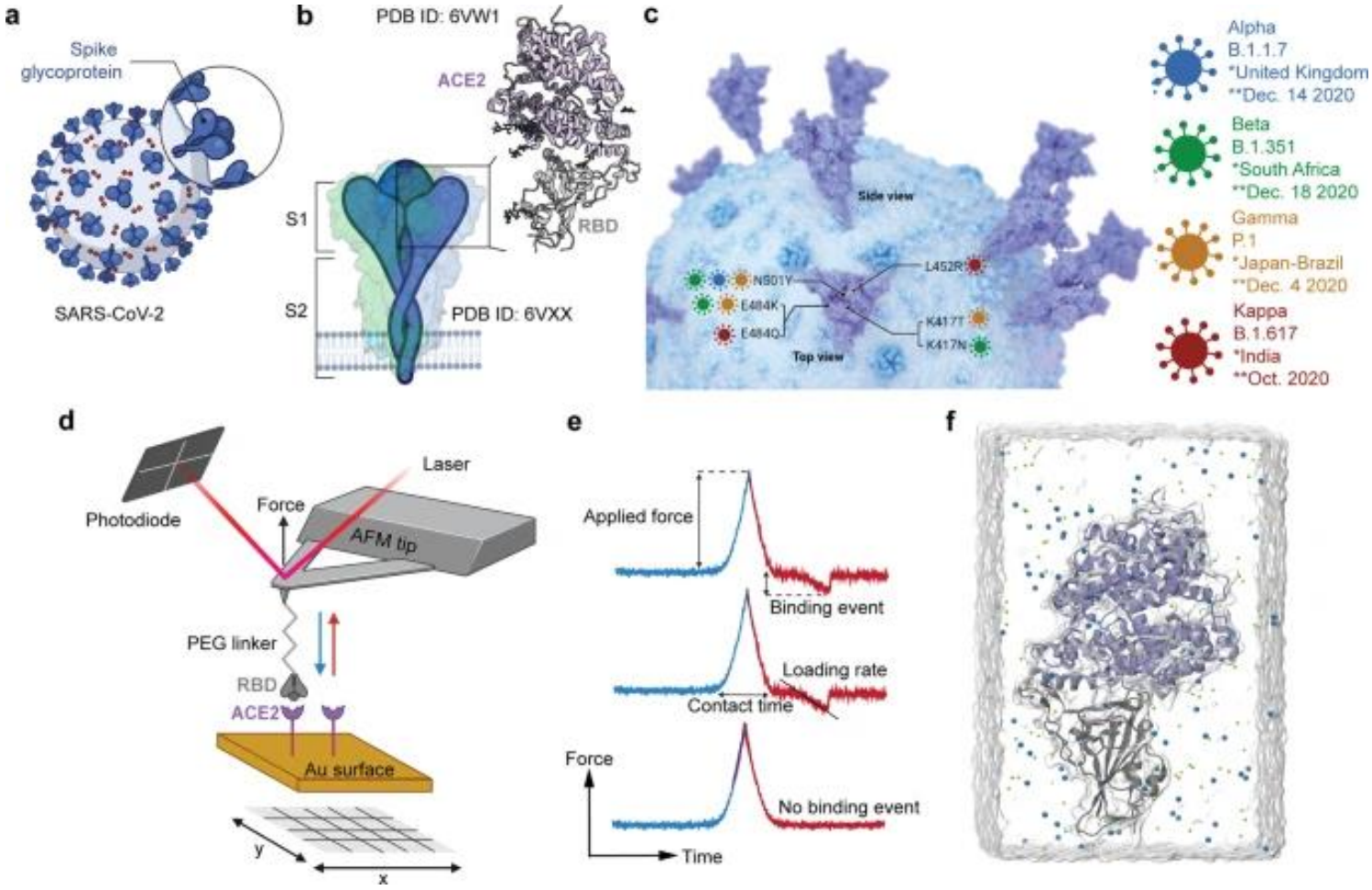


Liquid mode AFM hubs

Who's who in liquid-mode AFM



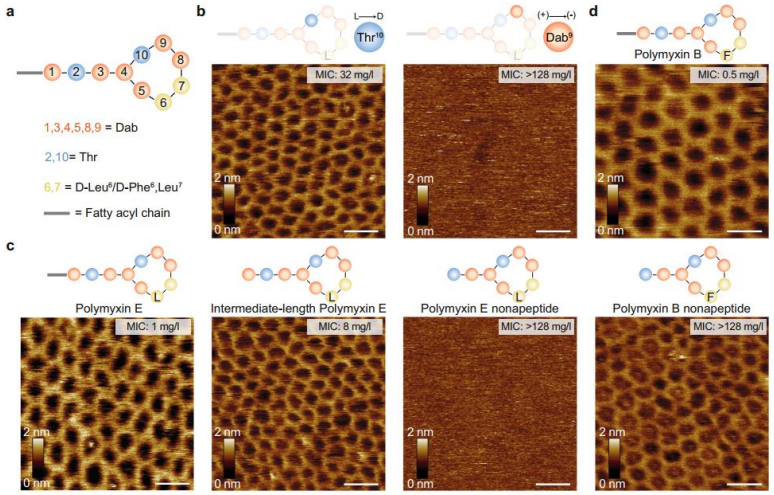




Molecular insights into receptor binding energetics and neutralization of SARS-CoV-2 variants

Melanie Koehler, Ankita Ray, Rodrigo A. Moreira, Blinera Juniku, Adolfo B. Poma ✉ & David Alsteens ✉

Nature Communications **12**, Article number: 6977 (2021) | Cite this article



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Antibiotic polymyxin arranges lipopolysaccharide into crystalline structures to solidify the bacterial membrane

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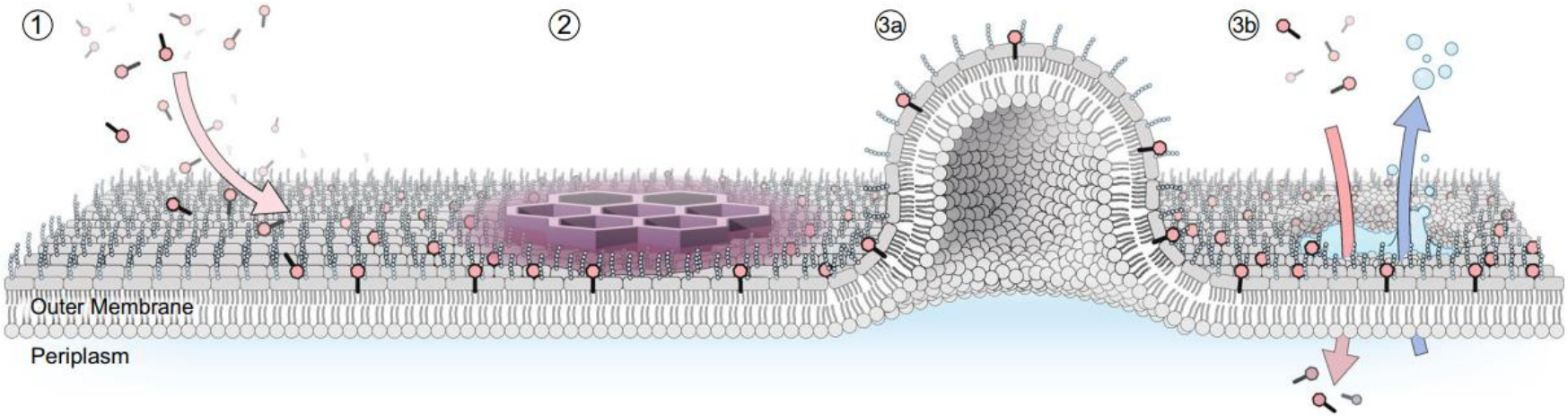

Fig. 7 | Model of the mechanism of action of polymyxins. 1 Polymyxins initially localize on the OM membrane of Gram-negative bacteria through electrostatic interactions with LPS. **2** On the membrane, polymyxins form hexagonal crystalline structures that increase the membrane surface area, decrease the membrane bilayer height, and stiffen the membrane. The altered mechanical properties of the OM lead to (**3a**) the formation of membrane bulging protrusions and (**3b**) membrane rupture, content leak, which allows progression to the inner membrane and subsequent bacterial death.

How coronavirus enters human cells

An example case of using ChimeraX to visualize proteins

UCSF ChimeraX

UCSF ChimeraX (or simply ChimeraX) is the next-generation molecular visualization program from the Resource for Biocomputing, Visualization, and Informatics (RBVI), following UCSF Chimera. ChimeraX can be downloaded free of charge for academic, government, nonprofit, and personal use. Commercial users, please see ChimeraX commercial licensing.