INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

AFM for biological application – Theory 1

Pieter De Beule

24 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

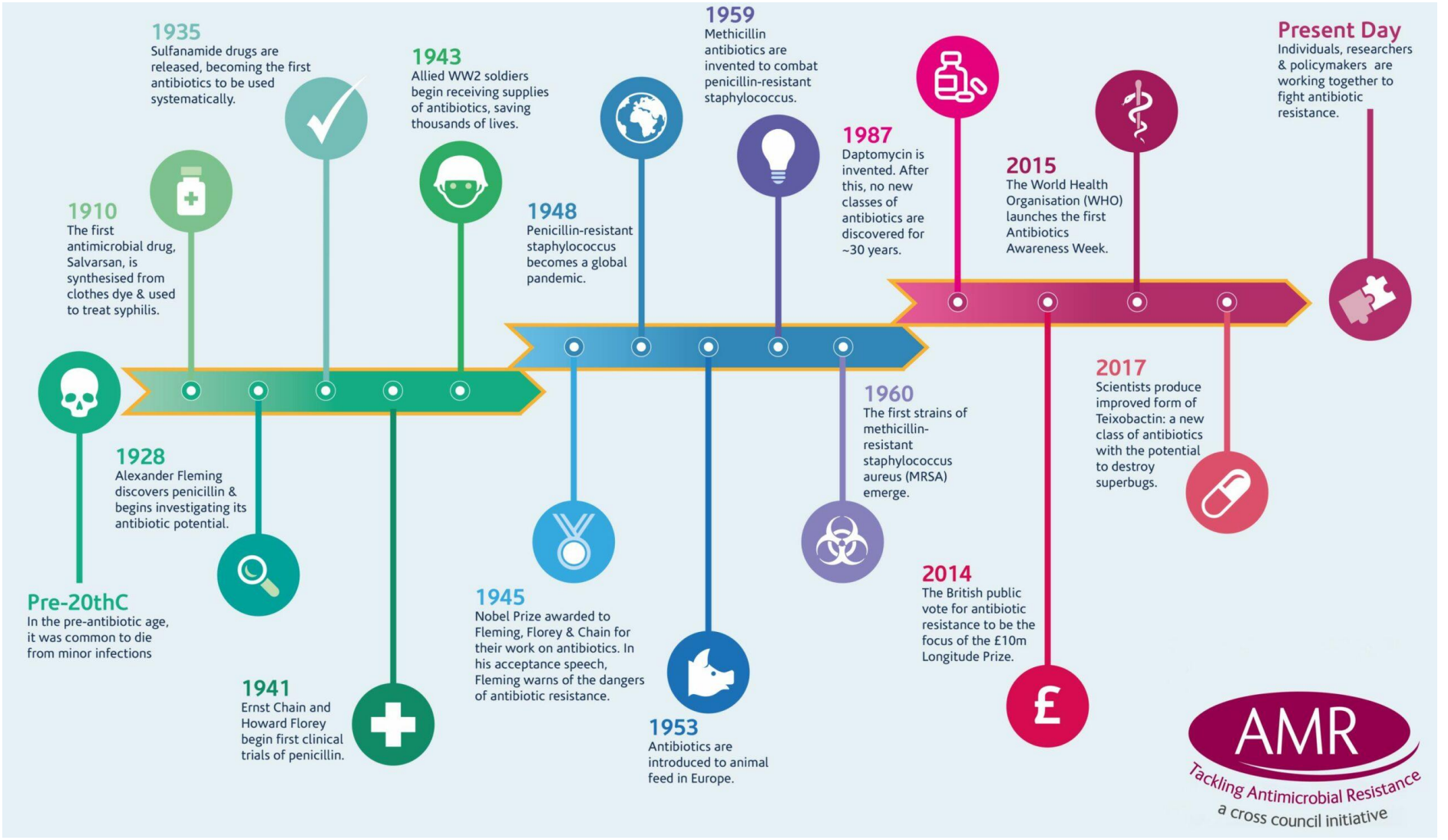
- Bio-AFM
  - Theory 1 – 15:30-17:30h – 24 October 2022 – UMinho Ed.1-1.18
  - Theory 2 – 15:30-17:30h – 31 October 2022 – UMinho Ed.1-1.18
  - ~~Laboratory 1 – 16:30-19:00h – 26 October 2022 – INL Lab~~
  - Laboratory 2 – 16:30-19:00h – 2 November 2022 – INL Lab

Biophysics is the field that applies the theories and methods of physics to understand how biological systems work.

Biophysics has been critical to understanding the mechanics of how the molecules of life are made, how different parts of a cell move and function, and how complex systems in our bodies—the brain, circulation, immune system, and others— work. Biophysics is a vibrant scientific field where scientists from many fields including math, chemistry, physics, engineering, pharmacology, and materials sciences, use their skills to explore and develop new tools for understanding how biology—all life—works.

Societal relevance of biophysics

A brief history of antibiotics and resistance



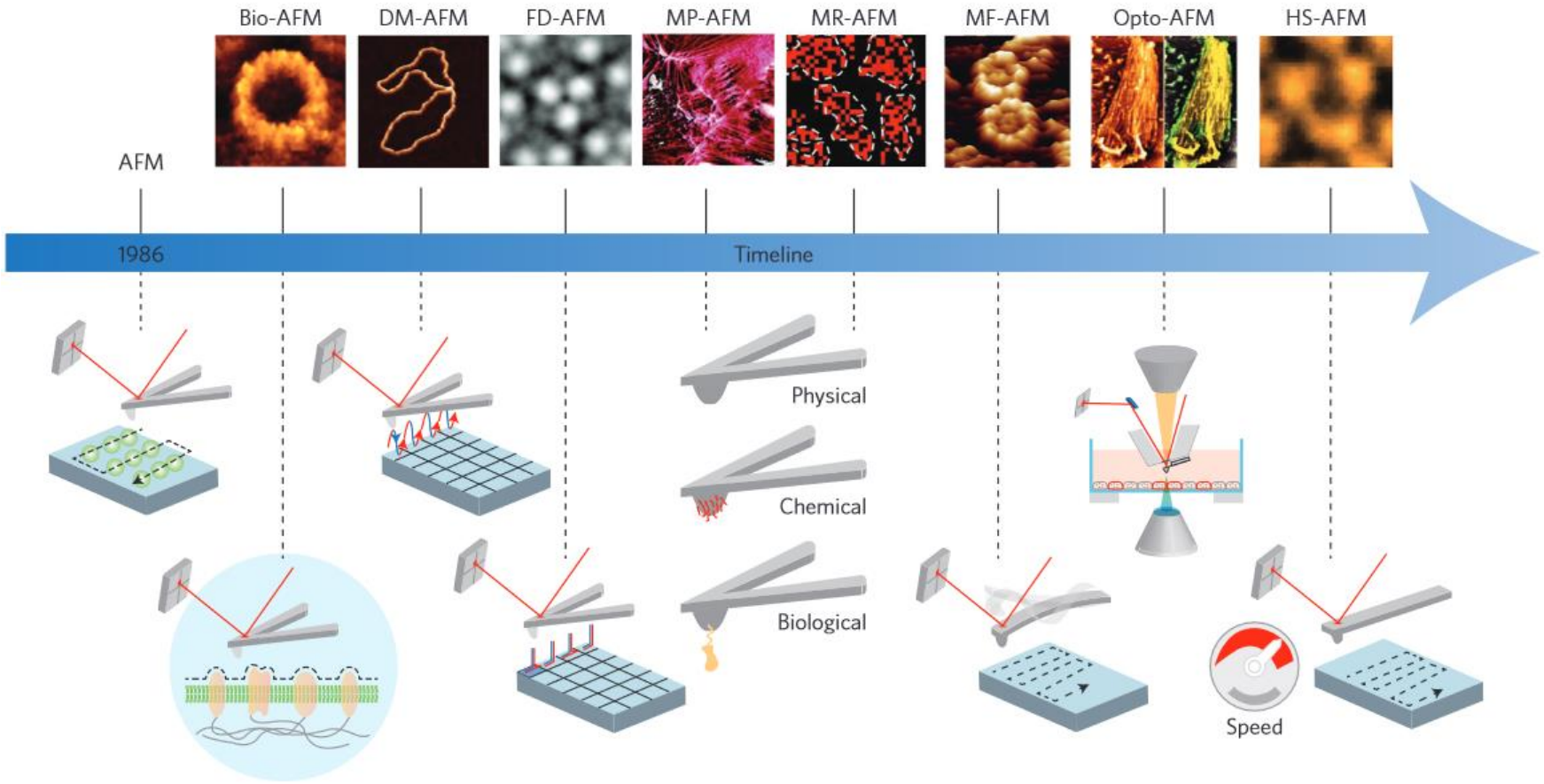
Imaging techniques in molecular and cell biology

Comparison

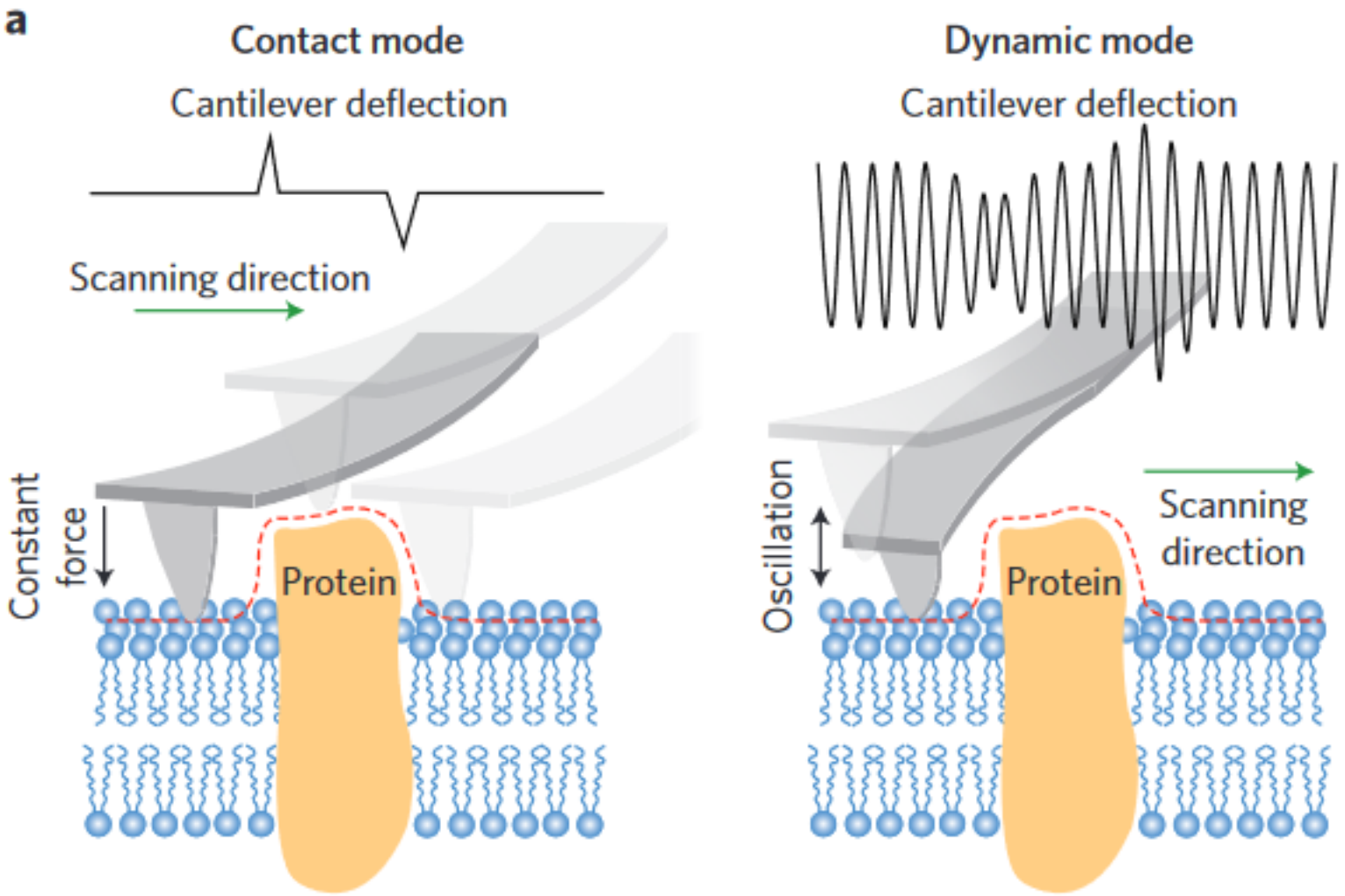
Technique/feature	Atomic force microscopy	Super-resolution microscopy (STED, PALM, STORM)	Transmission electron microscopy	Scanning electron microscopy
Resolution	≤1 nm–50 nm*	20–50 nm	0.2–10 nm	2–10 nm
Sample preparation and environment	Sample on support; physiological (buffer solution, temperature, CO_2)	Fluorescence labelling; physiological (buffer solution, temperature, CO_2)	Sample on grid; dehydrated (negative stain); vitrified (cryo-electron microscopy)	Freeze/critical point drying and metal shadowing
Artefacts	Tip, force, scanning	Bleaching, toxicity	Dehydration, ice crystal formation, beam damage	Dehydration, metal shadowing, beam damage
Advantages	Imaging under native conditions; no staining, labelling or fixation necessary; high signal-to-noise ratio; assessment of multiple physical, chemical and biological parameters	Access to three-dimensional cellular structures; high spatiotemporal resolution; monitoring biomolecular processes in life cells	Solves atomic structures of proteins; conformational snapshots of proteins and complexes; molecular-resolution structures within the cell	Imaging surfaces of tissues, cells and interfaces at nanometre-scale resolution
Limitations	Restricted to surfaces	Imaging restricted to fluorescence labels	No life processes	No life processes

Bio-AFM Imaging Modes

A novel approach to study biological structures

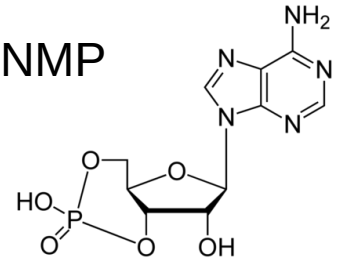


Source: Dufrêne, Y., Ando, T., Garcia, R. *et al.* Imaging modes of atomic force microscopy for application in molecular and cell biology. *Nature Nanotech* **12,** 295–307 (2017).

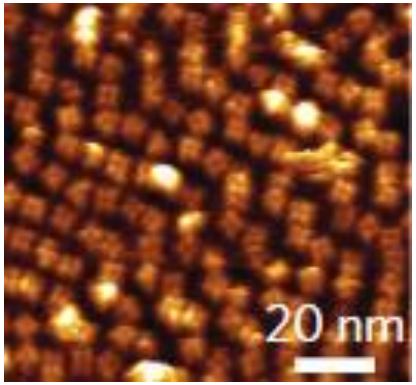

a
Contact mode
Cantilever deflection
Scanning direction
Constant force
Protein
Dynamic mode
Cantilever deflection
Oscillation
Scanning direction
Protein

Cyclic nucleotide (cNMP)-regulated potassium channels (MlotiK1) reconstituted into lipid membranes

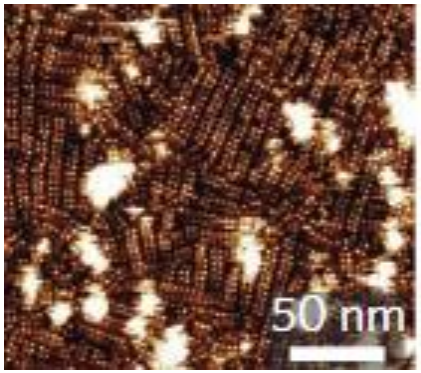
cNMP



Lotus corniculatus



Rows of densely packed rhodopsin dimers distributed in the native disc membrane extracted from rod outer segments of the eye



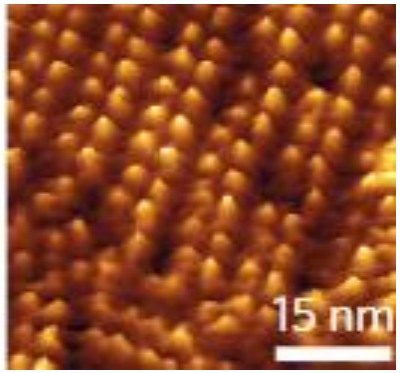
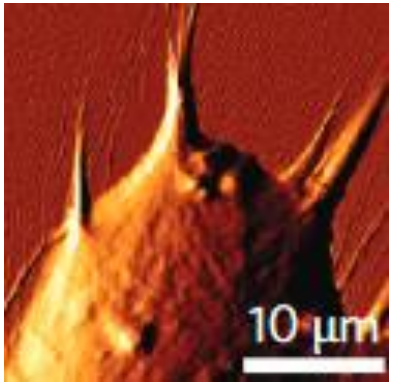
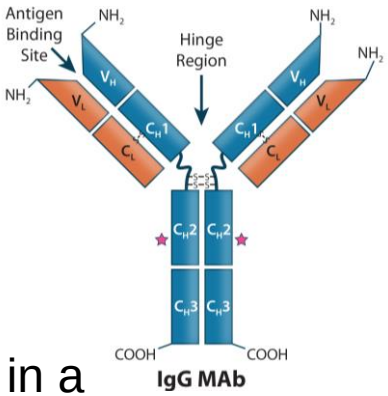
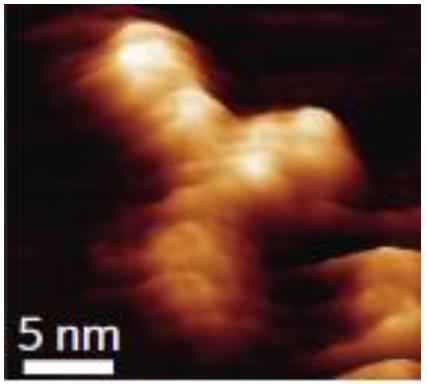


Image of a living SAOS-A2 cell bundling and pulling collagen fibrils coating a substrate. To maximize contrast, the exemplified image shows the deflection of the cantilever, which changes while contouring the sample.

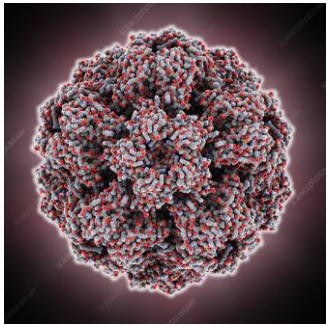


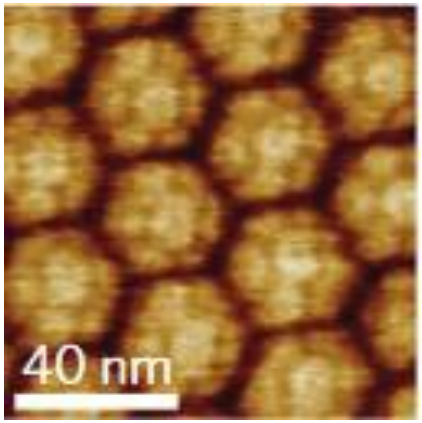
An IgG antibody absorbed to mica and visualized with frequency modulation mode



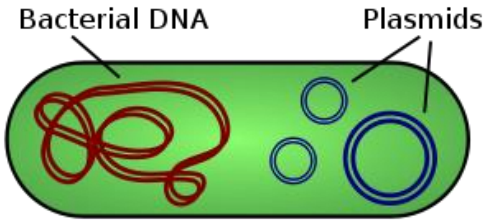


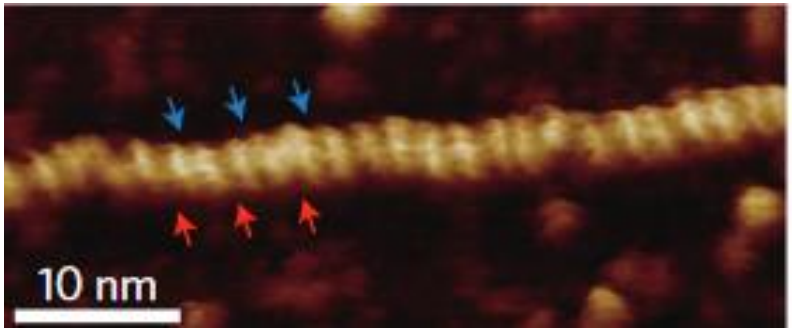
Single brome mosaic viruses packed in a crystalline assembly.

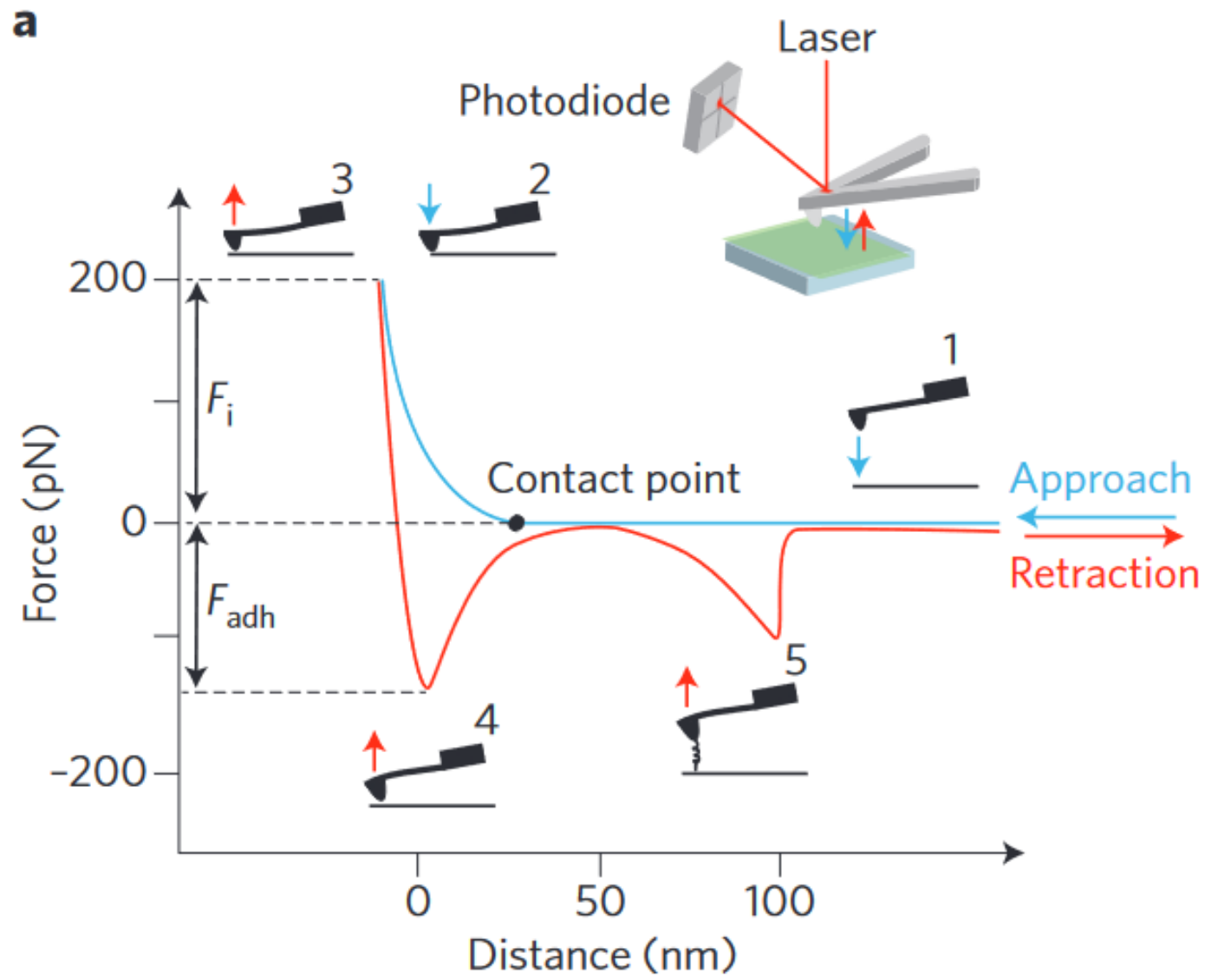




Circular plasmid DNA imaged in buffer solution by frequency modulation AFM. Red and blue arrows indicate major and minor grooves of the DNA, respectively.

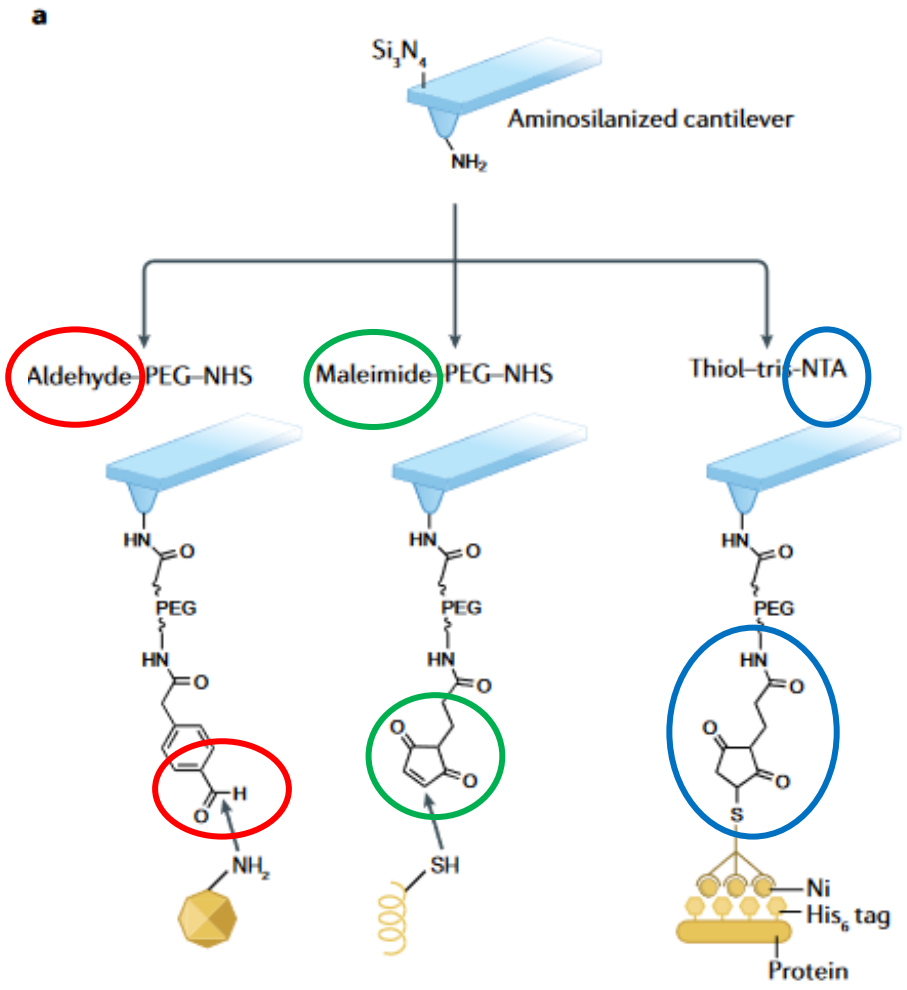


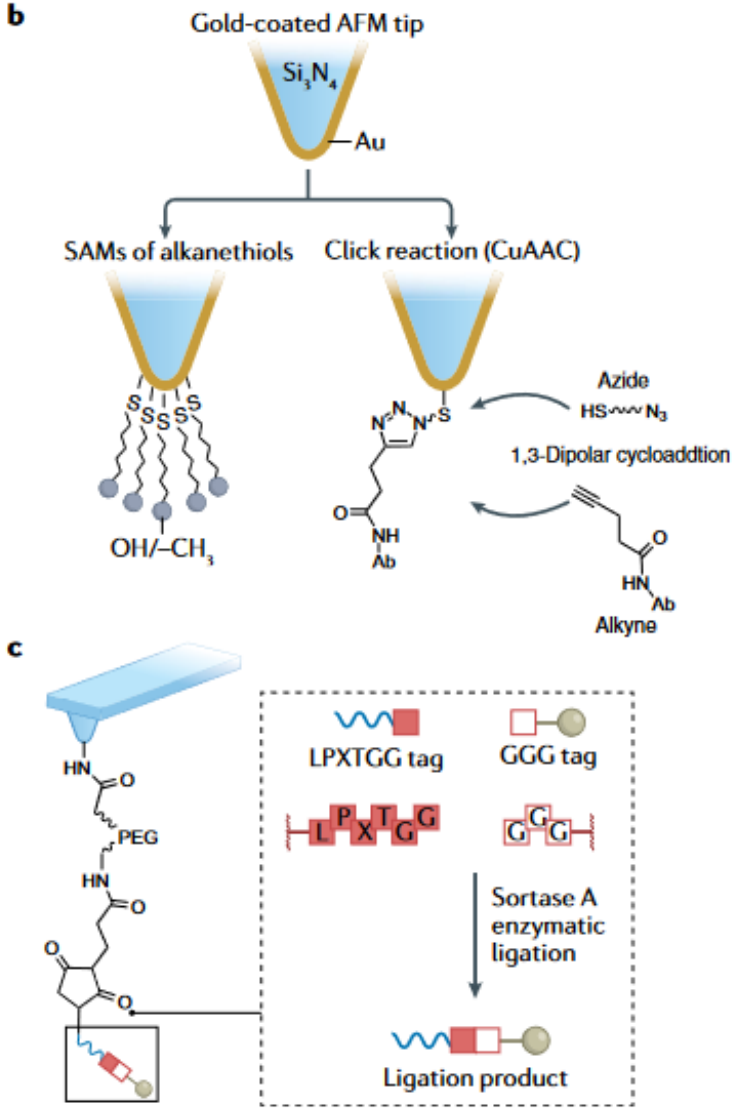



a
Laser
Photodiode
3
2
1
200
0
-200
F_i
F_{adh}
Contact point
Approach
Retraction
Force (pN)
4
5
0
50
100
Distance (nm)

Single-molecule force spectroscopy

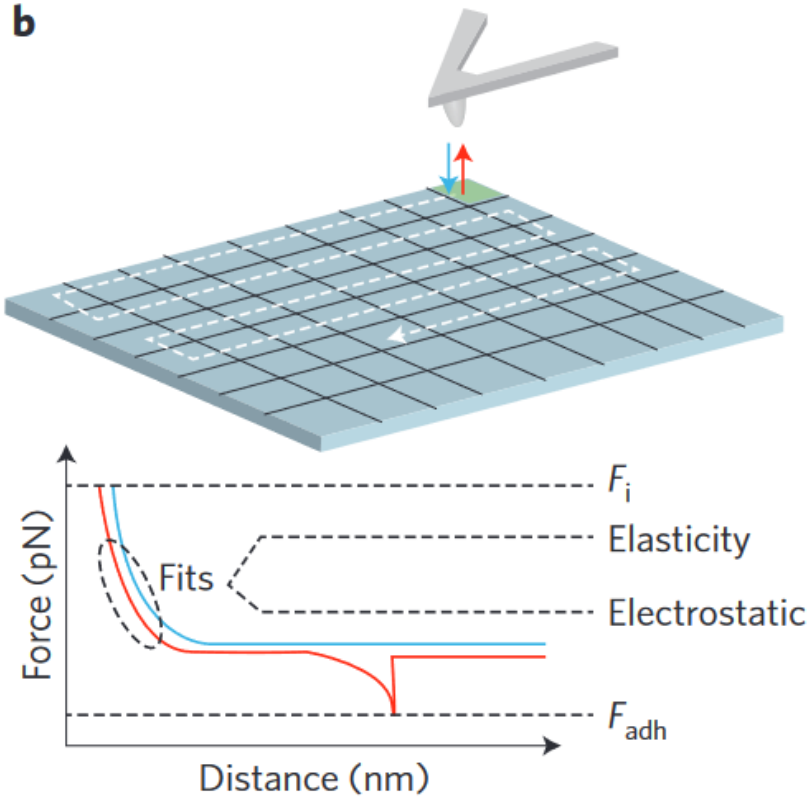
Surface functionalization strategies to anchor biomolecules

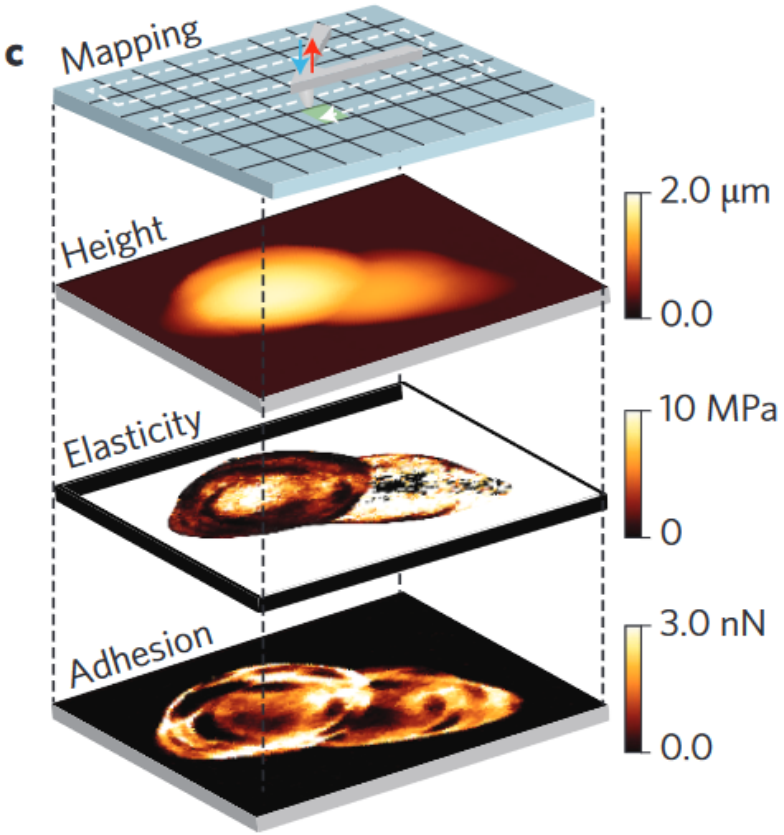




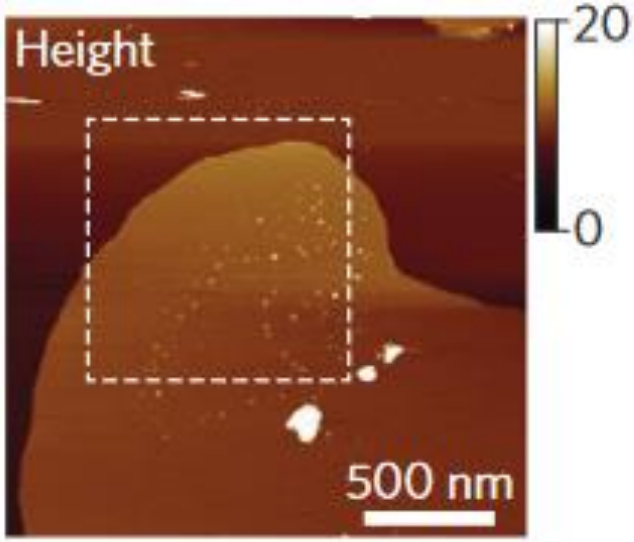
Force-distance curve mapping

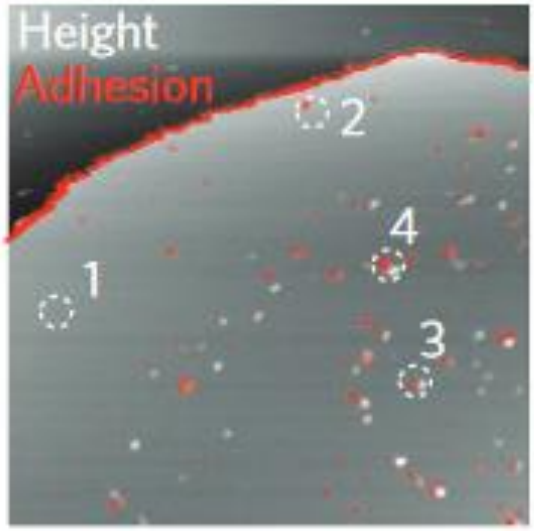
Multiparametric imaging

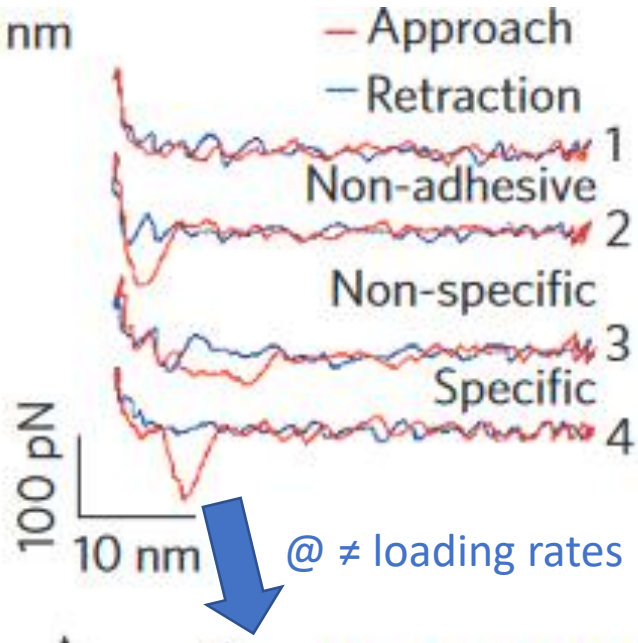




Thrombine receptors (PAR family) & SFLLRN-ligand functionalized tip







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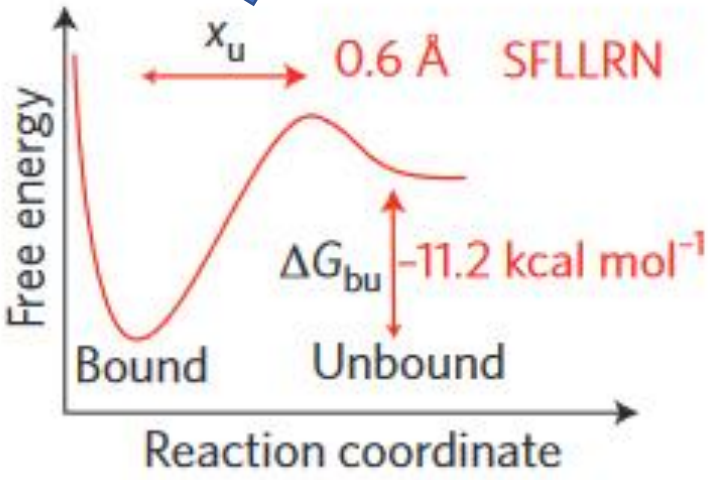

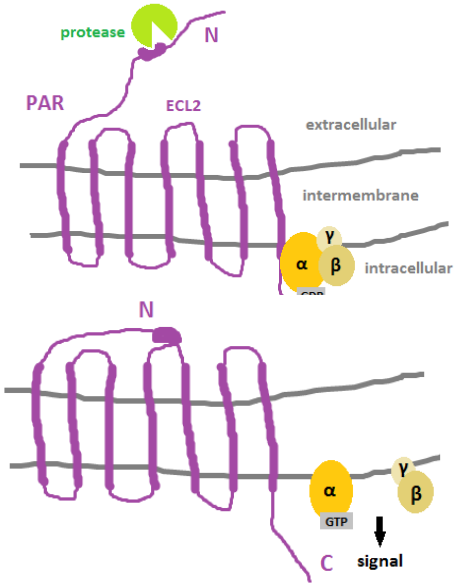

Learn more
YouTube
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