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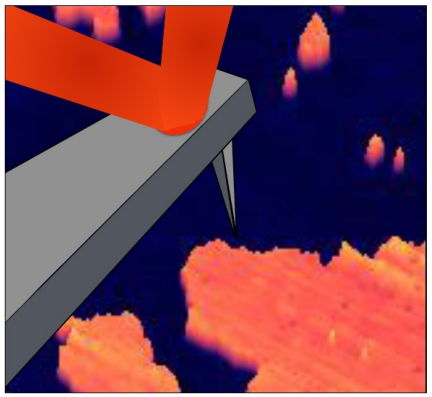
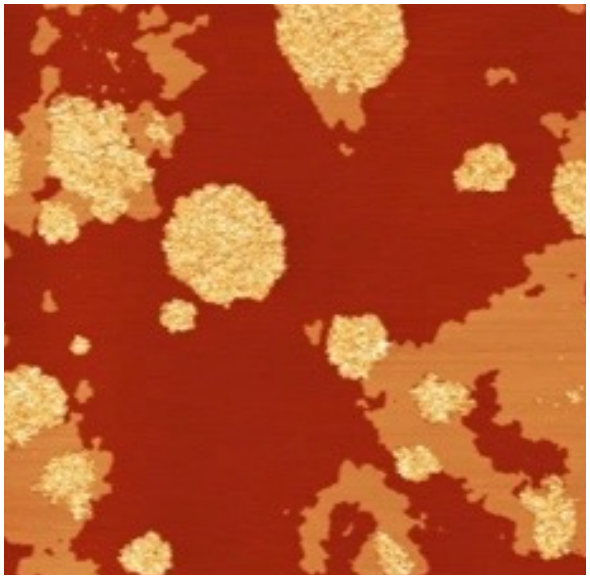
NanoInnovation Lab

Atomic Force Microscopy: biophysical applications





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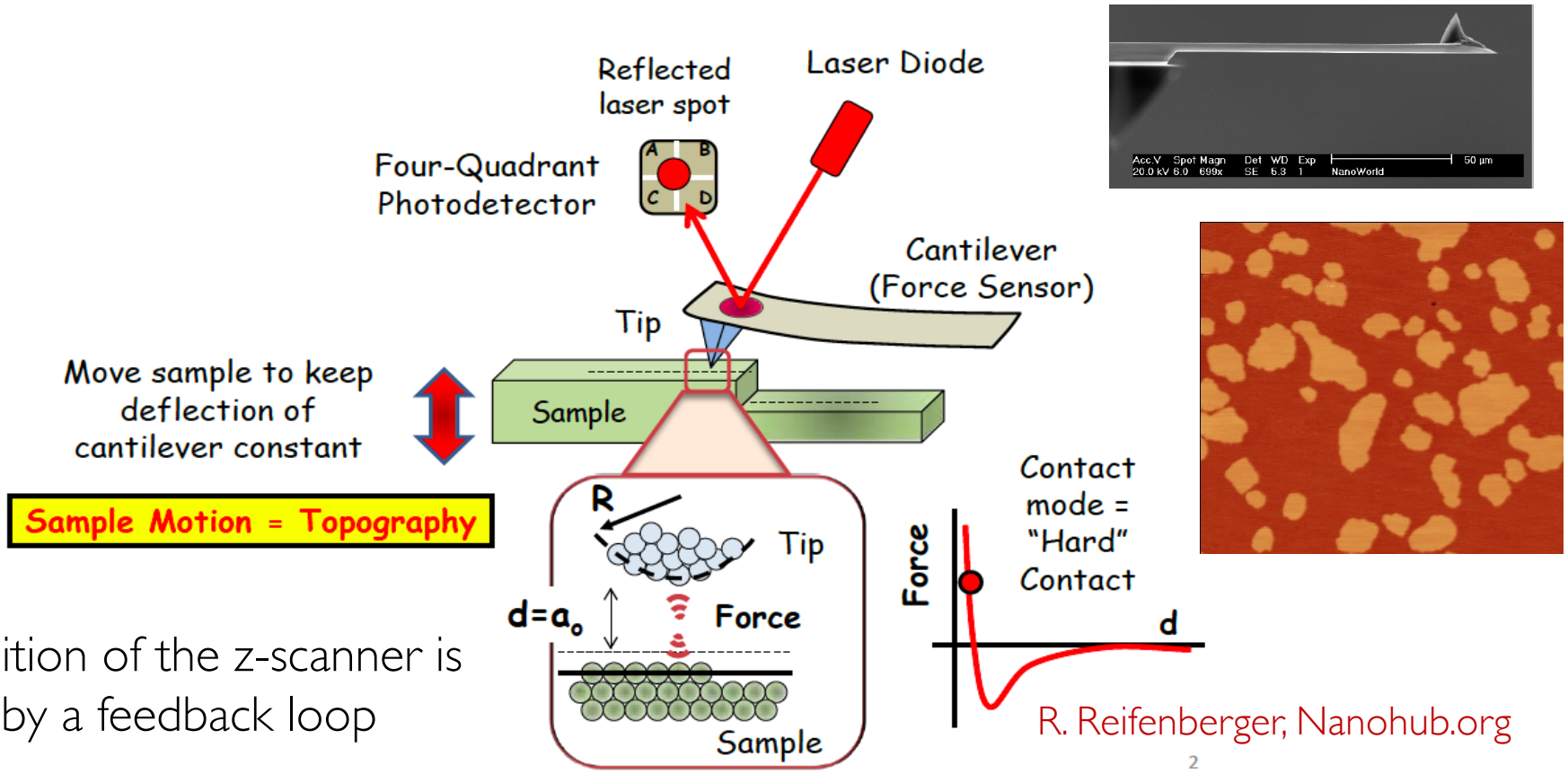
Atomic Force Microscopy



Height position of the z-scanner is controlled by a feedback loop

R. Reifenberger, Nanohub.org

AFM is a mechanical imaging instrument that derives the **3-D profile (topography)** and the physical properties of a surface, in liquid environment, by measuring the **INTERACTION FORCES** with a scanning, nanometer sized probe.

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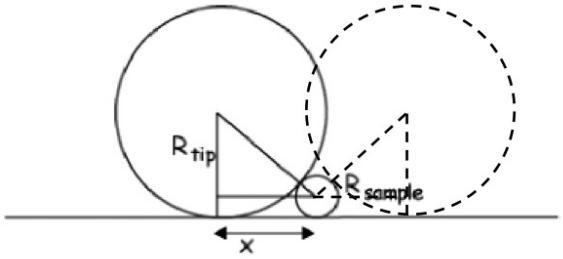
Atomic Force Microscopy

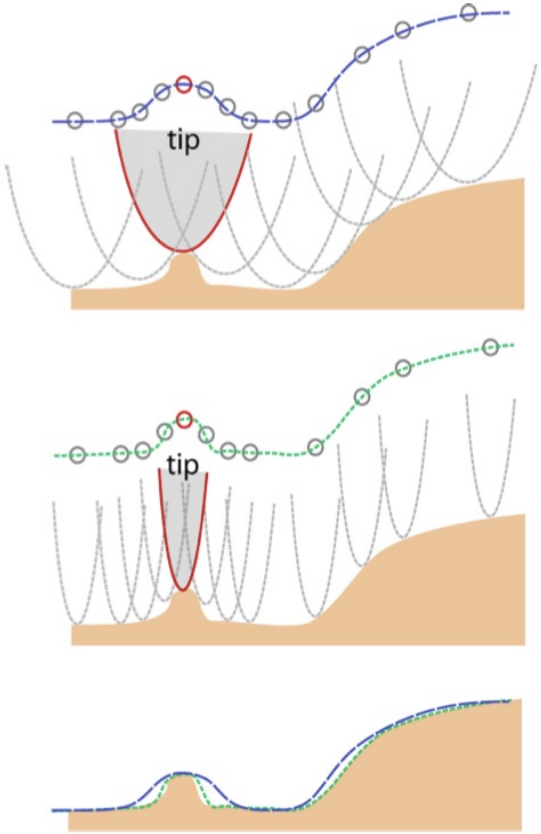


$$x^2 = (R_{tip} + R_{sample})^2 - (R_{tip} - R_{sample})^2$$

$$x^2 = R_{tip}^2 + 2R_{tip}R_{sample} + R_{sample}^2 - R_{tip}^2 + 2R_{tip}R_{sample} - R_{sample}^2$$

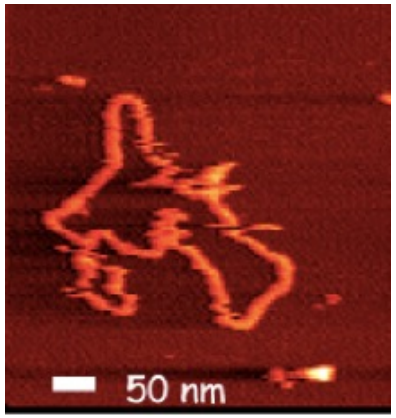
$$x = 2\sqrt{R_{tip}R_{sample}}$$

$$w = 2x = 4\sqrt{R_{tip}R_{sample}}$$



DNA: 2 nm.

tip ~ 20 nm => w = 25 nm
tip ~ 10 nm => w = 18 nm



The width w of an object is the convolution between tip and object size. **The height is true!**

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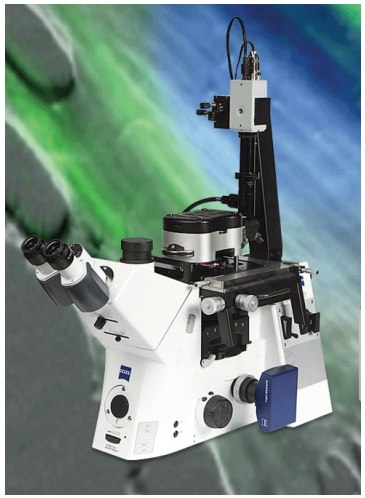
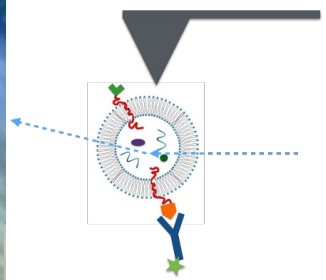
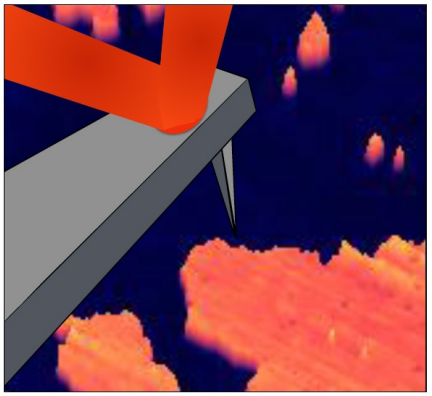
Atomic Force Microscopy: how fast?

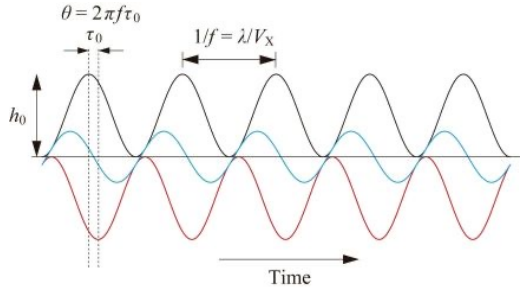


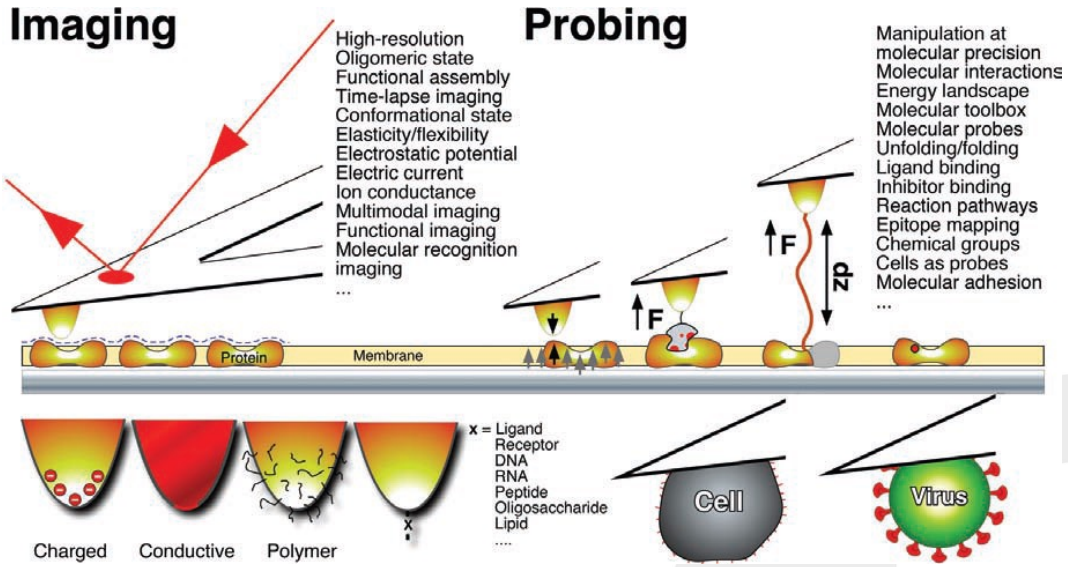


Correlative AFM and
Fluorescence Microscopy

Imaging in liquid







S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

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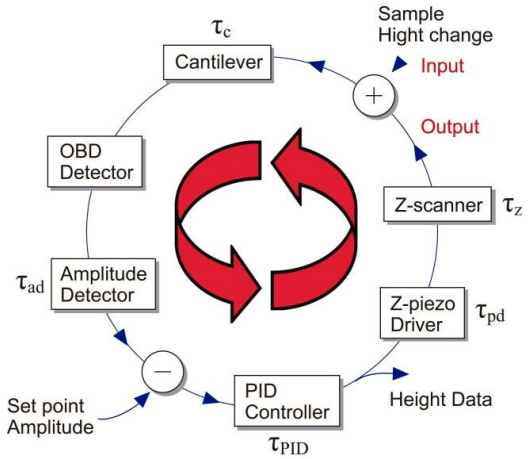
High-Speed AFM



http://biophys.w3.kanazawa-u.ac.jp/index.htm

High-speed AFM (HS-AFM, pioneered by **Prof. Toshio Ando,** Kanazawa University, Japan) allows for visualizing dynamic processes in nano-spaces, in liquid

(i) conformational changes of proteins during their functional activity,

(ii) processes of self-assembly, disassembly and aggregation,

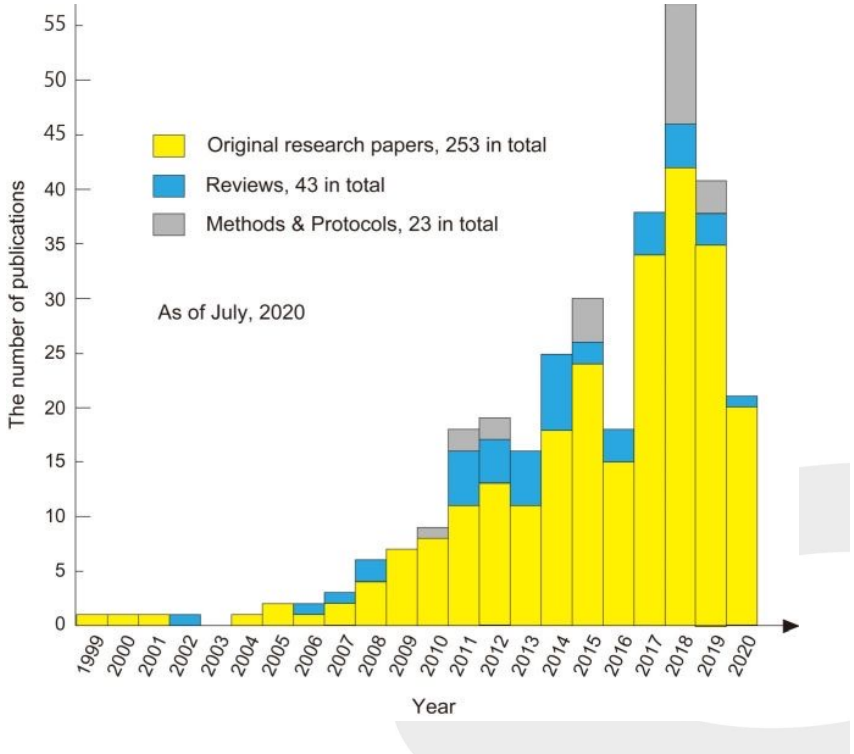
(iii) structural fluctuations and transitions, diffusion

(iv) **dynamic interactions** (association and dissociation),

(v) enzymatic reactions,

(vi) cellular morphological changes and dynamics of proteins on cell surfaces

(vii) dynamics in artificial systems made of biological materials.



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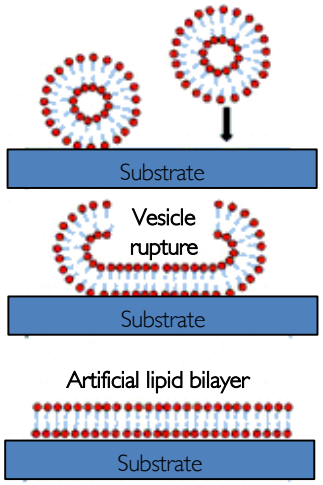
Supported lipid bilayers (SBL): standard AFM imaging

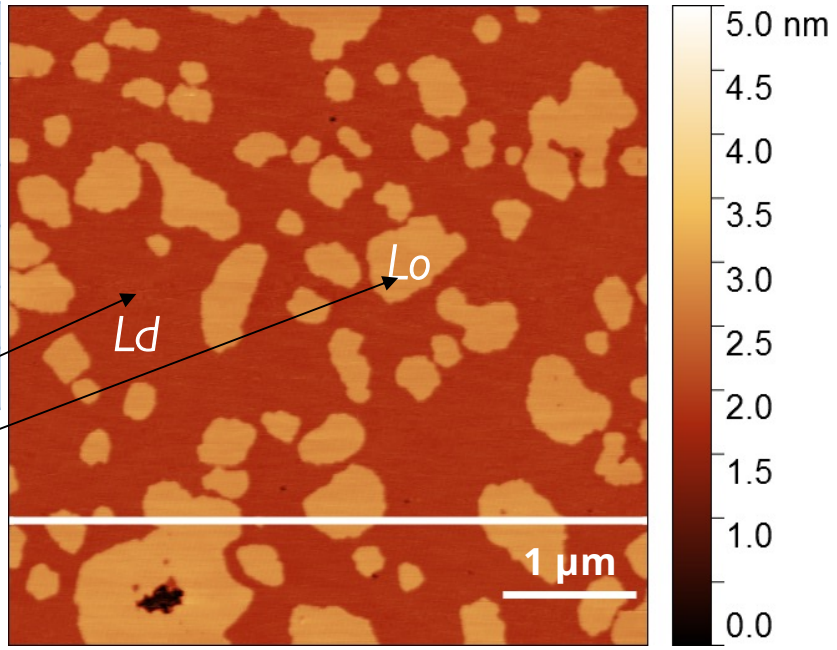
Raft-like artificial model membranes

➢DOPC + SM (66:33) + 5% Chol

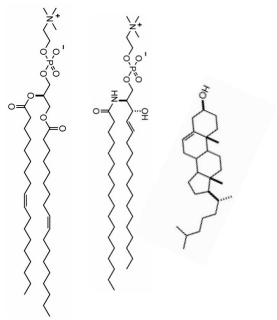
Vesicle fusion method

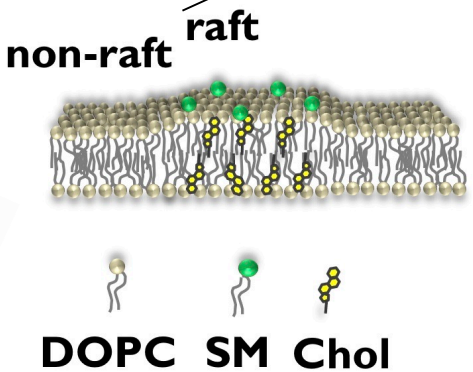


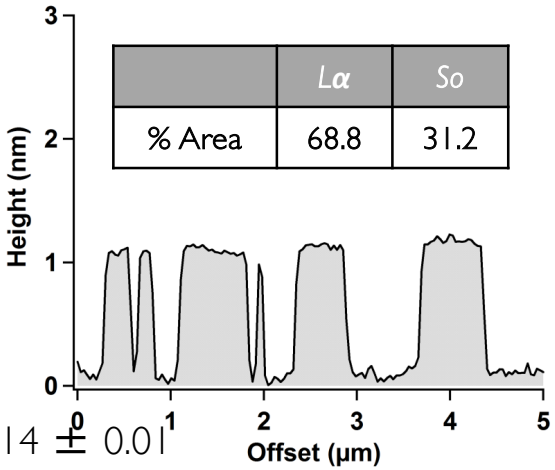
DOPC
1,2-dioleoyl-sn-glycero-3-phosphocholine
SM
Sphingomyelin
Chol
Cholesterol

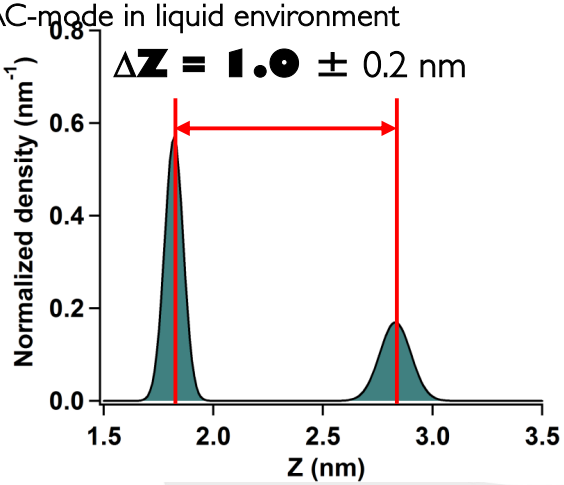


AFM imaging in dynamic AC-mode in liquid environment









surface roughness: Lα=0.16 ± 0.01 nm, Lo=0.14 ± 0.01

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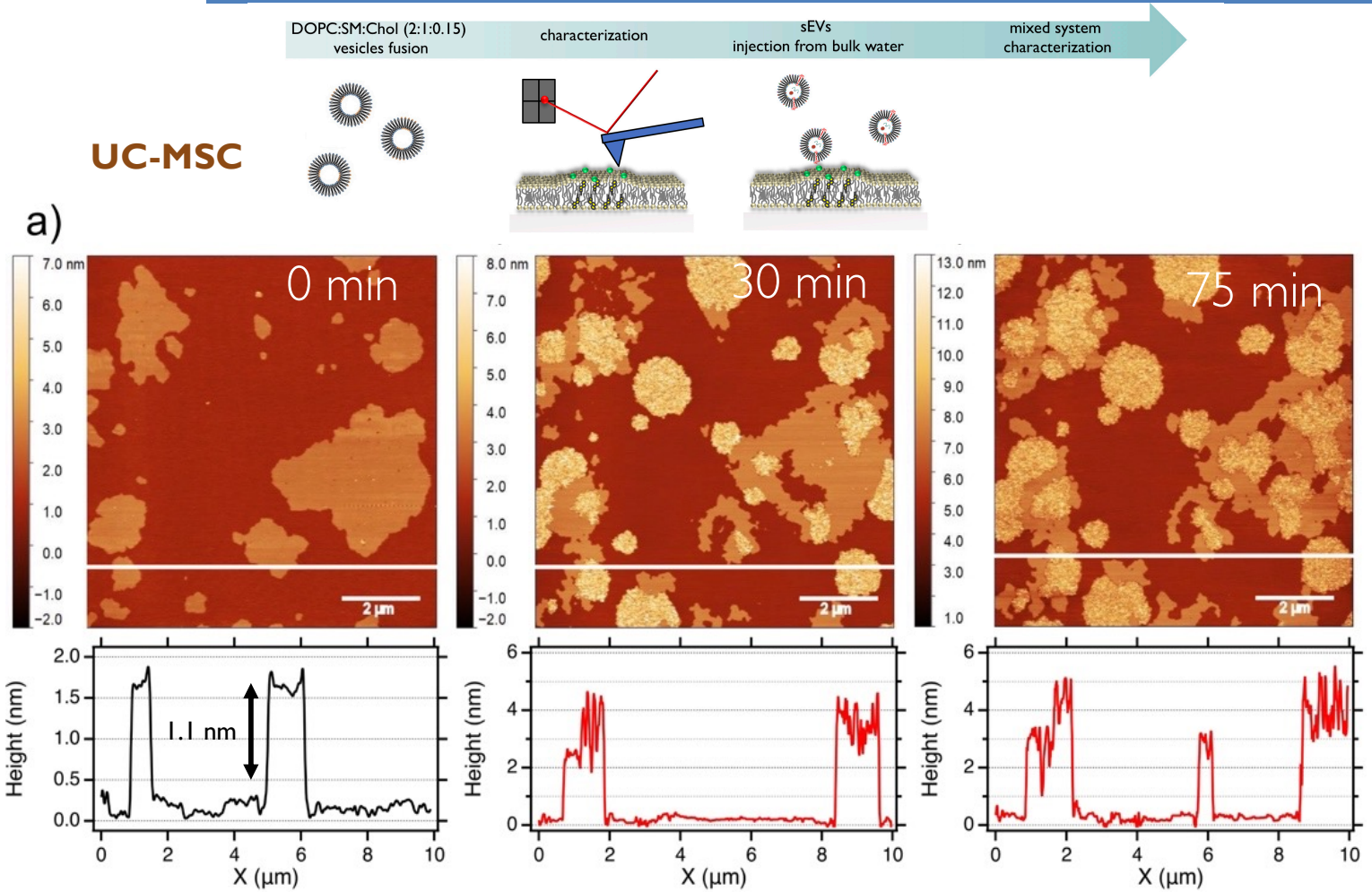
Extracellular vesicles-SLB interaction



- sEVs preferentially dock and break at phases borders: at thickness mismatches the free energy minimum enables favourable interactions without the need of large curvature deformations.
- Patches protruding 3-4 nm above SLB tend to expand in a more favourable fashion in L_d phase

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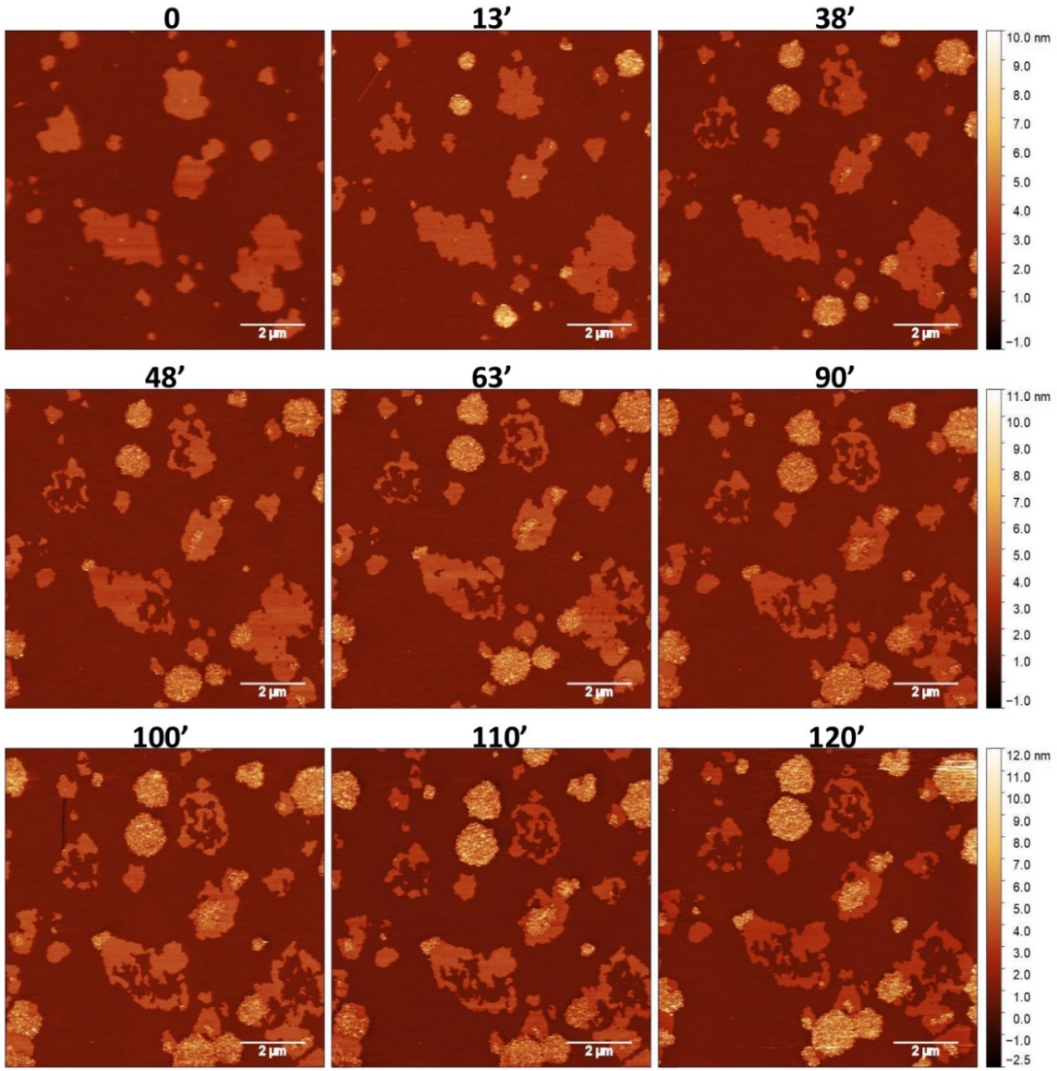
(HS)-AFM: EVs-SLB interaction

L_o phase re-shaping, borders granularity



From literature:
-> cholesterol depletion
-> components redistribution

Distinct processes occur:

1. **Fast diffusion of lighter elements** laterally migrating along phase boundaries

2. **Diffusion of bulkier sEVs** components mixing with target membrane

F. Perissinotto, V. Rondelli et al., Nanoscale, 2021, 13, 5224

EVs + deuterated PC SLB

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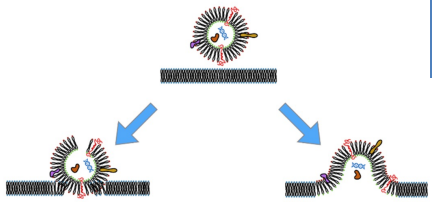
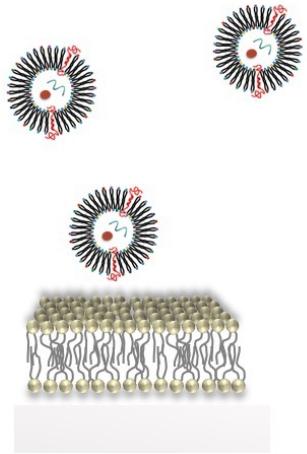
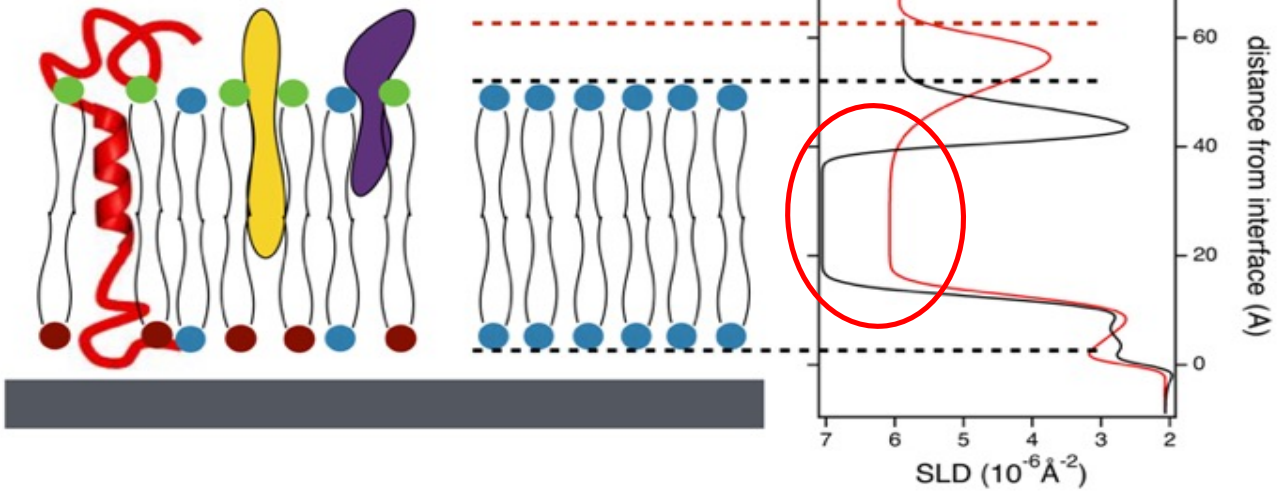
$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$

Neutron reflectometry







Playing with selective deuteration: protiated molecules in a ghost phospholipid matrix

- 20% volume penetration
- Change in contrast spans whole membrane thickness
- Asymmetry

	AFM ΔZ (nm)	NR h (nm)
PC	5.1 ± 0.6	4.2 ± 0.3
PC+EVs	6 ± 2	5.4 ± 0.3

F. Perissinotto, V. Rondelli et al., Nanoscale, 2021, 13, 5224

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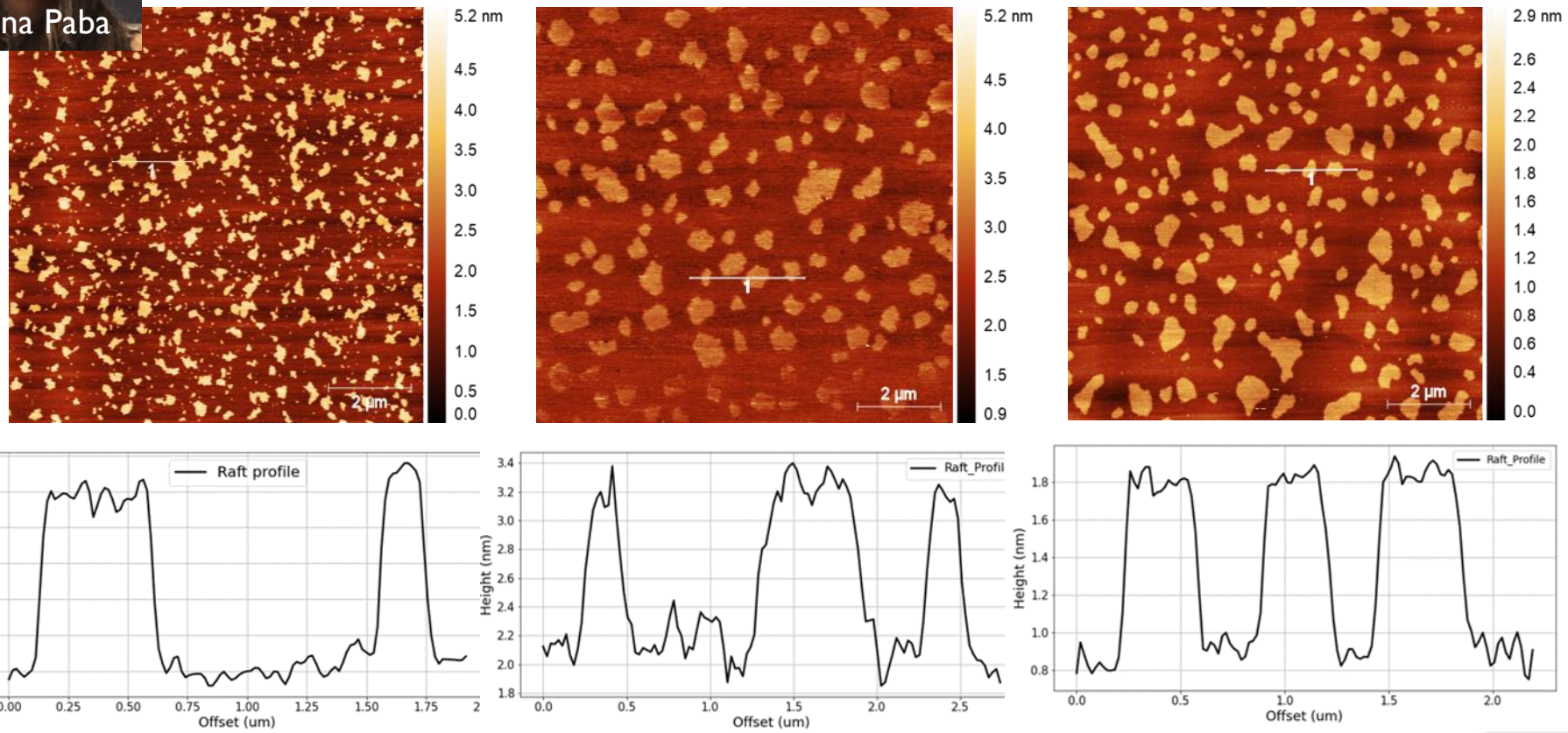
Chol modulation

DOPC, SM (2:1) with (a) 5, (b) 10, (c) 17 mol%



Thickening effect of cholesterol on the liquid-disordered DOPC phase resulted in a decrease of Lo/Ld height difference as well as increase of raft size

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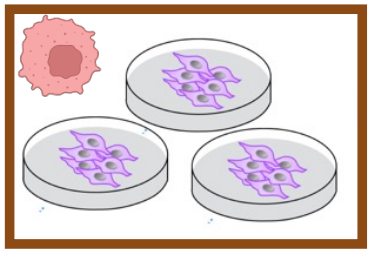
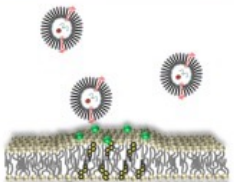
231-sEVs on chol rich SLB

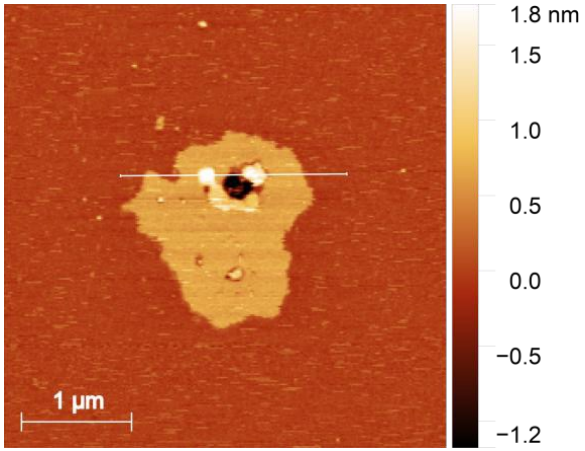
MDA-MB-231

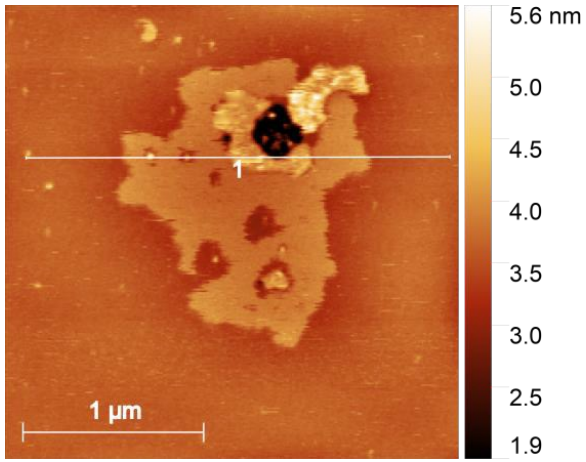
+

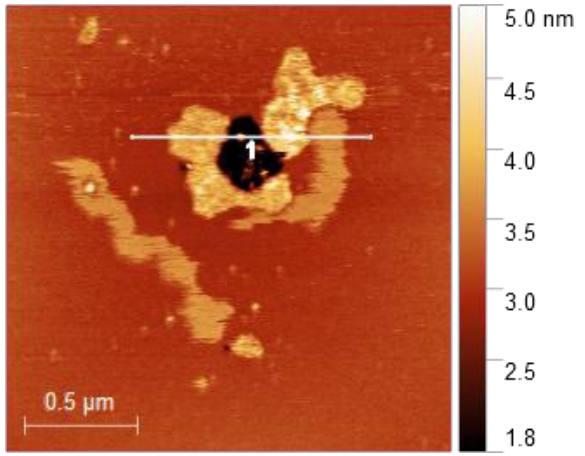




on 17% mol chol membrane







Time evolution

231-sEV uptake mainly affects the Lo phase (from its defects: no border!), with its progressive increase in fluidity toward a more disordered phase

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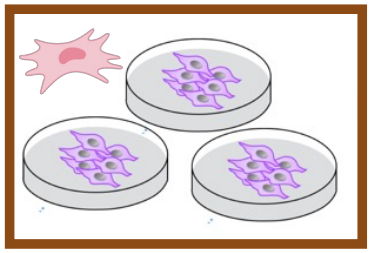
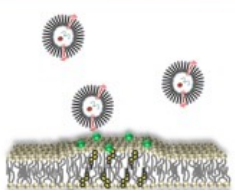
UC-MSC-sEVs on chol rich SLB

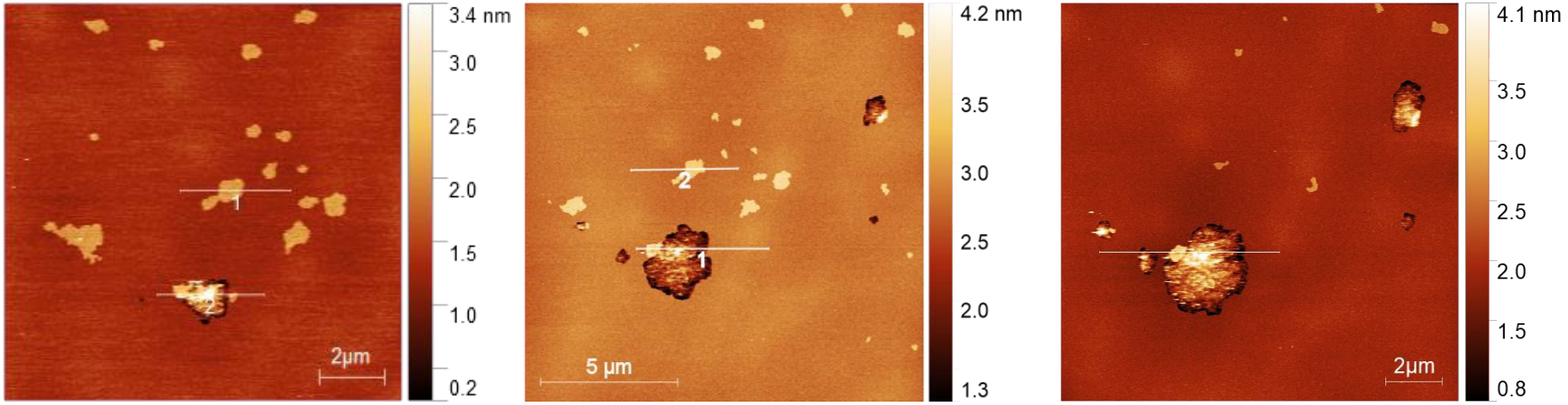
UC-MSC

+





on 17% mol chol membrane



Time evolution

As for 5% mol chol, here the interaction starts at the Lo/Ld phase separation.
However with time, Lo disorder increases, more than for Ld phase. Lo-to-Ld transition

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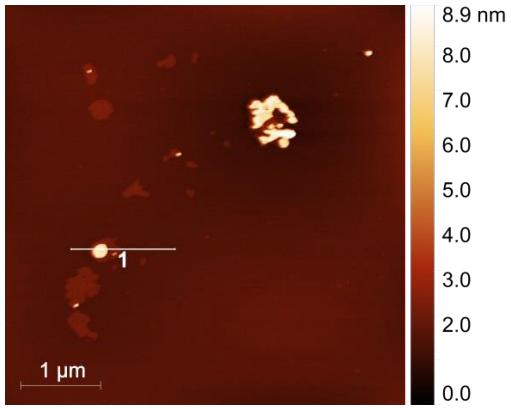
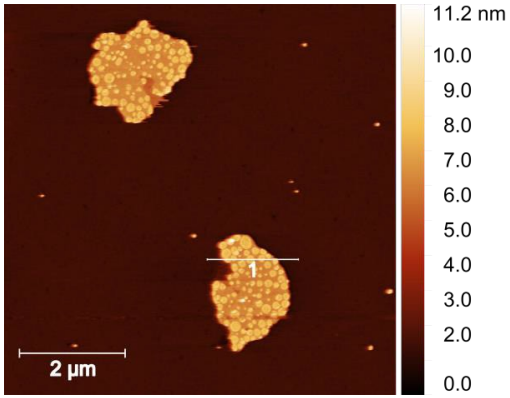
No chol

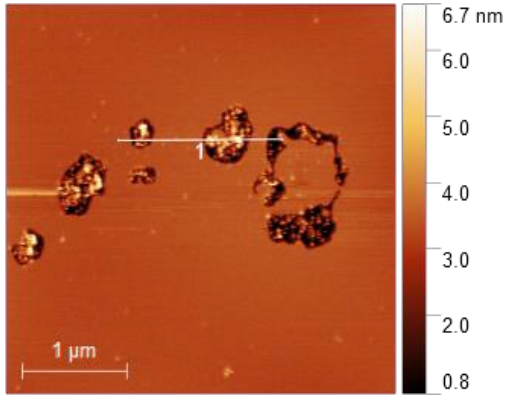
DOPC/SM (Ld/So) 2:1



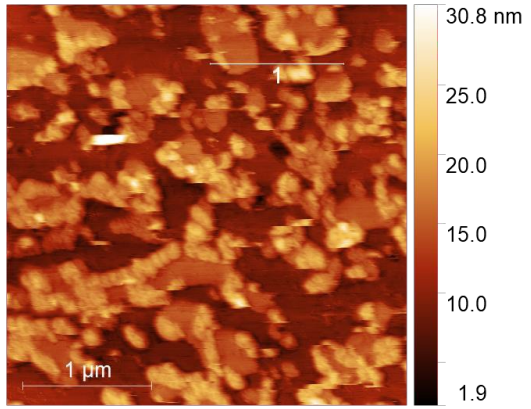
DOPC



DOPC/DPPC (Ld/So) 2:1



DPPC



EV uptake seems to be determined by the phase of the lipid bilayer, and the respective **degree of order/fluidity** of the lipids forming the docking domains, not by the chemistry.

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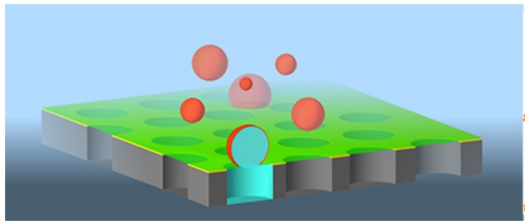
Conclusions

- Phase borders are docking sites for UC-MSC derived vesicles
- The area of EVs-membrane domains increases over time suggesting the formation of Initial nucleation seeds which act as docking sites for other EVs from solution
- EVs from different origin behave differently: we observed a higher affinity of MDA-MB-231 EVs for liquid ordered phase
- For SBL with no cholesterol, we saw the formation of a different intermediate with high physical affinity for So phase. EV-lipids 'bulge out' of the So phase.



- PERSPECIVES:
- Increase complexity by introducing reconstituted transmembrane proteins (i.e.caveoline) in pore-spanning membranes

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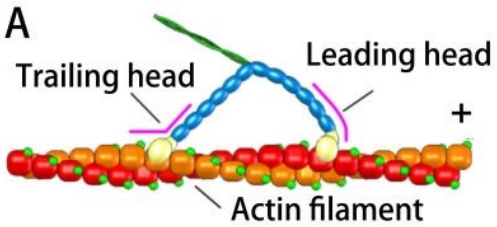
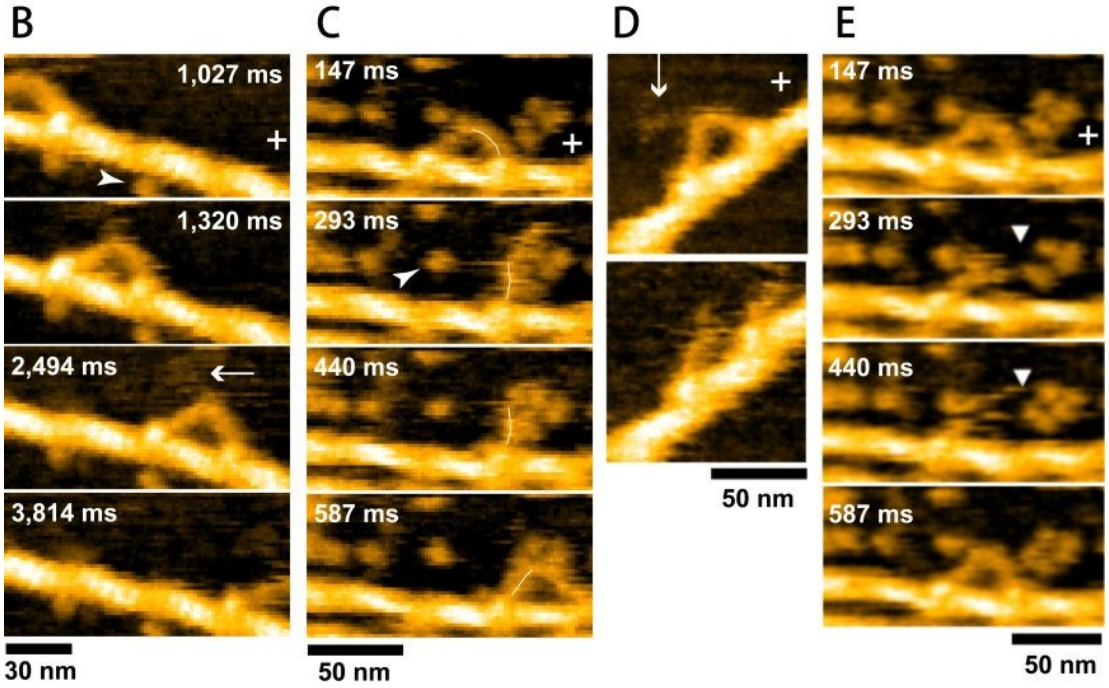
HS-AFM imaging of proteins examples



In every ATP hydrolysis cycle, M5 steps forward by ~36 nm hand-over-hand. Energy is coming from intramolecular strain-mediated retardation of ADP release from the leading head.



Open questions:
how the tension is generated?
how the energy liberated by ATP hydrolysis is used for mechanical work?
Visualized by HS-AFM!

Nature 468, 72–76 (2010)

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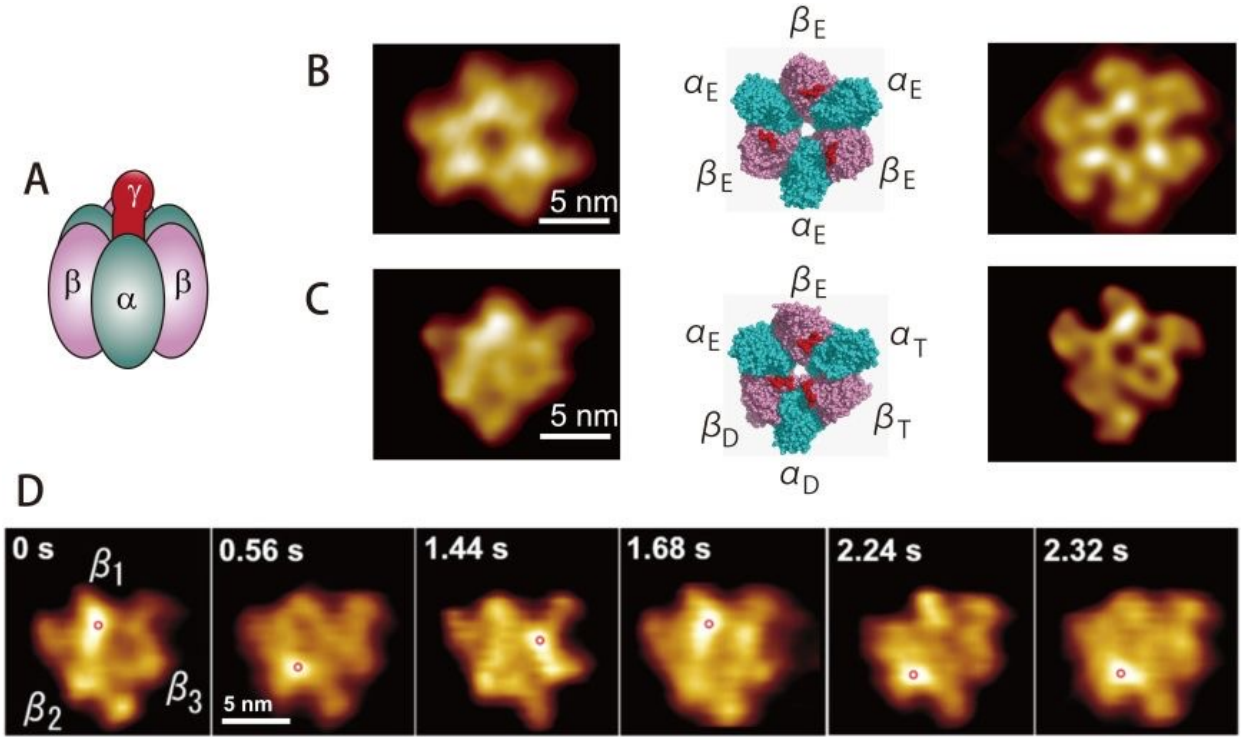
HS-AFM imaging of proteins: examples



Dynamic behavior of the **α3β3** ring of F1-ATPase filmed with HS-AFM. (A) Schematic of the **α3β3γ** complex. (B) AFM image of **α3β3** (left) and crystal structure (middle) in the nucleotide-free condition (left), and pseud-AFM image simulated from the crystal structure (right). (C) AFM image of **α3β3** in the presence of AMP-PNP (left), crystal structure of **α3β3** in the presence of nucleotides (middle), and pseud-AFM image simulated from the crystal structure (right). E, Empty; D, ADP; T, ATP. (D) HS-AFM images of **α3β3** in the presence of ATP. The red circles indicate the highest pixel positions in the respective images. Science 333, 755–758

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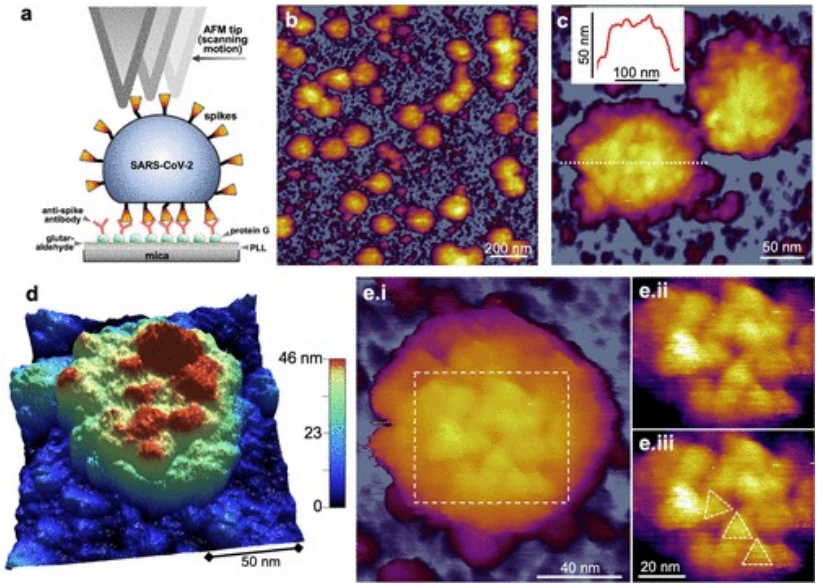
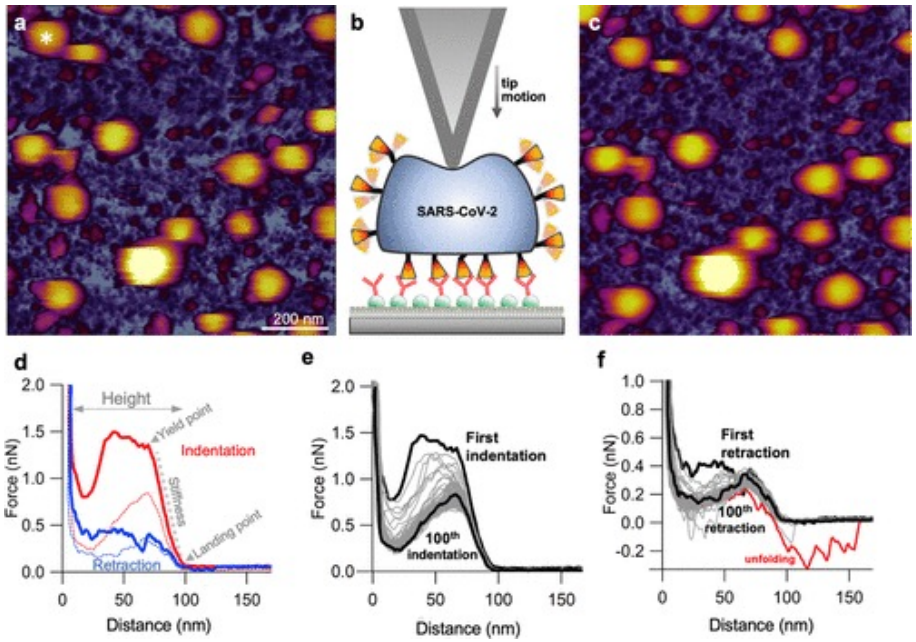
HS-AFM imaging of proteins: examples



SARS-COV-2 Spike dynamics



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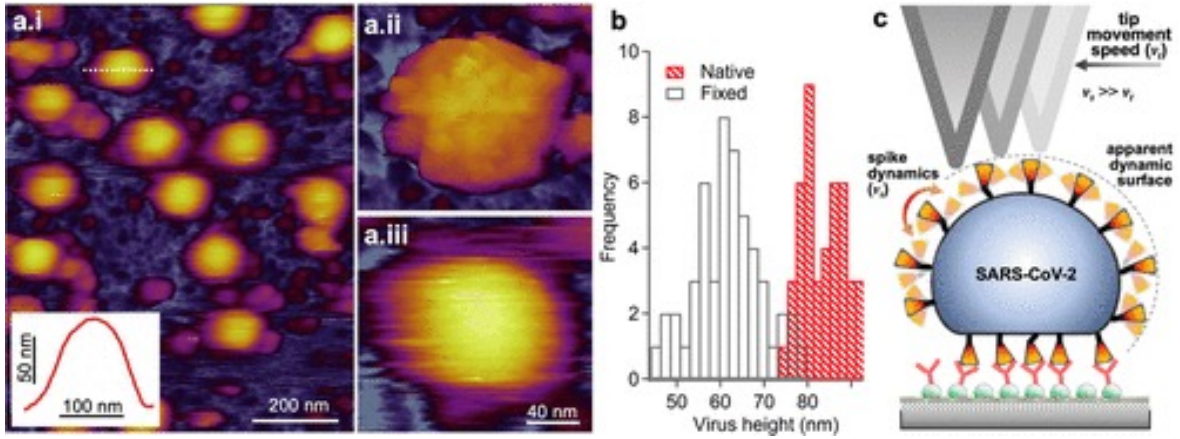

Virion highly dynamic, compliant, and resilient, with remarkable mechanical and global thermal stabilities.
Dynamics of the surface spikes may play important role in the unusually high infectivity of the virus.
Mechanical and self-healing properties may also ensure adaptation.

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