HS-NANOBIO-IMAGING

Etude dynamique et structurale de biomolécules par HS-AFM

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Journées Nationales Nanosciences et Nanotechnologies 2012

Objective

- Implementation of high-speed AFM (HS-AFM) developed by the Kanazawa's university in France
- Promote this technology and develop a commercial system by the end of the ANR project.
- Contribute novel knowledge about dynamic processes and structure-function relationships of individual molecules.

Growing expectation of:

- Improvement of high-speed AFM in term of dynamics and resolution.
- Advance in active devices and microfluidic for sensing dynamics of biomolecules;
- Characterization of supramolecular organization in model or native membranes;

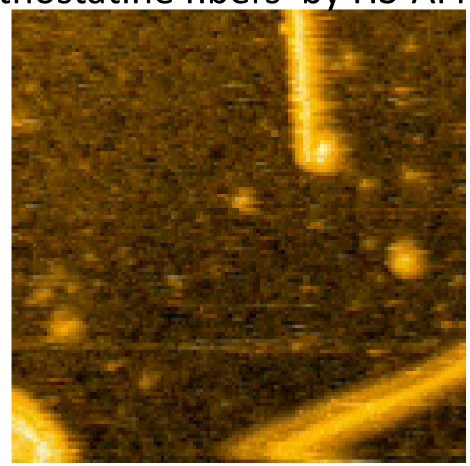
Context

1986	1990	1994	2006	2008	2012
STM	AFM	Force spectroscopy	HS-AFM Prototype v.1	HS-AFM consortium	Commercial HS-AFM v.1

High-Speed AFM
HS-AFM

High-Speed Atomic Force Microscope developed
by Pr. T. Ando since 2006 (Univ. Kanazawa, Japon)

Lithostatine fibers by HS-AFM



1 image = 1 sec

Actin filaments by AFM

1 image = 1 min

Consortium signed in 2008 between BRUKER (ex. VEECO Instr.) u. Kanazawa, u. Linz, Institut Curie, CBS, ICB

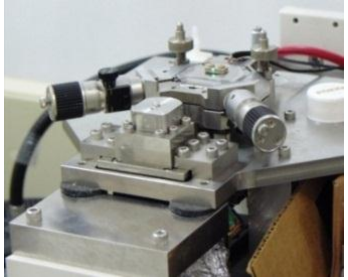
High Speed Atomic Force Microscopy - Improvements

Objectives:

Extend the capability of HS-AFM to cell investigation at 1-20 frames/s

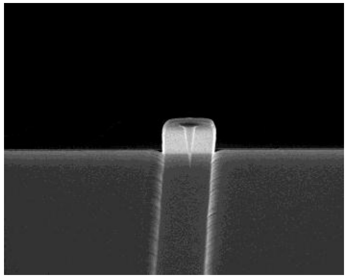
Develop a protocol for growing EBD tips in SEM

NEW IMPROVEMENTS



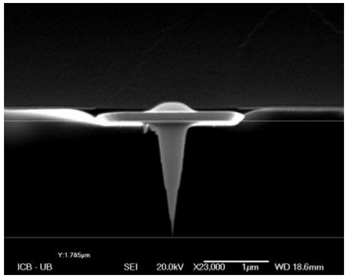
Wider scan range piezo-scanner

5x5 µm to 30x30 µm scan range
instead of 800x800 nm scan range initially

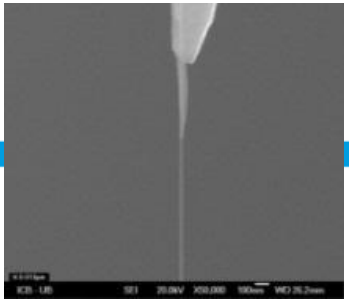


Protocol for reliable tip generation

OLYMPUS AC-10DS probe
L= 9 µm   w= 2µm   t= 130nm
k = 0.1 N/m   f= 0.6 MHz in liquid   Quality factor~2



NANOWORLD probe
L= 7µm w= 2µm t= 80nm
k = 0.1 N/m   f= 0.6 MHz in liquid   Quality factor~2



Preliminary miniaturized OLYMPUS AC-7DS probe
L= 6-7 µm   w= 2µm   t= 90nm
k = 0.2 N/m   f= 1.2 MHz in liquid   Quality factor~2

High Speed Atomic Force Microscopy - Improvements

Objectives:

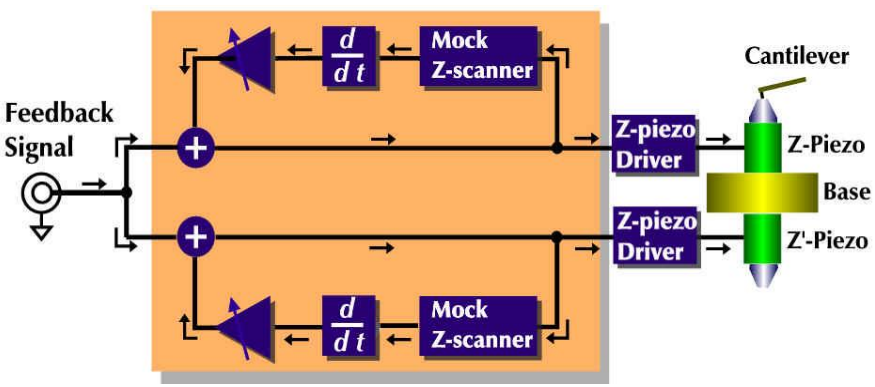
Increase the bandwidth of HS-AFM controller to use

- higher resonant cantilever frequency
- higher scan rate 50 frames/s

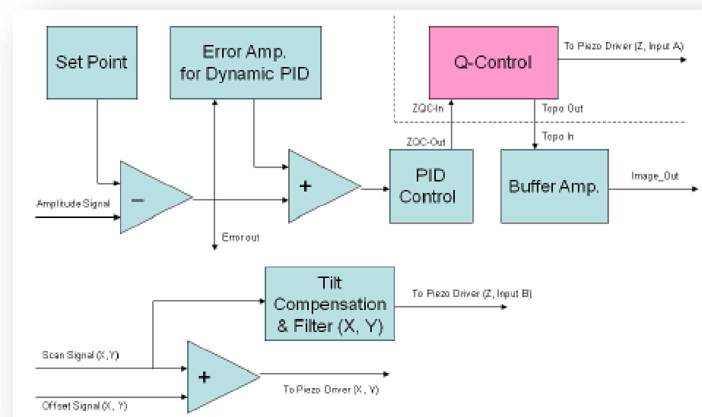
NEW IMPROVEMENTS

Ultra-fast dynamic AFM controller

ultra-fast dual-stage vertical positioner
(88 kHz) with control system



Block diagram of Z-piezo driver



High Speed Atomic Force Microscopy - Improvements

Objectives:

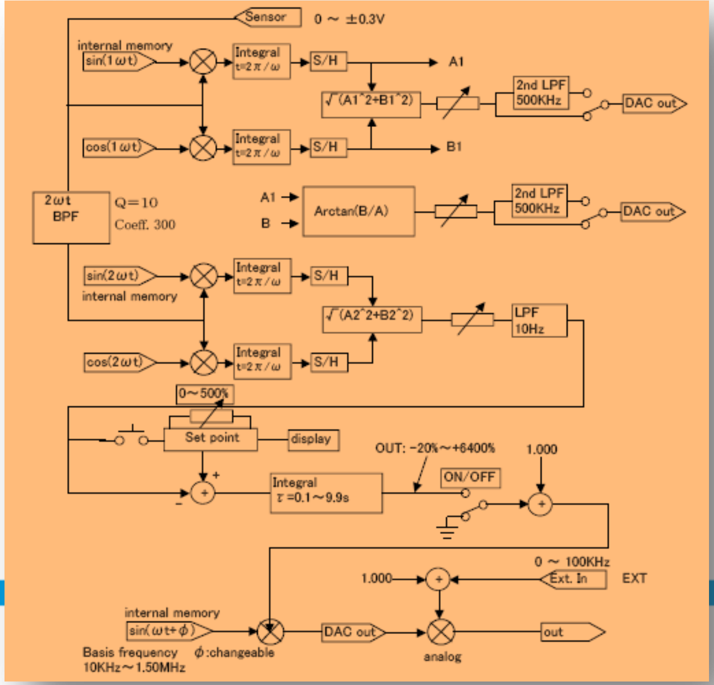
Design of ultrafast Fourier Analyzer for HS-AFM

NEW IMPROVEMENTS

Ultra-fast Fourrier analyzer

Wider frequency range 10kHz to 1.5 MHz

- Fourier Analyzer operates on analog signal at frequency 10kHz to 15 MHz
- Sine wave generator for excitation of piezo cantilever
- Basic phase component [Arctan(B/A)] calculated.
- Amplitude of 2nd harmonic component measured.
- Control the excitation output to maintain the level of amplitude of 2nd harmonic component.



Commercial achievement

HS-AFM lab development

2^{nd} version including new ultra-fast dynamic controller, Fourier analyzer, wider scanner, complete software on IgorPro interface, video analysis program, single computer with double display

HS-AFM1.0 – First commercial High-Speed Atomic Force Microscope developed based on the research achievements

RIBM

Also commercial version from BRUKER at 1 frame/s : Fast AFM

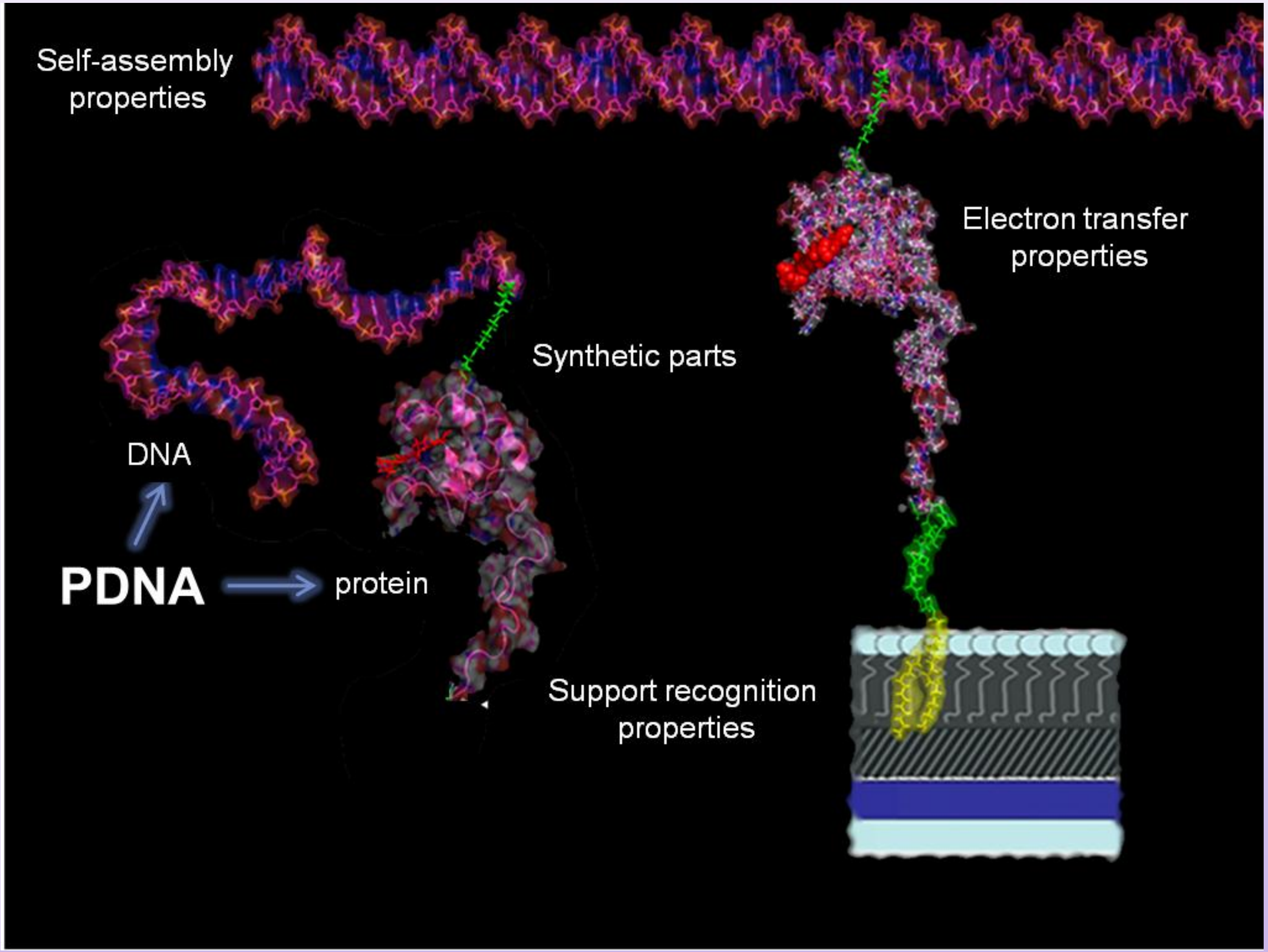
Dynamics of P-DNA blocks

SOME RESULTS ON PROTEIN-DNA INTERACTION

Study of protein-DNA interaction

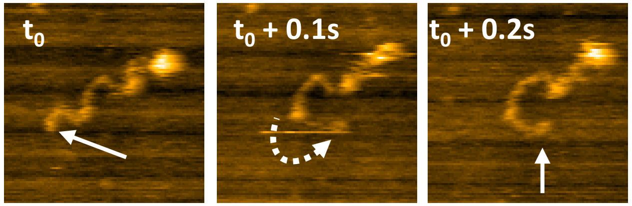
Study self-assembly properties of protein-DNA hybrid structures

- Specificity of cytochrome b5- DNA coupling?
- Effect of the substrate?



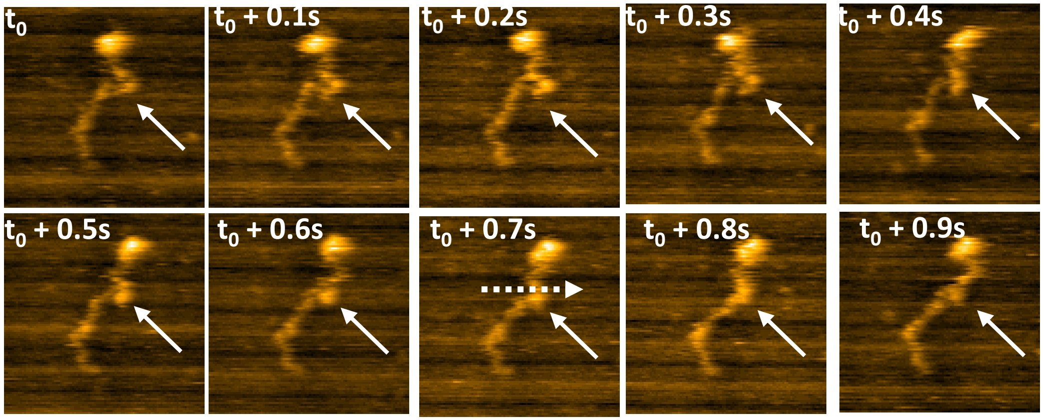
HS-AFM on P-DNA hybrid structure

- High scan rate : 1 to 25 frames/s
- Low tip oscillating amplitude: A_{tip} ~1nm
- Low interaction with the sample: $F=k.\Delta d/Q$~10pN
- Capacity to dissect dynamic processes of molecules



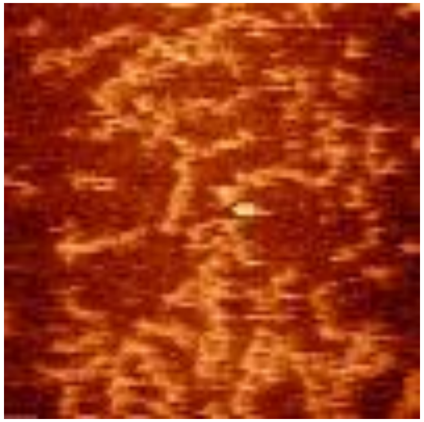
Conformational change

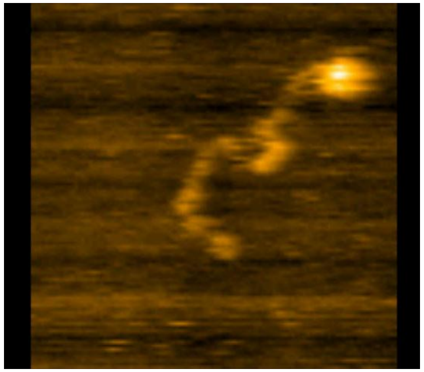
Rate: 800 nm/s



PDNA body reorganization

Rate: 50 nm/s





PDNA
protein-DNA hybrid structures
200 nm scan size
Rate = 10 frames/s
Buffer solution at pH 7.2

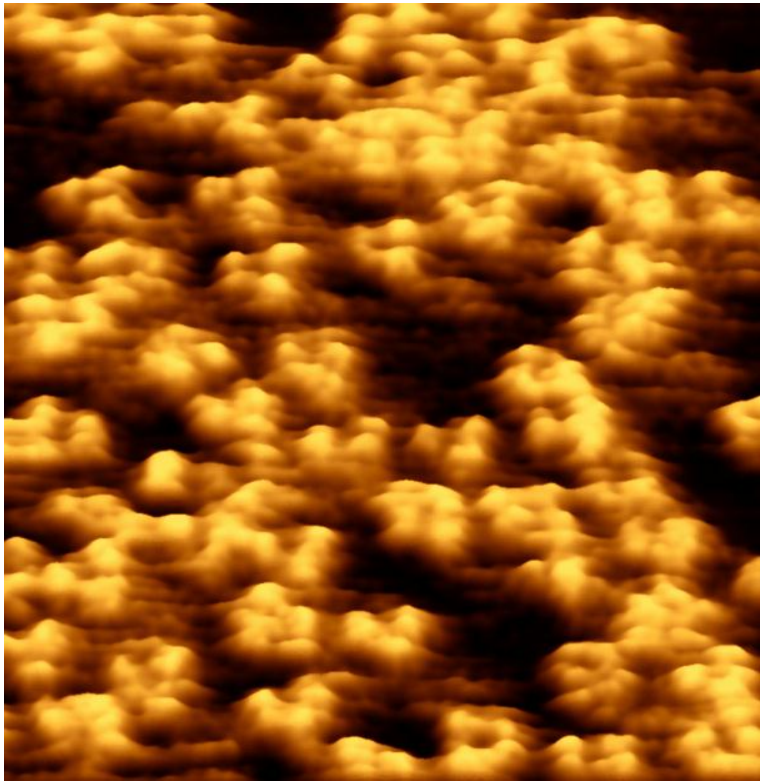
High-Speed AFM

Laisné et al., (2011) Bioconjugate Chem 22, 1824
Laisné et al., (2010) Nuclear Acid Res, 38 , 3817

Dynamics of porin proteins

SOME RESULTS ON PROTEINS

Watching single membrane proteins diffuse at high resolution



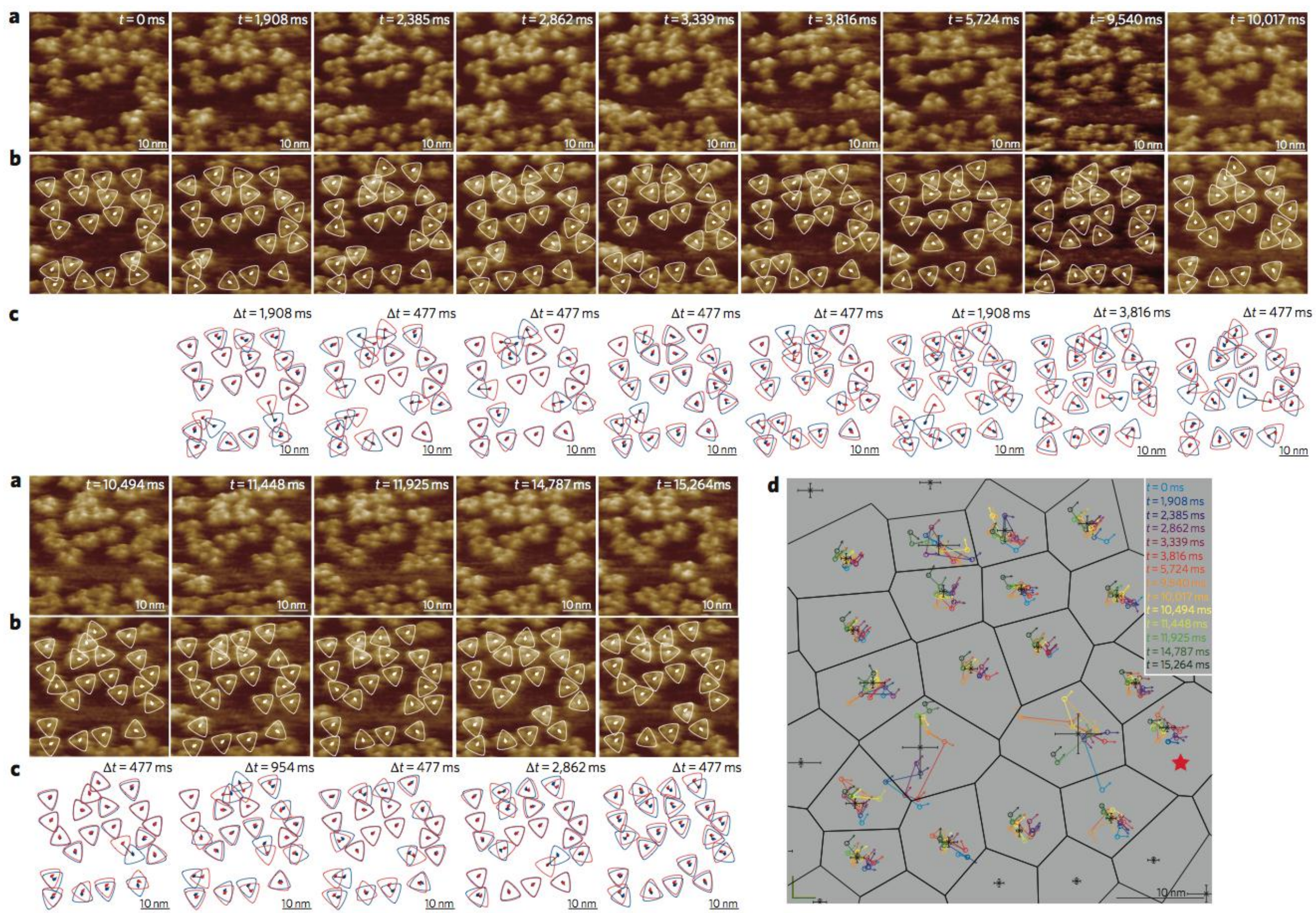
Outer membrane porin OmpF from Escherichia coli reconstituted into lipid bilayers of defined composition

nature nanotechnology LETTERS
PUBLISHED ONLINE: 8 JULY 2012 | DOI: 10.1038/NNANO.2012.109

Characterization of the motion of membrane proteins using high-speed atomic force microscopy

Ignacio Casuso[1], Jonathan Khao[2], Mohamed Chami[3], Perrine Paul-Gilloteaux[4], Mohamed Husain[1], Jean-Pierre Duneau[2], Henning Stahlberg[3], James N. Sturgis[2] and Simon Scheuring[1]*

HS-AFM movie frames showing the motion of OmpF trimers in the membrane



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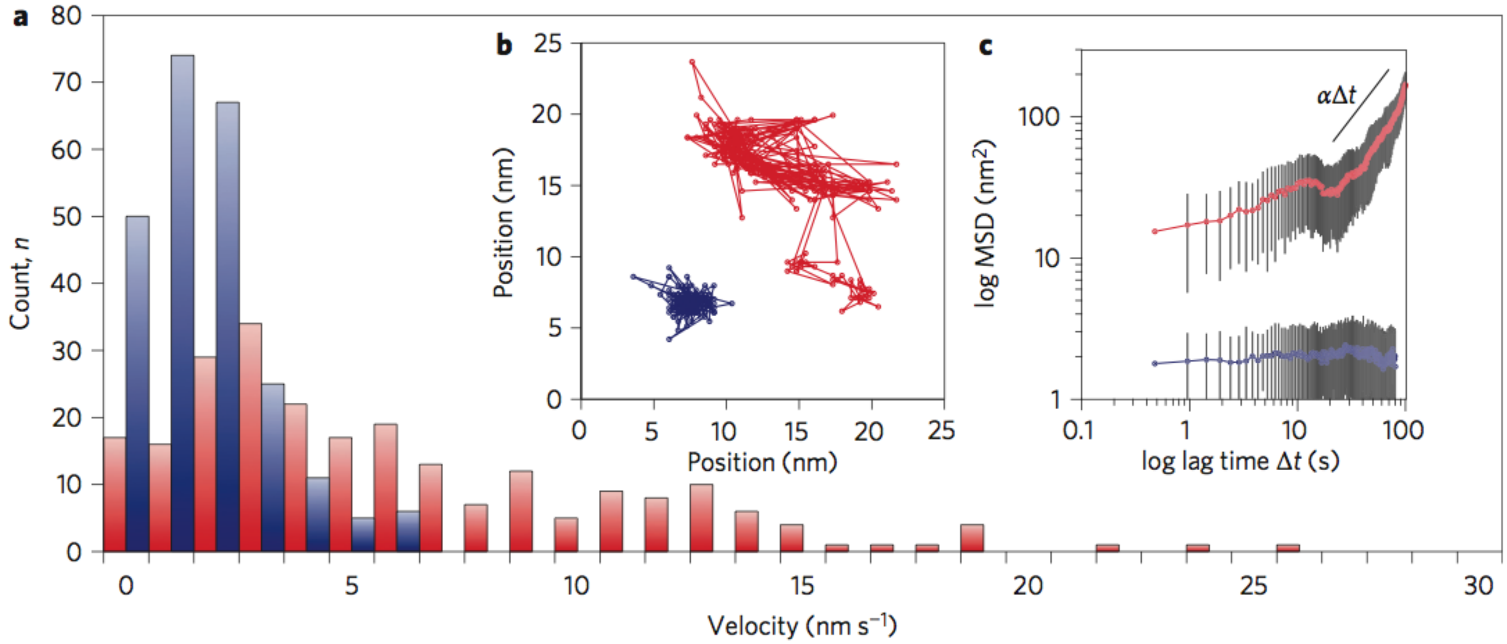
Diffusion analysis of OmpF



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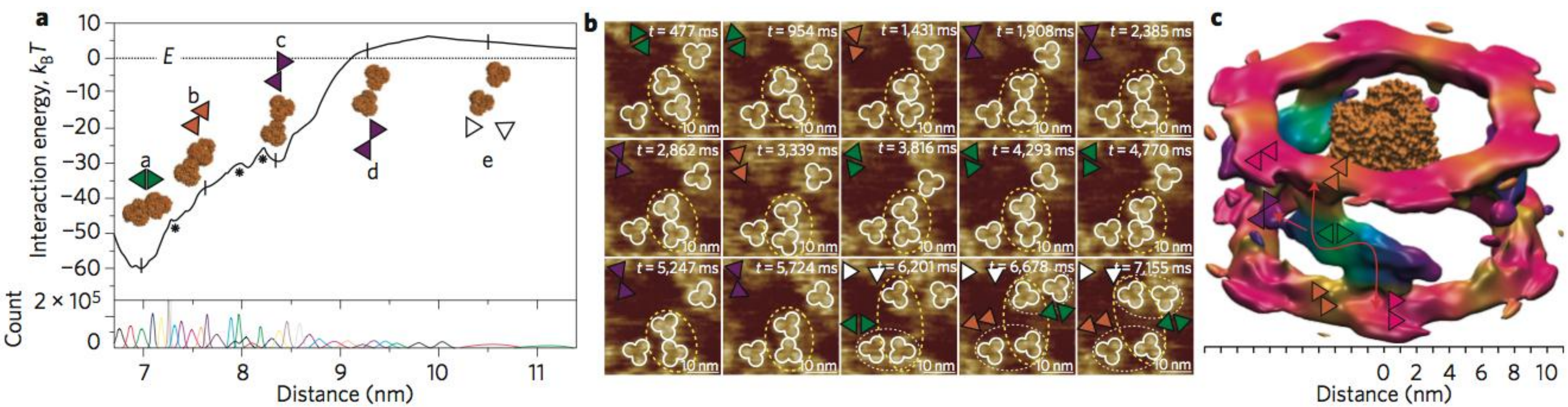
MDS and experimental evaluation of OmpF–OmpF interaction pathway and potential

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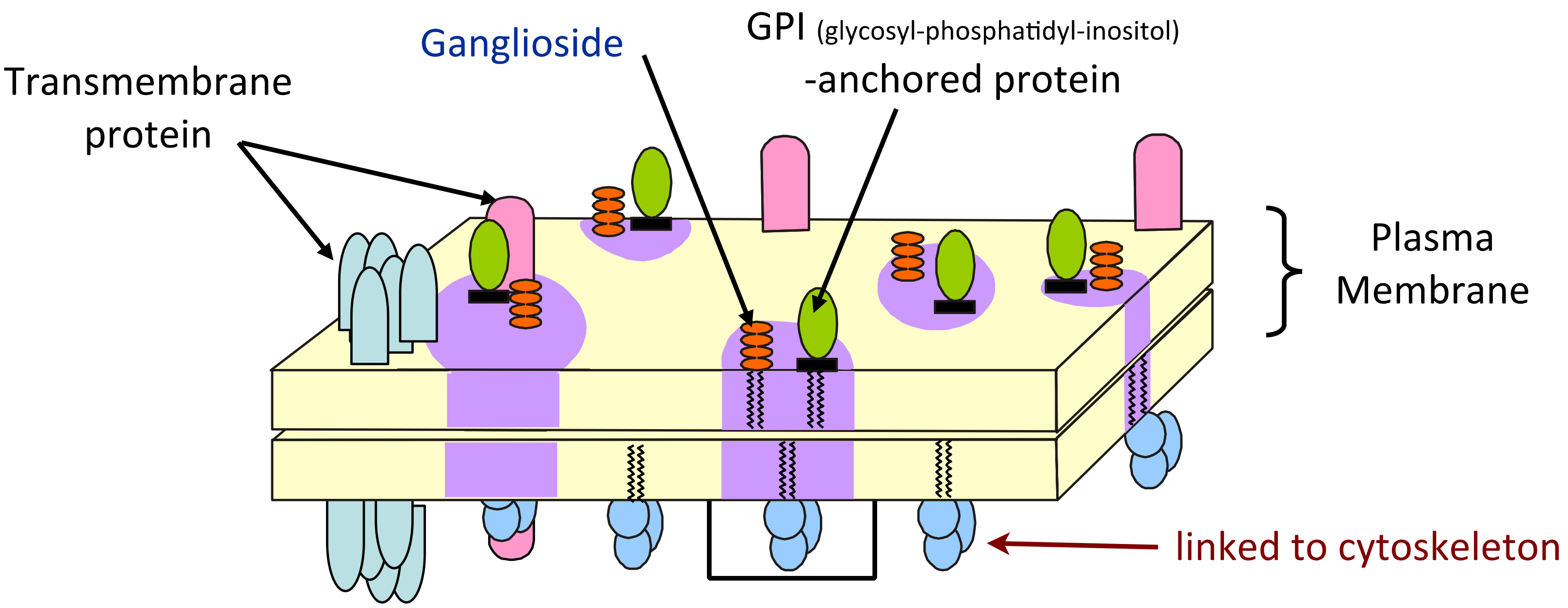
Dynamics of membrane nanodomains

SOME RESULTS ON MEMBRANE DYNAMIC

Structure and Dynamics of Membrane Nanodomains

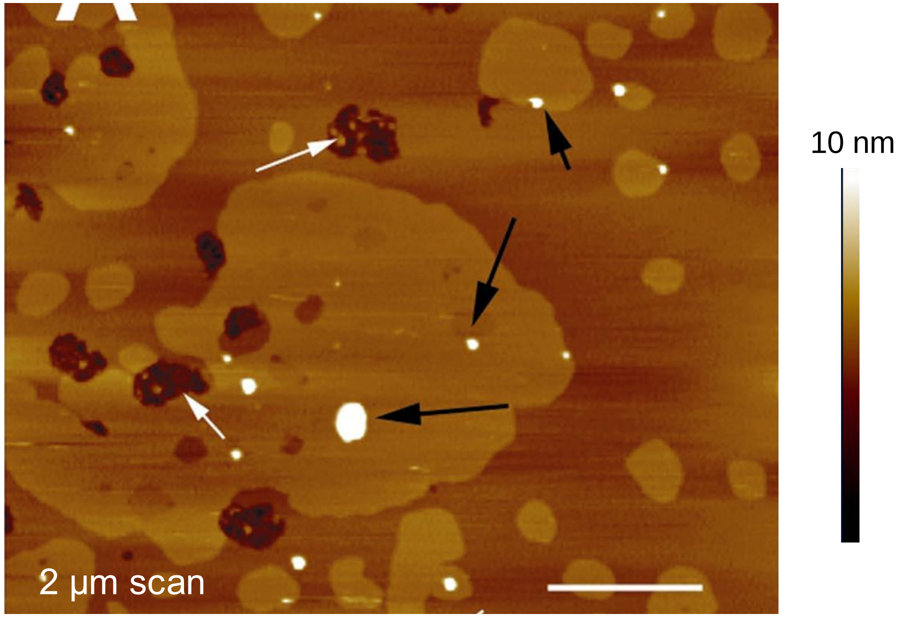
- Main goal: Understanding molecular mechanisms underlying lateral membrane organization in eukaryotic cells



Structure and Dynamics of Membrane Nanodomains

- Watching GM1 ganglioside membrane nanodomains within artificial membranes
  → Standard AFM

DOPC/DPPC + GM1 5%



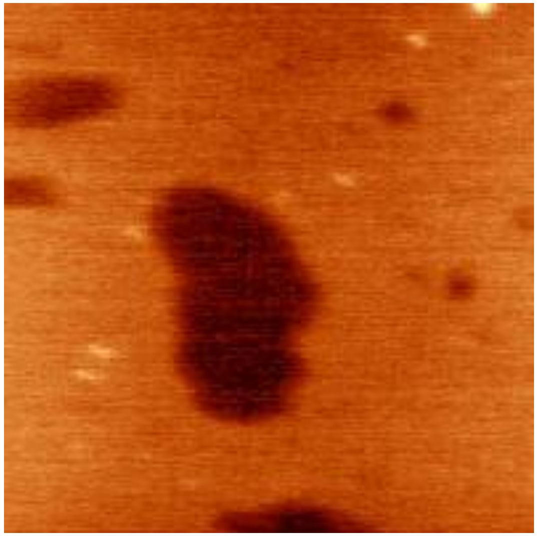
Size of GM1 domains: 30-50 nm protruding 1.2 nm above the membrane

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine (Tm=-18°C)
DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (Tm=41°C)

Structure and Dynamics of Membrane Nanodomains

- Watching GM1 membrane nanodomains within artificial membranes
  → High-Speed AFM

500 nm scan, 1 frame/s



∂DOPC/DPPC 1 nm

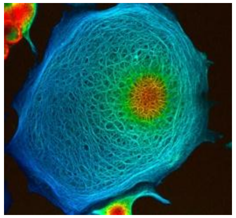
∂h = 1-2 nm
Ø = 20-30 nm

In the presence of GM1 in the lipid mixture ➔ GM1 nanodomains observed
1st frame of this movie clearly show the DOPC/DPPC phase transition
➔ DPPC phase protruding 1 nm above the fluid phase, and some GM1 domains
HS-AFM show that part of them are dynamics and still mainly localized in the gel phase

Dynamics of proteins on biomimetic membrane

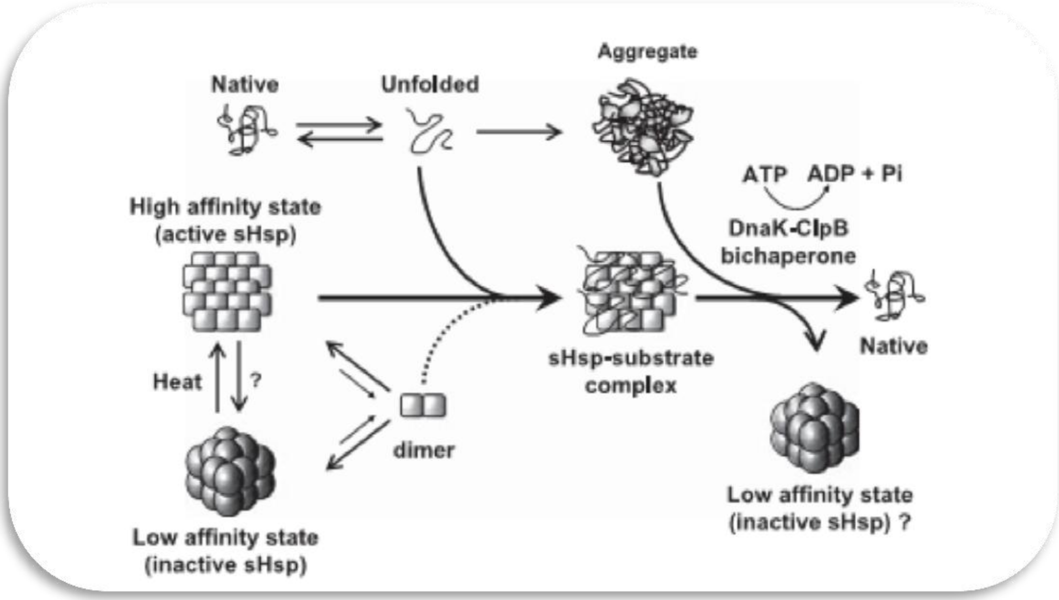
SOME RESULTS ON PROTEIN-MEMBRANE INTERACTION

Small heat shock protein smHsp Lo18



Function

- Reduces in vitro thermal aggregation of proteins
- Act as chaperones by binding to partially unfolded proteins in an ATP-independent manner, preventing their irreversible aggregation under heat shock
- Involved in an adaptive response allowing the maintenance membrane integrity during stress conditions in O. oeni cells



Number of amino acids: 148

Molecular weight: 16937.8 Da

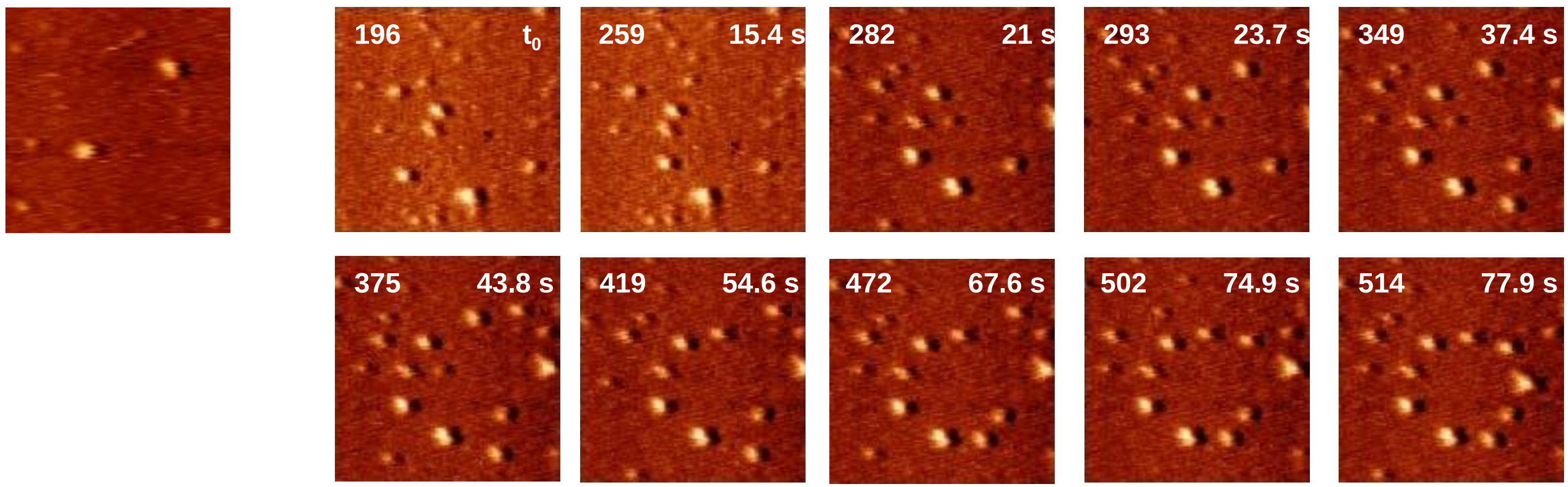
Theoretical pI: 5.10

Oligomerization process for smHsp Lo18

Oligomerization process

sHSP Lo18
300 nm scan size
Rate = 10 frames/s

pH 9 → pH 5



- Creation of hydrophobic bonds
- Complete process can take **80 s** to some minutes

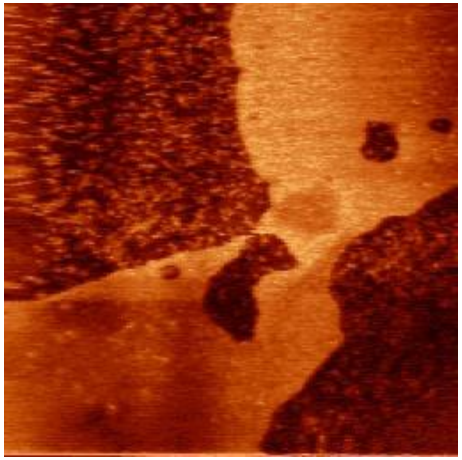
Action of stress on membrane dynamics

- ✓ Effect of chemical stress on membrane organization observed by HS-AFM

  Stress agent = benzylic alcohol

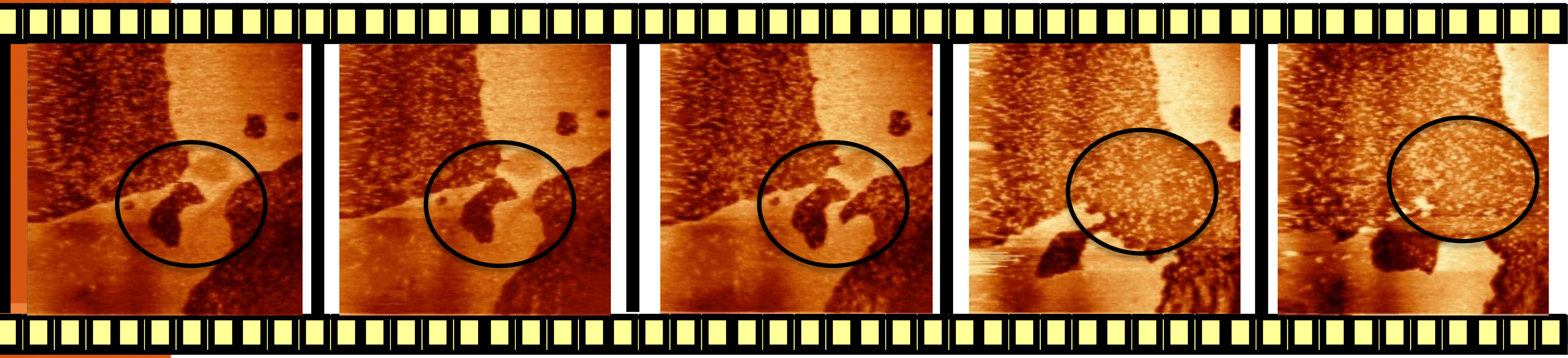
4 images / s

1 µm x 1 µm



Irreversible !

t_0 $t_0 + 4s$ $t_0 + 12s$ $t_0 + 20s$ $t_0 + 30s$



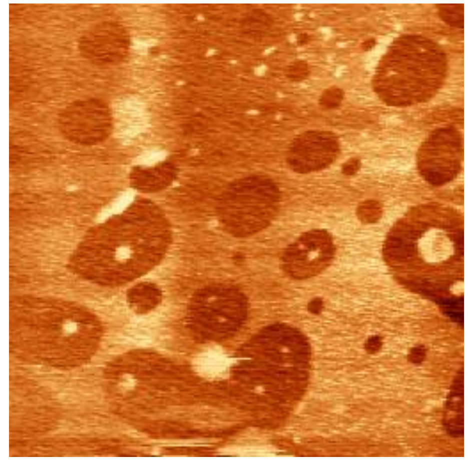
Dissociation of the membrane bilayer

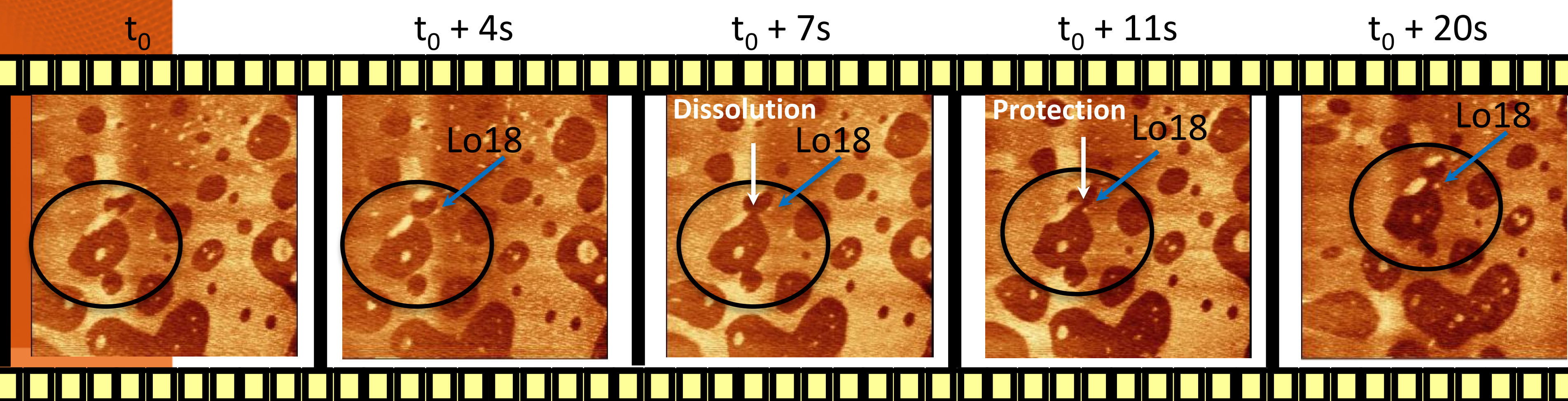
Action of protein on membrane dynamics

- ✓ Effect of protein sHsp Lo18 on chemical stress induced on membrane observed by HS-AFM

4 images / s

800 nm x 800 nm





Lipochaperon activity of sHsp Lo18 put in evidence

Valorisation

		Publications multipartenaires	Publications monopartenaires
International	Revues à comité de lecture	5	12
	Ouvrages ou chapitres	1	
	Communications orales		35
France	Revues à comité de lecture		
	Ouvrages ou chapitres		
	Communications (conférence)		9
Actions de diffusion	Articles vulgarisation		5
	Conférences vulgarisation		
	Autres		Interview radio / TV

Brevet 2011 : Optical component for integrating the optical microscopy into the atomic force microscopy maintaining maximal performance of the AFM. **N° EP 11 305 031.4 – Not licensed**

Contrats industriels : Sanofi-Aventis (Montpellier), ARMOR proteine (Rennes), CAPSUGEL (Colmar), GenFit (Lille)

Conclusions

Improvements:

- Ultra-fast dynamic controller,
- Fast Fourier analyzer,
- Wider scanner up to 30x30 µm scan range,
- Liquid cell adapted to exchange syringe pump
- IgorPro interface & video analysis program

Study of dynamics of biomolecules, cells, virus :

- Information on dynamics of proteins, oligomerization process,
- Information on the mobility ,the diffusion parameters of biomolecules
- Chaperone, lipochaperone activity could be observed
- Provide new insights into mechanics of bio-molecules interaction at nanometer scale

Actual developments:

- Integration of SERS analysis on HS-AFM
- Fluorescence analysis achieved on HS-AFM

Acknowledgements

High-speed Atomic Force Microscopy

- Kanazawa University
  - ***Dr. M. Ewald, Dr. N. Kodera, Dr. T. Uchihashi, Pr. T. Ando***
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  - ***P. Dosset, Dr. P.-E. Milhiet***
- Institut Inserm U1004
  - ***Dr. I. Casuso, Dr. S. Scheuring***
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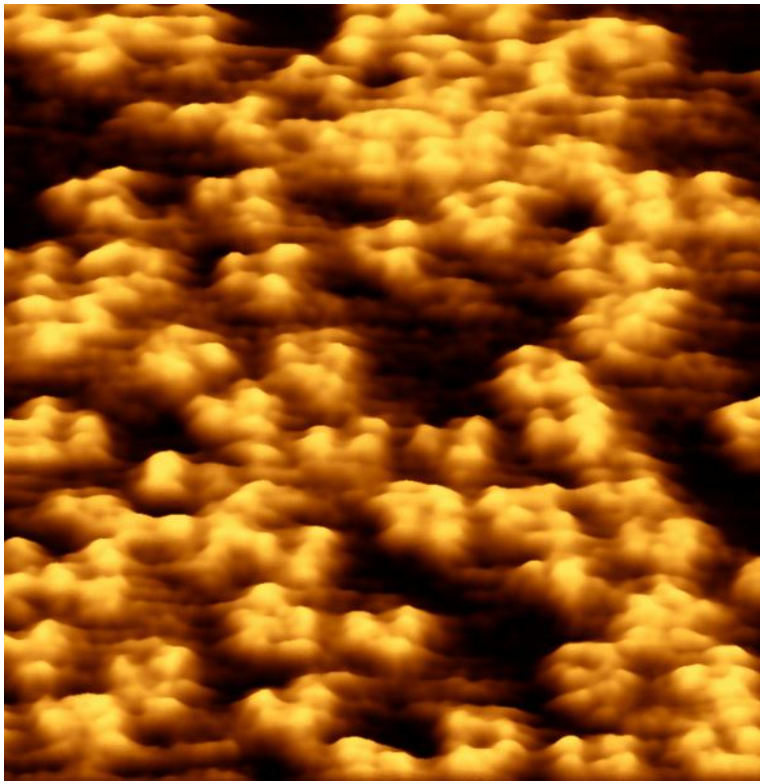
Grants:

Région Bourgogne PARI SMT1 Nano2bio

ANR PNANO 2008 HS-Nanobio-Imaging

Thank you for your attention

The “other feed-back loop”



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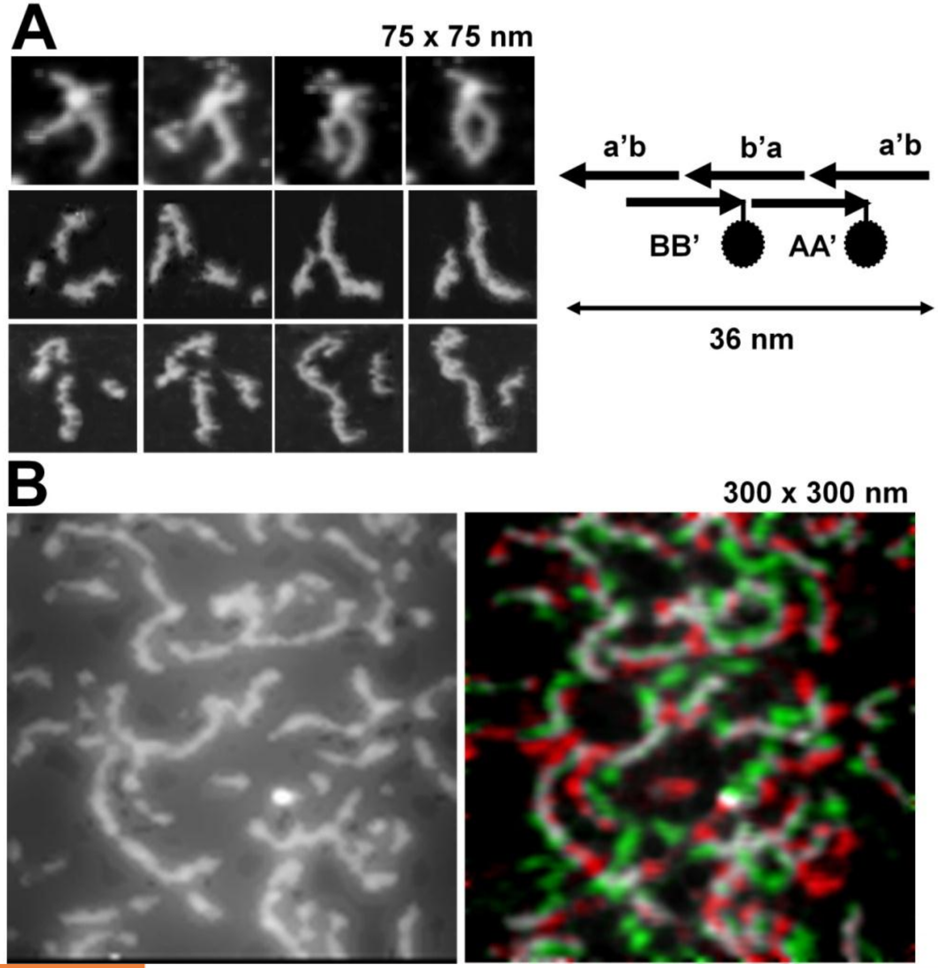
Dynamic AFM analysis of I-motif DNA polymer structures



(**A**) Defined polymer described on schematic

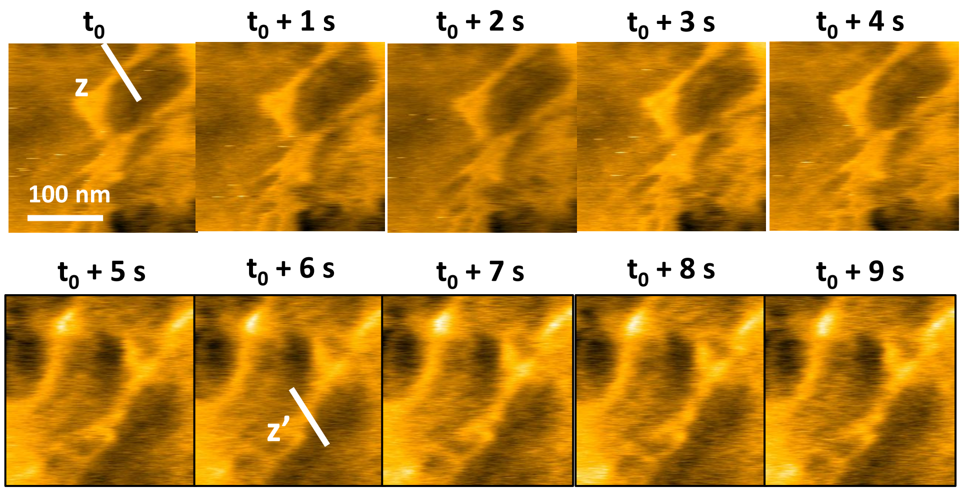
- ➢ Prepared from an equimolar mixture of

2 p-DNA and 3 complementary tiles,

- ➢ Visualization at 100 ms time sequence of selected single molecules.
  - Lane 1: mobility of two molecules trapped on a surface defect illustrated.
  - Lane 2 & 3: formation and dissociation of transient hybrids visualized.

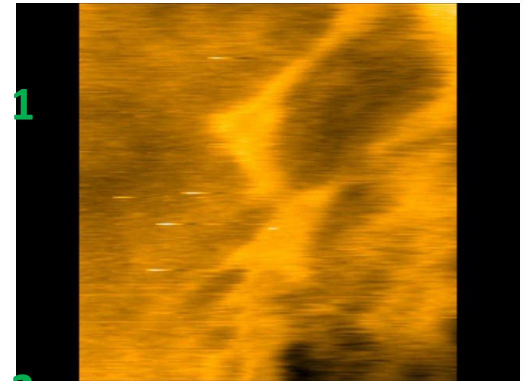
(**B**) Higher density population of the same constructs at a larger scale exhibit transient association giving rise to interacting longer polymers (left).
Overlay of two consecutive images (red and green) illustrating population dynamic on mica surface. Grey colour corresponds to immobile part of polymers.

Epidermal HaCaT cells observed by HS-AFM

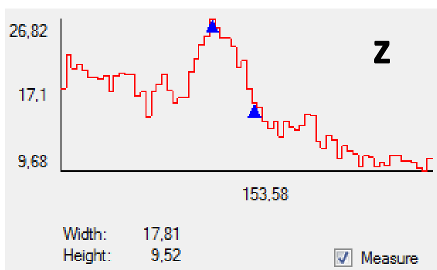


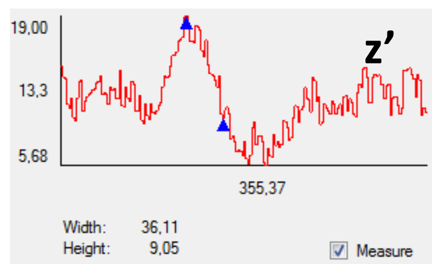
Zone 1

Zone 2



400 nm scan range
10 image/s



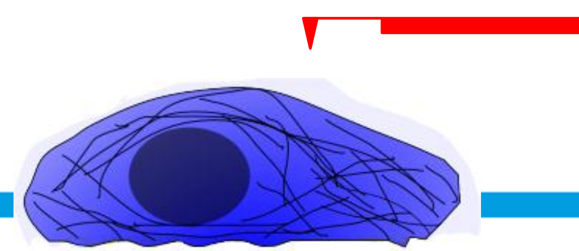


Mesure des sections z = 9.5 nm et z' = 9.1 nm

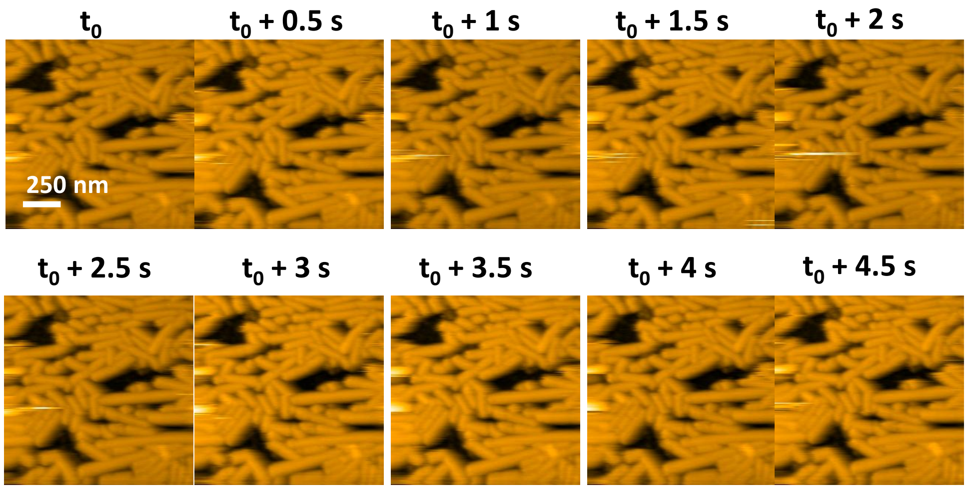
Cellules sur lamelles de verre
Tampon incomplet +
glyphosate [50 mM]

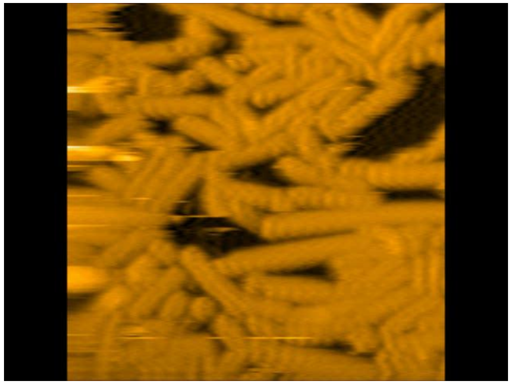
Filaments d'actine

- **Mise en place d'un protocole pour observation**
- **Caractérisation de filaments d'actine**

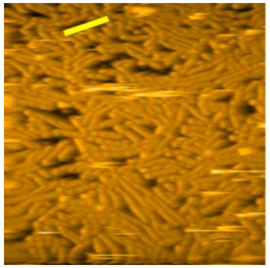


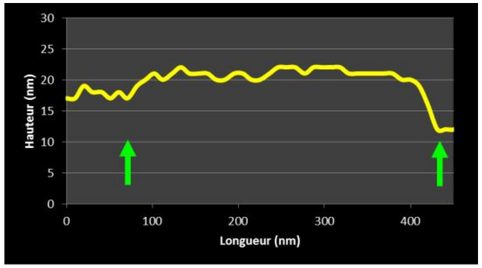
TMV virus observed by HS-AFM



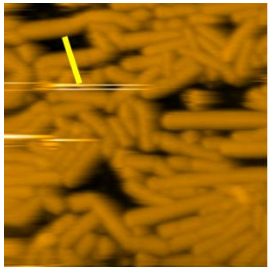


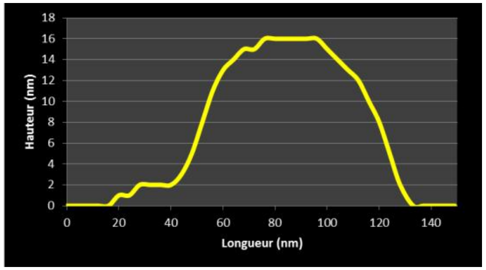
1 µm scan range
2 images/s
Traitement mica $NiCl_2$





Longueur du virus TMV : 335 nm





Hauteur du virus TMV : 16.2 nm