Grenoble | France

Qu'est ce qui perturbe le plus la topographie d'objets biologiques en imagerie AFM

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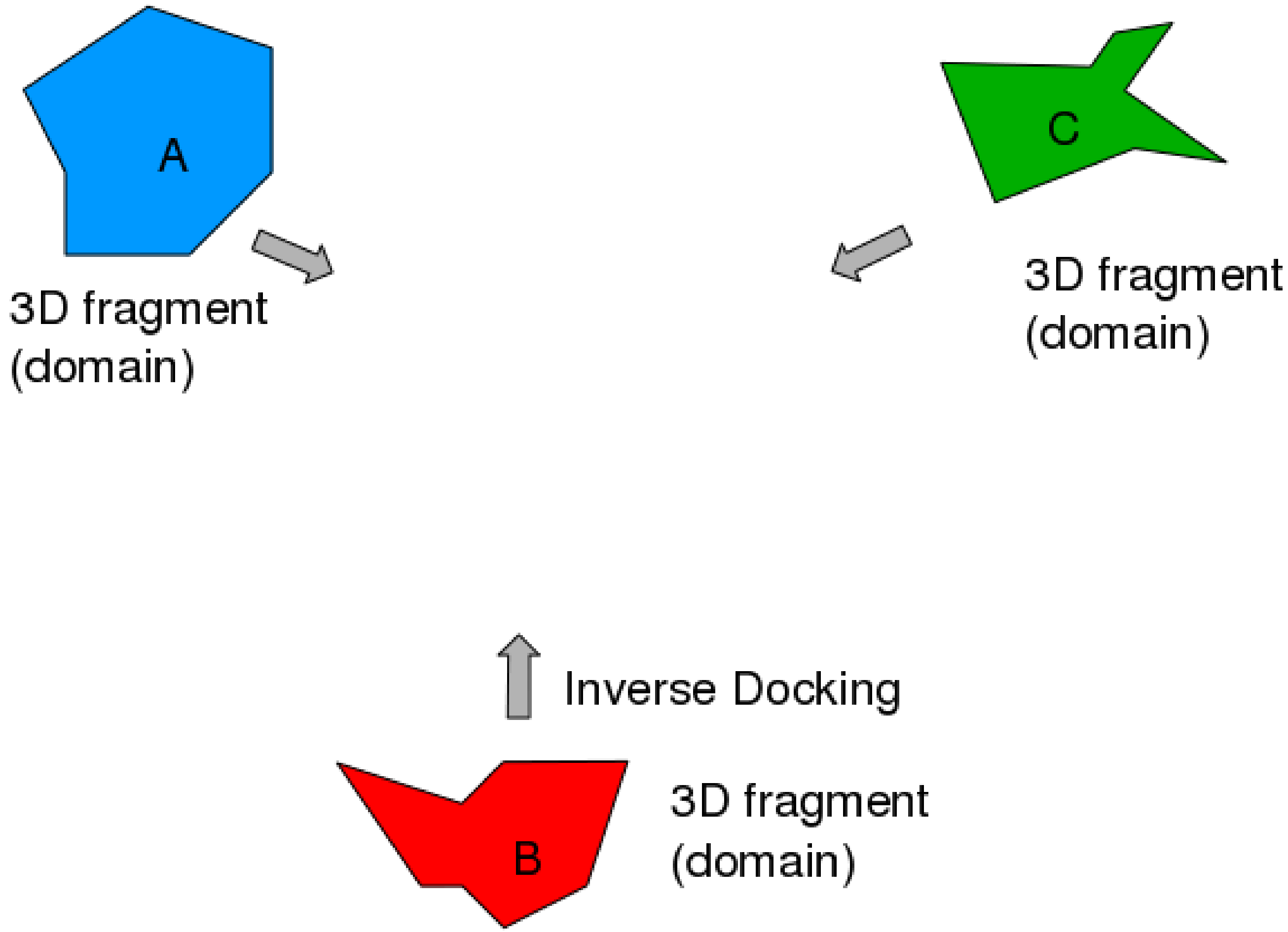
2015: AFM-assembly, an ambitious goal



What is our goal?

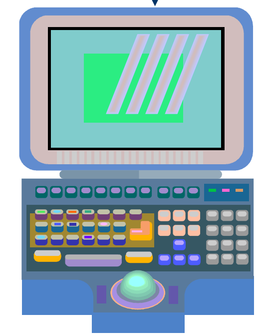
To image single isolated biological systems with the minimum perturbation

How do we minimize perturbations?

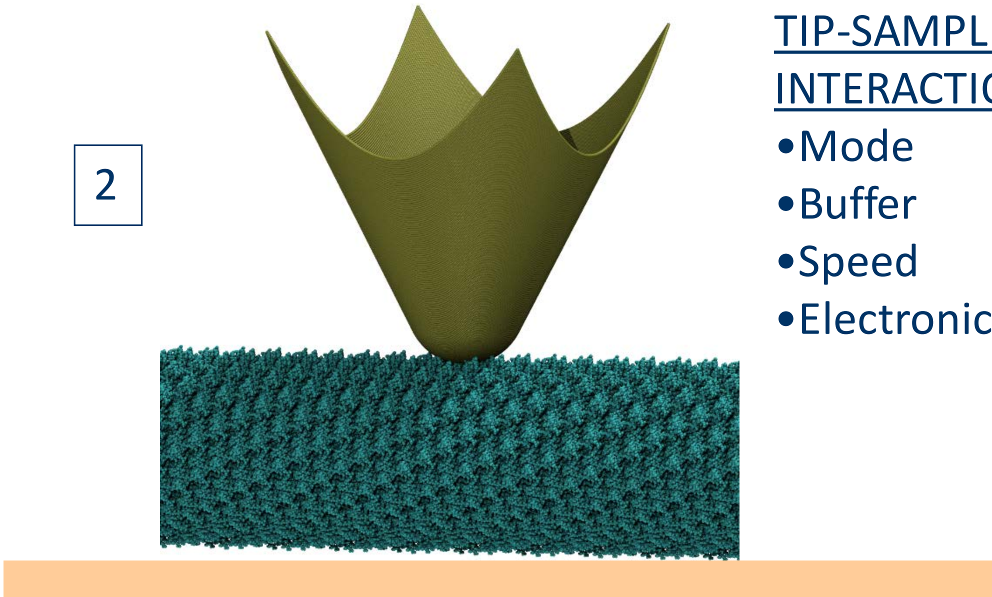
How to improve single molecule AFM imaging

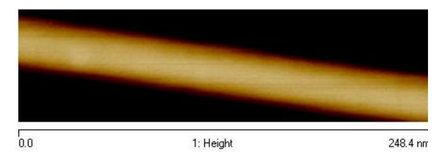
3

TIP-SAMPLE INTERACTION
- Mode
- Buffer
- Speed
- Electronics



TIP 2

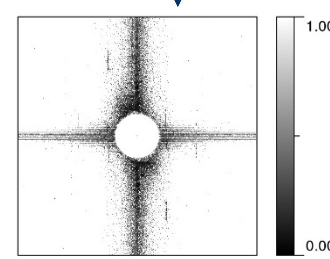




1

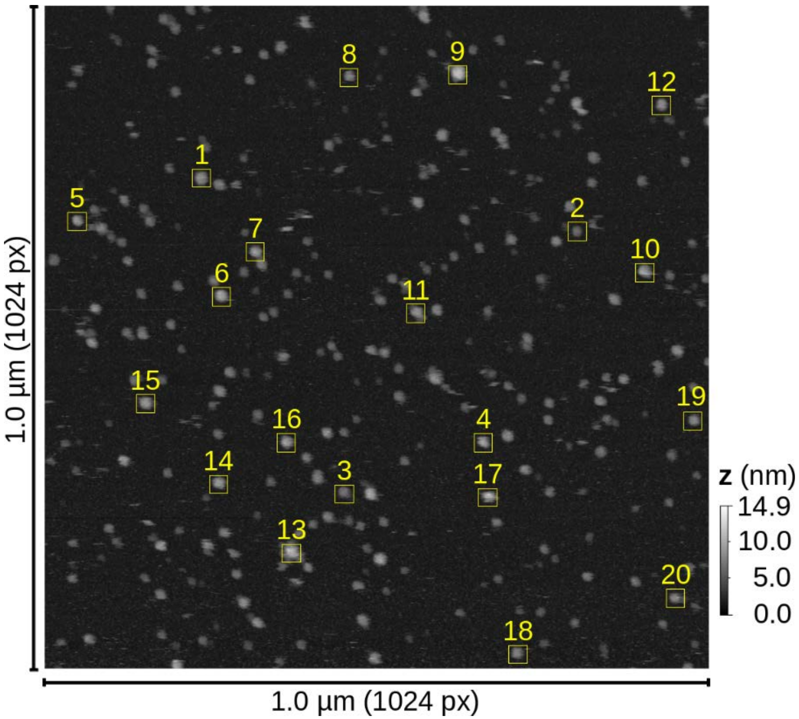
SAMPLE
- Substrate
- Molecular system
- Concentration

DATA PROCESSING 4

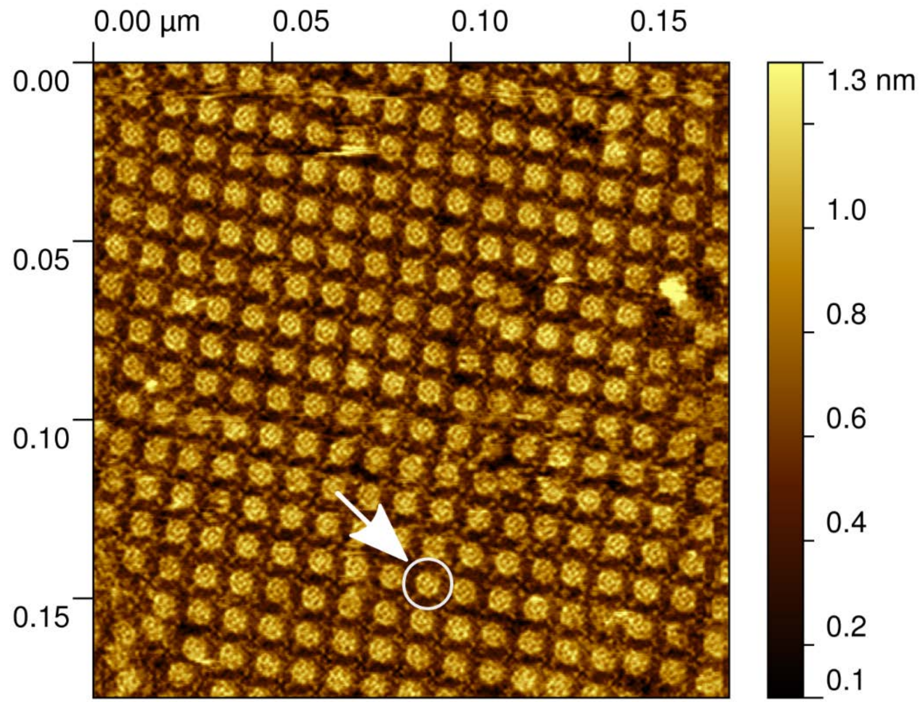


Chen S-wW and Pellequer JL (2011) *BMC Struct. Biol.* **11**: 7.

Working on the sample…

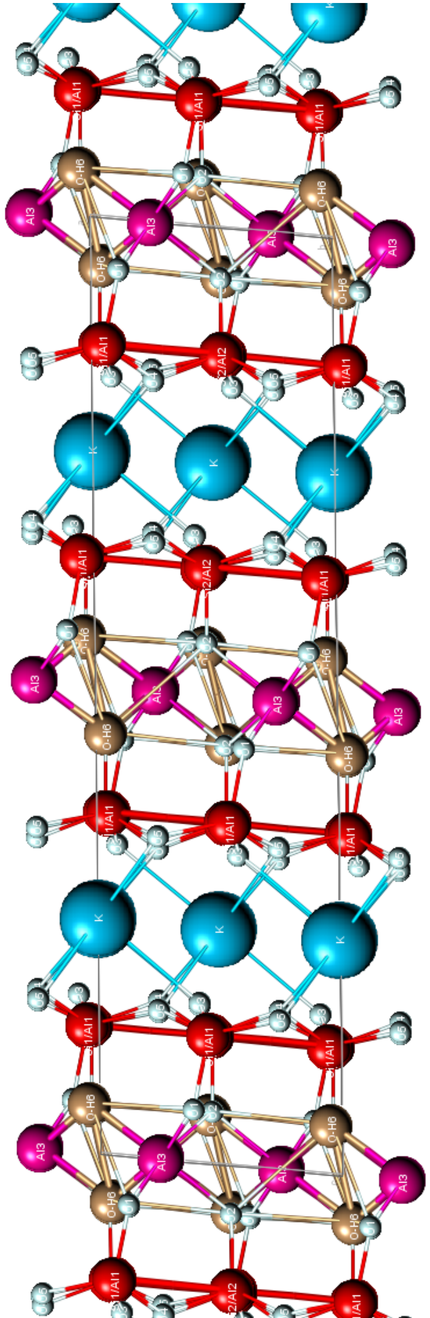


Chaves RC, Dahmane S, Odorico M, Nicolaes GAF and Pellequer J-L (2014) Factor Va alternative conformation reconstruction using Atomic Force Microscopy. *Thromb. Haemost.* ***112: 1167-1173.***

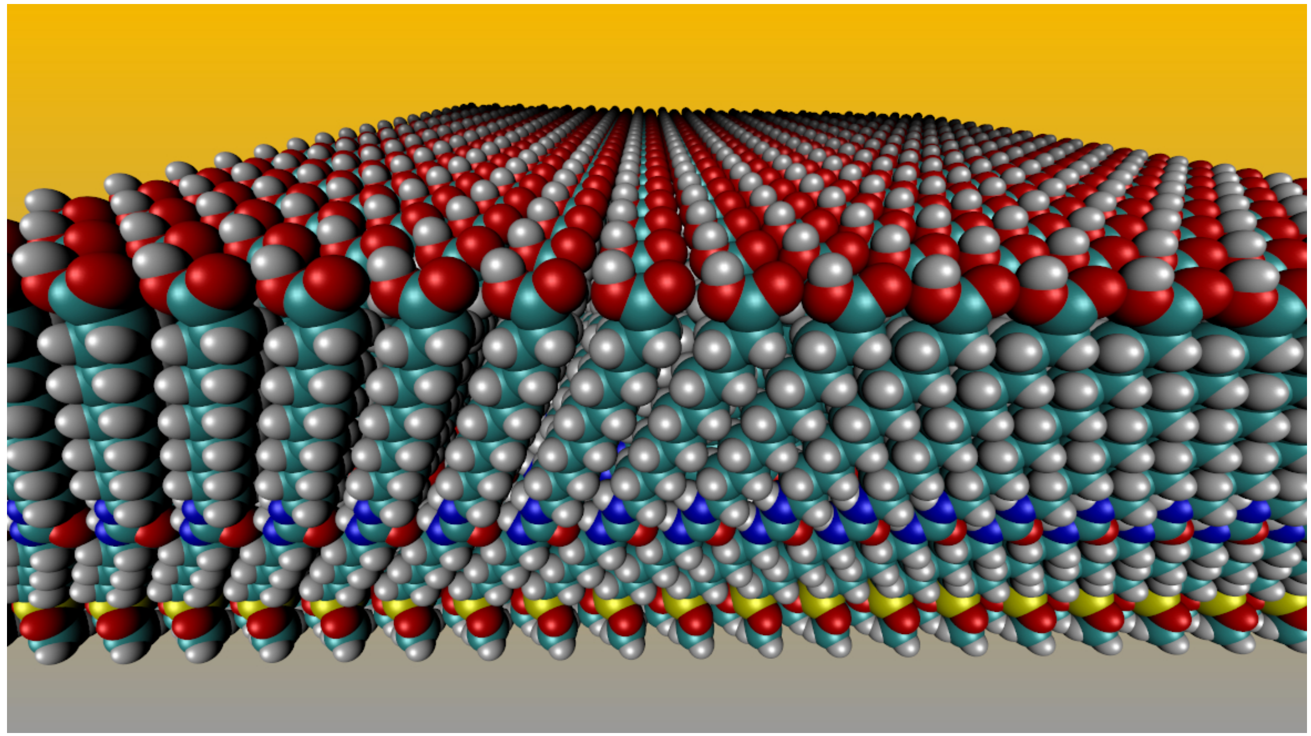


Scheuring, S., Ringler, P., Borgnia, M., Stahlberg, H., Damiel, M., Agre, P., and Engel, J. (1999). High resolution AFM topographs of the Escherichia coli water channel aquaporin Z. EMBO J. *18*, 4981-4987.

Working on the substrate…

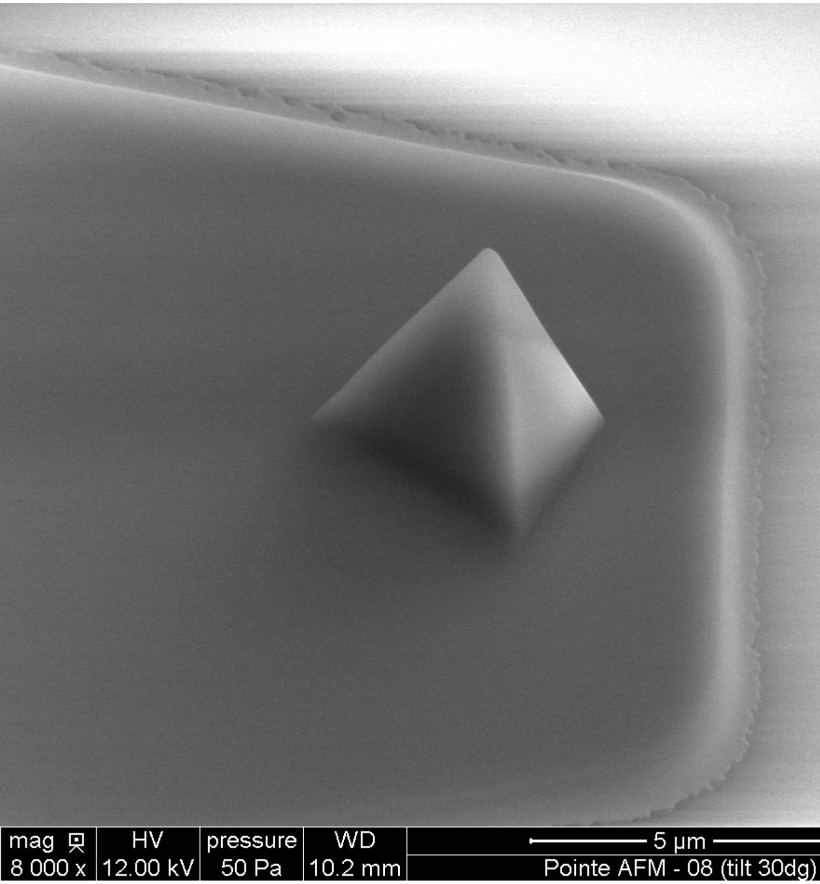


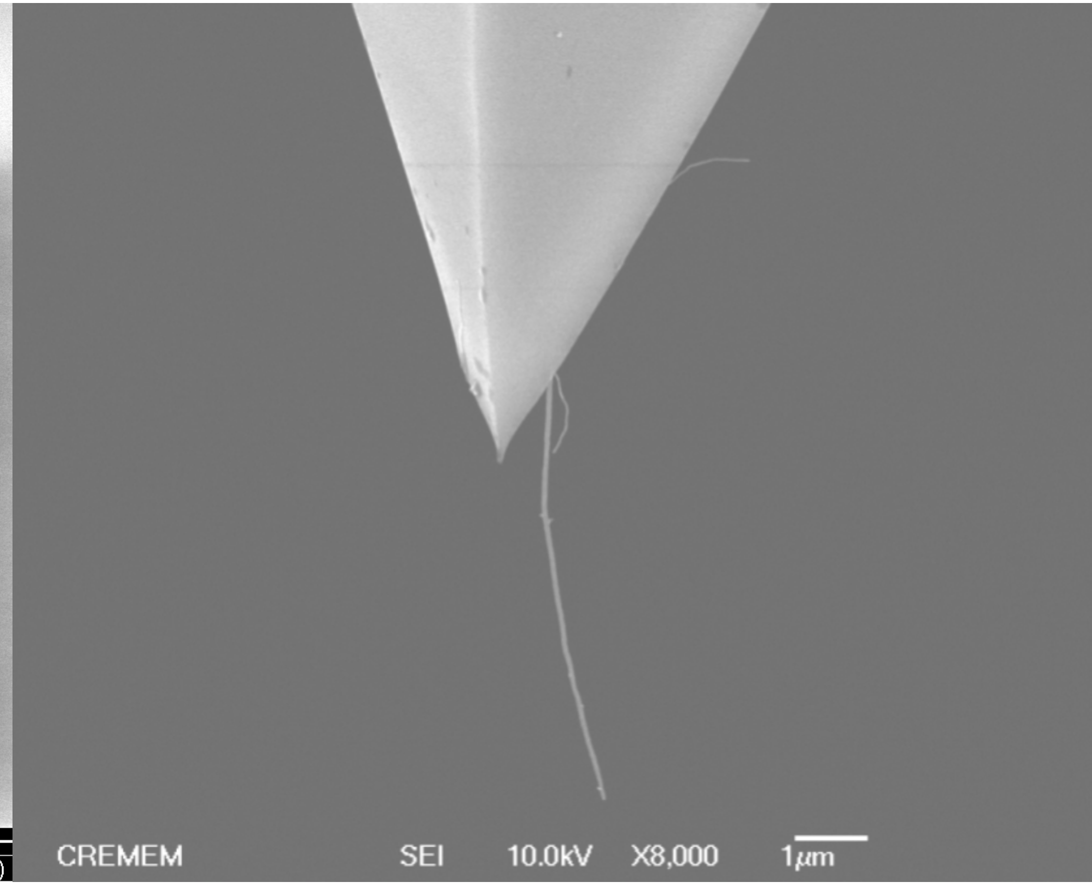
- Silicate mineral of aluminium and potassium: $KAl_2(AlSi_3O_{10})(F,OH)_2$
- Near-perfect basal cleavage yielding thin sheets
- Highly polar/charged surface
- Muscovite is the most common mica



http://webmineral.com/jpowd/JPX/jpowd.php?target_file=Muscovite.jpx

Working on the tip…





c.f. Matthieu, mercredi 17h30!

Tapping versus Peak-Force Tapping

- Dynamic mode
- Resonance Frequency (10-100 kHz)
- Amplitude set-point
- Since 1991

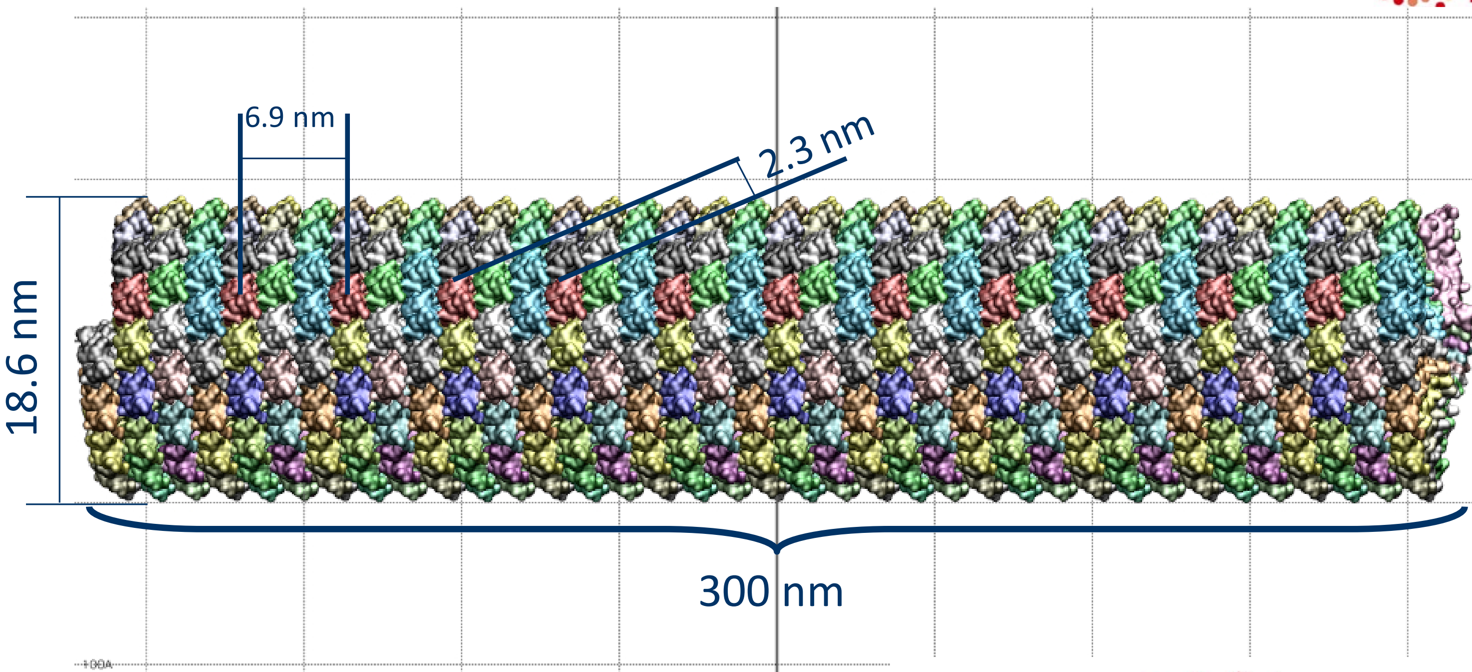
- Dynamic mode
- Off-resonance frequency (2 kHz)
- Force set-point
- Since 2012

Air versus Liquid

- Dried sample
- Residual water layer
- Easier to image
- Assumed to be denaturing

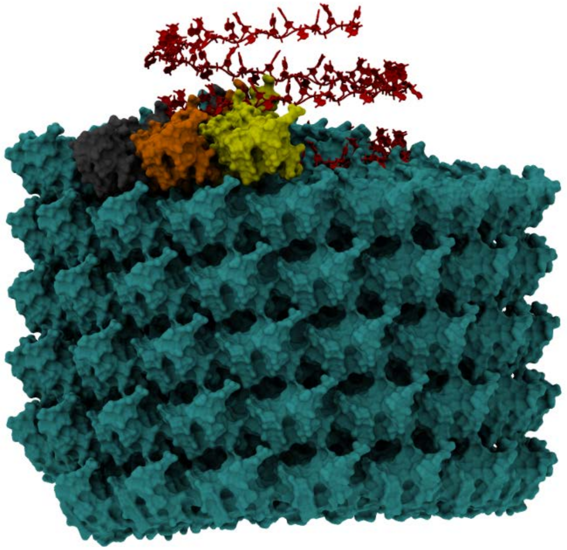
- Buffered sample
- No snap-in effect
- More difficult to image
- Assumed to be native

Lets go back to the fundamentals: TMV…

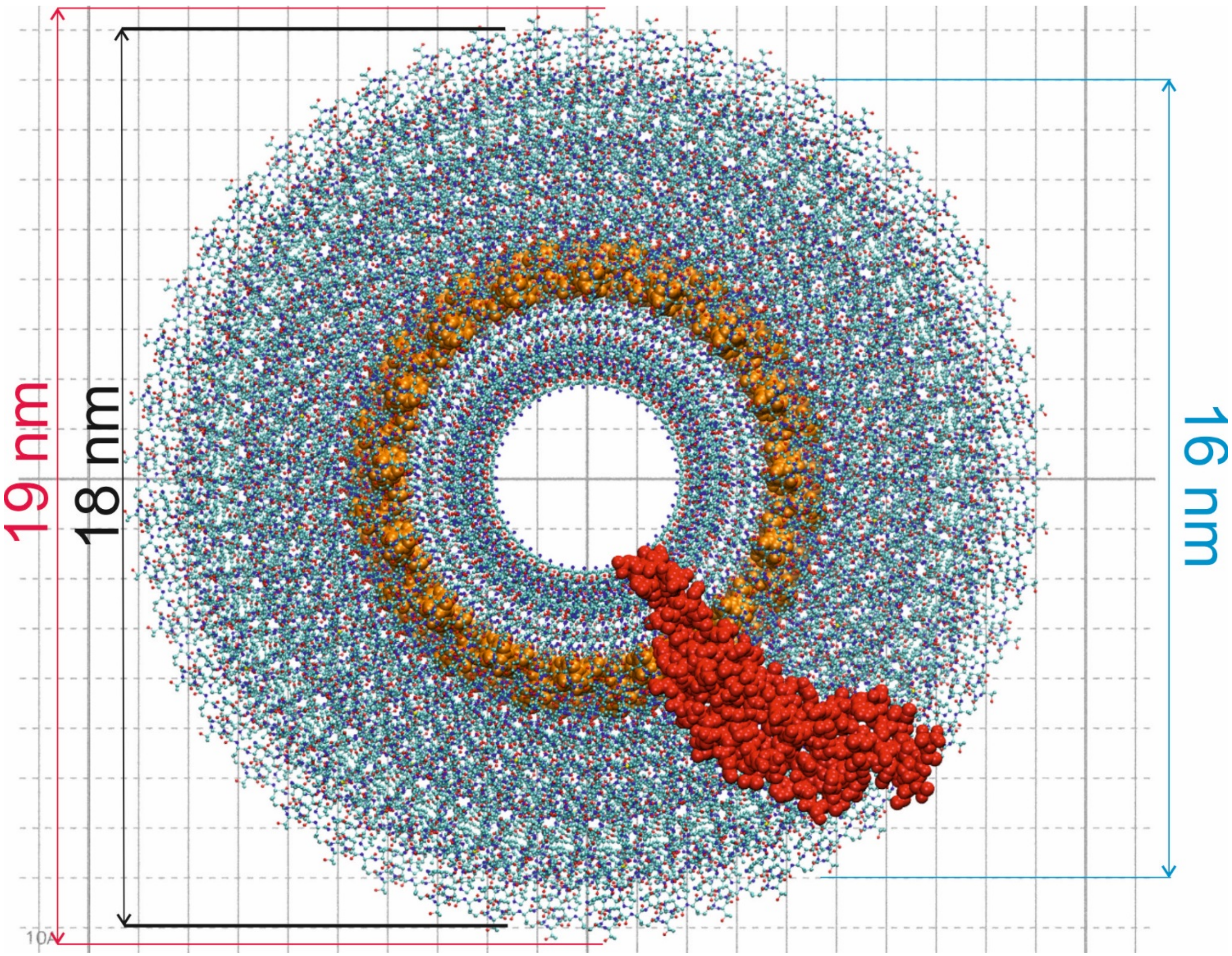


- Single stranded RNA virus
- Infects plants of the family Solanaceae
- Thermostable virus (up to 50°C)
- Easy to produce and purify

1VTM: Pattanayek R and Stubbs G (1992) *J. Mol. Biol.* **228**: 516-528.



Our target: the TMV height

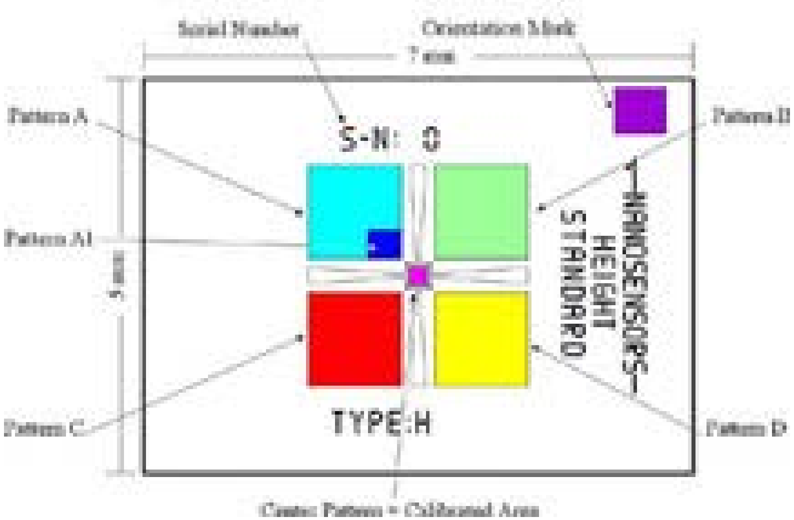


1VTM

What is wrong with TMV and AFM?

TMV height (nm)	Environment	Substrate	Imaging mode	Comments	Ref
19-23	Air	Mica	Contact	No calibration	(Drygin et al., 1998)
17-23	Air	HOPG	Tapping	No calibration	(Drygin et al., 1998)
~20	Air	Mica/HOPG	Tapping	Estimated value from scale bar in images	(Falvo et al., 1997)
16.8 – 18.6	Air	Glass	Tapping	Measured on aggregated viruses	(Maeda, 1997)
18.2 ± 1	Liquid	Mica	Tapping	Measured by cross-sections	(Schabert and Rabe, 1996)
18	-	HOPG	Non-contact	Measured by cross-sections, no statistics, with calibration	(Knez et al., 2004)
17.2	Liquid	Mica	Contact	Measured by cross-sections, single image	(Zhao et al., 2008)
17	Air	Blodgett film on silicon	Non-contact	No statistics	(Anselmetti et al., 1994)
15	Liquid	Mica	Tapping	Measured by cross-sections, no statistics, with calibration	(Knez et al., 2004)
12	Liquid	Gold	Contact	Measured by cross-sections, no statistics,	(Knez et al., 2004)

What about metrology?



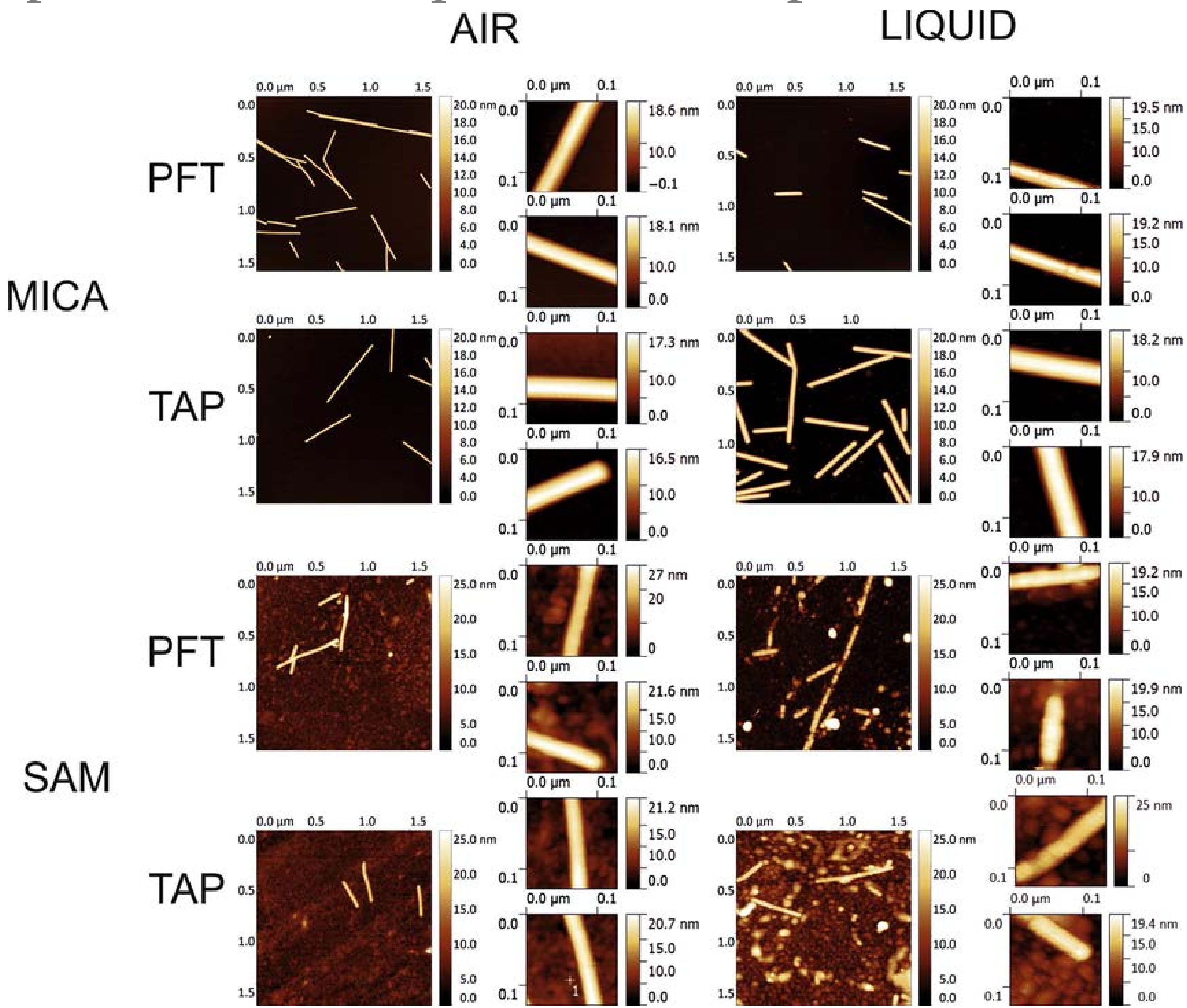
Height Standard with 8 nm nominal step height

Environment	PFT	TAP
AIR (ambient)	7.51 ± 0.09	7.41 ± 0.37
LIQUID (water)	7.38 ± 0.49	6.99 ± 0.59

[a] The nominal depth value used for calibrating scanners is 7.3 nm.

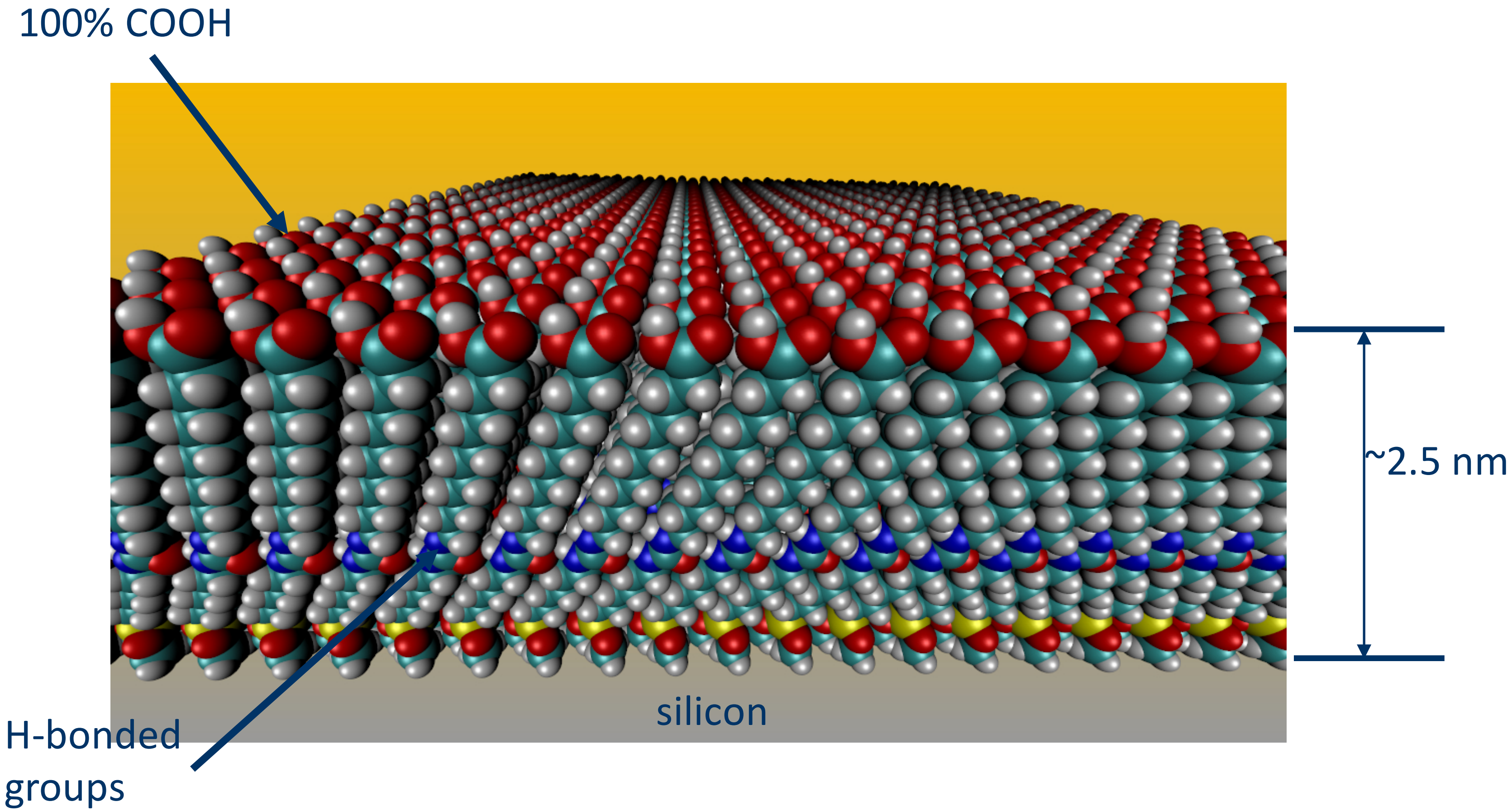
Godon C, Teulon J-M, Odorico M, Basset C, Meillan M, Vellutini L, Chen S-wW and Pellequer J-L (2017?) Conditions to minimize soft single biomolecule deformation when imaging with atomic force microscopy. *J. Struct. Biol.* ***197: in press.***

Experimental setup: 8 different parameters…



SAM: self-assembled monolayer

Trimethoxysilane-based self-assembled monolayers



provided by the laboratory of Dr. B. Bennetau (ISM, Univ. Bordeaux)

Results:

Environment	Substrate[a]	Imaging Mode[b]	TMV height (nm) Dataset 2015[c]	TMV height (nm) Dataset 2014d	TMV height (nm) Dataset 2013e
AIR	MICA	PFT	15.85 ± 0.37 (N_{crop}=47)[f]	18.48 ± 0.70 (Ncrop=30)	16.80 ± 0.24 (Ncrop=11)
		TAP	15.78 ± 0.35 (N_{crop}=29)	17.90 ± 0.67 (Ncrop=31)	15.95 ± 0.08 (Ncrop=15)
	SAM	PFT	19.73 ± 1.47 (N_{crop}=18)	19.58 ± 0.73 (Ncrop=23)	19.14 ± 0.33 (Ncrop=13)
		TAP	18.46 ± 1.17 (N_{crop}=16)	18.47 ± 0.52 (Ncrop=20)	18.13 ± 0.50 (Ncrop=21)
LIQUID	MICA	PFT	18.42 ± 0.19 (N_{crop}=28)		
		TAP	17.31 ± 0.23 (N_{crop}=19)		
	SAM[g]	PFT	18.55 ± 1.38 (N_{crop} = 6)		
		TAP	17.50 ± 1.02 (N_{crop} =28)		

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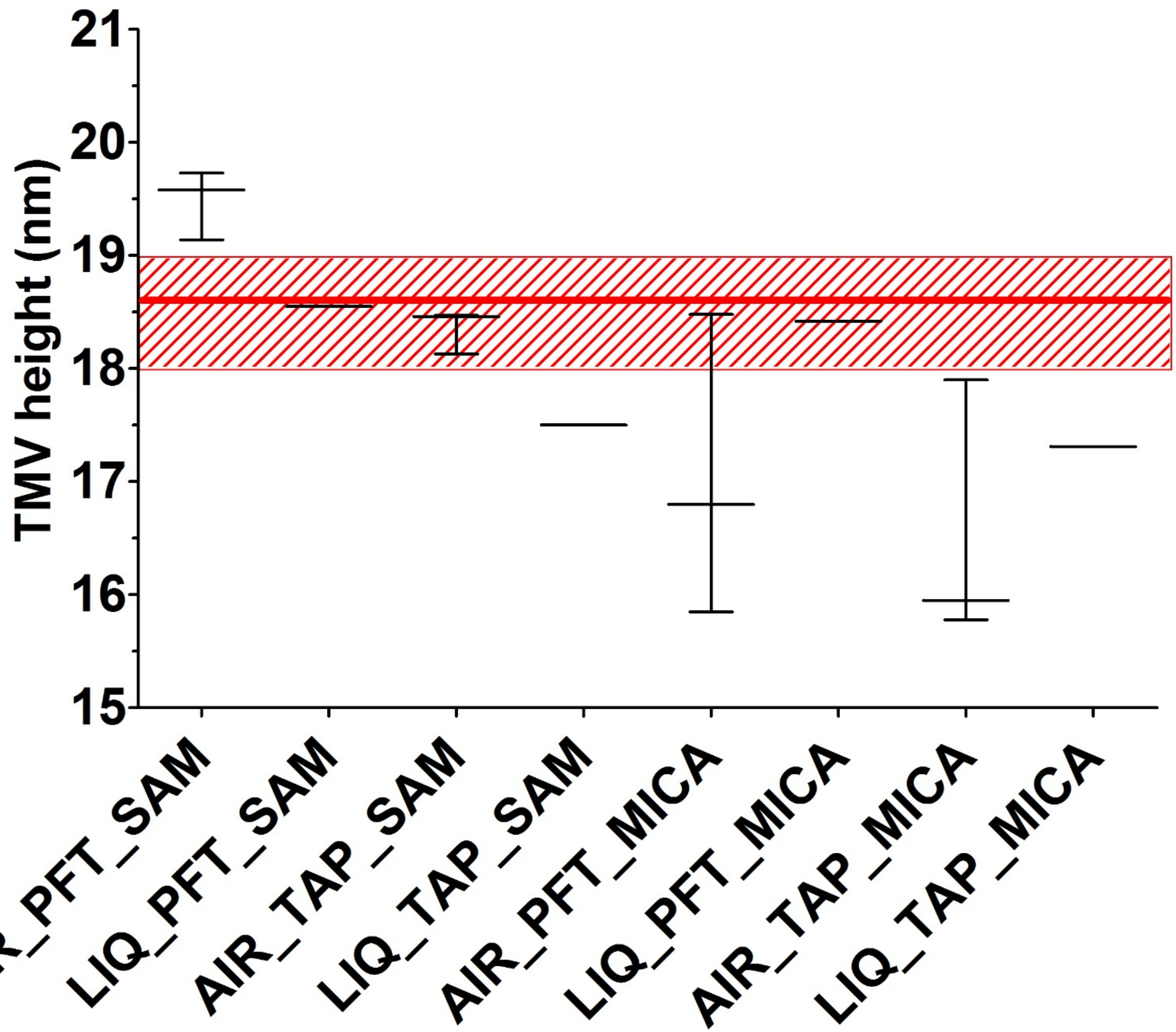
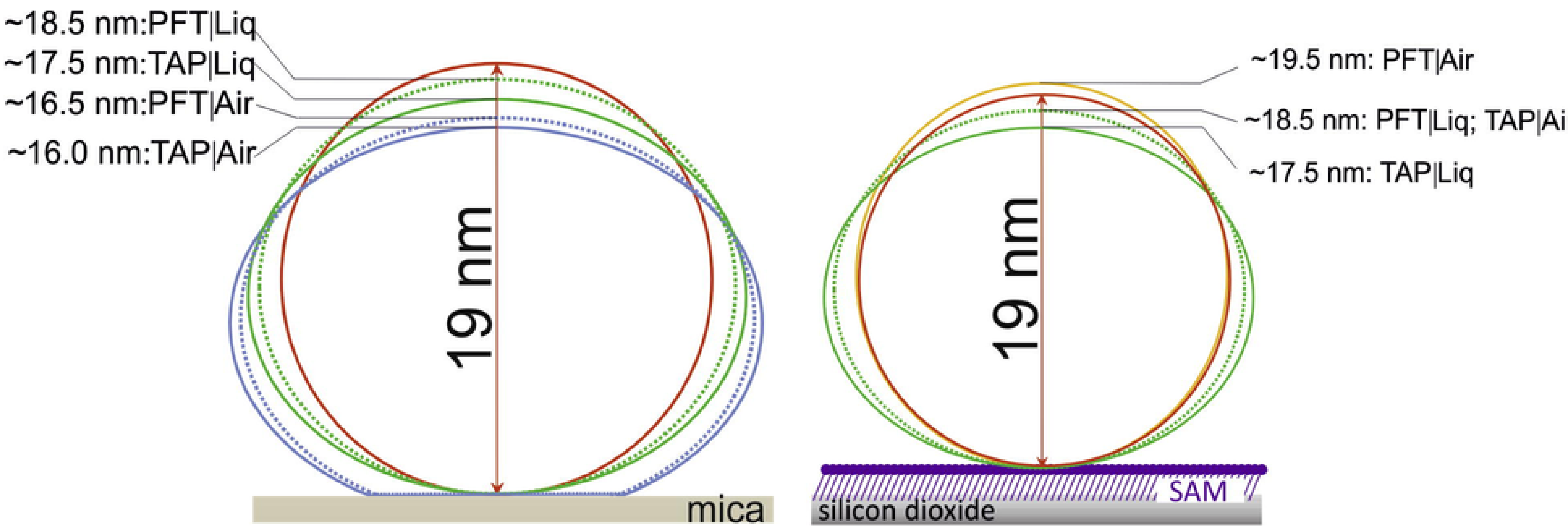
Results:

Global summary (3 years)



TMV height summary



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Conclusions

- Systematically, TMV height is lower in tapping mode than Peak-Force
- Otherwise, no other parameter is responsible for height loss during imaging
- Imaging in air is acceptable if SAM substrate are used
- Mica surface is only adequate when using liquid imaging

Take home message:

- Tapping (vs PFT) = 1/19 = 5.2 % loss in height
- Mica (vs SAM) = 1.5/17.5 = 8.5 % loss in height
- Air (vs Liquid) = ~2/19 = 10.5 % loss in height

Projet de standardisation des fichiers données AFM

Définition des méta-données en imagerie
Définition des méta-données en courbe de force

AFM BioMed Conference Krakow, Poland, September 4(5)-9, 2017

FIRST ANNOUNCEMENT

After Barcelona 2007 (Spain), Monterey 2008 (USA), Red Island 2010 (Croatia), Paris 2011 (France) and Shanghai 2013 (China), San Diego 2014 (USA), Porto 2016 (Portugal), AFM BioMed Conference has the pleasure to announce the 8th conference on AFM for Life Sciences and Nanomedicine, on September (4)5-9 September 2017 (including training) in Krakow, Poland.

The Conference co-organizers are the Institute of Nuclear Physics of the Polish Academy of Sciences and the Jagiellonian University.
The venue is in a new building near the Auditorium Maximum.

The Conference is chaired by **Prof. Małgorzata Lekka, Polish Academy of Science and Prof. Marek Szymonski, Jagiellonian University.**

Featured sessions / confirmed chairs:
Health and Diseases / **Ewa Wojcikiewicz** (Florida Atlantic University)
Bioimaging / **Alessandro Podesta** (Milano University)
Cellular Mechanobiology / **Wouter H. Roos** (University of Groningen)
Molecular Forces / **Martin Guthold** (Wake Forest University)

Organizing Committee

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Adam Engler	University of California San Diego, USA
Susana Sousa	INEB|i3S, Porto, Portugal

More details about the conference topics are available on the conference website:
http://www.afmbiomed.org

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Thank you for your attention