Nanoscale Studies of Plant Protective Membranes

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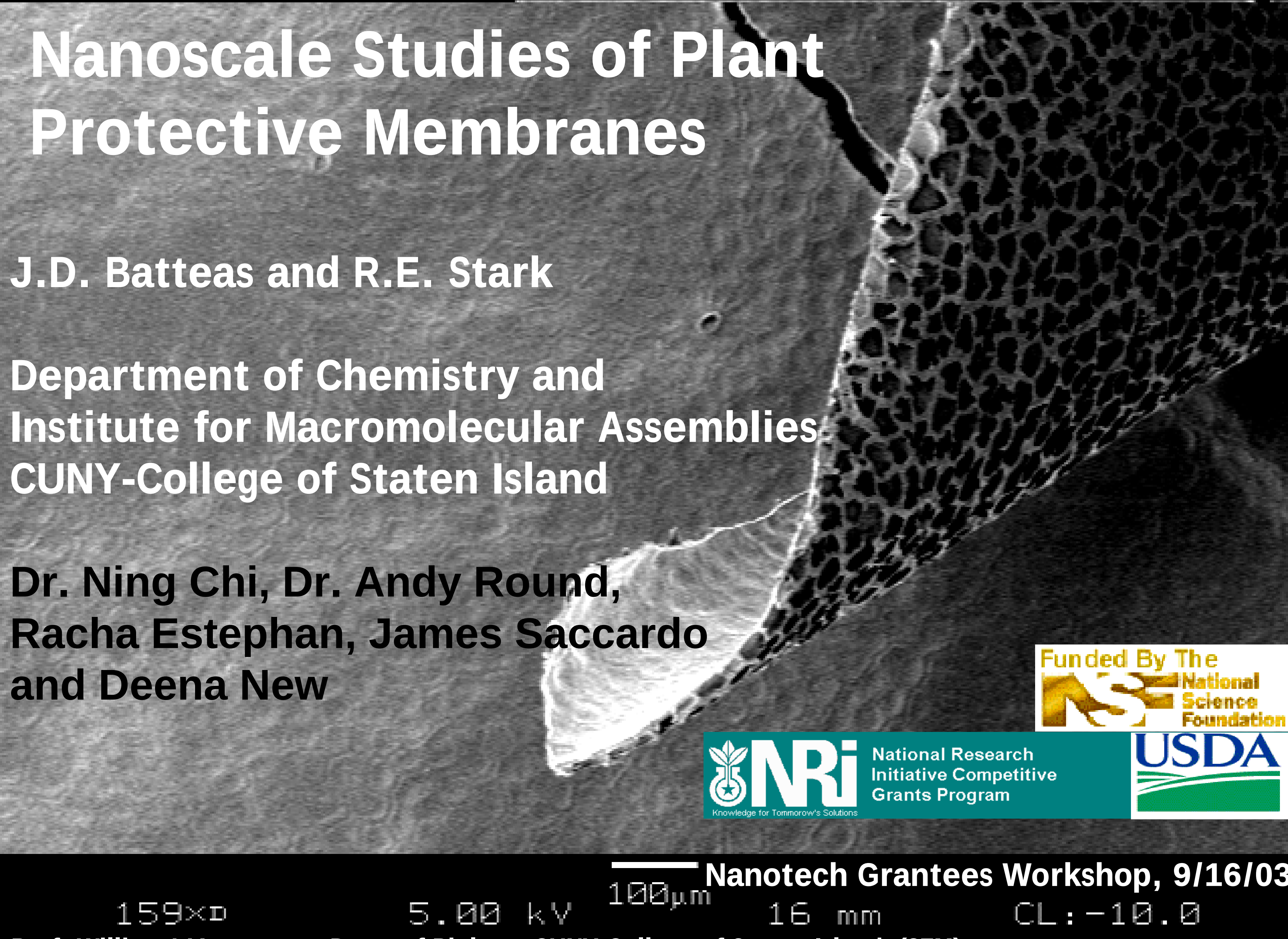

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BIOLOGICAL INVADERS

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Developing Nanoscale Approaches for the Investigation of Plant Surfaces and Interfaces

- **The outermost surfaces of the fruits and leaves of higher plants are coved by a cuticular membrane ~100 nm - 20 μm.**
- **Functions as the plant's primary protective barrier.**
- **Breakdown of this barrier by pathogens is associated with ~ $10 billon annual loss due to crop damage.[1a] (20 % worldwide loss in cash crops[1b])**

- Surface morphology

Important for wetting behavior.
3-D topology provides "ecological landscapes" for micro-organisms.

Mechaber et al., Mapping Leaf Surface Landscapes, PNAS, 93 (1996) 4600.

AFM
SEM

- Surface and membrane interfacial rheology

Critical for understanding fracture of the membrane allowing pathogenic access.

⟶ Focus on water and temperature. (surface vs. bulk?)

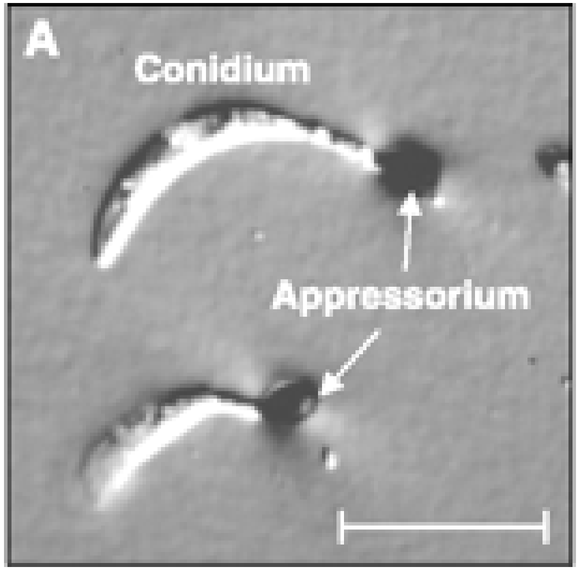
AFM
NMR

- Surface chemistry

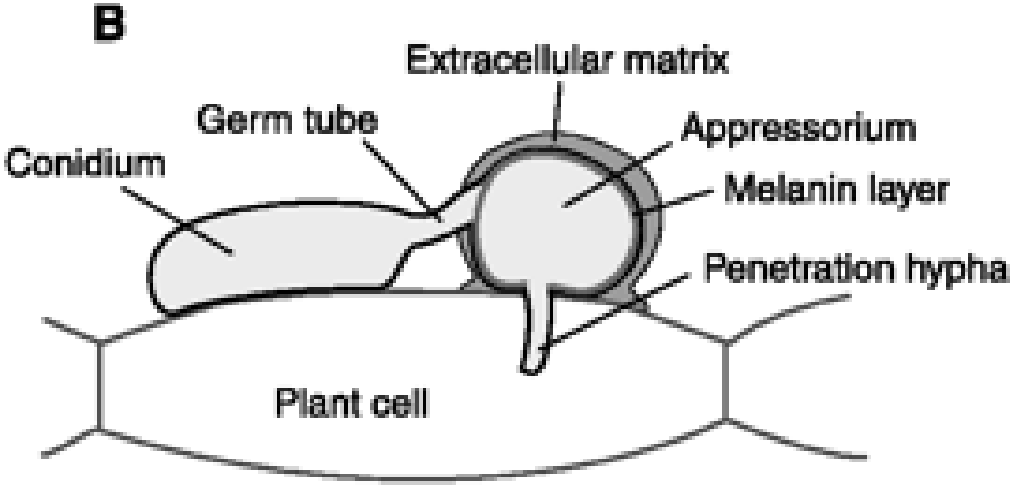
Important for waterproofing of plant tissues.
Influences adsorption of agrochemicals (wetting behavior).

AFM
SIMS

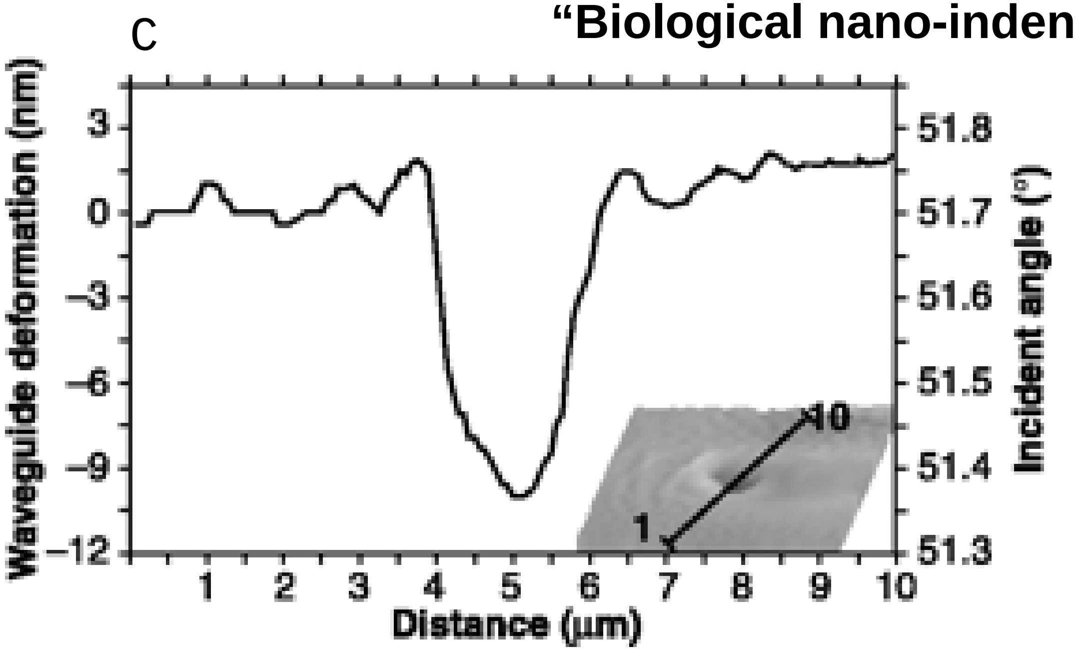
[1a] G.N. Agrios, *Plant Pathology*, Academic Press, New York, 1988; [1b] E.C. Oerke, H.W. Dehne, F. Schonbeck and A. Weber, *Crop Production and Crop Protection*, Elsevier, Amsterdam, 1994.



C. graminicola



“Biological nano-indenter”



Pathogenic Attack On Plant Surfaces

Issues:

1) **Adsorption of pathogens**
2) **Mechanical resistance**

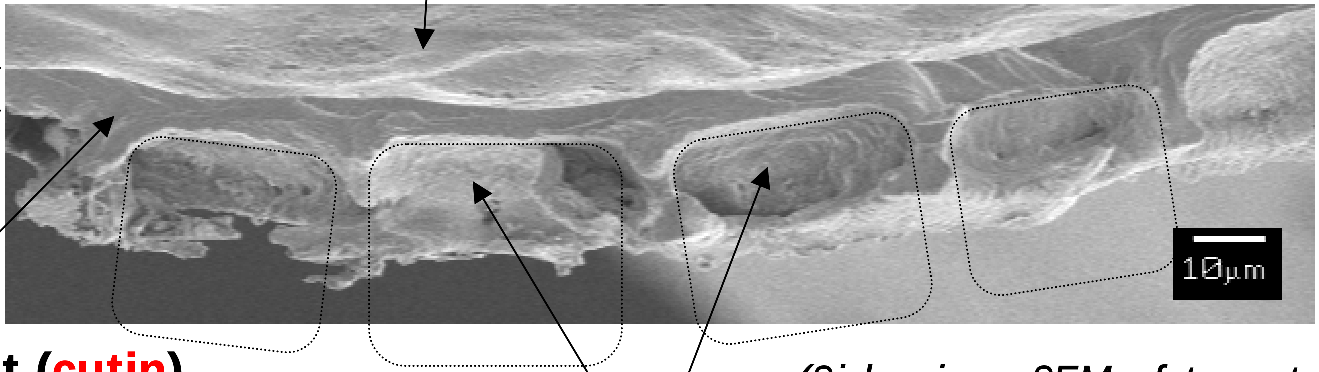
Adapted from Bechinger et al. Science (1999) 1896.

A closer look at the membrane . . .

Epicuticular surface (coated with thin layer of lipids for waterproofing)

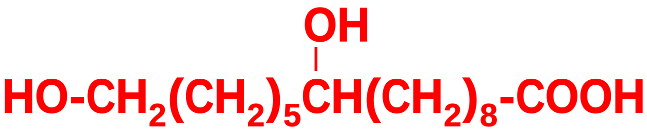
$CH_3\text{-}(CH_2)_n\text{-}CH_3$; $CH_3\text{-}(CH_2)_n\text{-}CH_2\text{-}OH$; $n \sim 30$

~ 6 μm thick



(Side view, SEM of tomato fruit cuticle)

Biopolyester support (cutin)



$$HO\text{-}CH_2(CH_2)_5\overset{\overset{OH}{|}}{C}H(CH_2)_8\text{-}COOH$$

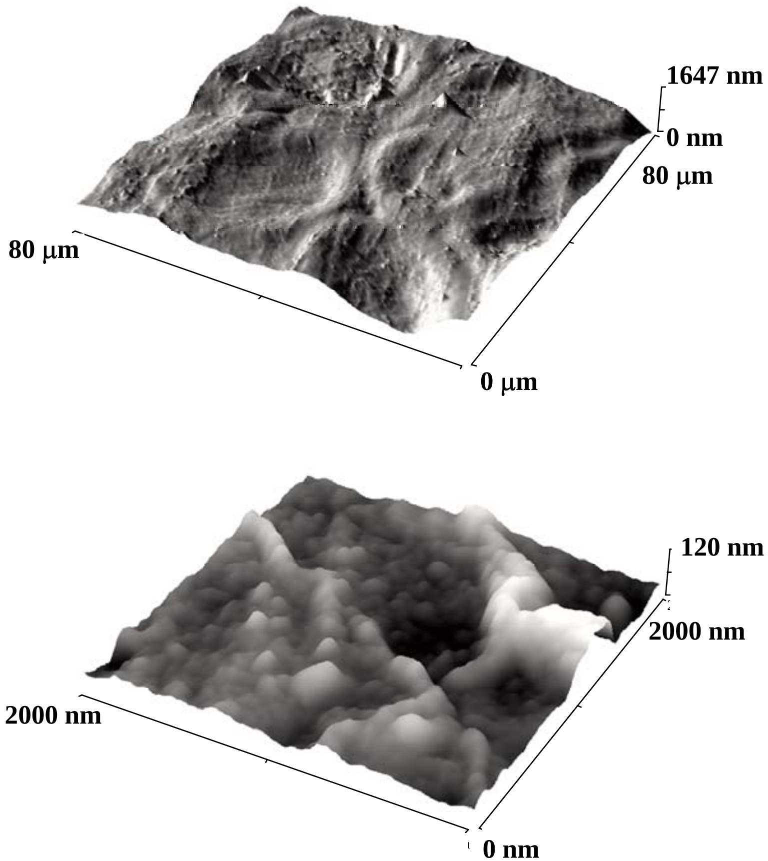
(also some embedded lipids pre-dominantly fatty acids)

Removed cells (Pectin degraded)

We examine a model system → tomato fruit cuticle (chemically simple)

1) Enymatically isolated to remove outer underlying plant cell
2) Investigate investigate influence of water on the surface and bulk rheology → intact membrane
   → isolated biopolymer support (lipids extracted)

Imaging of Plant Cuticular Surfaces by AFM . . .

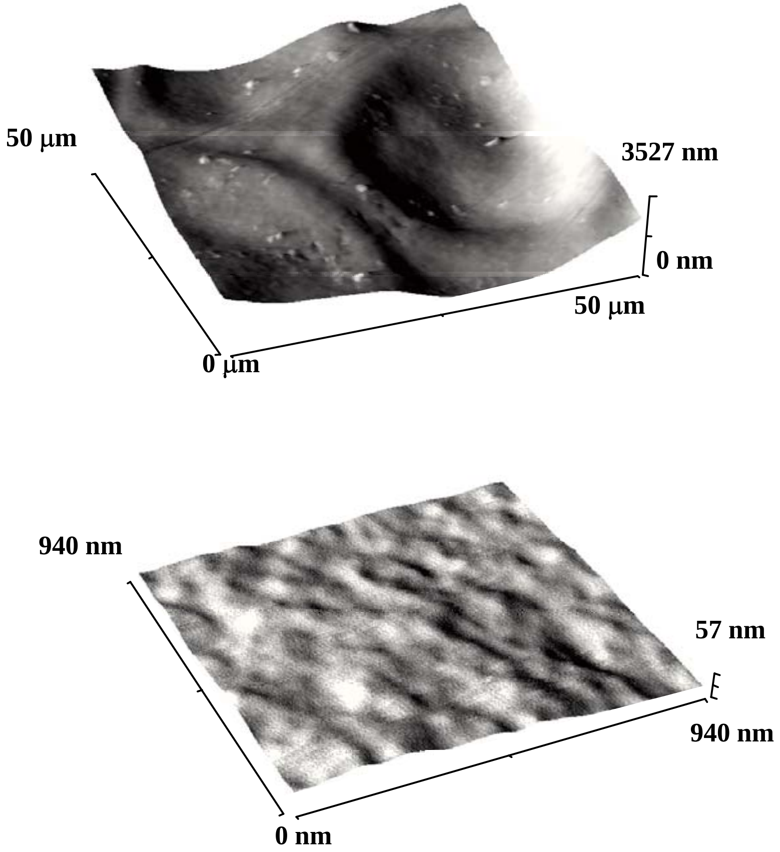


Intact Native Membrane

-Large scale cellular “imprints”

-Lipid clusters ~ 20 – 100 nm diamater

- Avg. roughness ~ 20 nm rms.



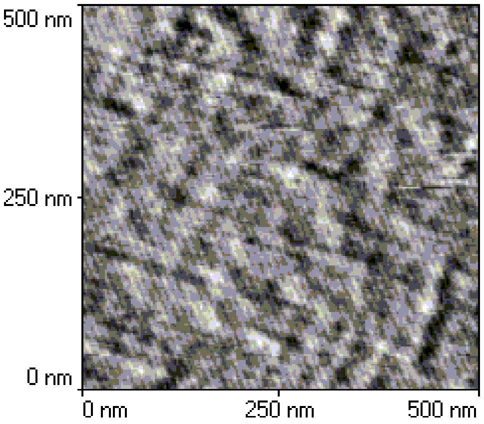
Isolated cutin

-Large scale cellular “imprints”

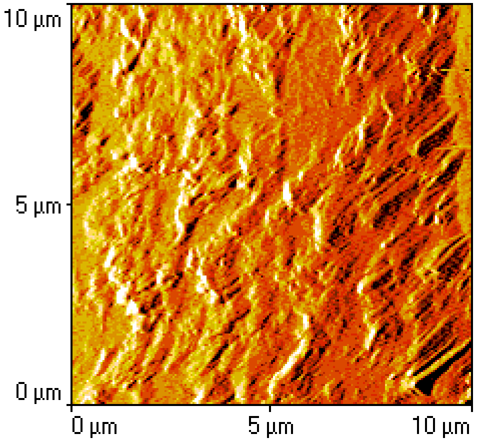
-No lipid clusters, fibrous, amorphous network.

-Avg roughness ~ 7 nm rms.

Interfacial Structure…



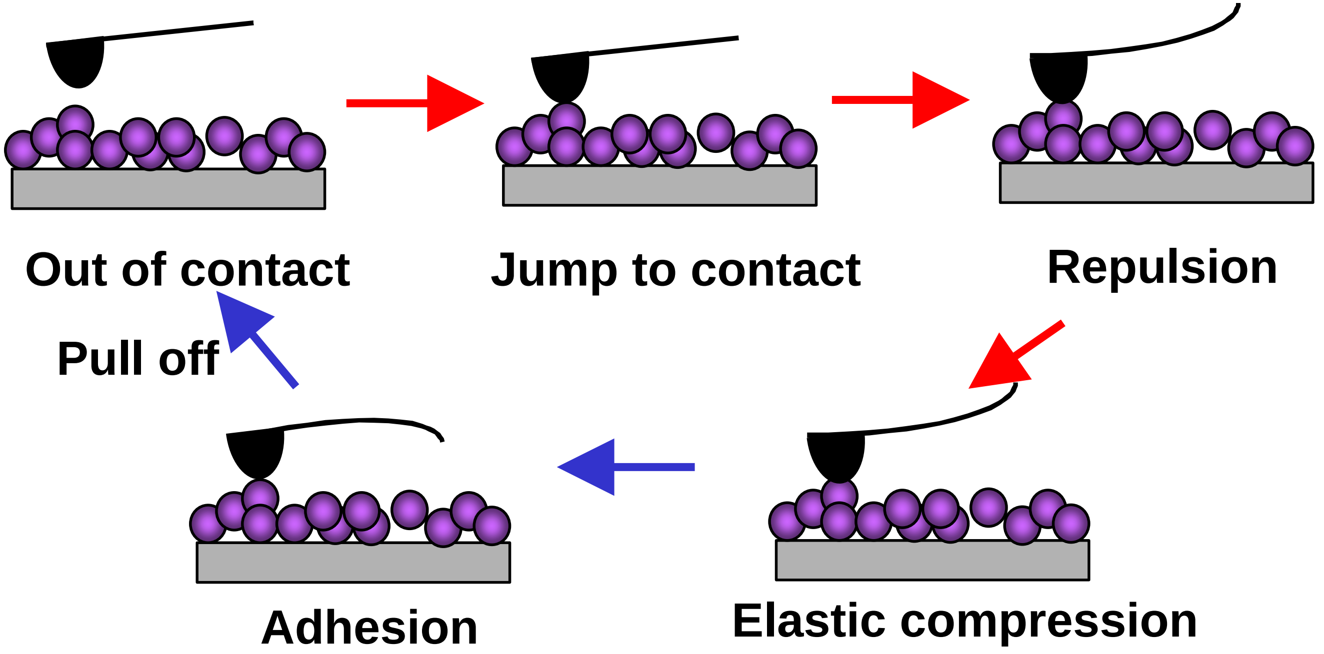
Pea leaf – polysaccharide Bundles (~ 20 nm)



Crystalline lipids on Pea Leaf

Pea leaf provided courtesy W. Abraham, Monsanto

Local Adhesion and Nanomechanics



Out of contact

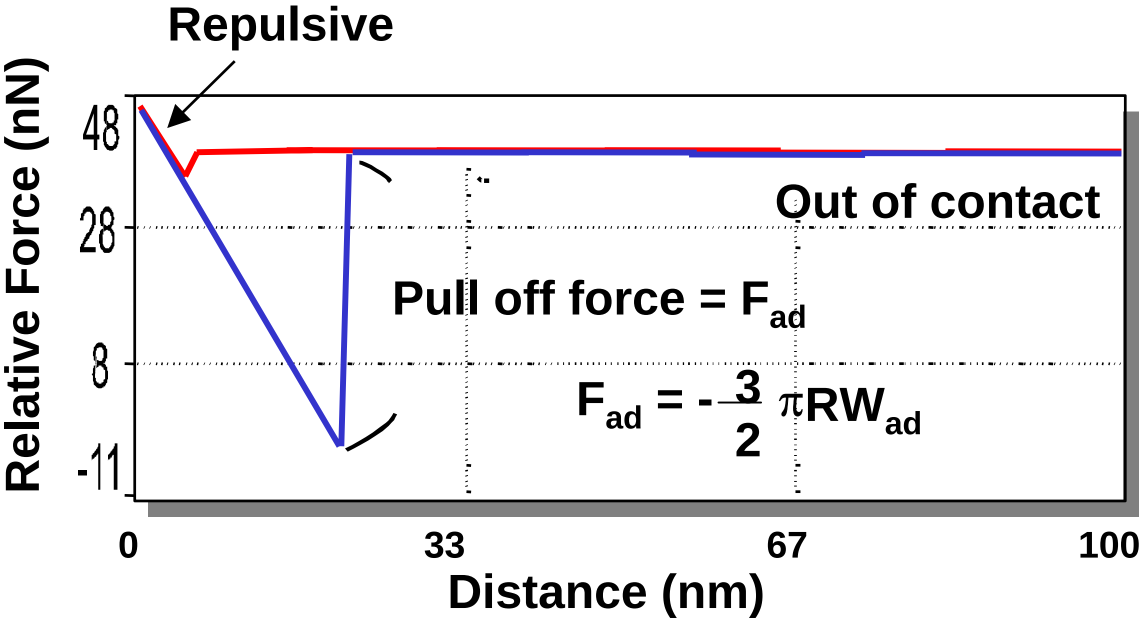
Jump to contact

Repulsion

Elastic compression

Adhesion

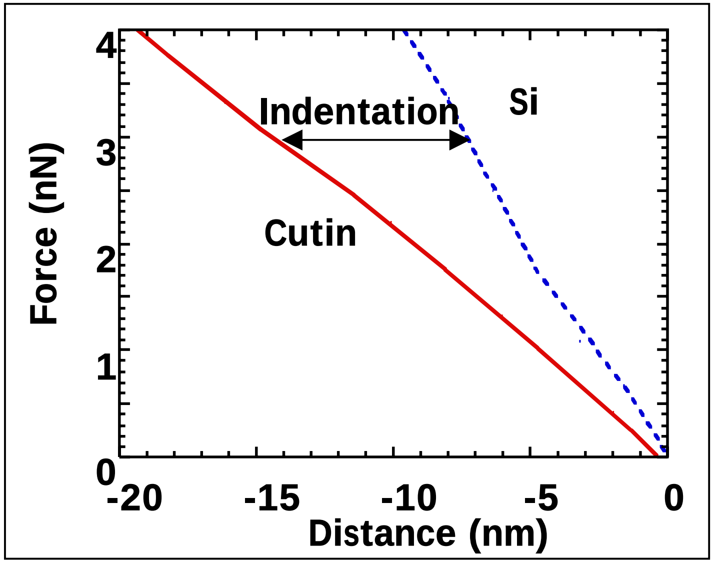
Pull off



Pull off force = F_{ad}

$$F_{ad} = -\frac{3}{2}\pi R W_{ad}$$

Nanoindentation...

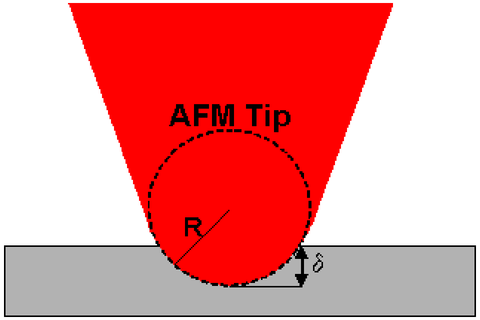


Compare force-distance curves for an ideally hard surface (Si wafer) to those from cuticular membrane.

Analyze indentation data using a simple Hertz contact mechanics model.

Hertz Contact Mechanics Model

$$A = \pi \left(\frac{R \cdot L}{K} \right)^{2/3}$$



$$K = \frac{4}{3} \left(\frac{1 - \nu_{tip}^2}{E_{tip}} + \frac{1 - \nu_c^2}{E_c} \right)^{-1}$$

L = load (N)

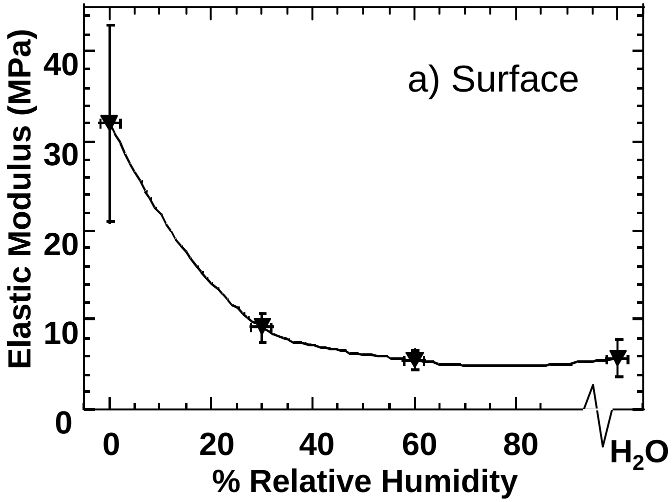
ν = Poisson ratio

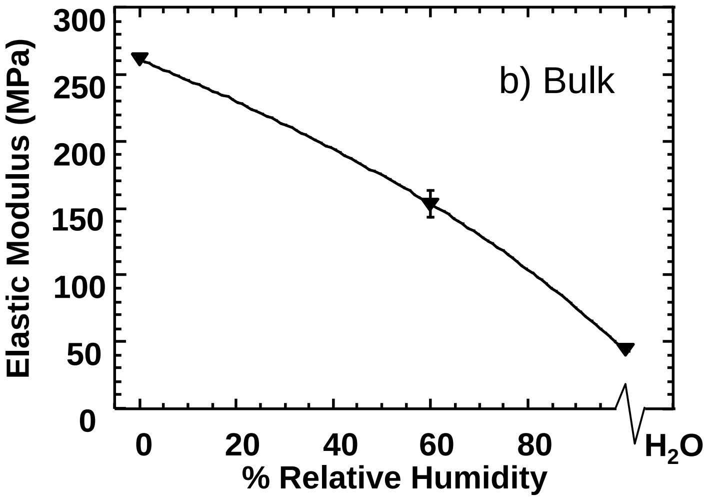
E = Elastic modulus

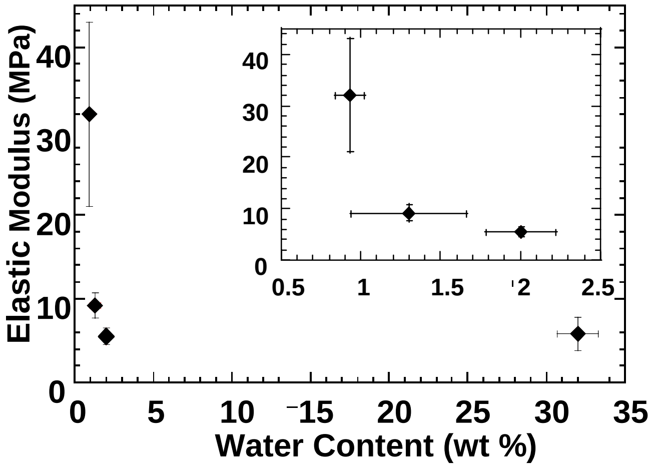
$$\boxed{E_c = \frac{3K\left(1 - \nu_c^{\,2}\right)}{4}}$$

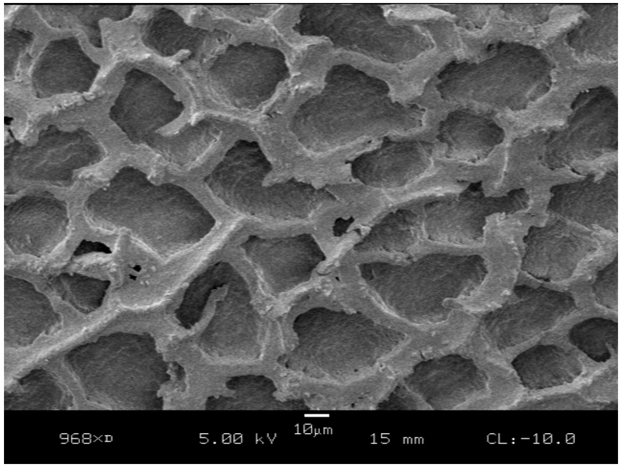
In the limit of E_{tip} >>> E_{cutin}

Elasticity studies of Isolated Cutin Membrane (no lipids) vs. water uptake. . .



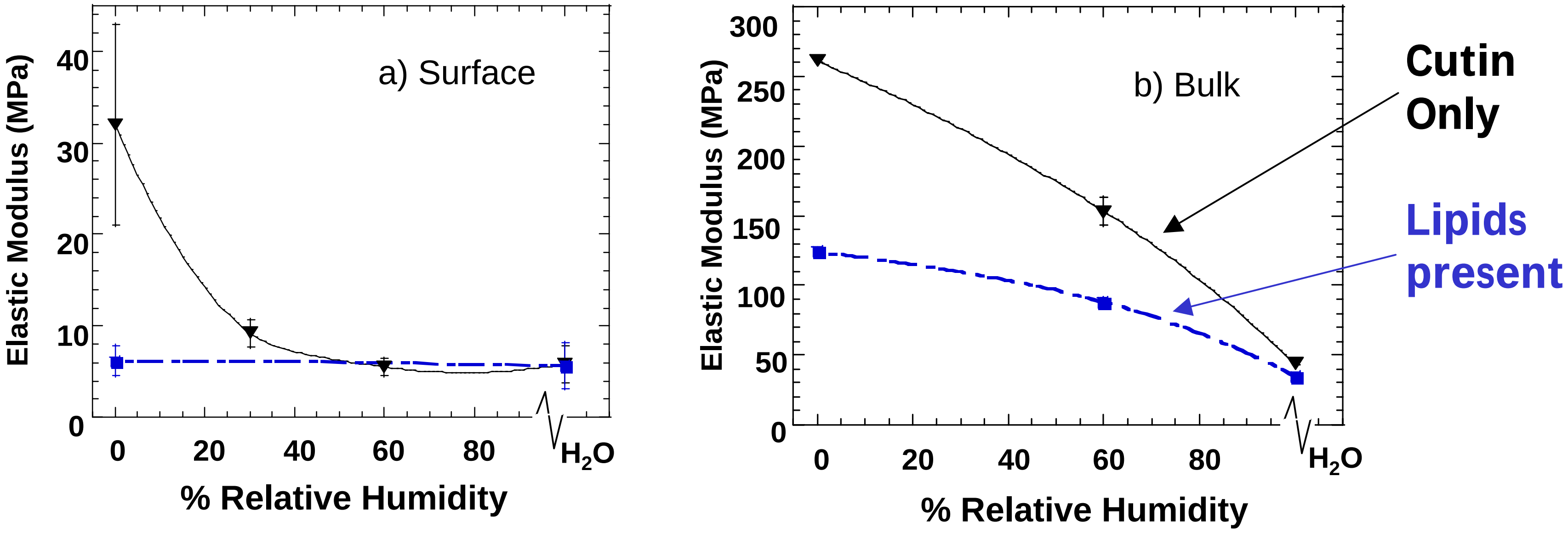






Networked structure enhances rigidity parallel to the surface.

Elasticity studies of the native membrane (w/lipids) vs. water uptake. . .

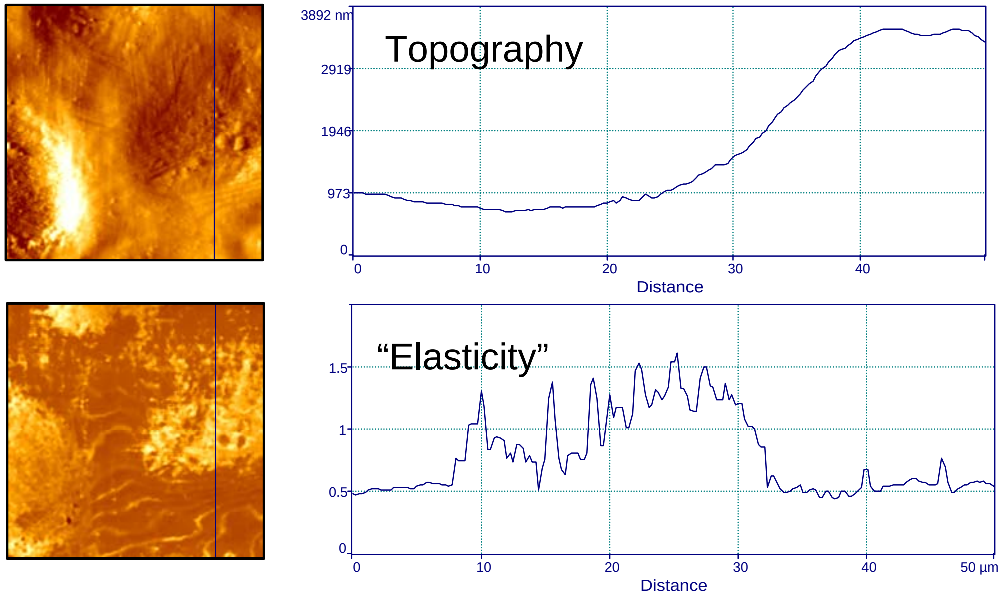


- AFM shows no sensitivity to water uptake → sees only the lipid covered surface.

  Suggests that the lipid coating is > 30 nm in thickness.

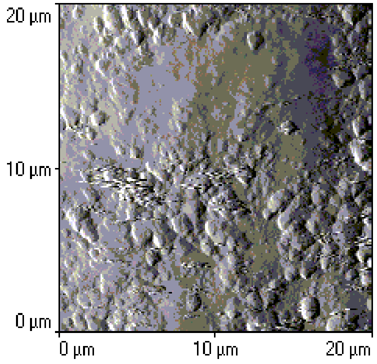
- Bulk response shows plasticizing impact of lipids and water uptake.

Modulated force and friction mapping of plant surfaces

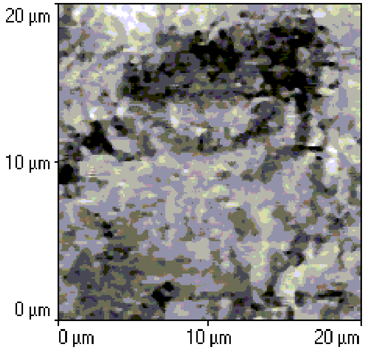


Spatial mapping of viscoelastic properties. Surface chemical heterogeneity.

Friction Contrast Map on Pea Leaf Surface

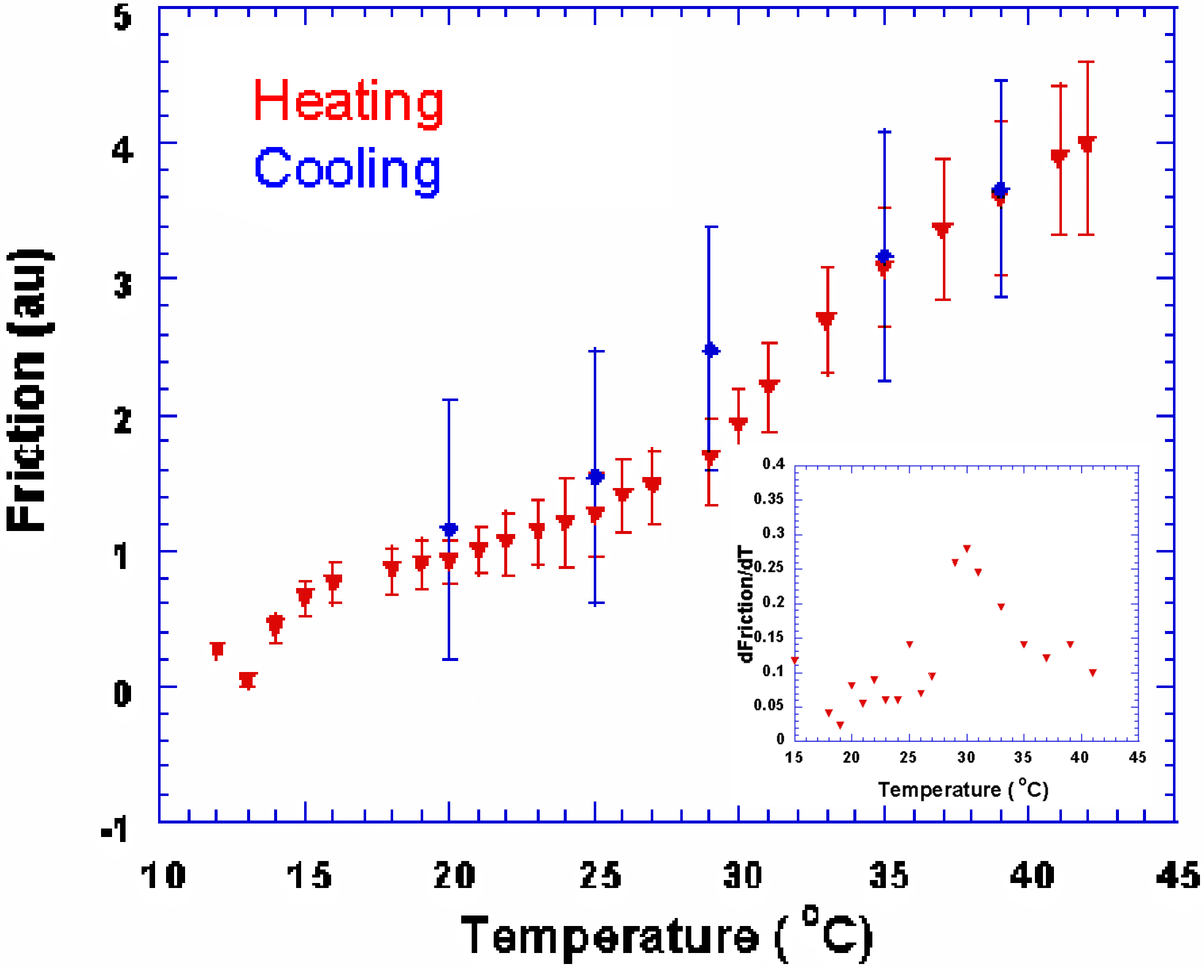


Topography



Friction

Probing Surface Thermal transitions. . .

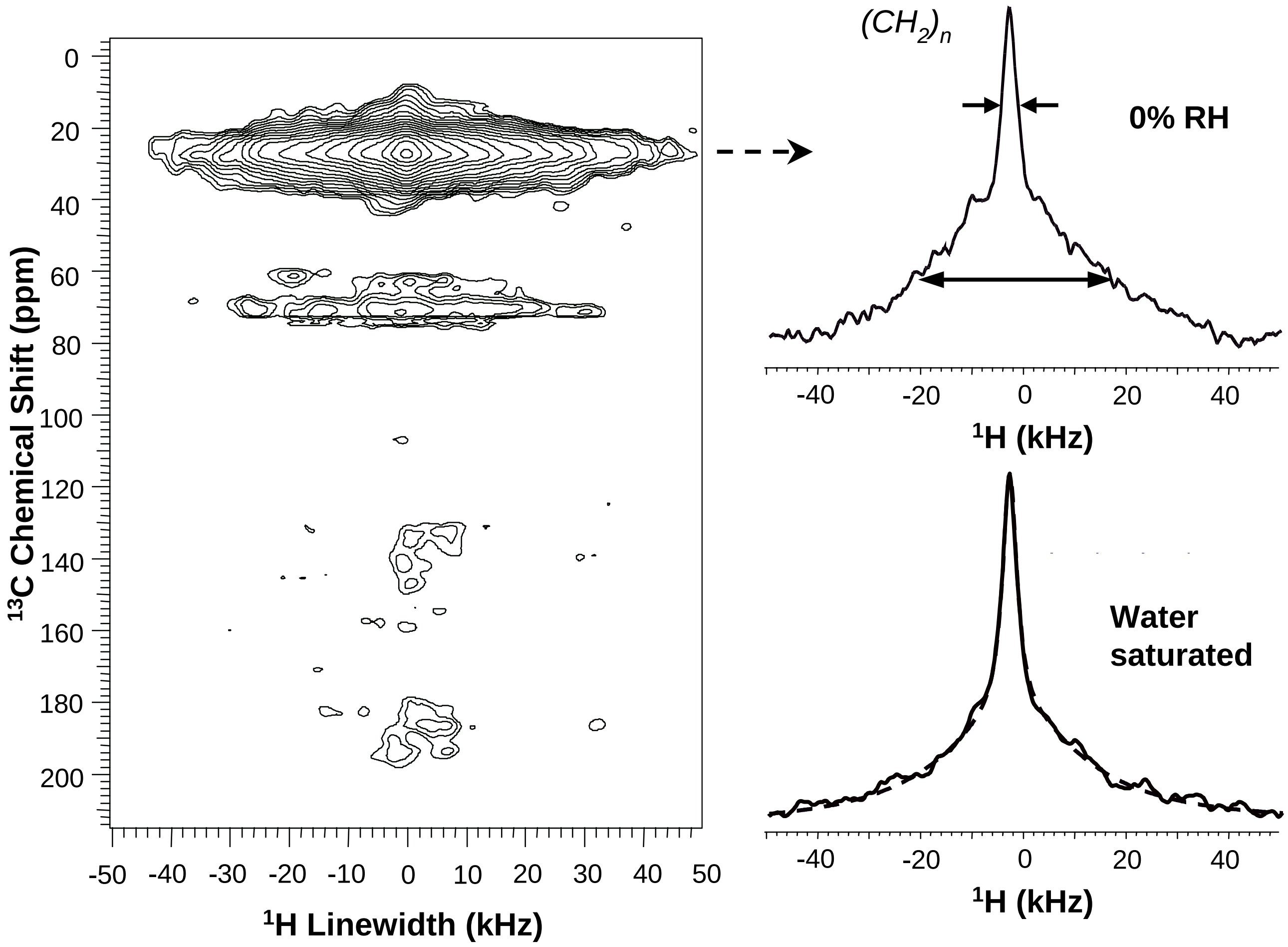


NMR Studies of Biopolymer Molecular Motions

Experiment	**Dynamics**	**Significance**	**Example**
$T_1(^{13}C)$	~ 75 MHz	Segmental motions (bulk modulus)	Cutin chains vs. cross-links Dry vs. wet
$T_1\rho(^{13}C)$	~ 50 kHz	Overall chain motions; impact strength	cutin/wax
$T_1\rho(^{1}H)$ 2D-WISE	~ 50 kHz	Spin diffusion (intimate mixing)	Cutin/wax association

Relationship between biopolymer structure and bulk mechanical properties.

2-D ^{1}H-^{13}C NMR WISE Measurements



- Use methylene lineshapes to discern information about flexibility.

NMR-Derived Flexibility of Bulk-Methylene Groups in Tomato Cuticle

^{1}H Linewidth*

Avg. wt% Water	Broad Component (kHz)	Narrow Component (kHz)	% Narrow Component[#]
0.9	38.2	5.3	9
1.3	36.2	5.7	12
2.0	32.3	4.9	12
32.0	31.3	4.7	18

Most significant changes in linewidth appear under the most saturated conditions.

Narrow component => relatively mobile CH_2 chain segments
Broad component => more rigidly bound CH_2 groups (i.e. near covalent crosslinks)

[#] From spectral deconvolution of narrow and broad components of the chain-methylene resonance; estimated uncertainties are 20%.

Rotating-Frame ^{13}C NMR Relaxations for Carbons in Tomato Cuticle

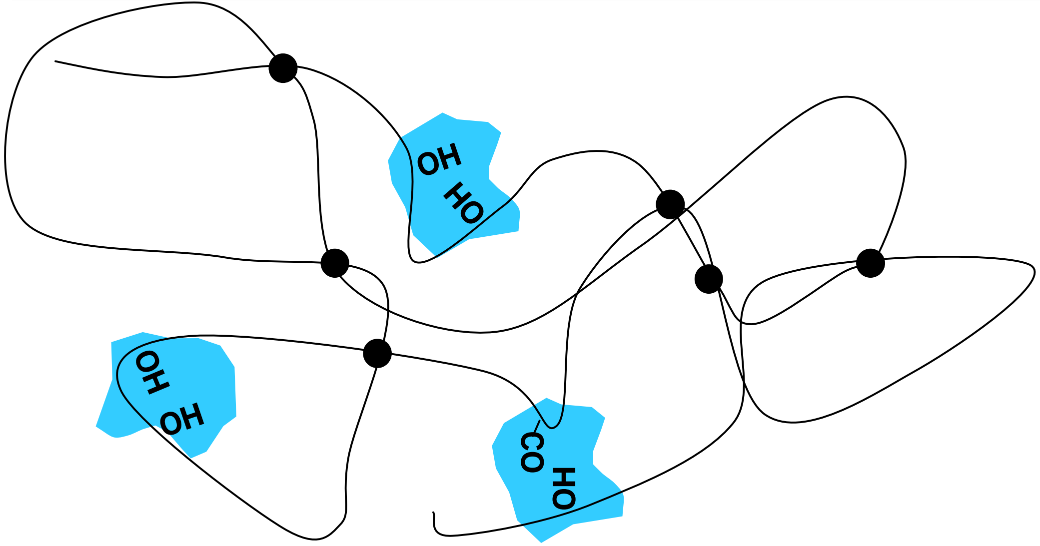
	$\langle T_{1\rho}(C)\rangle^*$, ms	
Avg. wt% Water	**$(CH_2)_n$**	***C*HOCOR, CHOH**
0.9	2.3 ± 0.1	5.5 ± 1.0
1.3	2.7 ± 0.2	5.2 ±1.6
2.0	2.8 ± 0.2	5.8 ± 1.5
32.0	2.0 ± 0.1	3.1 ± 0.3
	Main chains	Cross-links

NMR-Derived Cooperative Motions at Carbon Sites in Tomato Cuticle

	Average ^1H Rotating-Frame Relaxation Rate (ms^{-1})					
	Dewaxed Cuticle		**Lipid**	**Native Cuticle**		
Hydration	*Cutin* $(CH_2)_n$	CH_nO	Wax $(CH_2)_n$	*Cutin* $(CH_2)_n$	Wax $(CH_2)_n$	CH_nO
Dry (0% RH)	0.40	0.24	0.24	0.28	0.30	0.24
Saturated	0.48	0.18	0.050	0.38	0.32	0.15

Hydration alters slower <u>cooperative</u> motions in both dewaxed and native cuticles: more efficient for acyl chains.

Summary . . . and currect directions . . .



- Water uptake weakens hydrogen-bonded crosslinks affording increased chain mobility.

- Observed more rapidly by AFM due to surface saturation.

- Bulk rheology and AFM results compared at the extremes, are consistent with NMR WISE data.

- When present, lipids dominate the surface thermomechanical properties.

- Adsorption of model agrochemicals.

  1) Triton surfactacts

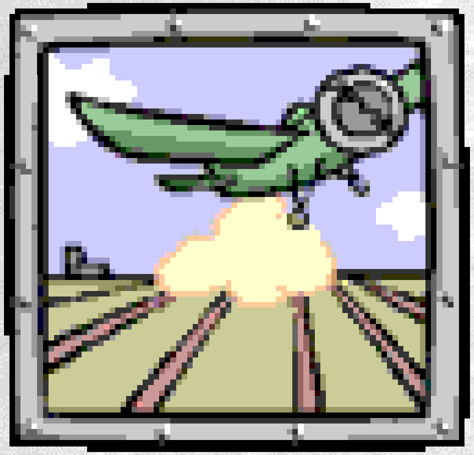
  2) Glyphosates

- Surface chemical mapping by SIMS

Agrochemical Delivery

Importance

- Agrochemical spray on leaves



Ways to enhance spreading

- Temperature
- Coating the surface
- Addition of a surfactant

----> Surfactant enhanced spreading

Alex Couzis, CCNY

Agrochemical Adsorption Studies

Issues of surface wetting

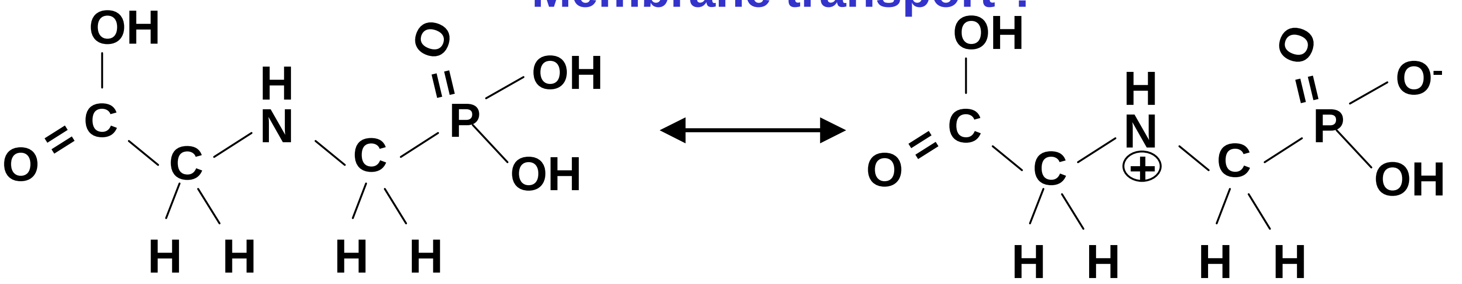
- spray dispersal – needs to be effective in wetting plant surfaces

=> need to directly probe surface wetting

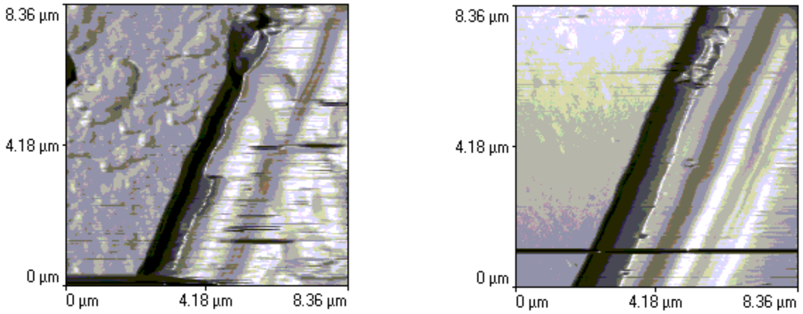
Is there preferential wetting ?

Interactions with surface lipids?

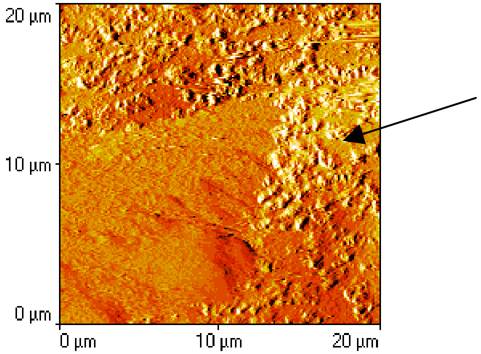
Membrane transport ?



Glyphosate – common herbicide (Roundup)



Before and after glyphosate adsorption

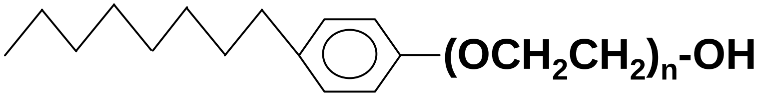


Surface lipids

Pea leaf cuticle

Dispersal agents:

Triton X-series



Non-ionic surfactants

n = 10 - 40

How do they impact wetting?

How do they impact foliar uptake?

e.g. 2-(1-naphthyl)acetic acid – TX-100 increases sorption by the cuticle.

Surface Modification of Smooth Surfaces to Demonstrate Super-Hydrophobicity

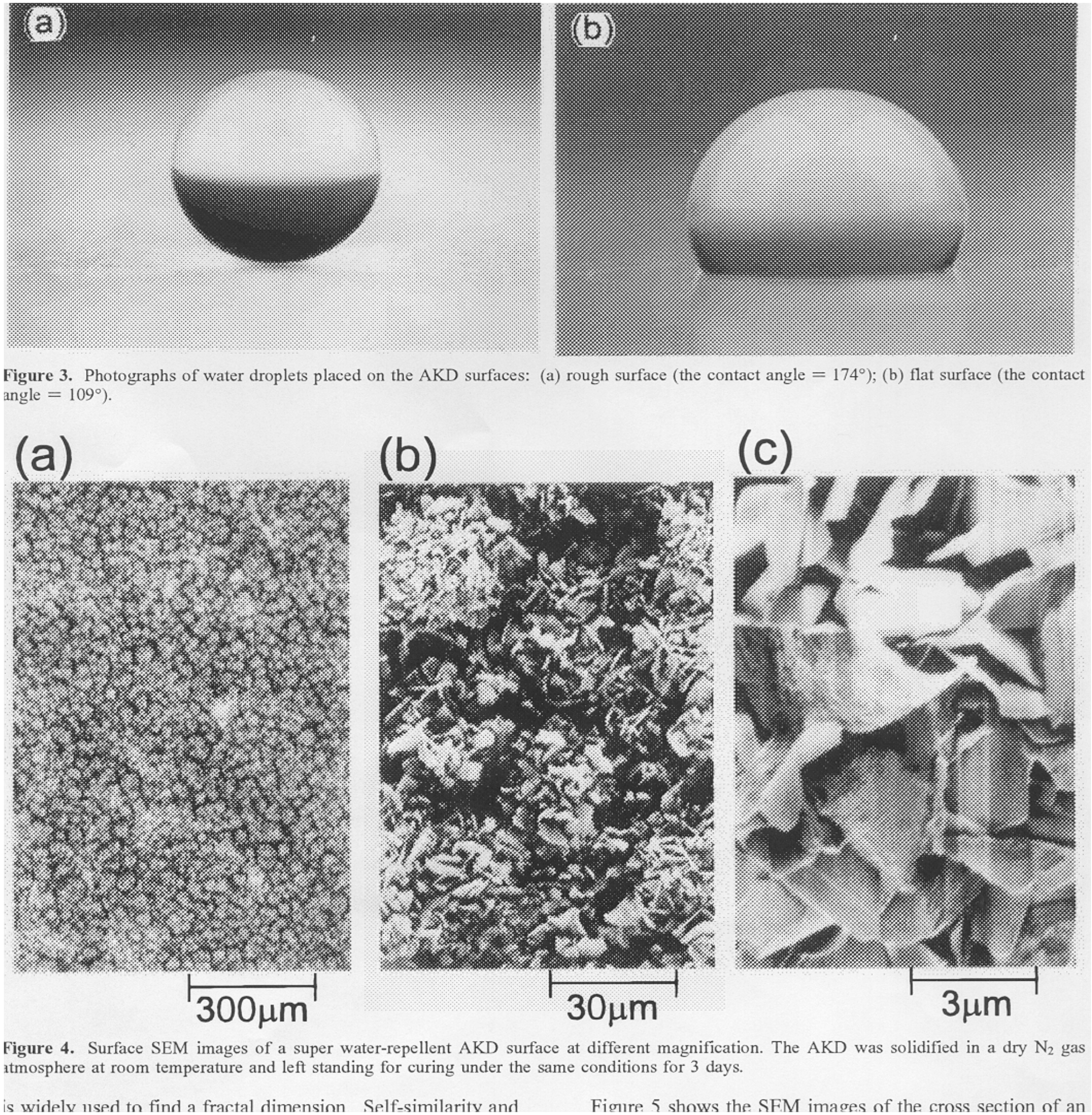


Figure 3. Photographs of water droplets placed on the AKD surfaces: (a) rough surface (the contact angle = 174°); (b) flat surface (the contact angle = 109°).

Figure 4. Surface SEM images of a super water-repellent AKD surface at different magnification. The AKD was solidified in a dry N_2 gas atmosphere at room temperature and left standing for curing under the same conditions for 3 days.

is widely used to find a fractal dimension. Self-similarity and

Figure 5 shows the SEM images of the cross section of an

Surface Modified using
Alkylketene dimer
from melt onto glass

Water Contact angle on:
- Flat Surface 109
- Fractal Surface 174 (max)

Tsuji et. al., J.Phys Chem.,1996

Drop Impact Dynamics

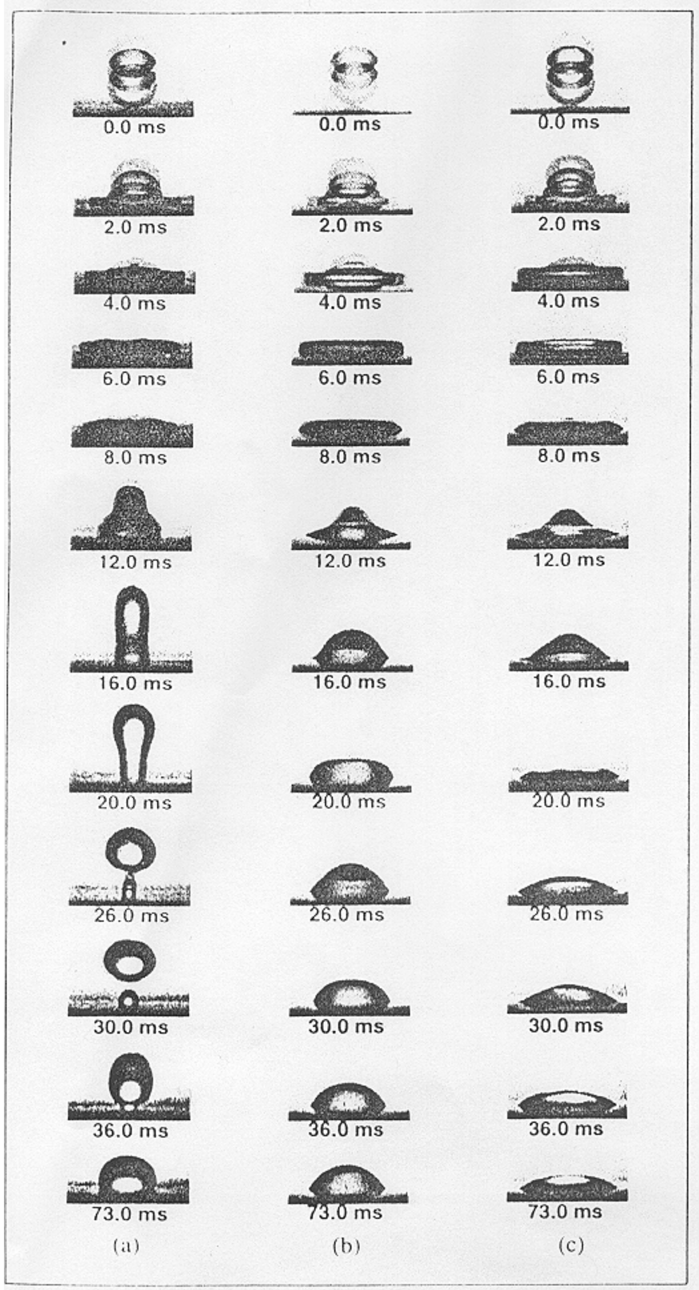
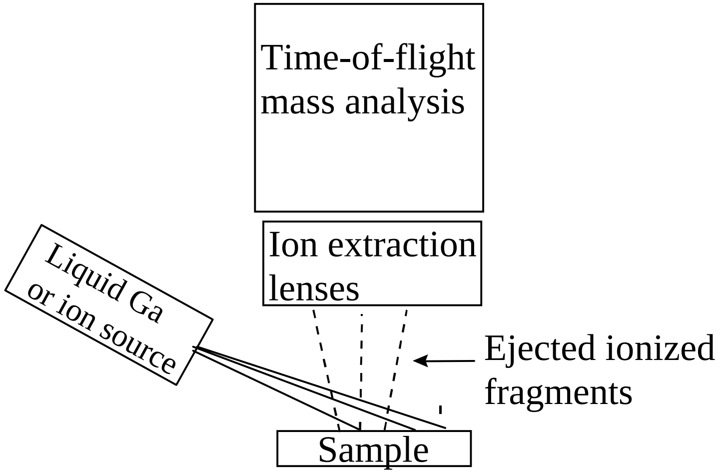


Figure 4. Impact of a 2.7 water droplet on (a) the paraffin wax block; (b) the stainless-steel plate; (c) the glass slide.

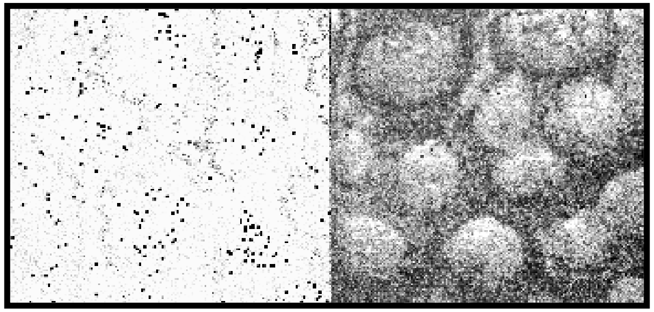
	Static Water Contact Angle
Paraffin	**97^0**
Stainless Steel	**67^0**
Glass	**37^0**

Tran et. al. AIChE 1997

Spatial Chemical Mapping: Secondary Ion Mass Spectroscopy (SIMS)



Hydrogen SIMS Chemical Map (100 x 100 μm)



Prior to lipid abrasion After lipid abrasion

1) Surface Chemical Mapping

- **Lateral resolution of ~ 100 nm**
- **Depth resolution of ~ 2 nm.**
- **Lipid abrasion or erosion by the environment. => Agrochemical wetting**

2) Depth profiling

- **Epicuticular lipid surface compositions (and thickness).**
- **Agrochemical intercuticular diffusion (Only bulk diffusion rates have ever been measured). => Early diffusion rates**
  => Concentration profiles
  => Diffusion pathways

In collaboration with Steve Schwarz, Queens College/CUNY

Applications of nanotech to agriculture . . .

1) Tools of nanotech applied to agriculture problems.
2) Apply design technologies for custom surfaces to agriculture.

Implications of nanotech to agriculture. . .

3) Design of metrology tools to assess crop protection mechanisms
   e.g. Genetic modification of cuticular barriers
   Spreading mechanisms of agrochemicals

4) Design of better measurement tools coupled with directed design of agrochemicals and genetic modification should directly impact need for agrochemicals.

Origin of Leaf Hydrophobicity

Cause: Waxy Outer Layer and Surface Roughness

- Wax Crystals on Epidermal Cells :Crystal Density determines actual hydrophobicity.

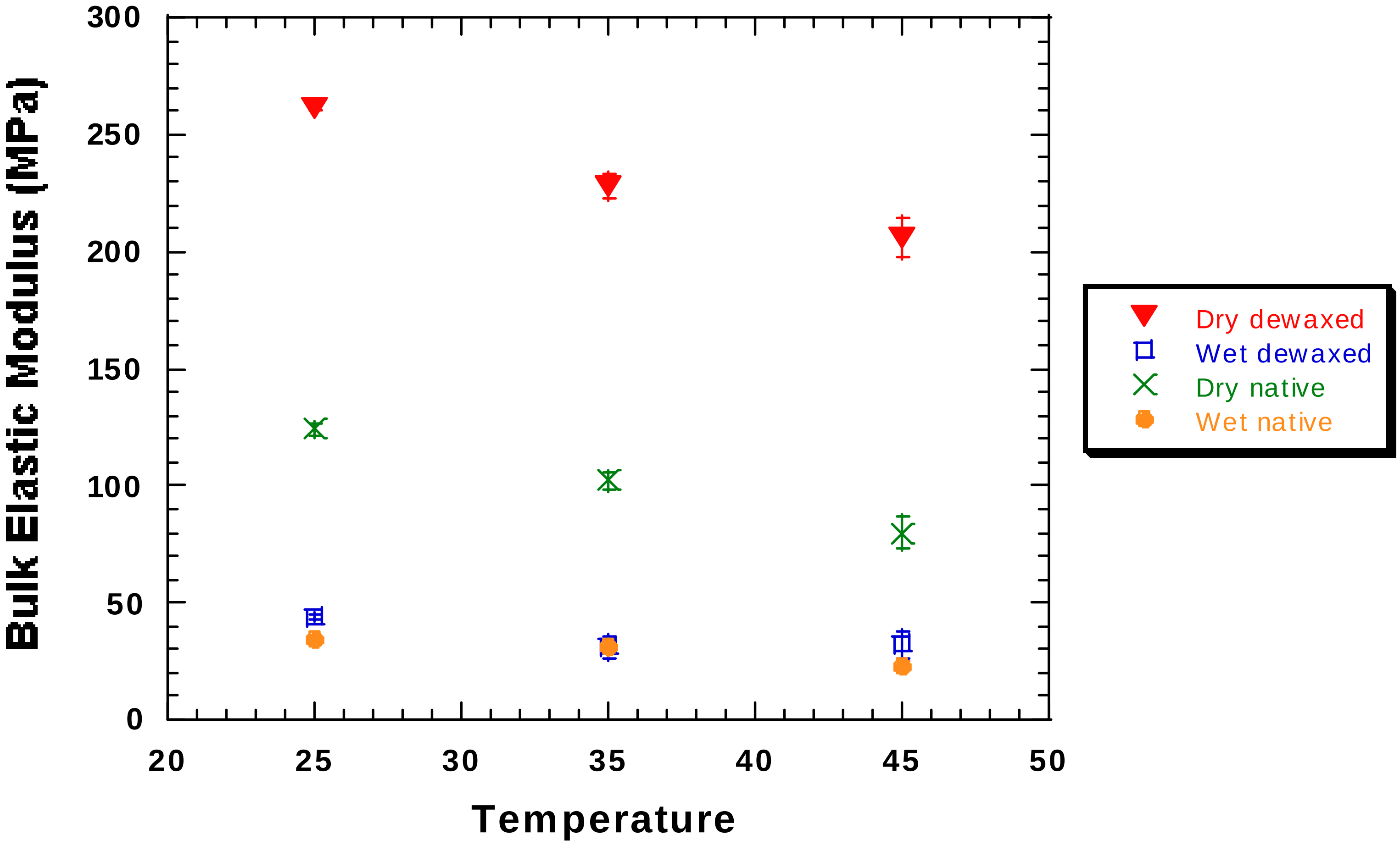
- Long hair like structures (trichomes) and bumpy protrusions induce surface roughness.

Effect: Nature's Self Cleaning Mechanism

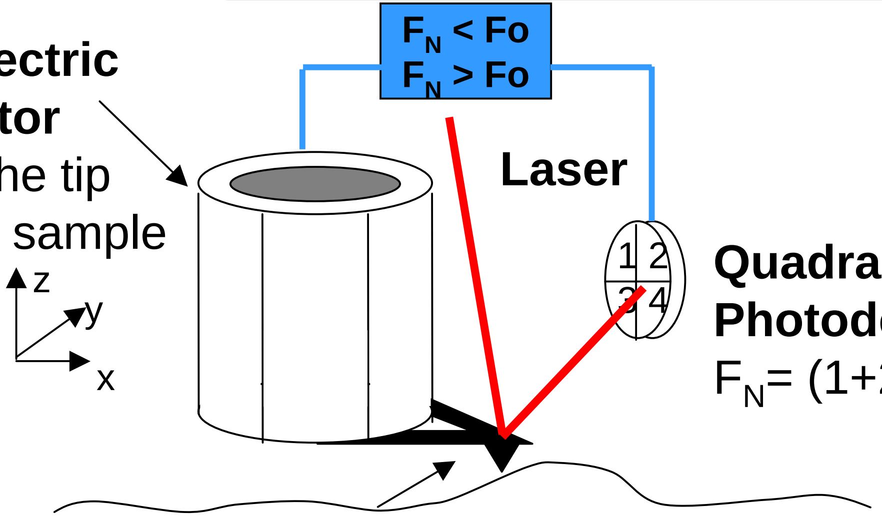
- Dirt particles (spores, disease fungi) adhere more strongly to water than the leaf and are consequently washed away.

- Lack of water on surface prevents disease organisms from germinating and growing as they cannot survive.

Temperature Dependence on Bulk Cuticular Elasticity



Atomic Force Microscopy (AFM)



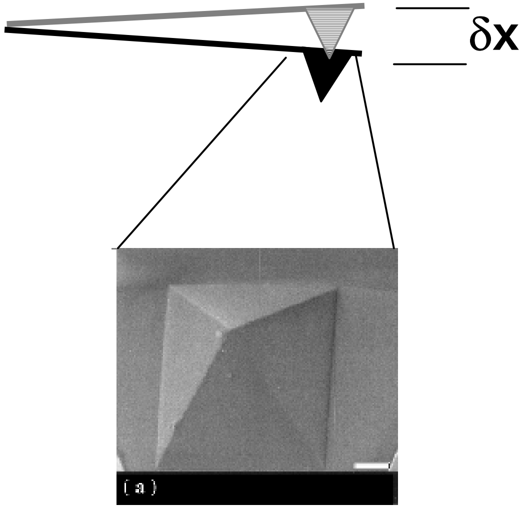
Piezoelectric Translator
(scans the tip over the sample surface)

Laser

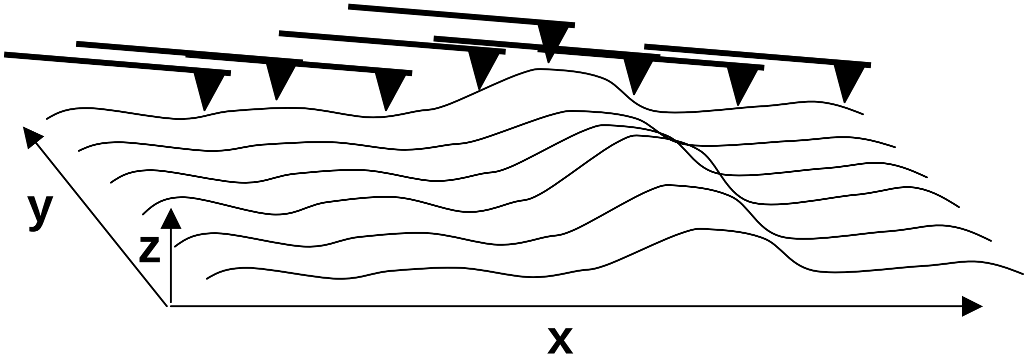
Quadrant Photodetector
$F_N = (1+2)–(3+4)$

Cantilever-tip

Lever:
$\delta F = -k\delta x$



Scanning in Contact: Tip follows the profile of the surface maintaining a constant lever deflection (constant force).



Forces acting on the tip provide the signal:
van der Waals; electrostatic, et

Sensitivity to intermolecular forces of ~ 10^{-12} N.