Nanoscale Studies of Plant Protective Membranes

J.D. Batteas and R.E. Stark

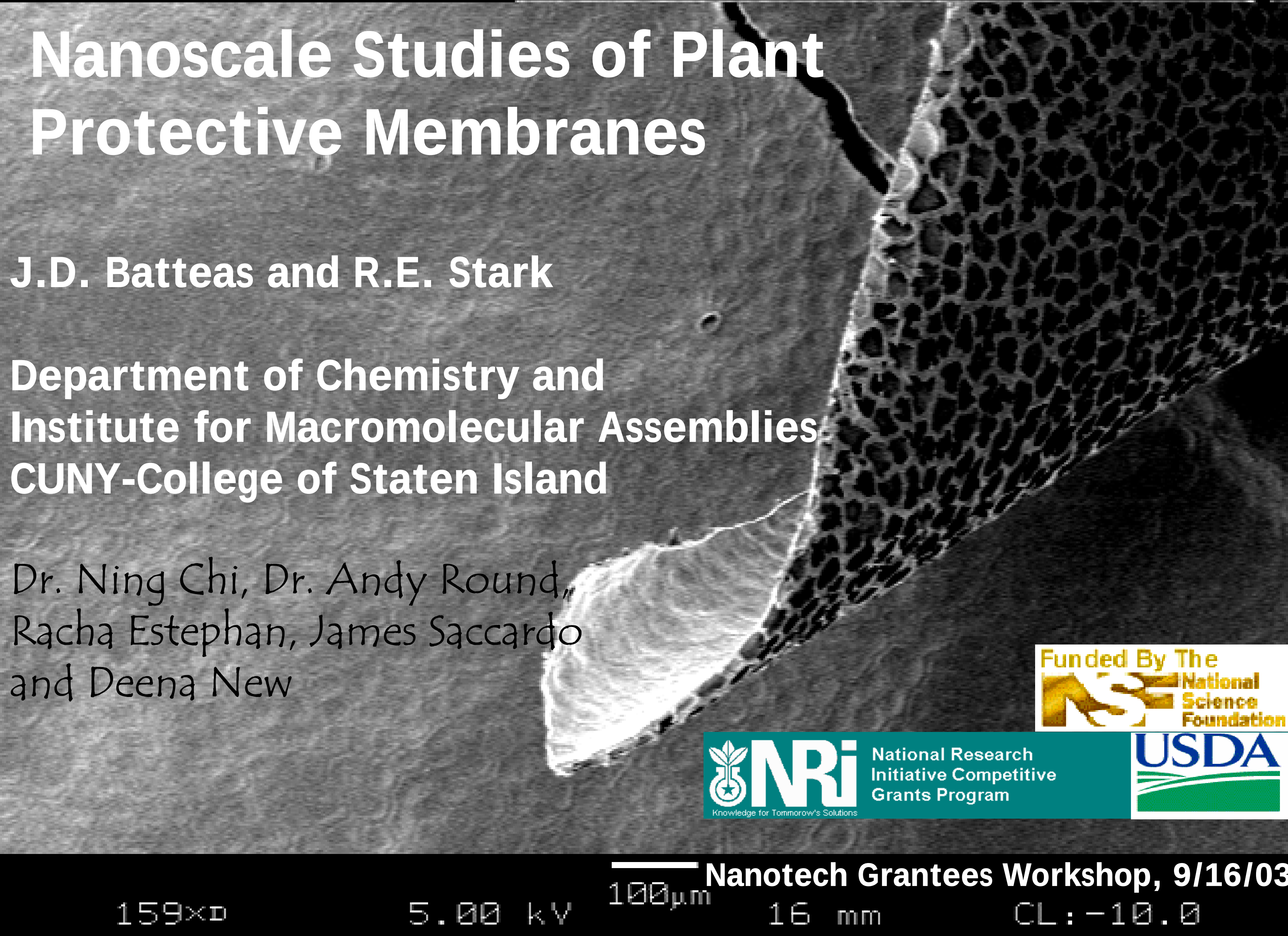
**Department of Chemistry and
Institute for Macromolecular Assemblies
CUNY-College of Staten Island**

Dr. Ning Chi, Dr. Andy Round,
Racha Estephan, James Saccardo
and Deena New

Funded By The
National Science Foundation

NRi
National Research
Initiative Competitive
Grants Program
Knowledge for Tommorow's Solutions

USDA



Nanotech Grantees Workshop, 9/16/03

Prof. William L'Amoreaux, Dept. of Biology, CUNY-College of Staten Island (SEM)

17 September 1999

Science

Vol. 285 No. 5435
Pages 1809–2020 $8

Biological Invaders

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

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

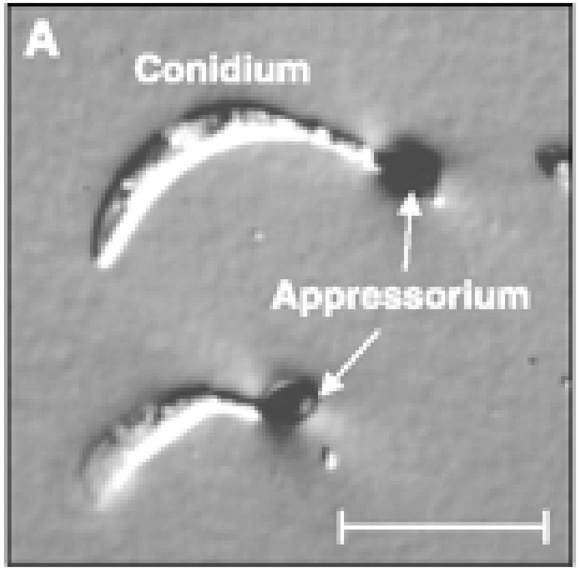
Important for waterproofing of plant tissues.

Influences adsorption of agrochemicals (wetting behavior).

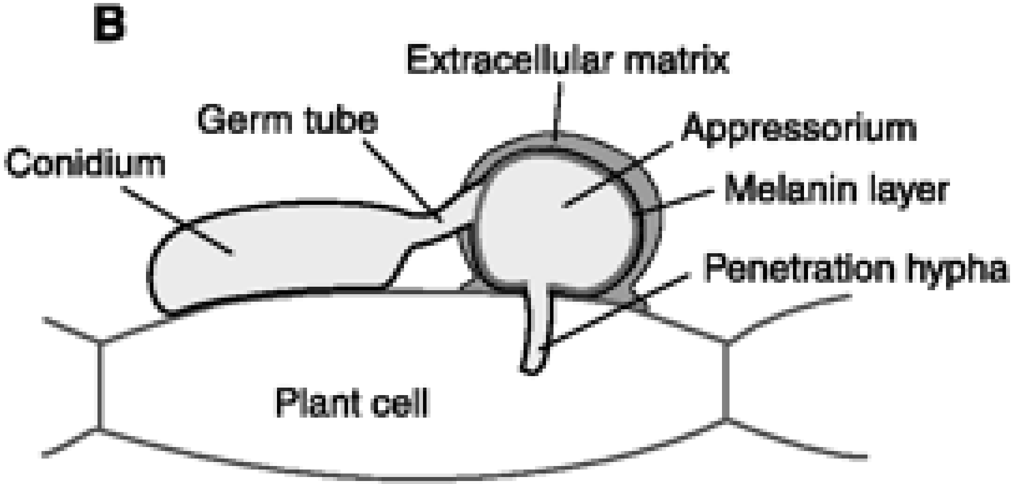
AFM
SIMS

[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.

Pathogenic Attack On Plant Surfaces

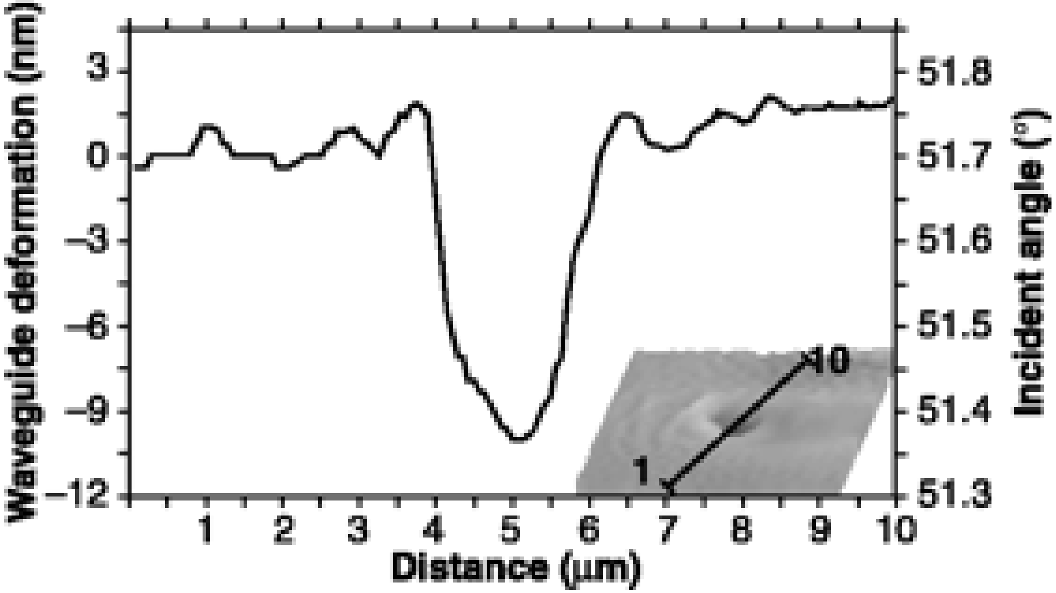


C. graminicola



"Biological nano-indenter"

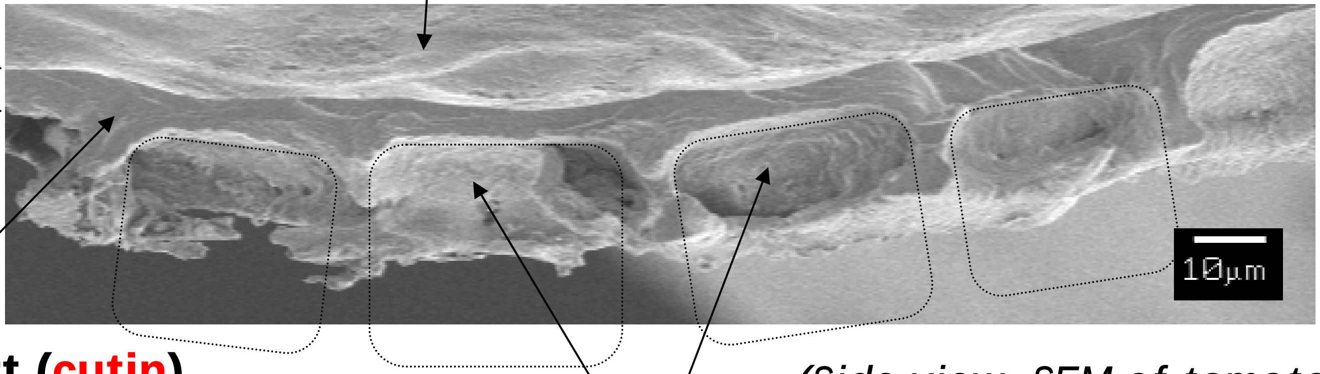
c



Issues:
1) Adsorption of pathogens
2) Mechanical resistance

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

A closer look at the membrane . . .

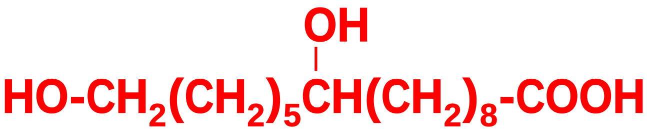


Epicuticular surface (coated with thin layer of lipids for waterproofing)

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

~ 6 µm thick

Biopolyester support (cutin)



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

(also some embedded lipids pre-dominantly fatty acids)

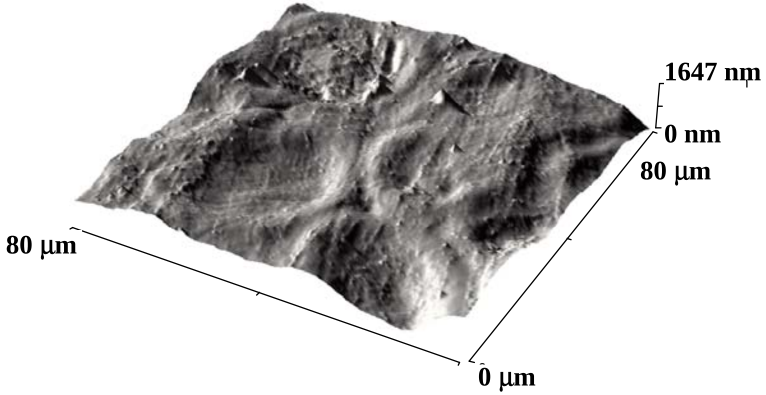
(Side view, SEM of tomato fruit cuticle)

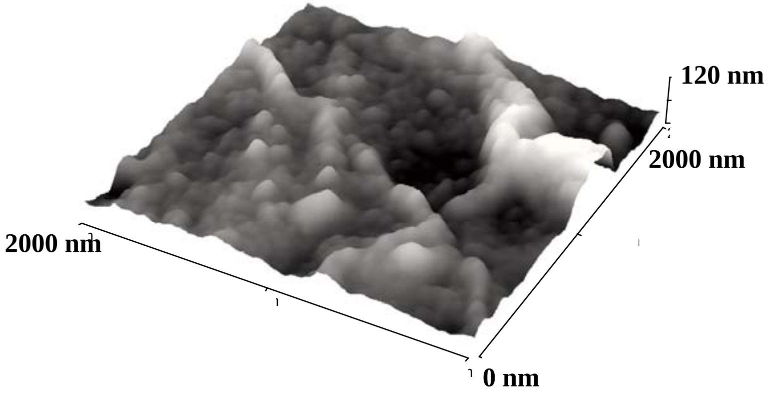
Removed cells (Pectin degraded)

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 . . .



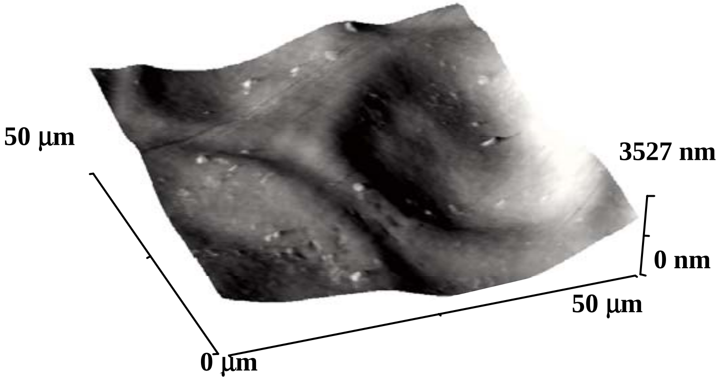


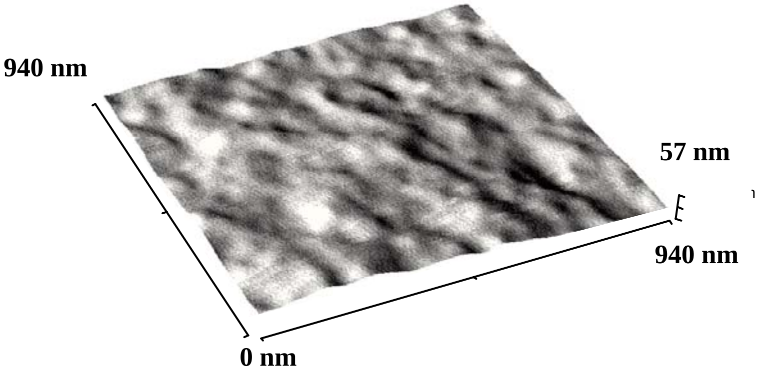
<u>Intact Native Membrane</u>

-Large scale cellular “imprints”

-Lipid clusters ~ 20 – 100 nm diamater

- Avg. roughness ~ 20 nm rms.





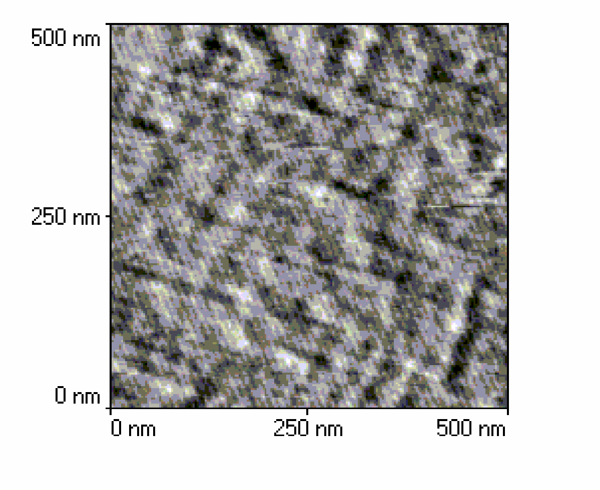
<u>Isolated cutin</u>

-Large scale cellular “imprints”

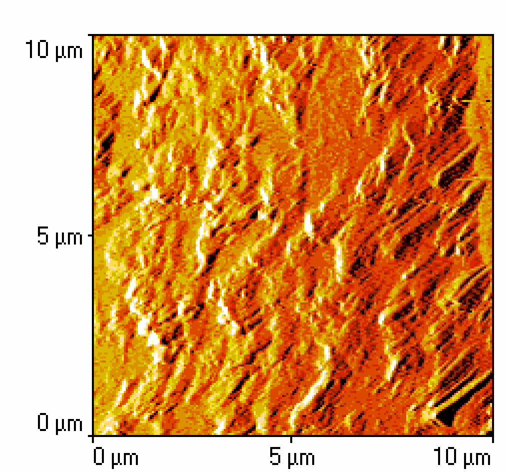
-No lipid clusters, fibrous, amorphous network.

-Avg roughness ~ 7 nm rms.

Interfacial Structure…



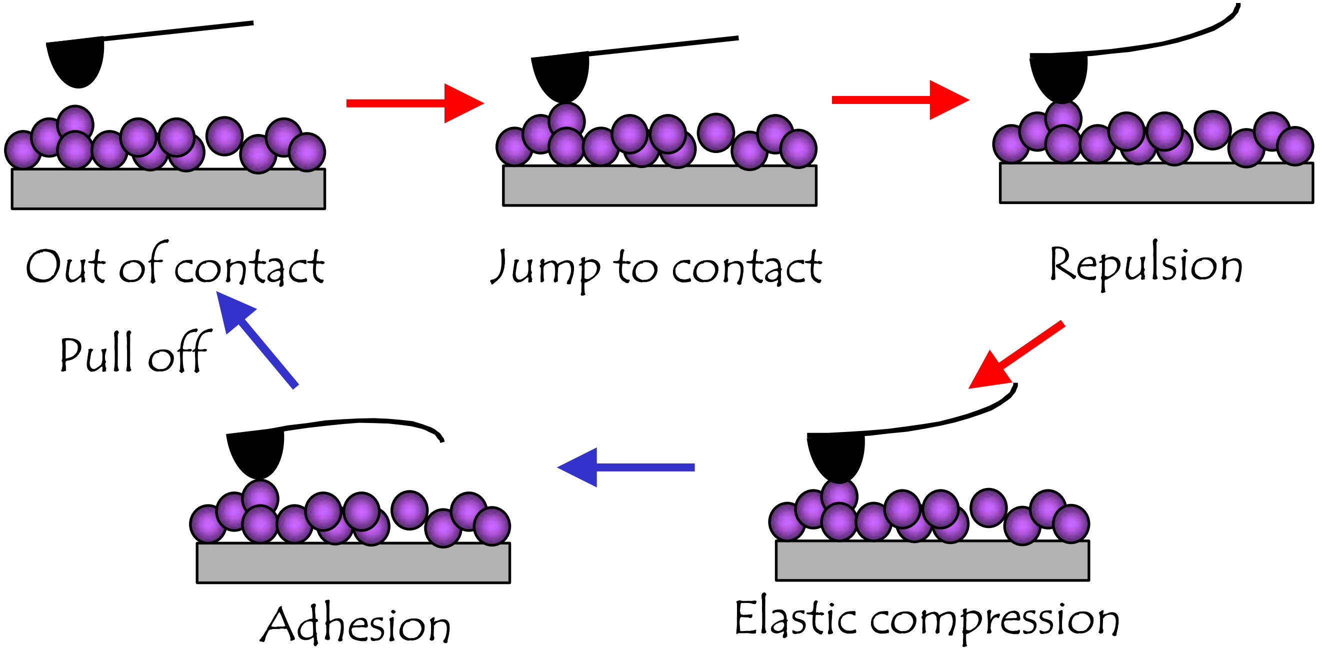
Pea leaf – polysaccharide Bundles (~ 20 nm)

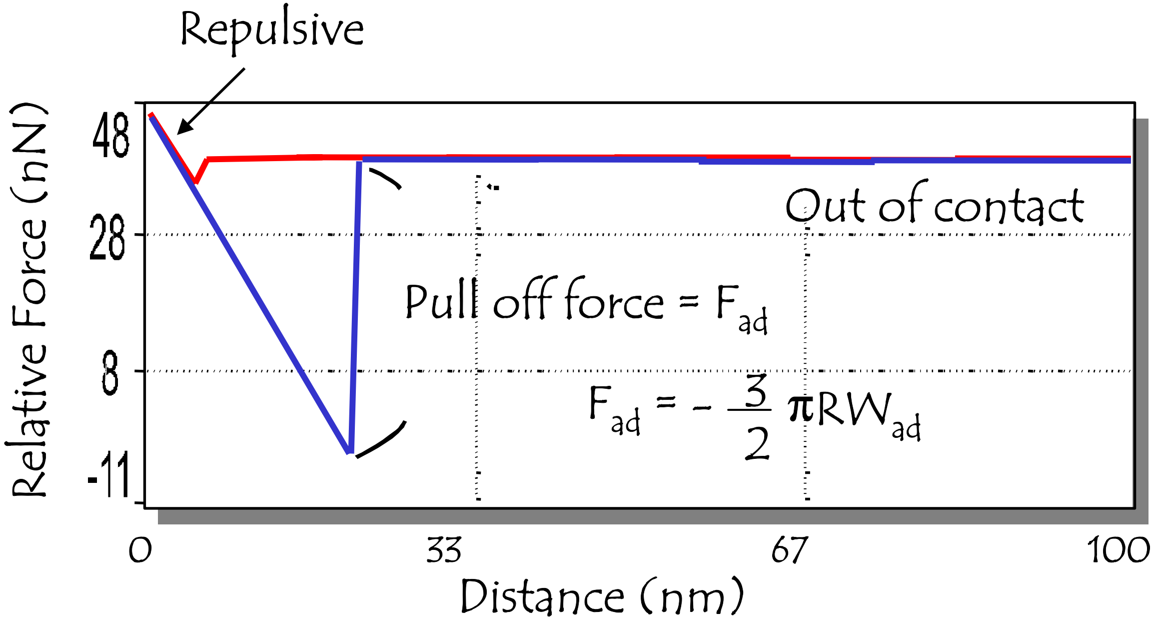


Crystalline lipids on Pea Leaf

Pea leaf provided courtesy W. Abraham, Monsanto

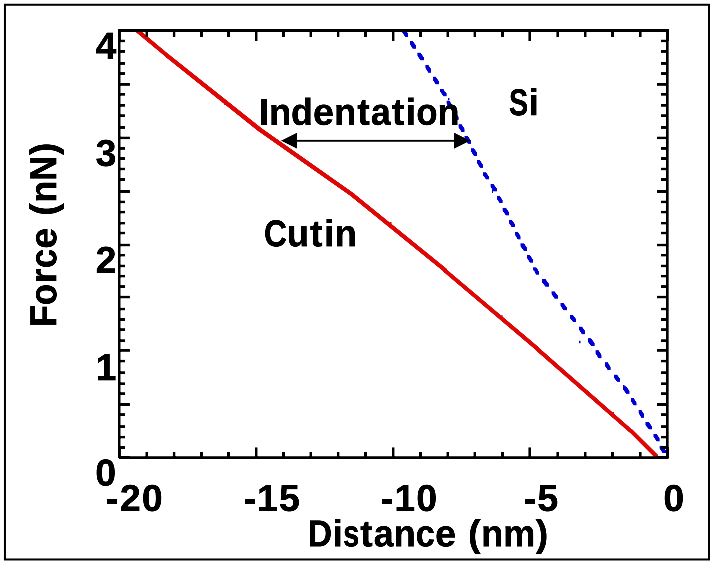
Local Adhesion and Nanomechanics





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

Nanoindentation...

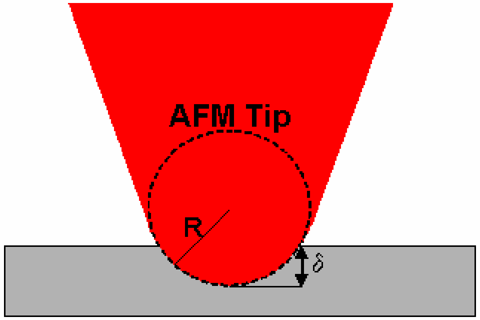


Compare force-distance curves for an ideally hard surface (Si wafer) to those from cuticular membrane.

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)

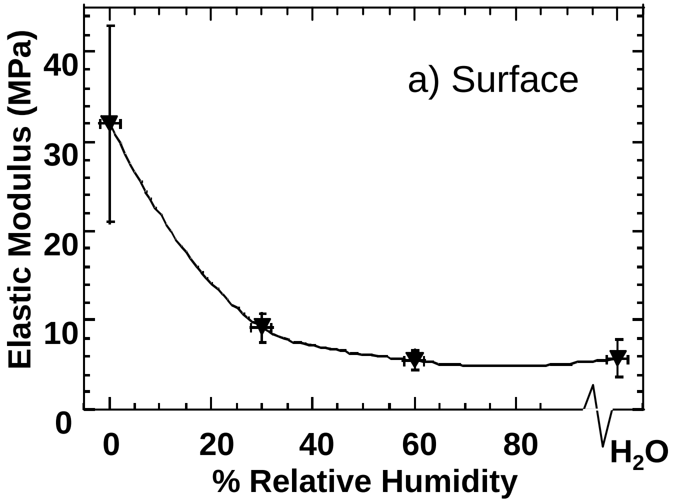
ν = Poisson ratio

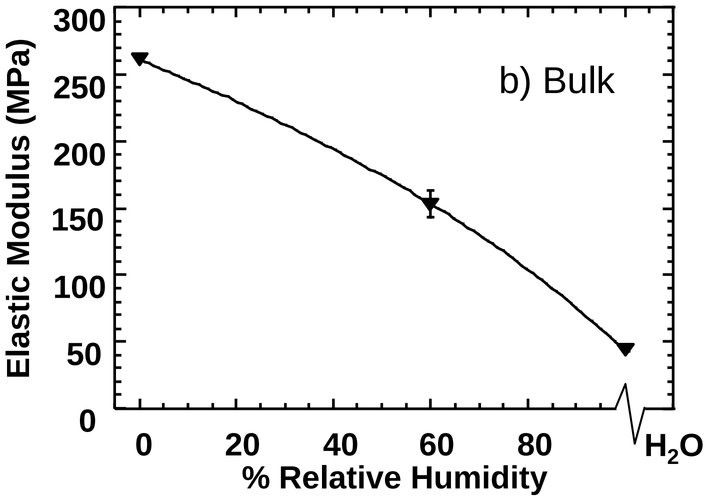
E = Elastic modulus

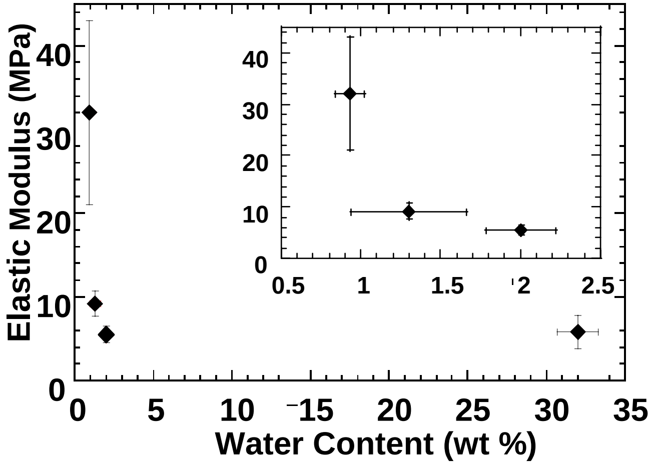
$$\boxed{E_c = \frac{3K\left(1 - \nu_c^2\right)}{4}}$$

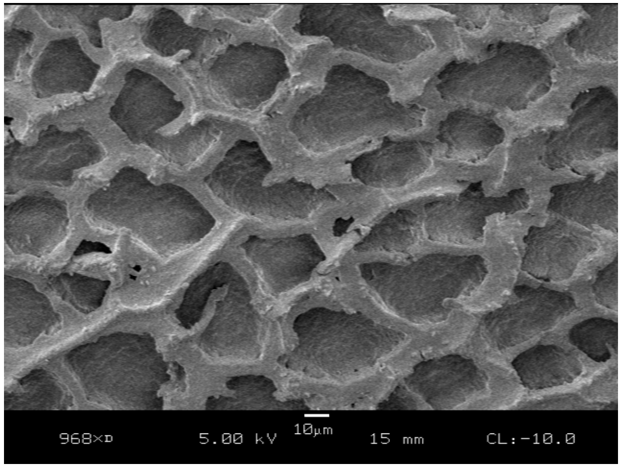
In the limit of E_{tip} >>> E_{cutin}

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



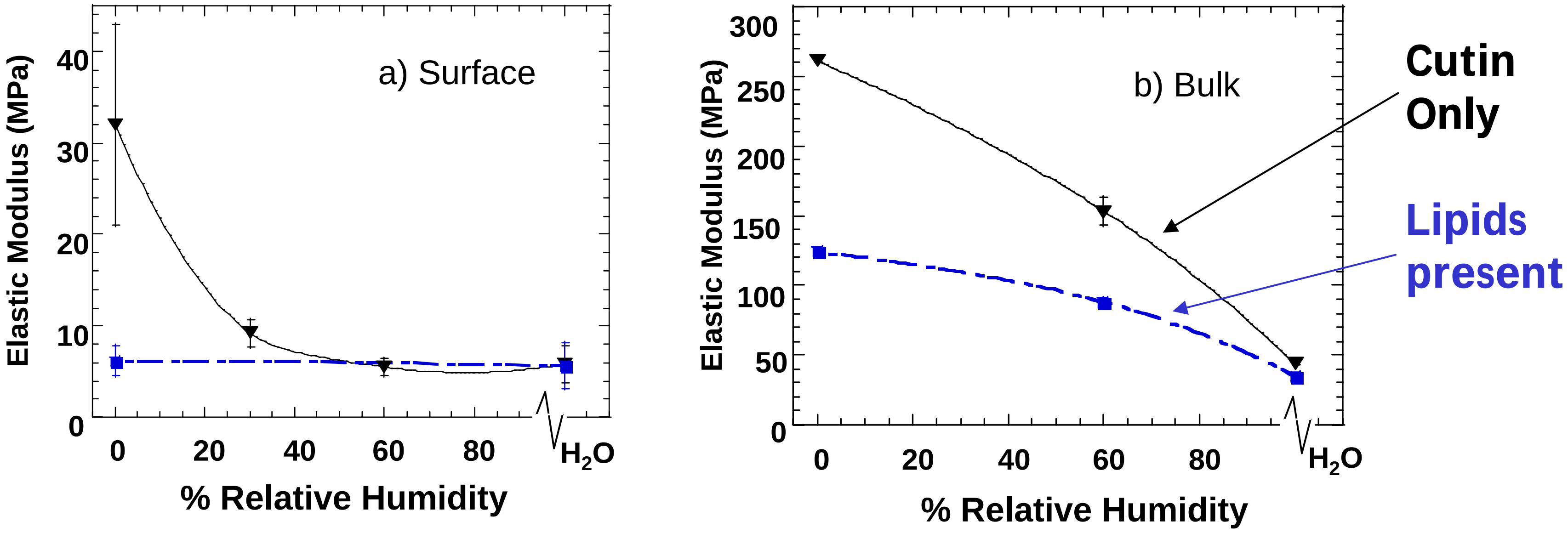






Networked structure enhances rigidity parallel to the surface.

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

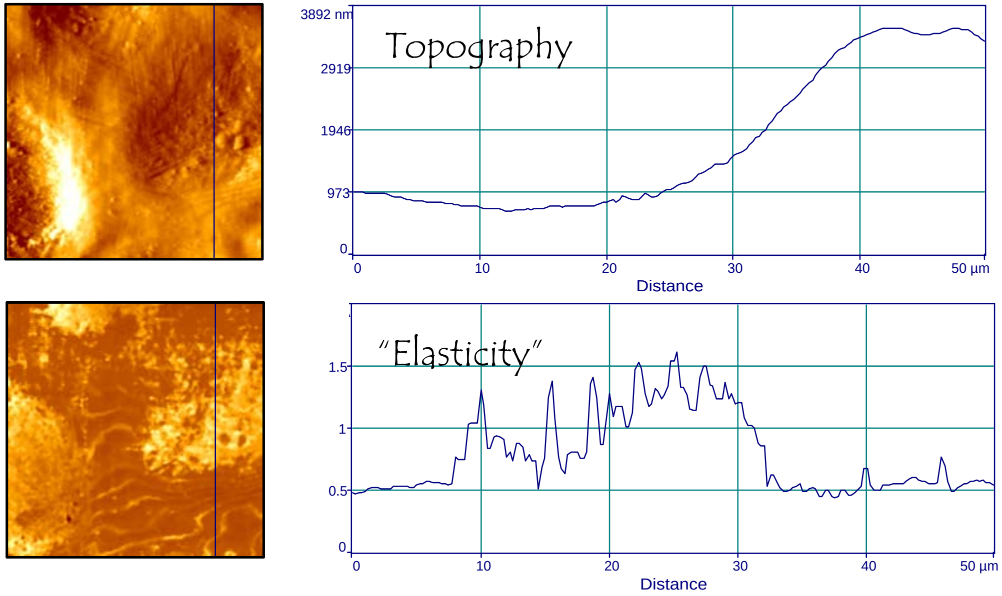


- AFM shows no sensitivity to water uptake ⟶ sees only the lipid covered surface.

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

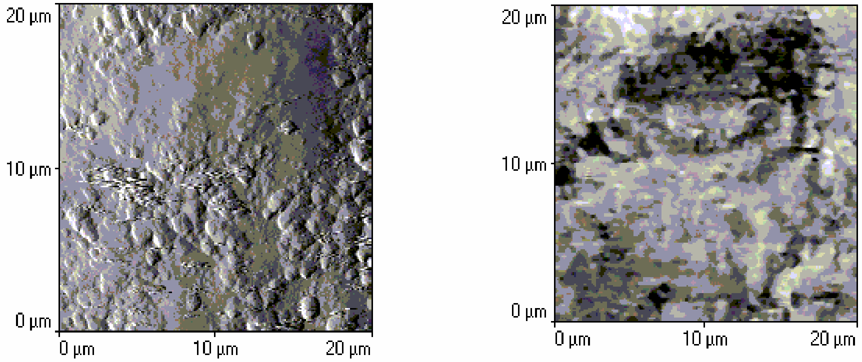
- Bulk response shows plasticizing impact of lipids and water uptake.

Modulated force and friction mapping of plant surfaces



Spatial mapping of viscoelastic properties. Surface chemical heterogeneity.

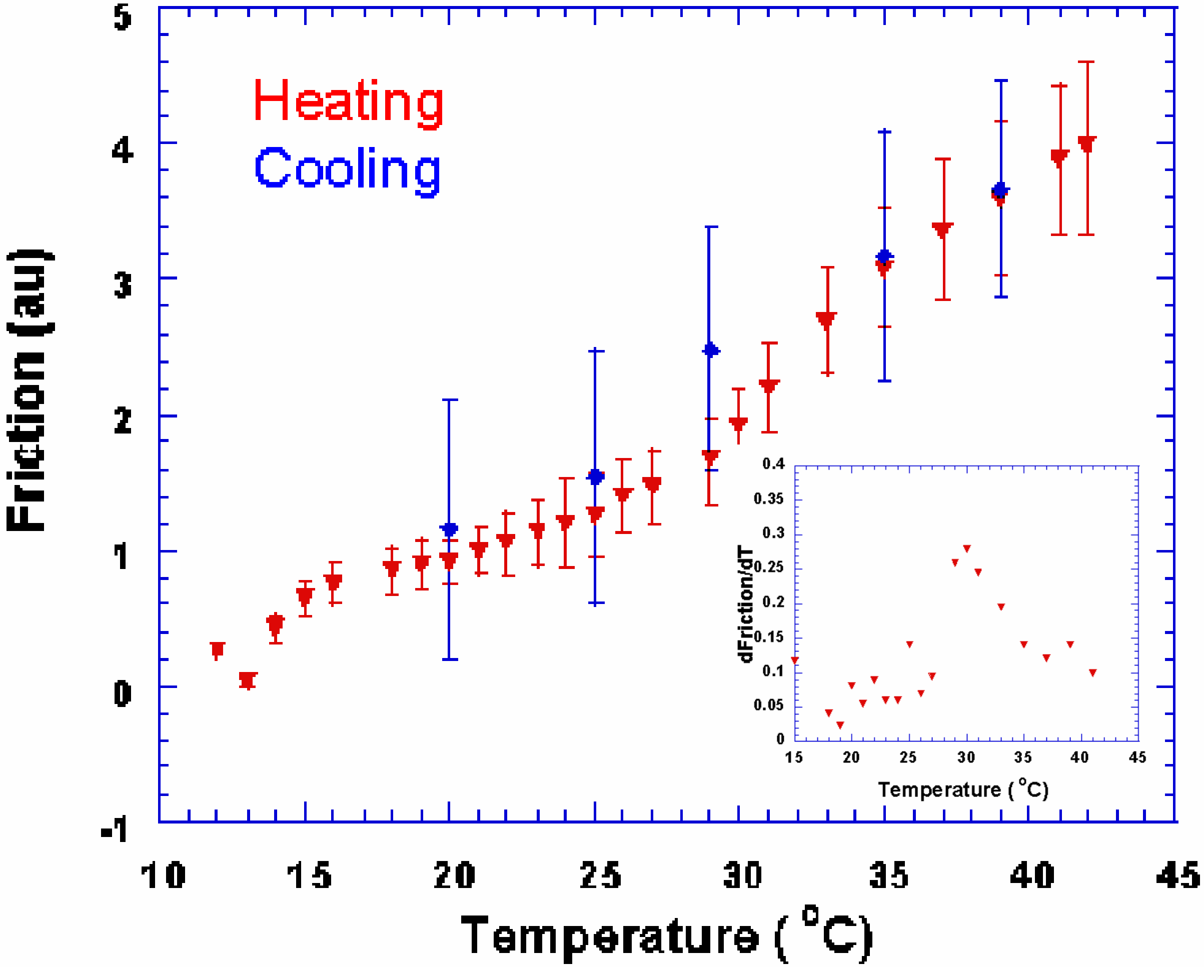
Friction Contrast Map on Pea Leaf Surface



Topography

Friction

Probing Surface Thermal transitions. . .

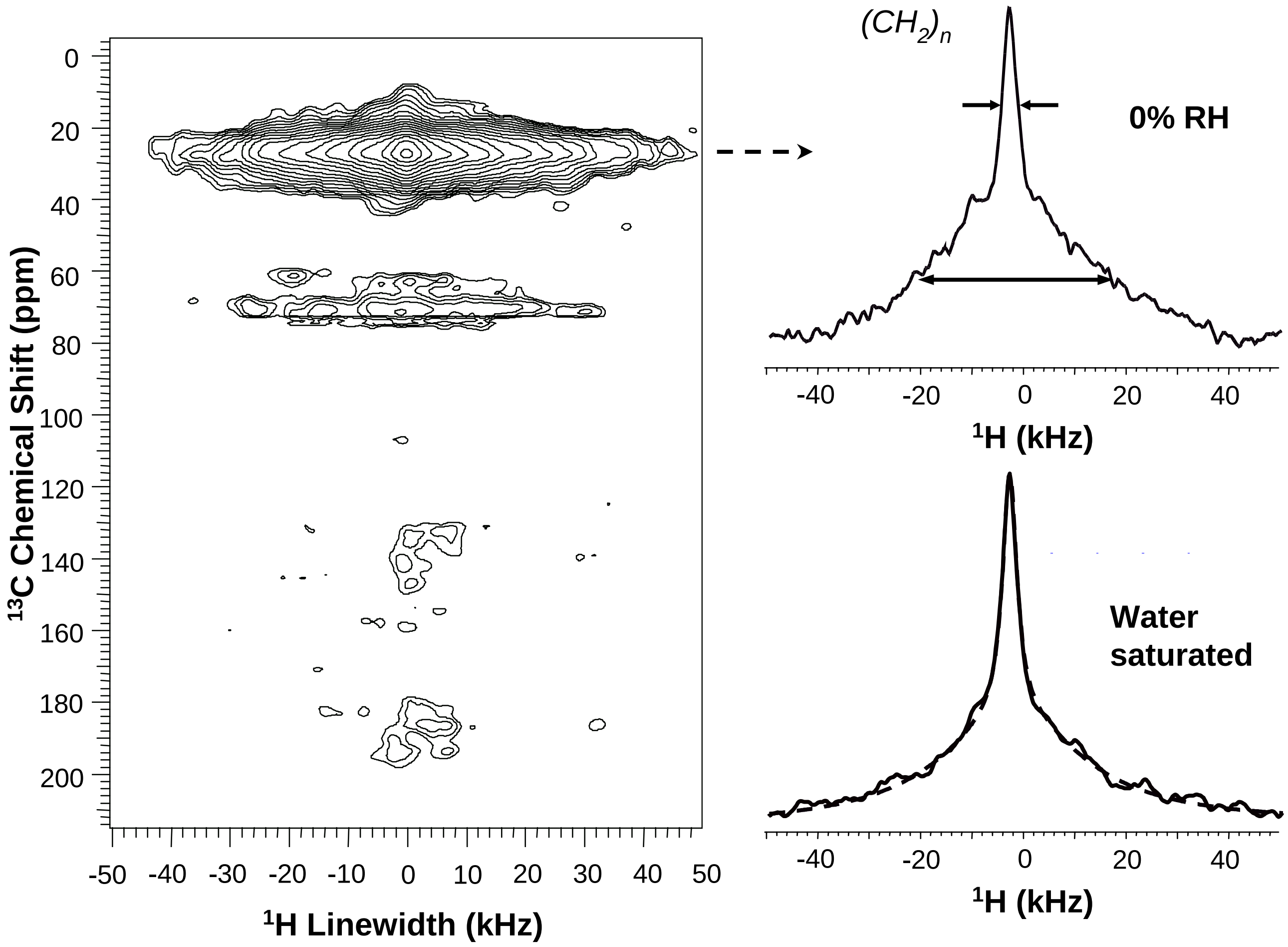


NMR Studies of Biopolymer Molecular Motions

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

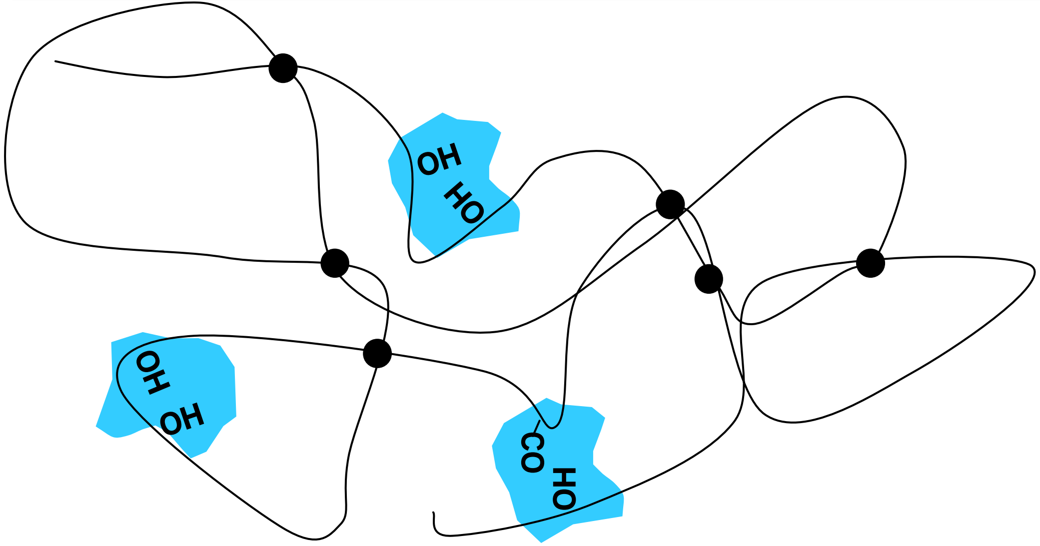
Cross-links

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 cooperative motions in both dewaxed and native cuticles: more efficient for acyl chains.

Summary . . .



- 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.

and currect directions . . .

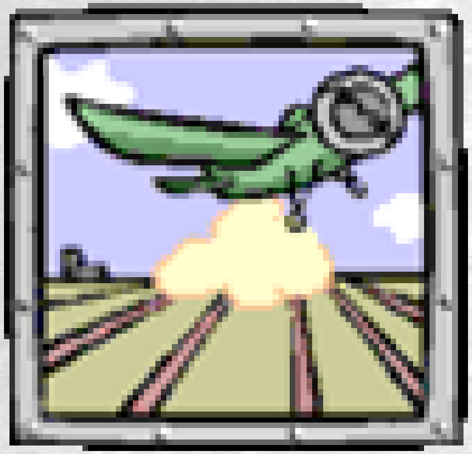
- Adsorption of model agrochemicals.

  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

Agrochemical Adsorption Studies

Issues of surface wetting

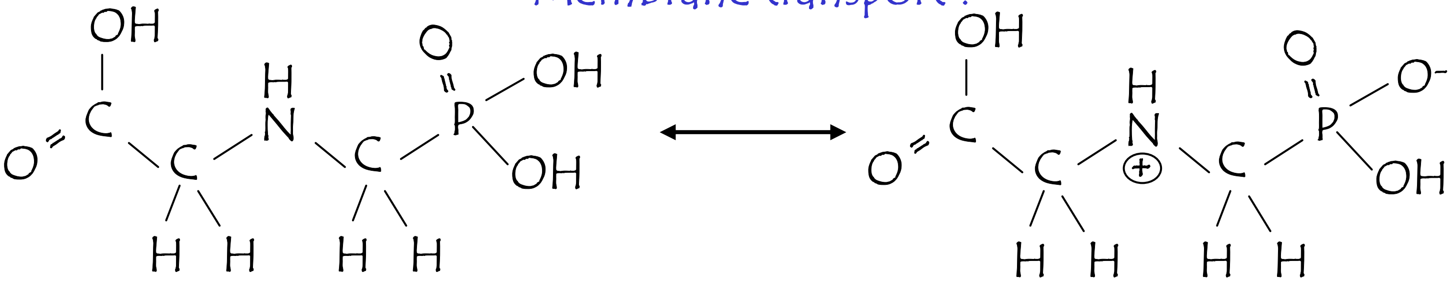
- spray dispersal – needs to be effective in wetting plant surfaces

=> need to directly probe surface wetting

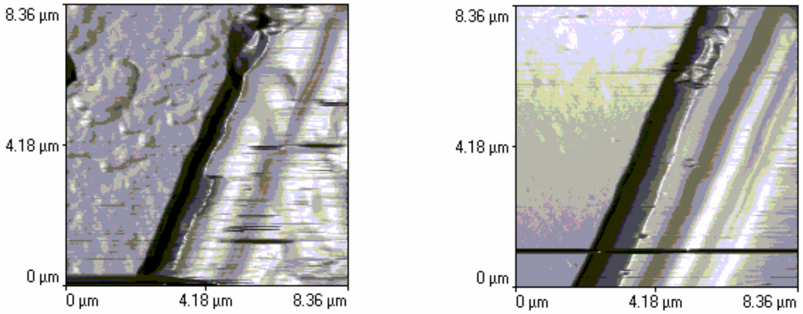
Is there preferential wetting ?

Interactions with surface lipids?

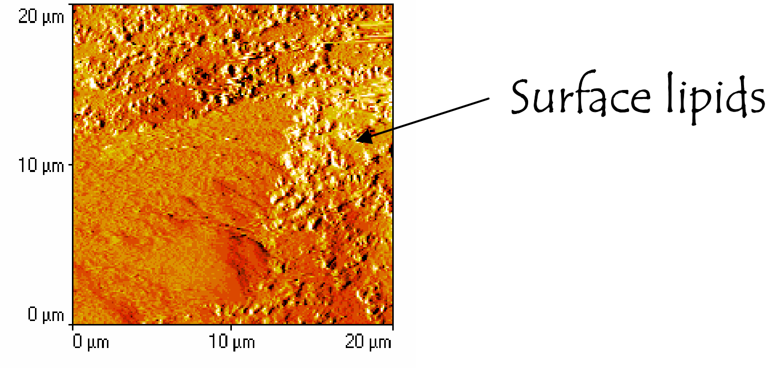
Membrane transport ?



Glyphosate – common herbicide (Roundup)



Before and after glyphosate adsorption

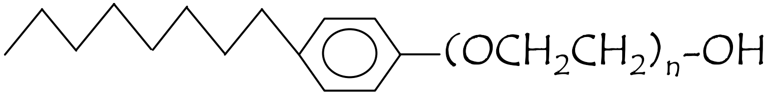


Surface lipids

Pea leaf cuticle

Dispersal agents:

Triton X-series



n = 10 - 40

Non-ionic surfactants

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 Surfaces

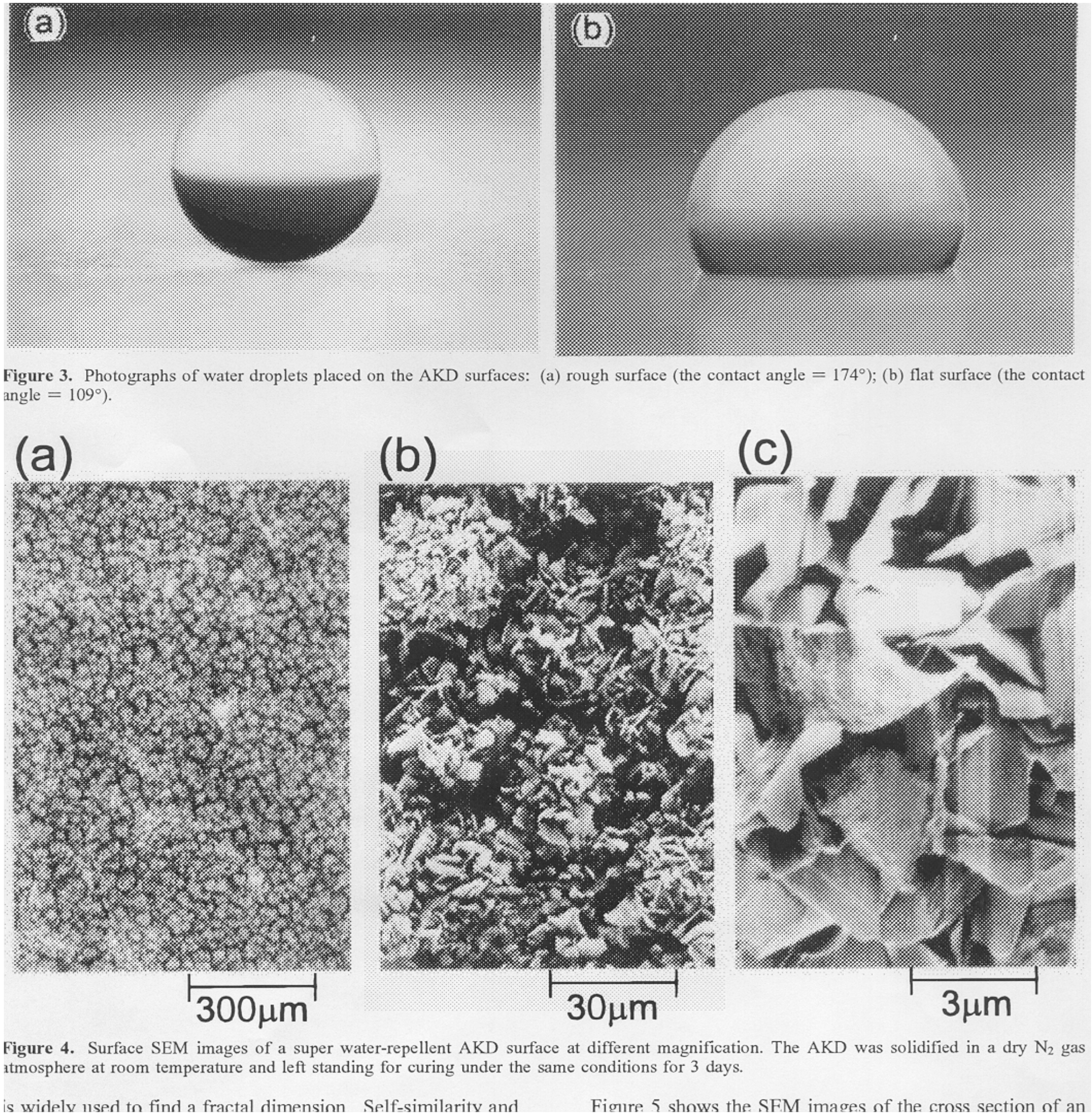


Figure 3. Photographs of water droplets placed on the AKD surfaces: (a) rough surface (the contact angle = 174°); (b) flat surface (the contact angle = 109°).

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

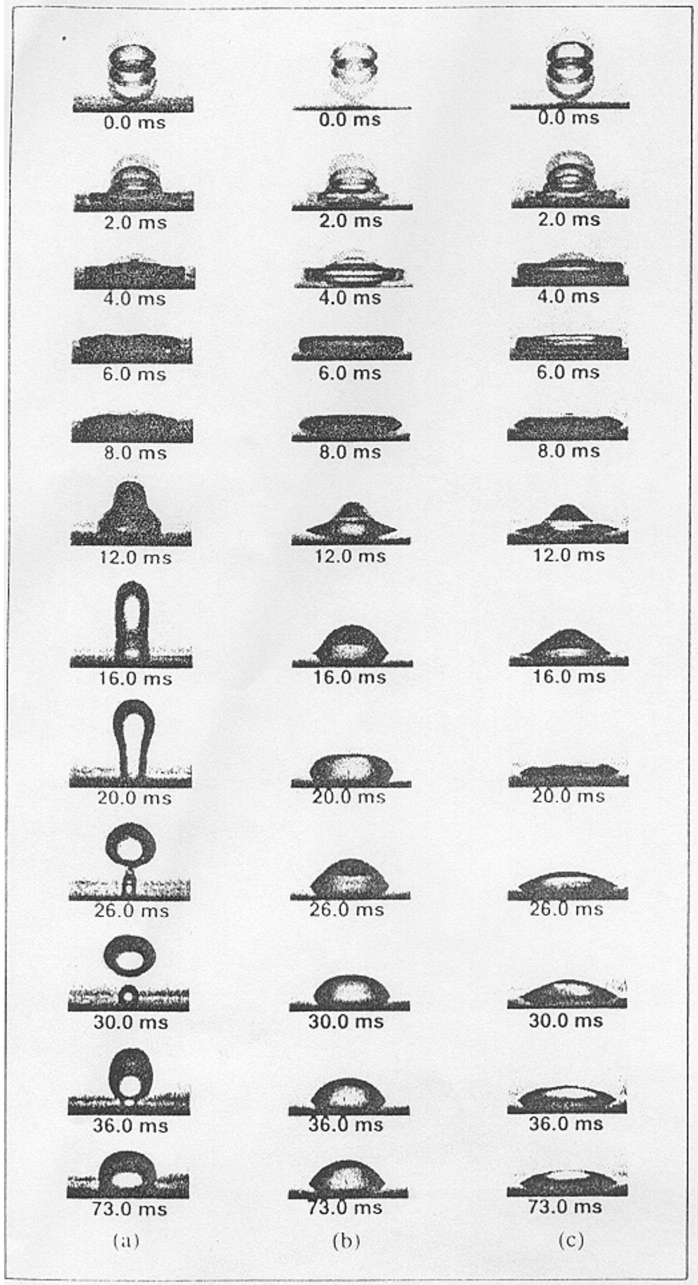
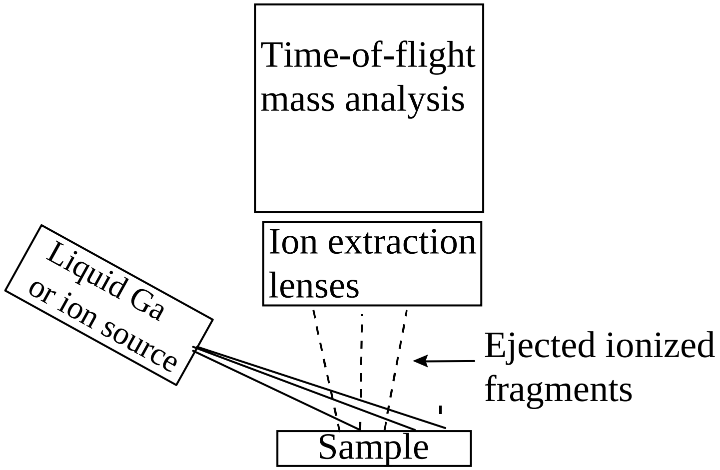


Figure 4. Impact of a 2.7 water droplet on (a) the paraffin wax block; (b) the stainless-steel plate; (c) the glass slide.

	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)


Time-of-flight mass analysis

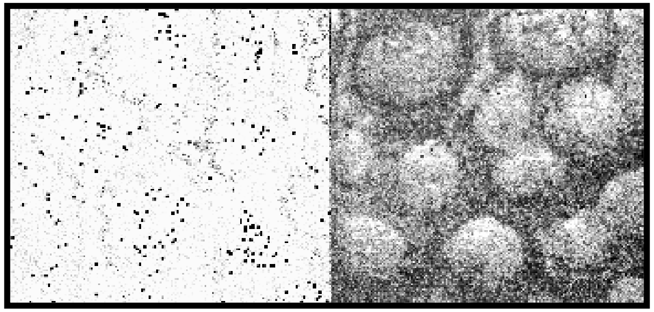
Ion extraction lenses

Liquid Ga or ion source

Ejected ionized fragments

Sample

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.