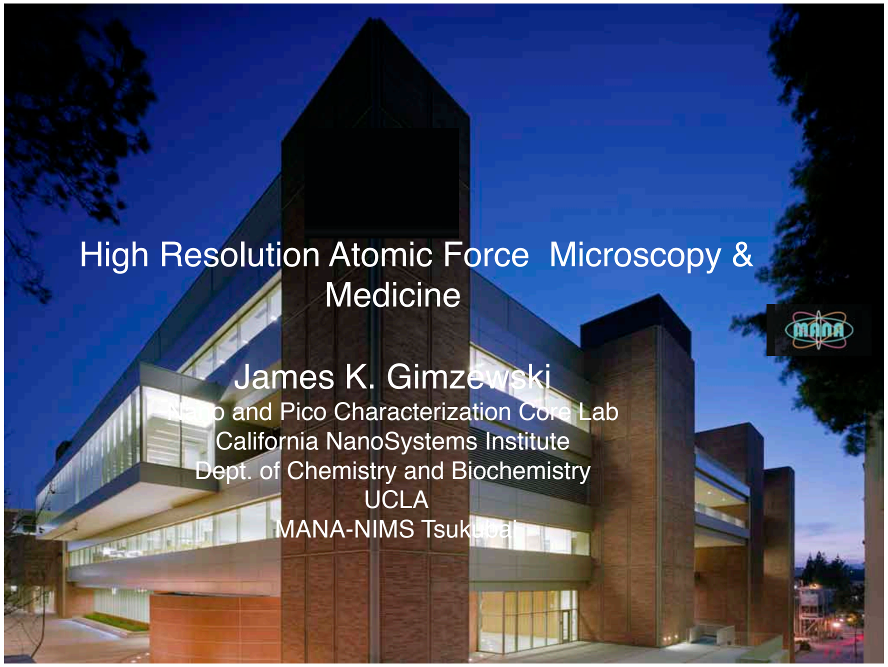


High Resolution Atomic Force Microscopy & Medicine

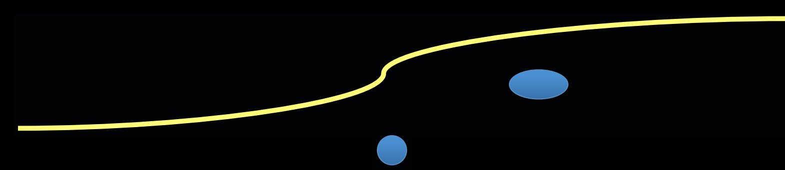
James K. Gimzewski
Nano and Pico Characterization Core Lab
California NanoSystems Institute
Dept. of Chemistry and Biochemistry
UCLA
MANA-NIMS Tsukuba

Life: networks to nanosystems

Nanotechnology enables the study of individual nano-components of life and their interactions in small world networks.

Life starts at the nanolevel !

Linear or globular 1nm-100nm



NANOMEDICINE

- **New , fast turnaround diagnostics**
  - **Genetic: DNA, mRNA ….**
  - **Cell Mechanics and metastasis**
  - **Natural nanoparticles: salivary exosmes, vaults, viruses**
  - **Nanoparticles for MRI, PET and other imaging**
- **Nano-therapeutics**
  - **advanced targeted drug delivery and gene therapy**
  - **Nanophotonics tumor elimination**
- **Theranostics (Pharma-codiagnostics)**
  - **diagnostic therapy for individual patients**
  - **Human variability to therapy and drug combinations**

Nanoscience and medicine

Correlating nanoscale to macro-physiological properties of disease

Technological transition from 'looking ' at images to advanced image recognition and real time data analysis

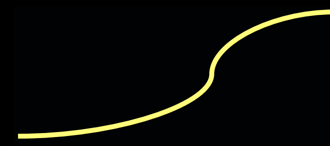
Incorporation of correlative techniques such as AFM-EM-Fluorescence etc

Enabling analysis of patient sample pools from human derived cell lines: correlation of variations in disease at point of care

Nano-components of life

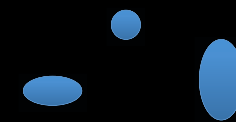
linear

- DNA, RNA
- Actin filaments
- Microtubules



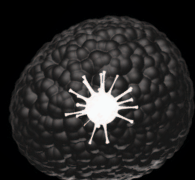
globular

- viruses
- exosomes
- vaults
- Drebrin



Bionanoparticles: Saliva Exosomes- as biomarkers for oral cancer

Small membrane vesicles(70-100nm) secreted into biological fluids such as blood and saliva
Possess defined set of membrane and cytosolic proteins
Bioactive- endocytosed by distant cells



Putative functions:

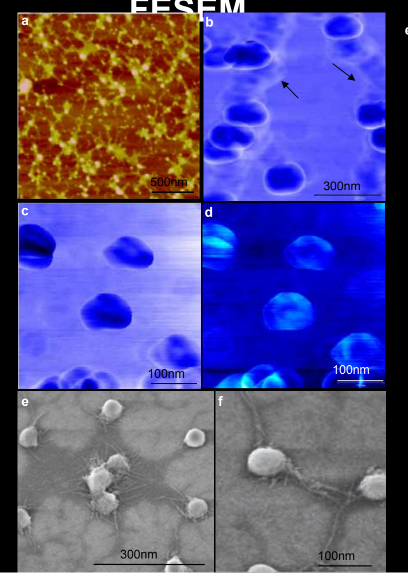
- Eliminate obsolete proteins during cell maturation
- Cell-cell communication
- Induction of immune tolerance

Potential applications:

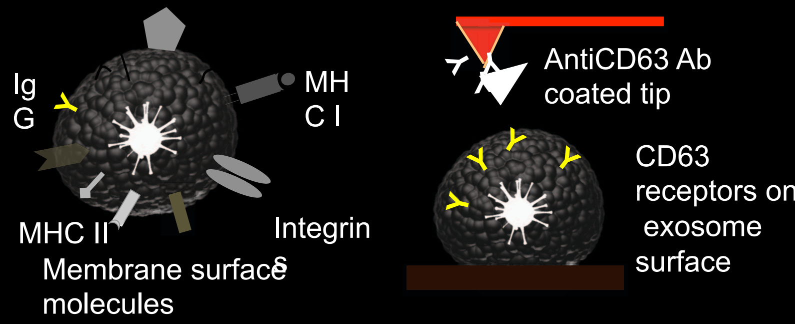
- Immunotherapy of tumor
- Immunosuppresion

S Sharma, JK Gimzewski, ACS Nano 2010

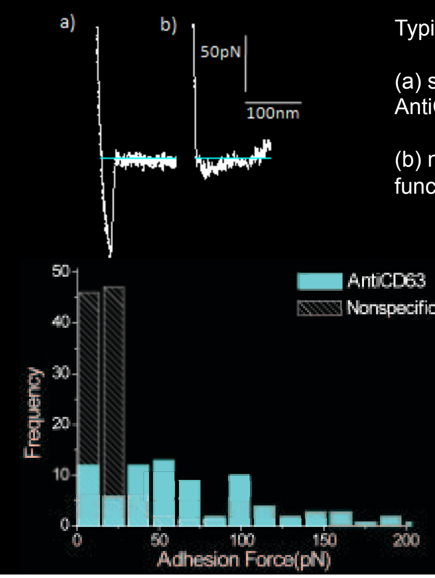
Ultrastructure of individual saliva exosomes observed under Tapping mode, AM-AFM and FESEM



Molecular Recognition Force Spectroscopy: CD63 receptors



Molecular force recognition spectroscopy

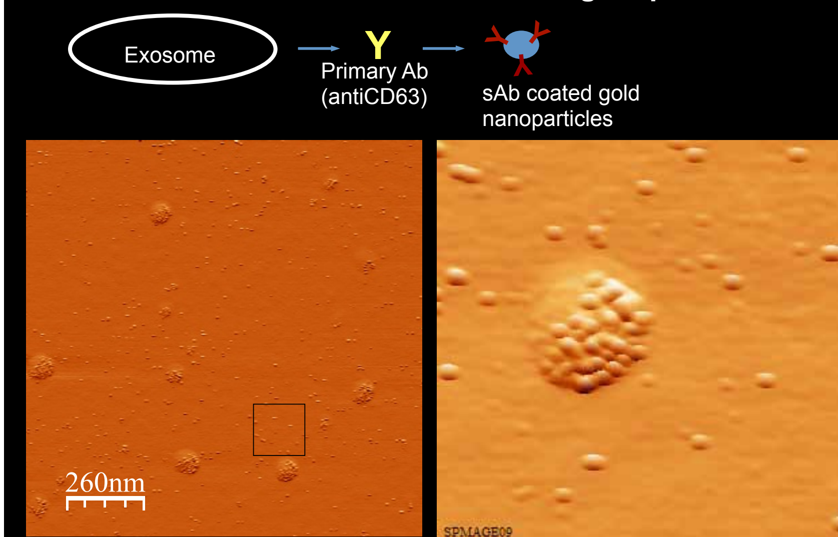


Typical force vs separation curves

(a) strong adhesive event between AntiCD63

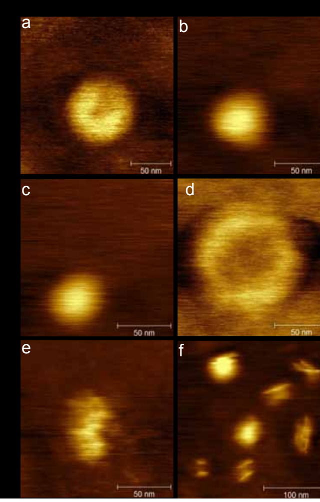
(b) no-event for non-specific Antibody functionalized tip and exosome

AFM based immunolabeling of exosomes extracted from human saliva with 4nm diameter gold particles



Reversible mechanical deformation and disintegration of single exosome imaging.

- **a,** Exosome size increases with decreases from a to b with decrease in imaging force applied. Vesicle size then increases from b to c and d with increasing applied force. **e,** Increasing the force to ~5nN, results in



CONCLUSIONS:

1. Exosomes- possess a a tri-lobed ultrastructure with unique biomechanical properties.

2.Epitope mapping of surface receptors via force spectroscopy and nanoparticle immuo-labeling can help distinguish cancer vs normal exosomes from easily assessable biological fluids such as saliva

3. Study of normal and cancer patients is underway

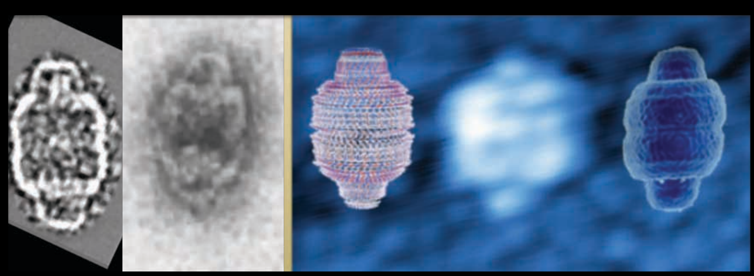
Vaults are found in all cells and show potential for new drugs 75 x 45 nm can be filled with DNA, polymers etc

9.5 MDa RNP
Composed of 3 proteins and 1 RNA
96 copies of 100 kDa Major Vault Protein (MVP)
75 nm x 45 nm exterior dimensions & hollow interior core
Synthetic 'designer' vaults can incorporate therapeutics and/or recognition site

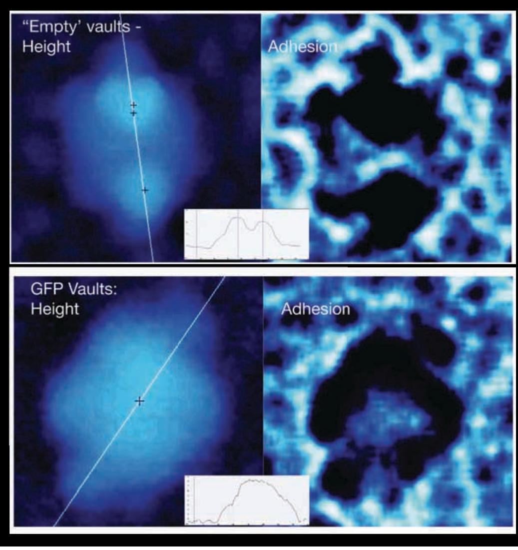
Variable structural mechanics of recombinant vault nanoparticles revealed by atomic force microscopy

Adam Z. Stieg, Valerie A. Kickhoefer, Haider I. Rasool, Leonard H. Rome and James K. Gimzewski

HUMAN VAULTS
Average Cryo TEM, Single CryoTEM, Model, Single AFM, Model II of Vaults: Correlative EM-AFM



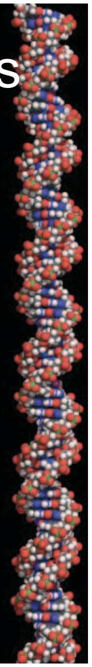
Designer Vault filled with GFP



Biopolymer Protein Interactions

- DNA – Restriction enzyme-AFM
  for PCR-free gene profiling

- Drebrin-Actin Nanomechanics potential relation to Neurodegenerative disease



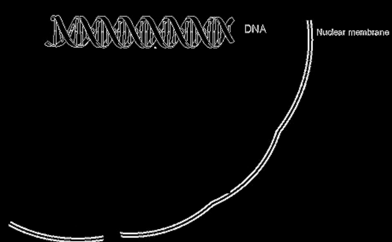
Gene Expression Profiling Using Atomic Force Microscopy

Carlin Hsueh, Jason Reed, Bud Mishra (NYU)
James K. Gimzewski

High speed Collaborations, Mervyn Miles
University of Bristol, UK
Veeco Santa Barbara, California

Gene Expression

A gene is transcribed into a messenger RNA (mRNA) transcript.



Measure here →

By examining which transcripts are present in a cell, it is possible to deduce which genes (and their related proteins) are expressed in a cell type.

Gene Expression profiling

Monitoring expression levels of genes simultaneously to study the effects of certain treatments, diseases, and developmental stages on gene expression.

Limitations

Sensitivity constraint:

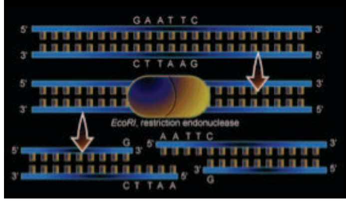
Each cell contains approx. *300,000* mRNA molecules

representing more than *30,000* different species

while each low abundance species may be present in only a few copies (<*20* copies) per cell

- Large amounts of material can be difficult to obtain in clinical and experimental settings
- Unequal replication from enzymatic amplifying process (ie PCR) can introduce serious biases
- Some mRNAs may cross-hybridize to wrong probes

Restriction Enzymes



Enzyme	Source	Recognition Sequence	Cut
EcoRI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
EcoRII	*Escherichia coli*	5'CCWGG 3'GGWCC	5'--- CCWGG---3' 3'---GGWCC ---5'
BamHI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
HindIII	*Haemophilus influenzae*	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'
TaqI	*Thermus aquaticus*	5'TCGA 3'AGCT	5'---T CGA---3' 3'---AGC T---5'
NotI	*Nocardia otitidis*	5'GCGGCCGC 3'CGCCGGCG	5'---GC GGCCGC---3' 3'---CGCCGG CG---5'

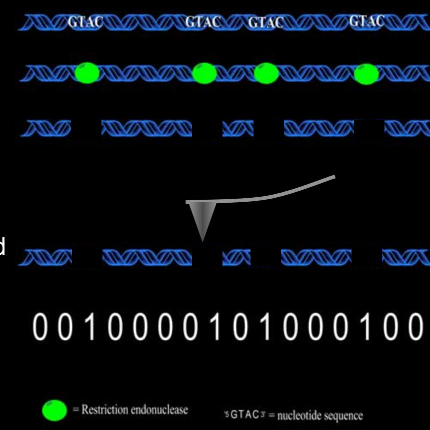
UCLA

Single Molecule Transcription Profiling using Atomic Force Microscopy

Use AFM to directly image and identify, through enzymatic tagging, individual gene transcript molecules.

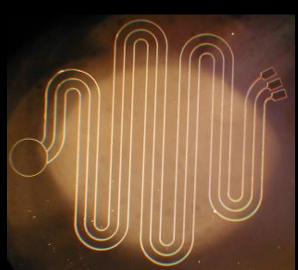
Generating single molecule, ordered restriction maps:

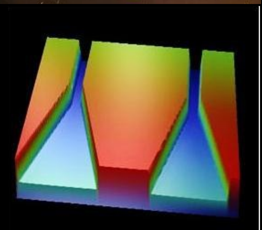
- DNA is fixed to the surface
- Order and distance between the cleavage sites is retained

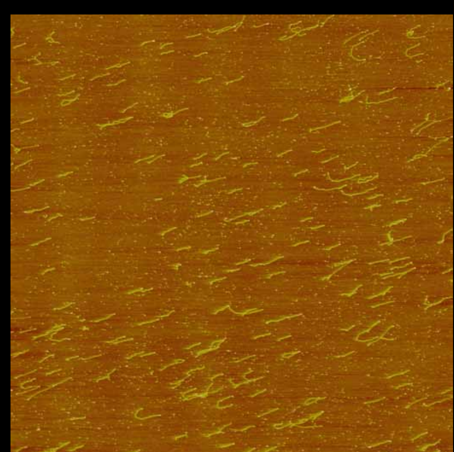


UCLA

Microfluidics



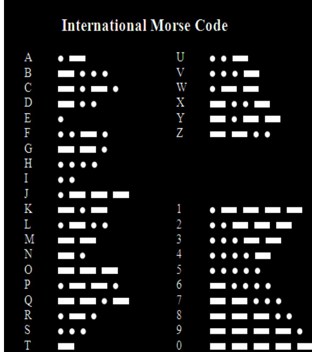


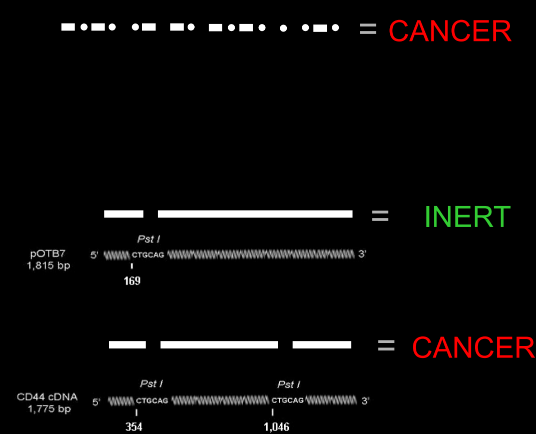


Sample preparation done on a single chip

UCLA

DNA Morse Code

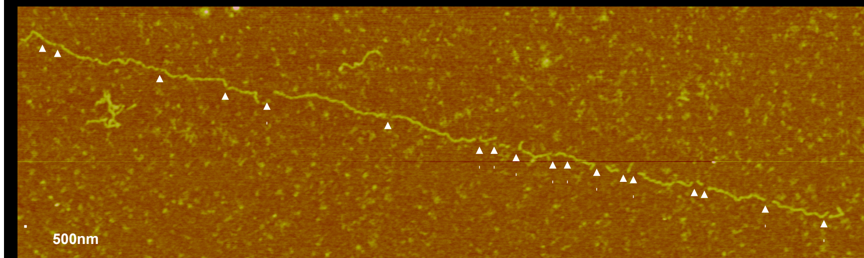




UCLA

Lambda DNA

- Lambda DNA pre-cut with claI = 16 frags. of varying sizes that are representative of molecule sizes in a single cell
- Fragments digested with restriction enzyme



Lambda/ClaI fragment digested with RsaI

UCLA

Software

AFM | AFMexplorer/MatLab | AFM | AFMexplorer/MatLab

10x10um scan survey
3-5Hz
1000x1000 pixels

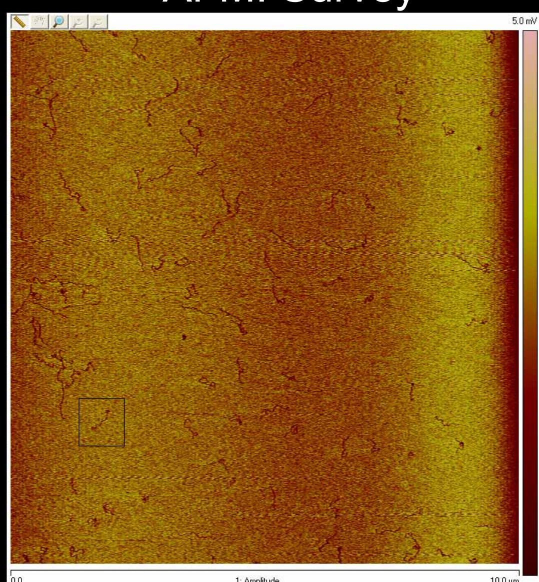
Molecule recognition
Region Of Interest (ROI)
Coordinate calculation

Detailed image of ROIs
~ 2x2um scan size
2Hz

Image analysis of ROI
(statistical algorithm)
Database matching

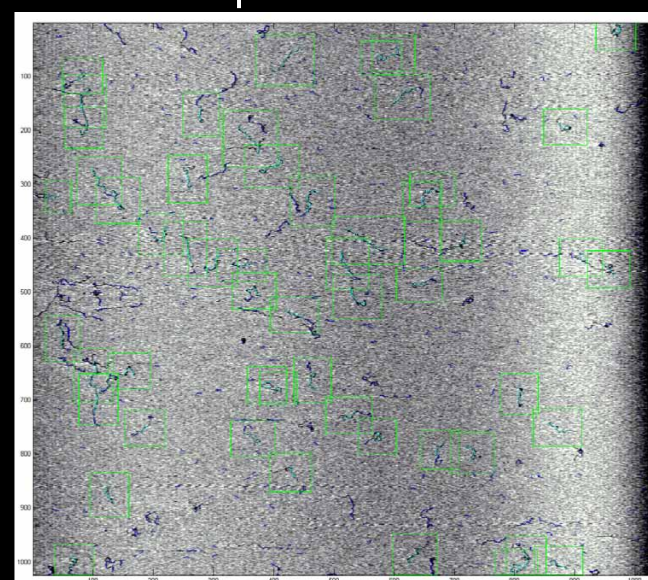
UCLA

AFM: Survey



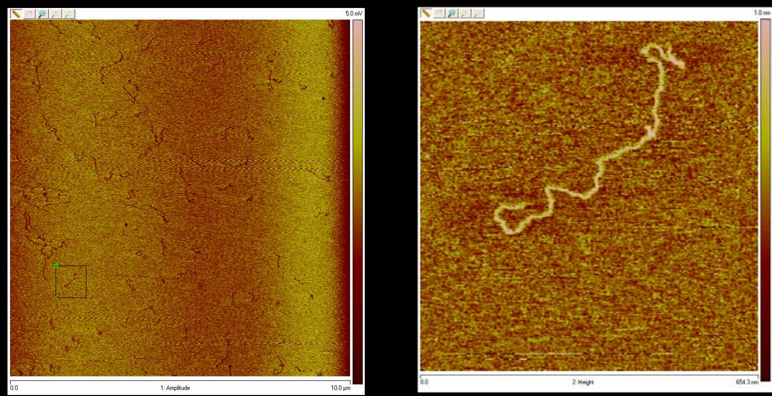
UCLA

AFM explorer/Matlab: ROI



UCLA

AFM: Detailed ROI

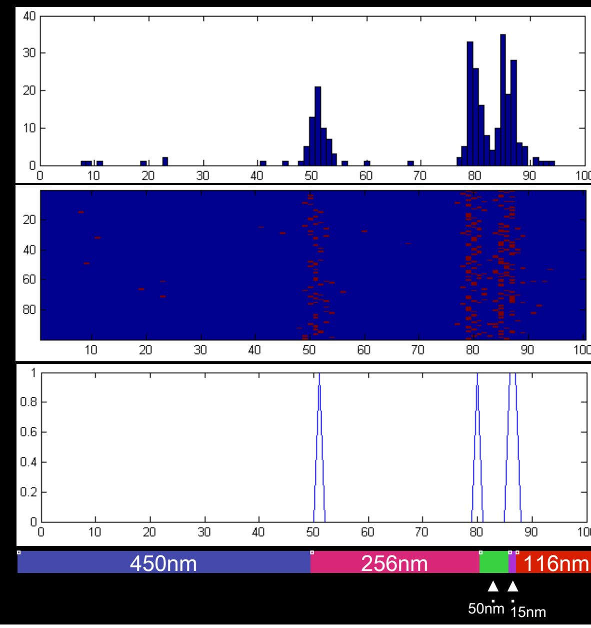


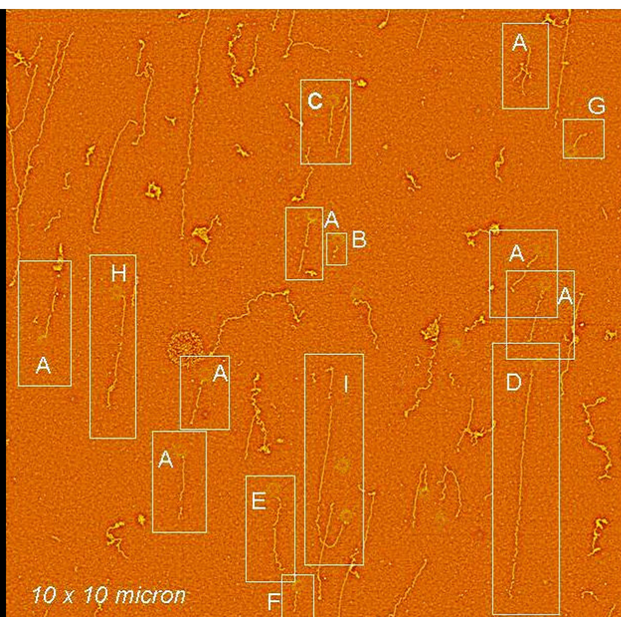
Histogram of label sites in experimental data

~115 molecules
106 matches
~ 9 false

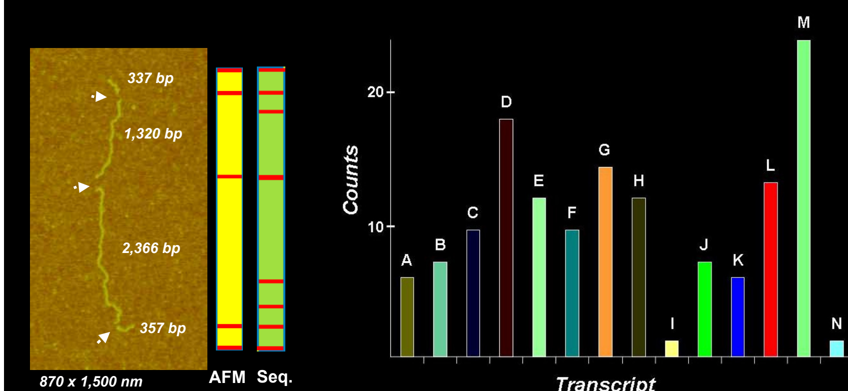
Algorithm scoring of experimental data

Map of NtBsmAI labeled pUC19



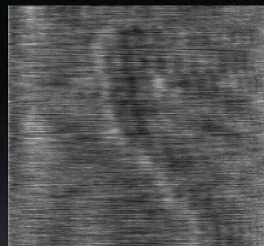


Individual species indicated by the letter in the box



We were able to identify all the species in the mixture with high confidence, and their relative abundance was roughly equal, as anticipated, within the variation expected from small sampling (median of 8 instances of each species from 15 images)

HS AFM of DNA



Mervyn Miles
Loren Picco

Malcolm Bremer
Mark Taylor

Astrophysics algorithms

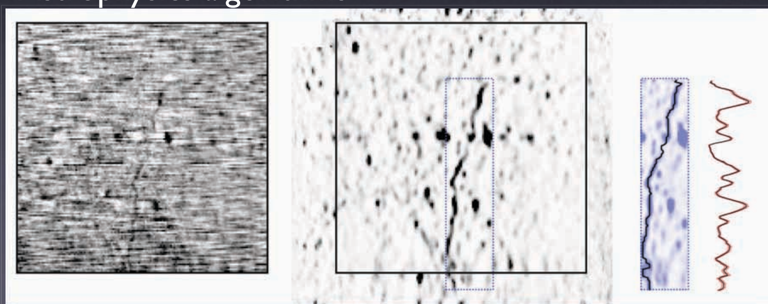
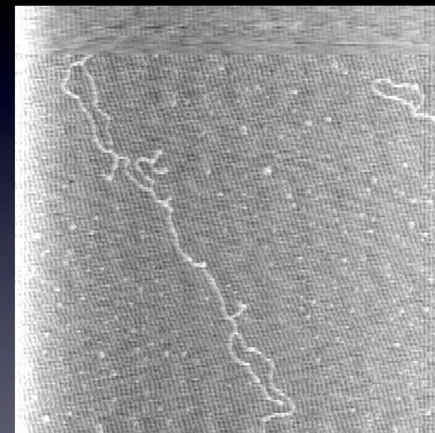


Image of DNA molecules deposited onto mica demonstrating the high-speed proof of principle



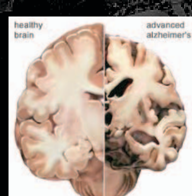
x2

Rob Harniman
Loren Picco
Mervyn Miles

Structural and nano-mechanical characteristics of Drebrin induced F-actin remodeling

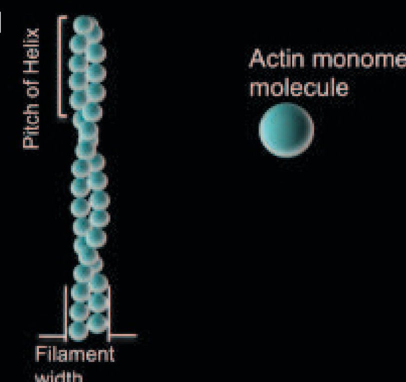
Shivani Sharma, Elena E. Grintsevich, Emil Reisler and James K. Gimzewski

During normal aging, the most dramatic decline was found in levels of synaptic proteins involved in structural plasticity (remodeling) of axons and dendrites. Alzheimer disease, the most common cause of dementia in the elderly, was associated with an additional 81% decrease in levels of drebrin, a protein regulating postsynaptic plasticity

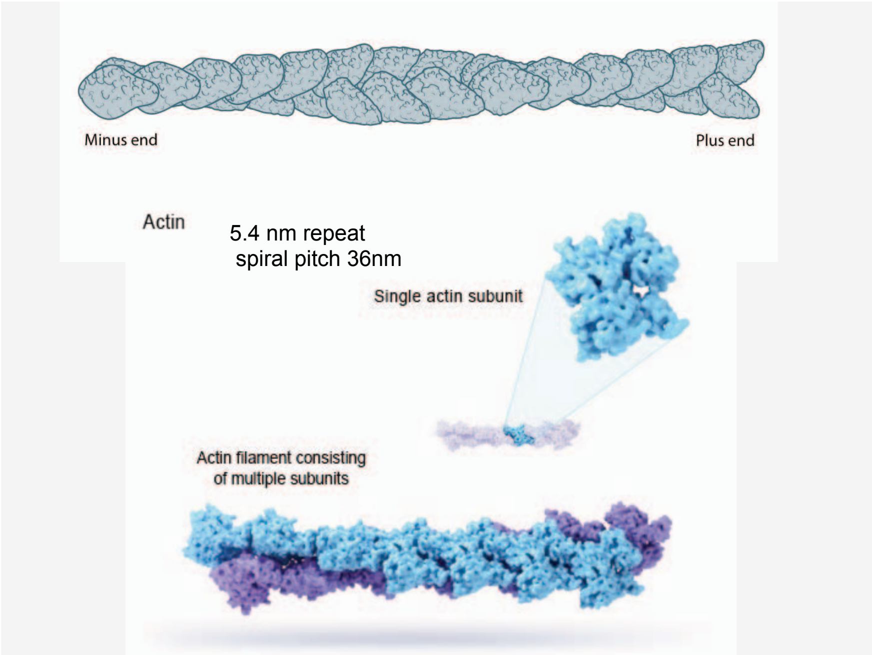


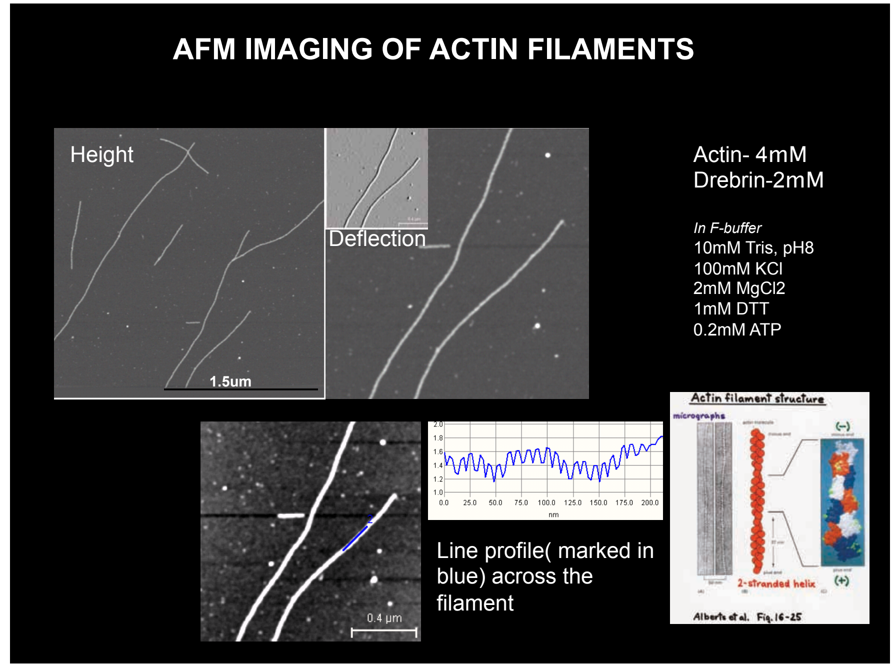
Biopolymer Protein Interactions

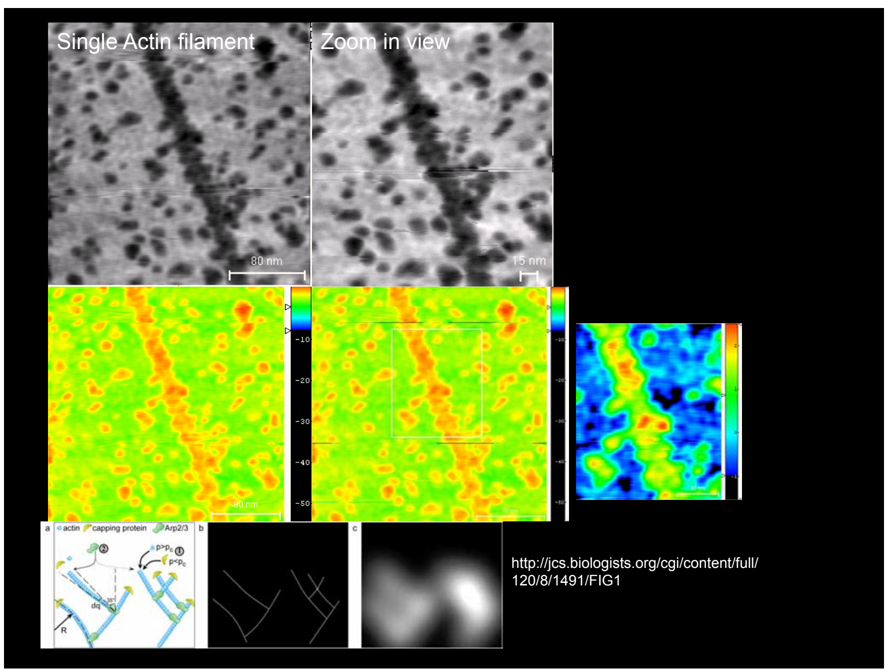
- DNA – Restriction enzyme-AFM for PCR-free gene profiling
- Drebrin-Actin Nanomechanics potential relation to Neurodegenerative disease

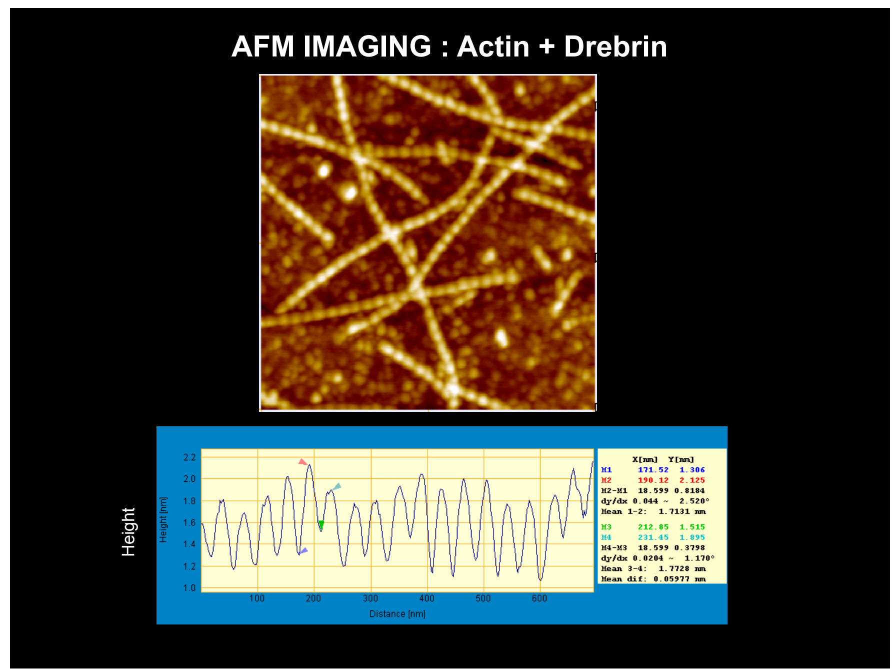


UCLA


Minus end
Plus end
Actin
5.4 nm repeat
spiral pitch 36nm
Single actin subunit
Actin filament consisting
of multiple subunits

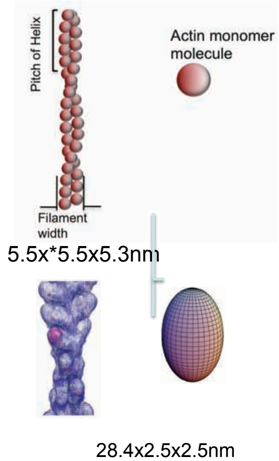

AFM IMAGING OF ACTIN FILAMENTS
Height
Deflection
1.5um
Actin- 4mM
Drebrin-2mM
In F-buffer
10mM Tris, pH8
100mM KCl
2mM MgCl2
1mM DTT
0.2mM ATP
0.4 µm
Line profile(marked in blue) across the filament
Actin filament structure
2-stranded helix
Alberts et al. Fig.16-25

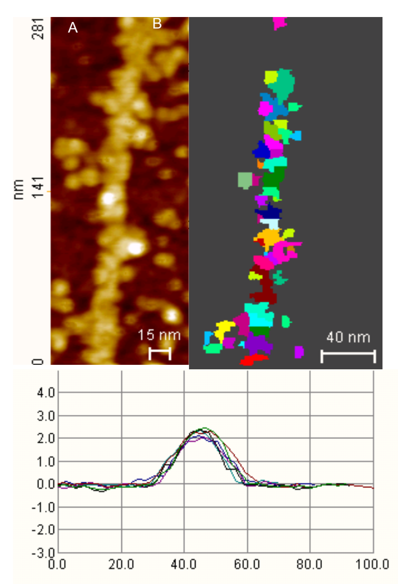

Single Actin filament
Zoom in view
80 nm
15 nm
http://jcs.biologists.org/cgi/content/full/120/8/1491/FIG1


AFM IMAGING : Actin + Drebrin
Height
Distance [nm]

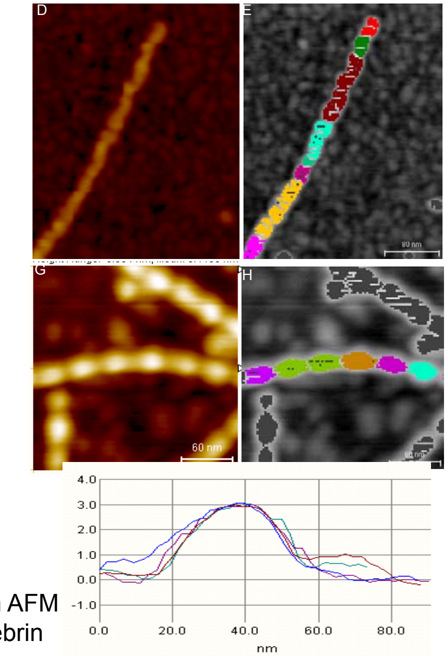
	(n=50)	Unbound F-Actin	Actin+ Drebrin	Actin+ Cofilin
1.	Filament width	20nm	40nm	
2.	Pitch of helix (periodicity)	36± 2nm	40± xx nm	29±xx nm 27nm*
3.	Persistence length lp (AFM)	7.05 um	10.99 um	1.47 um
4.	Lp (EM)*	17um(add ref)	NA	
5.	Lp (thermal fluctuations)$	9 um	NA	
6.	Lp (Fluoresence)&	9.8 um	NA	2.2 um
7.	Elastic modulus (Y)	$2.45x10^9$ Pa	$3.7x10^9$ Pa	$0.51x10^9$ Pa

Ref:
*JCB, vol 138,no 4, Aug 25, 1997, 771-781
$ JBC, vol 270, no 19, pp 11437-11444(1995)
& J. Mol. Biol.(2008) 381, 550-558





Histogram of molecular volume from AFM images of Bare actin and Actin + drebrin



1. *Drebrin plays a significant role in modifying the actin filaments and increases the mechanical strength of F-actin.* We show that drebrin mediates modification of F-actin by measuring the persistence length (Lp) and Elastic modulus of native and protein bound F-actin filaments imaged at nanometer scale resolution.
2. *Uniform periodicity, homogeneity and stoichiometry. How is the binding of drebrin influencing the actin mechanics…? How much force is required to unwind/ stretch the actin helix and make it stiffer.*
3. can we explain the effect by finite element modeling or use a tensegrity model ?

Acknowledgements

•Dr. Lisa Wesoloski, Dr. Masashi Kunitake (Kumamoto University), Dr. Franz Giessibl (University of Regensburg), Haider Rasool, Paul Wilkinson

[illegible]

[illegible]

[illegible] A&D TSM

[illegible]

[illegible]


شكرا
THE END
A
WB
Warner Bros.-First National
PICTURE