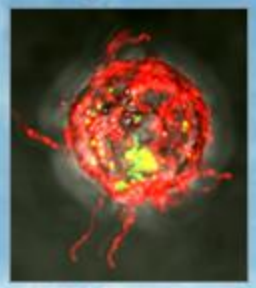


Biomimetically Engineered Nanomedical Systems

Demir AKIN, *D.V.M., Ph.D.*

Center for Cancer Nanotechnology Excellence-TR (CCNE-TR)

Radiology, MIPS, School of Medicine

Stanford University

Nanomedicine: WHY?

- Diagnostic tools of better sensitivity, specificity and reliability.
- In parallel or to integrate several analytical steps into a single miniaturized device with low sample volume requirements.
- Enables Personalized medicine
- Potentially Wireless, autonomous biocompatible medical devices
- Ultimately detect disease and intervene at single cell level

Sizes of target biological systems are at the nanoscale

Atom	0.1 nm
Water	0.2 nm
DNA (width)	2 nm
Protein	5-10 nm
Cell membrane	5 nm thick
Ribosomes/Microtubules	25nm
Nuclear Pore	70nm
Virus	40 – 200 nm
Bacteria	1,000 – 10,000 nm
White Blood Cell	10,000 nm

Nanotechnology: R&D Spending

Research in nanotechnology is rapidly growing!

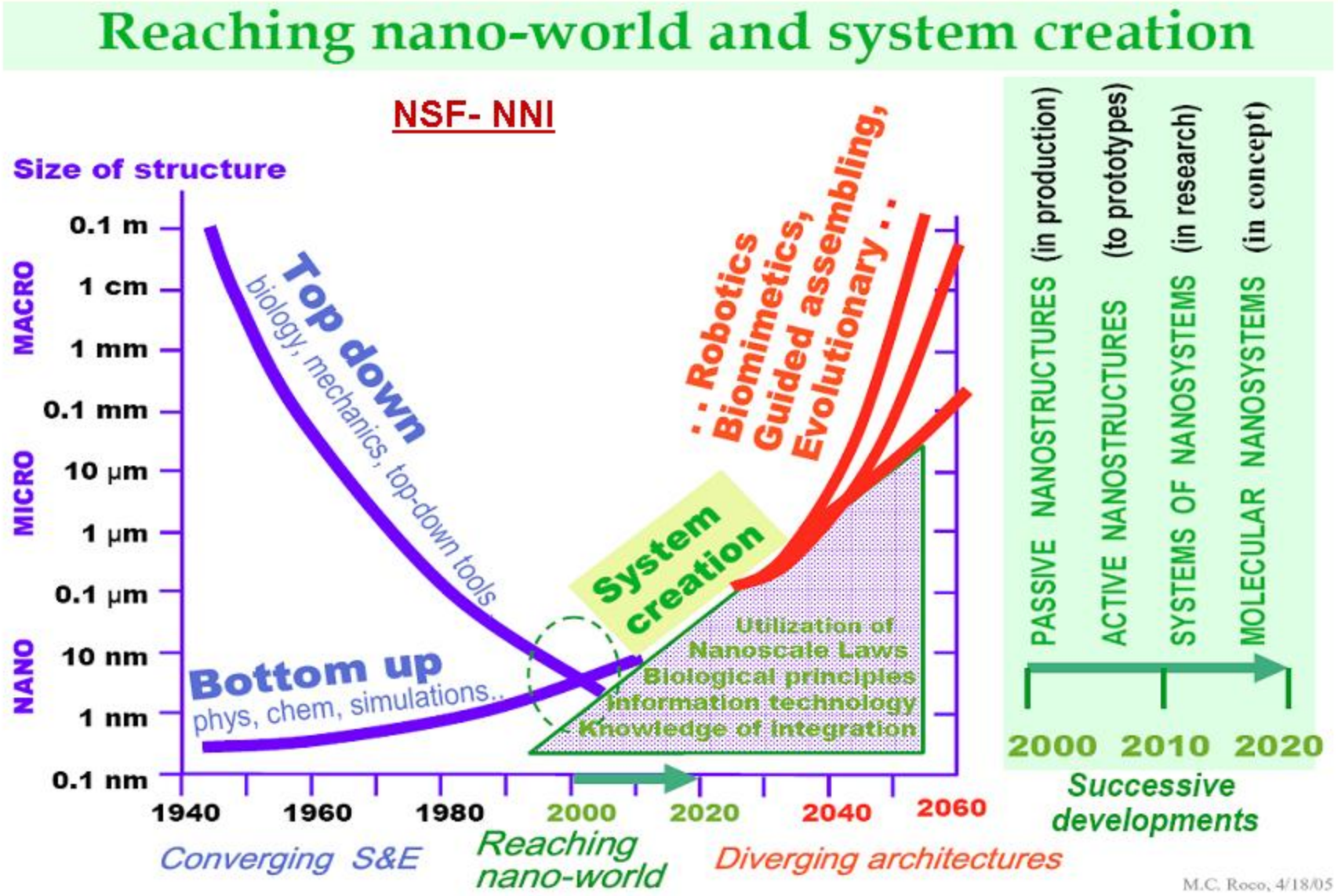
Estimated government sponsored R&D in $ millions/year

Fiscal Year	1997	2000	2001	2002	2003
W. Europe		200	270	400	
Japan	120	245	465	650	
USA	116	270	465	604	**710**
Others		70	110	380	520

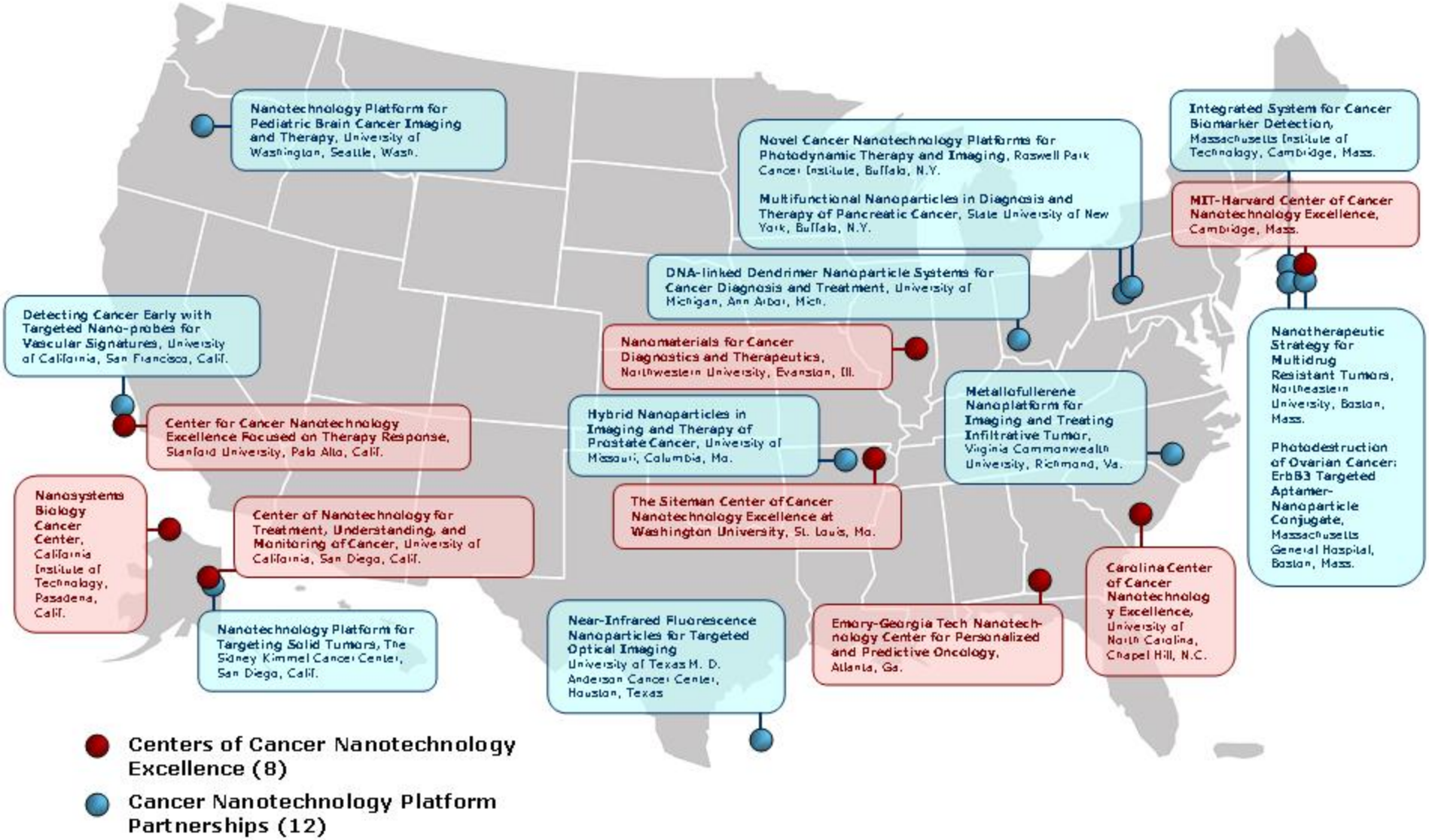
Projected $1 Trillion/year by year 2015!!
2 (7) million nanotech workers (0.8M in US)

MC Roco, NSF http://es.epa.gov/

Where are we now?



NCI Nanotechnology Alliance - Awards



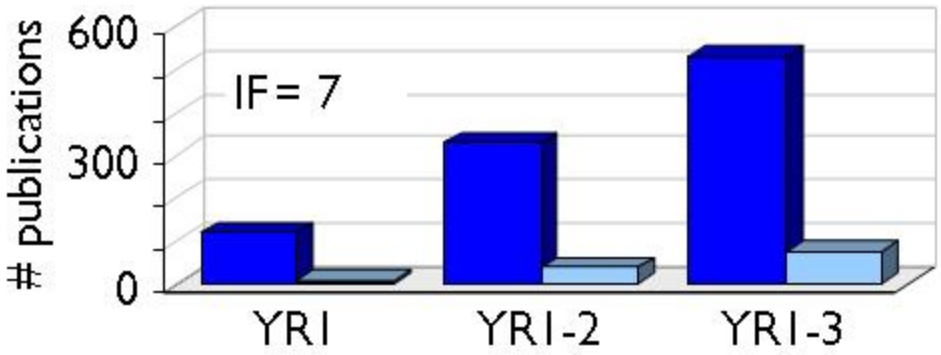
Major Accomplishments

CCNE Program Only (Since 2005)

NCI Alliance for Nanotechnology in Cancer

- **Scientific standing** – 606 peer-reviewed journal papers published with average impact factor ~7. Strong evidence of establishing joint projects: 43 publications involving multiple PIs.
- **Clinical translation** – 50 companies associated with the program in the space of diagnostics and therapy; 24 were formed in last 3 years. Developing strong intellectual property portfolio - 203 disclosures and patents filed.
  - 5 clinical trials associated with program projects
  - 2 companies completed pre-IND discussions with FDA
  - 17 IRB protocols for work with human samples in place.

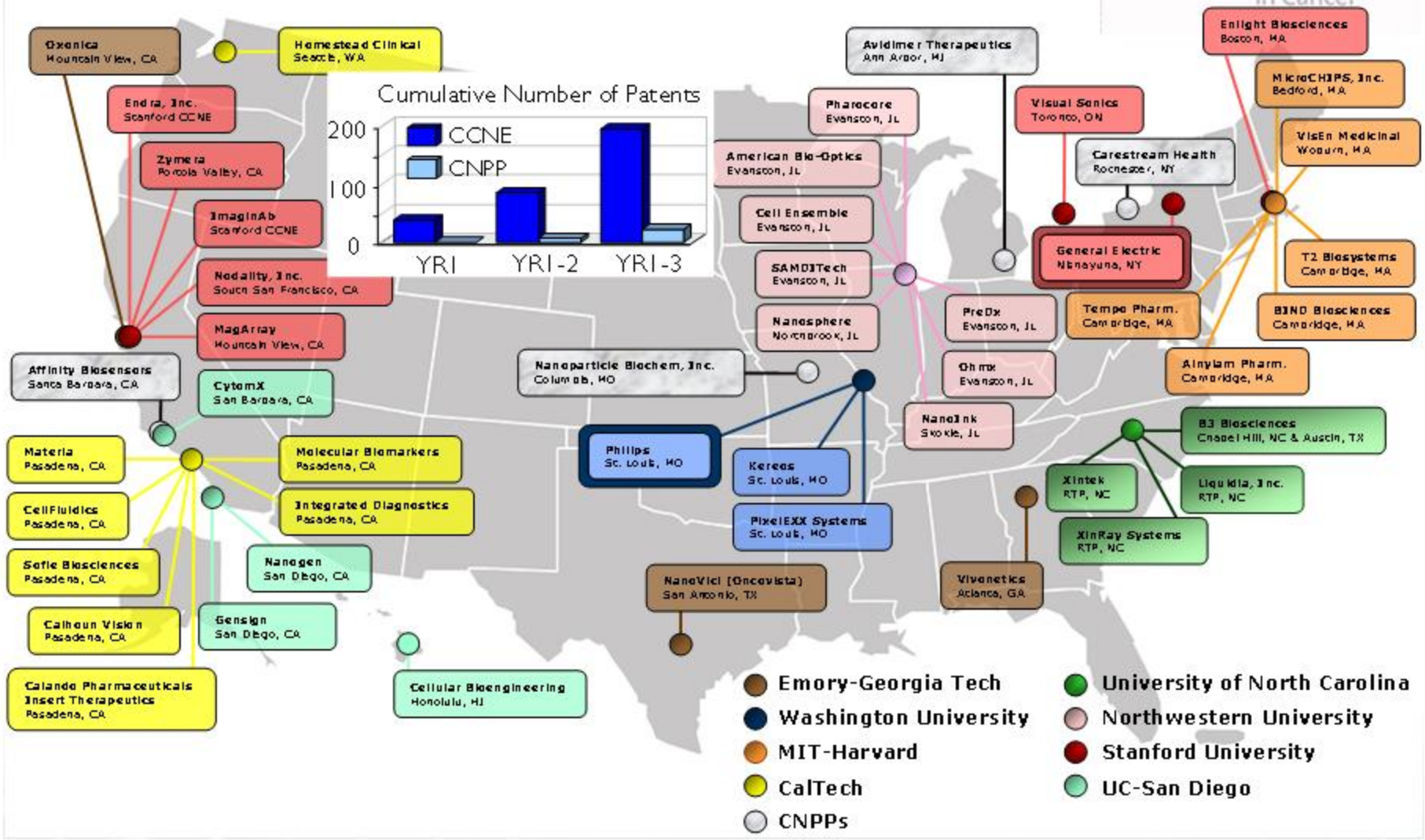
Cumulative Number of Publications



Partnerships with Industry – Technology Commercialization

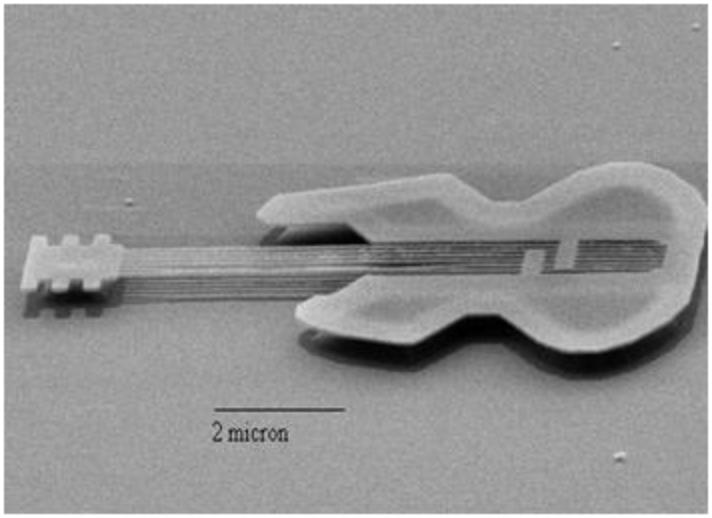
CCNE Program Only (Since 2005)

NCI Alliance for Nanotechnology in Cancer

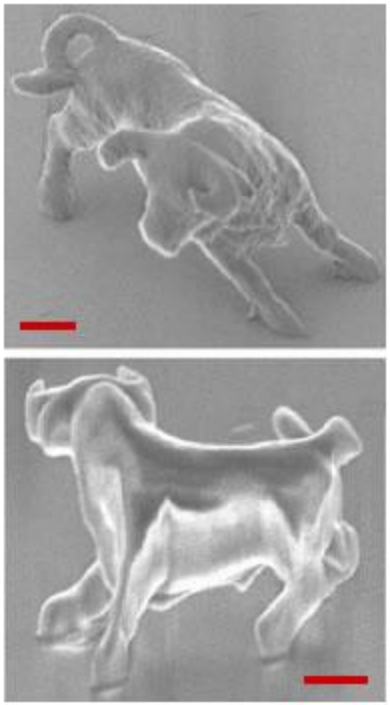


Where are we now?

Nano Masterpieces!

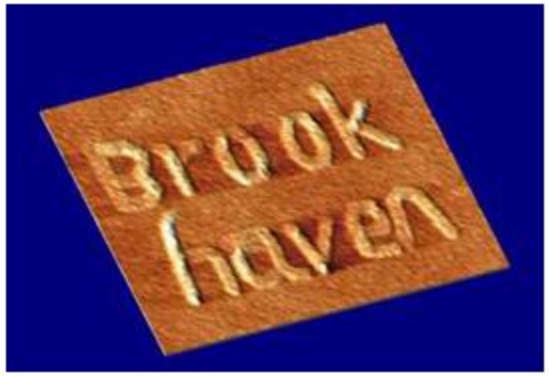


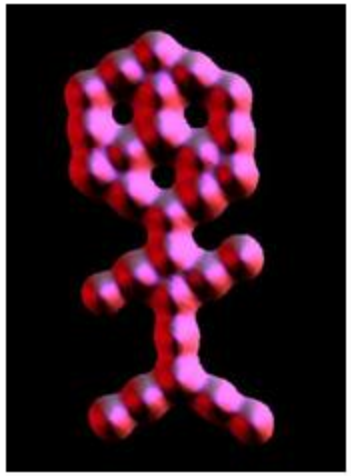
The world's smallest guitar is 10 micrometers long -- about the size of a single cell -- with six strings each about 50 nanometers, or 100 atoms, wide. *H. Craighead, Cornell, 1997*



Kawata, S., Sun, H.-B., Tanaka, T. & Takada, *Nature*, 2001.

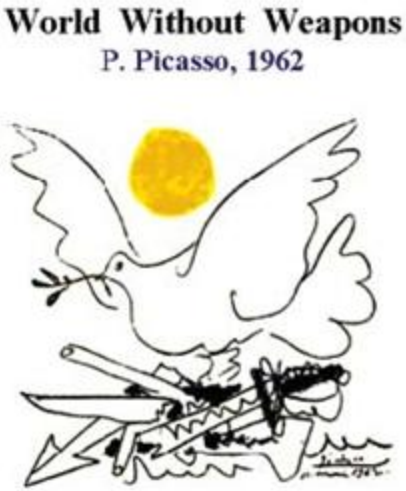
ElectroPen (150nm line width, 2005)

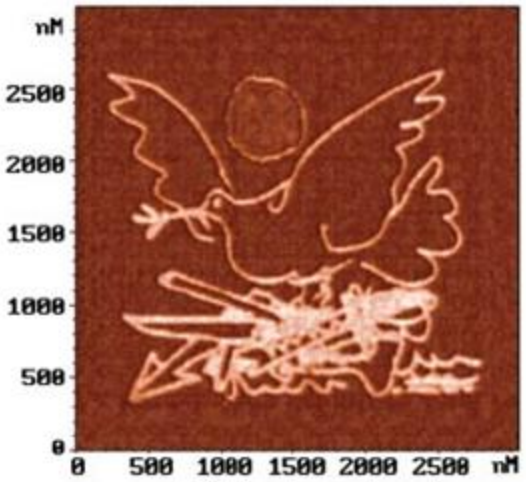




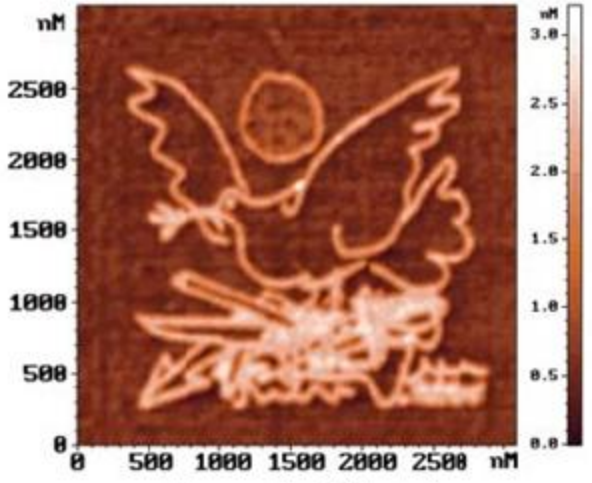
COman, 28 CO
IBM

Where are we now?

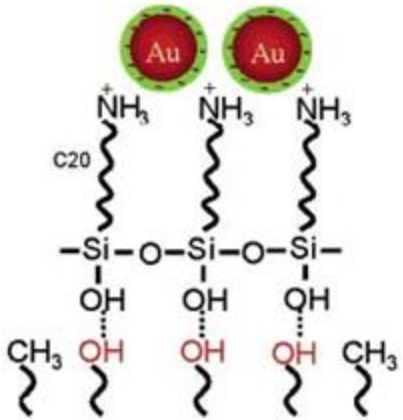


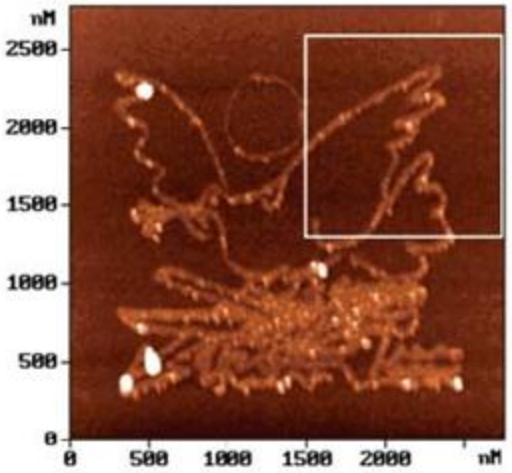


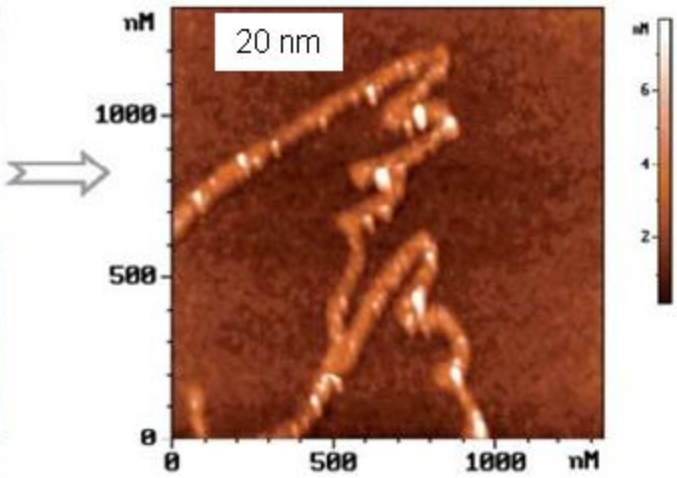
Friction (monolayer)



Topography (bilayer)



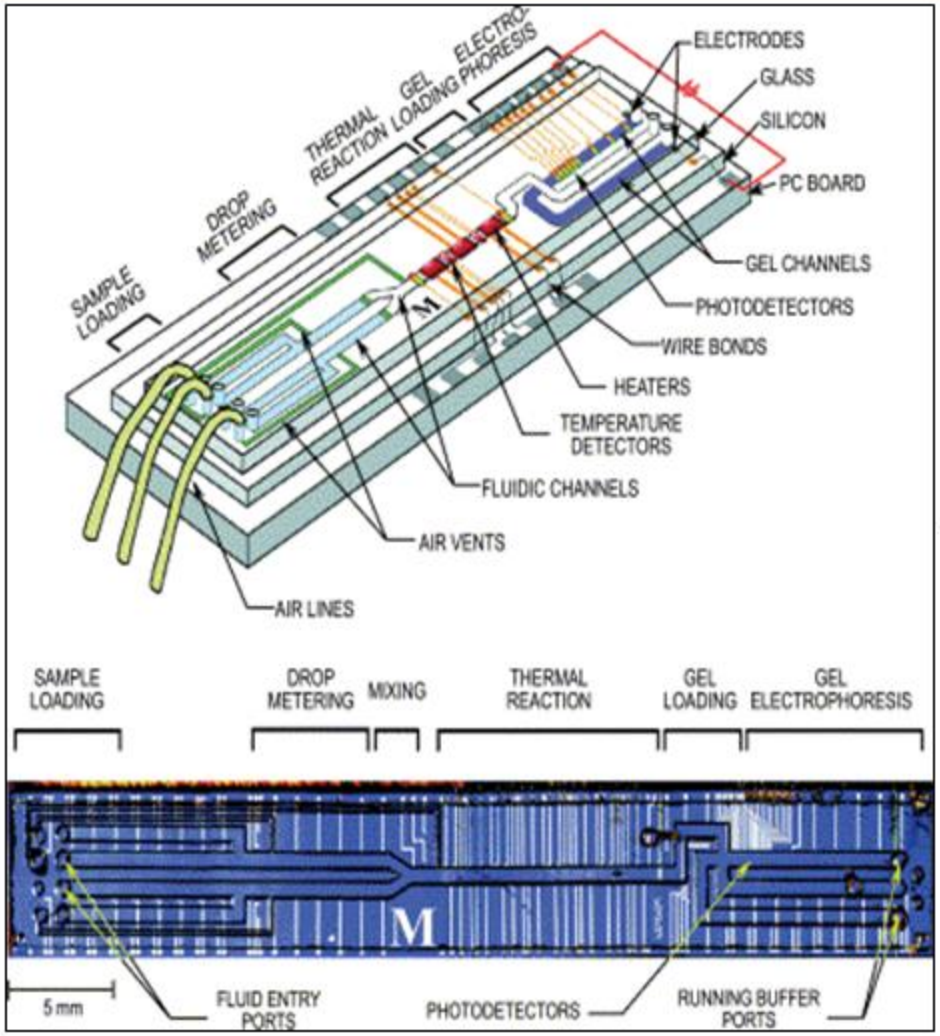




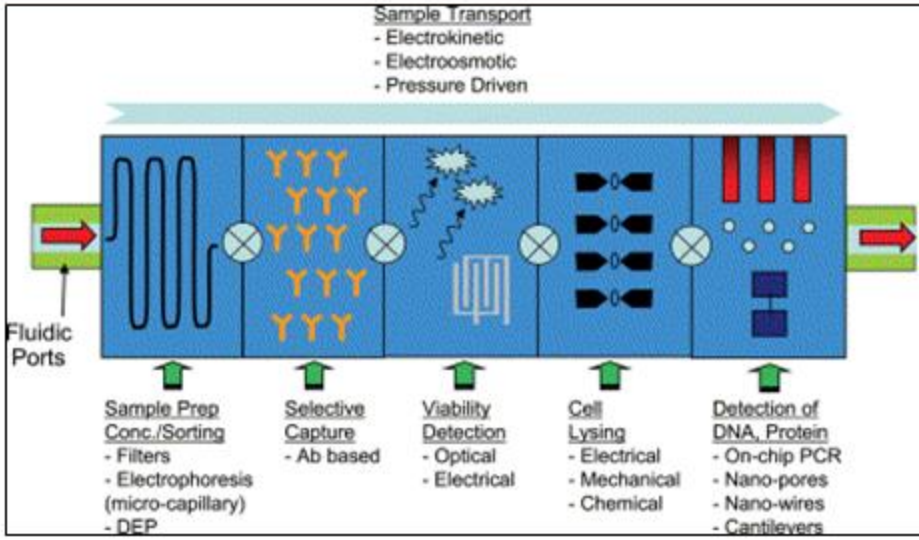
Topography (colloidal gold pattern)

http://www.nanopicoftheday.org/Images/NanoPicasso.jpg

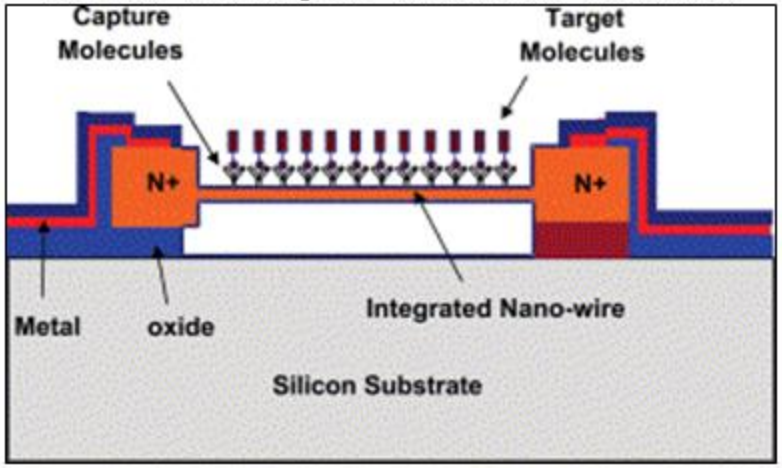
Where are we now? Microchips with nano-features



Madou et al, Science 282:1998484-487, 1998



Bashir, R. Adv. Drug Del. Rev. 56:1565-1586, 2004

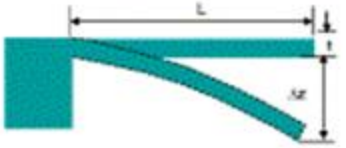


Elibol, O. et al., NanoLetters 2004

Nanomedicine: HOW? Key sensing methods

Mechanical Detection

Surface Stress Change Detection



$$\Delta z = 4\left(\frac{l}{t}\right)^2 \frac{(1-\nu)}{E}(\Delta\sigma_1 - \Delta\sigma_2)$$

- Δz = deflection of the free end of the cantilever
- L = cantilever length
- t = cantilever thickness
- E = Young's modulus
- ν = poisoni's ratio
- $\Delta\sigma_1$ change in surface stress on top surface
- $\Delta\sigma_2$ change in surface stress on bottom surface

Mass Change Detection

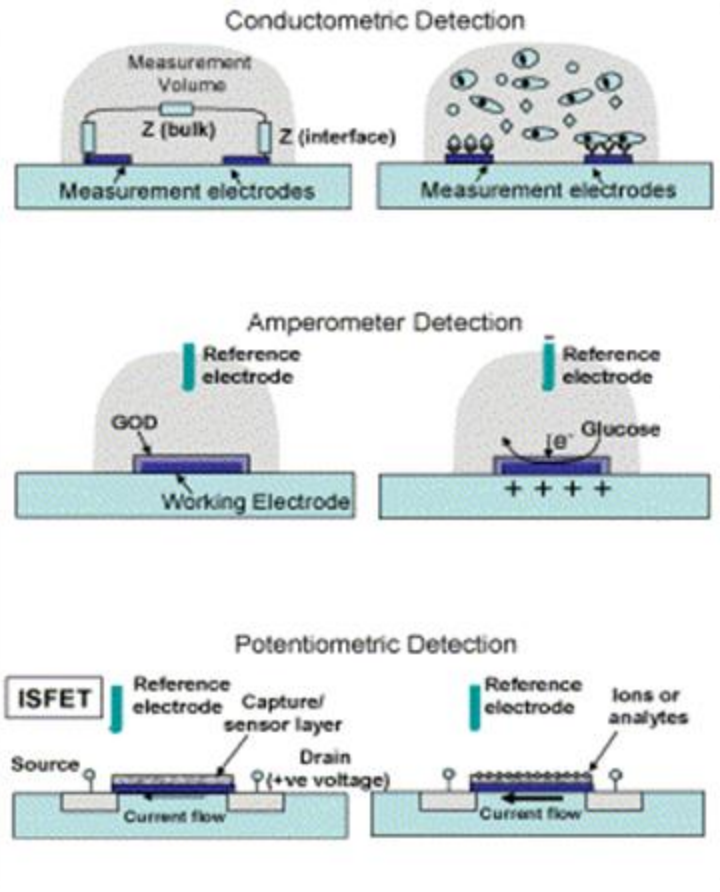
$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_o^2}\right)$$

- k = spring constant
- m = mass of cantilever
- f_o = unloaded resonant frequency
- f_1 = loaded resonant freguency

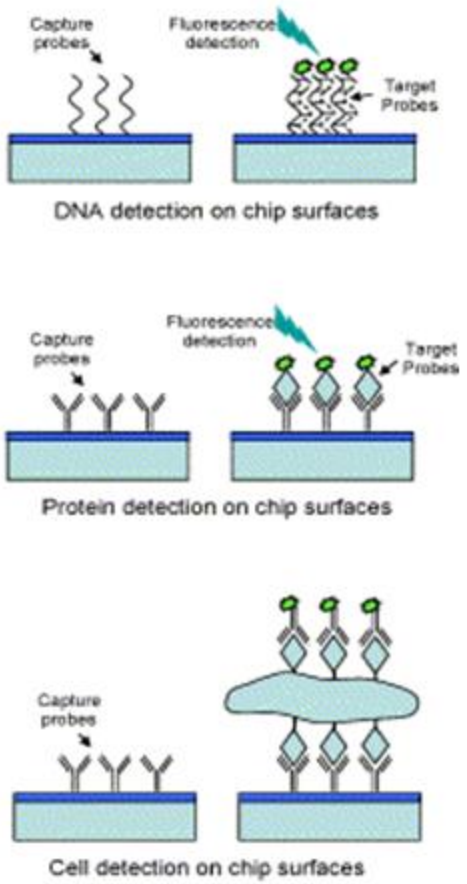
(a)

Electrical Detection



(b)

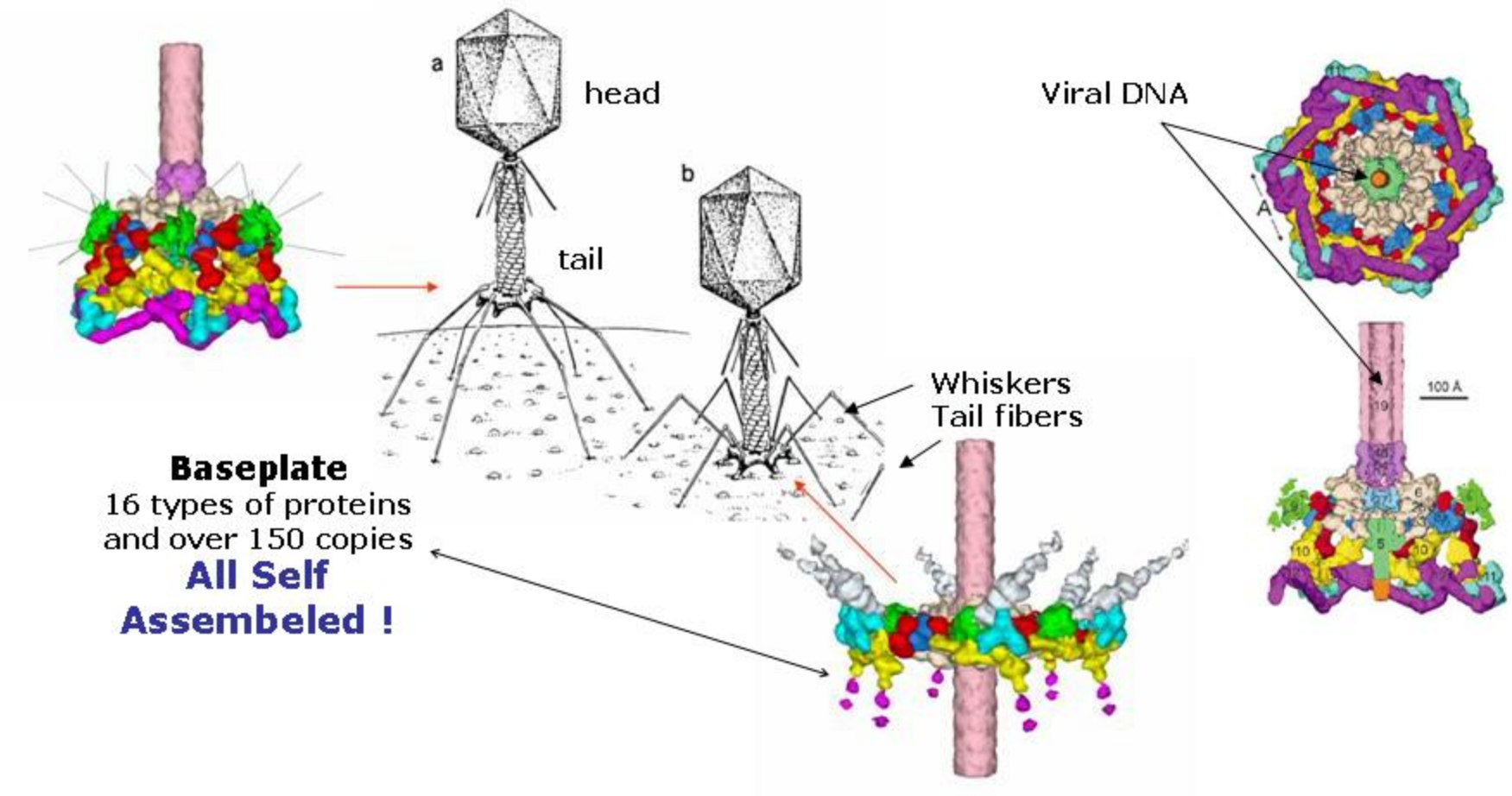
Optical Detection



(c)

Bashir, R. Adv Drug Deliv Rev. 56:1565-86, 2004.

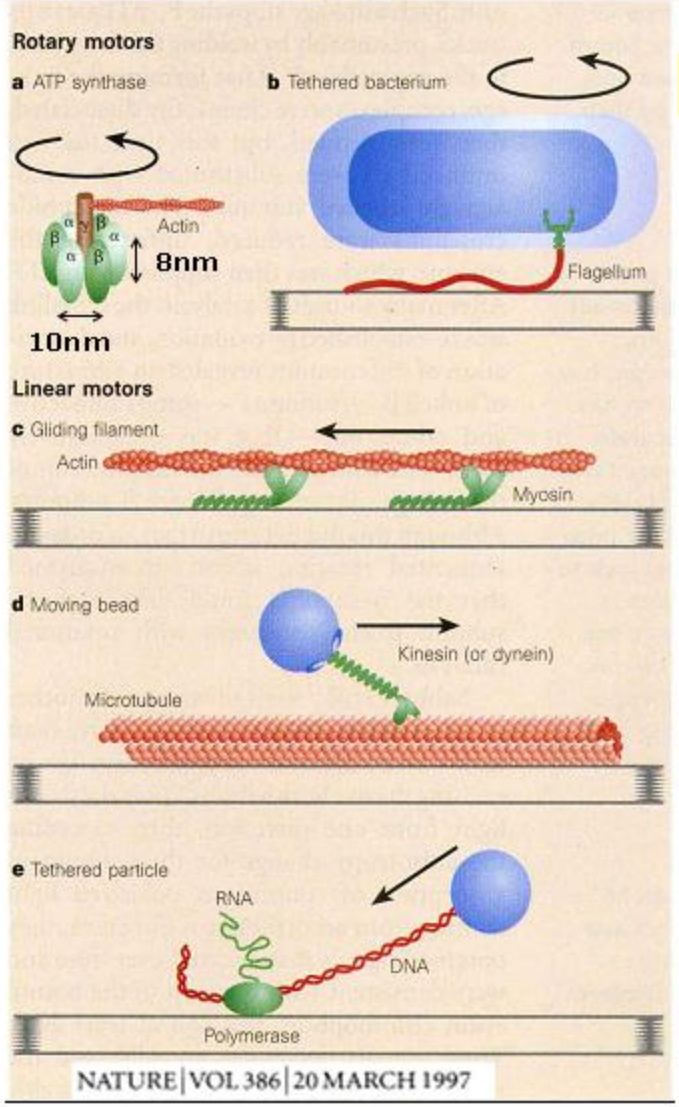
Nature as a model: Biomimetic Engineering



Bacteriophage T4

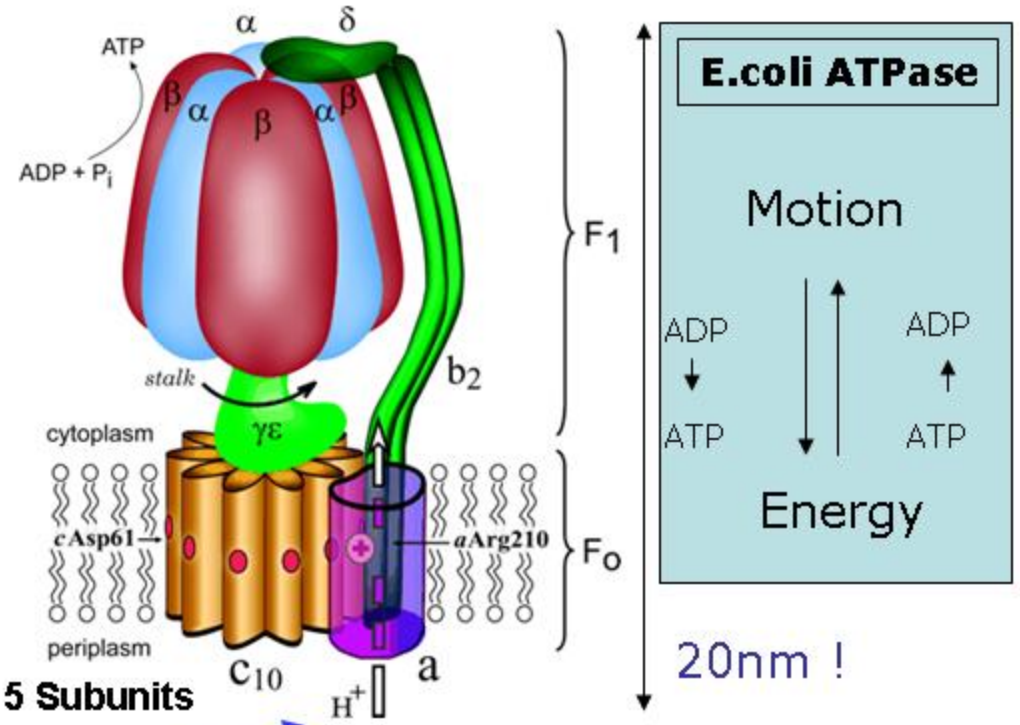
http://news.uns.purdue.edu/images/+2004/rossmann-baseplate2.jpg

Nature as a model: Biomimetic Engineering



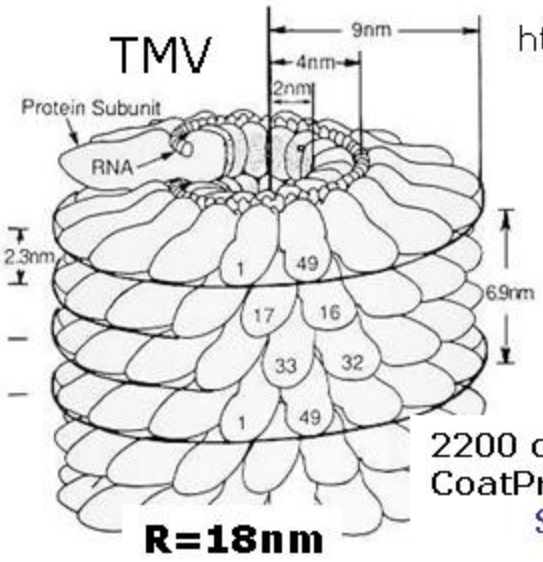
ATPase, ~20Hz, 280pN Torque, ~100%Efficiency!

Flagella, ~1000Hz, 100μms^{-1} bacterial speed

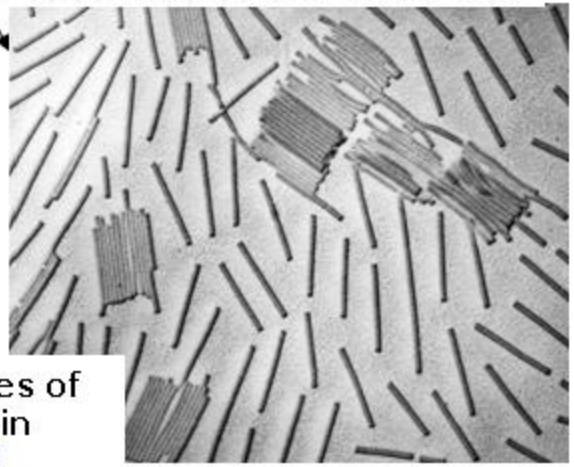


Biophysical Journal 86:1332-1344 (2004)
http://www.life.uiuc.edu/crofts/bioph354/lect10.html

Nature as a model: Biomimetic Engineering

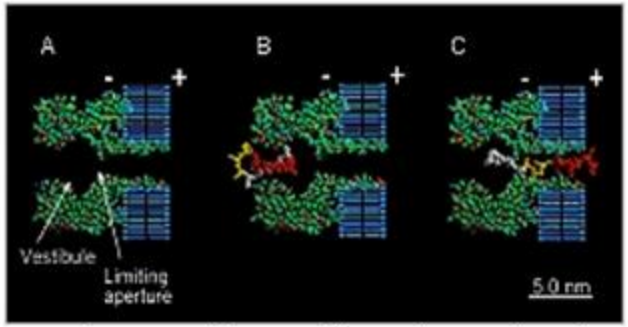


http://www.ncbi.nlm.nih.gov/ICTVdb/

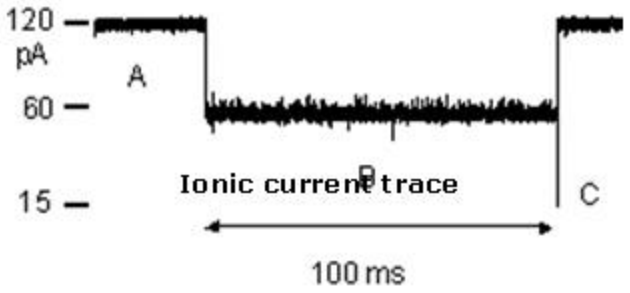


2200 copies of CoatProtein
SAM

Alpha hemolysin-homoheptameric

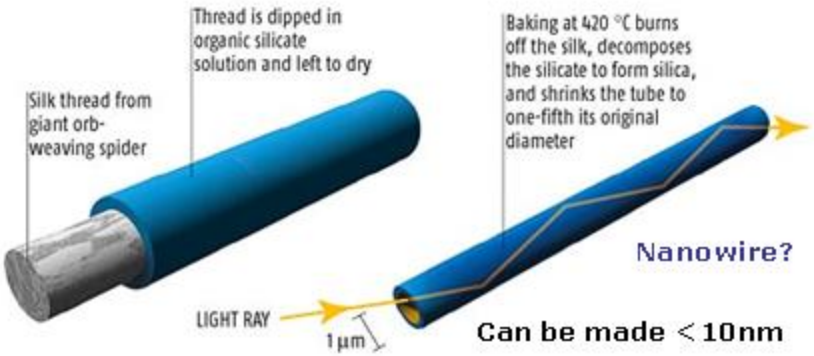


www.cbse.ucsc.edu/research/images/nanoanyimg2.jpg



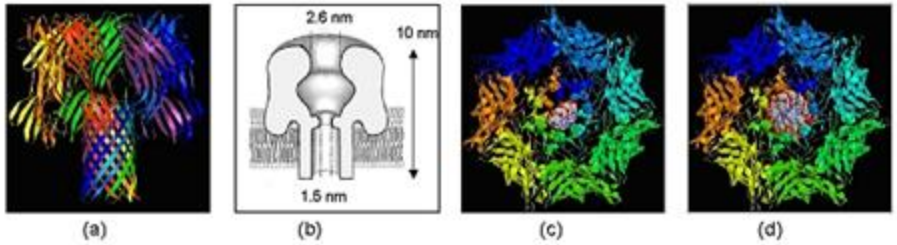
MAKING FINER FIBRES FROM SPIDER SILK

Hollow core allows total internal reflection of light, or allows fibre to be used as a silica nanotube



http://www.newscientist.com/data/images/ns/cms/dn3522

Deamer et al., Trends Biotechnol. 18, 147-51, 2000.



Research Areas

- **Single particle imaging:** AFM, FL-Nanoscopy, Confocal, BioLuminescence, NIRF
- **Nanomedical Robotics**
  - Integrated medical nanosystems that perform therapeutic interventions at the cellular level
  - Bioinspired Self Assembled Systems (bSAS)
- **Smart Nanodiagnostics and Smart Drugs**
  - Targeted CANCER drug delivery / Controlled Release
- **Biomimetic Engineering/BioMEMS/NEMS Devices**

  for Cell Analysis, Biosecurity
  - Protein, DNA and cell chips,
  - Polymer Devices, Rapid Prototyping
  - Molecular Diagnostics
  - Active BioNEMS—Cardiomyocyte:piezo devices
- **Systems Biology**
  - InSilico Cellular Communities (SilicoCytes)
  - Hybrid devices/Tissue Printing

Ideal TheraSensor

P53: *"Guardian of the Genome"*

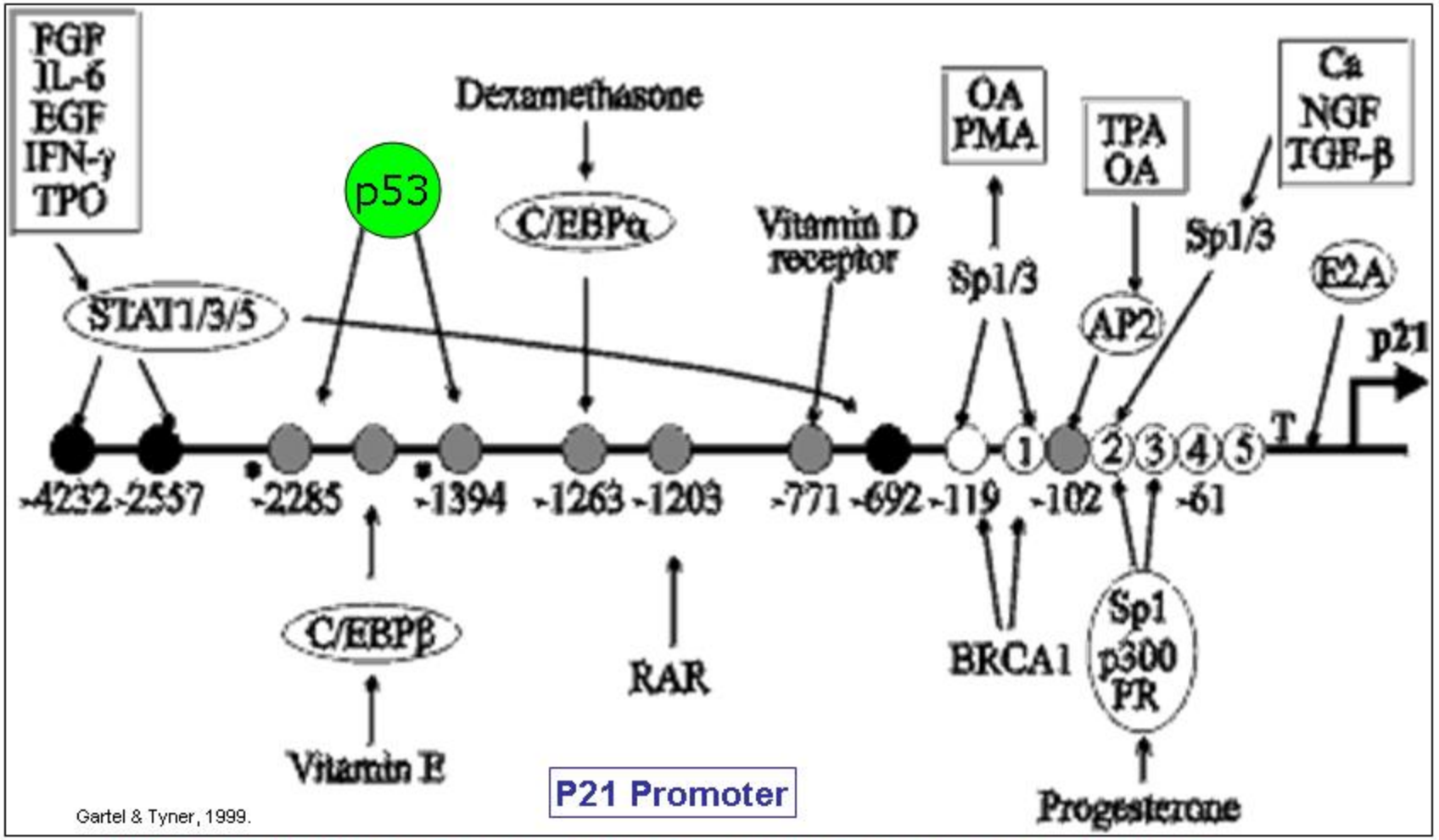
TrAD (1-42) indApop (80-94, JNK BD-97-116) DNABD (100-300) OligD/tet (307-355) dwnrDNABD (356-393)

- ~87% of all p53 muations are SNPs (Hainaut & Hollstein 2000).
- Of these, ~ 30% occur in six codons (175, 245, 248, 249, 273 and 282, Greenblatt *et al.* 1994)---the hotspot codons.
- These mutations are located in DBD, effecting protein-DNA contacts and the conformation of p53 protein (Guimaraes & Hainaut 2002)

Design and Engineer Drugs
- With Inherent Sensors
- As Prodrugs
- Multiparametric/Combined

Human p53 is 393 amino acids long and has ~five *domains*:
•An N-terminal transcription-activation domain (TAD), which activates transcription factors : residues 1-42
•A Proline rich domain important for the apoptotic activity of p53 : residues 80-94
•A central DNA-binding core domain (DBD): Residues 100-300.
•A homo-oligomerisation domain (OD) : residues 307-.355 Tetramerization is essential for the activity of p53 *in vivo*.
•A C-terminal involved in downregulation of DNA binding of the central domain : residues 356-393.

p21: cyclin-dependent kinase inhibitor 1A or CDKN1A



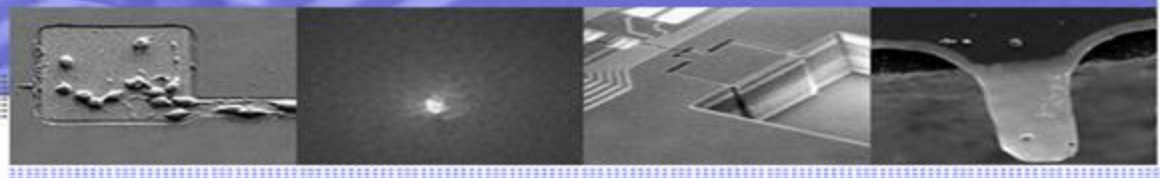
Biological Networks and Interactions are highly complex and interactive

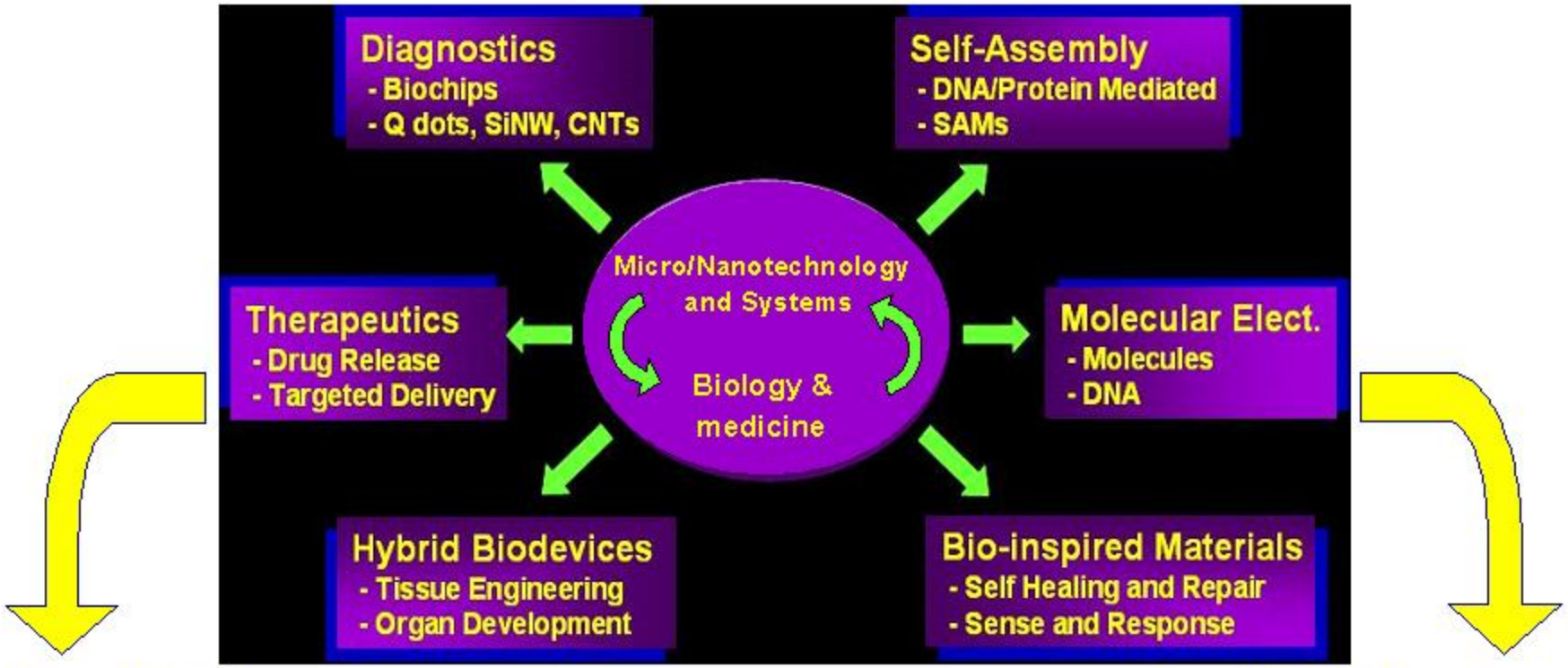
Collaborators/Acknowledgments

- **LIBNA:** A. Gupta, Dr. D. Morisette, A. Davila,
  O. Elibol, K. Park, P. Bajaj, A. Gafoor, P. Bajaj
  Drs. L. Yang, H. Li, J. Jang, S. Iqbal
- **Prof. R. Bashir**, UIUC, EE, BioE, MNTL
- **Prof. A.K. Bhunia**, K. Burkholder, Purdue FoodMicro
- **Prof. S. Mohammed**, C. Koon, Purdue Cancer Center
- **Prof. M. Ladisch**, Dr. T. Hwang, Purdue, BioE
- **Prof. J.P. Robinson**, J. Sturgis, K. Ragheb, Purdue BioE, BMS
- **Prof. S. Iqbal,** UT-Austin, EE
- **Prof. P. Guo**, U.Cinncinatti, BioE
- **Prof. C. Mao**, Purdue Chemistry
- **Prof. S. Clare**, IUPUI, SOM
- **Dr. D. Sherman, Purdue Life Sciences, EM**
- **Prof. L. Tsoukulas**+AISL, Dr. R. Gao
  - ECE, BME, Oncological Scincences Center
  - NIH-Bioeng., NSF, USDA, NASA

Director: Dr. Rashid Bashir

LIBNA is focused on research in BioMEMS & Bionanotechnology, in the areas of interface between micro, nanoengineering & life sciences



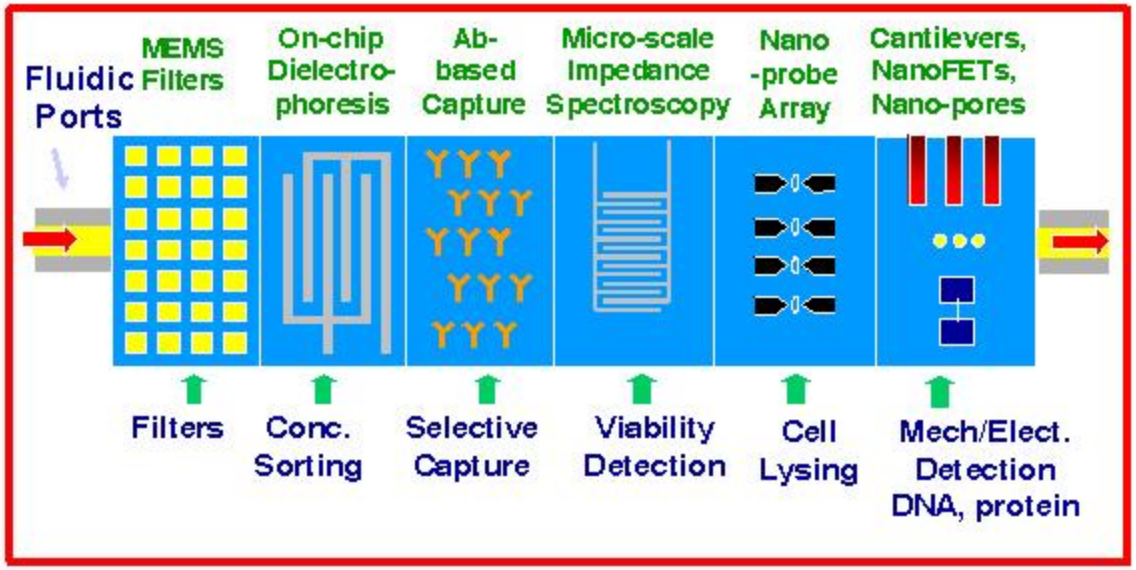


Novel Solutions for Frontiers in Medicine and Biology

Novel Solutions for Frontiers in Materials and Information Processing

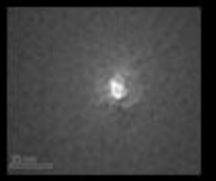
Integrated Chips for Study of Microorganisms and Cells

LIBNA



Lab-on-a-chip for Detection of Live Bacteria
Liu, Park, Li, Huang, Geng, Bhunia, Akin, Ladisch, Bashir

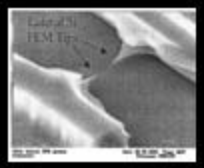
Nanopore Sensors for DNA Detection
Iqbal, Akin, Bashir



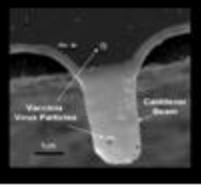
Dielectrophoresis Filters an Traps for Biological Entities
Li, Akin, Bhunia, Bashir

Silicon Nanowires as Biosensors
Elibol, Reddy, Nair, Akin, Bergstrom, Alam, Bashir

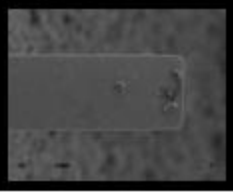
Trapping/Lysing of Bacteria/Viruses In Microfluidic Devices
Park, Akin, Bashir



Nano-Mechanical Cantilever Sensors for Detection of Viruses
Gupta, Akin, Broyles, Ladisch, Bashir



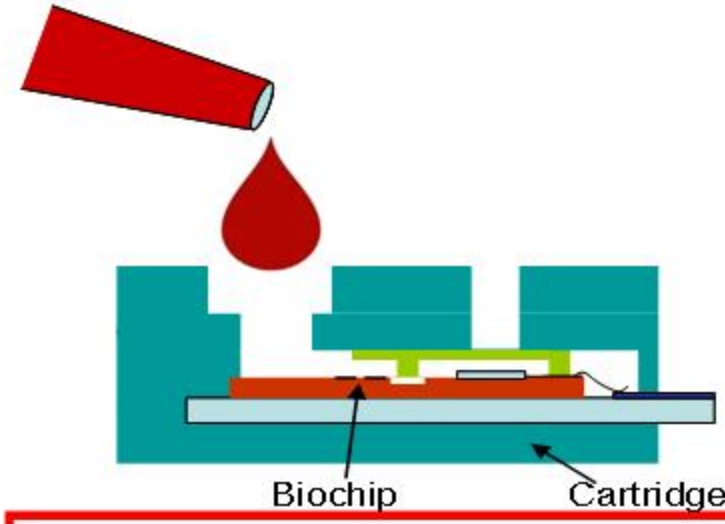
Micro-Mechanical Cantilevers for Detection of Spores
Davila, Walter, Akin, Aronson, Bashir

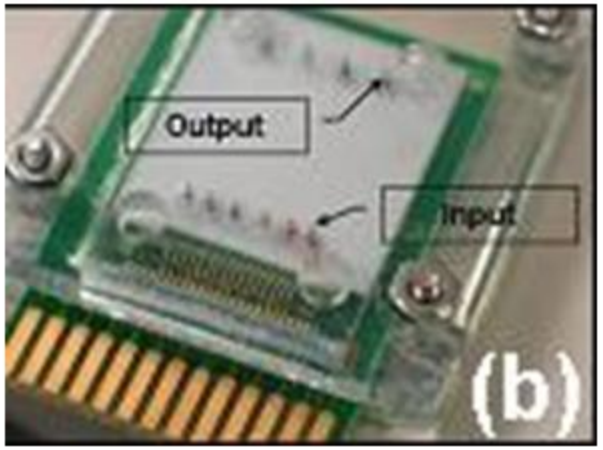


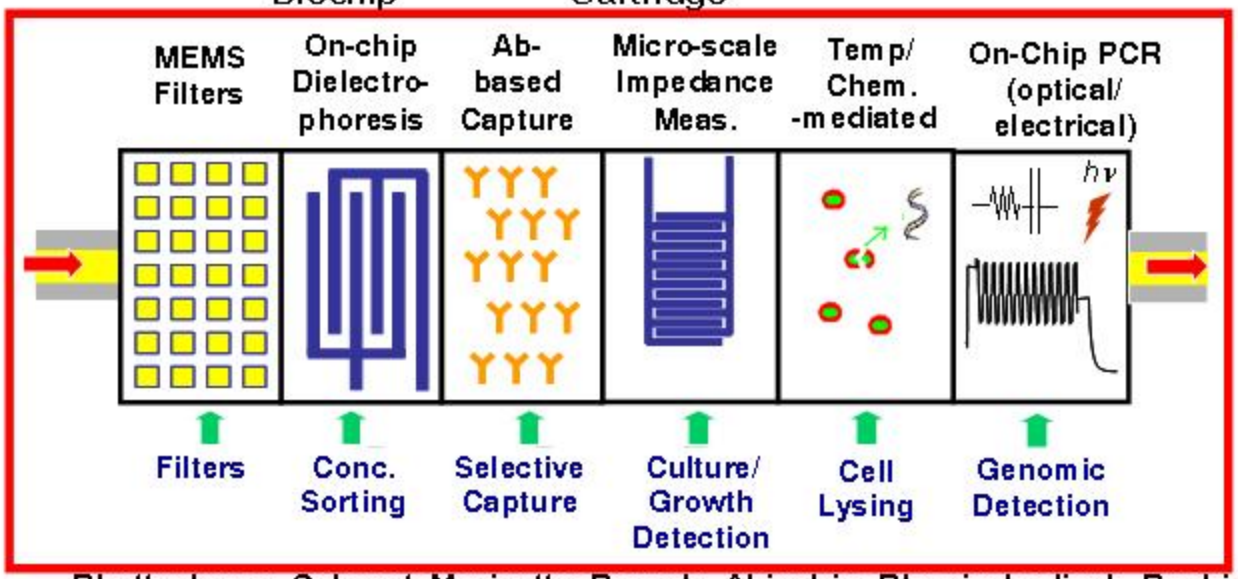
"Lab on a Chip" with microfluidics and micro/nanosensors

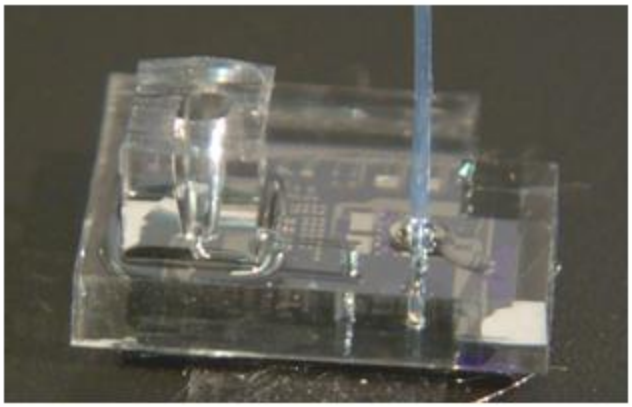
Lab on a Chip

LIBNA







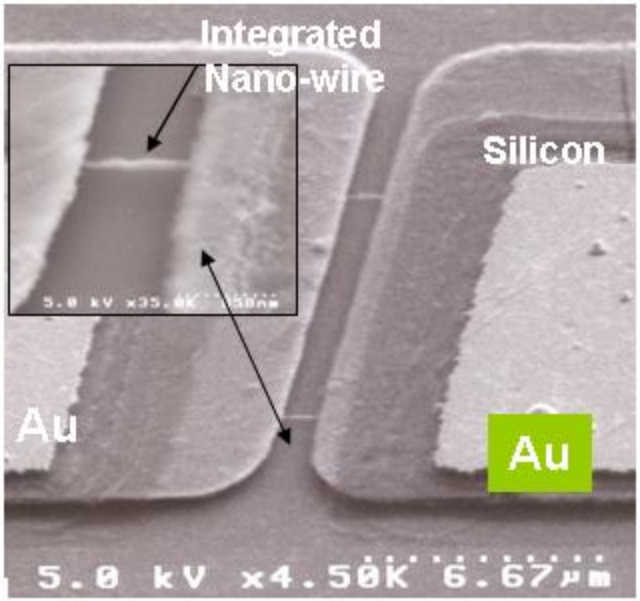


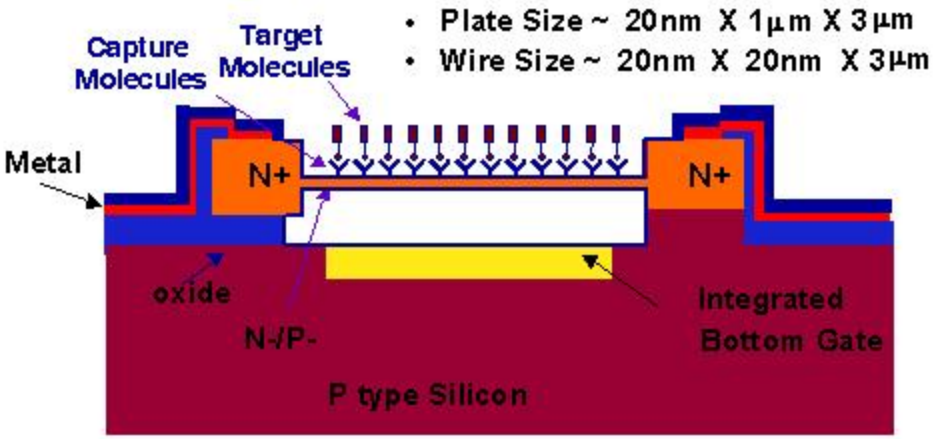
Bhattacharya, Salamat, Morisette, Banada, Akin, Liu, Bhunia, Ladisch, Bashir. *Lab Chip* 2008; 8: 7: 1130-6

Top-Down Silicon Field Effect Nanowire Arrays for Biomolecular Detection miRNA and Cancer BioMarkers

Objectives:

- Bio-sensors with electronic output
- Capability of dense arrays
- Direct Label Free Detection of Cancer relevant DNA and Proteins





Appl. Phys. Lett., Vol. 83, No. 22, 1 December 2003

Bashir et al

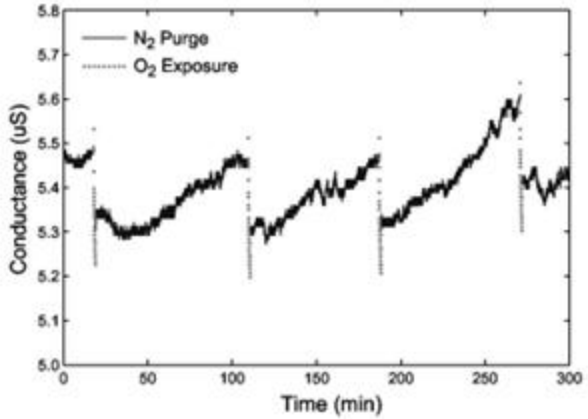
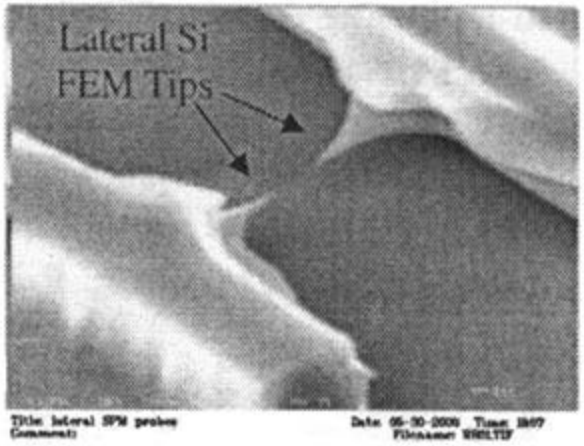
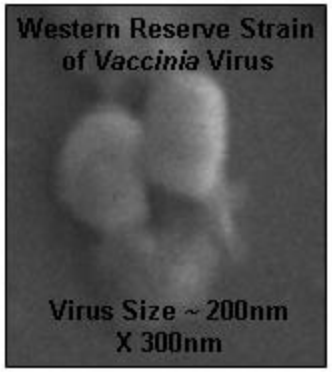


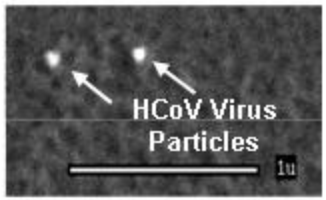
FIG. 3. Electrical response of the device upon exposure to oxygen (red dotted lines) and nitrogen (blue solid lines). Vertical axis shows the small signal conductance at 0 V dc bias and the horizontal axis the time in minutes. Experiment was performed at 82 °C of substrate temperature, and atmospheric pressure. Unlike nitrogen exposure, the conductance of the device decreases approximately 3% upon 1.5 min of exposure to oxygen. Note that the nitrogen purge steps recovers the oxygen sensitivity of the device.

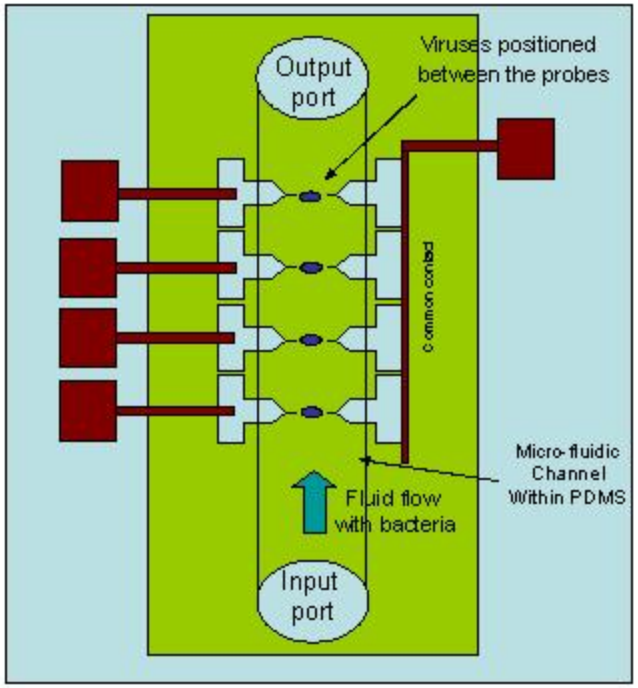
Genetic Manipulation/Lysis of Viruses, Bacteria, Cells in Nano-Probe Array

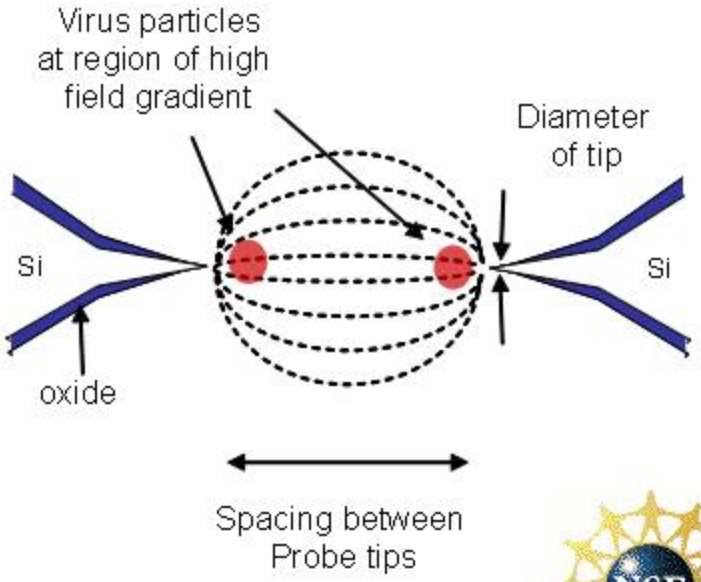
- **Handling and trapping of individual virus particles in nano-fluidic devices**
- **DEP mediated trapping and electroporation/lysis of viruses and bacteria**
- **Extraction and insertion of genetic material**









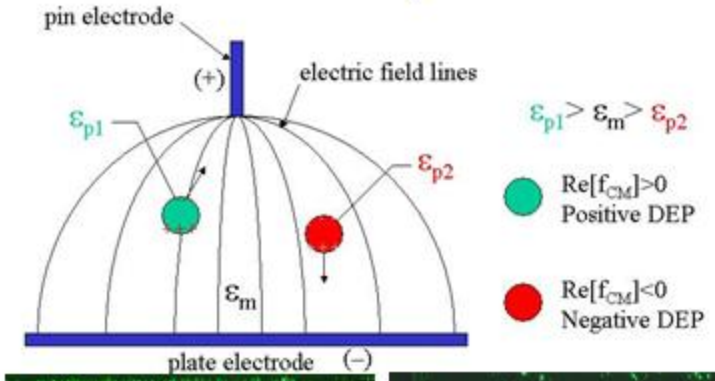


Park, Akin, Bashir. Biomed. Microdevices, 2007.

Nanoseparation/purification

Research Examples

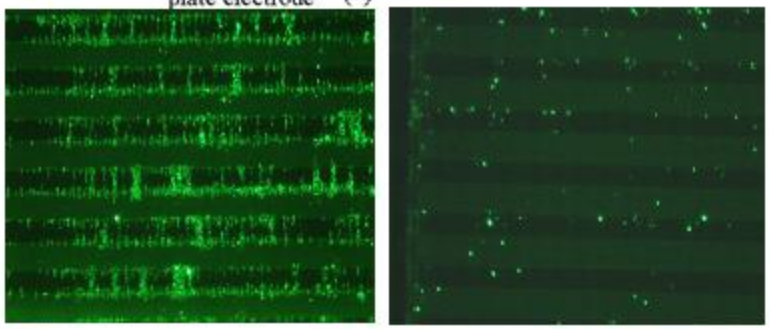
Dielectrophoresis

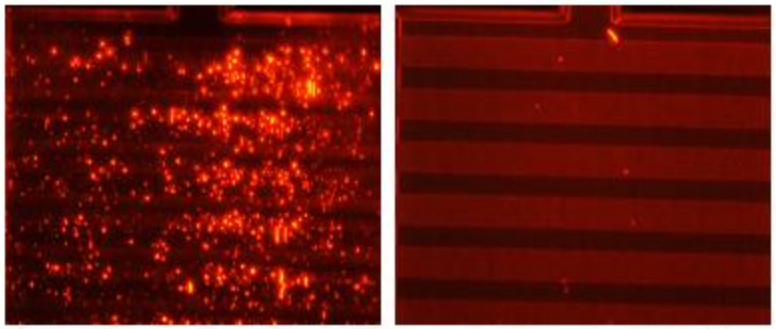


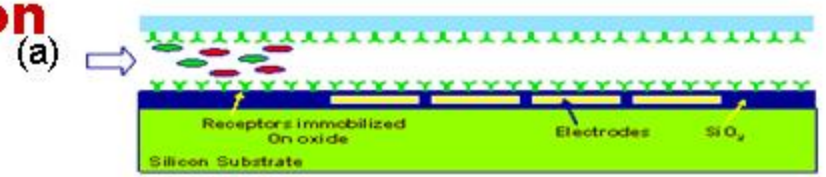
$\varepsilon_{p1} > \varepsilon_m > \varepsilon_{p2}$

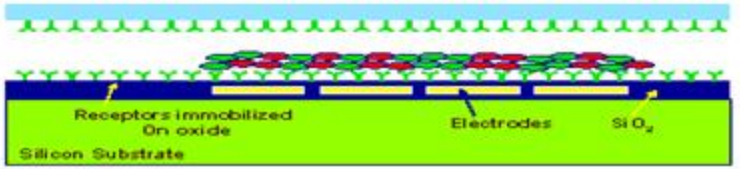
$Re[f_{CM}]>0$ Positive DEP

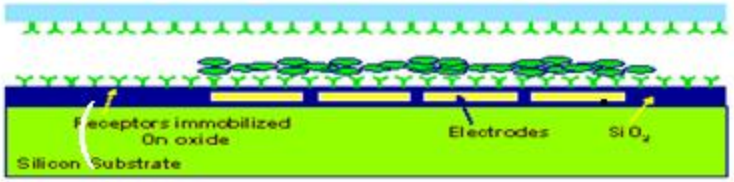
$Re[f_{CM}]<0$ Negative DEP



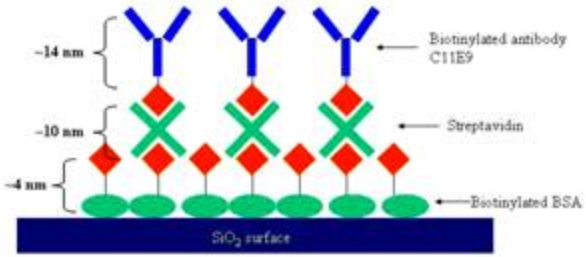




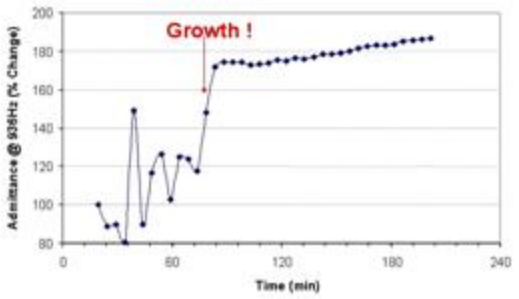




Lmono

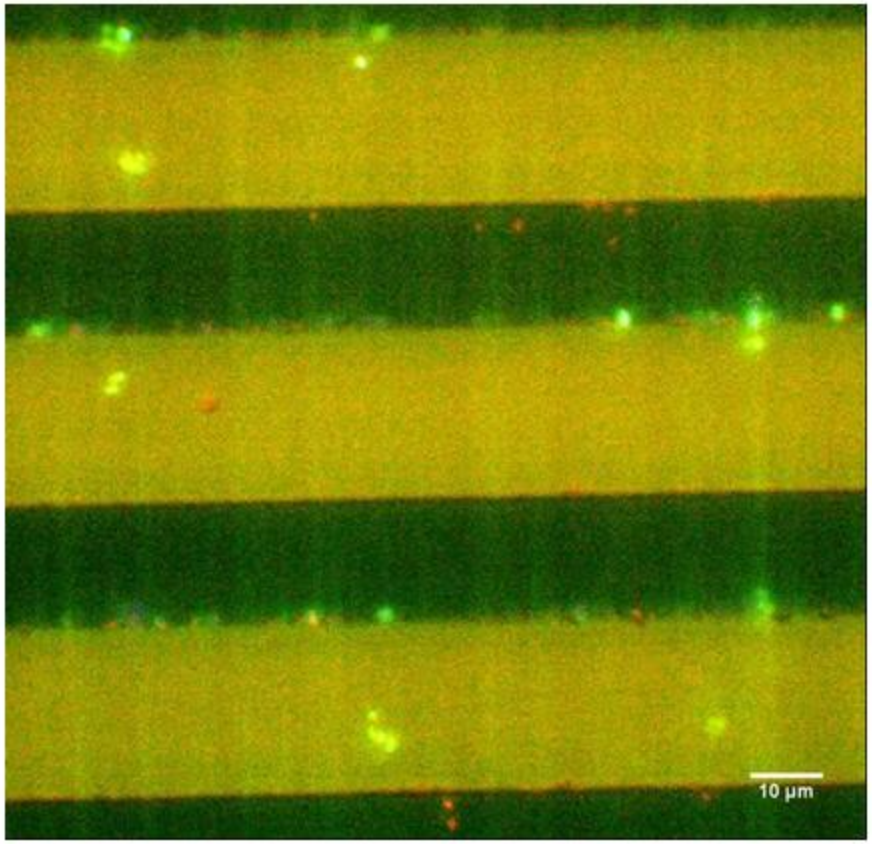


Ecoli



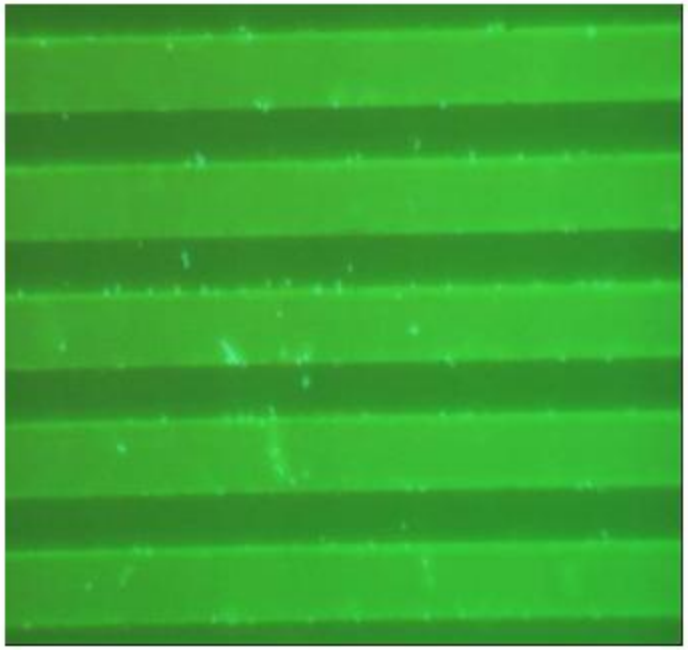
H. Li, L. Yang, D. Akin, T. Geng, A. Bhunia, T. T. Huang, M. Ladisch, R. Bashir, The 13th Solid-State Sens. Act. Microsys. Seoul, Korea, June 5th-9th, 2005.

Selective Capture and Separation by Dielectrophoresis (DEP)

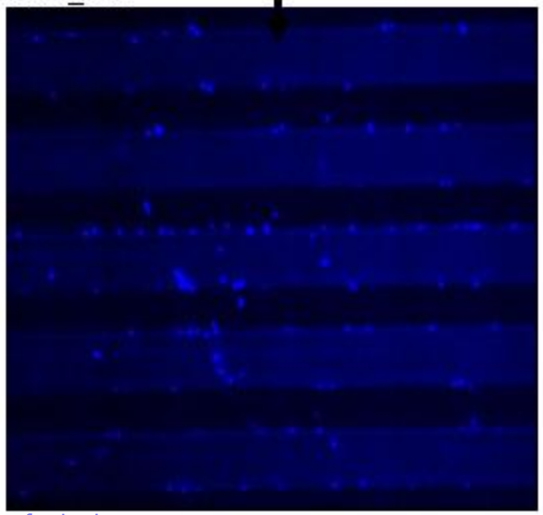


Adeno (red+blue) and VV(green+blue) collects but not Lm (green)

Also:
- **Dead vs Live Cells**
- **And Normal vs Cancer cells**



200x_2mhz_2vpp_triplefilter_triplemix_01ulmin_V
V stops_but not _Lm

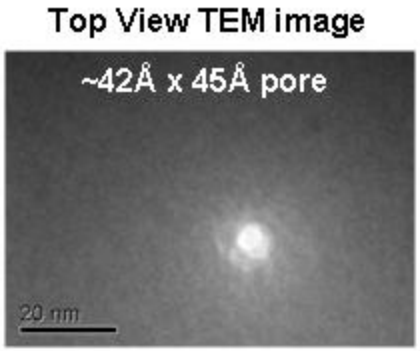


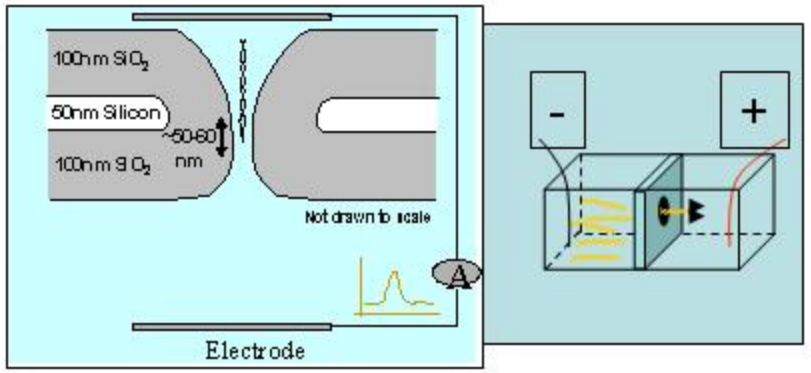
200x_2mhz_2vpp_1ulmin_V
Vstops_but not _Lm (Blue channel for the viruses)

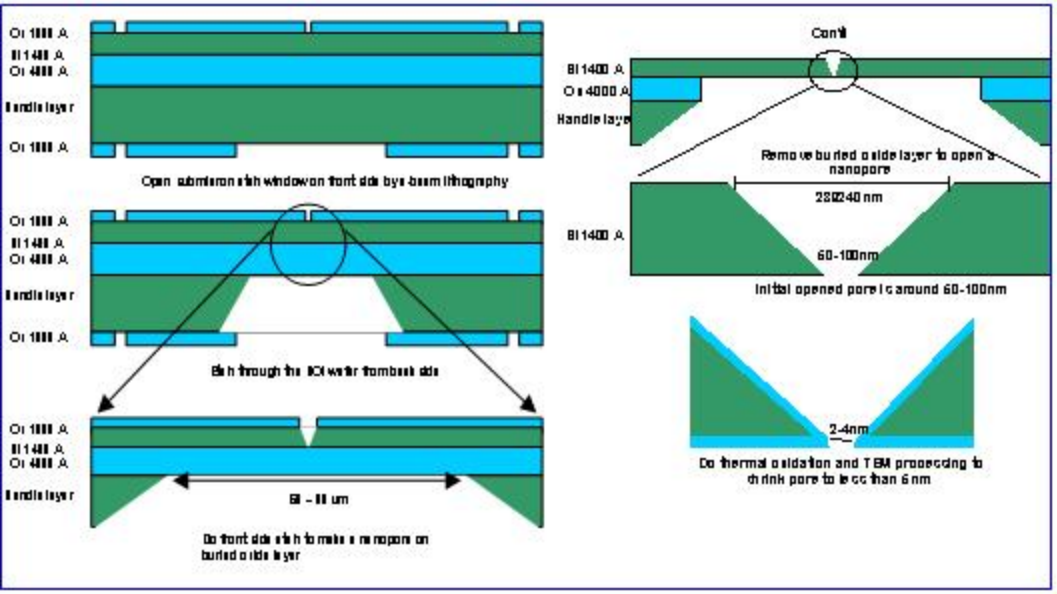
Nanopore Sensors for Label Free Detection of Single Molecule DNA/mRNA and Sequencing

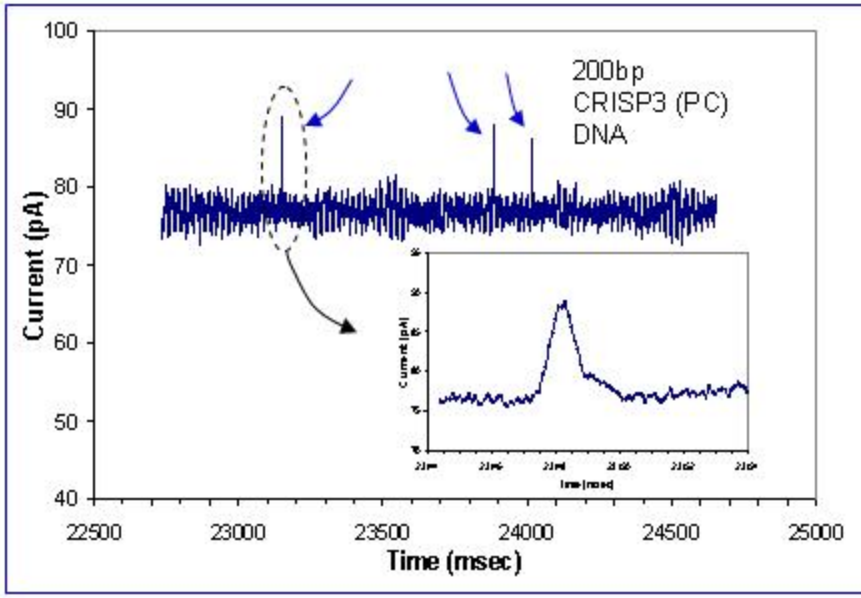
Iqbal, Akin, Bashir

- Frontiers in biology → Single molecule detection (and sequencing)
- Nanotechnology-based (top-down/bottoms up) are needed for making these approaches usable, and robust and form arrays of addressable pores.









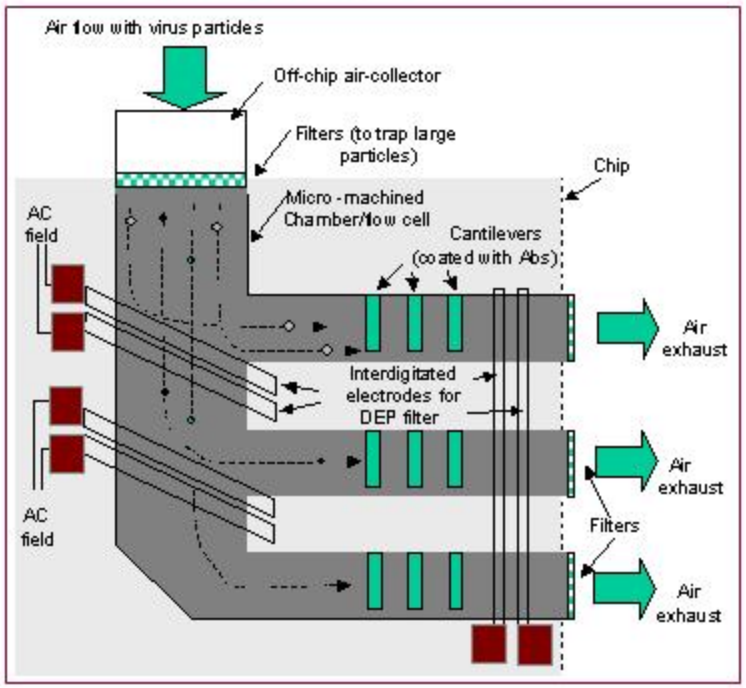
Iqbal, S., D. Akin and R. Bashir. Nature Nanotechnology, 2:243-248, 2007.

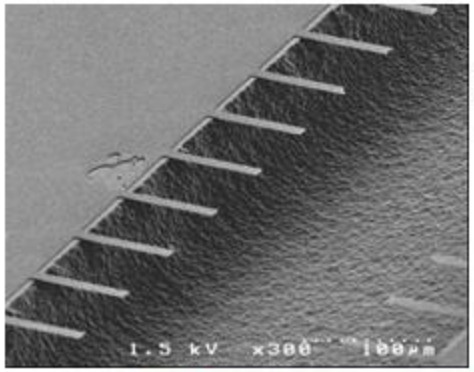
Nano-Mechanical Cantilever Sensors

Bashir, Akin, Ladisch, Broyles

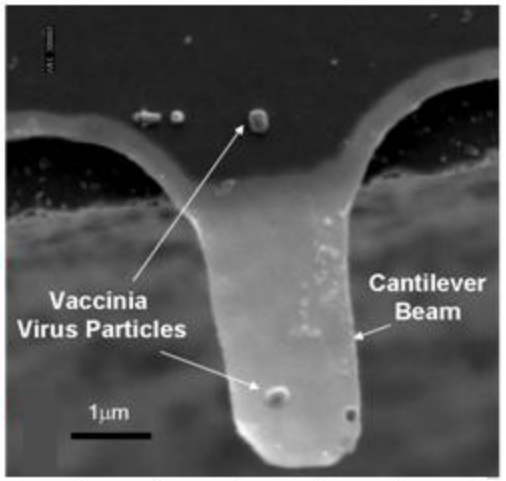
Objectives: To develop technology for the rapid detection of airborne pathogens

Concept Device Schematic



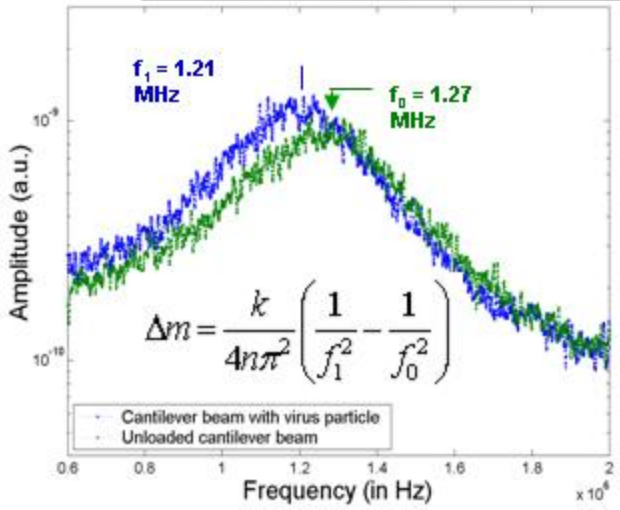


A cantilever (30nm x 1.5um x 4um) with a single vaccinia virus particle



Frequency Shift, Δf = 60 kHz
⇑ Mass change, Δm = 9 fg
⇑ This corresponds 1 vaccinia virus.

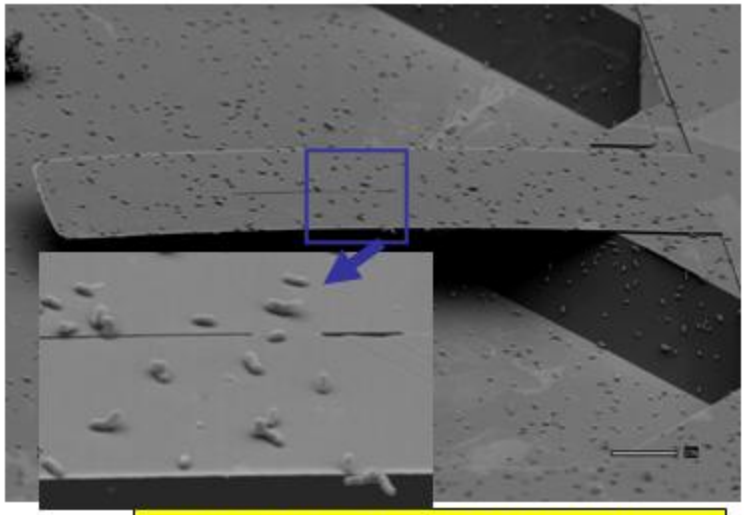
iso.avi
zoom.avi



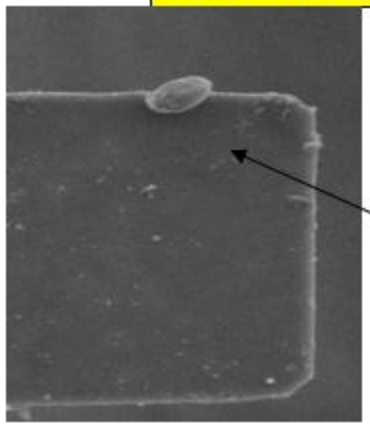
- A. Gupta, D. Akin, R. Bashir, APL, Vol. 84, No. 11, pp. 1976-1978, 15th March, 2004.
- L. Johnson, A. Gupta, D. Akin, A. Ghafoor, R. Bashir, Sensors and Actuators B, 2005
- Gupta, A., P.R. Nair, D. Akin, M, Ladisch, S. Broyles, M. A. Alam and R. Bashir. PNAS, 103:13362-13367, 2006

Nanocantilevers--- Nano "Scale": How sensitive are they?

A.Gupta, D. Akin, R. Bashir. J. Vacuum Sci. & Tech. B 22:2785-2791, 2004



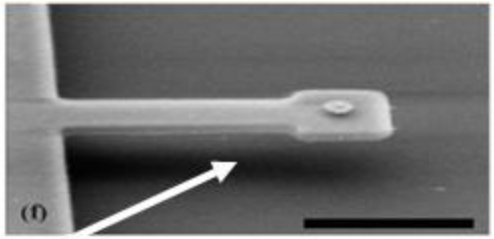
Listeria innocua ~85-132 fg/cell



B. anthracis spore
~78fg

A.Davila, T. Walter, A. Gupta, D. Akin,
A. Aronson, R. Bashir. Bacterial Spores Symp., 2004

Thiol Molecules on Au contact pads
6.3ag



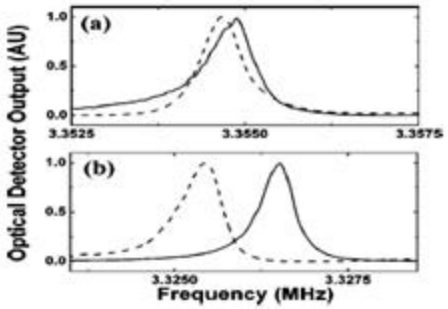
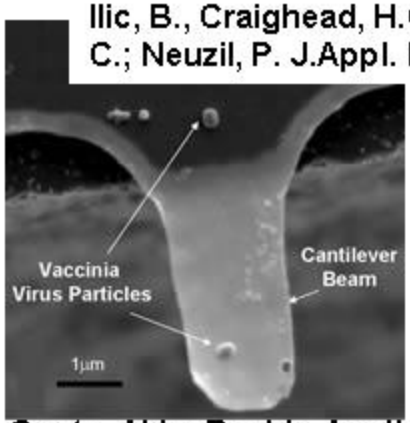


FIG. 14. Experimentally measured frequency spectra before (solid line) and after (dashed line) the adsorption of the thiolate on (a) 50- and (b) 400-nm-diam Au contact. Rectangular beam dimensions were $l = 10\ \mu m$, $w = 1\ \mu m$, and $t = 250$ nm.

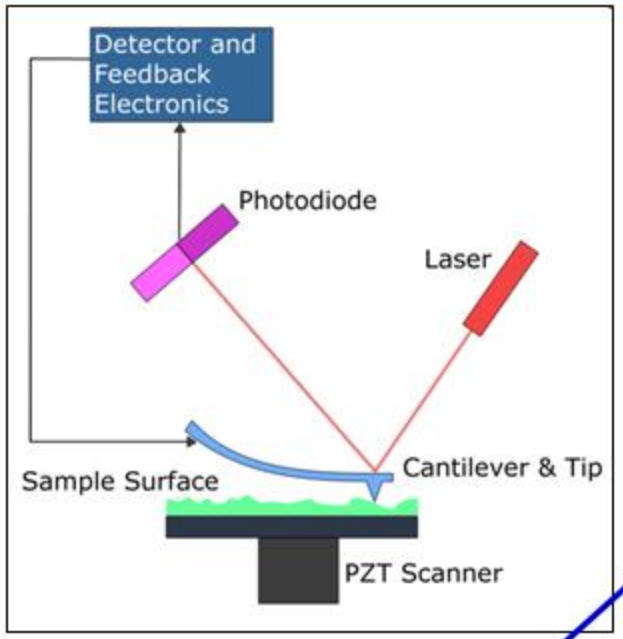
Ilic, B., Craighead, H.G.; Krylov, S.; Senaratne, W.; Ober, C.; Neuzil, P. J.Appl. Phys.,,95,2004,3694-703

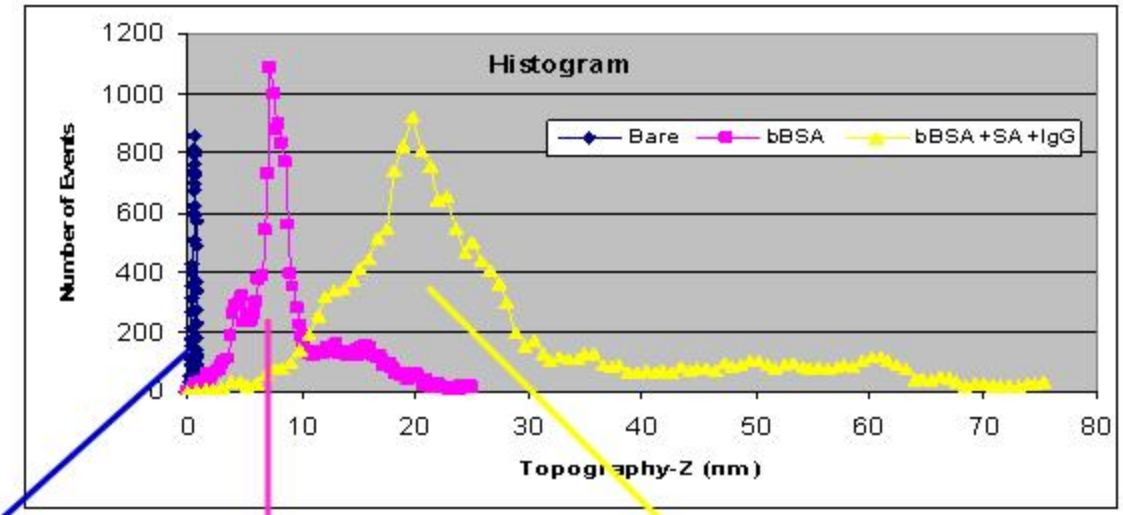


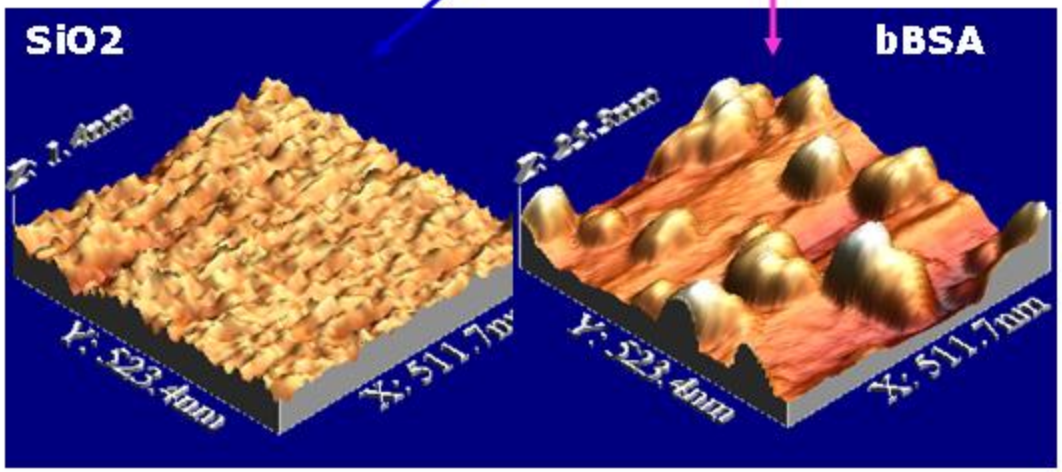
Vaccinia virus
~10fg/virion

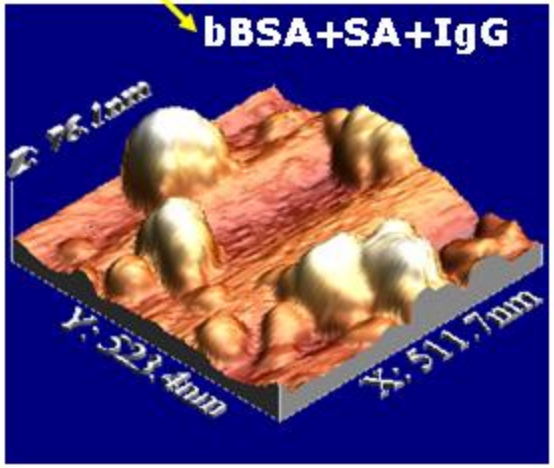
Gupta, Akin, Bashir. Applied Physics Letters, 2004.
Gupta, Akin, Bashir et al., PNAS 2006.

Single Particle Imaging, Biophysical/Mechano-elastic Analysis of Biomolecules by AFM







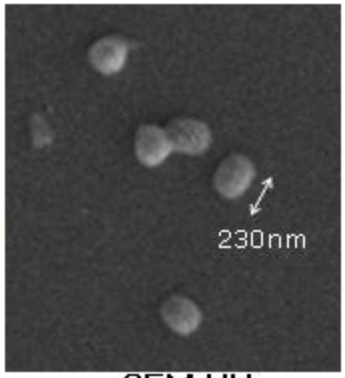


A. Gupta, D. Akin, R. Bashir et al., PNAS, 2006.

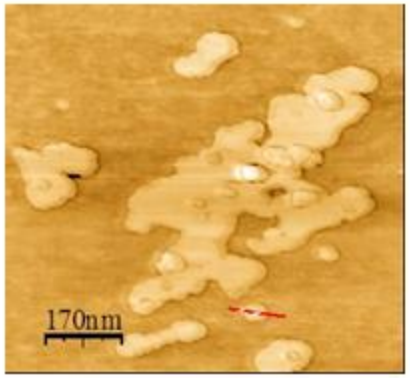
Single Particle Imaging and Biophysical Analysis: Enzymatic digestion of vaccinia virus virion and the release of condensed viral DNA molecules.

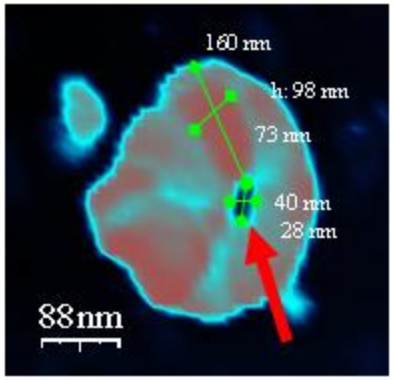
Research Examples

Intact VV--AFM

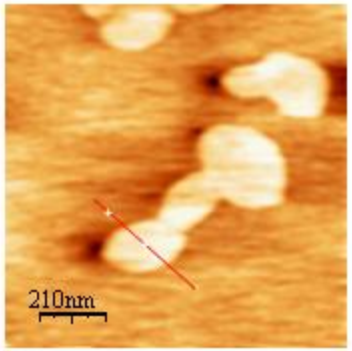


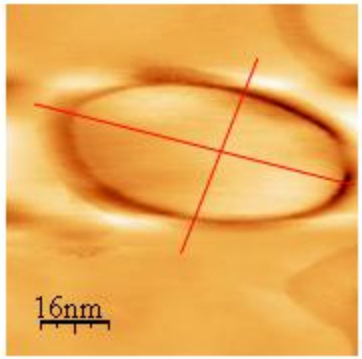
SEM VV



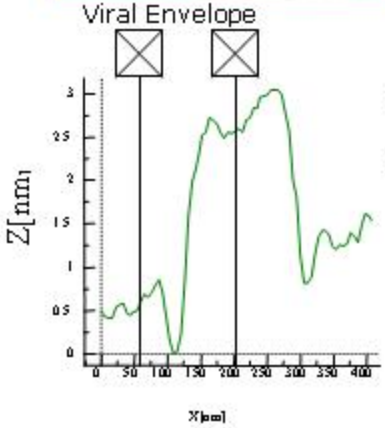


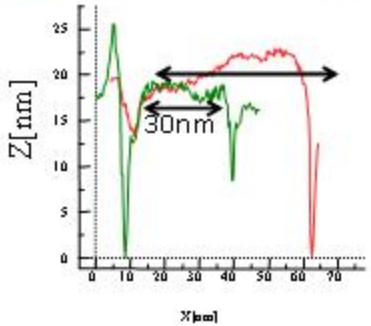
Pore

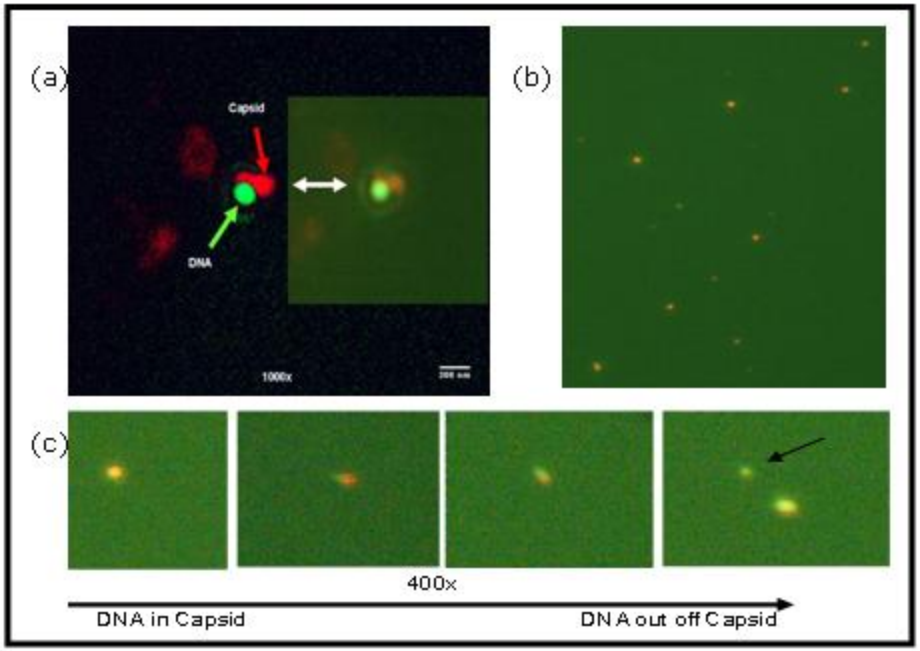




Viral Envelope



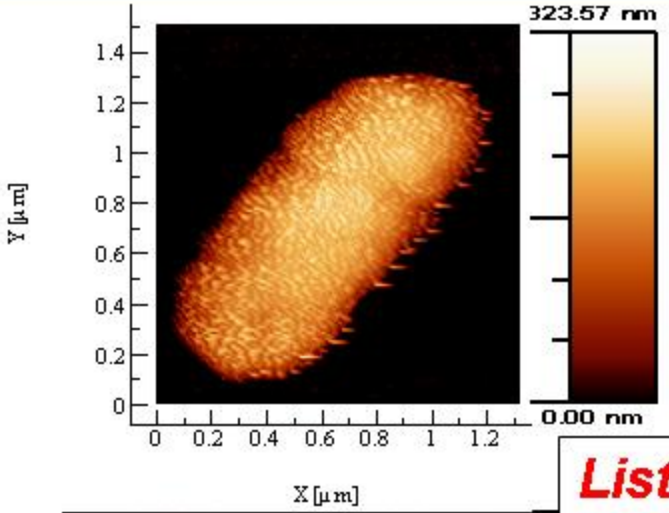




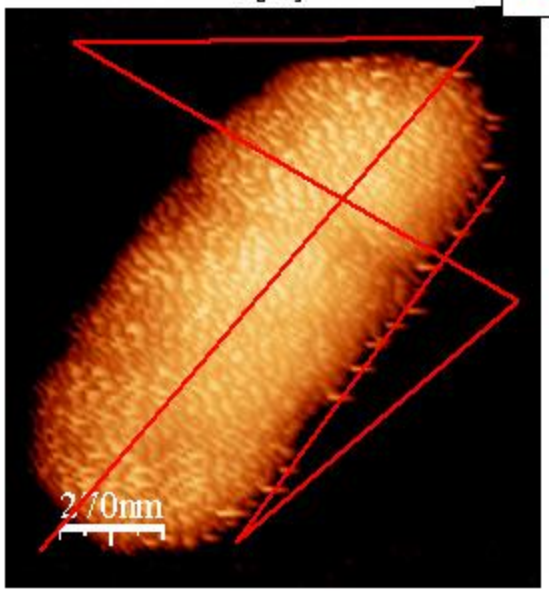
A. Ghafoor*, D. Akin*, R. Bashir, NanoBiotechnology, 2006.

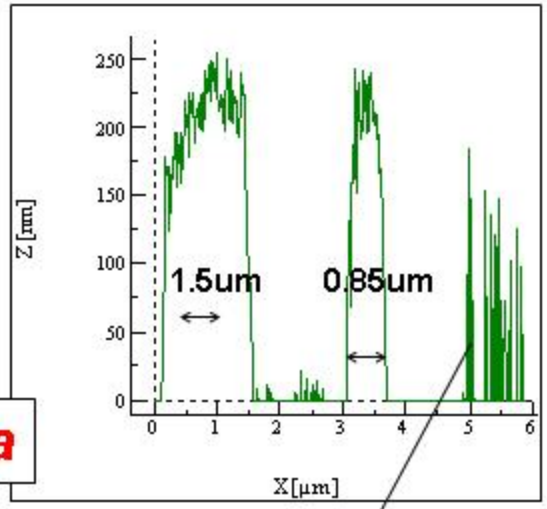
Single Particle Imaging: Microorganisms

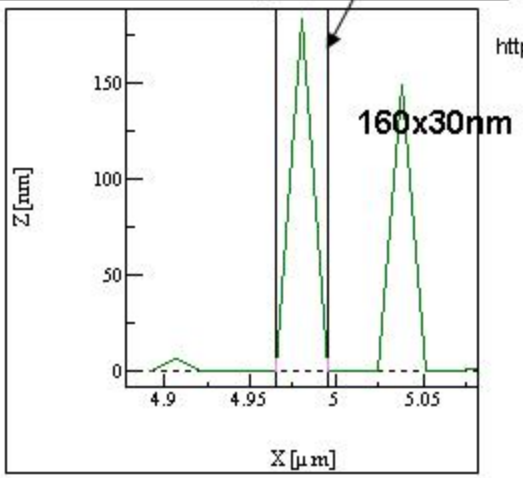
Research Examples



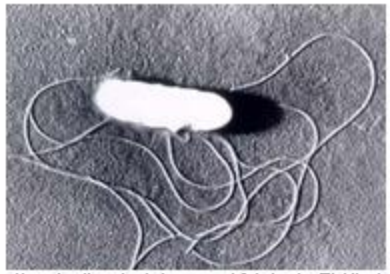
Listeria







Peritrichous



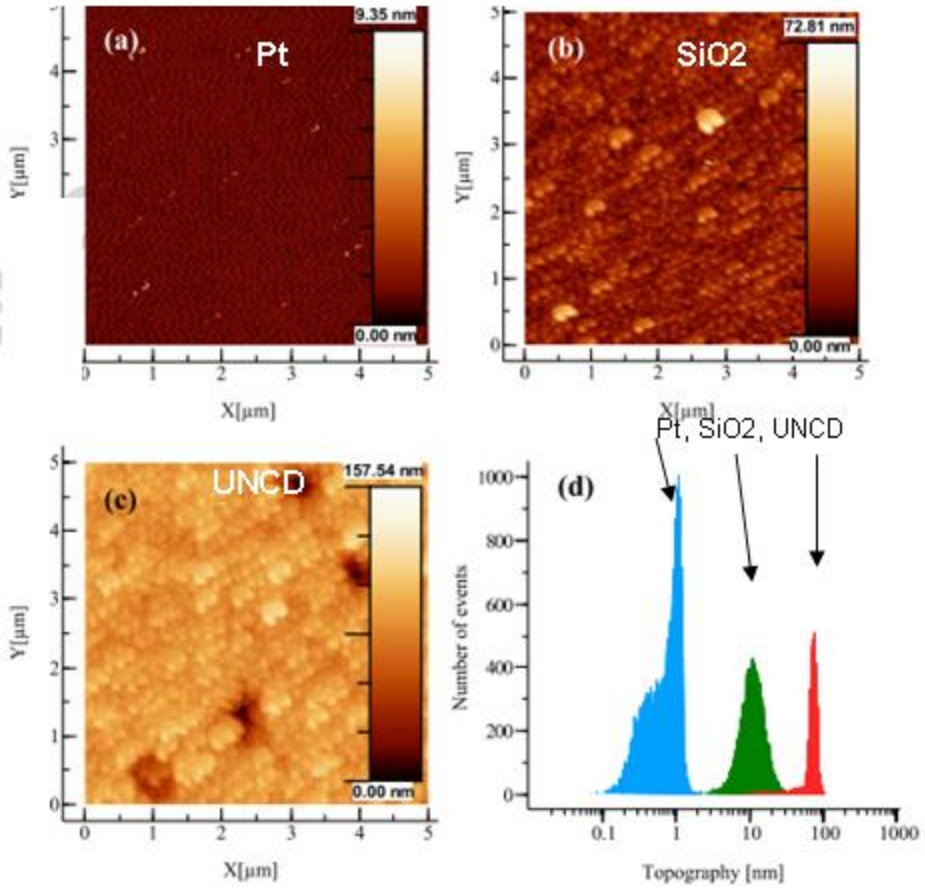
http://textbookofbacteriology.net/Listeria.EMfla.jpeg

- 20-25C Flagella
- 37C no Flagella

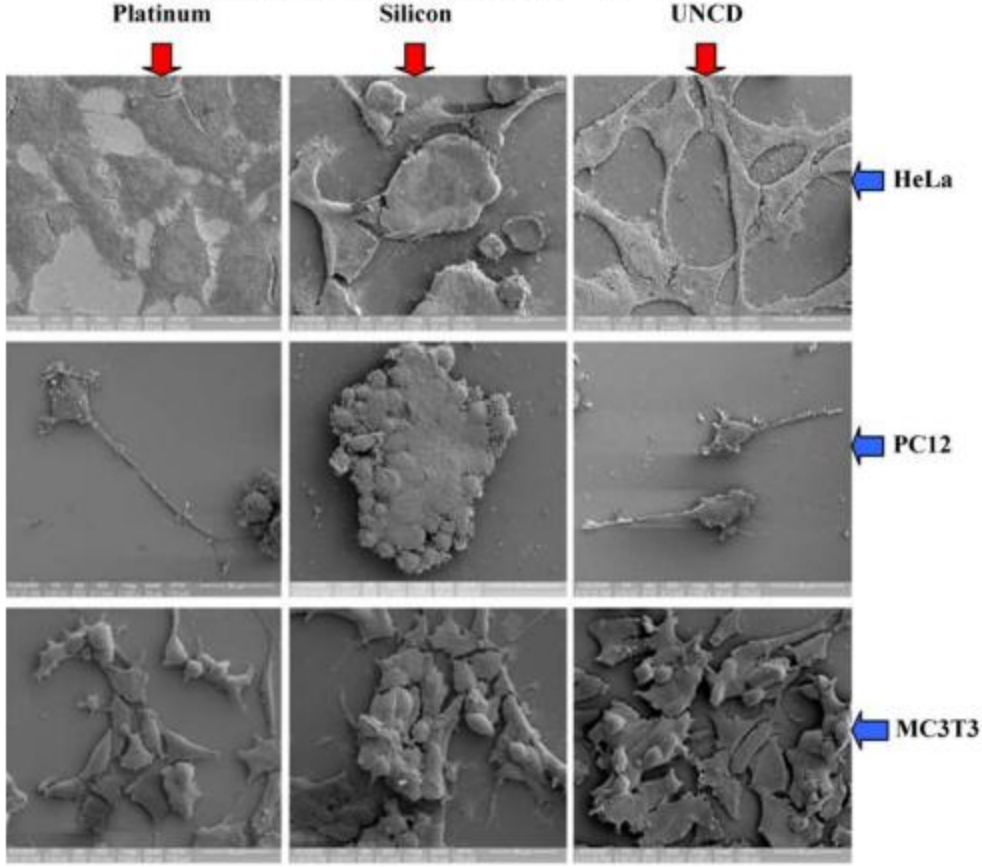
D. Akin, A. Gupta, R. Bashir, Manuscript in Preparation, 2008.

Biocompatibility of Nanomaterials:Ultra-Nanocrystaline Diamond (UNCD)

Surface Topology



Substrate Attachment



- Surface Roughness
- Surface Potential/Hydropathy
- Attachment
- Sustained cell growth
- Biocompatibility

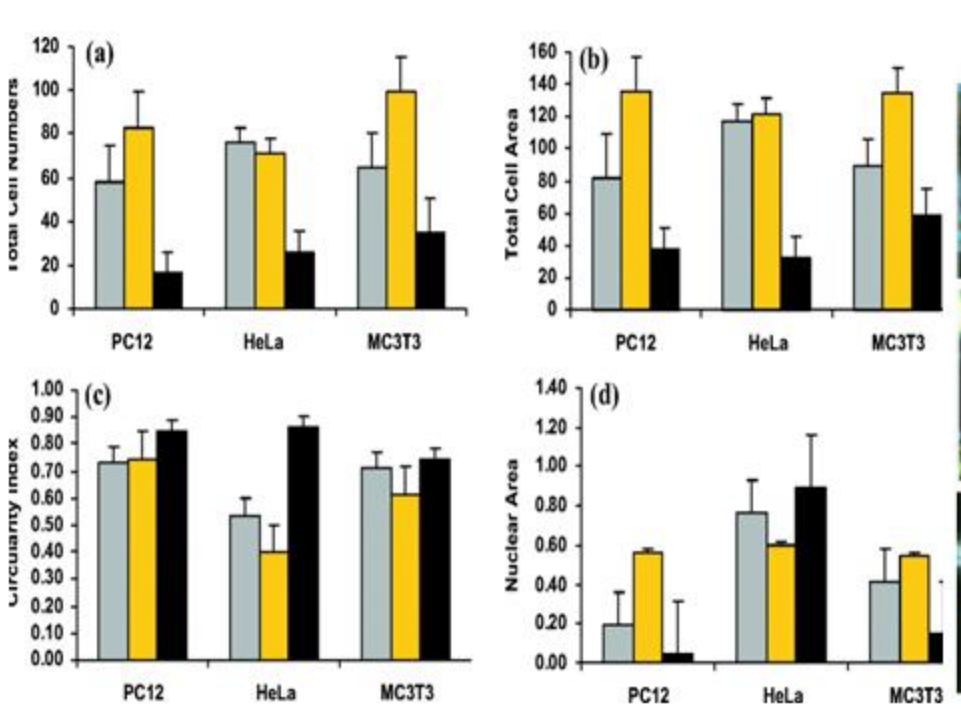
P. Bajaj, S. Bing, D. Akin, O. Auciello, D. Sherman, R. Bashir, Biomedical Microdevices, 9:787-794, 2007.

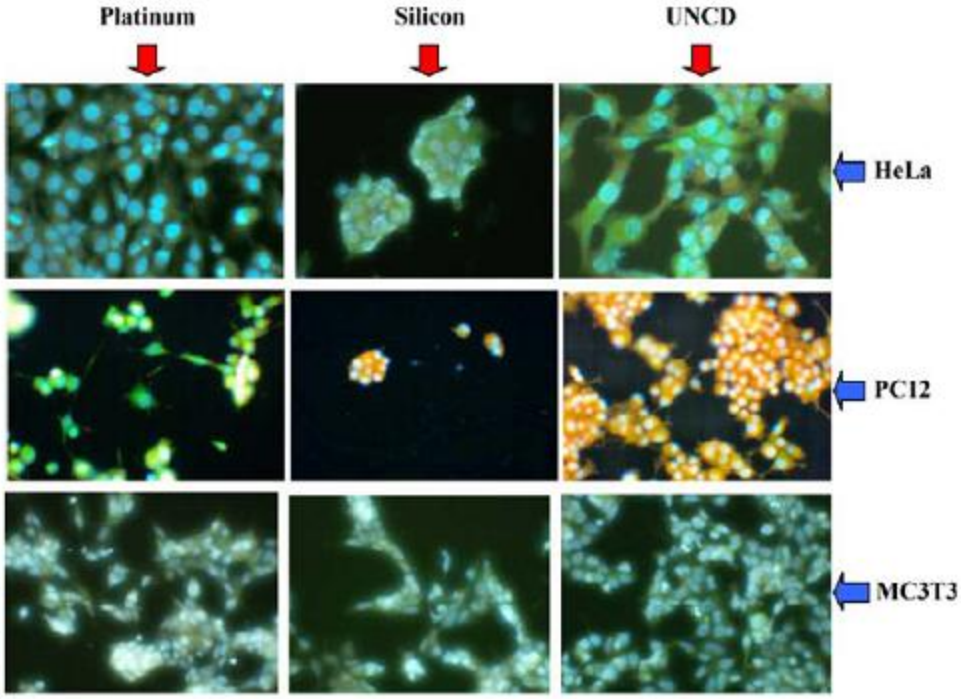
Collaboration Argon RL

Biocompatibility of Nanomaterials:Ultra-Nanocrystaline Diamond (UNCD)

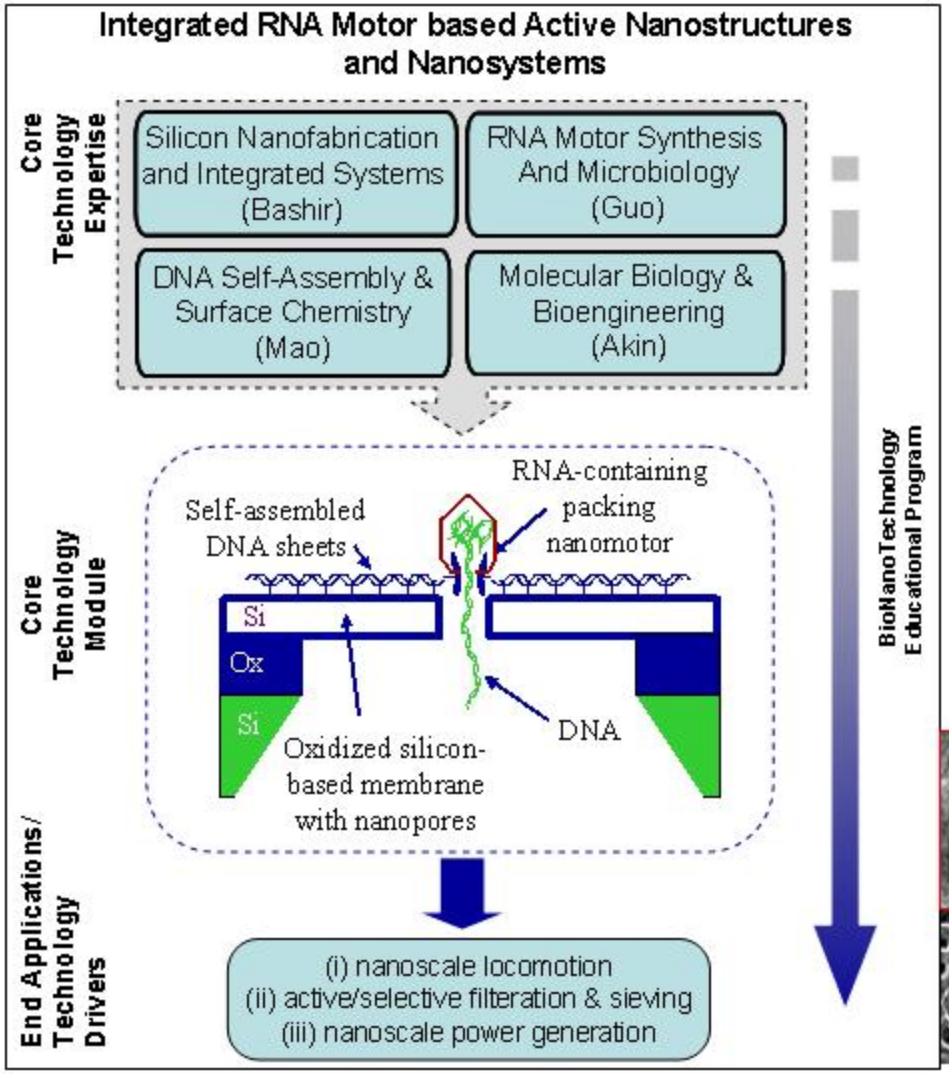
Surface features of the substrates that are important for attachment of various cells

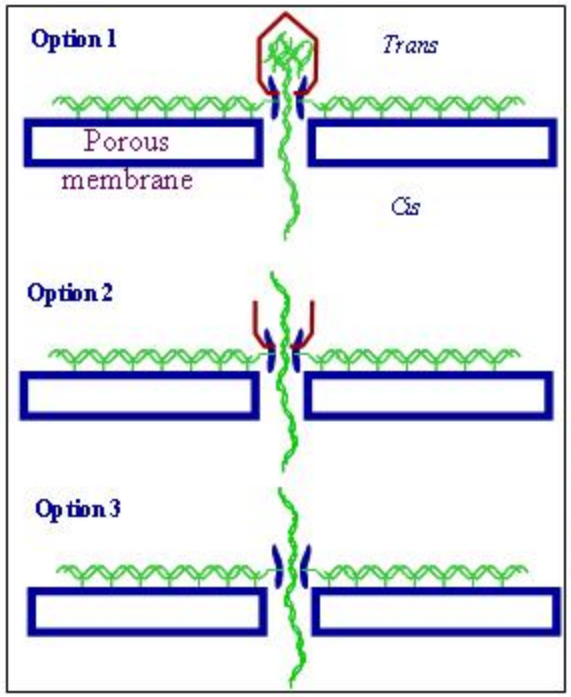
	Average height (nm)	RMS roughness (nm)	Contact angles (degrees)	Hydropathy
Si	1.0	0.4±0.4	30.4±3.21	Hydrophilic
Pt	15.8	6.1±0.1	83.6±2.2	Hydrophobic
UNCD	87.8	12.6±1.4	62.4±2.2	Hydrophobic

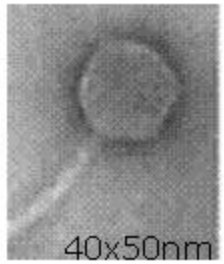


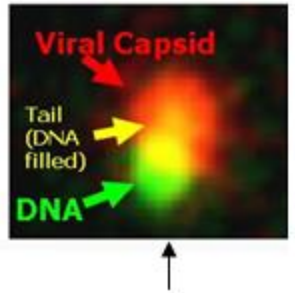


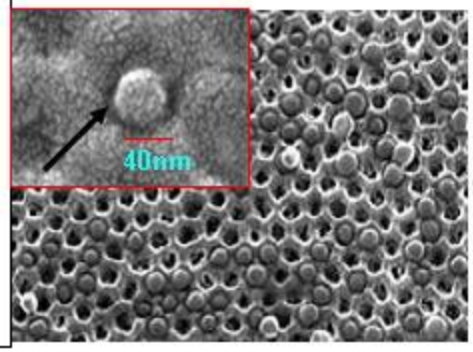
NIH, Nanomedicine Development Center, phi29 viral packaging motor

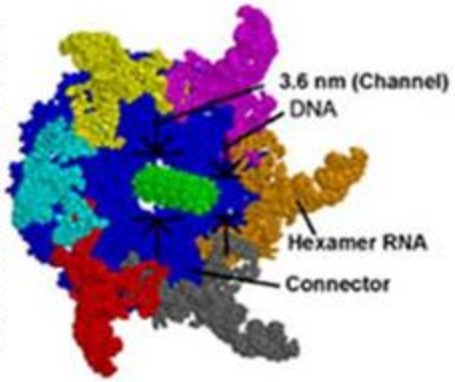








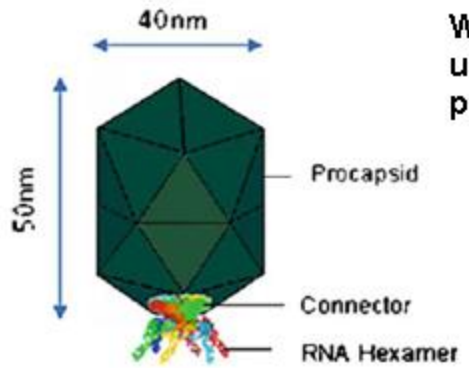




AlO_2- ALD Shrunk Nanopores

Nanometer Dimension Control: Atomic Layer Deposition based Anodized Aluminum Oxide Pore Size Reduction

Moon, Akin, Xuan, Ye, Guo, Bashir. Biomed. Microdevices, 2008.



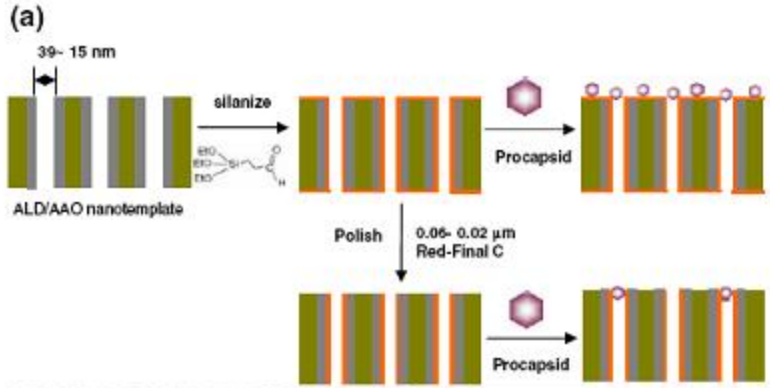
When packed under 60 atm pressure

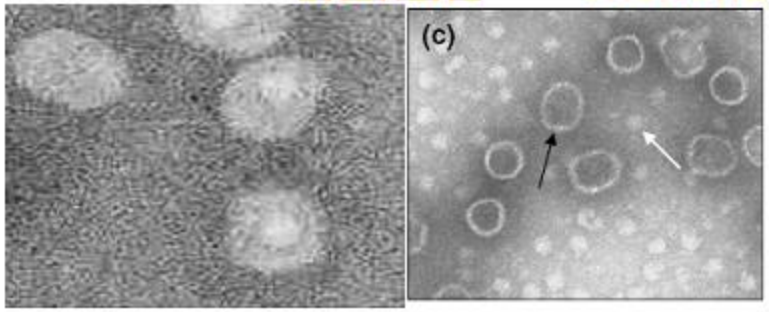
235x gp8

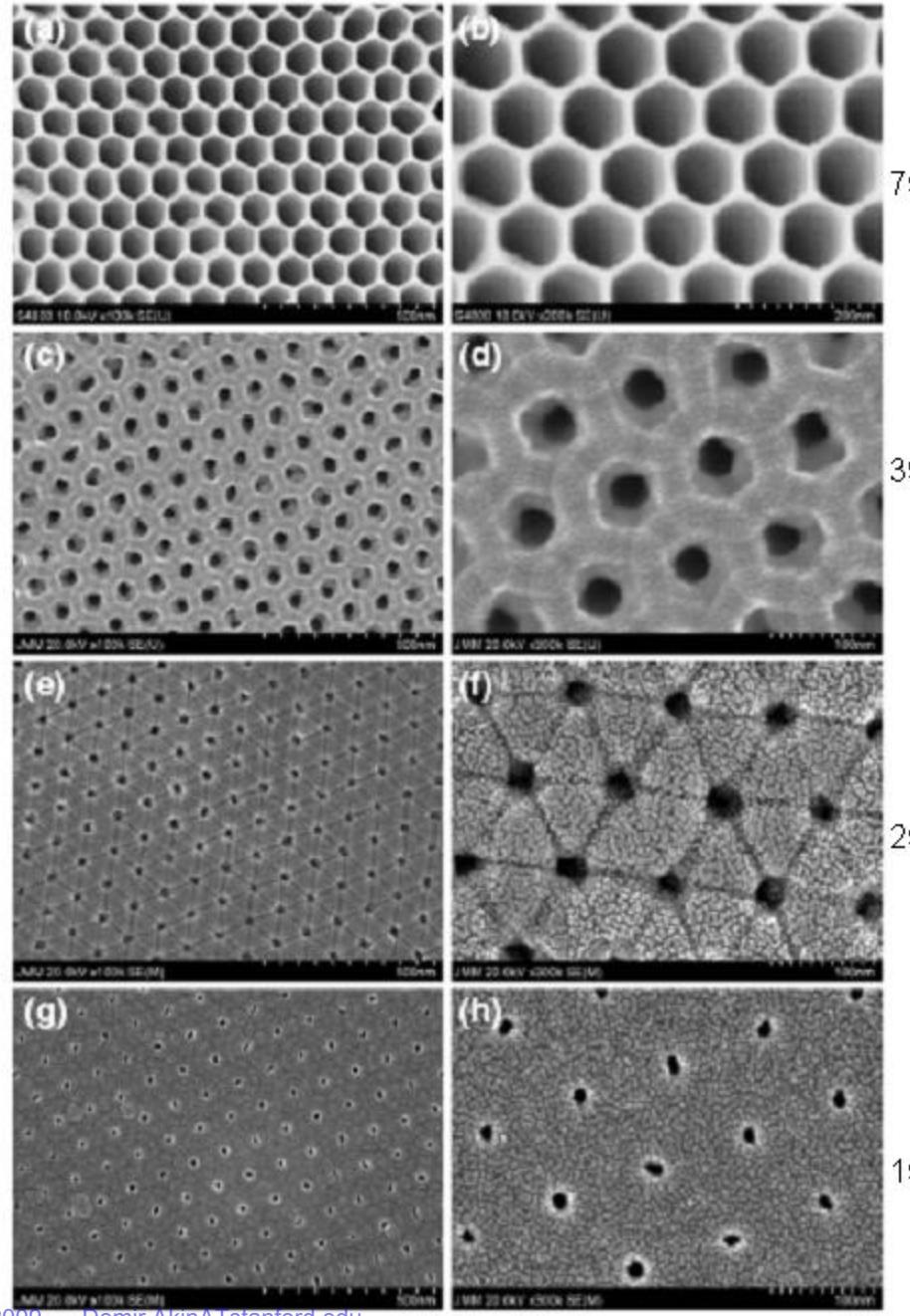
ATPase gp14,16

12x gp10

Phi-29 DNA Packaging Motor: Switchable, In Vitro complete Syhtesis, 20Kpb DNA (6μm)/~4min, 57pN, pRNA







PURDUE UNIVERSITY — Discovery Park — LIBNA — Birck Nanotechnology Center

Nanomedical Robotics and Smart Drug Delivery

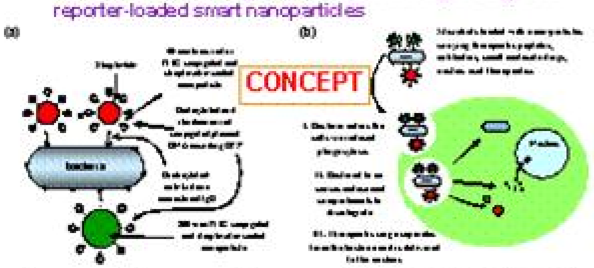
Demir Akin[1,2,3,4], Arun K. Bhunia[5], Sulma Mohammed[6], J. Paul Robinson[4,7], Rashid Bashir[1,2,3,4,8],

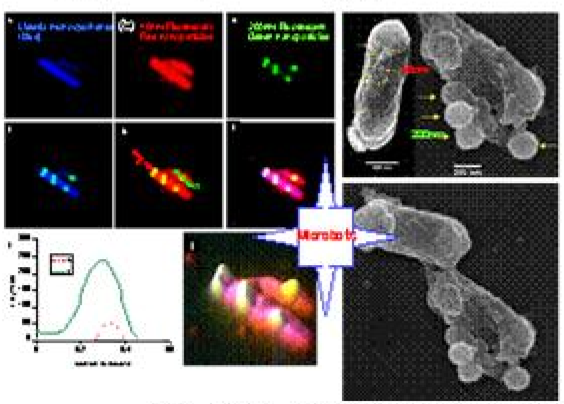
[1]LIBNA, [2]Weldon School of Biomedical Engineering, [3]Birck Nanotechnology Center, [4]Bindley Biosciences Center, [5]Department of Food Science, [6]Department of Comparative Pathology, [7]Department of Basic Medical Sciences, [8]School of Electrical Engineering,

OBJECTIVES

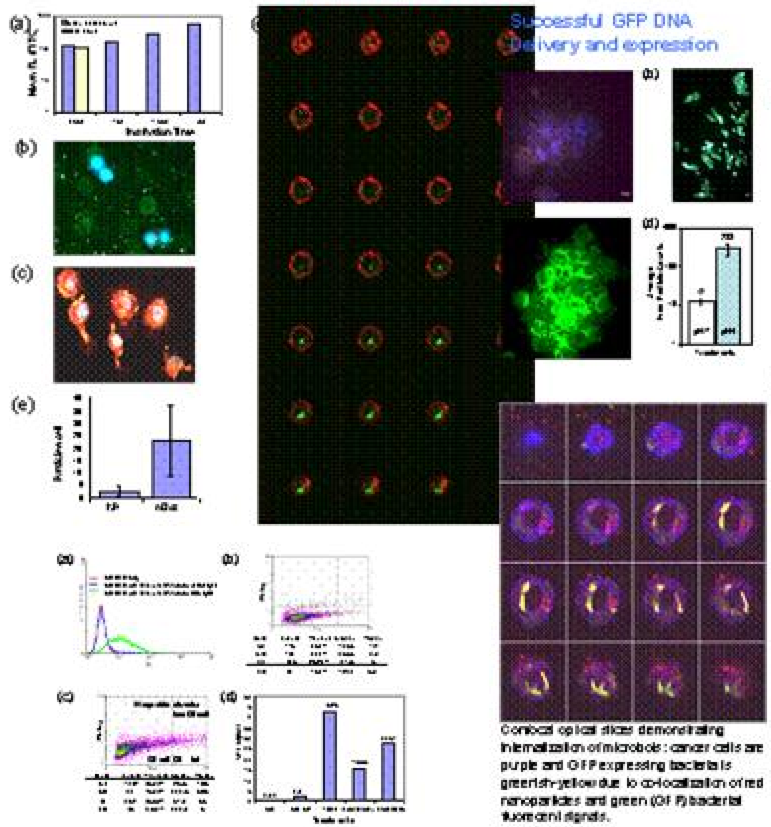
- Investigation of in-vitro and in-vivo delivery of synthetic smart nanoparticles and micro to nano scale diagnostic and/or therapeutic devices
- Investigation of monitoring and interventions of intracellular signaling networks in health and disease
- Development of a novel nanomedical platform (microbotics) for drug delivery into solid organ tumors
- Preparation for and Assessment of the initial foundations of nanomedical robotics and personal medicine

Microbots: Non-pathogenic bacteria with drug or diagnostic reporter-loaded smart nanoparticles





Demonstration and Quantification of *in-vitro* model drug delivery into numerous cancer cells

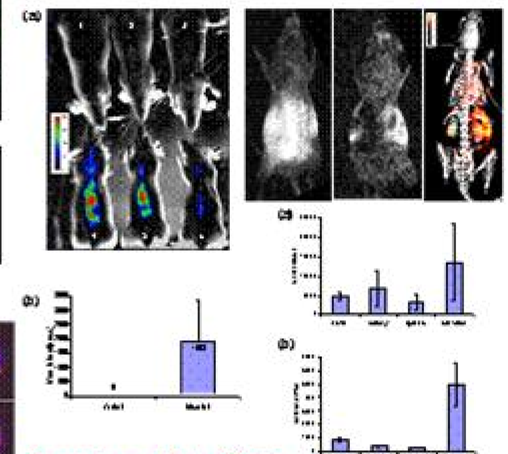


Confocal optical slices demonstrating internalization of microbots: cancer cells are purple and GFP expressing bacteria is greenish-yellow due to co-localization of red nanoparticles and green (GFP) bacterial fluorescent signals.

Flowcytometric analysis of microbot-mediated in-vitro delivery

Demonstration and Quantification of *in-vivo* model drug delivery into live animals

Successful Luciferase and SEAP Delivery into mice organs and bioluminescence live animal imaging of expression of the reporters



Conclusions and Future Studies

- Microbots can deliver cell impermeable large NPs in vitro
- Docked therapeutic cargo can escape and be targeted to subcellular locations
- Microbots can efficiently deliver smart nanoparticles docked with therapeutics into cells and organs of live mice *in vivo*.
- Engineering of intracellular environment dependent stimuli responsiveness to hybrid nanodevices and subsequent intracellular delivery of these smart devices are possible
- For future studies investigate microfabricated micro/nanoscale device delivery and real-time dynamic intracellular signal monitoring in depth
- Develop pathogenicity attenuated strains of the bacteria
- Demonstrate solid organ tumor penetration by microbots and selective therapy in a cancer model

References:

Akin et al., Bacteria-mediated delivery of nanoparticles and cargo into cancer cells. Nature Nanotechnology, 2:441-449, 2007.

Cancer: A metabolic and Immunological Perspective and A "Trojan Horse" based Treatment Approach

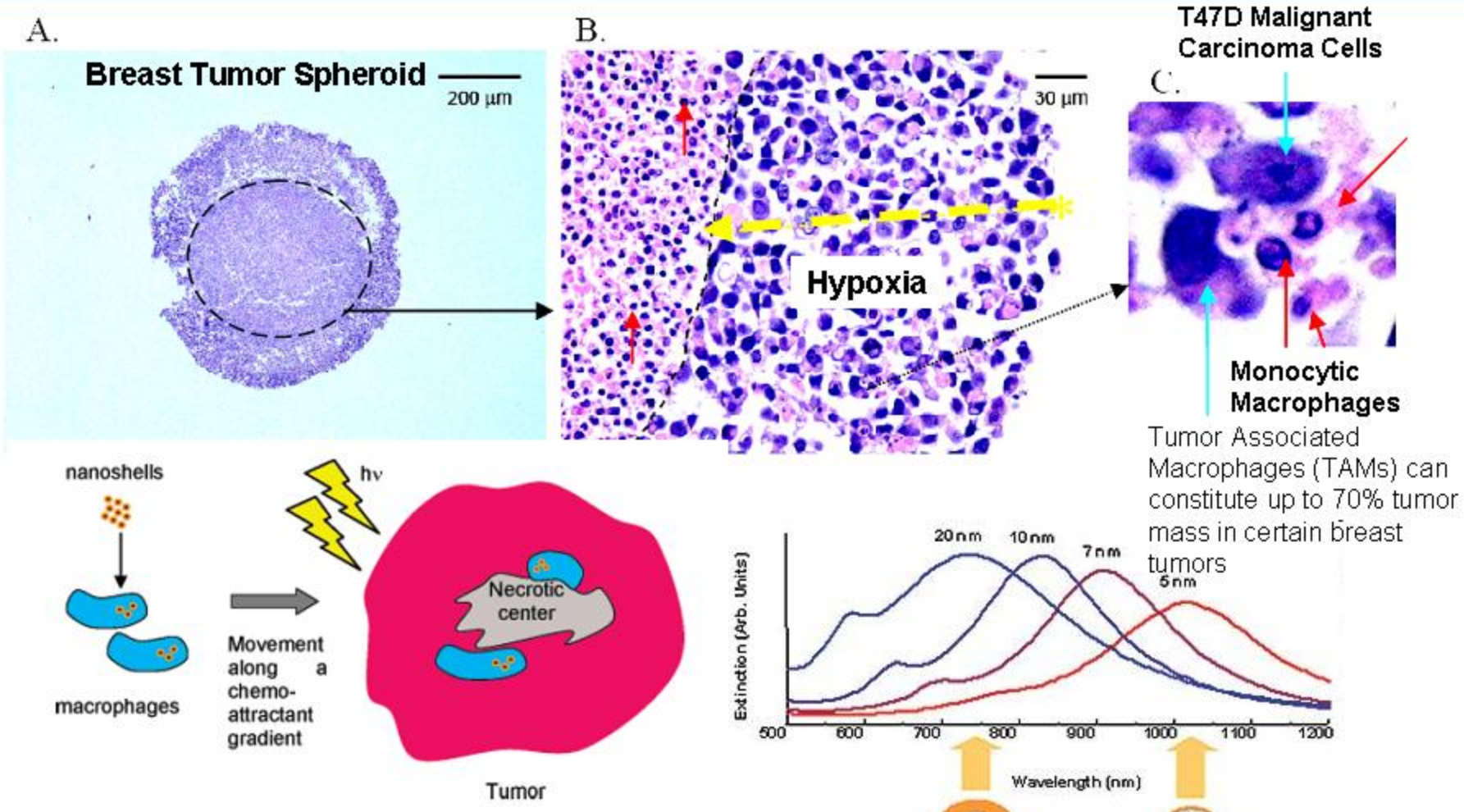
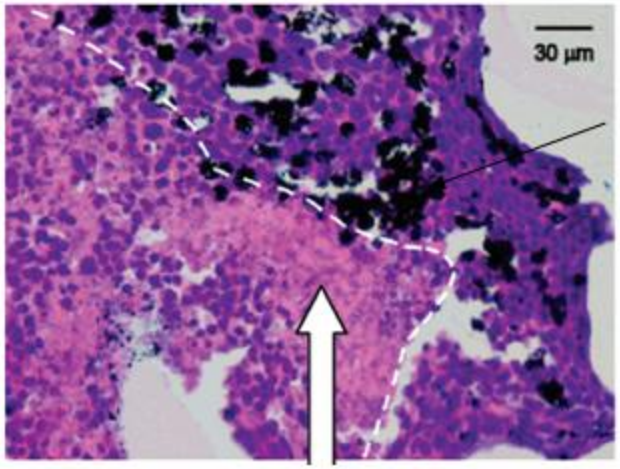


Figure 1. Schematic of Trojan Horse therapeutic nanoparticle delivery into the hypoxic region of tumor.

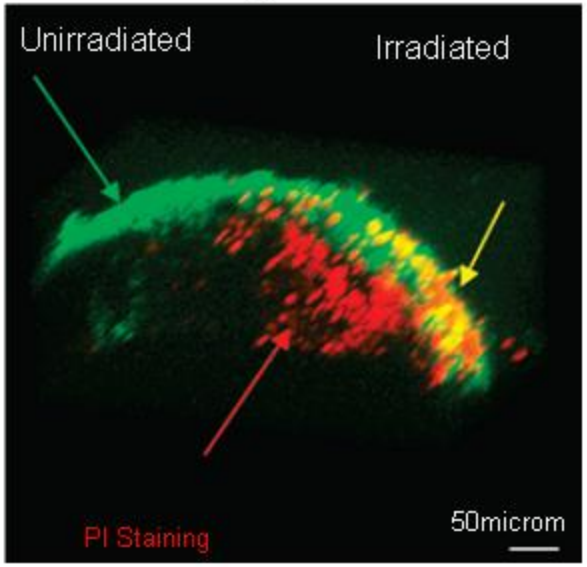
Choi, Akin, Bashir, Robinson, Clare et al., Nano Lett., 2007

Oldenburg et al., Chem. Phys. Lett., 288, 243-247,1998.

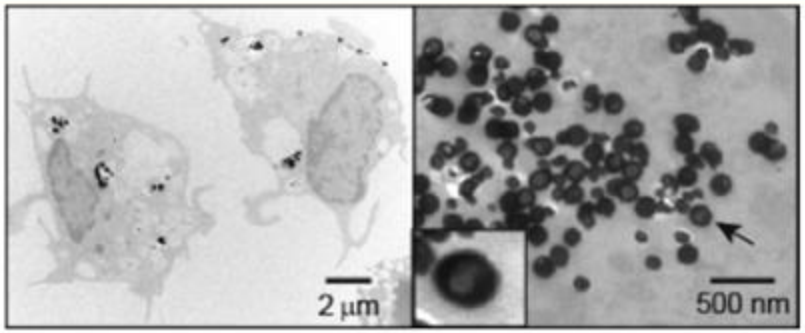
Cancer: A metabolic and Immunological Perspective and A "Trojan Horse" based Treatment Approach

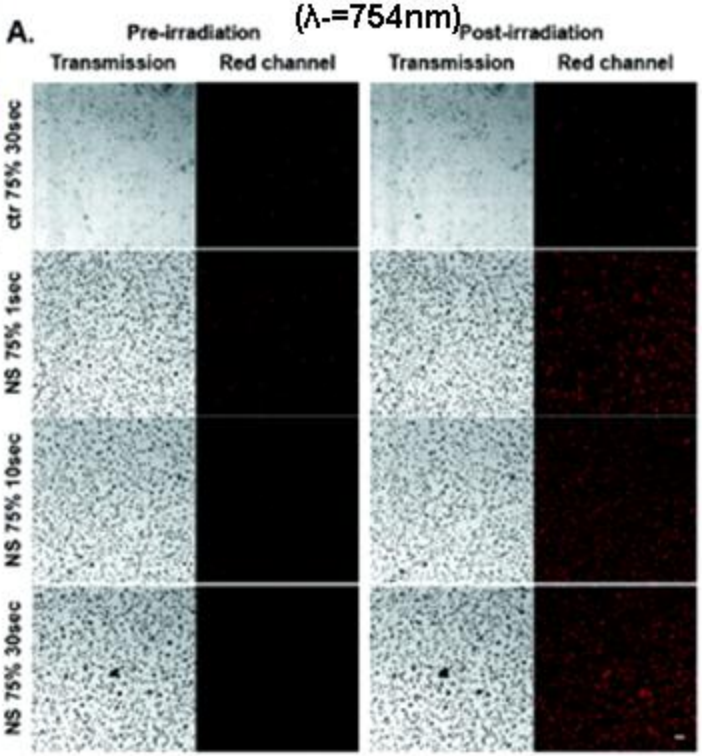


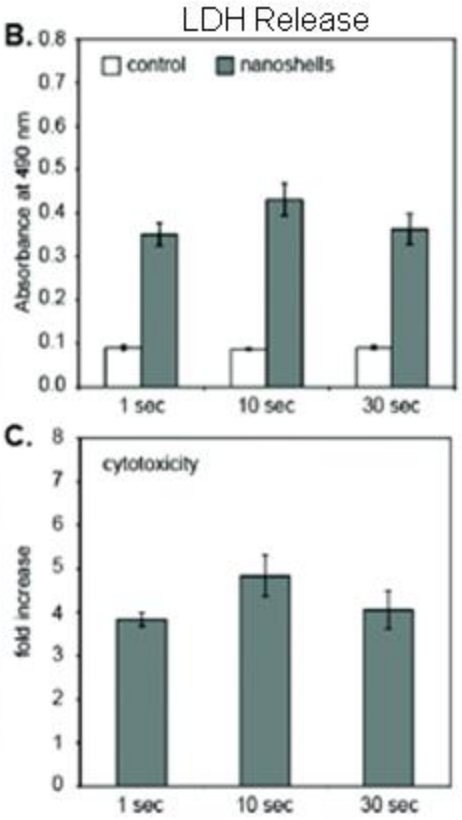
Necrosis/Hypoxia



Nanoshell-loaded macrophages

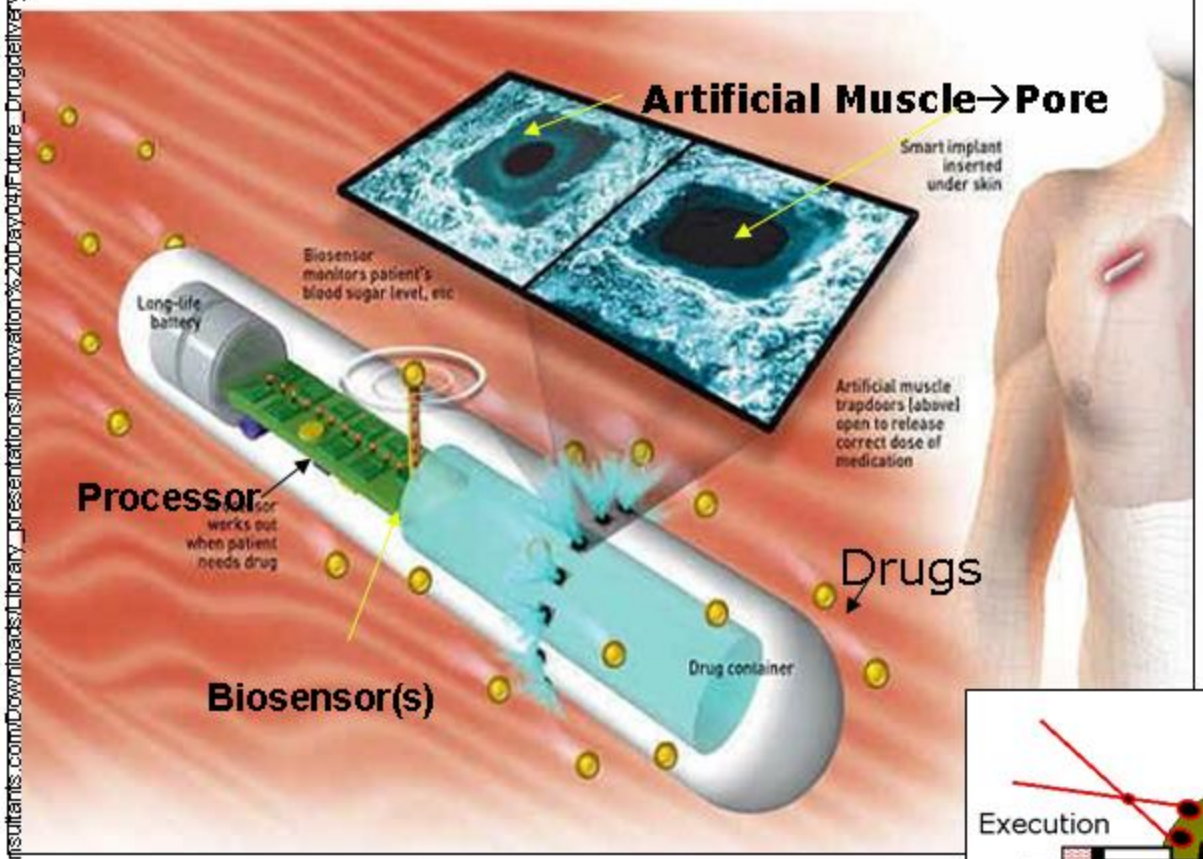






Future "Drugs"

Smart pills and implantables



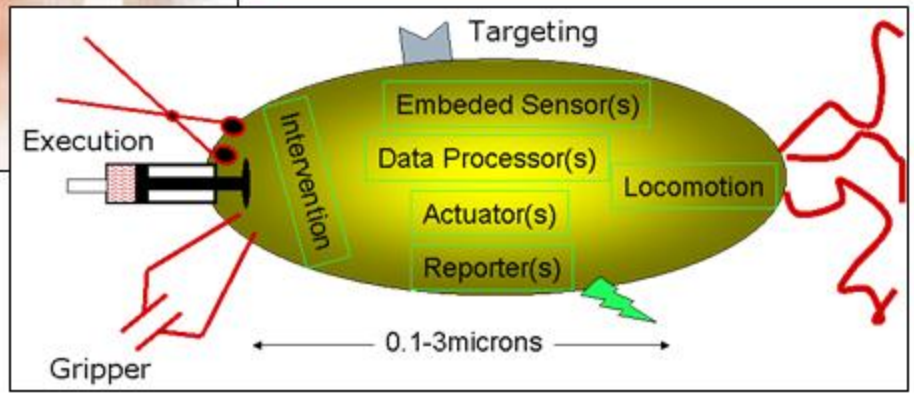
Fixed Micro/Nanosystems

↑

Nanomedicine

↓

Active Micro/Nanodevices



Akin et. al., in progress

www.cambridgeconsultants.com/DownloadsLibrary_presentations/Innovation%20Day04/Future_Drugdelivery.pdf