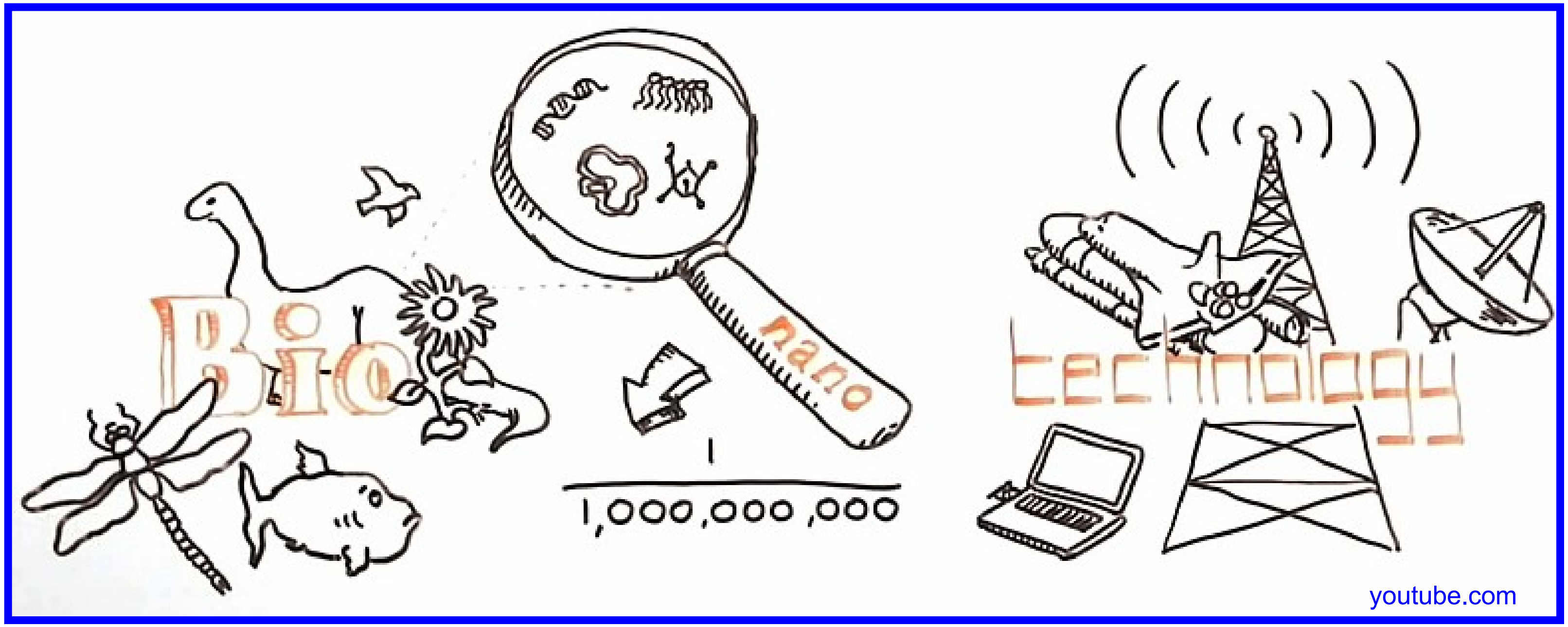
Nanobiotechnology

Petr Skládal
Department of Biochemistry, MU
RG Nanobiotechnology, CEITEC



Nanobiotechnology at MU

- at MU, we have started research in this area around 2005 (purchase of our first AFM Ntegra Vita at NCBR)
- significant expansion within CEITEC – Structural Biology research program – the Core Facility of Nanobiotechnology (5 AFM systems now)
- … we need to provide introduction to the future users of techniques available at this CF, as well as to educate our (PhD) students
- course is also incorporated to the Life Science PhD program at CEITEC, but opened to all students from MU – e.g. Dept. Biochemistry Biotechnology program

- Lecturers: Petr Skládal, Jan Přibyl, Zdenek Farka

Sylabus

- 1. Introduction. Science of Nano. What is nanobiotechnology.
- 2. Nanostructures. Carbon nanotubes, semiconductor nanoparticles - quantum dots. Metal-based nanostructures - nanowires and bioelectronics. Gold nanoparticles (nanorods, nanocages, nanoshells). Magnetic nanoparticles. Polymer nanostructures (dendrimers). Protein-based nanostructures - nanomotors from microbes and mammalian cells (myosin). Nanomachines based on nucleic acids.
- 3. Experimental technichues. Scanning probe microscopies (STM, AFM, SNOM, SECM, ...). Physical principles, basic and advanced measuring modes. Imaging of bioobjects - from atoms, molecules to cells and tissues. Combined techniques with inverted optical and fluorescence microscopes. Raman imaging. Biointeractions at the molecular level.
- 4. Self-assembling techniques. Separation, characterization and modification of nanoparticles. From natural to artificial structures. Nanolithography and nanomanipulations. Nanoparticles for biological labeling and cellular imaging. Nanobiosensors and nanobioanalytical systems. Microfluidics, cell sorting and lab-on-a-chip. Biochips and sensing arrays, nanodeposition of biomolecules.
- 5. Medical applications. Cytotoxicity of nanoparticles. Nanostructures in drug discovery, delivery and controlled release. Nanostructures in cancer research. Nanotechnology for tissue engineering and regenerative therapy.
- 6. Nanobiotechnology in commercial examples. Perspectives and conclusions.
- … to be improved …

Brief History

"There is plenty of room at the bottom."

R. Feynman, 1959 Caltech; Father of Nanotechnology

The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big"

Dr. Richard P. Feynman (1918-1988)

nano, Greek for "dwarf," means one billionth (x 10^{-9})

There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics – by Richard P. Feynman

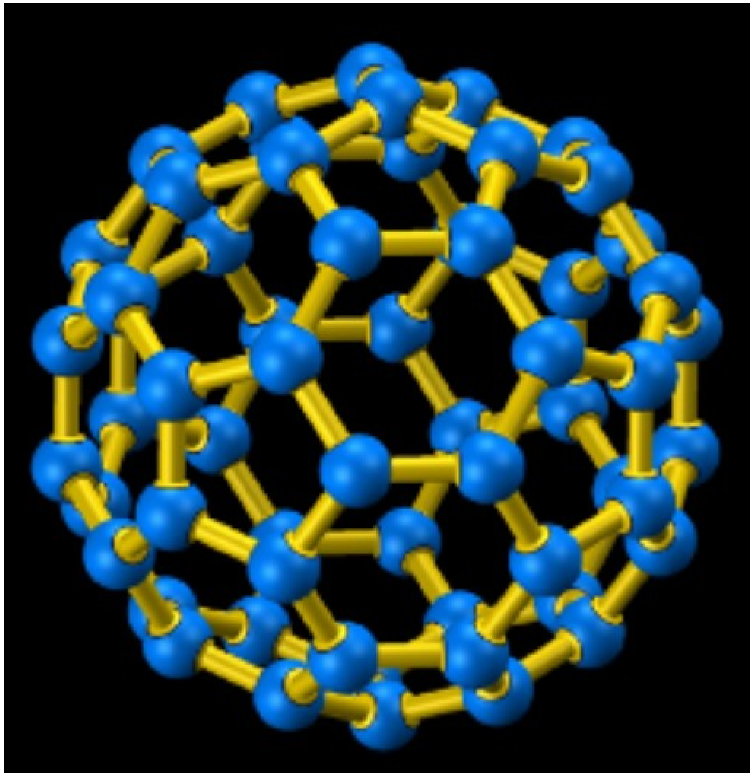
- the term "nanotechnology" appeared few years after Feynman's lecture
- his remarks are still relevant almost half a century after his original address
- related to us is Feynman' vision regarding the field of biology. Unlike many physicists of his time, Feynman realized how important biology is in the framework of nanotechnology
- The marvelous biological system.
  "The biological example of writing information on a small scale has inspired me to think of something that should be possible. Biology is not simply writing information; it is doing something about it."

- Rearranging the atoms
  "But I am not afraid to consider the final question as to whether, ultimately---in the great future---we can arrange the atoms the way we want; the very atoms, all the way down! What would happen if we could arrange the atoms one by one the way we want them"

- Engineering & Science Magazine, California Institute of Technology, Vol. 23/5, Feb. 1960

Richard Smalley

- a chemistry professor at Rice University, pioneered the field of nanotechnology and shared a Nobel Prize in 1996 for the development of bucky-balls (fullerene)



Dr. Richard E. Smalley (1943-2005, 1996 Nobel Prize in Chemistry)

Fullerenes

- a new form of carbon clusters was identified in 1985, graphite was vaporized using a laser
- closed and convex cage molecules formed by the arrangement hexagonal and pentagonal faces
- buckminsterfullerene, named after the architect R. Buckminster Fuller, **C60**, size is about 0.7 nm, excellent electrical and heat conductivity
- fullerenes that have 70, 76, 84, 90, and 96 carbon atoms were also found
- smallest possible fullerene, C20, consists of solely of 12 pentagons, generated from a brominated hydrocarbon, dodecahedrane, by gas-phase debromination, lifetime 0.4 ms
- potential use as nanocontainers, some antiviral activity
- elongated variants – carbon nanotubes (1991, Sumio Iijima), single and multiwall forms (SWCNT and MWCNT)

Eric Drexler

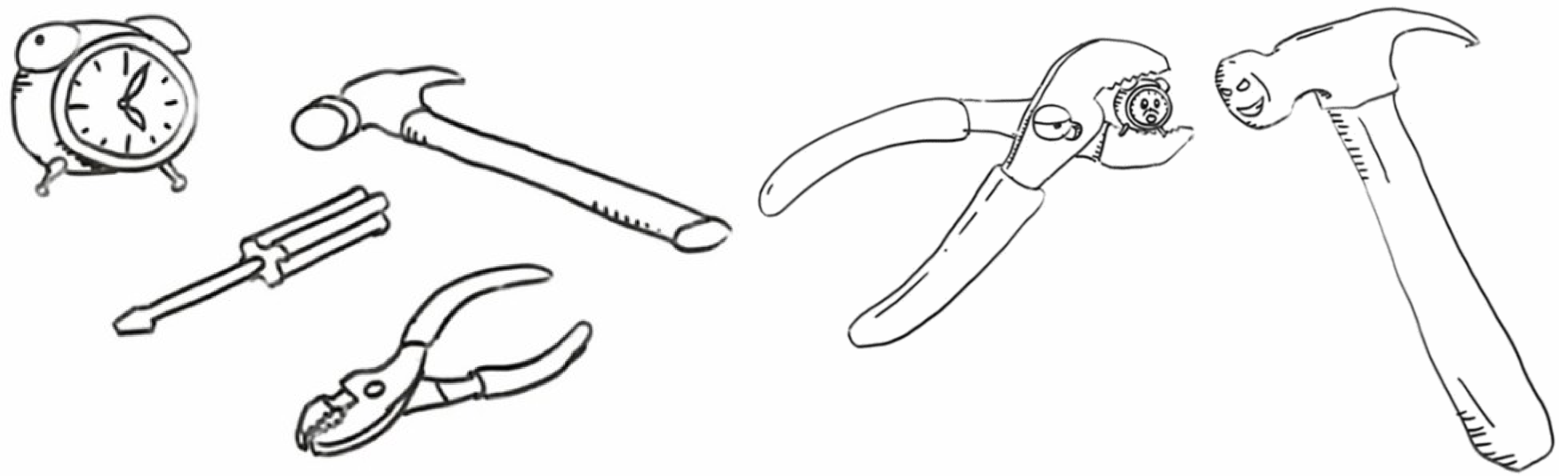
- **as a researcher, author, and policy advocate he has been one of the pioneers to focus on emerging technologies and their impact for the future**
- **founded the Foresight Institute, presently at Nanorex, a company that develops software for the design and simulation of molecular machine systems**
- **his thought provoking publications**
  - "Engines of Creation: The Coming Era of Nanotechnology,"
  - "Nanosystems: Molecular Machinery, Manufacturing, and Computation,"
  - "Unbounding the Future: The Nanotechnology Revolution"
- **made great impact by introducing the very topic of nanotechnology to many**

Dr. Eric Drexler (1955-)

Two-week revolution

- Drexler proposed constructing molecules by forcibly pressing atoms together into the desired molecular shapes - "mechanosynthesis" (parallels with macroscopic machinery and engineering)
- building objects in an assembly-line manner by directly bonding individual atoms
- central idea - construction of an assembler, a nanometer-scale machine that assembles objects atom-by-atom according to defined instructions
- creation of just a single working assembler would lead immediately to the "Two-Week Revolution
- still only an idea …
- other way - nanotechnology that looks to nature for its start; cells build thousands of working nanomachines, which may be harnessed and modified to perform our own custom nanotechnological tasks = Nanobiotechnology
- a slower process, but feasible

Tools for nanobio



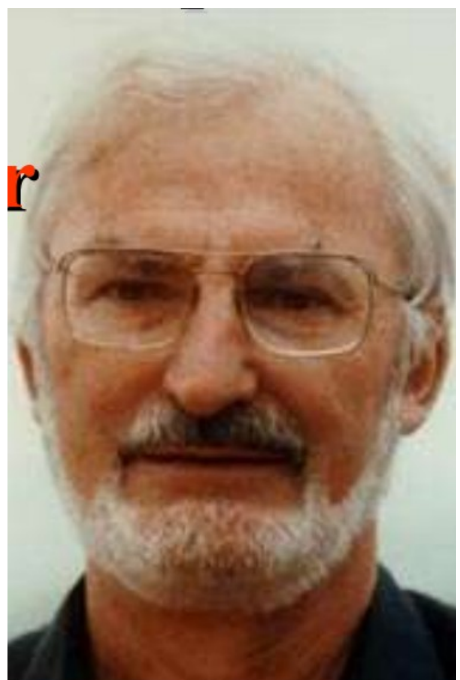
- bottom-up vs. top-down approach
- building innovative tools to study and manipulate biology at the nanometer scale
  - new tools
  - new materials
  - new devices
  - new knowledge

http://www.nanotech-now.com/columns/?article=097

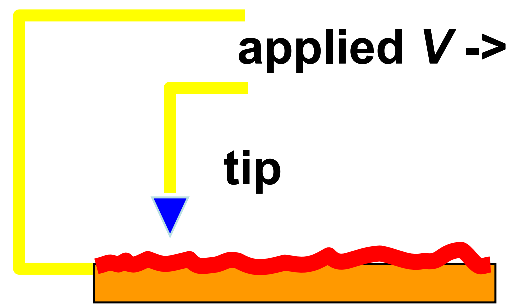
Scanning probe microscopies

- invention of the scanning tunneling microscope (**STM**) by Gerd Binnig and Heinrich Rohrer at IBM's Zurich Research Labs (1986 Nobel Prize in Physics)
- followed five years later by the invention of the atomic force microscope (**AFM**)

Gerd Binning



Heinrich Rohrer



$$I \sim V \exp(-A\Phi^{1/2}d)$$

Chad Mirkin

- Professor in the Institute for Nanotechnology at Northwestern University
- a pioneer in chemical modifications of nanosystems leading to breakthrough contributions to bionanotechnology
- explains the need to open our minds and change our attitude as we embark on learning this new field:

- ***"At the nano level atoms do not belong to any field of science. "***

- this conveys the extreme diversity and uniqueness of nanotechnology, while stressing the preparation required by those aspiring to contribute to it

- developed dip-pen nanolithography technique, uses AFM to introduce patterned molecules into surface

Dr. Chad Mirkin (1963-)

Definition of nanotechnology

- Nanotechnology can be difficult to determine and define
- realm of nanoscience is not new; chemists will tell you they've been doing nanoscience for hundreds of years
- stained-glass windows found in medieval churches contain different-size gold nanoparticles incorporated into the glass — creating orange, purple, red, orgreenish colors.
- Einstein, as part of his doctoral dissertation, calculated the size of a sugar molecule as one nanometer
- loosely considered, both the medieval glass workers and Einstein were nanoscientists …

Nanotechnology

- **research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1 - 100 nanometer range**
- **creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.**
- **ability to control or manipulate on the atomic scale**

… National Nanotechnology Initiative, USA

… alternatively:

- **design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scales**

Nanoscience

- study **of phenomena and** manipulation **of materials at atomic, molecular and macromolecular scales, where** properties differ significantly **from those at a larger scale**

Nanobiotechnology

- applies tools and processes of nano / microfabrication to build devices for studying biosystems
- biotechnology is the application of technological innovation as it concerns biological and life sciences. Nanobiotechnology incorporates biotechnology on the nano-scale
- is a subset of nanotechnology where the biological world provides the inspiration and/or the end goal. It is defined as atom-level engineering and manufacturing using biological precedence for guidance (Nano-Biomimetics) or traditional nanotechnology applied to biological and biomedical needs

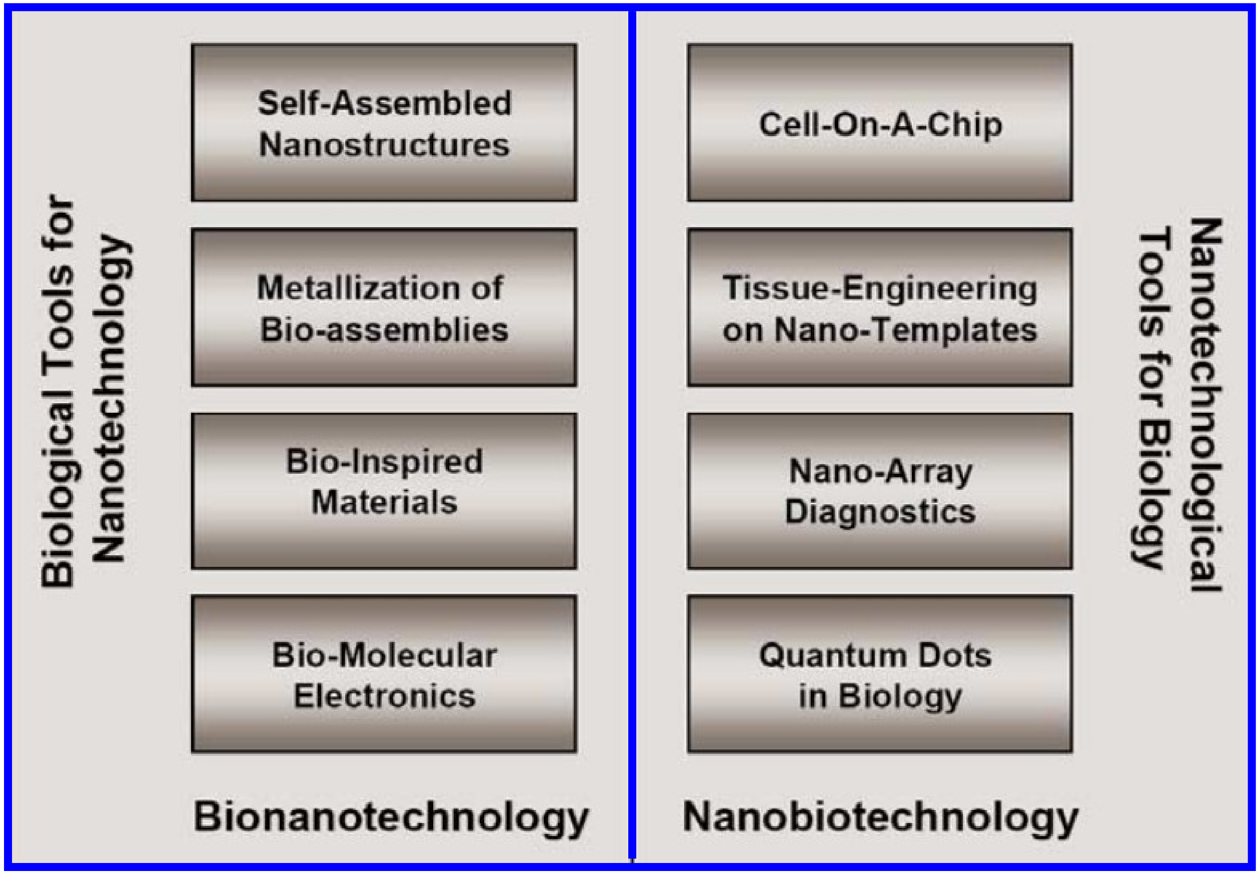
- slightly different is **Bionanotechnology**
- use of biological building blocks and the utilization of biological specificity and activity for the development of modern technology at the nano-scale
- focus on biological nanomachines

- Wikipedia:
- Nanobiotechnology, bionanotechnology, and nanobiology are terms that refer to the intersection of nanotechnology and biology

Wikipedia:

- Bionanotechnology generally refers to the study of how the goals of nanotechnology can be guided by studying how biological "machines" work and adapting these biological motifs into improving existing nanotechnologies or creating new ones
- Nanobiotechnology, on the other hand, refers to the ways that nanotechnology is used to create devices to study biological systems
- nanobiotechnology is essentially miniaturized biotechnology, whereas bionanotechnology is a specific application of nanotechnology
- for example, DNA nanotechnology or cellular engineering would be classified as bionanotechnology because they involve working with biomolecules on the nanoscale
- conversely, many new medical technologies involving nanoparticles as delivery systems or as sensors would be examples of nanobiotechnology since they involve using nanotechnology to advance the goals of biology

Nanobio- or Bionano- technology



- **Bionanotechnology as the use of biological assemblies for various applications that may not be directionally associated with biology**
- **Nanobiotechnology is the use of nano-science for specific biological applications**

- **Nanobiology** can serve as a description of both bionanotechnology and nanobiotechnology

- **Nanomedicine** is the medical application of nanotechnology. It ranges from the medical applications of nanomaterials and biological devices, to nanoelectronic biosensors, and even possible future applications of molecular nanotechnology such as biological machines.

- **Bionics**, which stems from [bi(o)- + (electr)onics] could be described as the application of biological principles and mechanisms to the design and fabrication of engineering systems
- not merely to copy nature but rather to understand its principles and use them as a stimulus and motivation for innovations
- bio-inspired technology may be more suitable name
- **Nanobionics** is applied at the nanoscale level

Convergence of disciplines

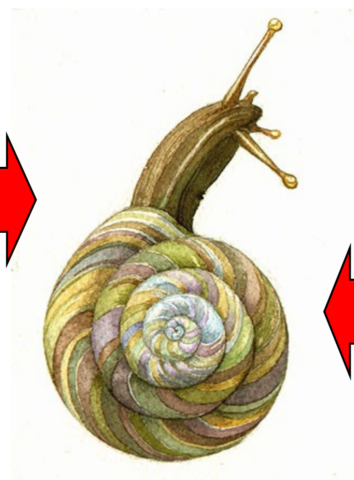
- and technology transfer from physics to the bio/medical sciences to solve Grand Challenges
- molecular:
  - structure analysis of single proteins (attosecond spectroscopy)
  - sequencing single DNA (nanopores)
  - understanding DNA damage and repair
- cellular:
  - molecular scale imaging / analysis of single, living cells
  - single molecule biochemistry in individual living cells
- medical:
  - detecting single, abnormal cells among healthy ones in tissues
  - developing non-invasive medical tools
  - understanding the cellular biochemistry of the brain

How small is small?
from macro to nano...

Object	Diameter (nm)
Hydrogen atom	0.1
Water molecule	0.3
DNA (width)	2.5
Cell membrane	5-9
Porin channel	4-10
Actin filament	5-9
Microtubule	25
Bacterial flagellum	12-25
Magnetosomes	35-150
Liposome vesicle	100 (min)
Bacterial cell	250 - 1,000
Spores	1,000 - 8,000
Red blood cell	6,000 - 8,000
Human hair	100,000

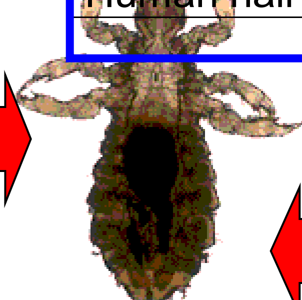
macro (m)

- bridges
- airplanes
- cars
- human beings
- birds



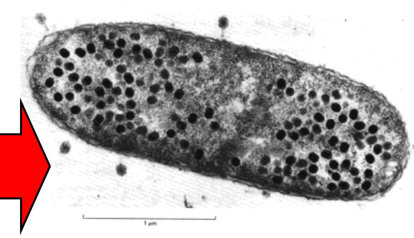
meso (mm)

- snails
- rice
- ants
- watch gears
- sand



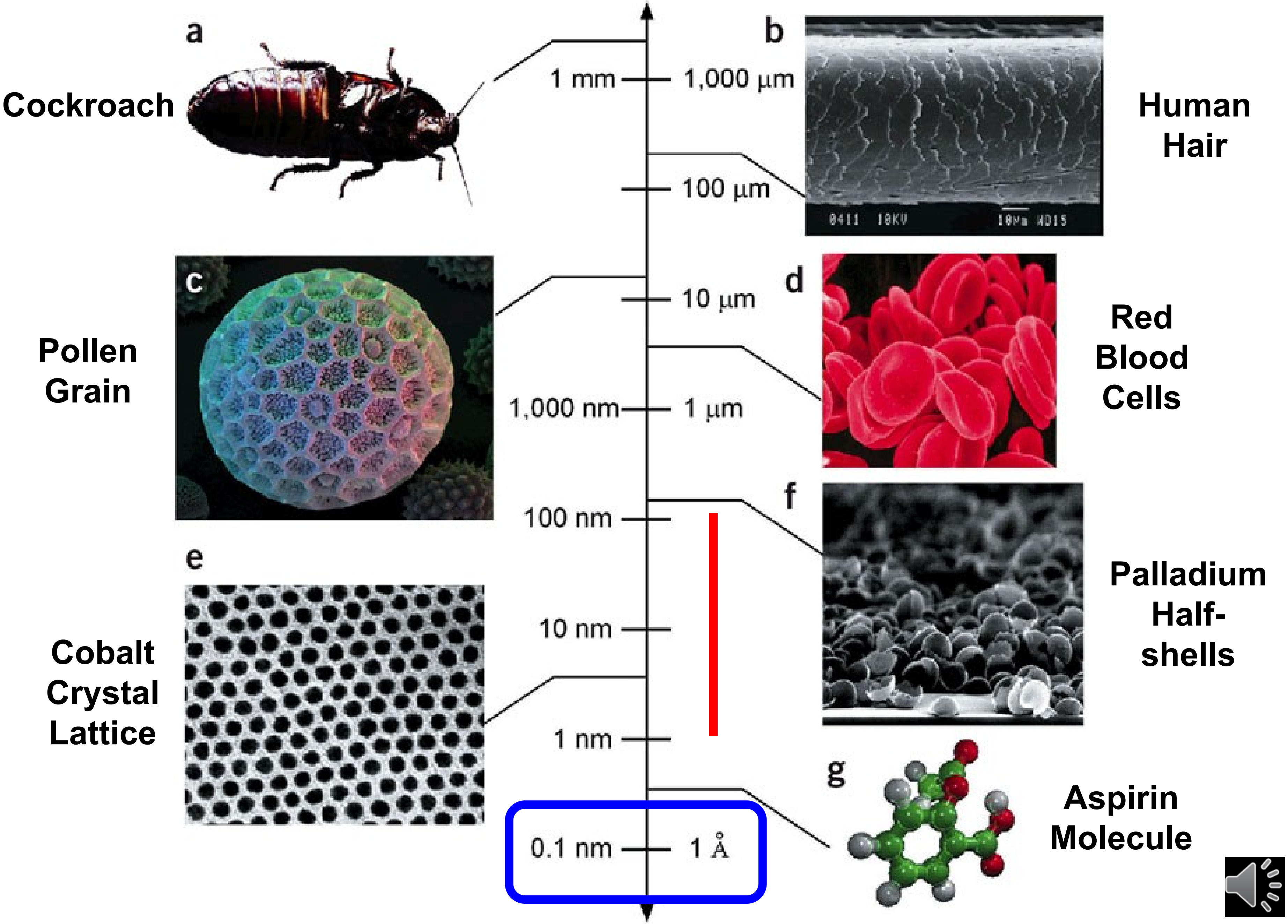
micro (um)

- hair
- dust
- pollen
- cells
- bacteria



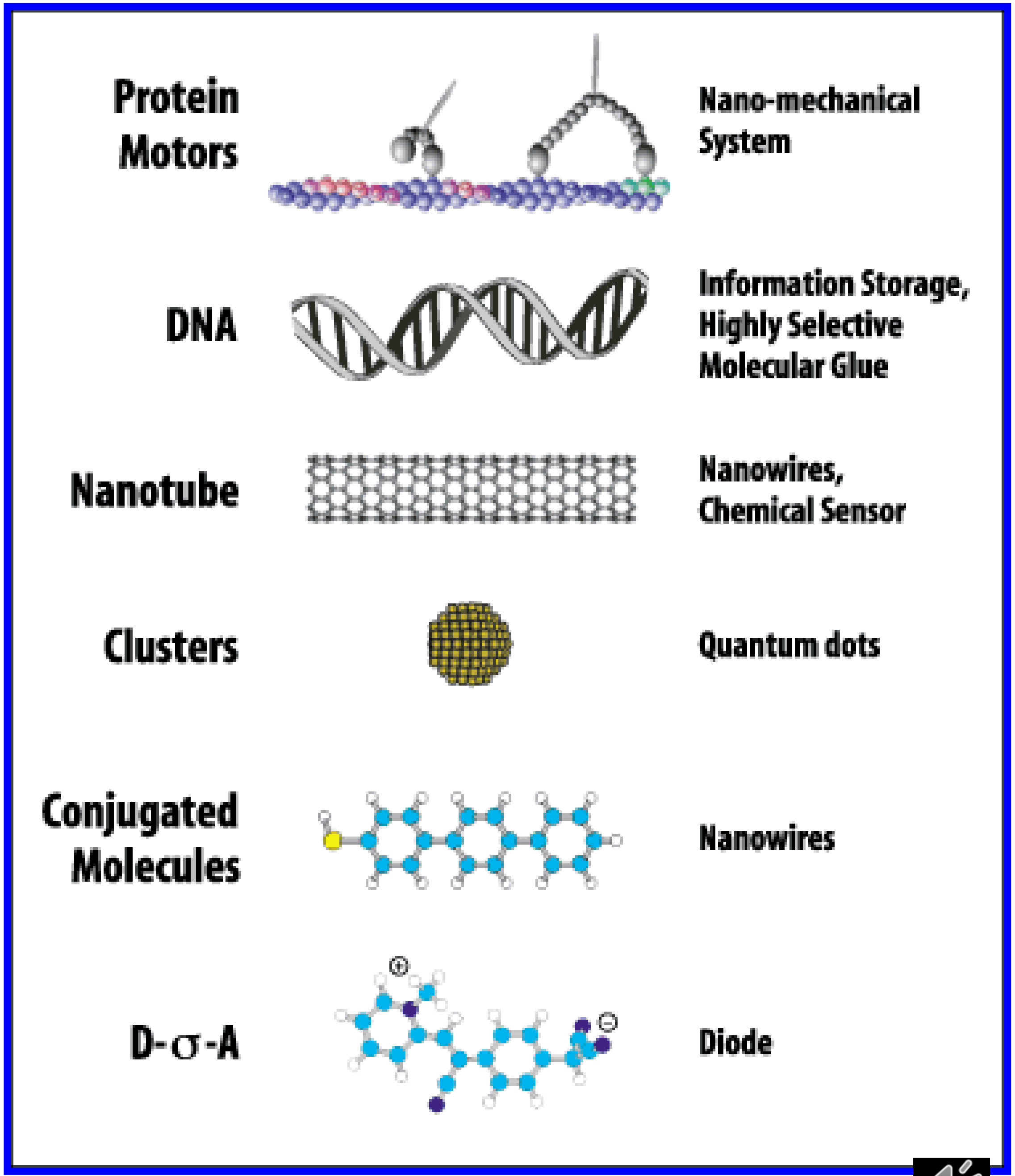
nano (nm)

- viruses
- nuclei
- transistors
- cell apparatus
- proteins


a
Cockroach
b
Human Hair
c
Pollen Grain
d
Red Blood Cells
e
Cobalt Crystal Lattice
f
Palladium Half-shells
g
Aspirin Molecule
1 mm
1,000 µm
100 µm
10 µm
1,000 nm
1 µm
100 nm
10 nm
1 nm
0.1 nm
1 Å

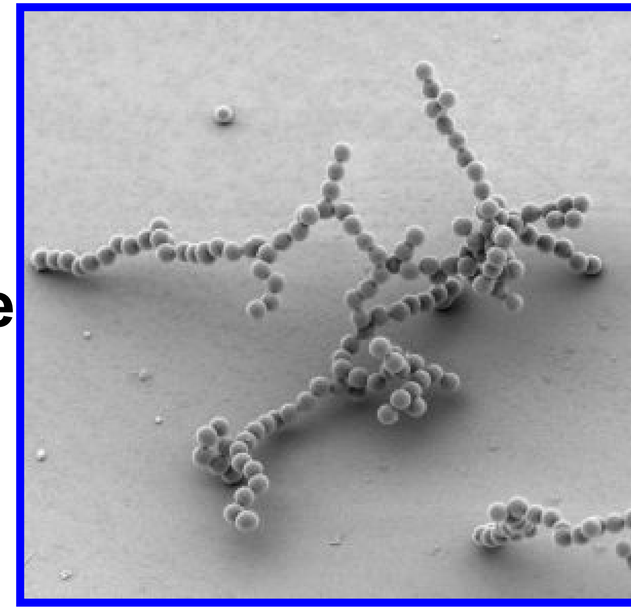
Nanobiotech systems

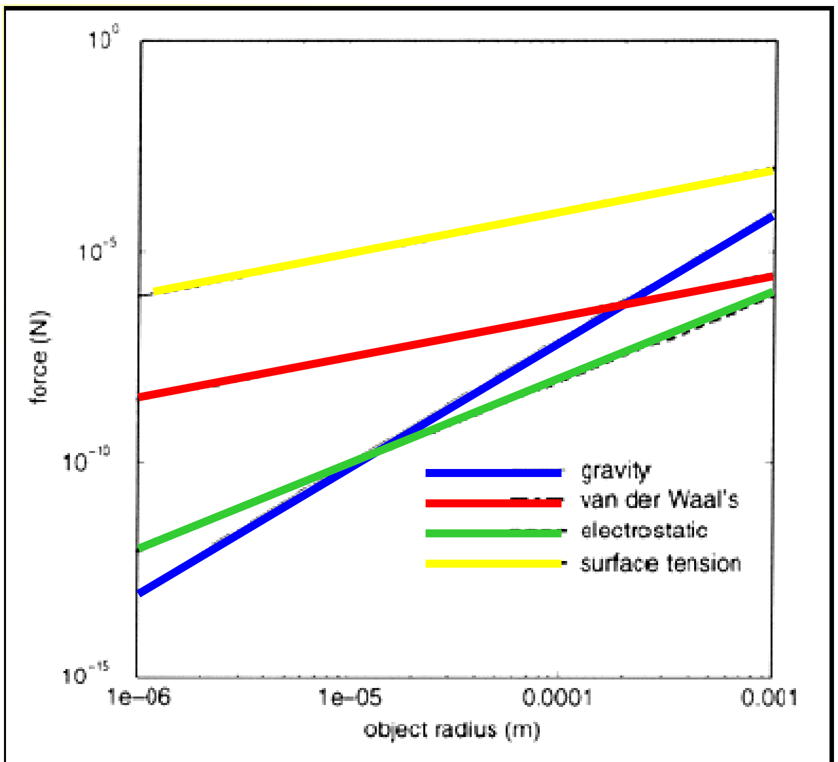
- some examples from different fields

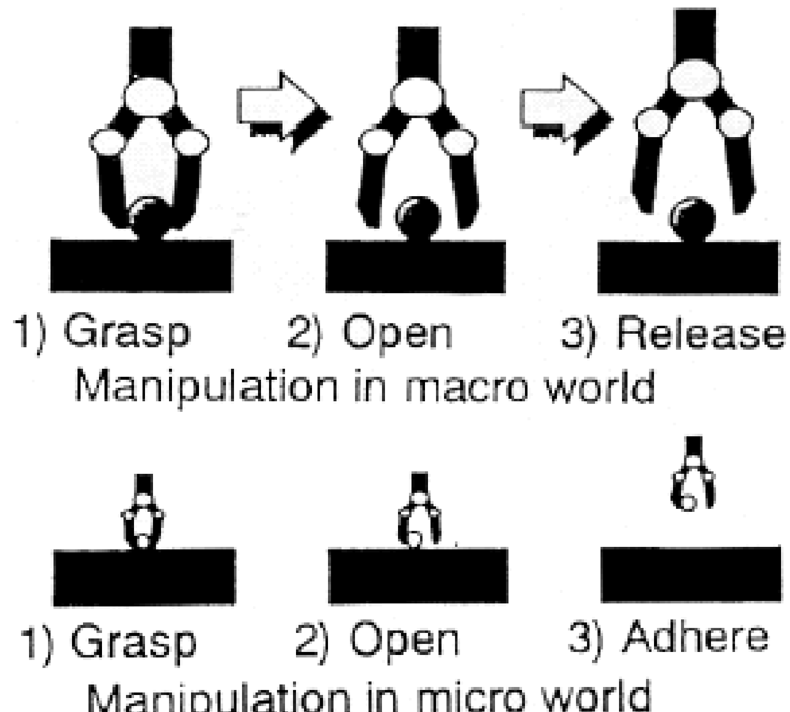


Scaling effects

If a system is reduced isomorphically, the changes in length, area and volume ratios alter the influence of various physical effects that determine the overall operation







Gravity and inertia are negligible at the nanoscale

- **macroscopic objects - dominated by properties of mass**
  - cm to m sizes - physical properties such as friction, tensile strength, adhesion, and shear strength are comparable to inertial and gravity forces
  - this balance changes, however, for larger or smaller objects
  - increase in inertia or weight can quickly overcome the increase in strength in a large structure such as a building (common sense to add extra support)
- **opposite effect for smaller and smaller objects**
  - um-sized objects (nanoparticles, individual cells) interact differently
  - inertia is no longer a relevant property
- **actions of small objects are dominated by their interaction with neighboring objects**
  - fine dust stays suspended in the air instead of dropping quickly
  - nano-objects in water undergo random Brownian motion
  - attractive forces between small objects are stronger than gravity

Scaling laws

Laws of physics make the small world look different.

Volume	$V \sim L^3$
Mass	$M \sim L^3$
Surface	$SA \sim L^2$
Strength	$S \sim L^2$
Force	$F \sim L^2$
Acceleration	$A \sim 1/L$
Frequency	$f \sim 1/L$
Power	$P \sim L^2$
Power density	$P \sim 1/L$

** Assumes constant mass density*

Voltage	$V \sim$ constant
E Field	$E \sim 1/L$
Resistance	$R \sim 1/L$
Capacitance	$C \sim L$
Current	$I \sim L$
Magnetic wire	$B \sim$ constant
Heat capacity	$C_v \sim L^3$
Heat flow	$dT/dt \sim 1/L^2$
Turbulence	$Re \sim L$

** Assumes constant voltage*

Typical small values

How the world looks to a “micro” sized device

Quantity	*Size = 100 µm*	*Size = 10 µm*
Typical volume	1 nanoliter	1 picoliter
Typical mass	1 microgram	1 nanogram
Typical force	10-100 nN (1-10 ug)	0.1-1 nN (10-100 ng)
Typical E field (at 1 V)	10,000 V/m	100,000 V/m
Typical frequency	10-100 kHz	0.1-1 MHz
Typical time constant	10-100 µsec	1-10 µsec

Some small scale phenomena

At the atomic level, we have new kinds of forces and new kinds of possibilities, new kinds of effects.

Richard Feynman

Surface tension

Surface tension force for 100 µm opening = 5.7 µN
Typical force for 100 µm device is 10 nN

Surface tension over 500x greater!

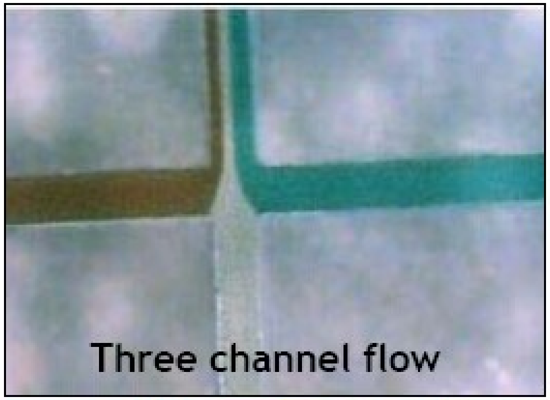
Surface tension
$P = \gamma / d$

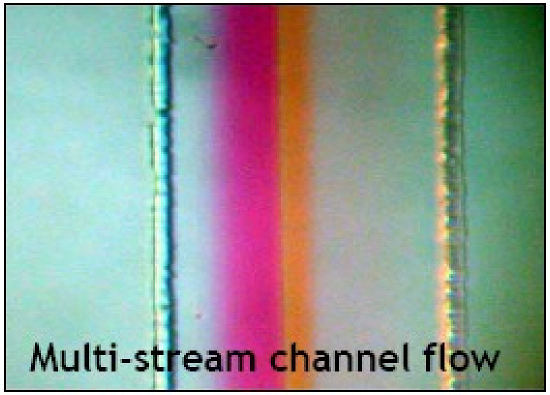
Practical significance: Surface tension **rules at the microscale**. Deal with

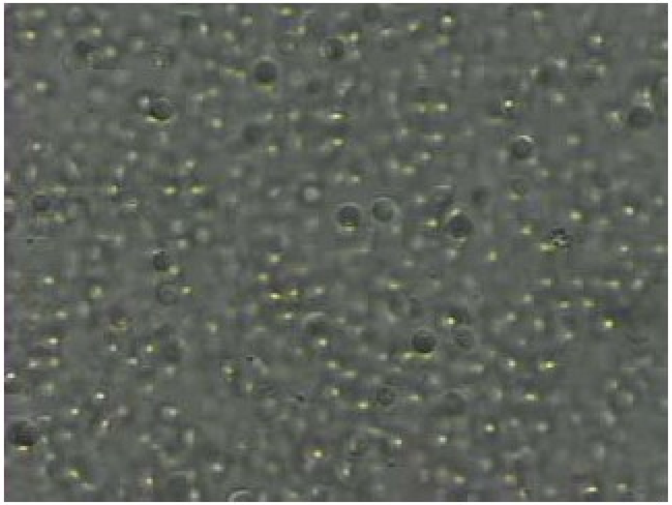
Laminar flow

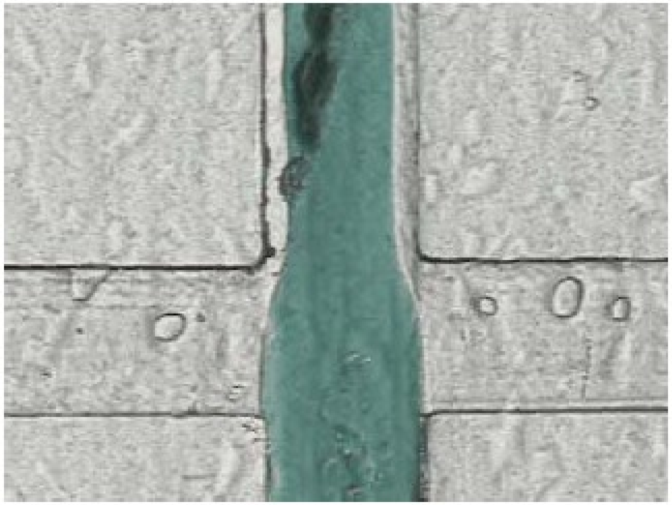
Reynolds Number (Re)scales as length.
Typical Reynolds Number for 100 μm device is Re ~ 0.1

Onset of turbulence is at Re ~ 2000


Three channel flow


Multi-stream channel flow


Blood flow


Three channel flow

Characteristic flows in micro channels are **smooth and viscous with no mixing.**

Rapid heat transfer

Surface area to volume is large at small scales.
Heat capacity depends on mass.

Heat transfer is very fast at small scales.

Temperature of small filament in 75 W light bulb is ~2500 °C.

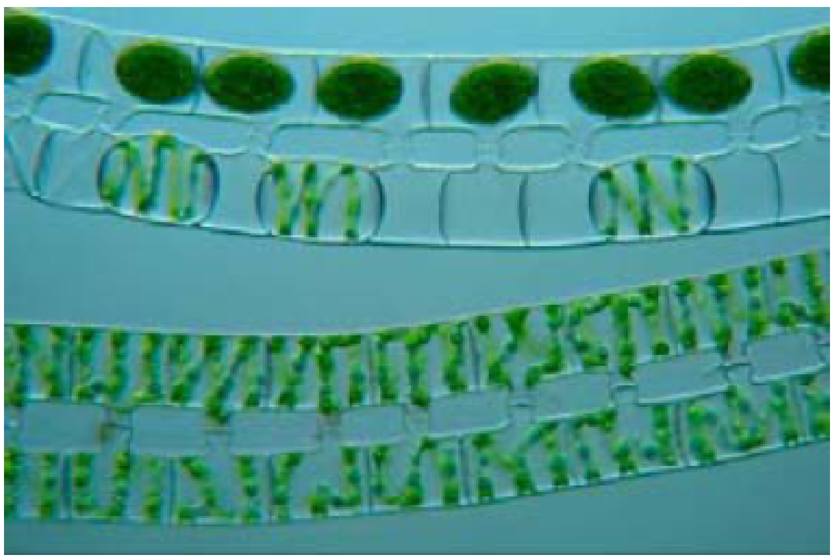
Ramp up time is ~20 ms.
Ramp down time is ~60 ms.

Heat flows through small devices quickly.
Hard to maintain temperature gradient.

Surface area / volume

Surface area to volume is large at small scales.
Mass flow saturates quickly in small volumes.

Equilibrium can be reached very quickly.



Micro-scale systems must utilize physical barriers (cell walls) to maintain concentration gradients.

Surface contamination is a serious issue at small scales.

Mass flows through small devices quickly.
Hard to maintain concentration gradient.

Scaling and diffusion

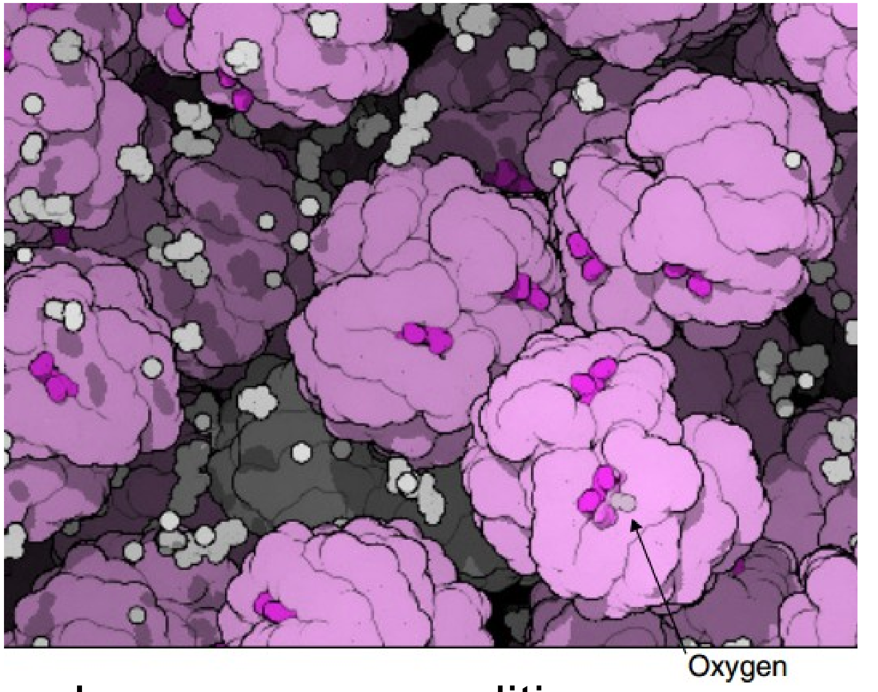
for a small molecule $D \approx 5x10^{-5}$ m^2/s

Volume	1 µL	1 nL (10^{-9} L)	1 pL (10^{-12} L)	1 fL (10^{-15} L)	1 aL (10^{-18} L)
Length of 1 cube side	1 mm	100 µm	10 µm	1 µm	100 nm
Time to diffuse over a cube side	500 s	5 s	0.050 s	0.5 ms	0.05 ms
Number of molecules in a 1 µ*M* solution	6×10^{11}	6×10^{8}	6×10^{5}	600	6

mixing by diffusion is very fast at the micro-level

In nature animals made up of more than a few cells cannot rely on diffusion any more to move materials within themselves. They augment transport with hearts, blood vessel, etc.

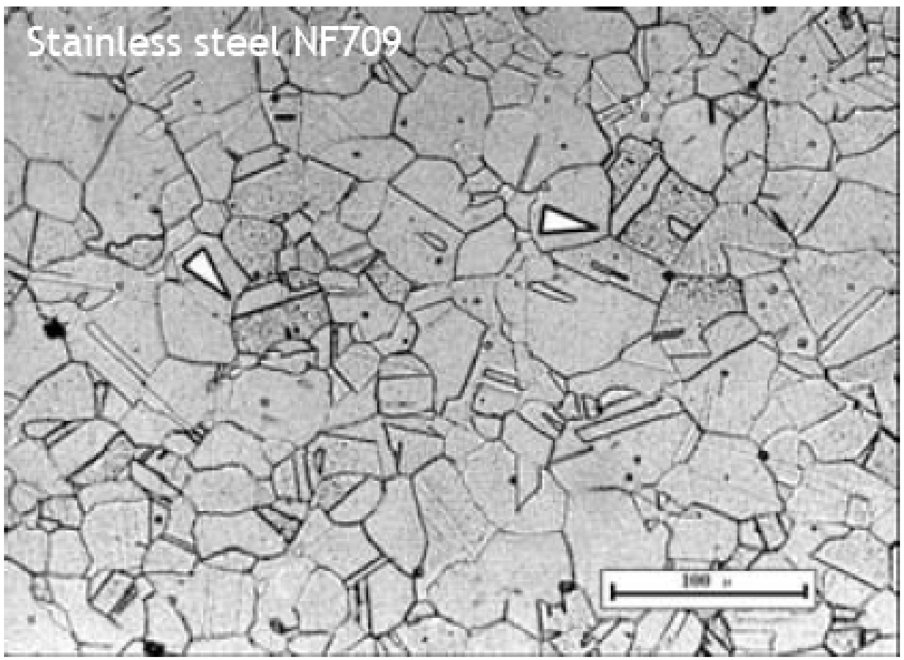
Oxygen storage



- **macro level**
  - high pressure **tanks**
  - delivered in a continuous stream in tubes
  - flow controlled by valves
- **nano level**
  - transported by individual molecules, low pressure conditions
  - molecules meet with the carrier – **hemoglobin** by random diffusion, resulting in formation of a complex
  - co-operative effects allow to gather oxygen efficiently when its content rise and discharge oxygen completely when its level drops

Loss of continuity

At sizes below ~50 μm, granularity of nature becomes relevant.
Many bulk-scale physical laws no longer accurate.

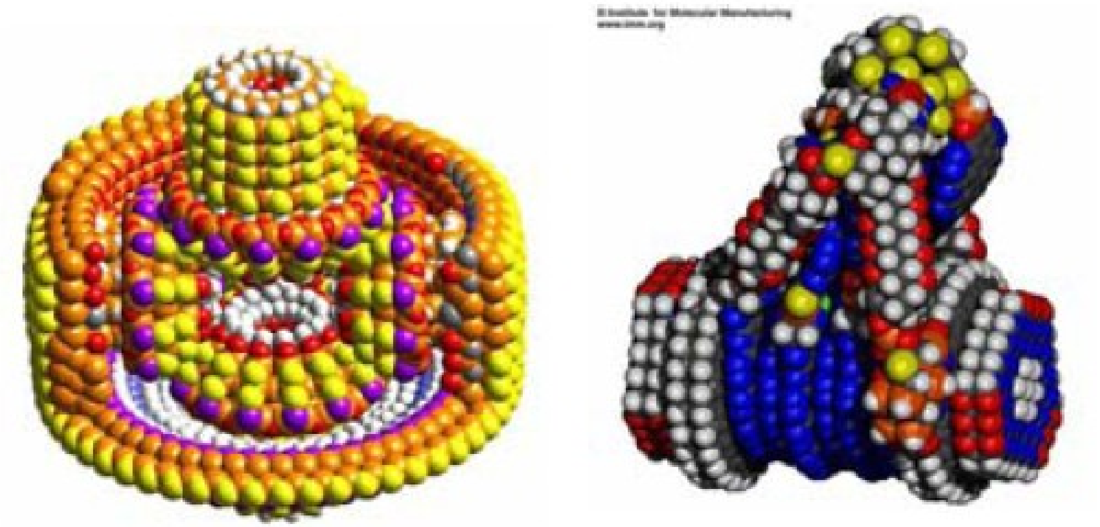


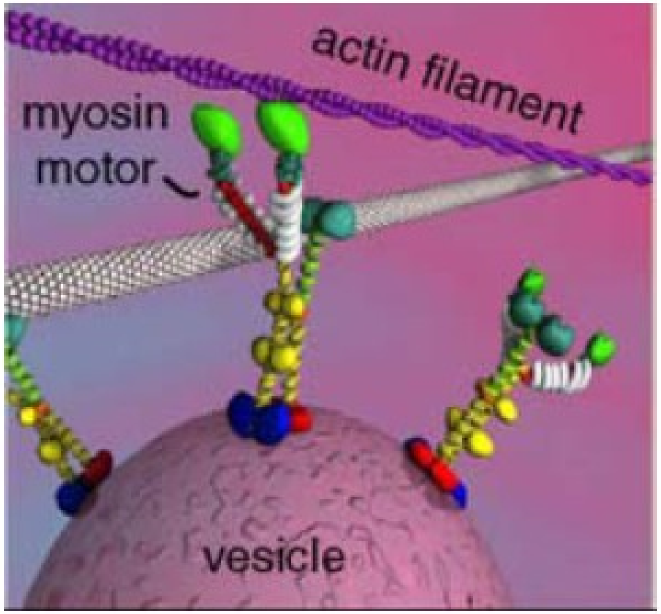
Metals and materials are not continuous materials. They have microscopic grain structure.

- Typical grain size is ~10 μm. Affects physical, thermal and electrical properties.
- Mean free path of N_2 at atmosphere is 60 nm. Affects dynamics in air. Example: Paschen effect.

Phenomena at the nanometer scale: atomic granularity

At sizes below ~100 nm, bulk properties are meaningless.
Atomic level understanding required.



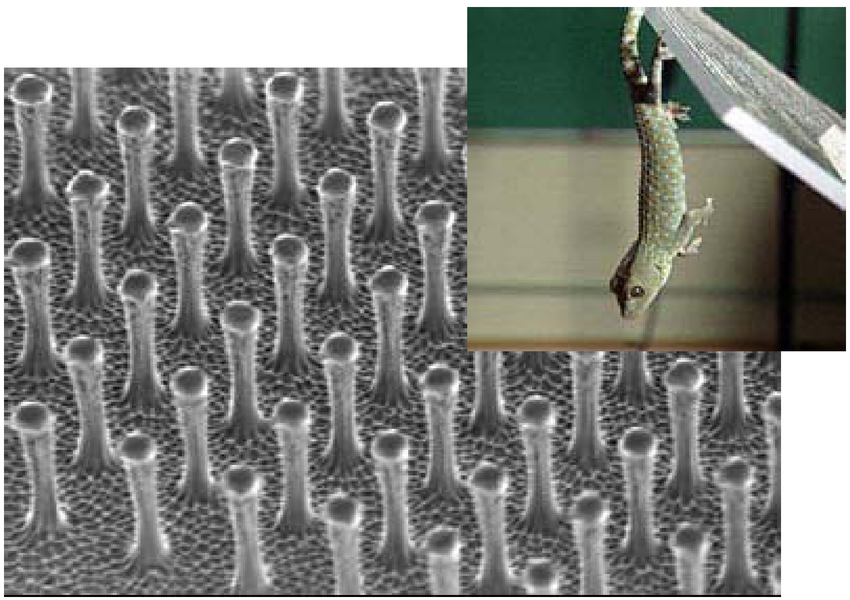


Hypothetical molecular motors (science fiction)
From: Institute for Molecular Manufacturing

Real molecular motors inside living cells

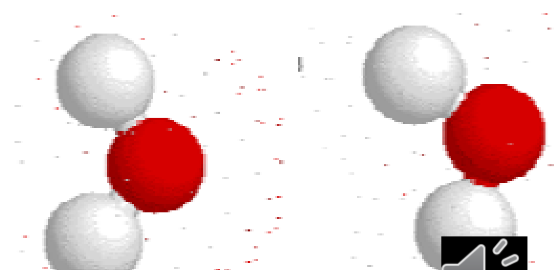
Surface forces

Residual forces, such as local electrostatic charging, hydrogen bonding, dipole interactions, and Van der waals forces play an important role.



Micro-machined gecko hairs (0.2 µm x 2 µm) made of plastic make dry tape and sticky fabric.

- Small scales allow very close interaction (e.g., Van der waals) and large surface interface.
- Dry state is favorable for electrostatic charging
- Wet/humid state mediates hydrogen bonding

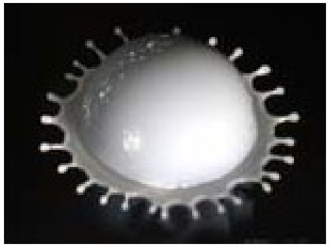


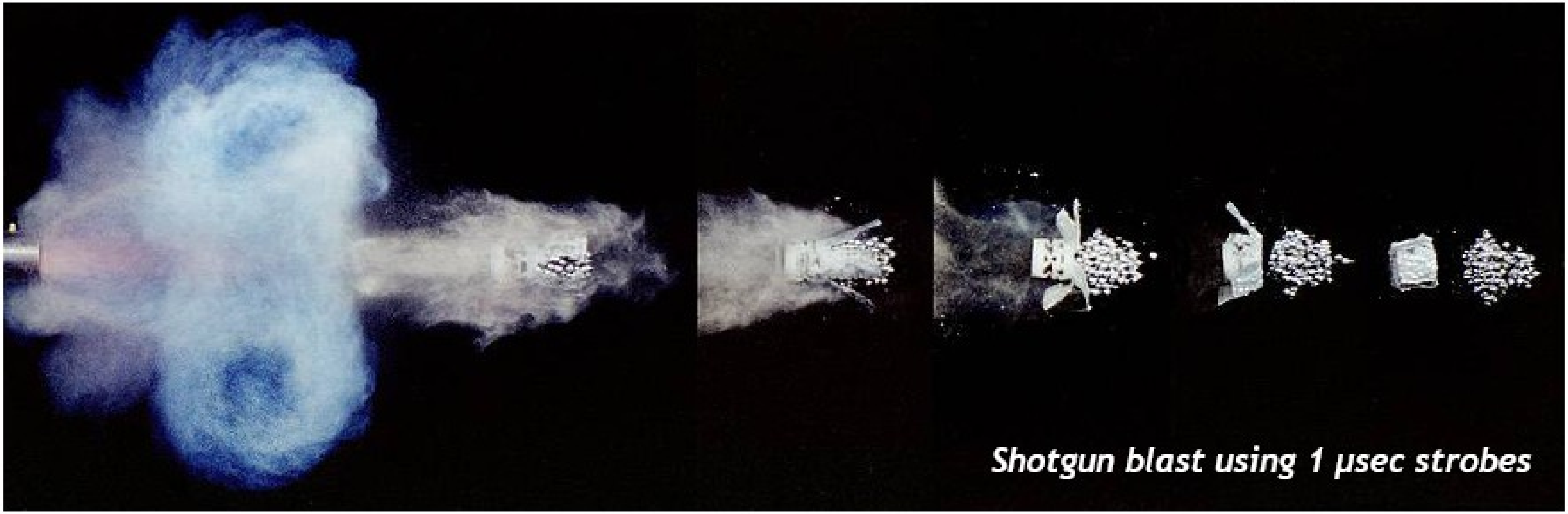
Hydrogen bonding

Fast time scales

Typical time constant is 10-100 μsec

(10 μsec = 10 mm of bullet fired from high speed gun)



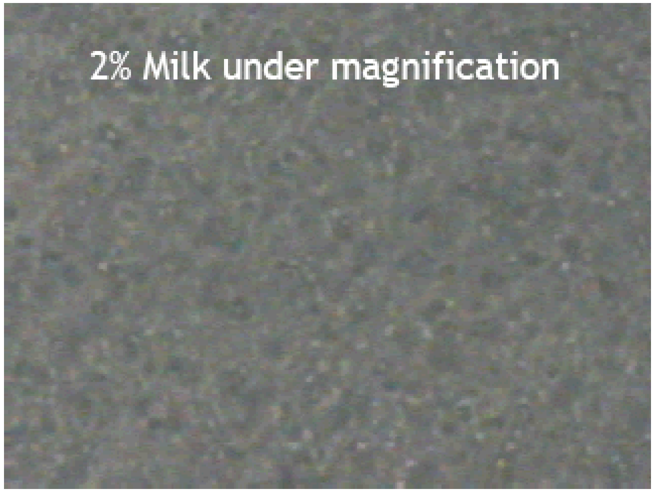


Shotgun blast using 1 μsec strobes

Hummingbird heart beat: 50 msec
Honeybee wing beat: 4 msec
Vibrating drop of water (100μm): 120 μsec

Some VERY small scale phenomena: Noise

At sizes below ~50 μm, thermal induced fluctuations are noticeable. Heat is not a continuous "fluid".



Microscopic flow of milk particles showing Brownian motion (random vibrations).

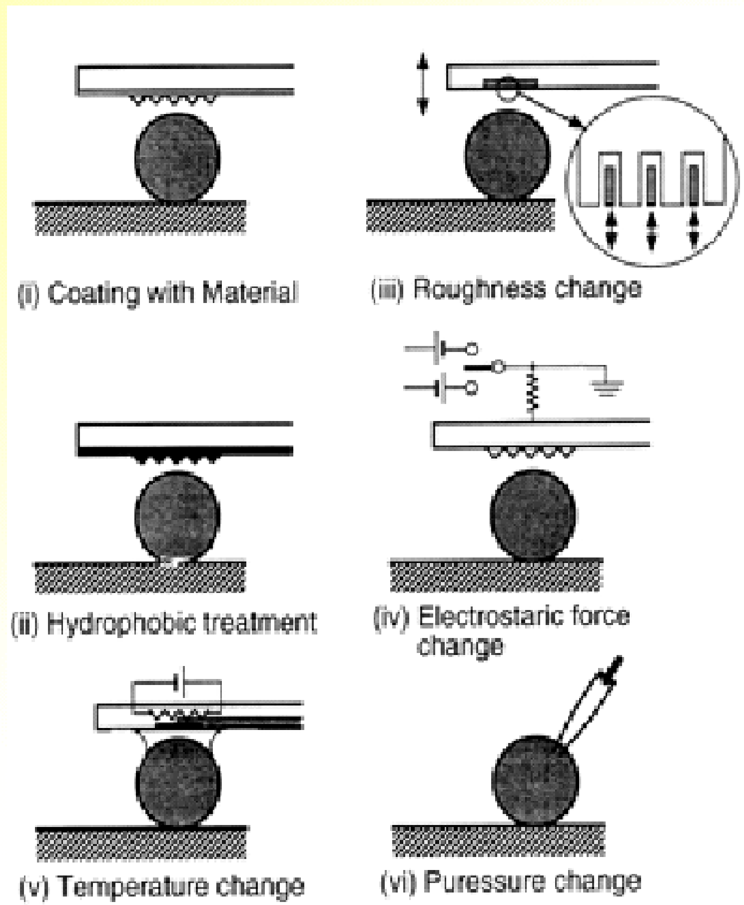
- Heat is not "fluid". Vibrations and Brownian motion add statistical fluctuation and noise.
- Thermal fluctuations depend on size and temperature.

How to deal with small objects: techniques to reduce adhesion

To reduce van der Waals forces

To reduce surface tension

To remove water molecules - reduce to surface tension



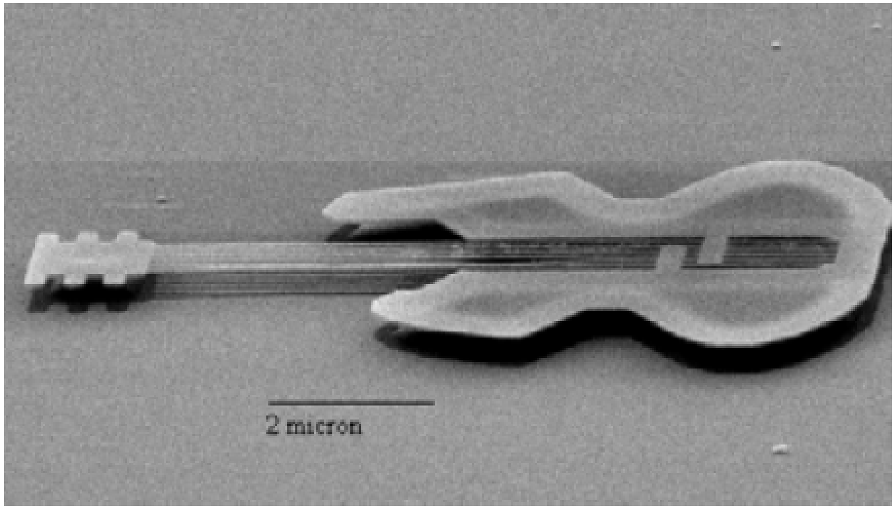
To reduce van der Waals forces

To eliminate static charges

Pressure generation

Engineering small: How small is small enough?

How small is small enough?



Miniature guitar micromachined in silicon at Cornell University, 1997. Fun demo.

- From engineering perspective, need to solve a real problem.
- After a point, integration more important than miniaturization.
- Academic research: Hubris and professional standing push for smaller.
- Technology for technology sake?

Engineering small: How small is small enough?

different perspectives, different communities

- **sub-miniature (~ 1 mm)**
  conventional precision engineering, primarily metals, glass

- **micro-electrical mechanical systems (MEMS~10-100 µm)**
  sensors and “useful” devices, primarily in silicon

- **nano-electrical mechanical systems (NEMS~100-1000 nm)**
  electronic devices, concept devices, primarily in silicon

- **nanotechnology (<100 nm)**
  mostly materials

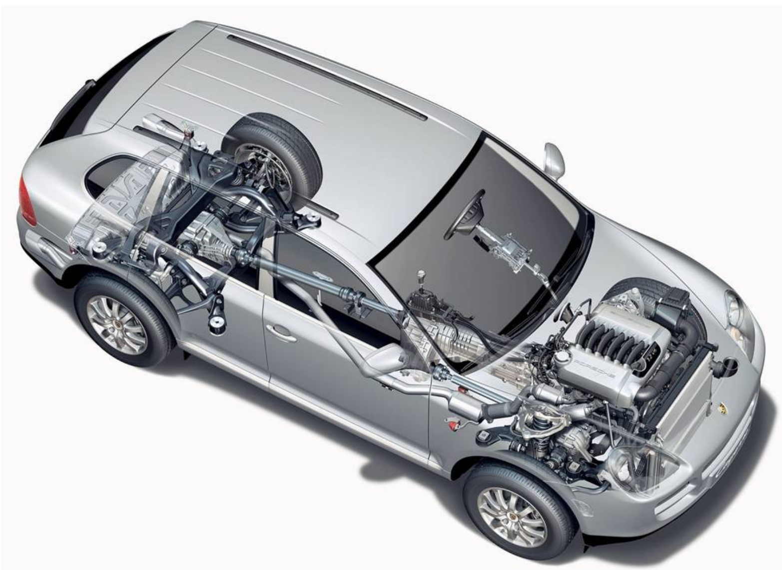
Engineered systems

how is a system engineered today?

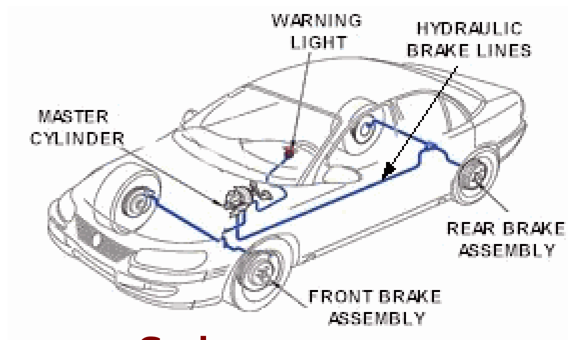
- **materials**
  basic building stuff, often with special properties processed in batch
- **components**
  simple parts designed to be put together
- **devices**
  assembly of components that can perform a simple function
- **sub-systems (modules)**
  assembly of devices designed to perform a complex function
- **systems**
  assembly of sub-systems designed to perform desired application

Engineered systems

Example system: automobile

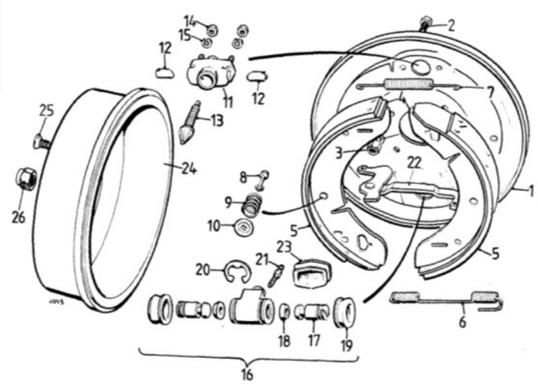


Final system: automobile



Sub-systems

Component

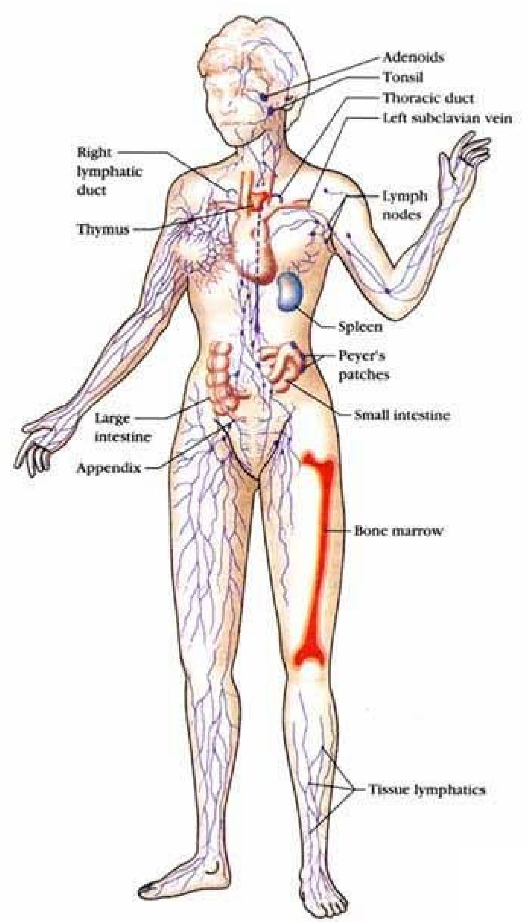


Device (brake)

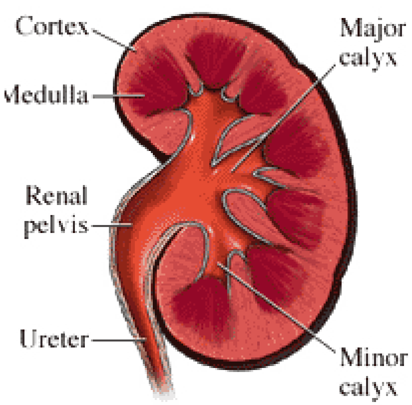
Material

Engineered systems

Example system: human body

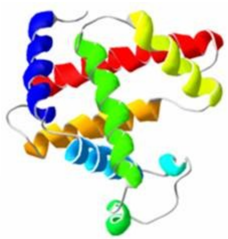


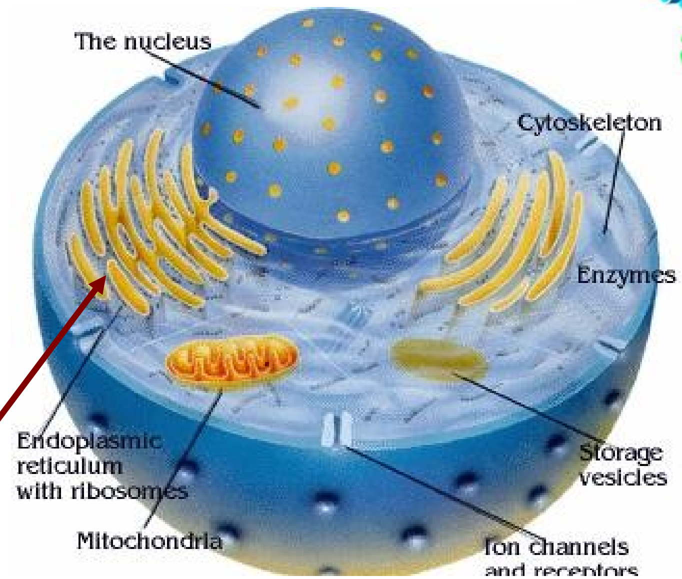
System: Human body



Sub-systems
(organs, cells)

Component
(proteins)





Device
(Ribosomes)

Material
(amino acids)

Engineered systems with nanotech

Engineered systems can take us to wonderful places…

Enabled by very small technology

The challenges of nanobiotechnology

Spatial fidelity

molecule < molecular structures < organelles < cells

1 nm	10 nm	100 nm	1 um	10 um
proteins	coated pits - caveolae		mitochondria	nucleus

Imaging:

atomic force microscopy

electron microscopy

optical microscopy

mass spectrometry

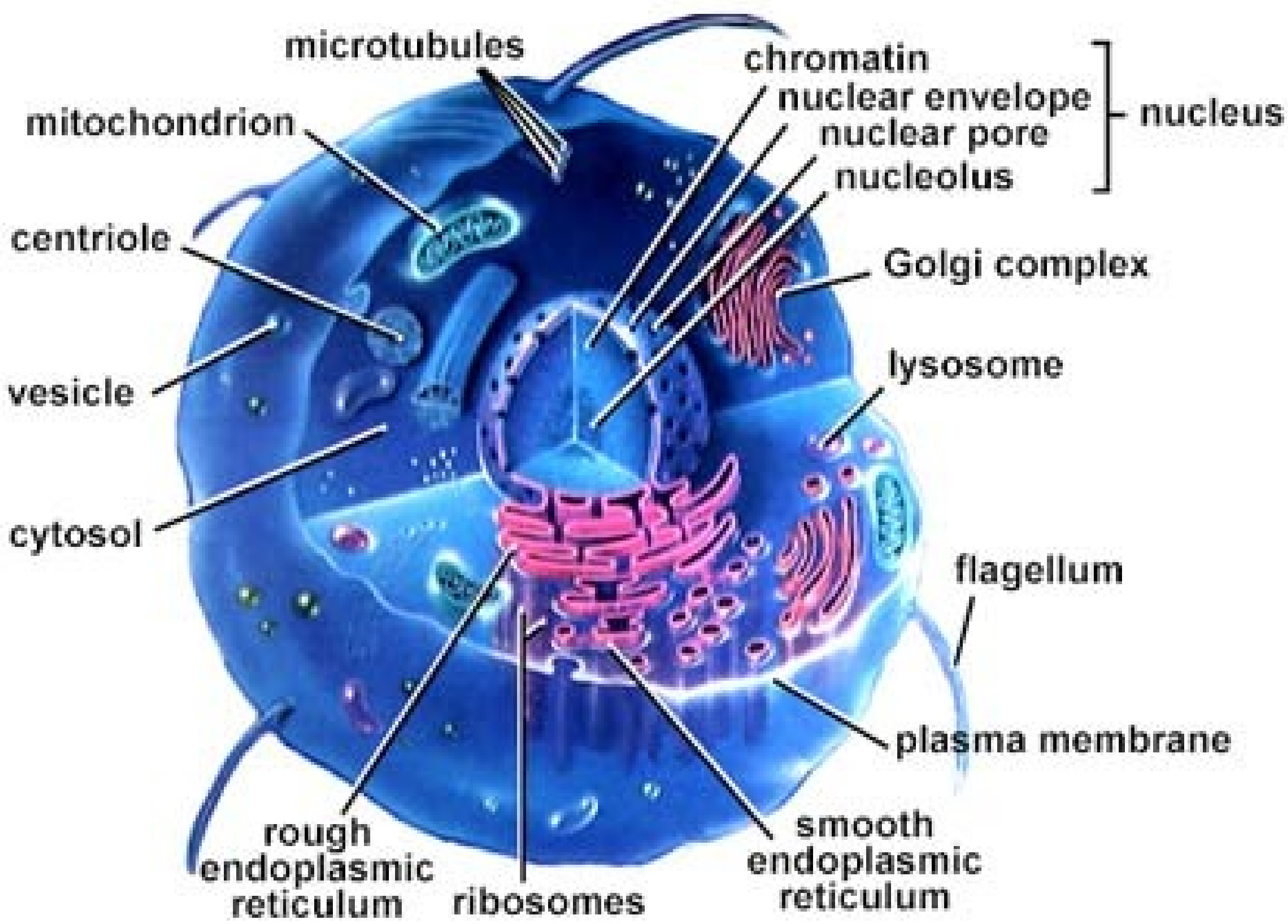
Spectroscopy:

optical (fluorescence) spectroscopies

Time scale of events

ps	- bond vibrations	triggering	physics
ns	- conformations		
us	- binding	associations	chemistry
ms	- reactions	flux	
s	- regulation	transport	
ks	- movement	regeneration	biology
Ms	- development	fetal Growth	
Gs	- life cycle	disease	

Heterogeneity



many compartments, many structures, many surfaces …

as many as 85% of biochemical interactions occur at or in a membrane

Complexity

a small number of needles:

1,000 – 100,000 copies of a particular protein
a few percent are modified at any given time - a few copies per cell?

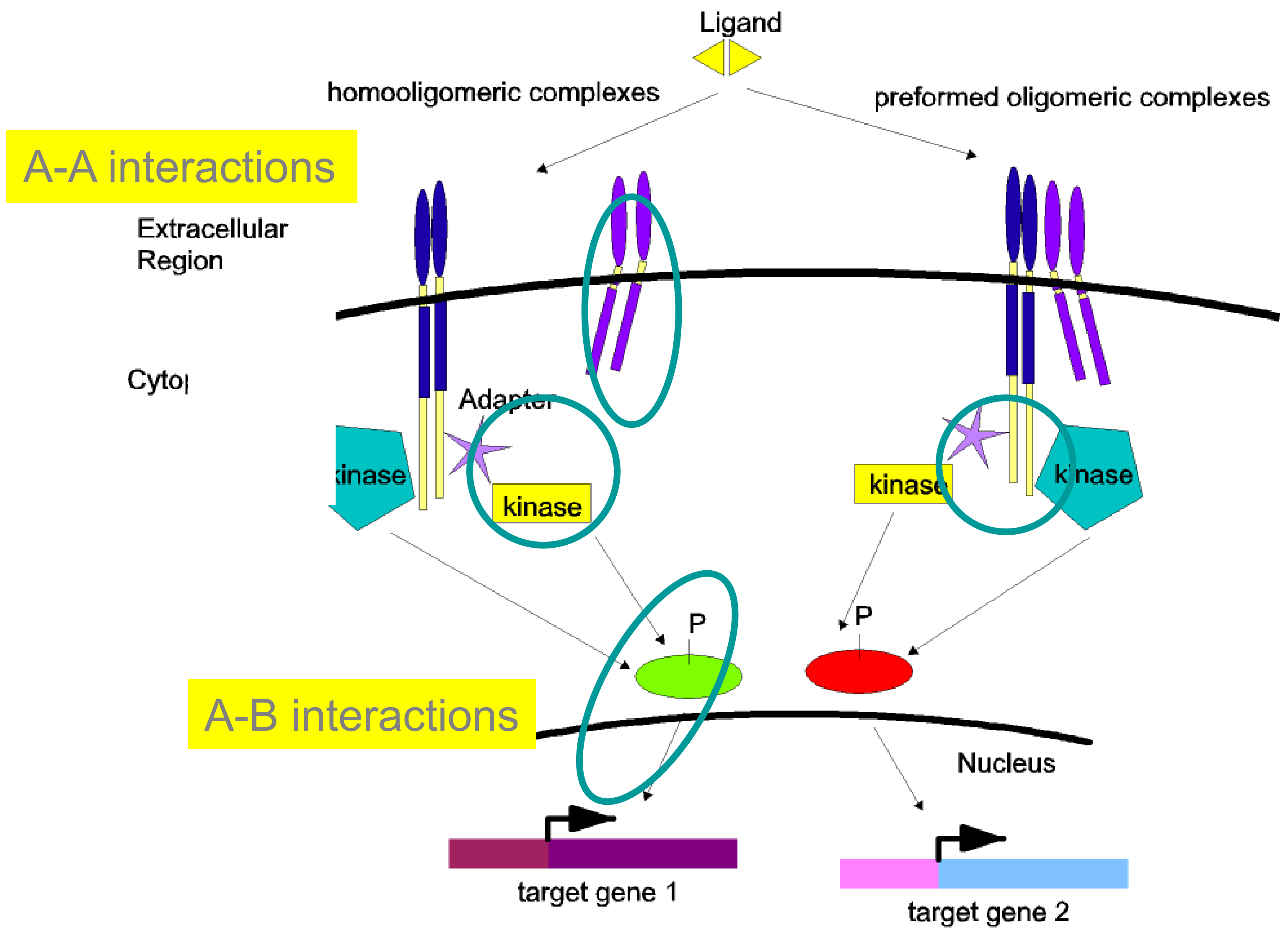
… in a very large hay stack:

~ 30,000 genes
~ 100,000 gene products
> 1,000,000 post-translationally modified proteins

must study specific intermolecular interactions in a mixture

Specificity

measure pair-wise interactions systematically - sequentially - exclusively and whether <u>transient or long lived</u>



Sensitivity

typical

Concentrations in a cell or Densities on a cell surface

N = 100,000 molecules

$V = 10 \times 10 \times 10\ um^3$

$= 1000\ fL = 1\ pL$

c = <u>100 molecules per um^3</u>

c ~ 100 nM

N = 100,000 molecules

$A = 10 \times 10 \times 6\ um^2$

$= 600\ um^2$

c ~ <u>100 molecules per um^2</u>

we need single molecule detection sensitivity to achieve sub-micron resolution

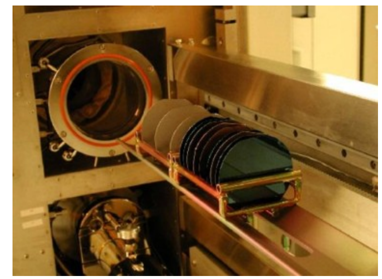
fluorescence can provide 100 counts per molecule per millisecon

Integration

Integrated Nanosystems

Macro world

Microsystems

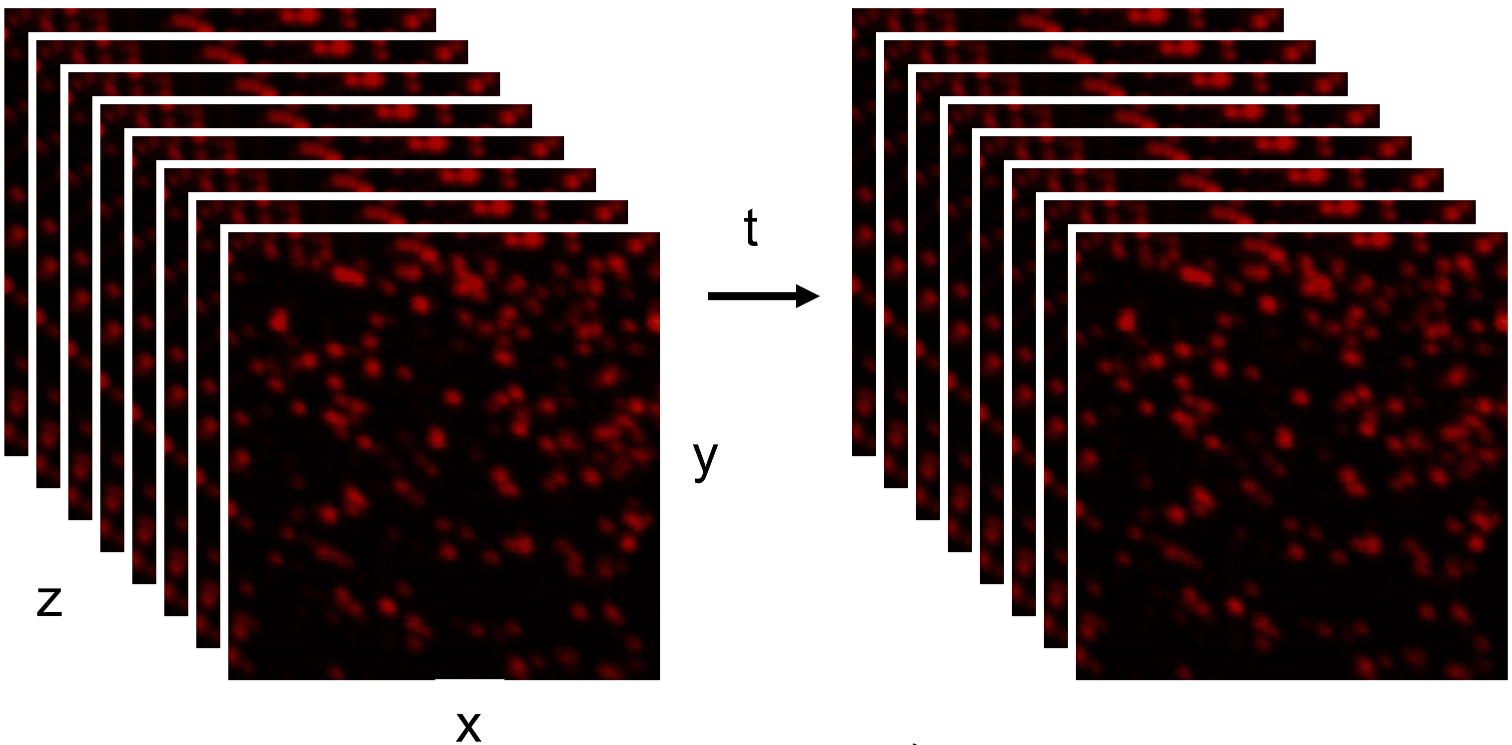


Nano world

Nano is an ***enabling*** technology; ***integration*** is the key!

Data management

probing in space and time leads to a lot of data



(512x512) x 20 x 100 = 512 MB per cell

100 cells per condition

1 CD per cell

1 hard drive per experiment

SIMPLE ANALYSES

Where to meet nano ...

- **security**
  - superior, lightweight materials
  - advanced computing - unbreakable security, biometrics
  - increased situational awareness - sensors
- **healthcare**
  - diagnostics – faster and cheaper, lab-on-chip, point-of-care, personal DNA mapping
  - novel drugs – specific targeting, on-site local treatment
- **resources**
  - energy – effective utilization, alternatives – nanocatalysts for solar generation of hydrogen from water
  - water – purification and desalinization

Nano at internet

- *nano.gov …* National Nanotechnology Initiative, USA
- *education.mrsec.wisc.edu/271.htm* … Univ. Wisconsin video labs
- *nanobio.cz* … our website with AFM resources (CZ)
- *nanocon.eu* … international nano-conference in Brno
- *nanotechweb.org* … IOP Institute of Physics
- *understandingnano.com* …
- *imaginenano.info* .. MathScience Innovation Center
- *nisenet.org* … Nanoscal Informal Science Education
- *nano-ed.org* … NSF-supported web community
- *trynano.org* … IEEE

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- *nanocon.eu* … international nano-conference in Brno
- *nanometrologie.cz* … Czech Metrological Institute (Gwydion)

Conclusions

- **modern science pushes the limits of**
  - creativity
  - understanding
  - technology
  - information management

- **need**
  - ability to ***collect*, *store* and *retrieve*** large data sets **rapidly**
  - ability to ***analyze* and *reduce*** information to comprehensible principles in ***real time***
  - ability to ***visualize* and *present*** conclusions and data
  - ability to ***interpret*** in context of biological problem