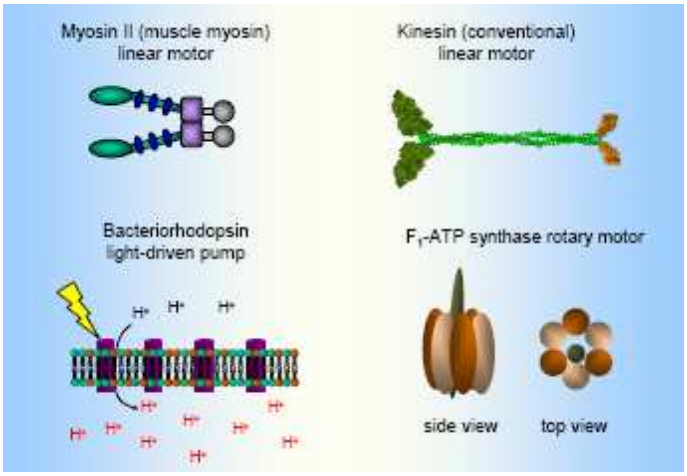
Biological systems

- … the technological point of view

- modular and replaceable parts
- molecular motors with specific targeting
- durable
- catalytic at ambient temperatures
- “bottom-up” manufacturing
- self-assembly
- genetically re-engineered
- mass produced

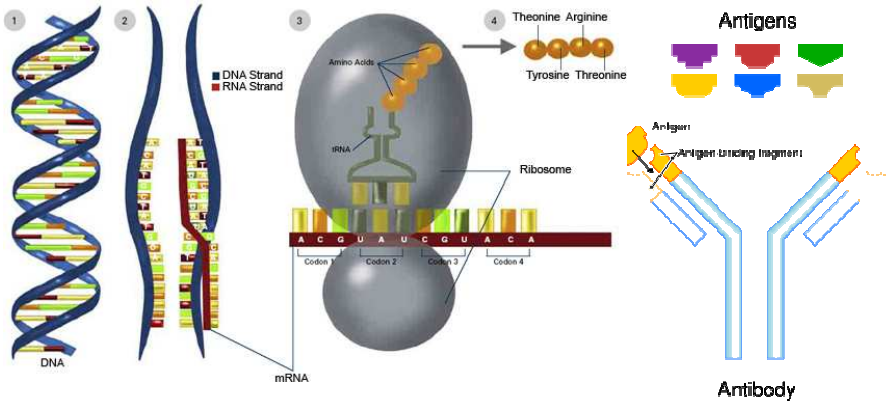
Biological systems, Continued



examples of nanoscale actuators and pumps - modular and replaceable parts
natural “components” possess a wide range highly desirable, intrinsic properties (e.g., thermostability, energy conversion, actuation, …)
can be isolated and engineered for integration as structural and functional modules of nanoscale materials and systems

Molecular motors with specific targeting

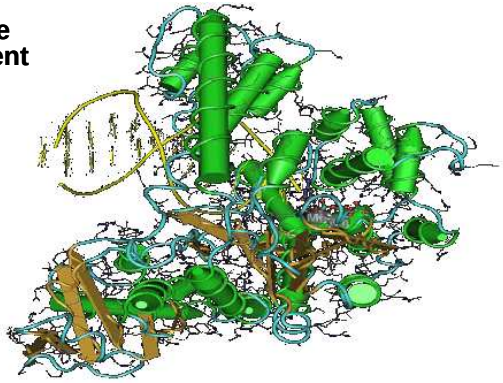
- circular, linear, and antibodies



- DNA polymerase … linear motor; antibody … targetting
- youtube.com (the inner cell)
- complication – working in water

Durable …

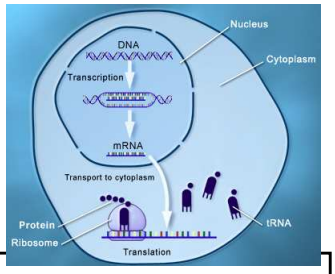
- **organisms can live in hostile environments - they possess proteins and other components that can withstand extreme conditions**
- **secondary structure – helices, sheets is a result of hydrogen bonding, a major factor in stability**
- **contributes to the enzyme's ability to work at high temperatures**
- **enzyme was cloned from a bacteria living in the thermal pools of Yellowstone National Park**
- **many common enzymes are catalytically active at ambient temperatures**

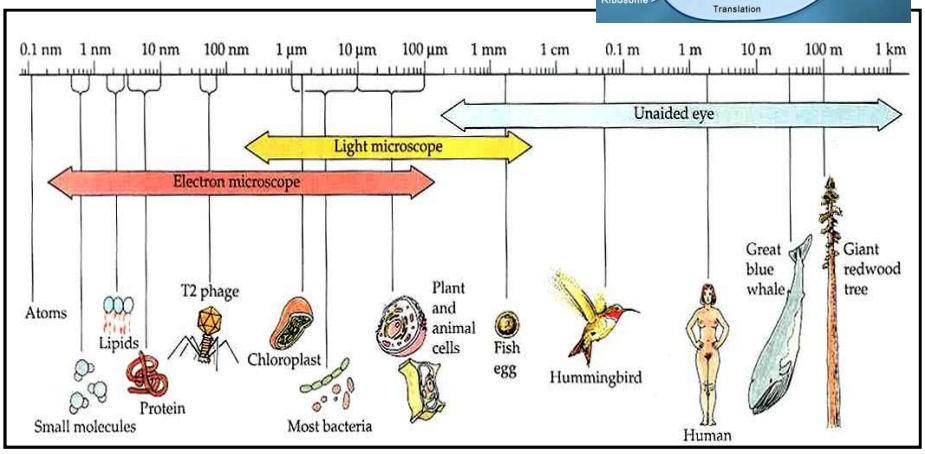


- Taq DNA polymerase
  www.dnai.org for details

“Bottom-Up” manufacturing

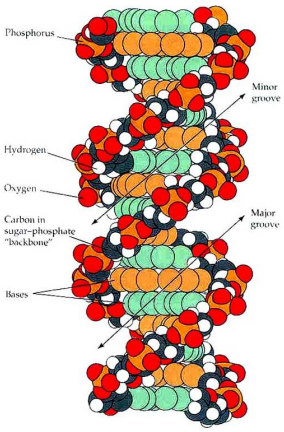
- biological systems are manufactured from “bottom up,” can self-assemble, be re-engineered and mass produced
- strategies from the nanometeric to metric scales

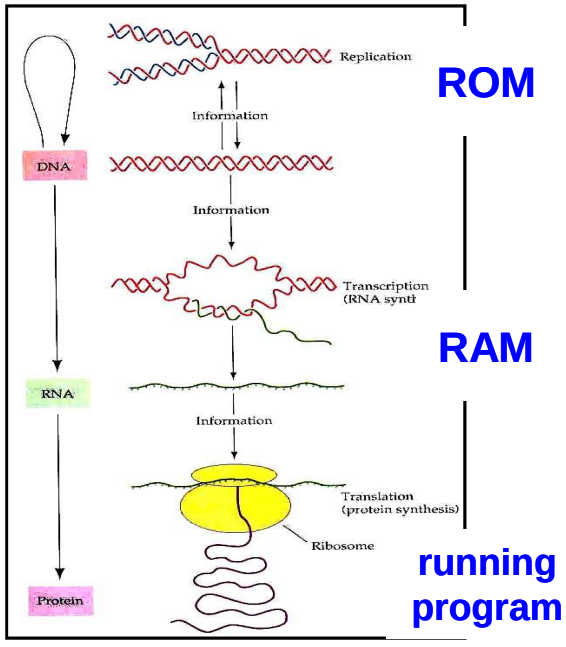




Levels of information in bio systems

- analogy with electronic data storage / manipulation



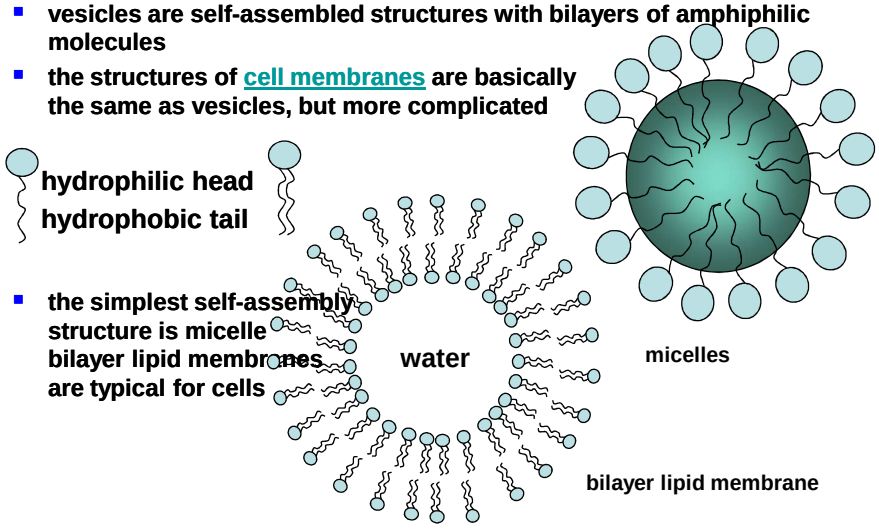


Self-assembling of biosystems

- **… assembly of molecules without use of outside source.**
- **Examples:**
- **proteins can assembly while being synthesized, some can reassemble after being denatured**
  - some require help from other proteins to assemble correctly (chaperons), some undergo post-translational modifications
- **our goal: understand, exploit, and engineer structural and functional biomolecules to assemble integrated nanomaterials and nanodevices with unique properties**

Amphiphilic molecules

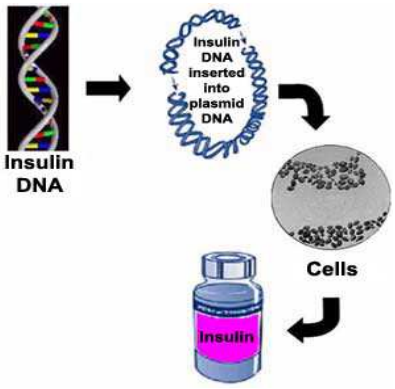
- **self-assembly occurs spontaneously in nature, e.g. in cells**
- **vesicles are self-assembled structures with bilayers of amphiphilic molecules**
- **the structures of cell membranes are basically the same as vesicles, but more complicated**



- **the simplest self-assembly structure is micelle bilayer lipid membranes are typical for cells**

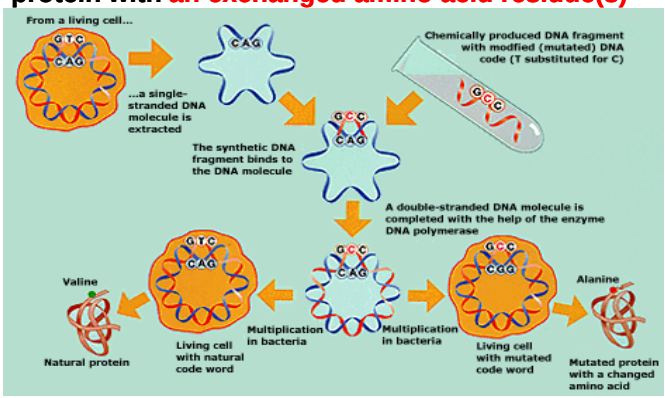
Genetic engineering … Insulin

- people suffering from diabetes need to watch their diet and inject insulin to control their blood sugar levels
- insulin originally taken from pancreas of pigs
- the DNA coding for human insulin was isolated from human cells, inserted into a small ring of DNA called a plasmid which was placed in production cells – large quantities easily available
- this recombinant insulin can then be purified and given to diabetics



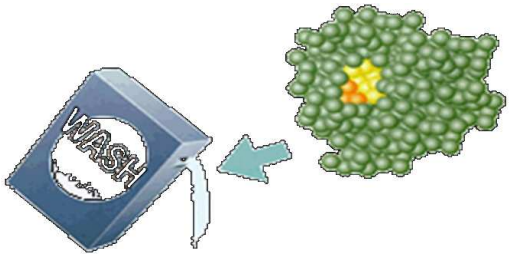
Genetic re-engineering

- site-directed mutagenesis technique
  - Nobel prize in chemistry to Michael Smith in 1993
- the information in the genetic material can be changed - a synthetic DNA fragment is used as a tool for changing one particular code word in the DNA molecule
- this reprogrammed DNA molecule can direct the synthesis of a protein with an exchanged amino acid residue(s)



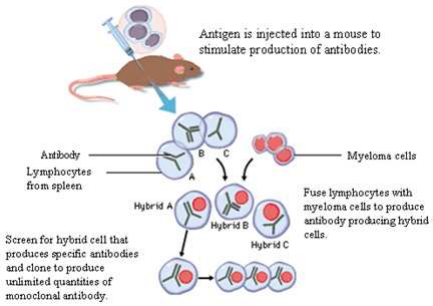
Protein engineering

- **protein design - to improve the stability or modify activity of enzymes**
- **washing process - by specifically changing an amino acid (orange) close to the catalytic region (yellow)**
  - the enzyme can afterwards survive the chemicals also needed to make our clothes clean
  - Genencor International - specifically changed amino acids in subtilisin (a fungal protein degrader), so that it would work better in the washing machine
  - fungi were treated with chemicals that causes changes in the their DNA code and then grown under “washing machine” conditions
- **this resulted in a fungi providing enzymes working well under those specific conditions**



Mass production

- **to produce antibodies, antigen is injected into a mouse**
- **immune cells (B cells – lymphocytes, from spleen) become amplified and produce polyclonal antibodies (Ab) to this target antigen**
  - limited source, undefined product, animal-dependent
- **better results – monoclonal Abs.**
  - isolated B cells are fused with myeloma cells (an immortal cell line)
  - the fused cells are screened to find the ones that produce the best antibody - specific for one site on a target
- **result - an immortal B cell line producing one type of antibody**
- **once the fused cells stabilize, they can be cultured indefinitely**

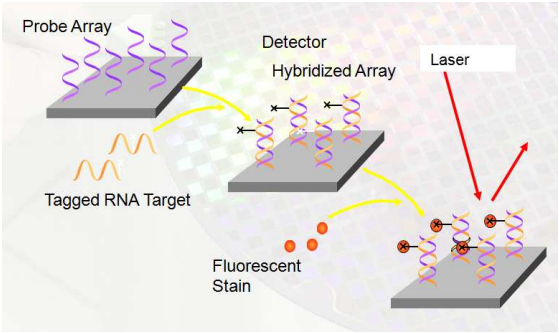


Current applications

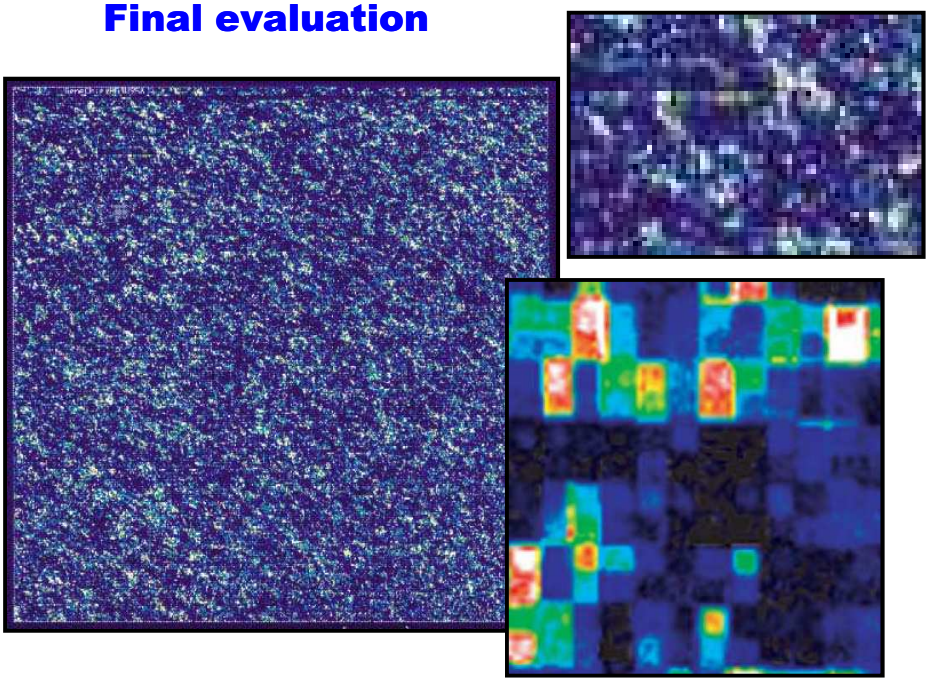
- **in vitro biological screening**
  - DNA, Hybridization, Detector Arrays
- **Lab-On-A-Chip**
  - PCR, Molecular Diagnostics
- **drug delivery and targeting**
  - In Vivo Molecular Medicine
- **sensors, biosensors**
- **smart medical devices**
  - dental, glucose, electric nose
- **Tissue engineering**
  - implants, transplantation

Biological screening

- **a gene is "expressed" when the protein is made (the gene is "on")**
  - since the process of making a protein starts with the making of the mRNA, its levels allow to quantify gene expression
  - useful to determine function of a gene as well as finding drugs to fight a disease
- **methods – hybridization of a chip with immobilised DNA probes with the sample, fluorescent evaluation**



Final evaluation



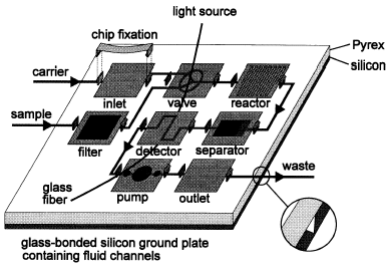
Areas of use

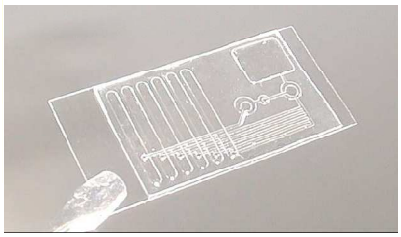
- **Agricultural Biotechnology**
- **Environmental Testing**
- **Food Testing**
- **Livestock Diagnostics**
- **Identity Testing**
- **Individualized Medicine**
- **Human Diagnosis**
- **Basic Research**
- **ethical issues:**
  - should we screen newborns before birth for conditions and diseases?
  - will everyone have access to screening?
  - what are social implications of newborn screening?

Lab-on-a-Chip

Basic idea

- **Miniaturize fluidic systems in the same way that electrical systems were miniaturized for the IC industry.**
- **Reduce a chemical laboratory in size so that it can be placed on a chip.**
- **Same or similar processes as in macro, but different technology**



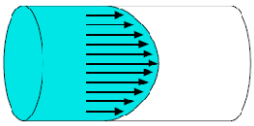


Active components

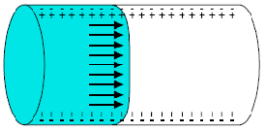
– Micropumps, microvalves, microsensors, micromixers, microreactors

Flow drive

hydrodynamic flow
Fluid flow profile
• Parabolic shape
• No-slip at walls



eletrokinetic flow
Fluid flow profile
Flat front shape
"Moving" walls



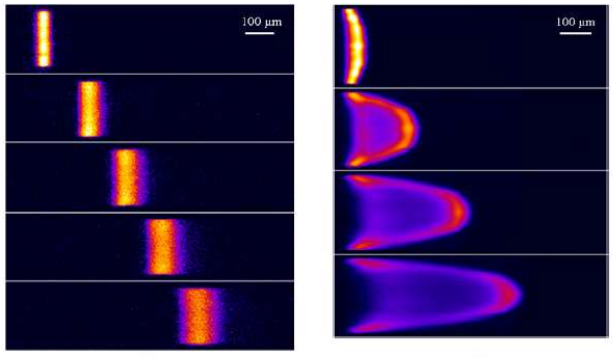


Figure 3.1: These images show a fundamental difference in the dynamics of sample dispersion between electroosmotically-driven and pressure-driven flows. This visualization was performed using a molecular tagging technique (caged fluorescence visualization described later on in the chapter) and shows the reduced sample dispersion for (a) electroosmotic flow (in a capillary with a rectangular cross section 200 µm wide and 9 µm deep) as compared to (b) pressure-driven flow (rectangular cross-section 250 µm wide and 70 µm deep). The images are adapted from recent work by Molho.[1]

Flow control

Electrokinetic focusing

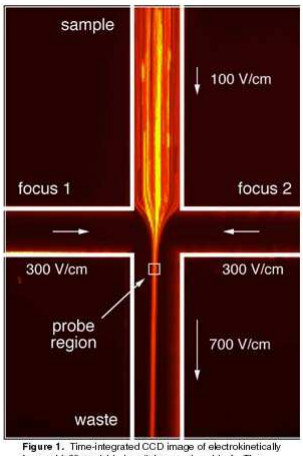
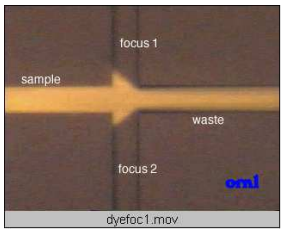
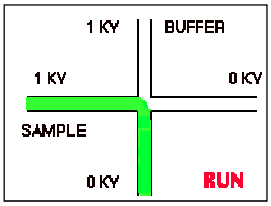


Figure 1. Time-integrated CCD image of electrokinetically focused 1.88 um labled particles on microchip A. The exposure time was 5 seconds with sample and focusing field strengths of 100 V/cm and 300 V/cm, respectively. Arrows depict direction of transport, and their lengths are proportional to averge fluid velocities in each channel.



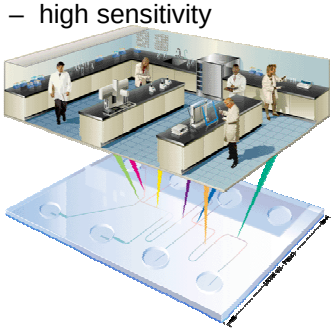
Gated valve

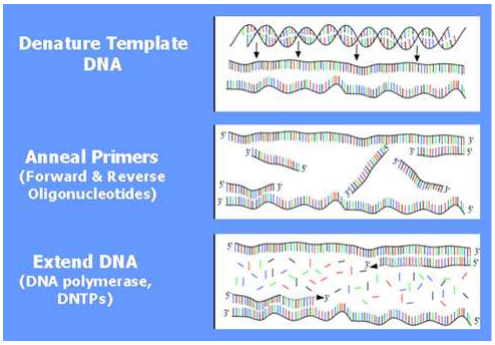


Flow cytometry

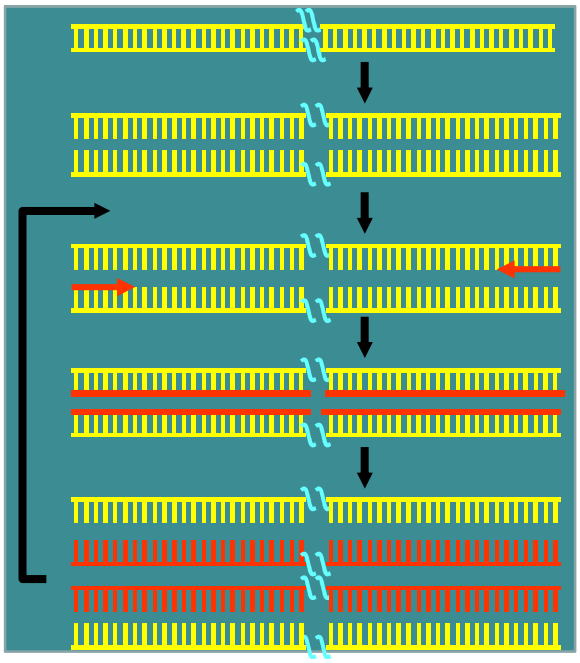
Lab-On-A-Chip

- **for most laboratory tests, the sample size is in the milliliter (ml) range, and the tests are done in a laboratory**
- **lab-on-a-chip:**
  - sample size can be very small
  - test could be possibly done at home (point-of-care)
  - eventually tests could even be embedded in a person
- **PCR reaction**
  - multiplication of target coppies
  - high sensitivity





PCR – temperature cycling



denaturate dsDNA
93°C

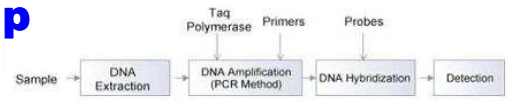
bind primers
37 - 55°C

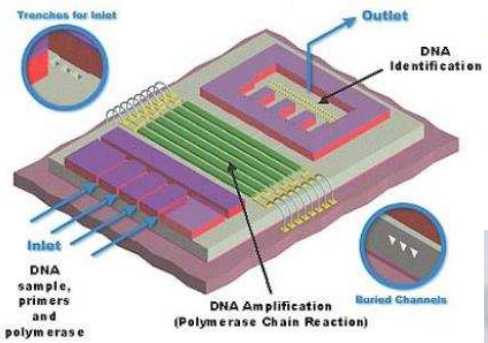
prolong primers
70°C Taq polymerase

denaturate dsDNA
93°C

repeat the cycle

PCR on chip

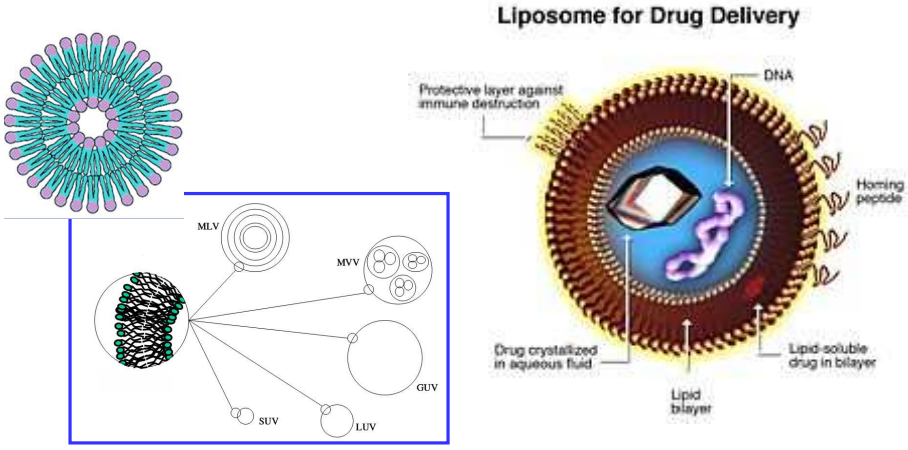




- Continuously flowing sample
  - well-defined temperature controlled zones
- Pattern of chip
  - determines the relative time sample is exposed to each

Drug delivery and targeting

- **delivery – liposomes**
  - microscopic fluid-filled pouches (vesicles)
  - could be used to release anticancer or antimicrobial compounds
- **targeting – specific recognition**
  - target specific cell types based on surface proteins



Smart Medical Devices

- **glucose sensing – blood glucose meters**
- **small computerized machines that "read" concentration of glucose**
- **not completely comfortable …**
- **American Diabetes Association, ~21 million Americans (7% of the population) have diabetes**
- **annually > $132 billion in direct and indirect costs**
- **of these 21 million, there are > 175,000 type 1, often referred to as juvenile diabetes, diabetes patients under 20 years of age**
- **globally, more than 1 in every 600 children has type 1 diabetes**
- **incidence in children and adolescents is increasing by ~3 percent annually**
- **~ 28 percent of these patients in the US use insulin to control their disease … need to measure glucose levels**

Recent trends

LifeScan's new product, One Touch Vario, incorporating technology developed by Universal Biosensors (UBI) and manufactured in Rowville, Melbourne, Australia, launched in the Netherlands in January 2010:

- 0.45µl
- Side-loaded sample
- No Code
- Results in 5 seconds
- Accurate to within ±15%

The Agamatrix Presto launched June 2008:

- No coding
- Comparatively inexpensive
- Sample size 0.5µl

- 31 March 2010 - AgaMatrix, Inc. and Sanofi-Aventis announce long-term agreement for the development, supply and commercialization of blood glucose monitoring (BGM) solutions

User interface

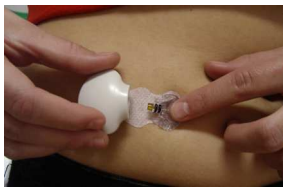
Link to your smartphone

• MyGlucoHealth for Nokia owners test marketed: Within 4 weeks downloaded 40,000 times by users in 130 countries

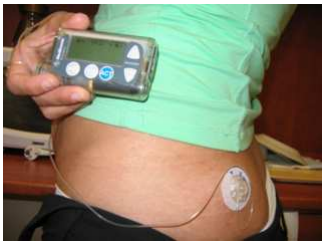
• Lifescan followed the iPhone route

Automated solution …

- insulin pump controlled by continuously operating glucose sensor
- "inject" the sensor
- link to the data transmitter
- pair with the insulin delivering pump

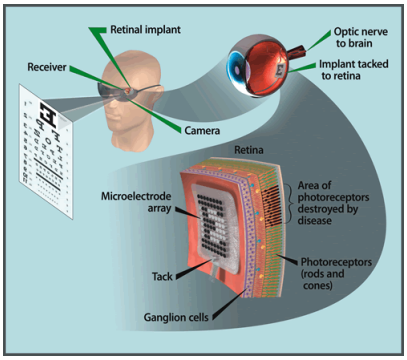


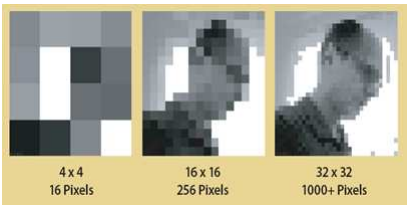
- comfortable, user independent



Tissue engineering

- **implants - in retinal diseases, photoreceptor cells are destroyed**
- **artificial retina device transmits signals directly to the optic nerve**
  - camera and microprocessor mounted in eyeglasses.
  - receiver implanted behind ear.
  - electrode studded array tacked to retina.
  - wireless battery pack worn on belt for power.





The Future of Nano-Biotechnology

- **diagnostic vs. therapeutic medicine**
- **why play “catch-up” when you can prevent it or diagnose it early and fix it fast!**
- **prevention and early diagnosis has always been cheaper than therapeutics**

Market view …

Measures of Nanotechnology's maturation

- **R&D surging: global nanotech R&D $1 trillion estimated for 2015**
- **term "nano" approaches ubiquitous status in U.S. society and media (>18,000 citations in U.S. media in 2005).**
- **"gold rush" for nano-patents continues**
  - over 4,000 U.S. patents issued to 2006
- **perhaps most importantly, nanotechnology commercialization is moving forward at a rapid rate…**
- **products include paints, coatings, sporting goods, sunscreens, cosmetics, personal care products, stain-resistant clothing, food and food packaging, and light emitting diodes used in computers, cell phones, and digital cameras**

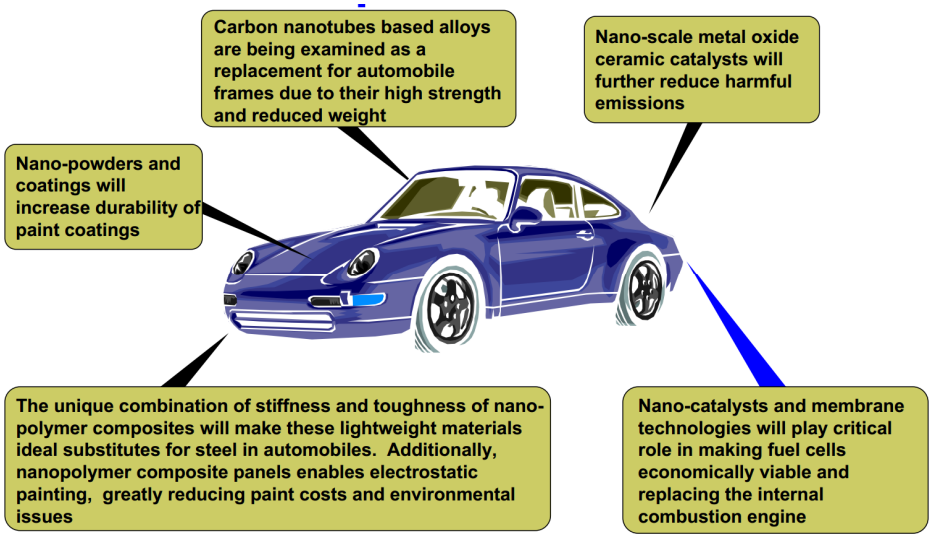
Potential applications .. pharma / chemicals

- **pharmaceuticals and medical products**
  - new and more effective drug compounds
  - nearly perfectly targeted drug delivery
  - diagnostics, sensors, and assays
  - DNA sizing and sequencing
  - bioelectronics
  - bio-warfare protection
  - antibacterial dressings and coatings
- **chemicals and basic materials**
  - ultra-lightweight, high-strength, precision-formed materials
  - nano-composite polymers for structural and electronic applications
  - membranes and filters for costeffective desalinization of water
  - thermal and optical barriers
  - ink jet materials
  - high efficiency and novel catalysts
  - neo-composite cements
  - wrinkle / stain / water resistant textiles

Potential applications .. electronics / enargy

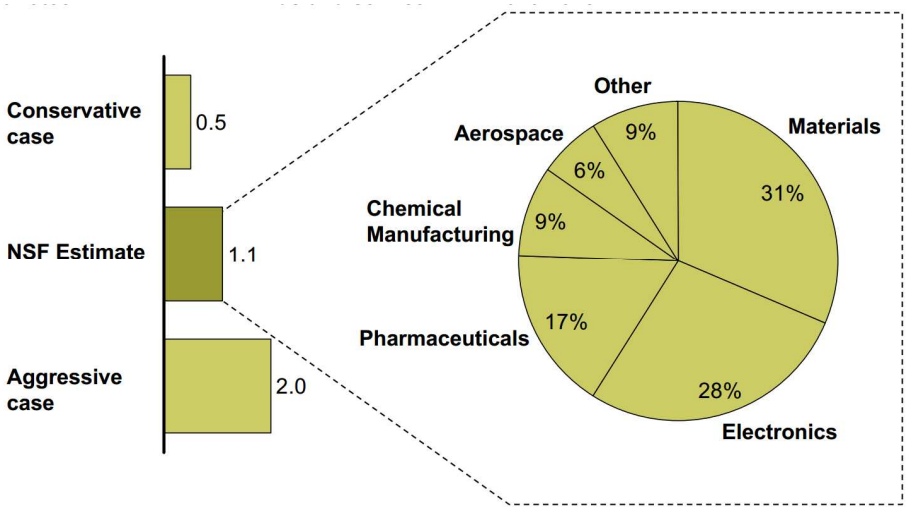
- **consumer electronics and computing**
  - miniaturized supercomputers
  - terabit non-volatile memory
  - pervasive computing
  - low voltage and high brightness displays
  - faster semiconductors and microprocessors
- **energy**
  - thin film photovoltaics for cost effective solar energy
  - cost competitive fuel cells for automotive applications
  - micro fuel cells for portable power applications
  - high capacity, rapid charge batteries
- **tools and methods**
  - scanning probe microscopy
  - software, simulation
  - directed self assembly and lithography

Pervasive penetration in the long-term scale … automotive example



Estimation of market size

- nanotechnology related goods and services – by 2010-2015, USD trillions



Key drivers limiting penetration

Consideration	Effect	Examples
Technological complexity	Integration requirements and interdependency delay penetration for complementary designs	New chip architectures require integration with peripheral providers
Product life cycle	Limits integration of new and improved components into systems	Automobile (3-4 years) Aerospace (decades)
Regulatory limitations	Rigorous regulatory approval process limits adoption, success rate	•FDA approval •FAA approval
Investment requirements	Creates significant switching cost, raising required value for innovation to penetrate	CMOS chip fabrication facilities (> $ 2B)

Market impact will take time

technological complexity

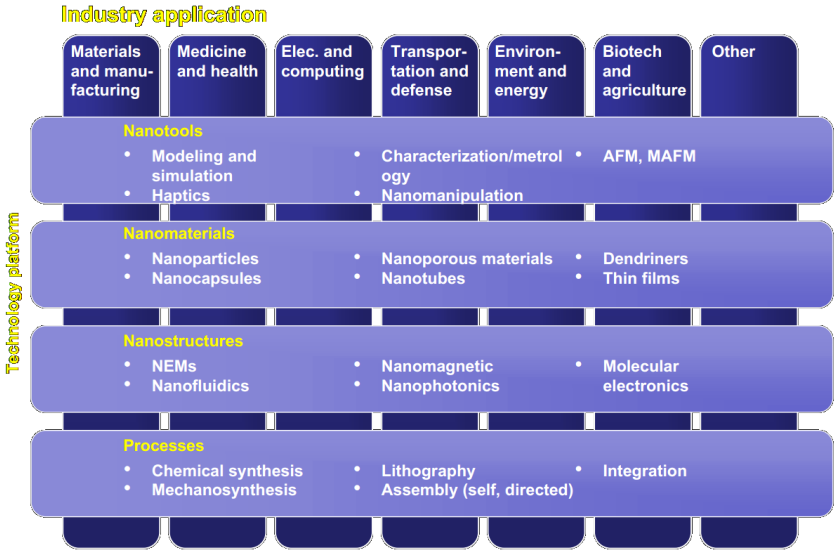
Time frame (years)	0 - 3	3 - 7	7+
Systems	-	Non-volatile memory	Biomimetic materials, Bioelectronics Nanowire memory and logic
Structures and devices	Displays	Sensors, diagnostics, assays / Fuel cells / Solar cells	Novel therapeutics through functionalized dendrimers, nanotubes, other nanoparticles for targeted drug therapy
Passive / materials	Nanoparticles / Bulk composites / Coatings / Catalysts / Tools	High performance nanocomposites, ceramics, metals	?

regulatory complexity and product life cycles

Why nano-bio-tech is different

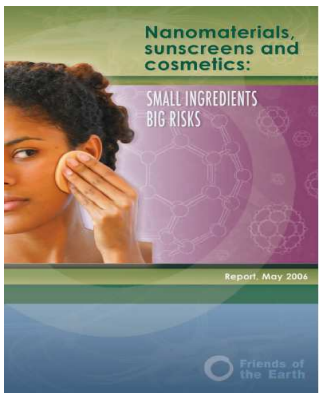
- **players will "own" the innovations on multiple levels:**
  - patent protected (IP ownership)
  - require proprietary know-how
- **significantly lower barriers to market entry for most applications than e.g. biotech**
- **opportunities for near term revenue will allow both attackers and incumbents to develop robust "bootstrap" models**
- **concentrated in university and academic / central labs**
- **secure strategic assets and maintained options for growth**
- **non-traditional competitors may emerge**

Asses relevance of each platform



Personal care products

- Friends of the Earth: *Nanomaterials, Sunscreens and Cosmetics* Report
- largest single category (125 products) is health and fitness (including sunscreens, cosmetics, and other personal care products)
- at least 116 cosmetics, sunscreens, and personal care products containing nanomaterials commercially available



Nano-silver consumer products

- brooms, food storage, refrigerators, air filters, drywall, paint, medical coatings, sports clothes, washing machine
- Samsung's *Silvercare Washer*
- Shaper Image's *Fresher Longer Miracle Food* storage

The time for action is now

- **materials engineered or manufactured to the nano-scale exhibit different fundamental physical, biological, and chemical properties**
  - quantum physics effects
  - exponentially increased surface area
- **these new properties ("nano-ness") create unique and unpredictable human health and environmental risks**
  - increased surface area creates increased reactivity and enhanced intrinsic toxicity
  - size creates unprecedented mobility to human body and environment

Human health risks

- **enhanced toxicity: some nanoparticles shown to cause DNA mutation, structural damage to mitochondria and even cell death in laboratory studies.**
  - Nanoparticles of titanium dioxide and zinc oxide (cosmetics and sunscreens) photoactive in studies, producing free radicals and causing DNA damage
  - Carbon fullerenes ($Carbon_{60}$): Adverse impacts on aquatic species and low levels have been found to cause damage to human liver cells
- **unprecedented mobility:**
  - due to size, nanoparticles more easily taken up by the human body and can cross biological membranes, cells, tissues and organs more efficiently than larger particles
  - once in the blood stream, nanomaterials can be transported around the body and can be taken up by organs and tissues including the brain, heart, liver, kidneys, spleen, bone marrow and nervous system.
  - entry through inhalation or ingestion; jury still out on ease of skin penetration

A new class of manufactured non-biodegradable pollutants

- **pathways: during manufacturing, transport, use, or disposal (e.g., nano-cosmetics or other nano-personal care products: washed off in the shower and join water waste streams.)**
- **environmental impacts:**
  - ***mobility* -** ability to persist; reach places larger particles cannot; move with great speed through aquifers and soils; settle slower.
  - ***transportation* -** large and active surface for absorbing smaller contaminants that could "hitch a ride" over long distances
  - ***reactivity* -** interactions with substances present in the soil could lead to toxic compounds
  - ***durability and bioaccumulation*** - nano-aluminum and stunted plant growth; nano-silver and microorganisms
- **management challenges: detection and removal?**
  - New protocols and cost-effective technologies for measuring, monitoring, and controlling nanomaterials are required

New nano-specific toxicity testing paradigms are required

- **European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR):**
  - "Experts are of the *unanimous* opinion that the adverse effects of nanoparticles cannot be predicted (or derived) from the known toxicity of material of macroscopic size, which obey the laws of classical physics."
- **U.K. Royal Society and Royal Academy of Engineering:**
  - "Substances made using nanotechnology should be considered *new chemicals* and undergo extra safety checks before they hit the market to ensure they do not pose a threat to human health."
- **Food and Drug Administration agency in USA**
  - believed that the existing battery of pharmacotoxicity tests is *probably adequate* for most nanotechnology products that we regulate. Particle size is not the issue.
  - publics strongly agains

Petition Focus: Nano-sunscreens

- **sunscreens are classified by FDA as human drugs and should be subject to rigorous pre-market regulation**
- **red flags regarding free radical creation and DNA damage; unanswered questions about skin penetration.**
- **despite their unique dangers and patented differences, FDA currently considers nano-sunscreens equivalent to bulk material sunscreens.**
- **petition calls for nano-sunscreen recall until manufacturers submit and FDA reviews pre-marketing testing data proving the drugs' safety and efficacy.**

- **requirements:**
  - comprehensive nanomaterial-specific regulations
  - new paradigms of nano-specific toxicity testing
  - clasification of nanomaterials as new substances
  - mandatory nanomaterial product and ingredient labeling
  - compliance with the National Environmental Policy Act (NEPA)

What should be done

- **<u>learn from the past</u>: regulatory agencies must act quickly if they hope to avoid repeating the mistakes of past regulatory failures of "wonder" materials or technologies (e.g., asbestos, CFCs, DDT, PCBs)**
- **<u>adequae regulation</u>: a regulatory framework is needed that protects workers, the general public and the environment from the impacts of nanomaterials throughout their lifecycle**
- **<u>much more robust EHS study</u>: adequate, publicly available, independent, peer-reviewed safety studies on the environmental and health impacts of nanomaterials**