• **Study of the controlling of matter on an atomic and molecular scale. Generally nanotechnology deals with structures sized between 1 to 100 nanometer in at least one dimension.**

• **The ability to design systems with defined structure and function on the nanometer scale.**

  - Involves developing materials, devices within that size, and analytical tools (methodology), which can be used for analysis and measurement on a molecular scale

• **Interdisciplinary area:**

  Physics, Chemistry, Material science, Electronics, Chemical Engineering, Information technology
Nanotechnology plays by different rules
Analytical methods and Nano-sized materials

• Analytical tools
  - AFM, EM

• Nano-sized materials

  Unusual and different property
  - Semiconductor nanocrystals :
    Size-dependent optical property

  - Nanoparticles
    Ferromagnetic, Superparamagnetic, Gold
    Carbon nanotubes, Dendrimers, Grephene
Future implications of Nanotechnology

• Nanotechnology may be able to create many new materials and devices with a vast range of applications, such as in medicine, biomaterials, electronics, and energy production.

• Nanotechnology raises many of the same issues as with any introduction of new technology, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics.
Nano-Biotechnology

• Integration of nano-sized/structured materials, nano-scale analytical tools, and nano-devices into biological sciences for development of new biomaterials and analytical toolkits as well as for understanding life science

• Use of bio-inspired molecules or materials

  Typical characteristics of Biological events/materials
  - Self assembly
  - Highly efficient : high energy yield
  - Very specific ; extremely precise

• Bio-molecules
  DNA, RNA, Aptamers, Proteins, Peptides, Antibody, Virus
• **Development of tools and methods**
  - More sensitive
  - More specific
  - Multiplexed
  - More efficient and economic

• **Implementation**
  - **Diagnosis and treatment of diseases**
    - Rapid and sensitive detection (Disease biomarkers, Imaging),
    - Targeted delivery of therapeutics
    - Theranostics
  - **Drug delivery: Chemical drug, siRNA**
  - **Understanding of life science**
Major research fields

• New molecular detection and imaging system to probe nanoscale processes occurring in human organs: To diagnose cancer or any other diseases in early stages, to treat diseased tissues/cells more effectively → quantitative understanding of how biological systems work

• Quantitative analytical tools: Technologies to derive a detailed understanding how cell functions are regulated and how they can be manipulated in a predictable manner

• Physical model of the cell as a machine: To understand how the components of a cell work together → provides new insights into the physical relationships between cellular components and functional irregularities that trigger pathological abnormalities
Examples

- Nano-Biodevices
- Nano-Biosensors
- Medical use (Imaging with nanoparticles)
- Analysis of a single molecule/ a single cell
Issues to be considered

- Synthesis or selection of nano-sized/structured materials
- Functionalization with biomolecules or for biocompatibility
- Integration with devices and/or analytical tools
- Assessment: Reproducibility, Toxicity
- Implementation
The size of Things

Nanodevices:
- Nanopores
- Dendrimers
- Nanotubes
- Quantum dots
- Nanoshells
How are nano-biomaterials fabricated?

Two main approaches: "bottom-up" and "top-down"

- **Bottom-up**: Materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition.
  - Needs research on self-assembly

- **Top-down**: Nano-objects are constructed from larger entities without atomic-level control.
  - Impossible to fabricate nano-objects less than 50 nm
NanoBiotech was initiated by the development of AFM that enables imaging at atomic level in 1980
A cantilever with a sharp tip (probe) at its end that is used to scan the specimen surface.

When the top is brought into a close proximity of a sample surface, force between the tip and sample leads to a deflection of the cantilever.

Deflection is measured using a laser spot reflected from the top surface of the cantilever into an array of photodiodes.

One of the foremost tools for imaging, measuring, and manipulating matters on a nanoscale.
Feedback mode

- If the tip was scanned at a constant height, the tip would collide with the surface, damaging the surface.

- A feedback mechanism to adjust the tip-to-sample distance to maintain a constant force between the tip and the sample.

Imaging modes
- Contacting mode
- Non-contacting mode
- Tapping mode
<table>
<thead>
<tr>
<th>VEECO TESPA®</th>
<th>VEECO TESPA-HAR®</th>
<th>NANOWORLD SuperSharpSilicon®</th>
</tr>
</thead>
</table>
| Tip length : 10 \( \mu \text{m} \)  
Radius : 15~20 nm | Tip length :10 \( \mu \text{m} \)  
(last 2 \( \mu \text{m} \) 7:1)  
Radius : 4~10 nm | Tip length :10 \( \mu \text{m} \)  
Radius : 2 nm |

- Tip length : 10 \( \mu \text{m} \)
- Radius : 15~20 nm

- Tip length : 10 \( \mu \text{m} \)
- Radius : 2 nm
Example showing the resolution of protein structure by AFM

Image of ATP synthase composed of 14 subunits
The Accurin nano-drug BIND-014 encases a toxic payload, docetaxel, (red) in a layer of biodegradable polymers (grey), and uses molecules on the surface (blue) to target the tumour. A long polymeric string that spontaneously folds to form a particle. Phase II trials for treatment of lung and prostate cancer.
• Nanoparticles to deliver small pieces of RNA to cancer cells, where they decrease expression of certain genes in a method called RNA interference.

• Question about whether nanotechnology is worth the high price tag that accompanies its production: it can cost ten times more than conventional treatment.

“New nanoparticle-based drug delivery will be expensive and it has to be justified by improved therapeutic outcomes
Biomedical & Biological Sciences :

- Ultra-sensitive imaging of biological targets under non-invasive in-vivo conditions
- Fluorescence, positron emission tomography, Magnetic resonance imaging
- **Ultra-sensitive imaging**
  - Cancer detection, cell migration, gene expression, localization of proteins, angiogenesis, apoptosis

- MRI : powerful imaging tool as a result of non-invasive nature, high spatial resolution, and tomographic capability
  Resolution is highly dependent on the molecular imaging agents
  → Signal enhancement by using contrast agents : iron oxide anoparticles
Optical Properties Of Quantum Dots

a) Multiple colors

b) Photostability

Quantum Yield ≥ 60 ~ 70 %

c) Wide absorption and narrow emission

d) High quantum yield

Single source excitation
In Vivo Cell Imaging

QD + Antibody conjugates

Organelle

Antigen

Organelle

3T3 cell nucleus stained with red QDs and microtubules with green QDs

- Multiple Color Imaging
- Stronger Signals

Wu et al. Nature Biotech. 2003 21 41
In Vivo Cell Imaging

Live Cell Imaging

Quantum Dot Injection

▶ Red Quantum Dot locating a tumor in a live mouse

Cell Motility Imaging

◀ Green QD filled vesicles move toward to nucleus (yellow arrow) in breast tumor cell

Alivisatos et al., Adv. Mater., 2002 14 882
Förster (or Fluorescence) Resonance Energy Transfer (FRET)

- Non-radiative energy transfer from an excited energy donor to an energy acceptor
- Dipole – dipole interactions
- Energy transfer efficiency between two probes:
  - Degree of spectral overlap between donor emission and acceptor absorption
  - \( \sim 1 / (\text{separation distance})^6 \)
  - \( \sim 10 \text{ nm} \)

\[ E = \frac{R_o^6}{(R_o^6 + R^6)} \]

\( R_o : \) Förster distance at which the energy transfer is about 50%

- Detection of a target analyte
- Imaging (localization)
- Analysis of biomolecular interactions
Analysis of Biomolecular interaction by FRET

CFP : Cyan Fluorescent Protein
YFP : Yellow Fluorescent Protein
New format based on FRET between QDs and AuNPs

- **Model system**
  - *Streptavidin-biotin* interaction: Avidin as a target analyte
  - QDs with Ex and Em wavelengths of 460 nm and 605 nm
  - Use of interaction between SA-QDs and Biotin-AuNPs

- **PL quenching of SA-QDs by Biotin-AuNPs due to energy transfer**
  - Externally added *Avidin* prevents biotin-AuNPs from binding to SA-QDs
  - PL intensity of SA-QDs is recovered

Oh et al., *JACS* (2005)
Protease assay based on FRET between QDs and AuNPs

Essential for the dynamic regulations of cell function and aberrations
- Involved in major human diseases (cancers, apoptosis, and inflammation)
- Protease inhibitors are known to be drug candidates
- Matrix metalloproteinases (MMPs), Caspase-3, Thrombin

**Peptide-conjugated AuNP**

- **Peptide** (CRPLALWRSK-bio)
- **Au** monomaleimide AuNP 1.4 nm in diameter

**QD-AuNP Nanoprobe**

- **QD**
- **Au** monomaleimide nanogold 1.4 nm
- **peptide** (CRPLALWRSK-bio) 3.8 nm
- **QD-SA** 11.3-13.8 nm
- 15-20 nm (5-10 SA/QD)

*Kim et al., Anal. Chem. (2008)*
Specificity of proteolytic cleavage

Peptide sequences for proteases

Thrombin : Biotin-GKGGLVPR-GSGC
MMP-7 : Biotin-KSRWLA-LPRC
Casp-3 : Biotin-GRRGDEVD-GGGRRC
Multiplexed Assay of Proteases

**Thrombin inhibitor**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>QD Color</th>
<th>QD Type</th>
<th>QD Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride</td>
<td><a href="image">chemiimage</a></td>
<td>QDs</td>
<td>QDs + Pep-AuNPs</td>
</tr>
</tbody>
</table>

**MMP-7 inhibitor**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>QD Color</th>
<th>QD Type</th>
<th>QD Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minocycline</td>
<td><a href="image">chemiimage</a></td>
<td>QDs</td>
<td>QDs + Pep-AuNPs + Proteases</td>
</tr>
</tbody>
</table>

**Caspase-3 inhibitor**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>QD Color</th>
<th>QD Type</th>
<th>QD Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-Val-Ala-Asp fluoromethyl ketone</td>
<td><a href="image">chemiimage</a></td>
<td>QDs</td>
<td>QDs + Pep-AuNPs + Inhibitor</td>
</tr>
</tbody>
</table>
Inherent capabilities of molecular recognition and self-assembly

Attractive template for constructing and organizing the nano-structures

Proteins, toxin, coat proteins of virus etc.
α-Hemolysin: Self-Assembling Transmembrane Pore

- A self-assembling bacterial exo-toxin produced by some pathogens like *Staphylococcus aureus* as a way to obtain nutrients \(\rightarrow\) lysis of red blood cells

  α-hemolysin monomers bind the outer membrane of susceptible cells.

- The monomers oligomerize to form a water-filled heptameric transmembrane channel that facilitates uncontrolled permeation of water, ions, and small organic molecules.

- Rapid discharge of vital molecules, such as ATP, dissipation of the membrane potential and ionic gradients, and irreversible osmotic swelling leading to the cell wall rupture (lysis), can cause death of the host cell.
- Mushroom-like shape with a 50 Å beta-barrel stem
- Narrowest part (1.4 nm in diameter) of channel at the base of stem
Biotechnological applications: Stochastic sensors

- A molecular adaptor is placed inside its engineered stem, influencing the transmembrane ionic current induced by an applied voltage.
- Reversible binding of analytes to the molecular adaptor transiently reduces the ionic current.

- Magnitude of the current reduction: type of analyte.
- Frequency of current reduction intervals: analyte concentration.
Stochastic sensors
a : Histidine captured metal ions (Zn$^{+2}$, Co$^{+2}$, mixture)
b: CD captures anions (promethazine, imipramine, mixture)
c : biotin ligand
- Transmembrane pore can conduct big (tens of kDa) linear macromolecules like DNA or RNA
- Electrophoretically-driven translocation of a 58-nucleotide DNA strand through the transmembrane pore of alpha-hemolysin
- Changes in the ionic current by the chemical structure of individual strands
- Nucleotide sequence directly from a DNA or RNA strand

- A single nucleotide resolution
Nanoparticle-based Bio-Bar Codes for the ultrasensitive detection of proteins

Science, 301, 1884-1886, 2003
Magnetic Particle Probe

Target Protein

Nanoparticle Probe with Barcode DNA

Separation using magnetic field & Barcode DNA dehybridization

Barcode DNA

Signal Amplification by PCR

Chip-based detection of Barcode DNA

a : complementary capture DNA
b : non complementary capture DNA

atto molar detection!!

300 aM 30 aM 3 aM control
Detection of biomolecular binding by microcantilever

- **Label-free detection of biomolecules**
- **Methods for measuring the bending of micro-cantilever**
  - **Optical**
    - Less amenable to monolithic integration and multiplexed detection because of difficulties in laser alignment and power management
    - Interference with turbid or opaque fluidic and smoky environment
  - **Piezoresistive**
    - Compatible with aqueous media and parallel cantilever arrays
    - Piezoresistors cover a large length of the cantilever, and high doping levels are required → the stress measurement is not localized
    - → thermal and electronic noise, thermal drift, non-linearity in piezo-response
    - More than 50 nm bending is required

The piezoresistive effect describes change in the electrical resistivity of a semiconductor or metal when mechanical strain is applied
Nanomechanical cantilever arrays

Fig. 1. (A) The preparation of an active cantilever biosensor array and an illustration of the basic principle of nanomechanical label-free biodetection are shown. (Inset) The incubation of individual gold-coated cantilevers (dimensions: 500 × 100 × 1 μm) in microcapillaries, each containing a different solution of thiolated probe DNA. The schematic illustrates how target DNA injected into solution will hybridize sequence-specifically to its complementary partner immobilized on a particular cantilever. Hybridization generates a compressive surface stress, which causes the cantilever to bend with respect to a reference probe-coated cantilever, giving rise to a differential bending signal, Δx, of 10 nm. (B) Absolute deflection signals from an eight-cantilever array were monitored in real time. By convention, a positive deflection signal corresponds to the downward bending of the cantilever (away from the gold coating) because of the generation of a compressive surface stress. A negative signal corresponds to the upward motion of the cantilever resulting from a tensile surface stress. Changes in environment (index of refraction, temperature, etc.) might result in lever movements, but then all levers react simultaneously to the changes (see discussion in the Fig. 2 legend). Throughout the process, the absolute deflection of individual cantilevers was recorded, and simultaneously we extracted the differential signal (e.g., the deflection of the

Linear position detector: beam-deflection
With an accuracy of 0.1 nm

Mckendry et al., PNAS, 99, 9783-9788 (2002)
Virus-enabled synthesis and assembly of nanowires for lithium ion battery electrodes

- Li ion battery: anode, cathode, electrolyte
  Anode: Graphite, Cathode: layered oxide (lithium cobalt oxide, lithium iron phosphate, lithium manganese oxide etc.)

- Smaller and more flexible Li ion batteries

- Methods to assemble battery materials

- Dimensionally small batteries: Nanoparticles, nanotubes, nanowires as well as several assembly methods based on lithography, block copolymer, layer-by-layer deposition

- Nanostructured materials: Improvement of the electrochemical property of Li ion batteries

- Monodisperse, homogeneous nanomaterials and hierarchical organization control are needed to maximize the potential

*Nam et al., Science (2006)*
M13 virus

- Helically wrapped by ~2700 copies of major coat protein (p8) around its single-stranded DNA, with minor coat proteins (p3, p6, p7, and p9) at each end of the virus: Total genome size; 6,407 nucleotides (900 nm long)

- Protein engineering:
  - Coat proteins are used for expression and selection of peptides or proteins with high binding affinity for a target: Affinity maturation: display of a library → selection by a panning process:

- A new approach to the construction of functional hetero-structured nanomaterials and monodisperse, highly crystalline nanowires
Phage display

- Used for the study of protein–protein, protein–peptide, and protein–DNA interactions based on bacteriophages (viruses that infect bacteria)
- A gene encoding a protein of interest is inserted into a phage coat protein gene, causing the phage to "display" the protein on its outside while containing the gene for the protein on its inside, resulting in a connection between genotype and phenotype.
- These displaying phages can then be screened against other proteins, peptides or DNA sequences, in order to detect interaction between the displayed protein and those other molecules.
- In this way, large libraries of proteins can be screened and amplified in a process called in vitro selection, which is analogous to natural selection.
- Selection of a high-affinity binder from a library by repeating the cycle
- Antibodies, proteins, or peptides
Predictive-based design

Fusion of tetra-glutamate (EEEE-) to the N-terminus of the major coat p8 protein
- A general template for growing nanowires through the interaction of the glutamate with various metal ions

Favorable interaction with the positively charged electrolyte polymer
→ assembly of nanowires

(Ex) Design of cobalt oxide nanowires
- Incubation of the virus templates in aqueous cobalt chloride solution
- Cobalt oxide has large reversible storage capacity arising from displacement reactions: ~ three times larger capacity compared to carbon-based anodes currently used in commercial batteries
- Homogeneous and high-crystalline nanowires: 141.7 m²/g
Schematic diagram of the virus-enabled synthesis and assembly of nanowires as negative electrode materials for Li ion batteries. Rationally designed peptide and/or materials-specific peptides identified by bio-panning were expressed on the major coat p8 proteins of M13 viruses to grow Co$_3$O$_4$ and Au-Co$_3$O$_4$ nanowires.
(A) TEM image of virus-templated Co3O4 nanowires. (B) High-resolution TEM image of a Co3O4 viral nanowire. Electron diffraction pattern (Inset, upper right) confirmed that the crystal structure was Co3O4. (C) Charging-discharging curves for a virus-mediated Co3O4/Li half cell cycled between 3 and 0.01 V at a rate of C/26.5. C was defined as eight Li ions per hour. (D) Specific capacity versus cycle number for the same cell. (E) TEM images of differently nanostructured Co3O4 viral nanowires (F) TEM images of the assembly of discrete Co3O4 nanocrystals on the p8 proteins.

Reversible capacity : 600 ~ 750 mA-hour/g
Twice that of current carbon-based anodes
Characterization of the hybrid nanostructure of Au nanoparticles incorporated into Co3O4 nanowires. (A) Visualization of the genetically engineered M13 bacteriophage viruses. P8 proteins containing a gold-binding motif (yellow) were doped by the phagemid method in E4 clones, which can grow Co3O4. (B) TEM images of the assembled gold nanoparticles on the virus. (C) TEM image of hybrid nanowires of Au nanoparticles/Co$_3$O$_4$. (D) Specific capacity of hybrid Au-Co3O4 nanowires. (E) Cyclic voltammograms of hybrid Au-Co3O4 and Co3O4 nanowires at a scanning rate of 0.3 mV/s

Specific capacity of hybride : ~ 30 % greater than Co$_3$O$_4$ nanowires
New hybrid material electrodes

- Systematic and controlled arrangement of two distinct nanomaterials
- Increase in the electrochemical properties through the cooperative contribution of each material
- Design of composite material

Hybride Au-Co$_3$O$_4$,
- Gold NPs: high electronic conductivity
- Isolation and expression of gold-binding peptide motif (LKAHLPPSRLPS) with major coat p8 protein
- Assembly of bifunctional viruses expressing both Au- and Co$_3$O$_4$-specific peptides with the virus coat
- Production and random package of two types p8 proteins:
Two-dimensional assembly of $\text{Co}_3\text{O}_4$ nanowires driven by liquid crystalline ordering of the engineered M13 viruses. 

(A and B) Phase-mode atomic force microscope image of macroscopically ordered monolayer of $\text{Co3O4}$-coated viruses. The Z range is 30°

(C) Digital camera image of a flexible and transparent free-standing film of (LPEI/PAA)100.5 on which $\text{Co3O4}$ viral nanowires are assembled into nanostructured monolayer with dimensions of 10 cm by 4 cm.

(D) Capacity for the assembled monolayer of $\text{Co}_3\text{O}_4$ nanowires/Li cell at two different charging rates.

Linear poly(ethylene imine)/Poly(acrylic acid)