Enzyme Immobilization on Protein Scaffolds

"Self Assembly of Cellulases on the **Stable Boiling Protein 1**"

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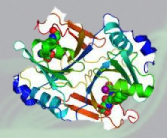
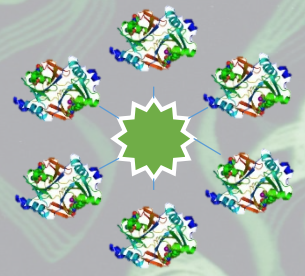
Why Enzyme Immobilization?

An Example.....

For 1 reaction cycle:



1 g of Free Enzyme



1.2 g of Immobilized Enzyme

0.4 g Enzyme

0.8 g Supporting Material

!Assuming that the Immobilized Enzyme retains its activity for 3 reaction cycles!

$3000/g enzyme

$2000/g I.E. per 3 R.C.

$1200 per 3 R.C.

$3000/g enzyme

$1500/g S.M.

COST

$3000 per 1 R.C.

$800 1 R.C.

$400 per 1 R.C.

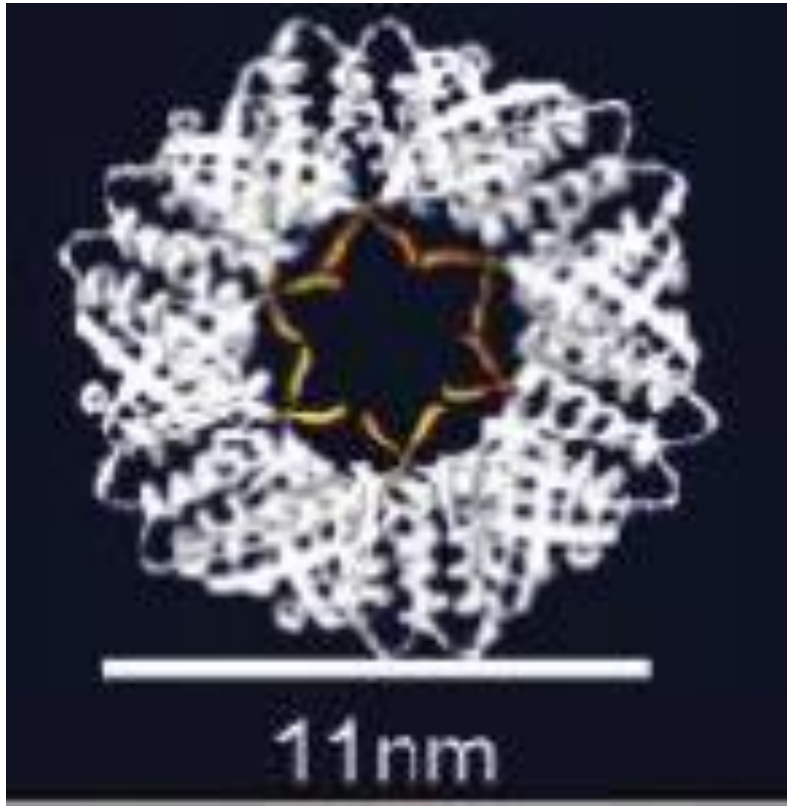
Protein Scaffolds

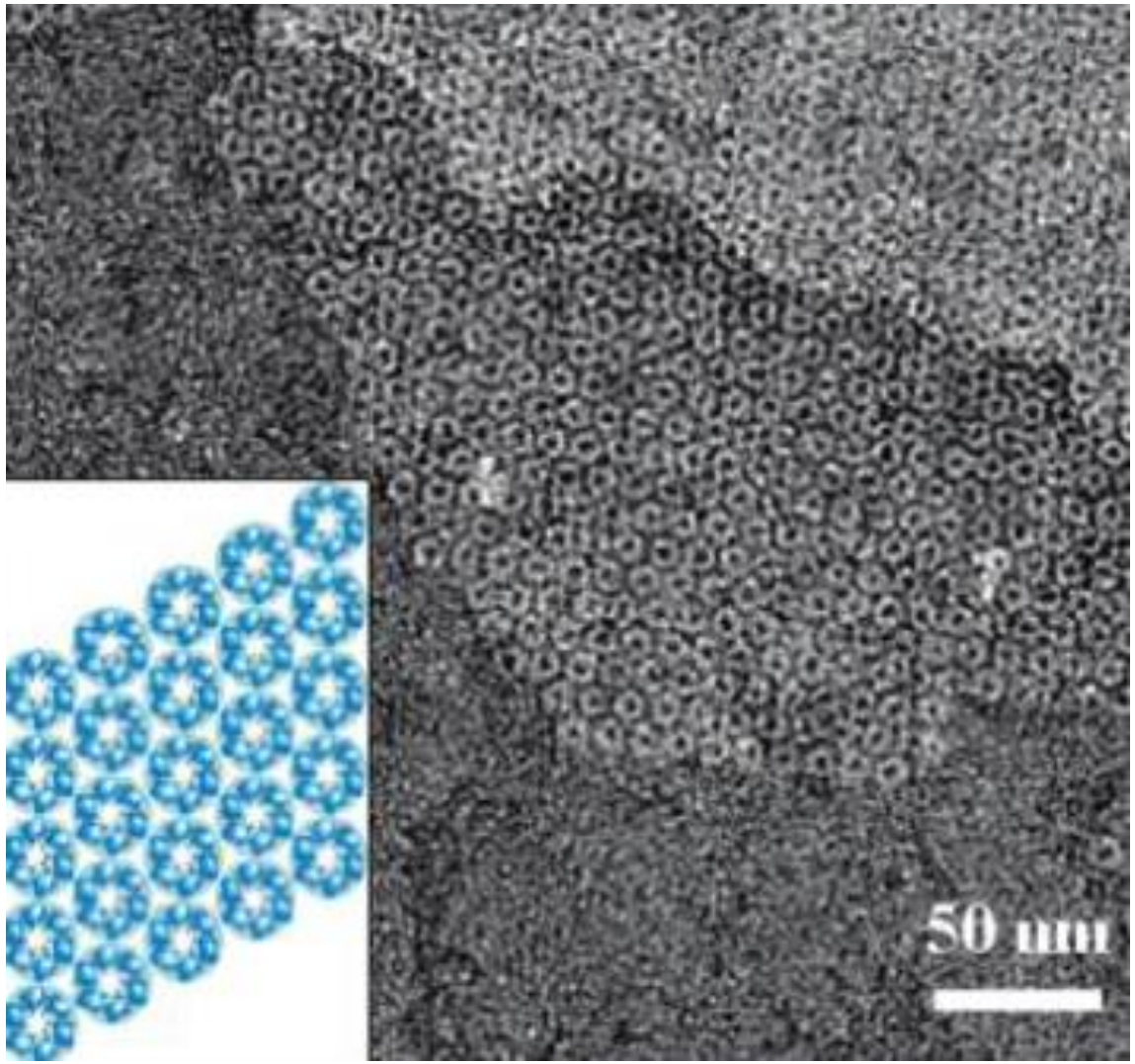
A biological tool for enzyme immobilization…

- These are proteins that have the ability to self-assemble into nanostructures with well defined 2D or 3D structure
- These nanostructures can be utilized as **"scaffolds"** where enzymes can be immobilized specifically via self-assembly
- A recently discovered protein scaffold is the **Stable Boiling Protein 1** isolated from *Populus tremula* plants

Stable Boiling Protein 1

Recently isolated from *Populus tremula* plant



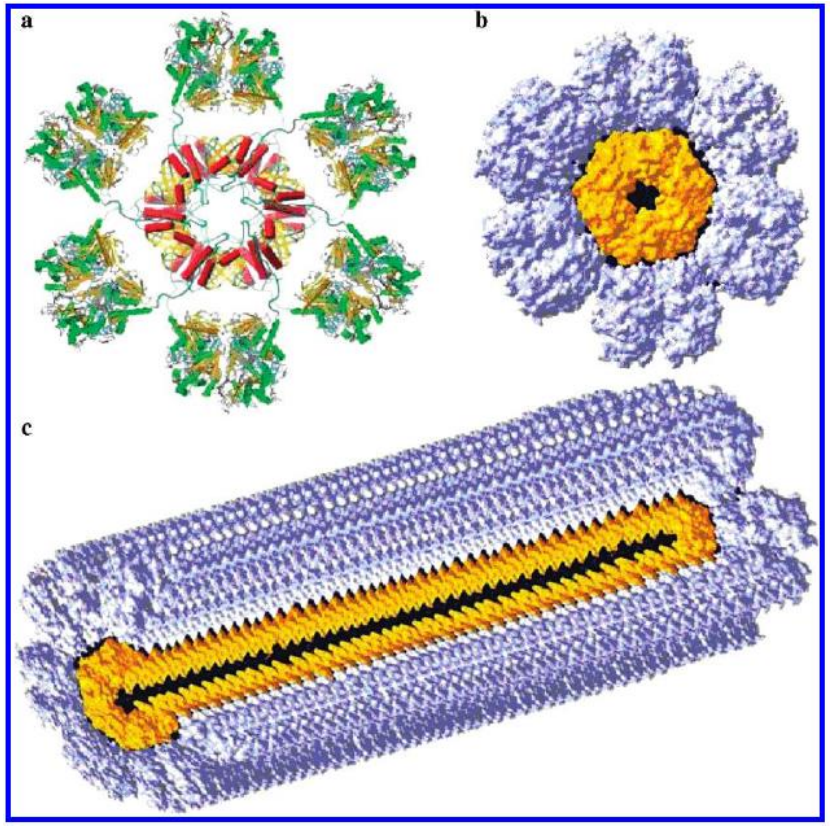


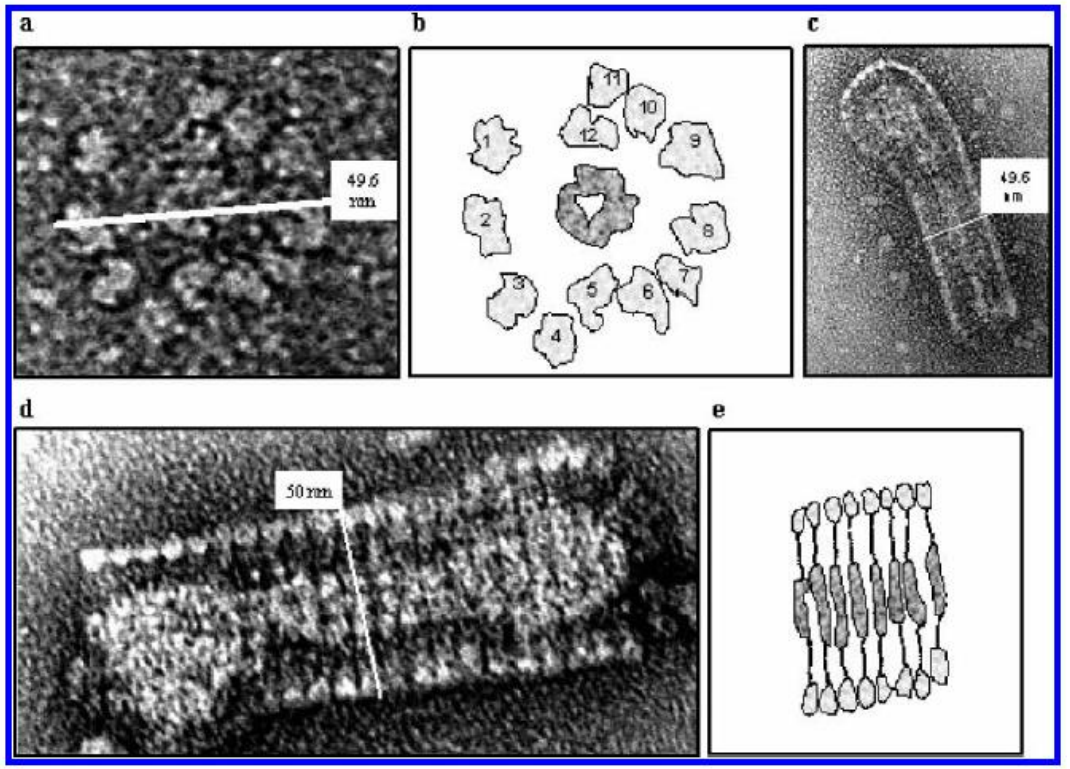
Medalsy, I., O. Dgany, M. Sowwan, H. Cohen, A. Yukashevska, S. G. Wolf, A. Wolf, A. Koster, O. Almog, I. Marton, Y. Pouny, A. Altman, O. Shoseyov and D. Porath (2008). "SP1 Protein-Based Nanostructures and Arrays." Nano Letters **8**(2): 473-477.

Stable Boiling Protein 1

Recently isolated from *Populus tremula* plant

LEFT: An illustration of GOx-L-SP1 complex. **(a,b)** SP1 dodecamer in the center and six GOx dimers encircling it. **(c)** Dodecamers clinging together to form an enzyme nanotube particle. (the SP1 and the GOx files can be found in the PDB database 1TR0 and 1GPE respectively).



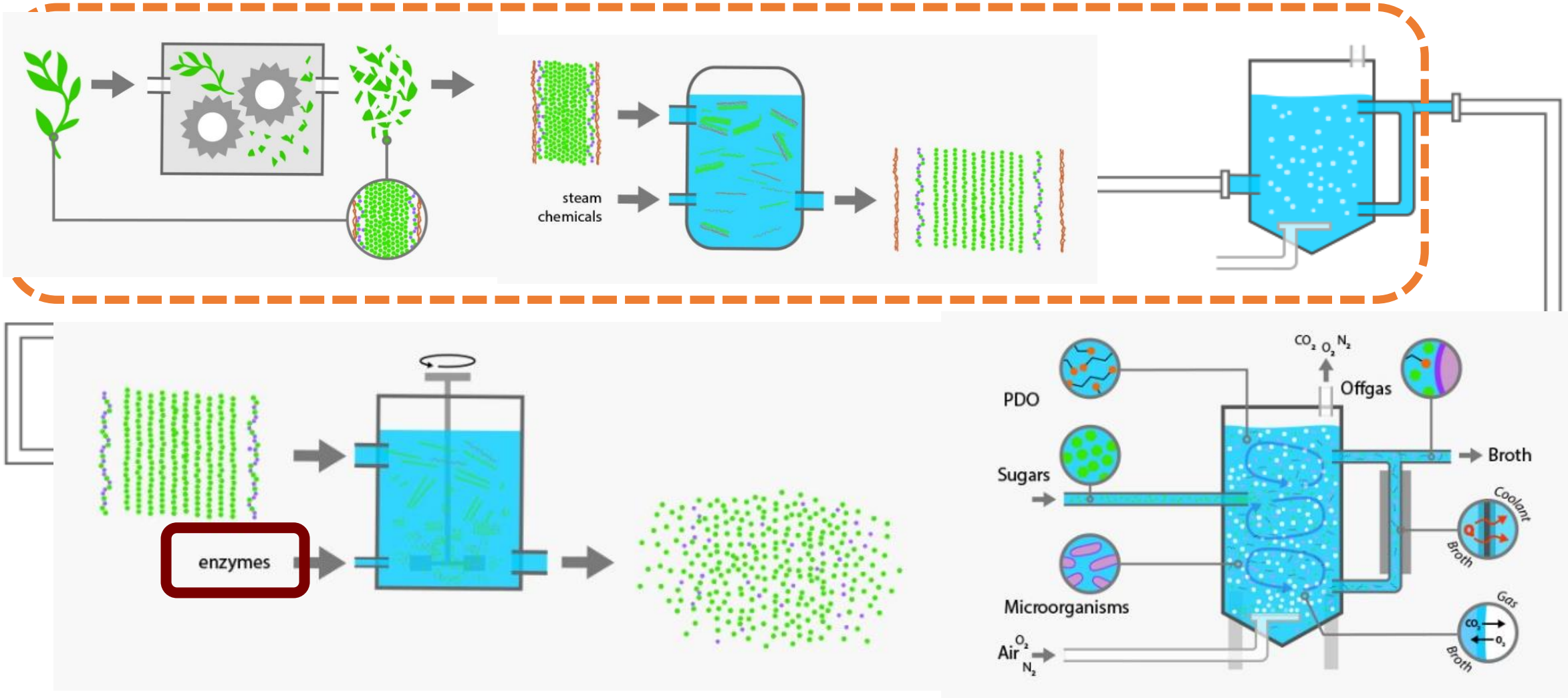


Right: (a) Transmission electron microscopy imaging of GOx-L-SP1. **(a)** GOx-L-SP1 complex, 12 GOx monomers around the SP1 dodecamer (49 nm diameter). **(b)** Graphical representation of the complex. **(c,d)** Multimers: dozens of dodecamers clinging together to form an enzyme nanotube particle. **(e)** Graphical representation of the enzyme nanotube particle.

Heyman, A., I. Levy, A. Altman and O. Shoseyov (2007). "SP1 as a Novel Scaffold Building Block for Self-Assembly Nanofabrication of Submicron Enzymatic Structures." Nano Letters **7**(6): 1575-1579.

The Cellulases

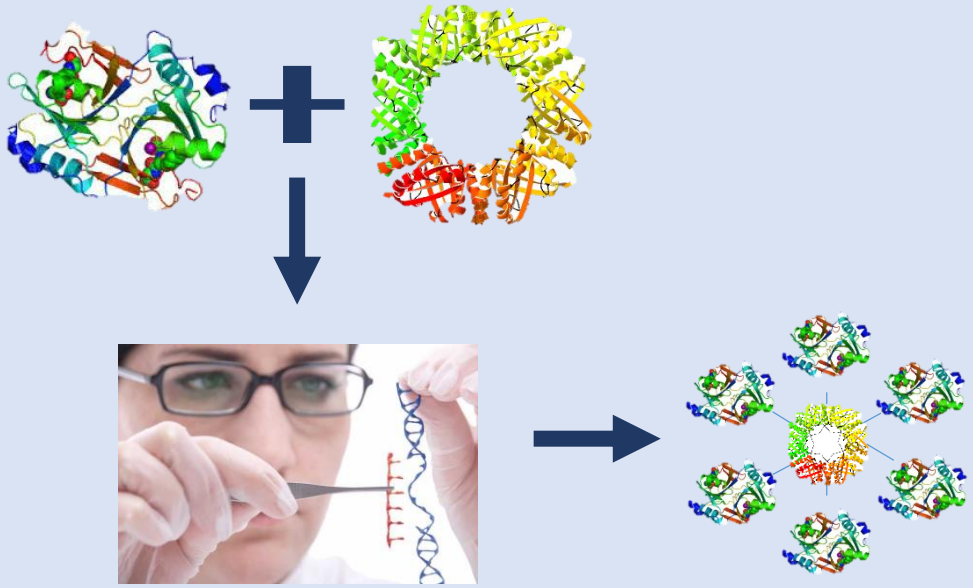
Their usefulness in biomass processing



The Proposed Idea

"A brief discussion…"

The potential of Molecular Cloning in Biocatalysis

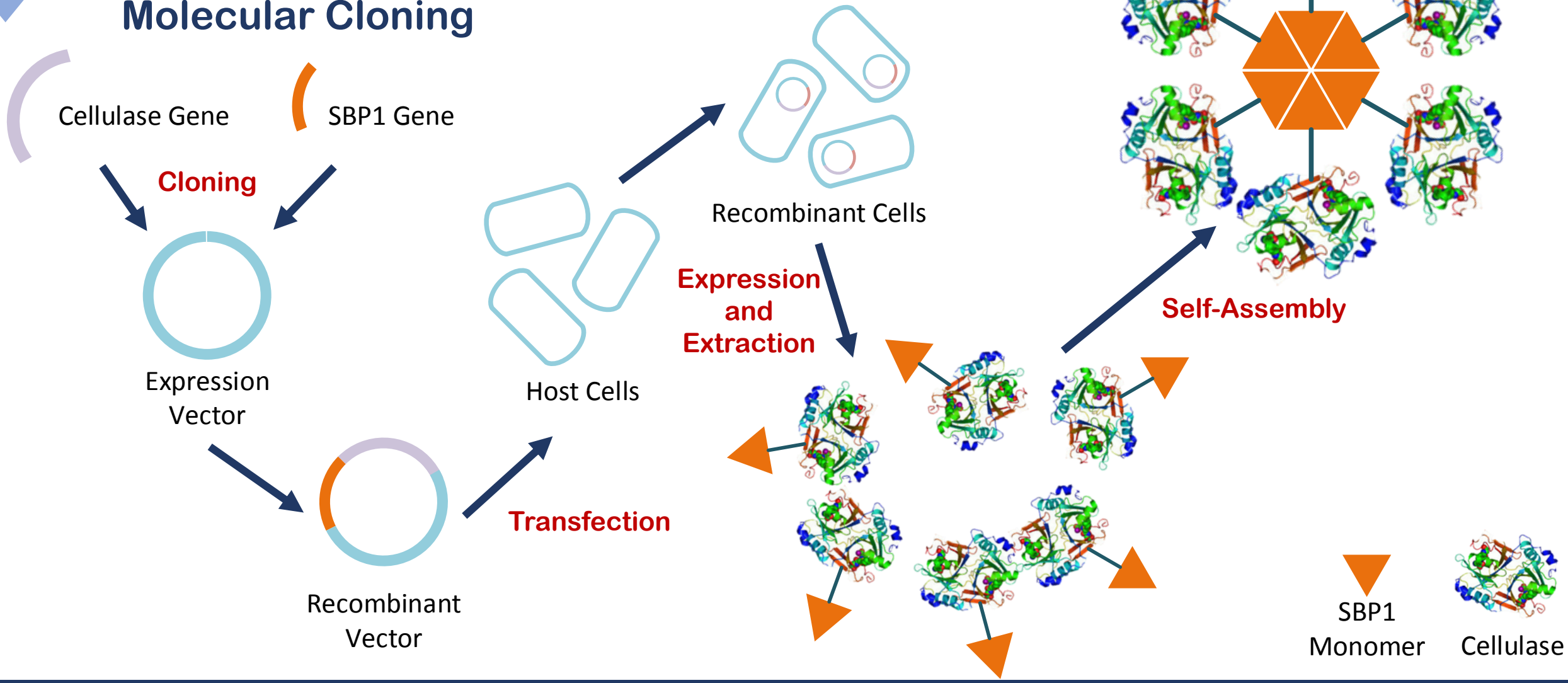


Immobilization of Cellulases on Stable Boiling Protein 1

A biomolecular engineering approach…

Research Design

Cellulase Immobilization Scheme by Molecular Cloning



Concluding Remarks

- Utilization of the “stable boiling protein 1” as a protein scaffold for cellulases
- The immobilized cellulases on SBP1 will form a “nanotube” structure via self-assembly
- A biomolecular engineering approach in assembly of nanobiocatalysts

Biotechnology for Biofuels

Enzyme Immobilization on Protein Scaffolds

"Immobilization of Cellulases on Stable Boiling Protein 1"

Any Questions, Comments or Suggestions?

Please don't hesitate to ask….

-End of Presentation-

Thank you for your attention!

microRNA: Insight for nanotechnology and tissue regeneration

PRESENTER:
IMAN ADIPURNAMA (D10204811)

Introduction: DNA – thread of life –

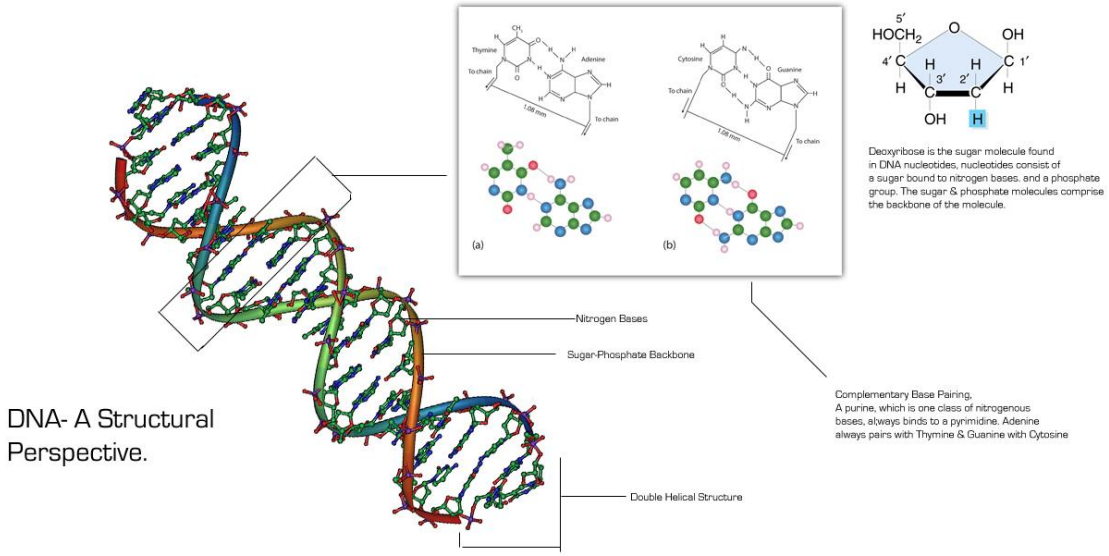
DNA has the ability to orchestrate all the complex chemistry that is essential to turn single cells that carry a sufficiently complex genome to extremely complex multicellular organisms.

Everything that we are, except whatever behaviours we may learn due to the environment, is down to DNA and processes that act on DNA.

Genomics sequence

Gene expression

“Human Genome Project”

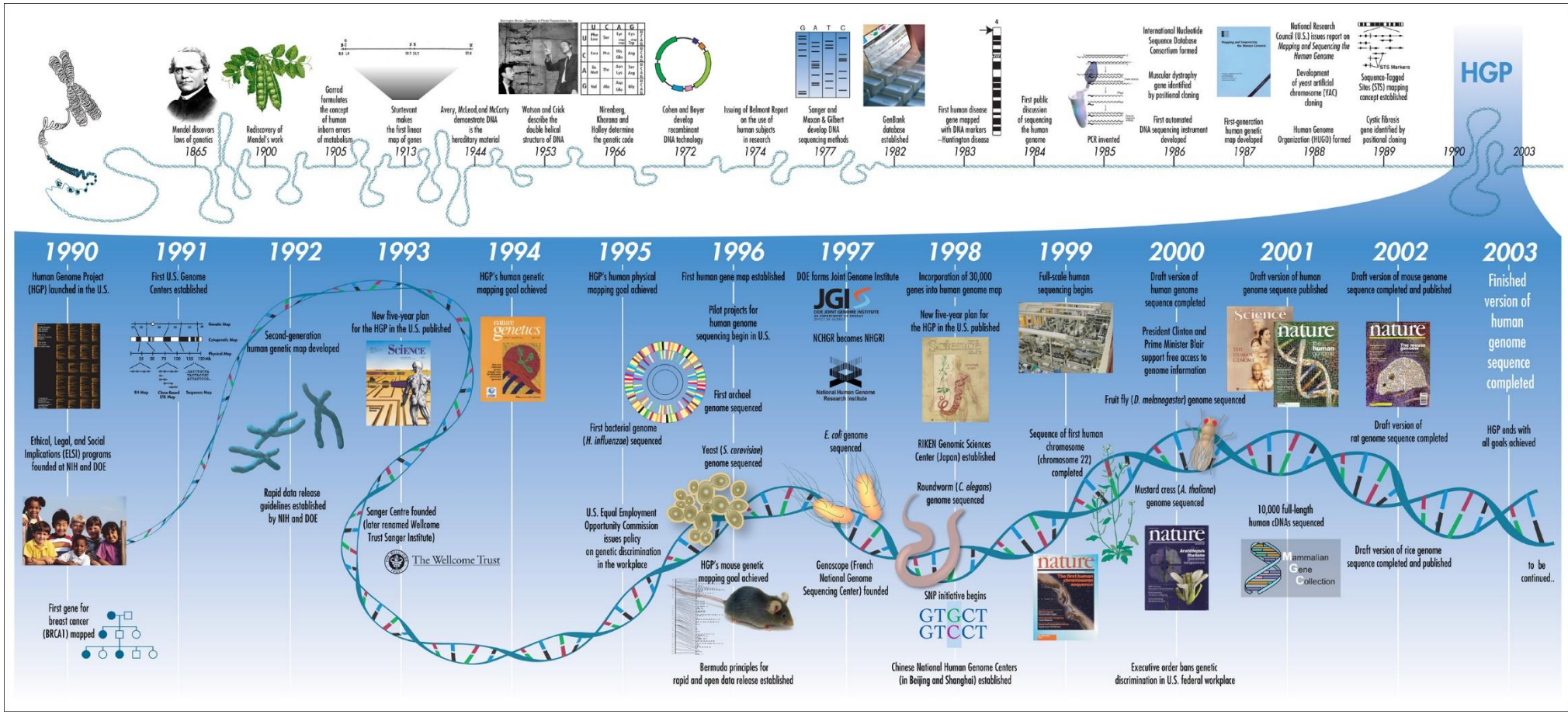


Introduction: Human genome project

How we will heal? How we will age?

"The next decades of medicine and health care will be about using technologies and keeping the human touch in practicing medicine. Everyone's genomes will be sequenced to access personalized treatments."

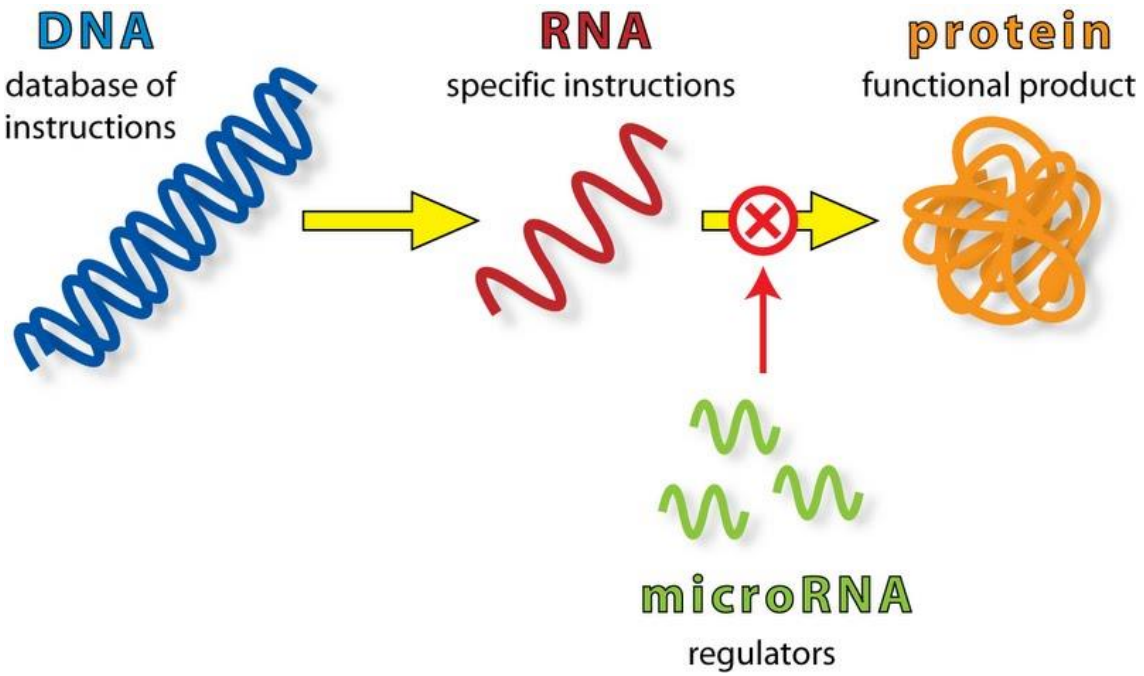
-Bertalan Mesko, *medical futurist*.



microRNA

MicroRNAs (miRNAs) are a class of small noncoding RNAs that play an **important role** in **posttranscriptional gene regulation** which affecting a multitude of biological processes including cell proliferation, differentiation, survival and motility.

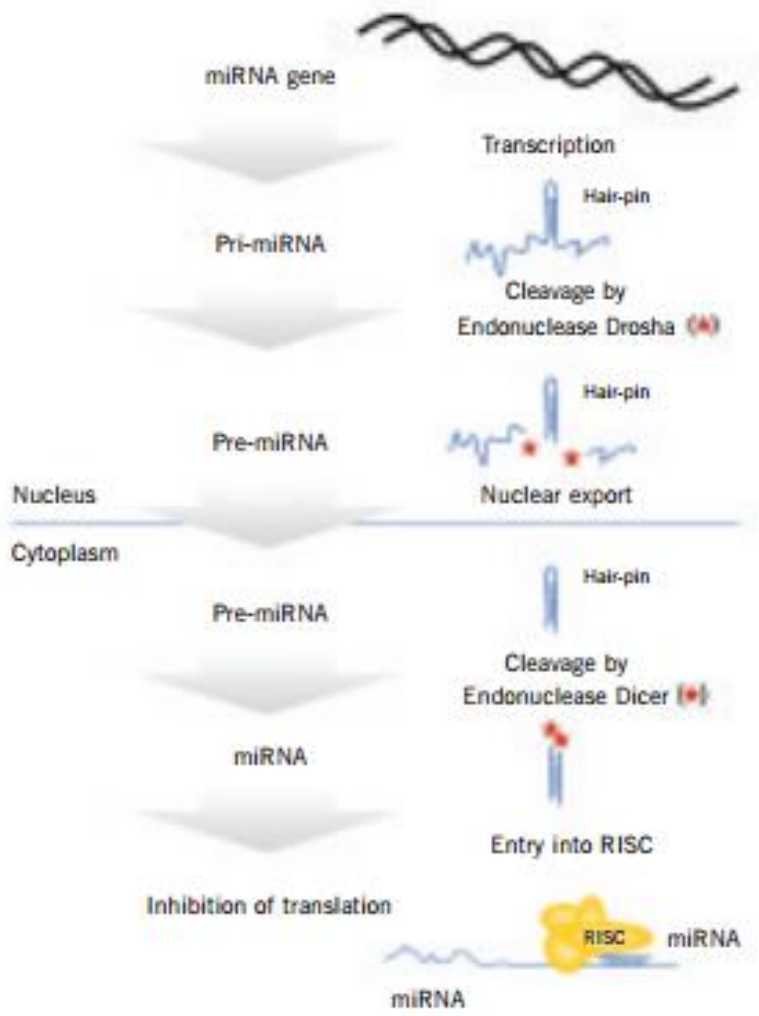
*miRNAs regulate the expression of at least **half of all human genes**. These single-stranded RNAs exert their regulatory action by **binding messenger RNAs** and **preventing their translation** into proteins.*



Mechanisms of miRNAs

Initially synthesized as longer precursors (**pri-miRNA**), miRNAs are processed through a series of stages to mature, **cytoplasmic miRNA** duplexes of ~22 nucleotides in length. Their regulatory activitities conferred upon loading into the RNA-induced silencing complex (**RISC**) in which one strand of the RNA duplex guides the RISC to its target **mRNA**. **Gene expression** is **downregulated** by inhibiting translation, targeting the mRNA for **degradation**, or a combination of both.

[microRNA formation and function - YouTube.MKV]



miRNAs potential

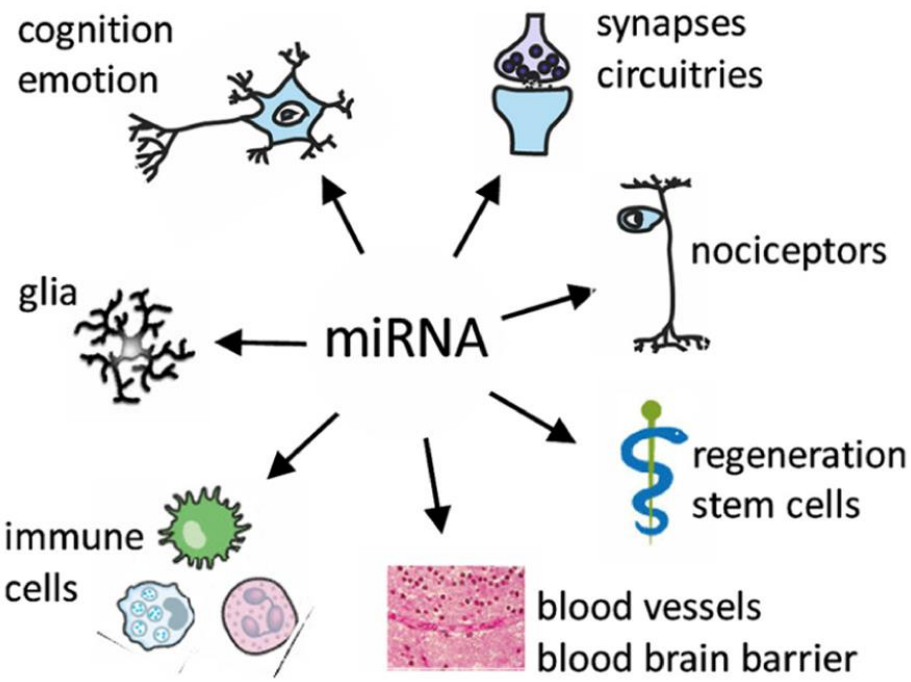
Many of the miRNAs identified to date have been associated with **cancer**. A comparison of tumour tissue with normal tissue has shown that **miRNA genes** are frequently **located** at **fragile sites** of the **human genome** and **subject to chromosomal rearrangement, gene amplification** and **deletion**.

Table 1: miRNAs in therapeutic development

miRNA	Indication	Status of development
miRNA antagonists		
miR-122	Hepatitis C virus	Phase 2 clinical trials
miR-208/499	Chronic heart failure	Preclinical development
miR-195	Post-myocardial infarction remodelling	Preclinical development
miRNA replacement		
miR-34	Cancer	Preclinical development
let-7	Cancer	Preclinical development

miRNAs potential

Therapeutic miRNA regulation has been thoroughly studied and **widely established in cancer** research but its impact and the therapeutic prospects of miRNAs in the pain field are largely unexplored. Manipulation of miRNAs offers the possibility to **control** multiple targets including **neuro-immune interactions**, **nociceptive processing** and **cognitive pathways.**

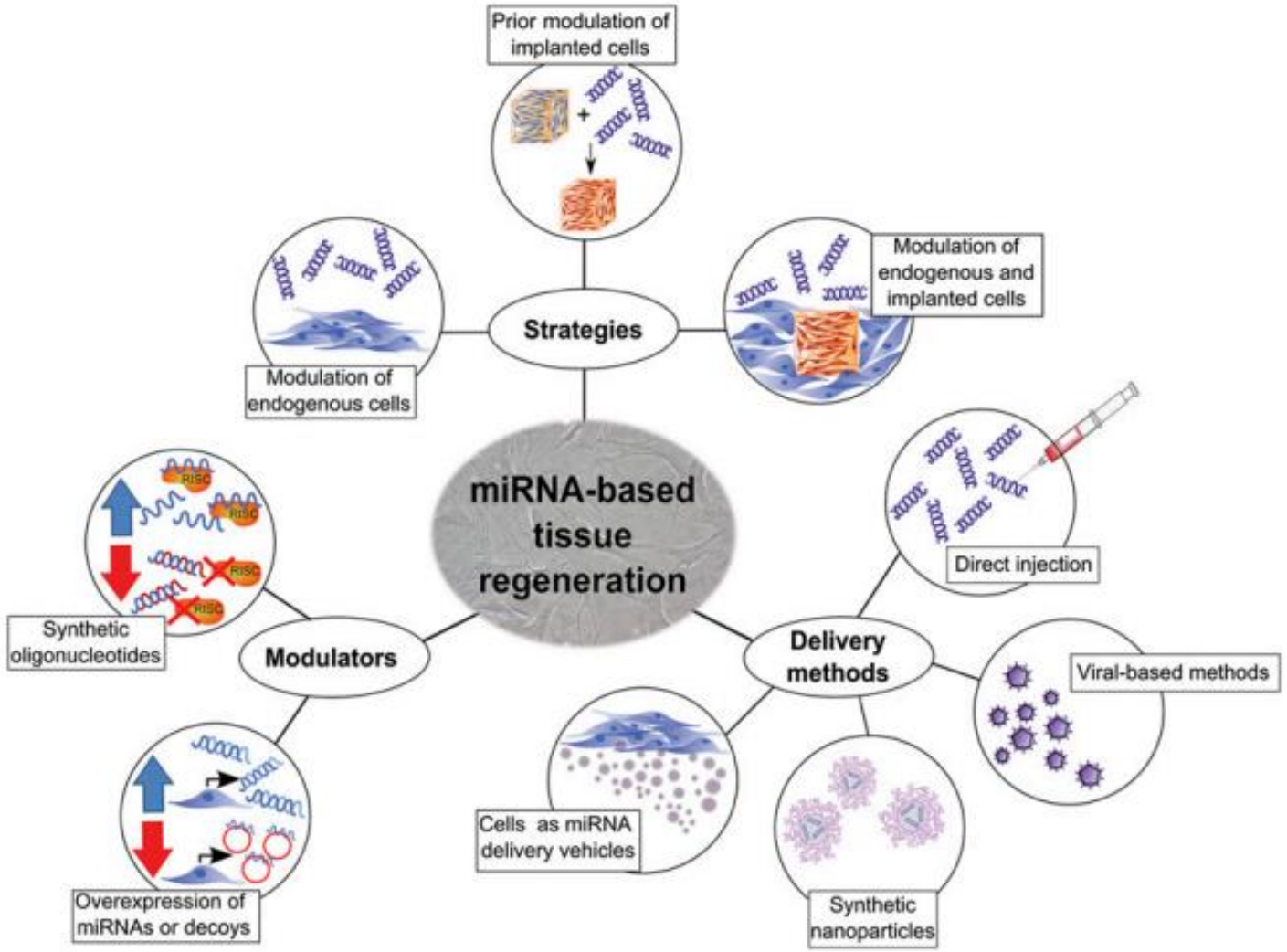


Micro-RNA based tissue regeneration

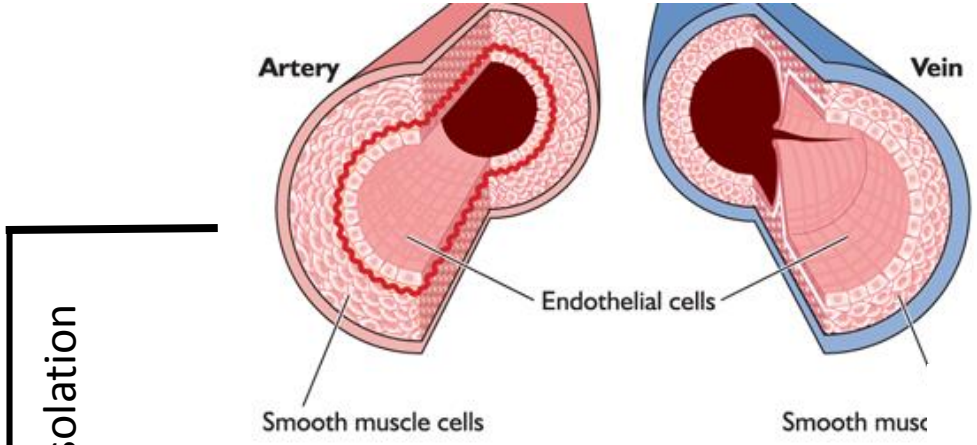
Strategies using miRNAs for tissue regeneration therapies include altering endogenous cellular activity, directing the behavior of (stem) cells incorporated into tissue-engineered constructs, or targeting both implanted and endogenous cells.

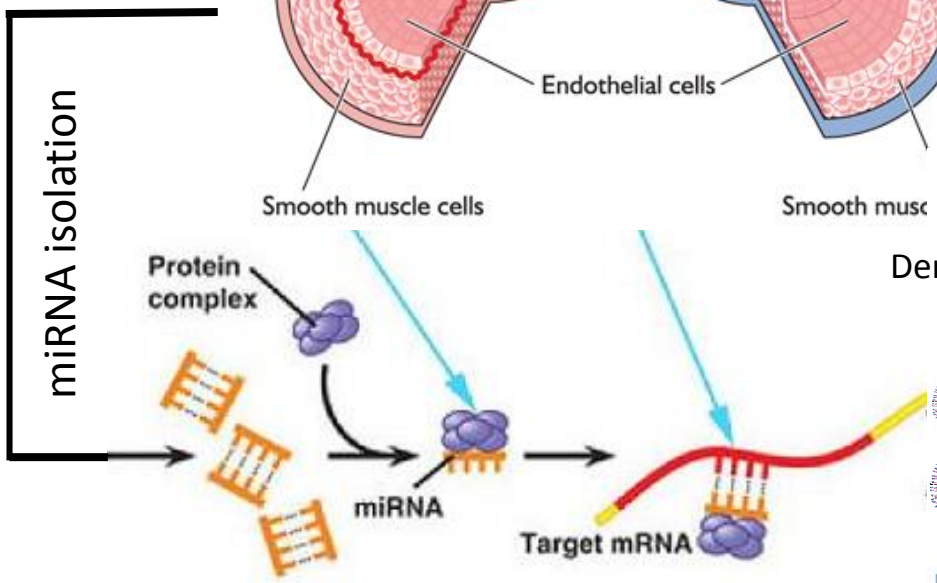
Modulation of miRNAs can be achieved by the delivery of miRNA mimics or anti-miRs and by overexpressing miRNAs or miRNA sponges in the cell type of interest.

Methods for delivery of such modulators include simple injection, viral overexpression, delivery via synthetic nanoparticles, or delivery from cells via extracellular vesicles.



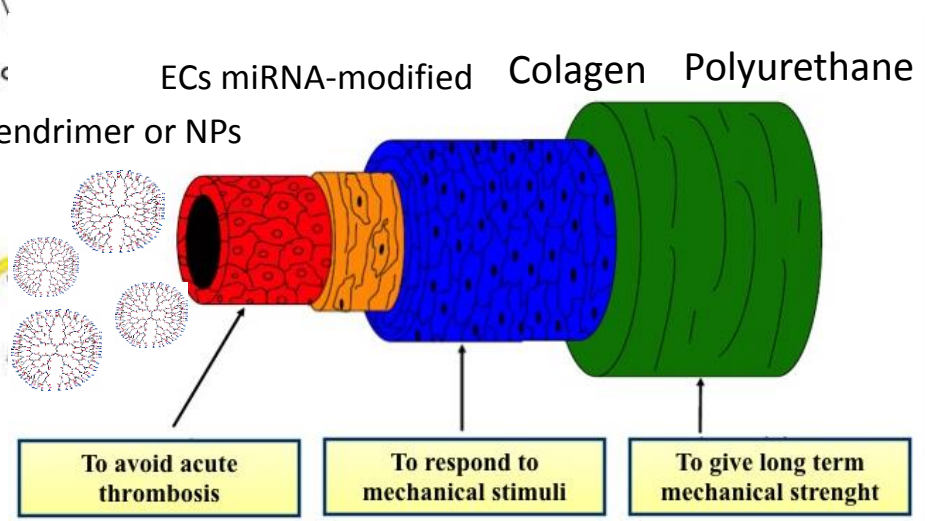
Strategies for Blood vessel engineering





One problem in blood vessel engineering that often happened is thrombosis

(Tissue Factor Pathway Inhibitor) TFPI-2
Regulating miRNA: miR-616



Promising Future

Personalized medicine.

There is significant potential to harness miRNAs to direct tissue regeneration, either via endogenous repair mechanisms or by directing the activity of implanted cells in cell therapies.

The promise of miRNA-based regeneration is vast, because the right candidate holds the promise to target multiple levels of relevant cellular signaling pathway.

Attractive candidates for clinical use because miRNAs can be pharmacologically targeted and has small size compared with proteins.

Both miRNA biology and delivery technologies hold significant promise in the future.

Literature cited

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Frith, J.E., Porello, E.R., Cooper-White, J.J. Concise Review: New Frontiers in MicroRNA-Based Tissue Regeneration. STEM CELLS TRANSLATIONAL MEDICINE 2014;3:1–8

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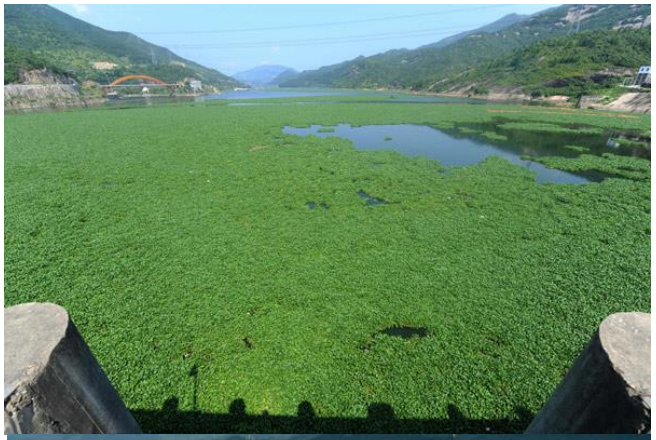
www.fireflybio.com/introduction_to_microRNA

exploreable.wordpress.com



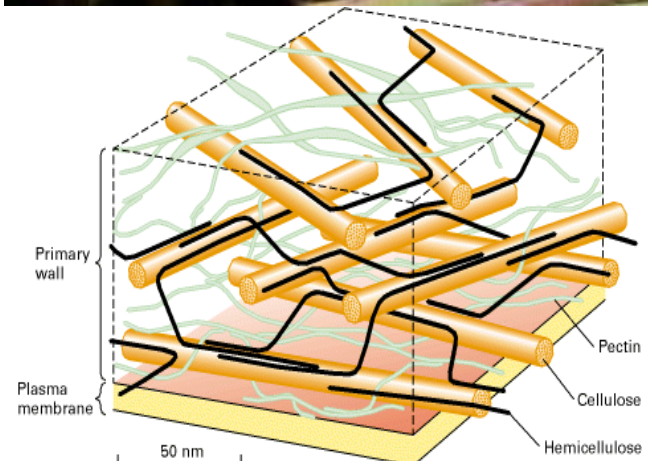
Cellulose chemistry

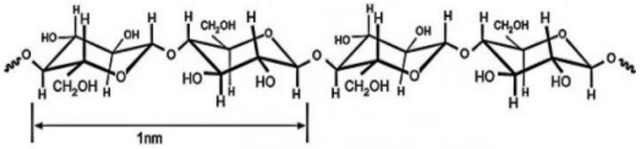
Synthetic organic chemistry

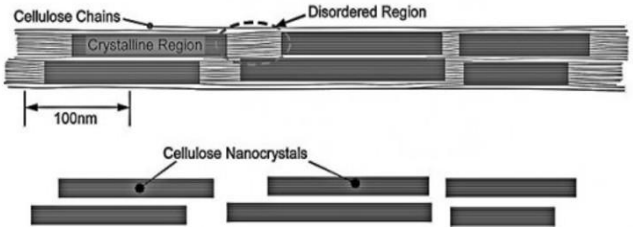
Product development

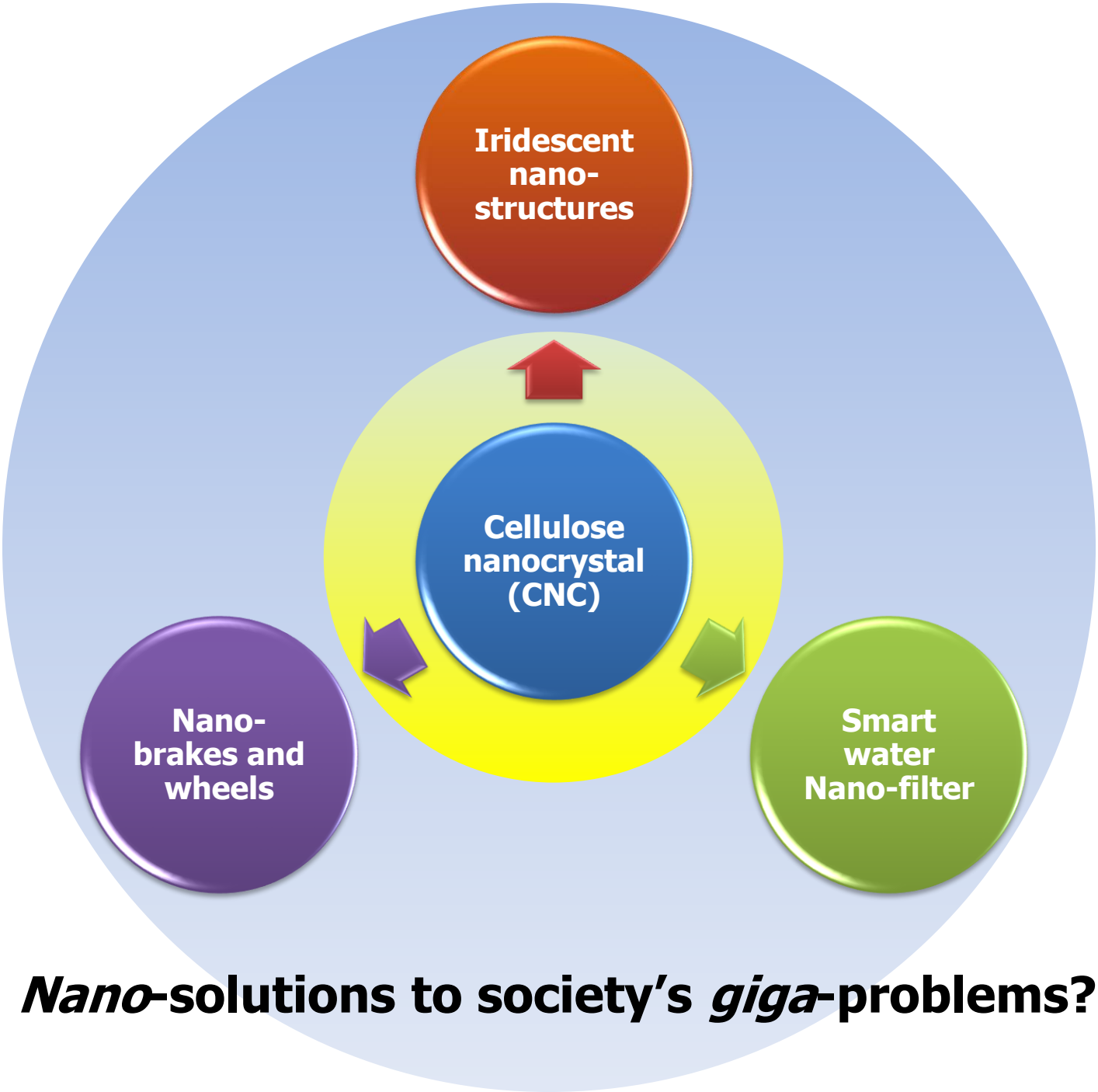
Textile Science and Engineering

NANOTECHNOLOGY









***Nano*-solutions to society's *giga*-problems?**

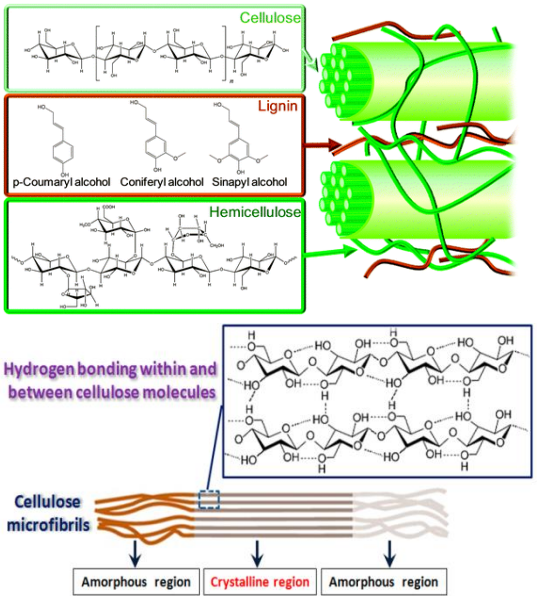
Cellulose nanocrystal (CNC)

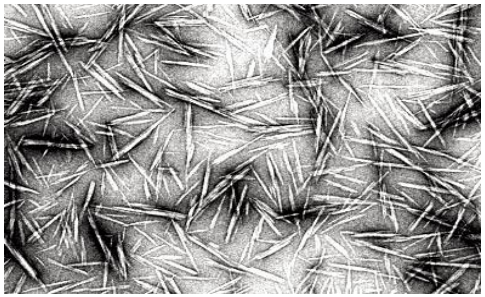
Harness cellulose nanocrystals from abundant raw material *e.g.* agricultural by-products *(Top-down approach)*

Bananas, at **9,162 tonnes**, remained the largest export with a 25.1 per cent share. *(Source: http://www.fruitnet.com/asiafruit/article/160771/taiwan-targets-pineapple-export-gains)*

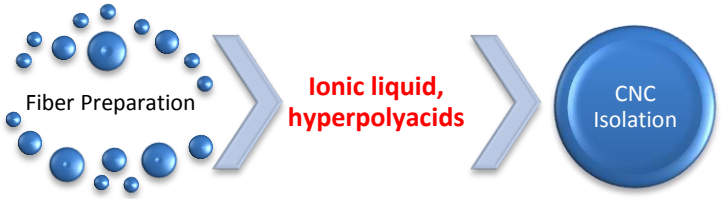
Taiwan exported a total of **4,746 tonnes** of **pineapples** in 2013, marking an per cent increase on the previous year, climbing by 30 per cent to net NT$142 (US$4.69m)
(Source: http://www.fruitnet.com/asiafruit/article/160771/taiwan-targets-pineapple-export-gains)

The Science





The Core Idea



- Pre-treatment
- characterization

- Optimization of reaction conditions
- characterization

Remarkable properties

- High aspect ration (length-to-width ratio)
- Tensile strength = 500MPa
- Stiffness-= 140-220GPa
- Strength/weight ratio=8x stainless steel
- High conc. of –OH in the surface
- Self-assembles
- Natural

Socio-economic Relevance:

Farm-level fiber recovery → Fiber processing → CNC extraction → Application

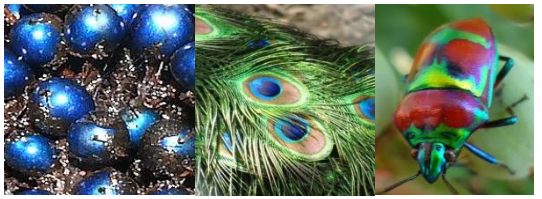
Novelty of the Idea

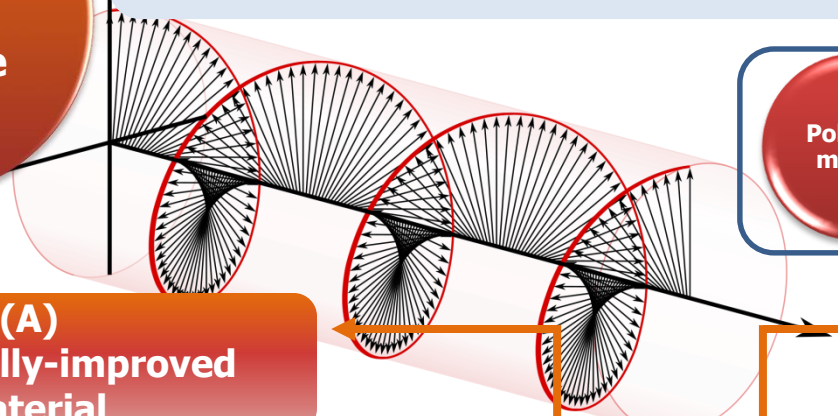
1) New raw materials i.e. by-products
2) New cellulose material technologies
3) New CNC extraction approach
4) *Green*-orientation:material&process

Naturally-abundant and untapped raw material of nanomaterial towards exciting applications!

Iridescent Nano-structure

Takes advantage of the self-assembling property of cellulose nanocrystals (CNC)





Polymer matrix + **Cellulose Nano-crystal** = **Iridescent nano structures**

(A) Structurally-improved material

Structure

1) Electrospun, film

Variables

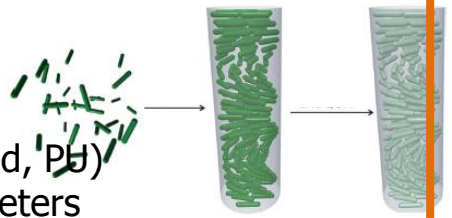
1) Matrix (latex-based, PU)
2) Processing/parameters

Expected properties:

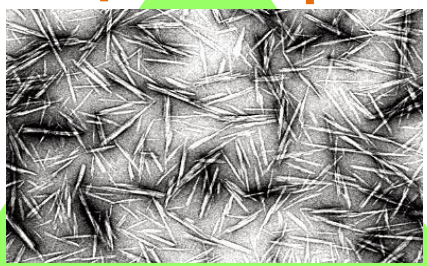
1) Improved mechanical properties (brittle probably for PF resin)
2) Left-handed helical orientation of the CNC in the material
3) Iridescence (birefringence)

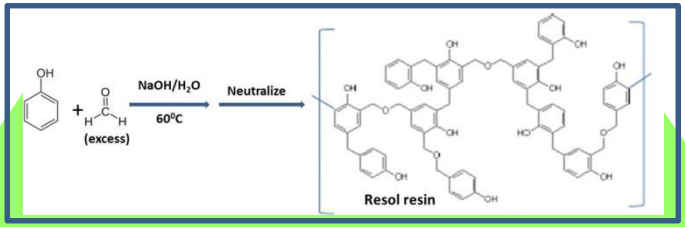
Potential Applications:

1) **Durable iridescent textile fiber for light-harnessing**
2) **Iridescent paints, coatings, surfaces**



CNC-discharge





(B) Mesoporous materials

Structure

1) Electrospun, film

Variables

1) Matrices (other plastic resins)
2) Processing/parameters

Expected proeprties:

1) Mesoporous film
2) Swellable/tunable
3) Left-handed helical orientation of the CNC in the material
4) Iridescence (birefringence) but **flexible**

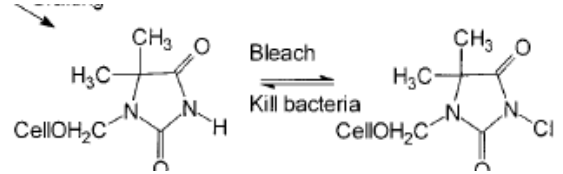
Potential Applications:

1) Durable light-guided water and ion security feature against counterfeiting.

***Smart* water nano-filter**

Practical functional water *ultra-filters*.

The Biocidal Property



Ind. Eng. Chem. Res. **2001**, *40*, 1016−1021

The Element Sequestration (*e.g* B)

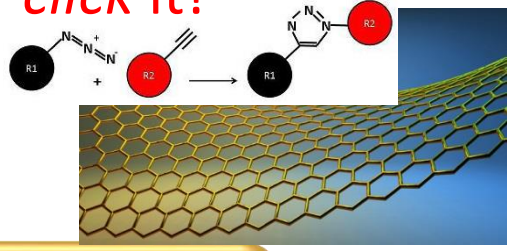
Graphene + CNC + Fe3O4 or β-CD = Nano sorbent

Socio-economic Relevance

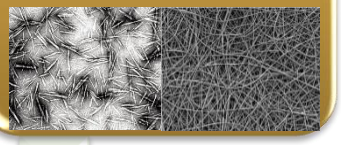
Filter and Sorbent production → Production and Sale/Deployment → Massive impact on water challenged communities

CNC-functionalization (graphene, magnetic NP, cages)

click it!

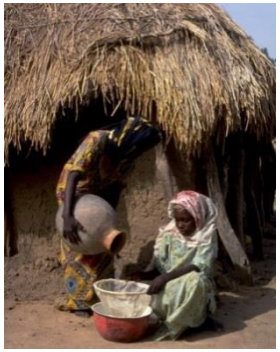


Ultra-Electrospinning



Yarn spinning
Fabrication
Product development
Performance verification

Ultra-filtration



The Idea

1) Durable because *covalently bound*
2) Regenerable by simple household chlorine bleach
3) Biocidal (across a wide range of bateria and fungi)
4) Safe and reliable and simple system –small-scale and upscale

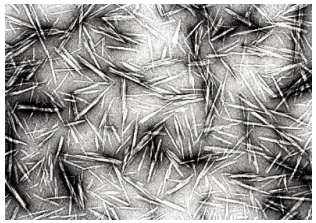
Potential Application/ Impact:

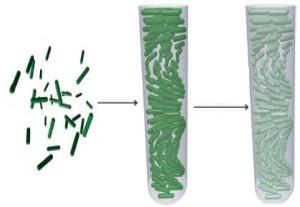
(1) Durable, rechargeable, biocidal, practical ultra-filters for unsafe drinking water!

(2) Effective use of salt water as drinking .

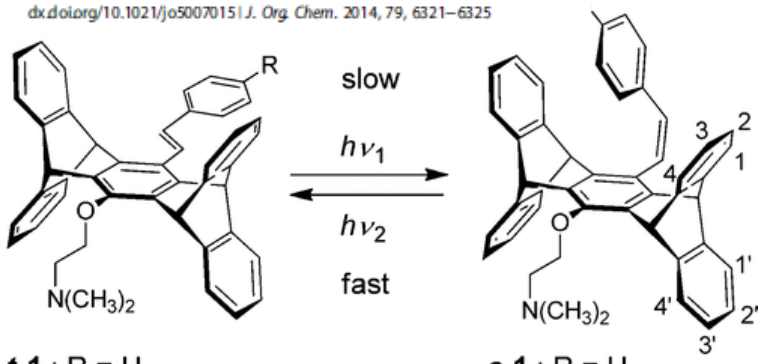
Nano- **brakes and wheels**

Develops an ABS-like mechanism for CNC in their self-assembly.





The Science:
Introduce light-controlled way to introduce/ halt motion of CNC particles

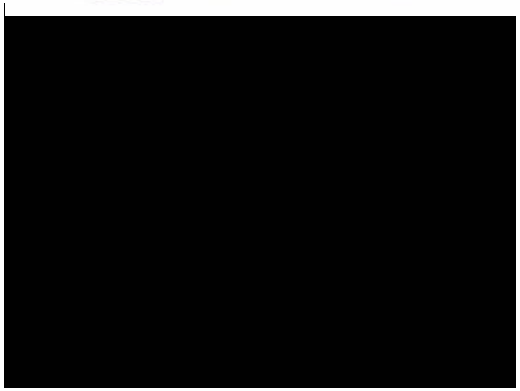


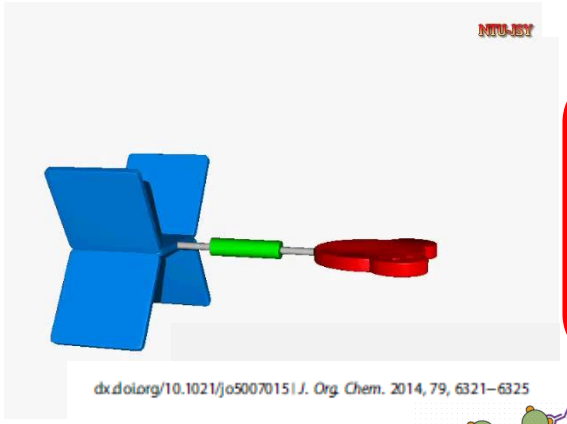
***t*-1** : R = H
***t*-2** : R = 4-C_6H_4CN

***c*-1** : R = H
***c*-2** : R = 4-C_6H_4CN

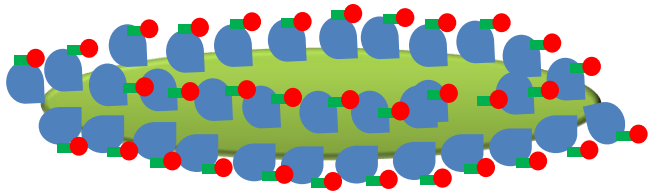
brake-off

brake-on



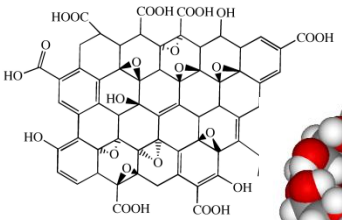


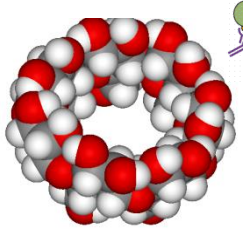
My Idea: ***Illustrated***

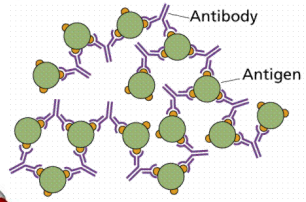


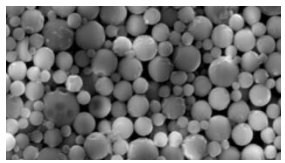
Key Features:
Light-controlled Brownian motion of the CNC's

Novelty of Idea:
Naturally-derived NP platform that is robust, motile and is light-controlled!









Potential Applications:

1) **Light-gated control**
2) **Sensors and detection material**
3) **Biomaterials**
4) **Remote nano-diagnostic**
5) **Drug delivery**
6) **Remote nano-surgery/therapy (PDT)**

sustainable

facile

relevant

Introduction to Nanotechnology B
(Project Idea)

JULIUS L. LEAÑO JR.
TIGP Nano-Chemistry

My *nano* solutions for today's giga problems!

Biotransformation of Artonin E (Optimized by SWCNT glass bead)

LAI YEW SENG

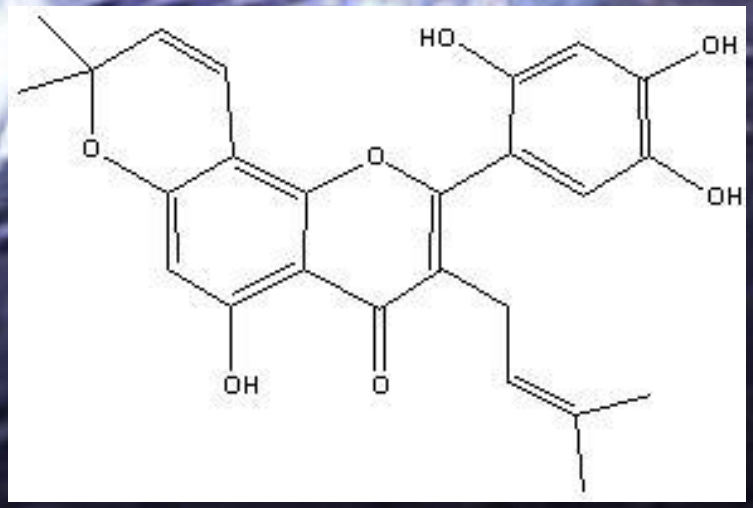
- Biotransformation involves the modification made by an organism on a chemical compound in which catalyzed by the enzyme present in the microorganism

- operate in non-extreme pH and near room temperature

- high stereospecificity and the products produced are usually less toxic than the parent compound

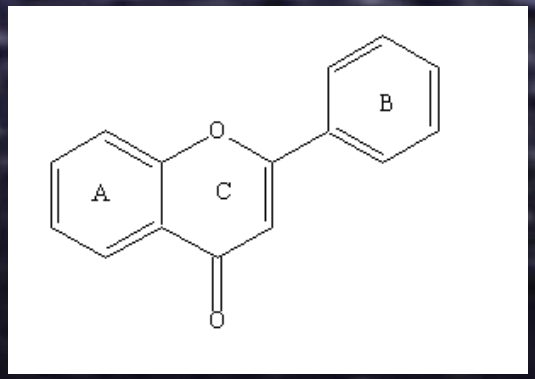
Artonin E

- extracted from the bark of *Artocarpus.tesymanii*
- molecular formula of $C_{25}H_{24}O_7$



- *Artocarpus sp tree*

- Flavonoid, polyphenolic compounds
- include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones
- act as antioxidants due to the ability to chelate iron and to scavenge reactive oxygen species (Ueno *et al.*, 1984; Robak and Gryglewski, 1988; Afanas'Ev *et al.*, 1989; Jovanovic *et al.*, 1994)
- basic molecular structure of flavonoids compounds consist of an aromatic A ring and an oxygen-containing heterocyclic C ring attached by carbon-carbon bond to aromatic B ring (Beecher, 2003)



Problem Statement

- Low productivity of the transformation product
- Low efficiency of the biotransformation reaction

Objective

- Increase the productivity of the transformed product
- Increase the efficiency of the biotransformation reaction

Methodology

Preparation of Single Walled Carbon Nanotube's Glass Bead

the purchased glass beads with 5 mm of diameter will be cleaned for 30 min in a solution composed of H_2O_2 (30%) and H_2SO_4 (18 M) with the ratio of 1:2

↓

washed with distilled water and dried under a N_2 flow

↓

commercial SWCNT with purity >90% and outer diameter 1-2 nm will be previously dispersed in a $CHCl_3$ solution

↓

then deposited on the glass bead by drop casting

↓

SWCNT can be sterilized by autoclaving at 121 ◦ C for 15 min and immersing them in 3% H_2O_2 solution for 10 min, followed by washed in sterile distilled water to remove residual H_2O_2

Fungus colonization on SWCNTs Glass Bead

Aspergillus aculeatus will be grow in Erlenmeyer flask with *SWCNTs glass bead*

↓

Incubate and shaked at 200rpm at 30°C, 4days

↓

The colonization of fungus strain can be view by AFM

↓

The modified SWCNTs glass bead will be added to the biotransformation reaction with the particular concentration of artonin E

↓

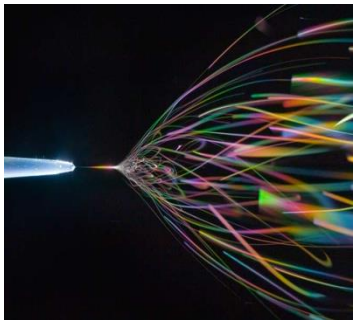
Transformed product will be further identified by HPLC and NMR; study the antioxidant property

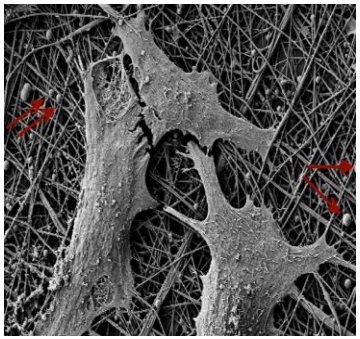
Expected Result

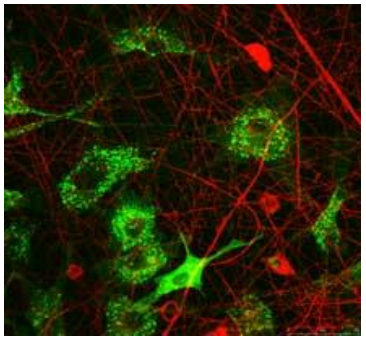
- The productivity of the transformed product was increased
- The efficiency of the biotransformation reaction was promoted

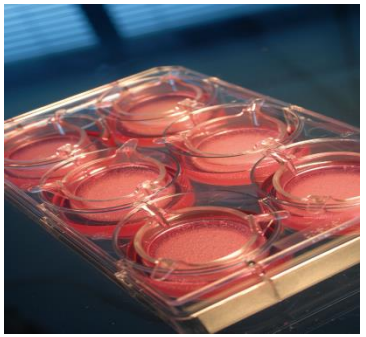

Thank You

January, 13th, 2015









Biomimetic Electrospun Nanofibrous Structures for Tissue Engineering

Student Project

Student Name: Mai Khaleel

Instructor: Dr. HosseinKhani

Outline

- Introduction.
- Techniques of Nanofibers for Tissue Engineering.
- Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering.
- Conclusion.

Introduction

- What is a Nanofiber?

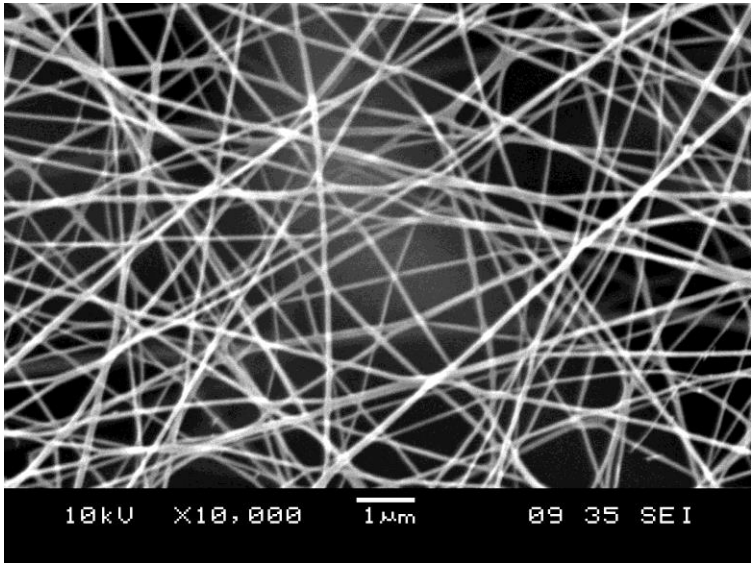
Fiber with diameter in nanometer range. Many types of polymers were processed into nanofibers of 50 to 1000 nanometers in diameter.

- Nanofibers Properties

High surface to weight ratio

Low density

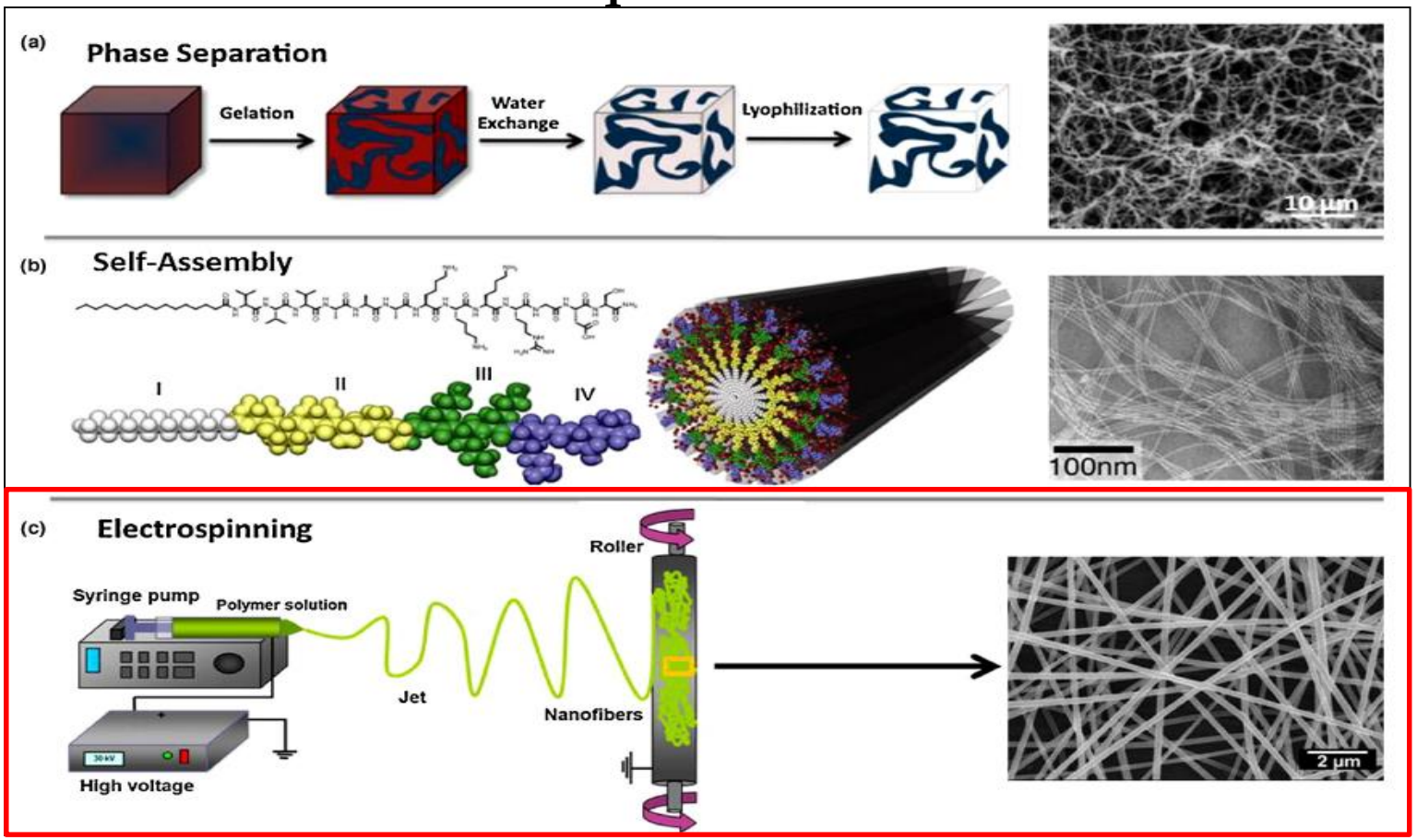
Large surface area to mass



http://www.nano109.com/products.php?productcategoryId=39&productId=153

Introduction

- Nanofibers Techniques



R.J. Wade, J.A. Burdick, Mater. Today 15 (2012) 454.

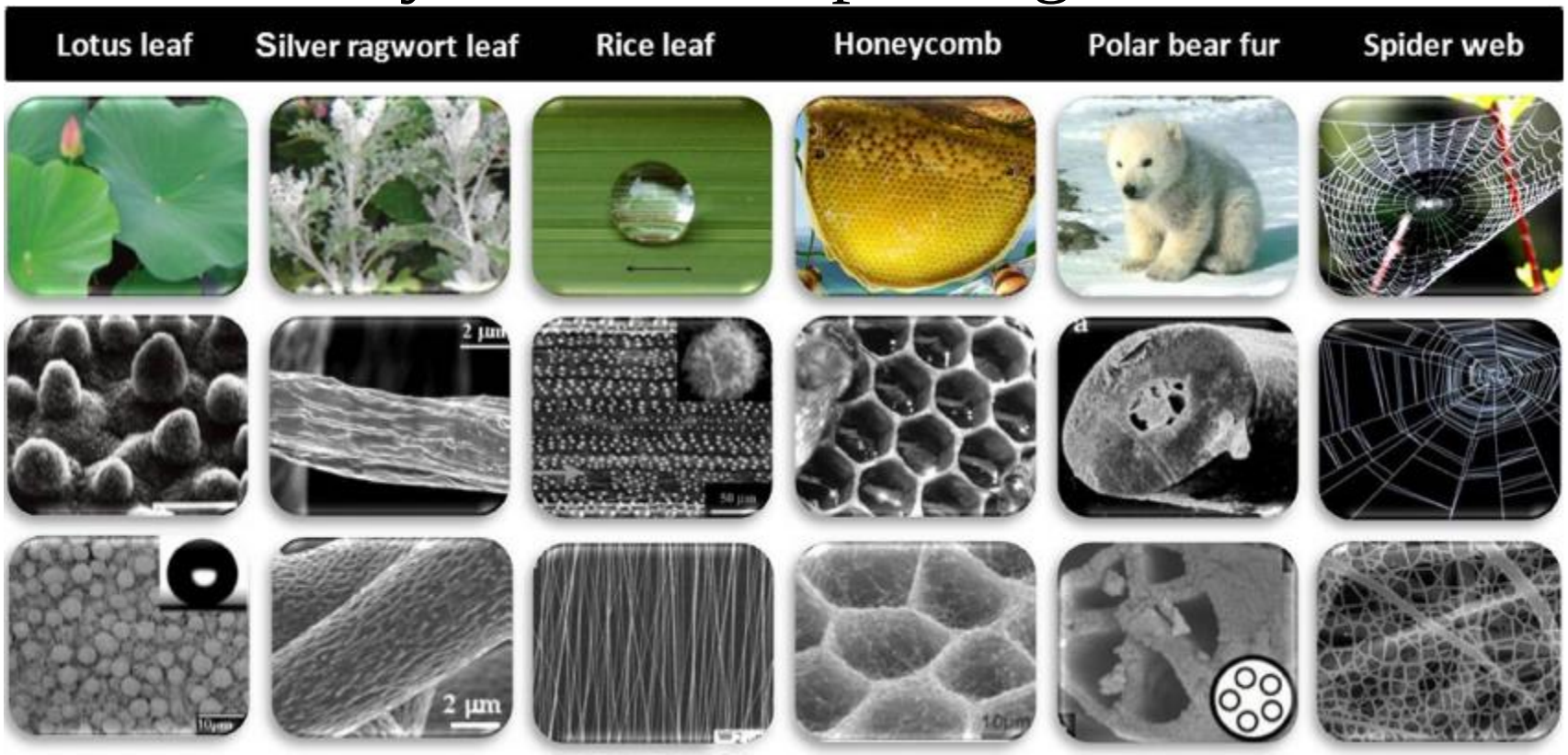
J.B. Matson, et al. Curr. Opin. Solid State Mater. Sci. 15 (2011) 225.

P.X. Ma, R.Y. Zhang, J. Biomed. Mater. Res. 46 (1999) 60.

J.D. Hartgerink, et al. Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5133.

Introduction

- Biomimicry via Electrospinning



B. Ding, et al. J. Mater. Chem. 21 (2011) 12784.
X. Zhang, et al. J. Mater. Chem. 18 (2008) 621.
W. Barthlott, C. Neinhuis, Planta 202 (1997) 1.
L. Jiang, et al. Angew. Chem. Int. Ed. 43 (2004) 4338.
Y. Miyauchi, et al. Nanotechnology 17 (2006) 5151.
Z.G. Guo, W.M. Liu, Plant Sci. 172 (2007) 1103.
L. Feng, et al. Adv. Mater. 14 (2002) 1857.
D. Li, et al. Nano Lett. 3 (2003) 1167.
G.D. Yan, et al. Langmuir 27 (2011) 4285.
R.E. Grojean, et al. Appl. Optics 19 (1980) 339.
Y. Zhao, et al. J. Am. Chem. Soc. 129 (2007) 764.

Introduction

- Nanofibers Technology
- Techniques of nanofibers for tissue engineering.

- Tissue engineering

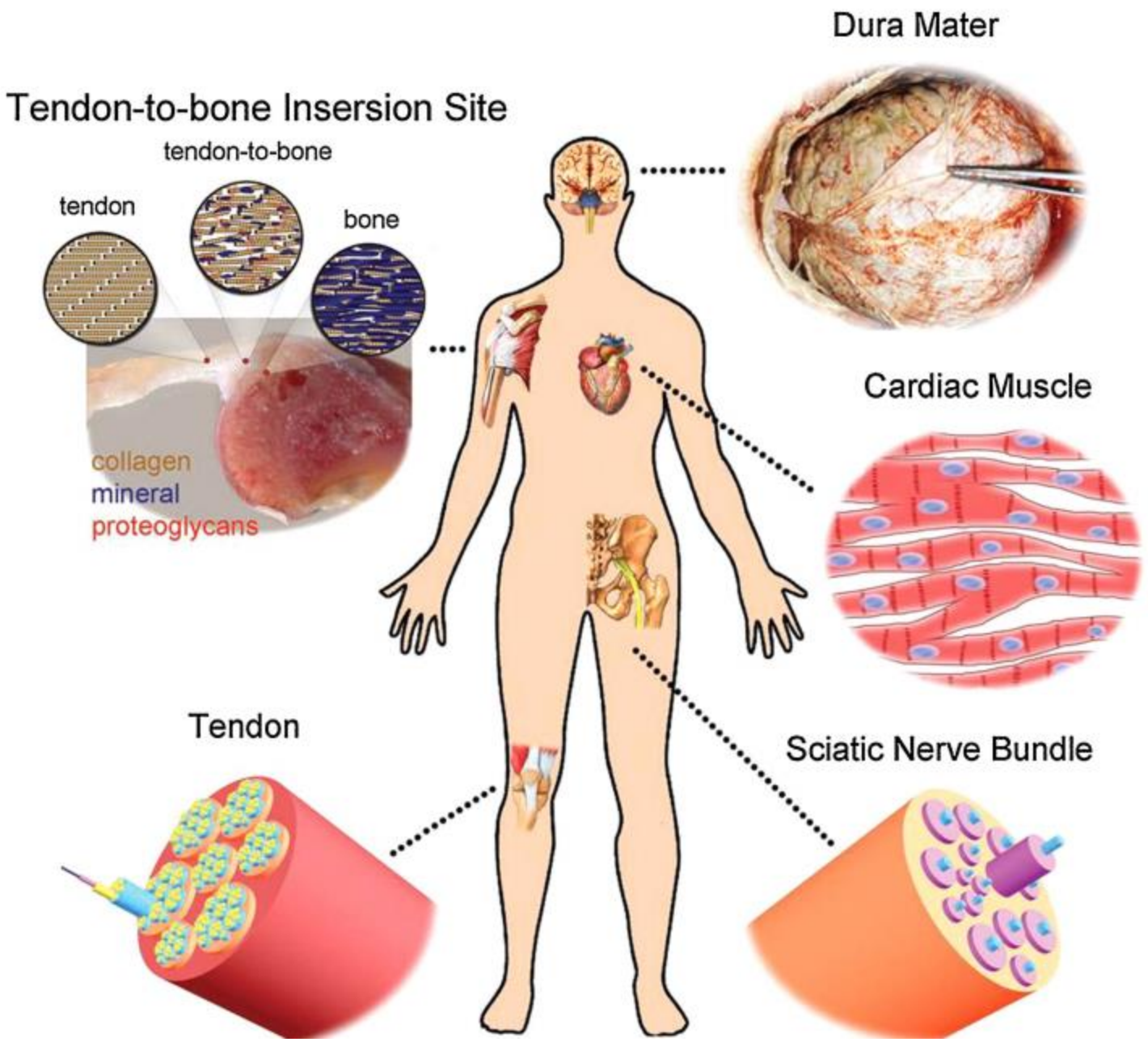
Interdisciplinary field addressing the improvement, repair, or replacement of tissue/organ function.

- Scaffolds → Artificial Extracellular Matrix

Biomaterials, which may be natural or artificially derived, providing a platform for cell function, adhesion and transplantation.

Techniques of Nanofibers for Tissue Engineering.

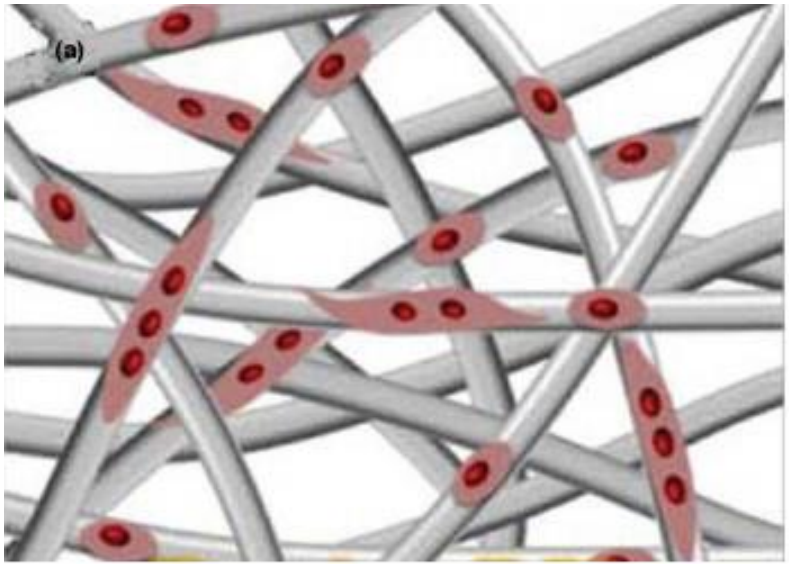
- Superior capacity in shaping cell morphology.
- Guiding cell migration.
- Affecting cell differentiation when compared to other types of scaffolds both in vitro and in vivo.

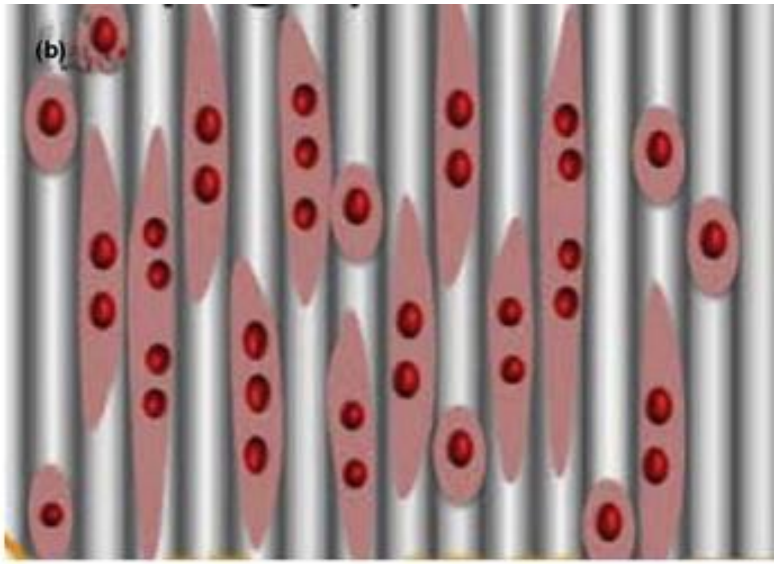


W. Liu, et al. Adv. Health. Mater. 1 (2012) 10.

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

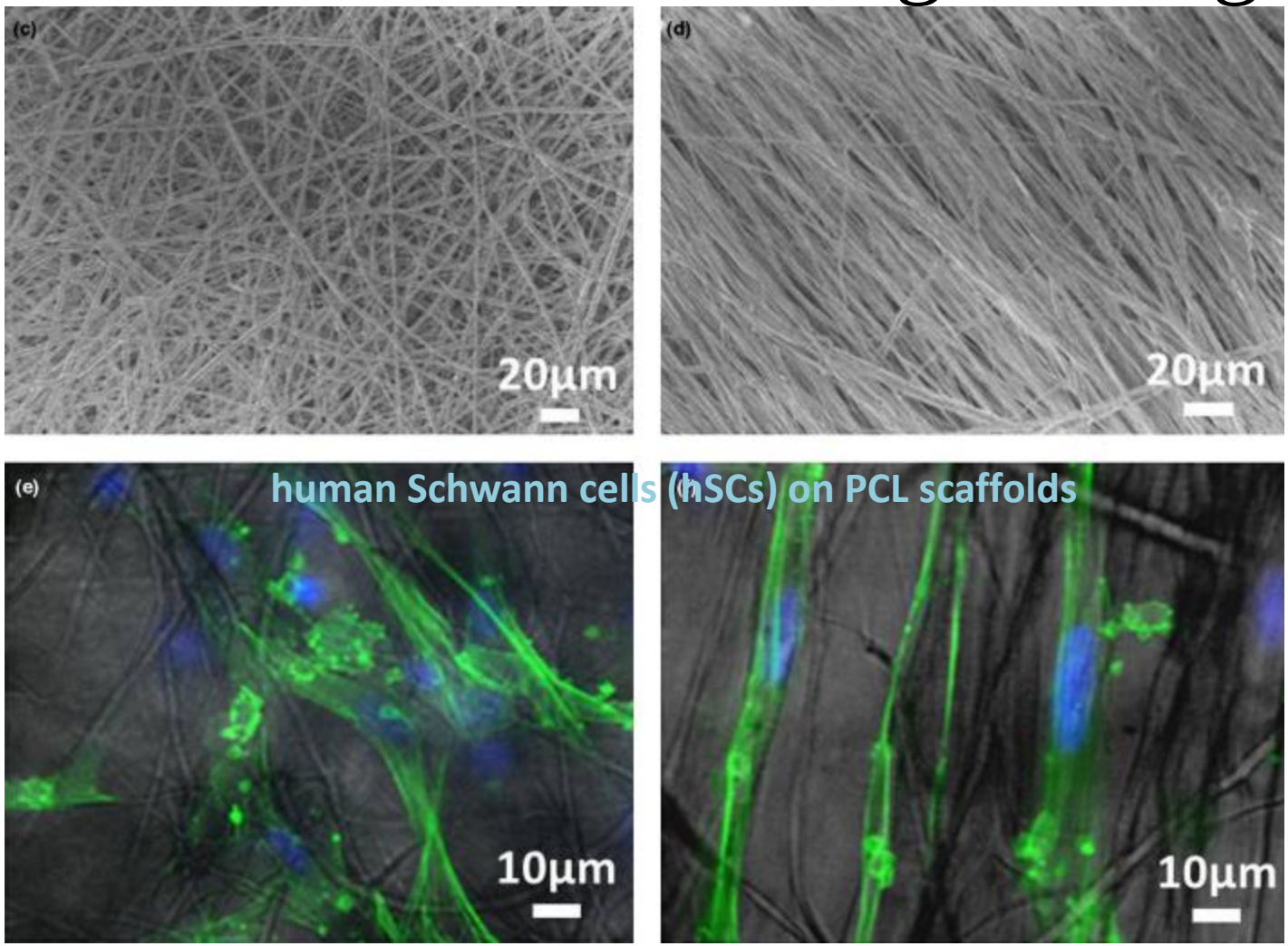
- **Why Alignment?**
- An aligned electrospun nanofibrous scaffold can guide the migration and extension of cells.





C.Y. Huang, et al. Biomaterials 33 (2012) 1791.
L.S. Wan, Z.K. Xu, J. Biomed. Mater. Res. A 89 (2009) 168.
M. Chen, et al. Acta Biomater. 9 (2013) 5562.
J. Xie, et al. ACS Nano 4 (2010) 5027.

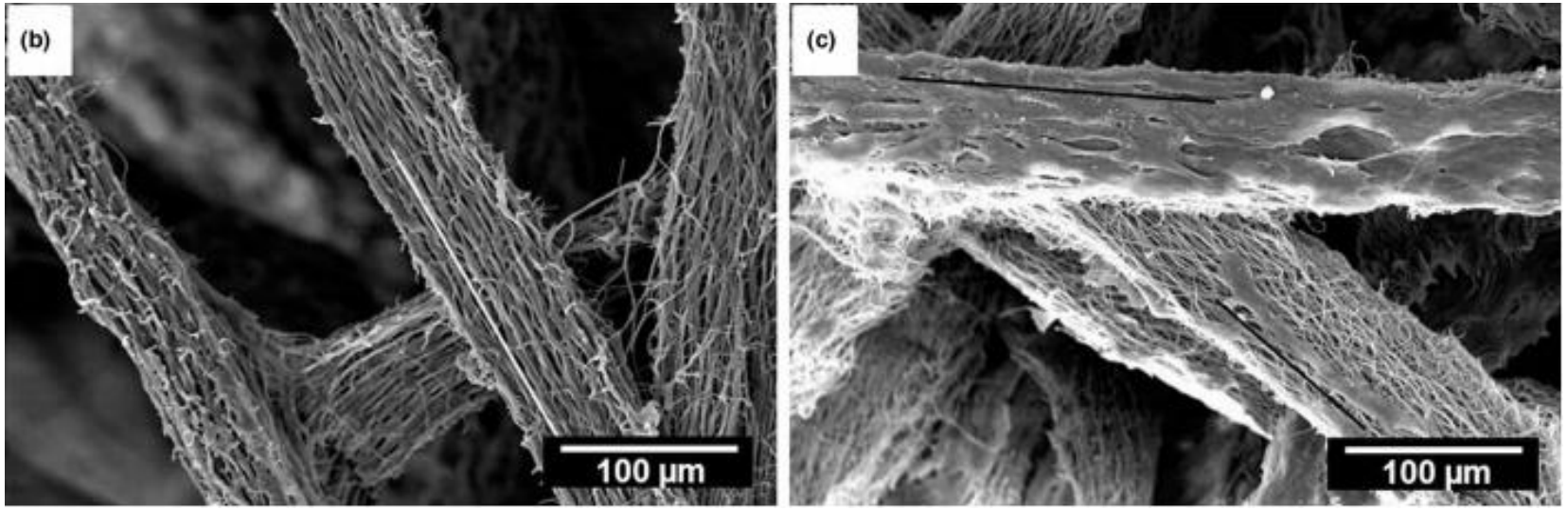
Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering



S.Y. Chew, et al. Biomaterials 29 (2008) 653.

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

1. Bone Tissue Engineering.

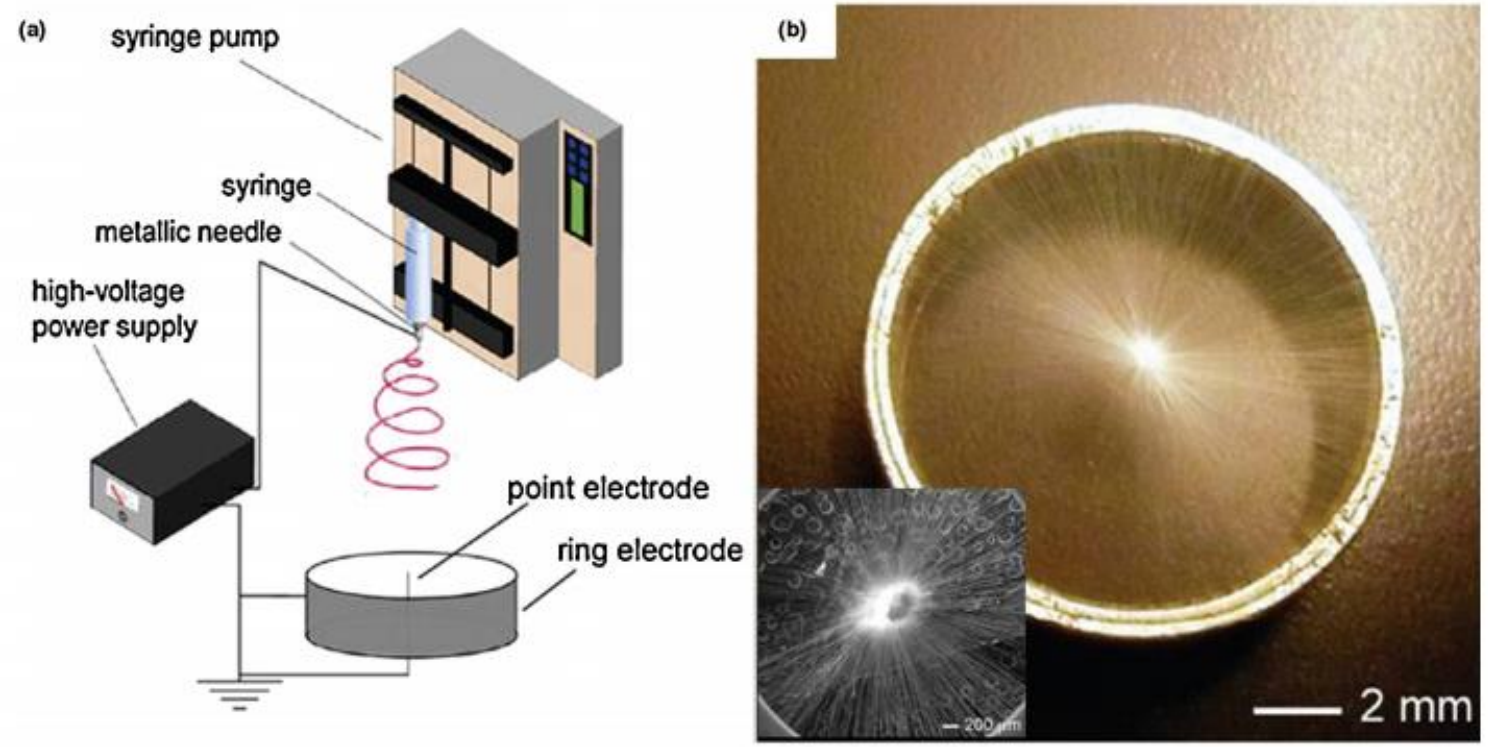


(b) 3D macroporous nanofibrous (MNF) scaffold from aligned electrospun nanofibrous yarns for bone tissue engineering. (C) Human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) well attached on the 3D MNF scaffolds and the cells changed their original rounded shape to elongated and spindle-like shapes.

Y.Z. Cai, et al. J. Biomed. Mater. Res. A 100 (2012) 1187.

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

2. Dura mater.

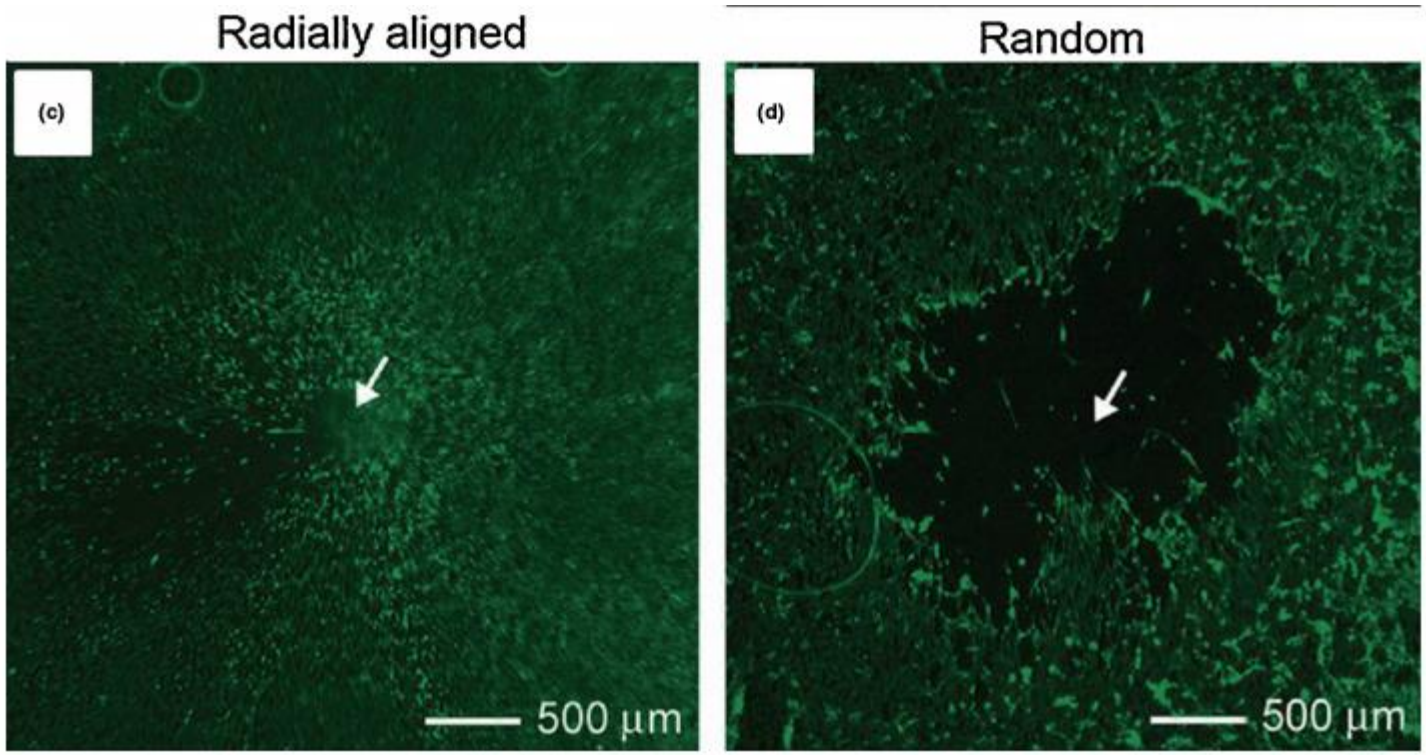


(a) Electrospinning setup for generating scaffolds consisting of radially aligned nanofibers. (b) Photograph of a scaffold of radially aligned nanofibers directly deposited on the ring collector. Inset of (b) shows the SEM image of the radial alignment nanofibers.

J. Xie, et al. ACS Nano 4 (2010) 5027

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

2. Dura mater.



(c, d) Fluorescence micrographs comparing the migration of cells when dura tissues were cultured on scaffolds of random and radially aligned nanofibers, respectively, for 4 days.

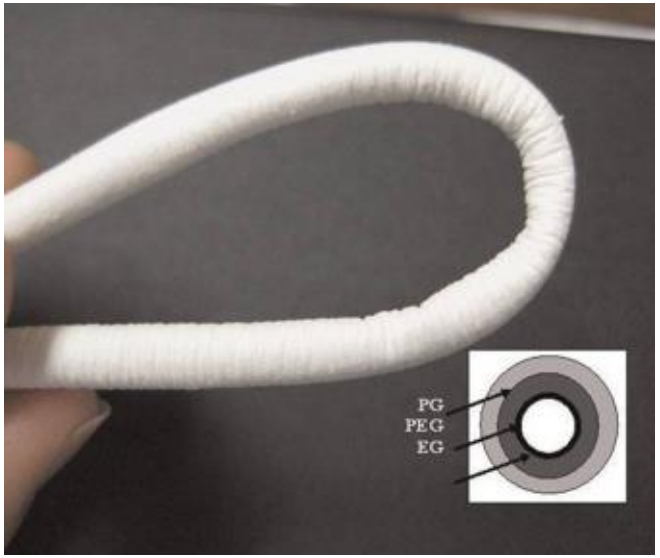
J. Xie, et al. ACS Nano 4 (2010) 5027

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

3. Tubular conduit scaffold.

- Vascular tissue regeneration**.**
- Nerve tissue engineering.

Imitates the complex matrix structure of native arteries.

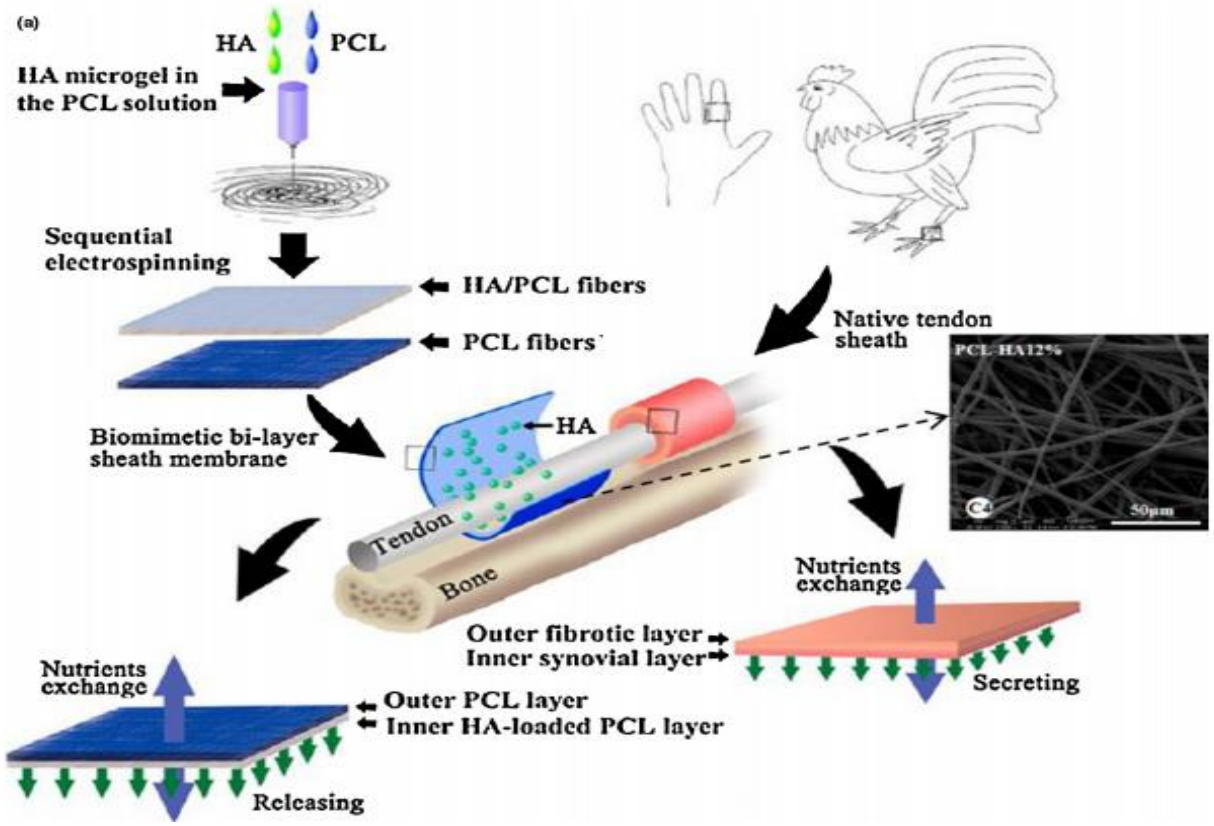


Photograph of tubular conduit of 20 cm length and 4 mm inner diameter. Inset is a schematic showing the trilayer tubular conduit (EG/PEG/PG) with spatially designed layers of elastin/gelatin (EG), PDO/elastin/gelatine (PEG), and PDO/gelatine (PG). The lumen layer is rich in protein and outer layers are rich in PDO.

V. Thomas, et al. Biotechnol. Bioeng. 104 (2009) 1025.

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

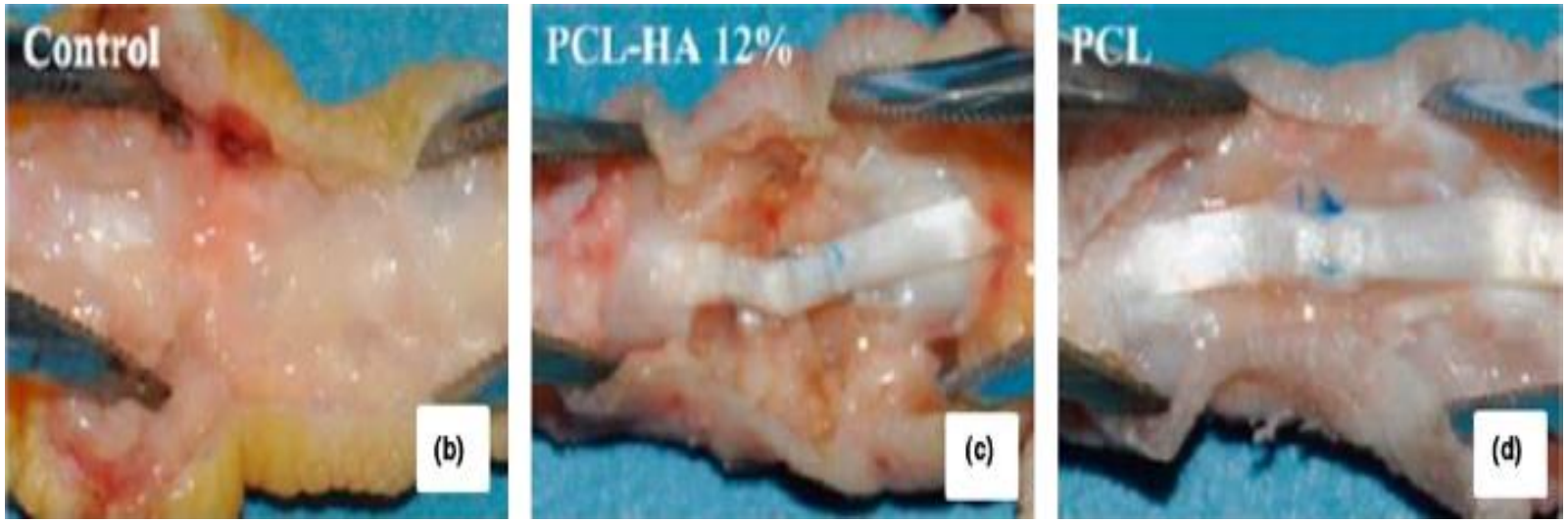
4. Biomimetic tendon sheet membrane.



S. Liu, et al. Biomacromolecules 13 (2012) 3611.

Applications of Aligned Nanofibrous Scaffolds in Tissue Engineering

4. Biomimetic tendon sheet membrane.



Gross evaluation of a chicken model of flexor digitorum profundus tendon repair after 21 days.

S. Liu, et al. Biomacromolecules 13 (2012) 3611.

Conclusion

- Electrospinning has emerged as an extremely promising method for the preparation of tissue engineering scaffolds.

- Electrospinning offers advantages for the preparation of scaffolds in terms of:

1. Resembling the fibrillar structures of ECM.
2. Large surface areas.
3. Ease of functionalization, and controllable mechanical properties.

Thanks for Attention

NANOTECHNOLOGY FOR ENHANCED OIL RECOVERY

MARIO LEONARDUS

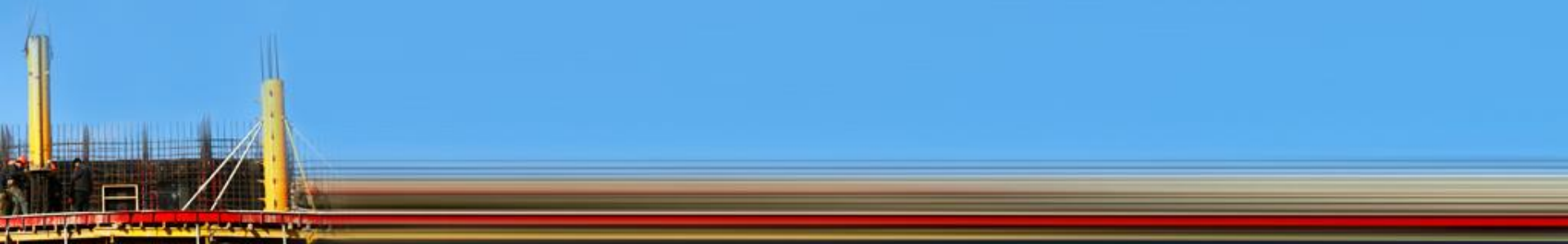
D10322806

Course: Introduction to nanotechnology

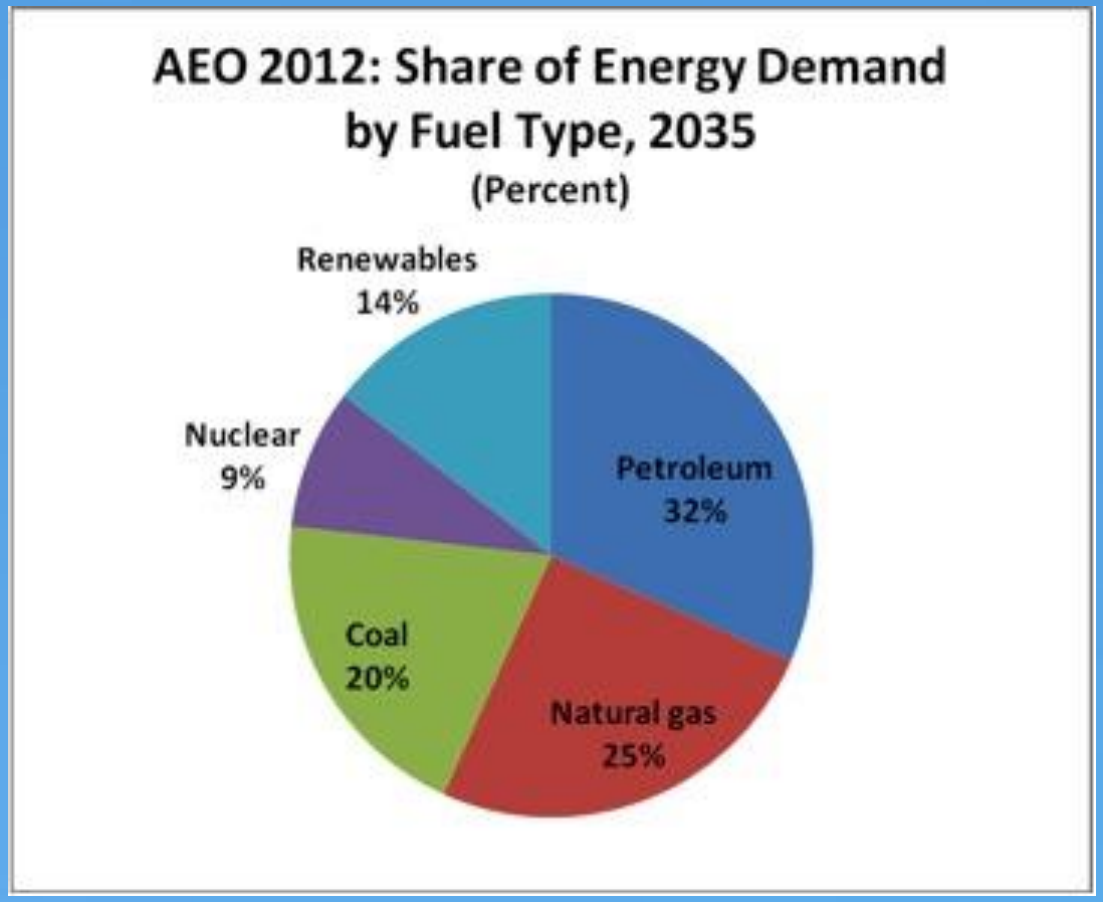
Assoc. Prof. Hossein Kosseinkhani

OUTLINE

- Introduction
- Enhanced Oil Recovery (EOR)
- Challenge
- Idea
- Innovation
- Target



INTRODUCTION



---Annual Energy Outlook 2012---

ENHANCED OIL RECOVERY

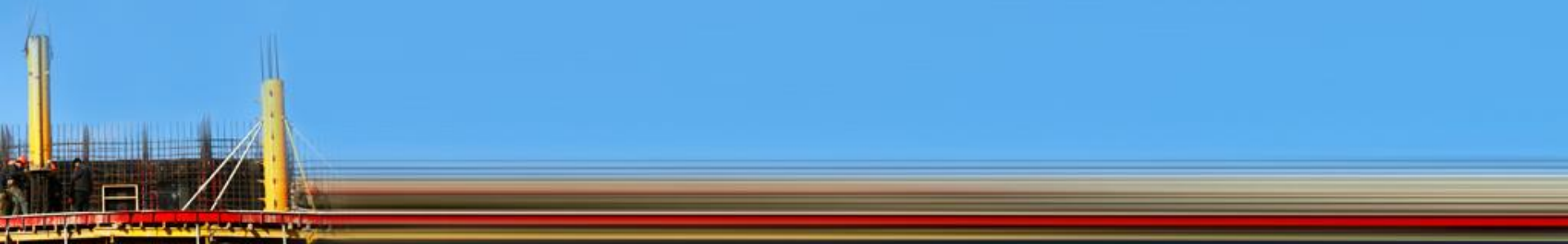
Enhanced Oil recovery?

"Enhanced oil recovery is oil recovery by injection of gases or chemicals and/or thermal energy into the reservoir."

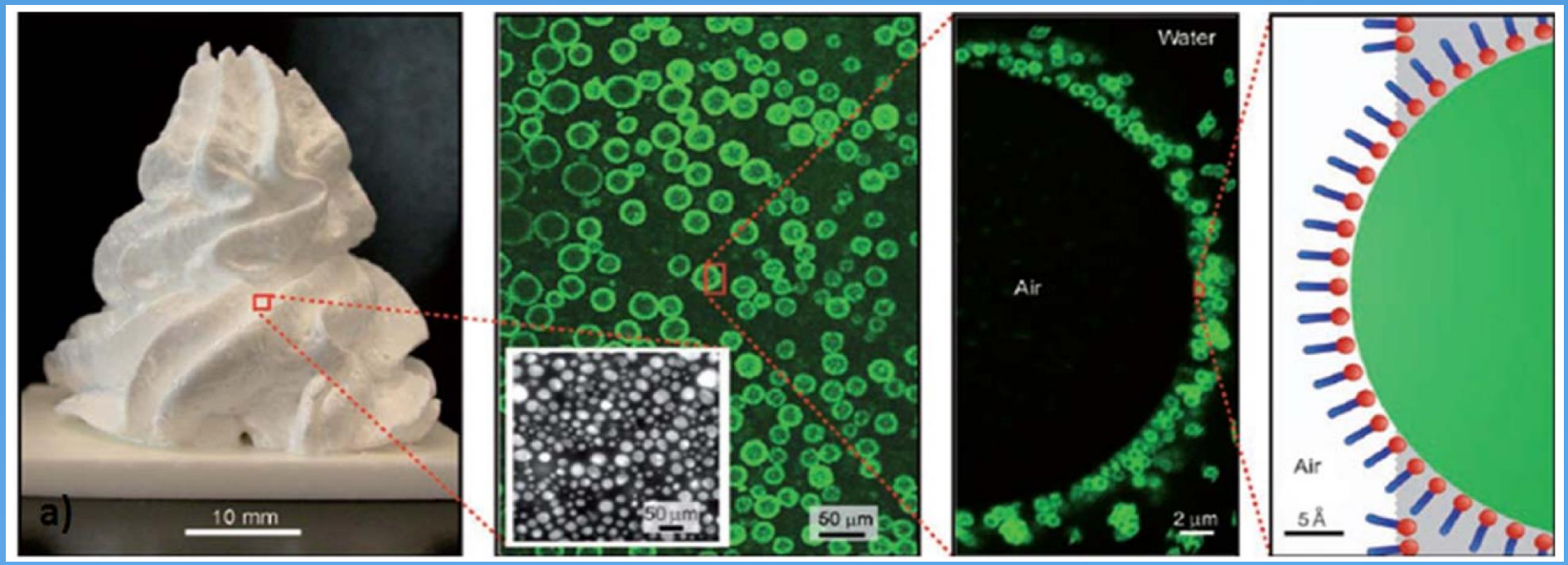
JJ. Sheng, "Modern Chemical Enhanced Oil Recovery

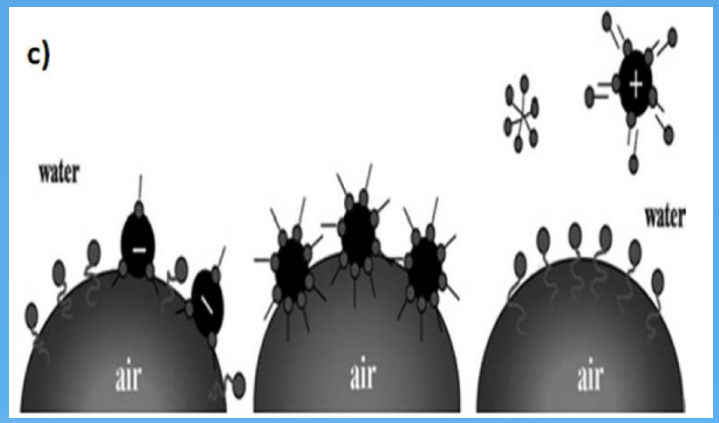
CO_2

Methods	
Thermal reco	e-to-Heel Air
Miscible floo	, solvent
Chemical floo	oam, and their combinations
Microbial	



CHALLENGE





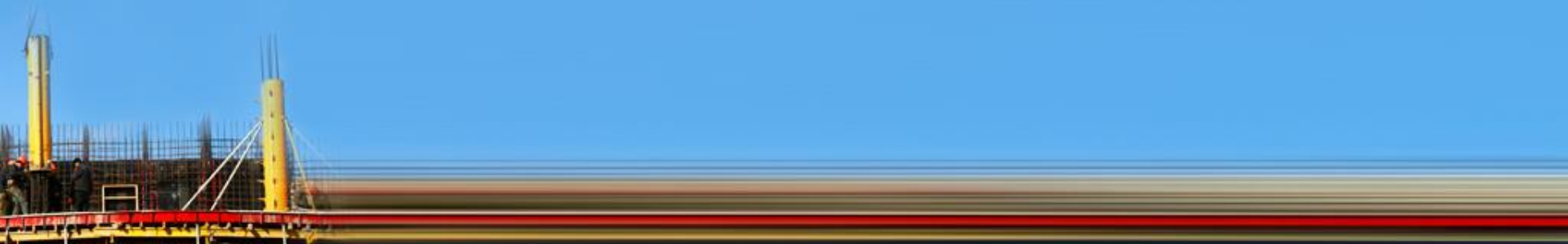
CHALLENGE

+

- Better sweep efficiency

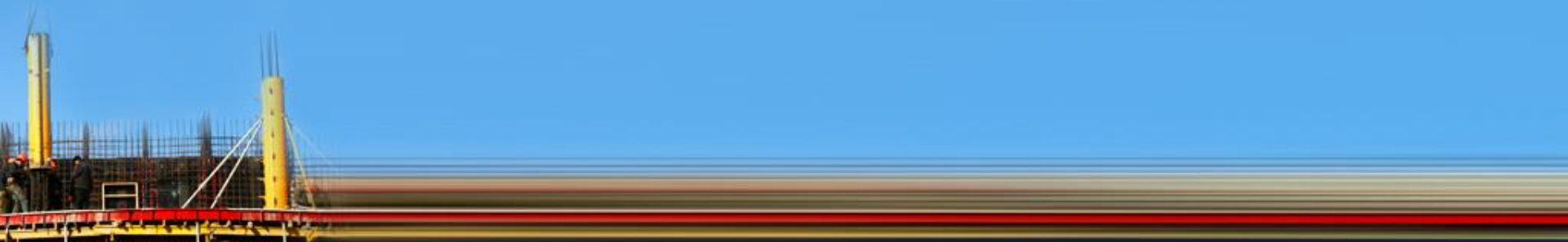
−

- Require continuous regeneration
- Degradable on the harsh condition
- Increased material cost due to the rocks adsorbing of surfactant



IDEA

Replacing
the surfactant
to make foam



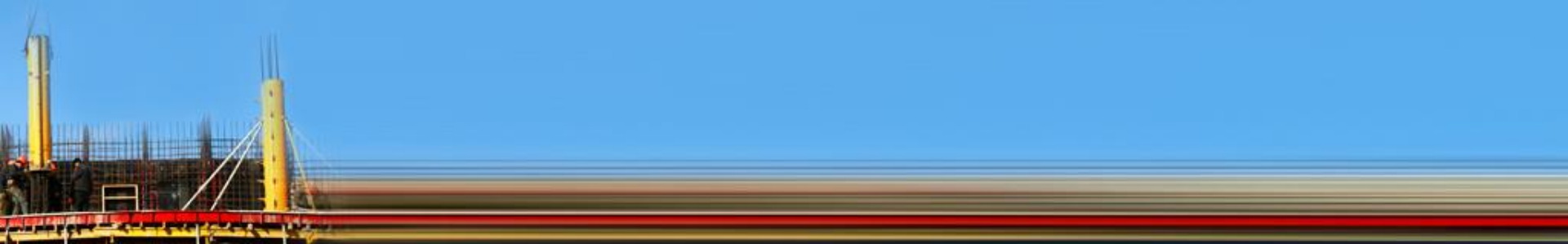
INNOVATION

- Nanoparticle for stabilize the foam

→ Solid state, have potential to withstand the high-temperature

→ Small size, can be trasported without straining in pore throats

- Nano carrier for CO_2 made by nanoparticle and polymer.



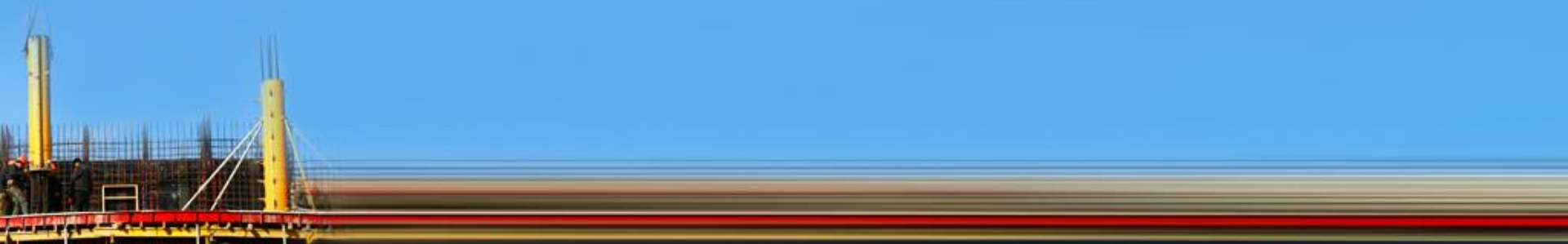
TARGET

Researc

reasing

ction

Oil sustainability




Thank you...

Silver and Ag@Au Core Shell SERS Nanotags for Breast Cancer Detection

Presenter : Septila Renata

Introduction To Nanotechnology B

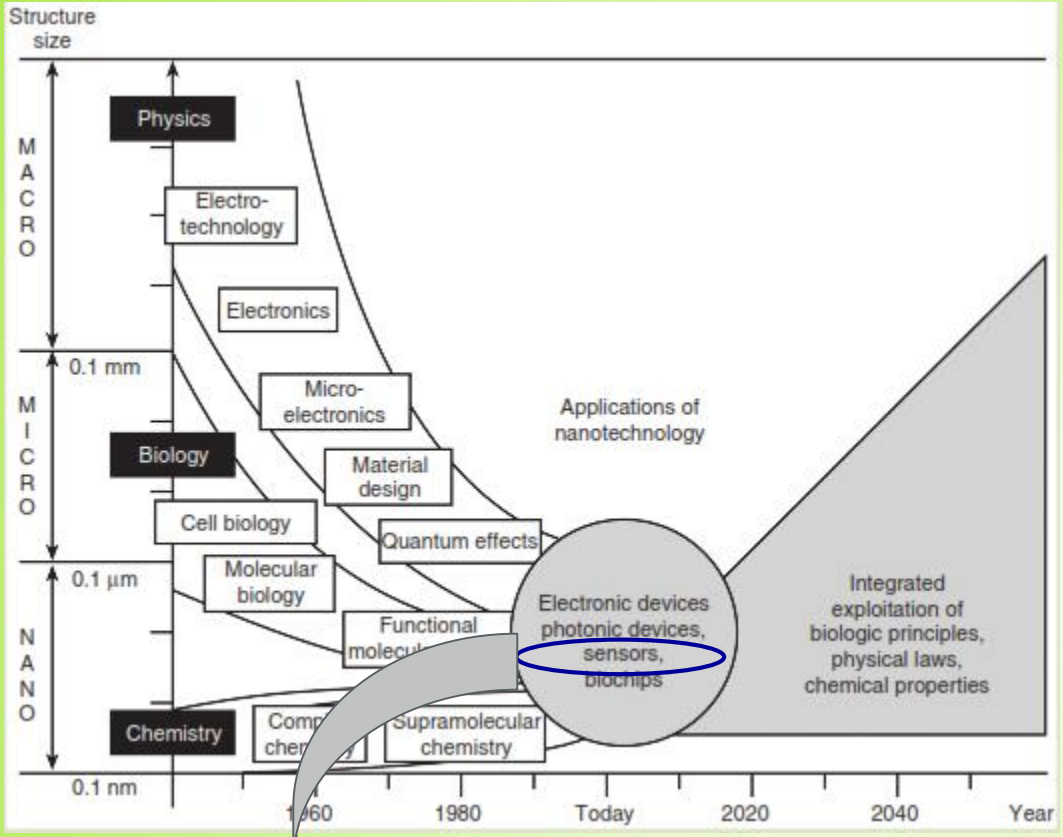


Figure I.1 Convergence at the nanoscale (source: VDI-Technology Center, Future Technologies Division –APEC Center for Technology Foresight, Thailand)

Breast Cancer

Biomedical

- **Diagnosis**
- **Drug Delivery**
- **Treatment**

Why Breast Cancer ??

In men and women

Rank	Cancer	New cases diagnosed in 2008 (1000s)	Percent of all cancers
1	Lung	1608	12.7
2	Breast	1384	10.9
3	Colorectum	1235	9.8
4	Stomach	989	7.8
5	Prostate	899	7.1

In women

Rank	Cancer	New cases diagnosed in 2008 (1000s)	Percent of all cancers
1	Breast	1384	22.9
2	Colorectum	571	9.4
3	Cervix uteri	530	8.8
4	Lung	516	8.5
5	Stomach	349	5.8

Source: GLOBOCAN 2008 database (version 1.2) http://globocan.iarc.fr

3 The earlier a cancer is detected and treated, the better a patient's chance of cure.

Breast Cancer

Type of Breast Cancer :

- Non-invasive breast cancer

This cancer is found in the ducts of the breast and has not developed the ability to spread outside the breast

- Invasive breast cancer

Has the ability to spread outside the breast, about 80% of all cases of breast cancer

TYPE OF BREAST CANCER BASED ON SPECIAL RECEPTOR

1. Hormone receptor (estrogen or progesterone receptor) positive

. Give information about prognostic and predictive factor

. 75% of all breast cancers are “ER +”. And about 65% of these are also “PR +”

. ‟ER-negative” breast cancers are more aggressive and unresponsive to anti-estrogen

2. HER2 positive

. HER2 proteins are growth factor on breast cells. Normally, HER2 receptors help control how a healthy breast cell grows, divides, and repairs itself. But sometime the HER2 gene doesn't work correctly and makes too many copies itself

. 20% to 25% of breast cancers are “HER2-positive”

. HER2 positive Breast Cancer : Have a lot of HER2 protein

. HER2 negative Breast Cancer: Have little or no HER2 protein

“HER2-positive” breast cancers tend to grow faster and are more likely to spread and come back compared to HER2-negative breast cancers

3. 5 Triple negative to receptors for estrogen, progesterone, or HER2

How to describe a breast cancer

1. TNM stage to describe the stages of breast cancer
2. Tumor morphology
    1) Type of breast cancer - ductal, lobular, etc
    2) Vascular lymphatic invasion (VLI)
    3) Perineural invasion (PNI)
        VLI and PNI indicate aggressive behavior
3. Special receptor
    1) Hormone receptor :
        estrogen or progesterone receptor (ER or PR)
    2) Her2/neu

HER 2

Why HER2 (Human Factor Receptor 2) important in cancer diagnosis ?

HER2 plays a pivotal role in therapeutic for breast cancer patient

- Patients with a HER2-positive status are generally associated with a worse prognosis and a higher rate of disease recurrence compared with patients with a HER2-negative status
- HER2 determine the drug that can apply to breast cancer patient. Herceptin, well tolerated for HER2-positive breast cancer patients, but may cause cardiotoxicity for HER2-negative breast cancer patients

Human Breast Cancer Cell Lines

Different human breast cancer cell lines expressed different known solid cancer stem cell markers

Marker/ Cell line	MB468	MB231	HCC1937	T47D	MCF7	ZR75	SKBR3	MB361
ER	-	-	-	+	+	+	-	+
PR	-	-	-	+	+	-	-	-
HER2							o.e.	o.e.
CD44	+++	+++	+++	++	++	+	+	+++
CD24	+++	-	++	+++	+++	+++	+++	-
$CD44^{+}/CD24^{-/low}$	-	+++	++	-	-	-	-	+++
CD133	+++	-	-	-	-	-	-	-
PROCR	-	++	-	-/+	-	-	-	++
ABCG2	-	-	-	-	+	-	-	-
CXCR4	-	-	-	-	-	+	-	-
ESA	+++	++	+++	+++	+++	+++	+++	+++
ALDH	+	+	+	-	+	+	++	-

The ER/PR (+/−) and HER2 overexpression (o.e.) status were adapted from Neve et al (2006)[44].
−, not detectable.
+, <5%.
++, 5–70% of the cells express the marker indicated.
+++, >70% of the cells express the marker indicated.
doi:10.1371/journal.pone.0008377.t001

Hwang-Verslues WW, Kuo W-H, Chang P-H, Pan C-C, et al. (2009) Multiple Lineages of Human Breast Cancer Stem/Progenitor Cells Identified by Profiling with Stem Cell Markers. PLoS ONE 4(12): e8377. doi:10.1371/journal.pone.0008377
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0008377

Immunohistochemistry

Fluorescence in situ hybridization

DRAWBACK

Time consuming

Not suitable for the direct detection of living cells or tissues

SERS Nanotags

Ag & Ag@Au NPs

Advantage :

- Low detection concentrations
- Rapid measurement
- High sensitivity for targeting HER2-overexpressed breast cancer cells.

Surface-Enhanced Raman Scattering (SERS)

1928 C.V. Raman discovered "Raman Effect" of inelastic scattering

1974 Discovery of enhanced Raman signals (10^5-10^6) from molecules adsorbed on roughed Ag surfaces.
Mechanism is attributed to enhanced surface area for adsorption.

1977 Debate begins over the exact mechanism of signal enhancement.

M. Fleischmann, *et al.*, Chem. Phys. Lett., **26** 163 (1974)
D.L. Jeanmaire, R.P. Van Duyne, *J. Electroanal. Chem.*, **84** 1 (1977)
M.G. Albrecht, J. A. Creighton, *J. Am. Chem. Soc.*, **99** 15 (1977)
S. Schultz, *et al.*, *Surface Science*, **104** 419 (1981)
M. Moskovits, , *Reviews of Modern Physics*, **57** 3 (1985)
K. Kneipp, *et al.*, *Chem. Rev.*, **99 2957** (1999)

Surface-Enhanced Raman Scattering (SERS)

SERS Advantage :

- **Molecular fingerprinting**

Unique vibrational spectra distinguishes molecules

- **Multiplexed sensing**

Plasmon resonances allow for sensor tenability

- **In vivo applicability**

Near-IR excitation and biocompatability allow

- **Femtomolar and beyond**

Single molecule spectroscopy is possible

- **Sensitive and surface selective**

SERS Enhancement :

- **Electromagnetic enhancement**

Dependent on the presence of the metal surface roughness features

- **Chemical Enhancement**

Involves changes to the adsorbate electronic states due to chemisorption of the analyte

S.M. Nie, *et al.*, *Science*, **275** 1102 (1997)
http://www.oxonica.com/diagnostics/diagnostics_sers_imaging_applications.php

SERS Substrate

ADVANTAGE Ag NPs

- Inexpensive relative to other materials
- Has good chemical and physical properties
- Has high extinction coefficient (100 x greater than gold)
- Ag nanoparticle provide significant enhancement of Raman scattering (greater than gold)

Disadvantage :

Difficult to handle it in salt solution and in sulfur component

Develop Ag@Au core shell NPs

Double advantage :

-High enhancement from silver core
-Stable in salt solution and sulfur component from gold shell

SERS Nanotags

Introducing Raman label compounds onto noble metal (Ag NPs and Ag@Au NPs)

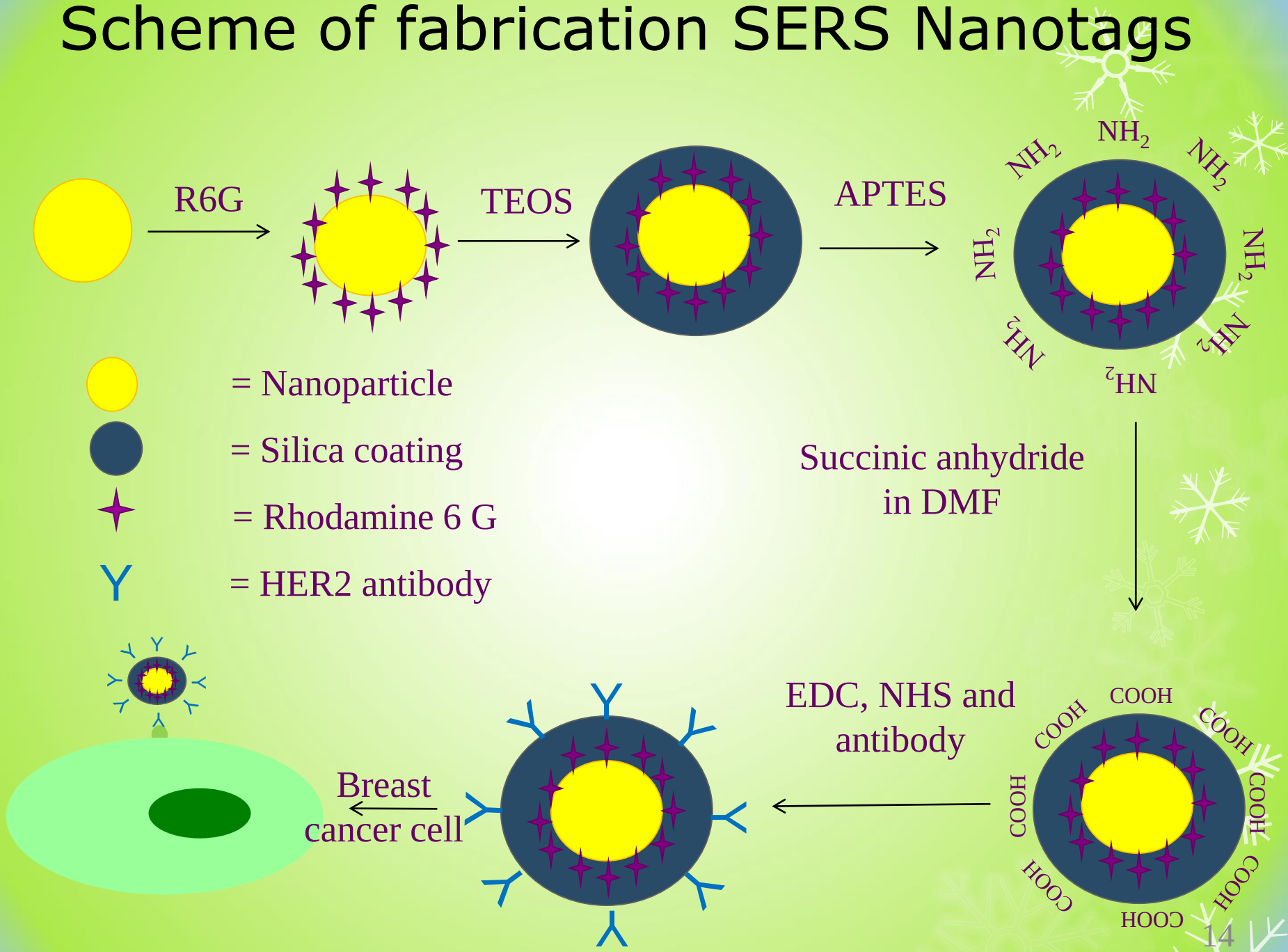
Electromagnetic enhancement

Metal nanostructure is used to amplify the Raman scattering signal for ultrasensitive detection

Chemical Enhancement

Raman label generates the Raman spectral signature used to identify a biomolecular interaction

Scheme of fabrication SERS Nanotags




THANK YOU
Danke
Merci
Gracias
Grazie
Obrigado
Köszönöm
Mahalo
Arigato
Спасибо
Multumesc
Dziękuję
Tak
Paxмет
Prasom

Detection of cancer biomarkers in serum using a hybrid mechanical and optoplasmonic nanosensor

P. M. Kosaka, V. Pini, et.al, *Nature Nanotechnology*, 9, **December 2014**, 1047-1053.

By: Shemsia M. Hudie

January 13, 2015
National Taiwan University

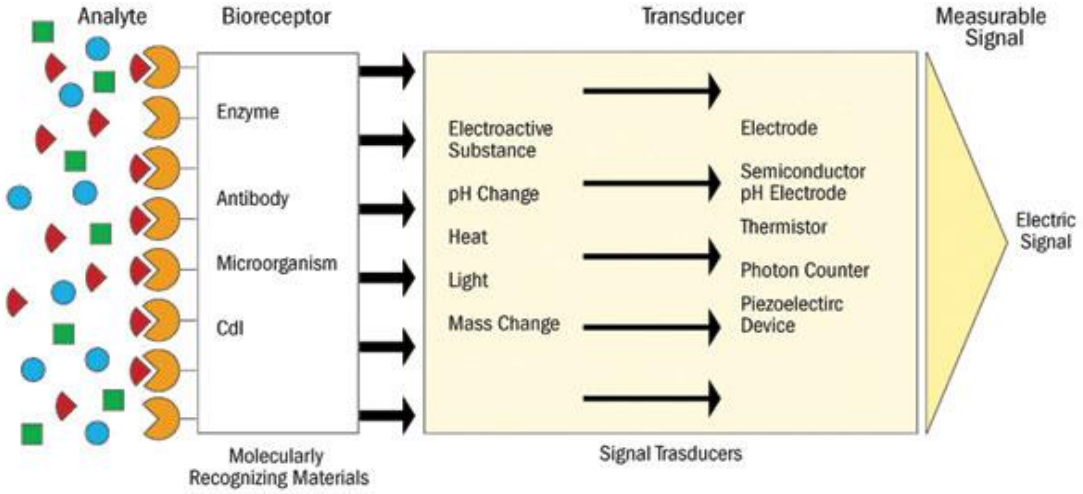
Out Line

- Introducetion
- Methodology
- Results and Characterization
- Conclusion

Introduction

- Blood contains a range of protein biomarkers that could be used in the early detection of disease.

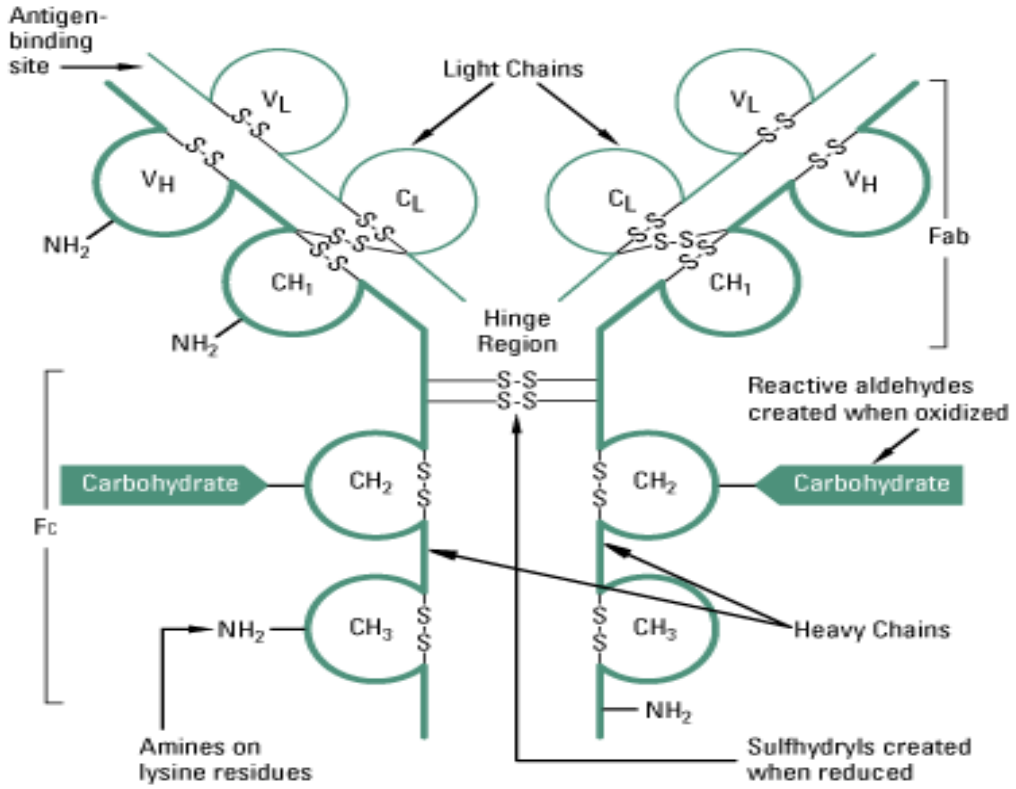
- A biosensor is an analytical device which is used to determine the presence and concentration of a specific substance in a biological analyte.

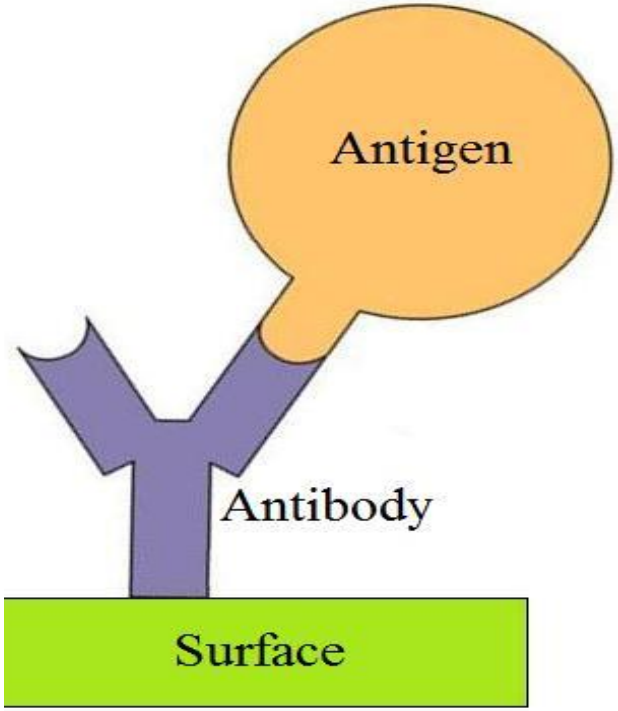


- Important parameters
  - reproducibility
  - sensitivity
  - detection limit

Introduction

- Antibody → Antibodies are biological molecules that exhibit very specific binding capabilities for specific structure (antigens).

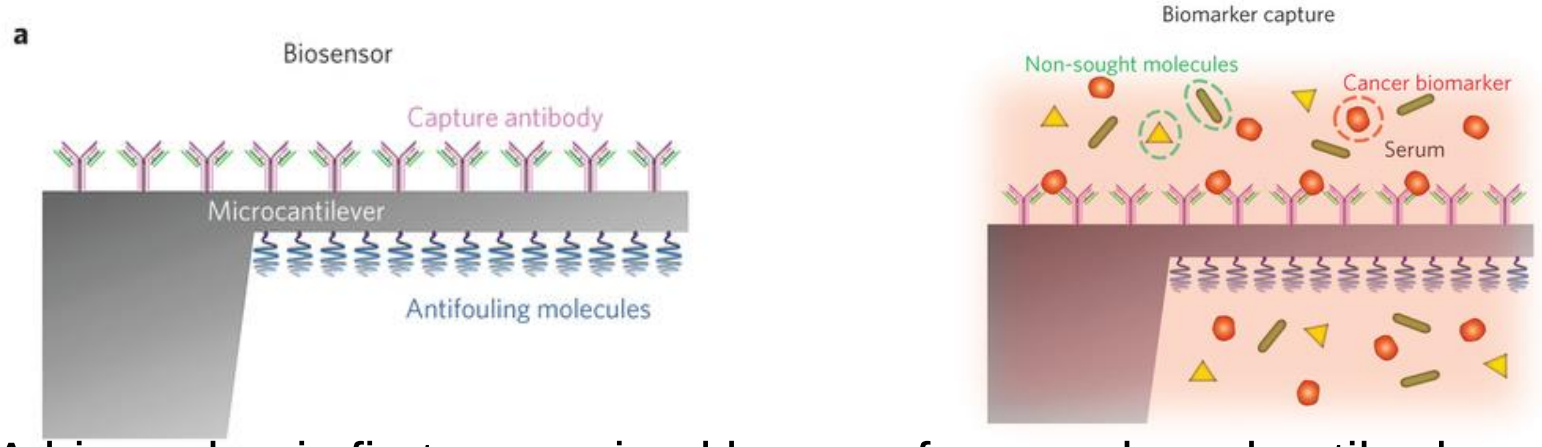




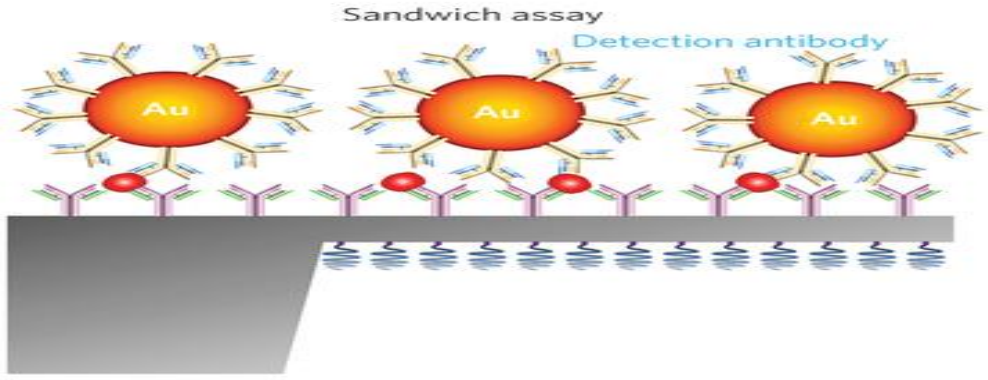
- Antigen → It can be recognized by antibody.

Methodology

- A sandwich assay that combines mechanical and optoplasmonic transduction which can detect cancer biomarkers in serum at ultralow concentrations.

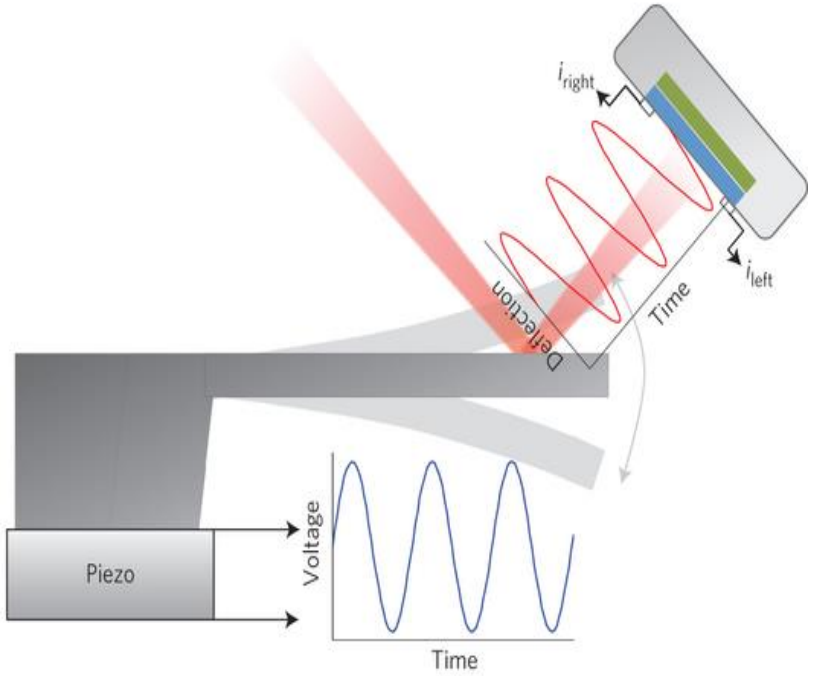


- A biomarker is first recognized by a surface-anchored antibody and then by an antibody in solution that identifies a free region of the captured biomarker.

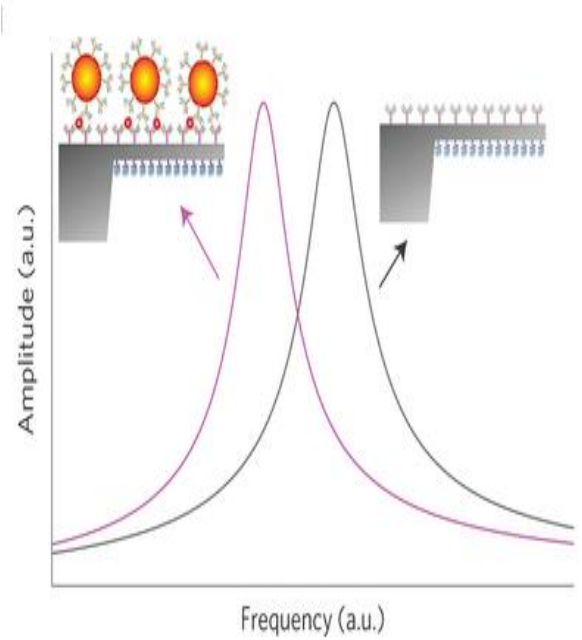


- Second antibody is tethered to a gold nanoparticle (this acts as a mass and plasmonic label)

- The two signatures detected by means of a silicon cantilever that serves as a mechanical resonator for ‘weighing’ the mass of the captured nanoparticles and as an optical cavity that boosts the plasmonic signal from the nanoparticles.



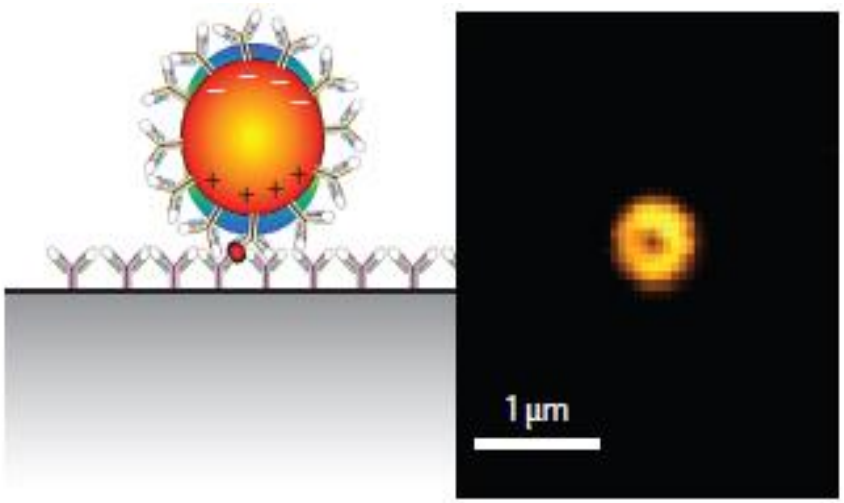
- Optical beam deflection method for Measuring the cantilever vibration.



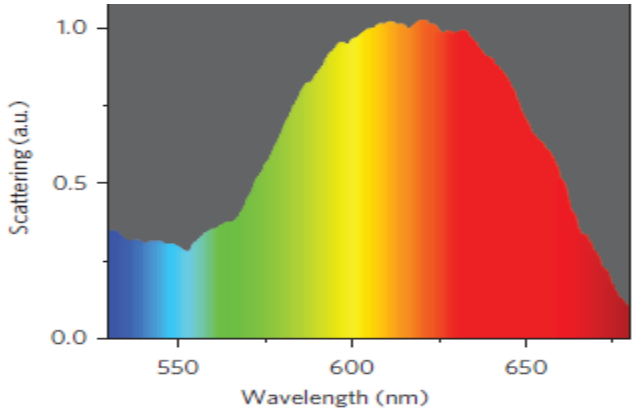
- The effect of the nanoparticle mass loading on the resonance frequency of the cantilever. The resulting downshift oof the resonance frequency is proportional to the added mass.

Characterizations

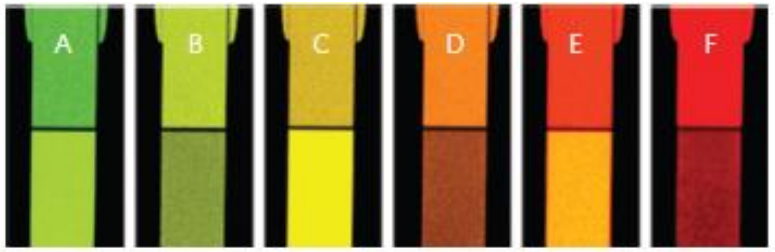
- SPR images of the nanoparticle immobilized



Optical dark-field image of a single nanoparticle

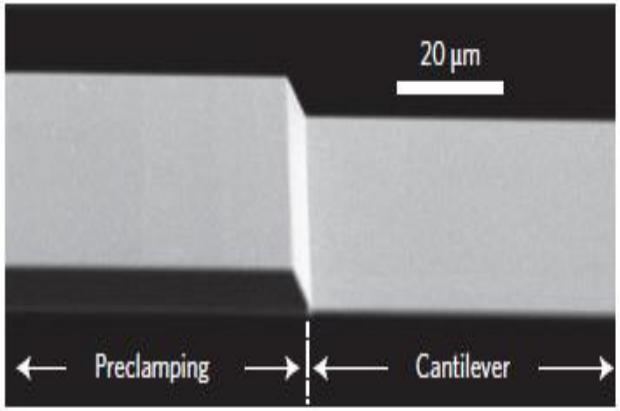


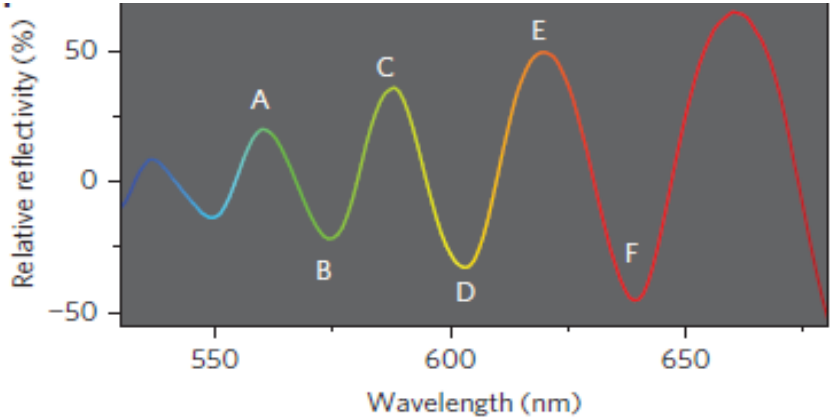
Scattering spectra of a single nanoparticle



Bright-field images at different illumination wavelengths

- SEM images of microcantelevers

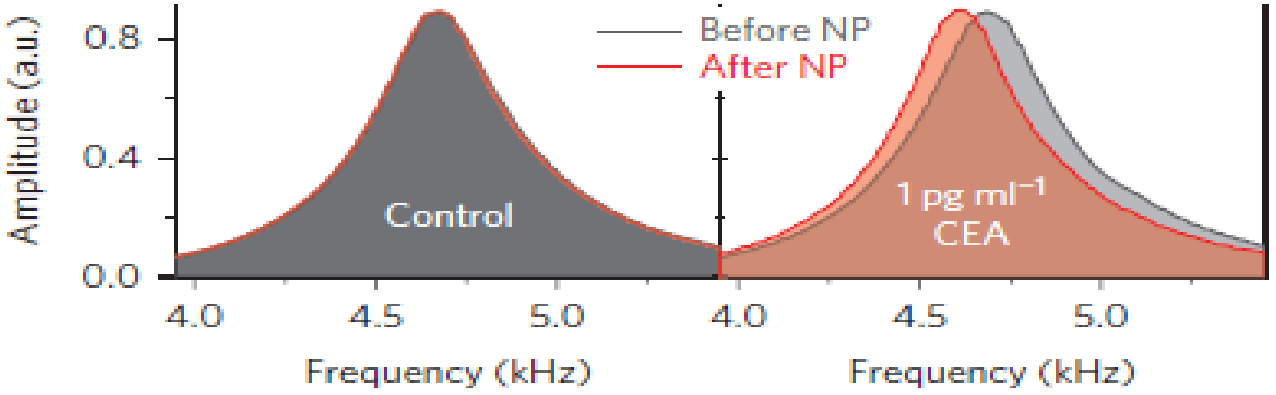


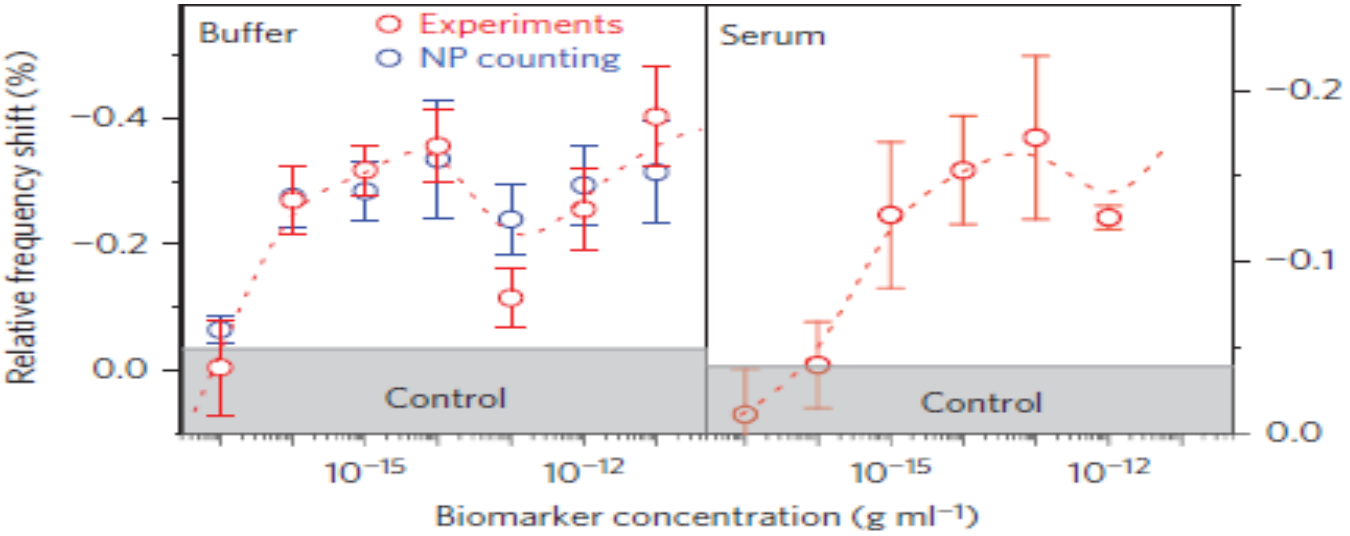


Relative reflectivity in the cantilever as a function of the wavelength

Detection of CEA cancer marker

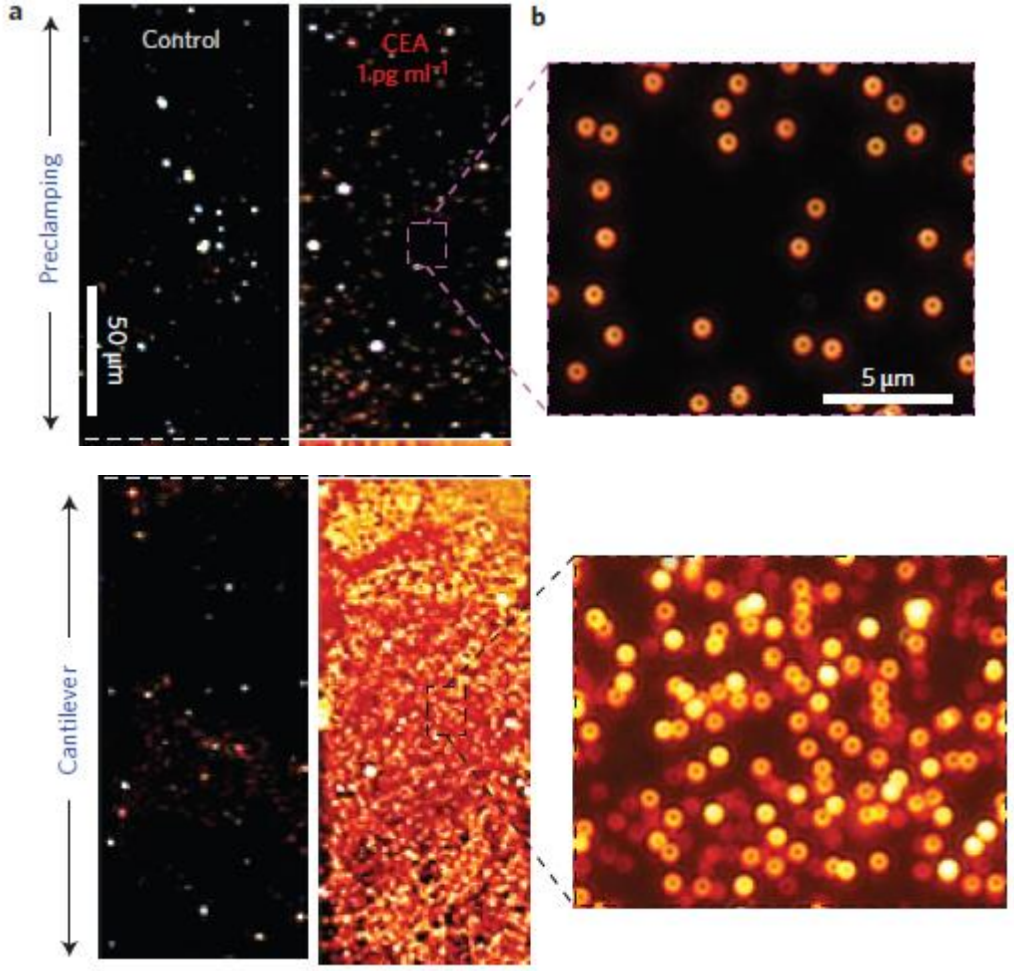
- Mechanical resonance frequency of a silicon cantilever before and after the recognition step



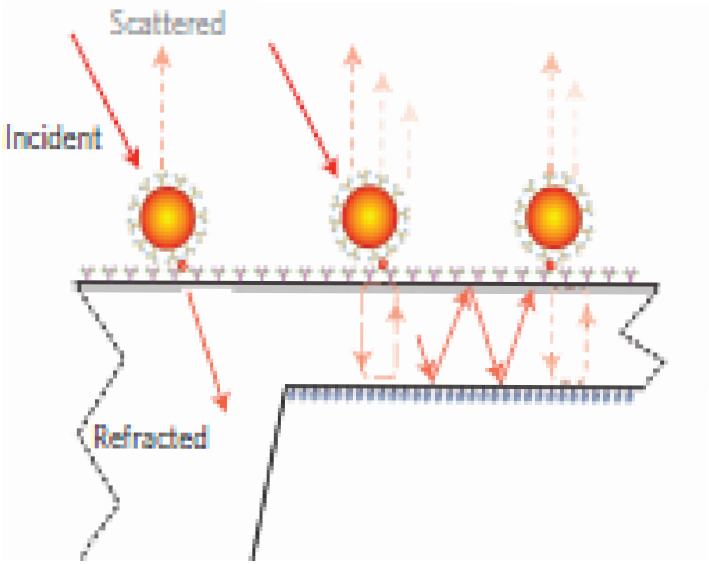


Plasmonic detection of the CEA protein biomarker

- The gold nanoparticles used in the sandwich assay feature plasmon resonances associated with collective electron oscillations in the nanoparticle.

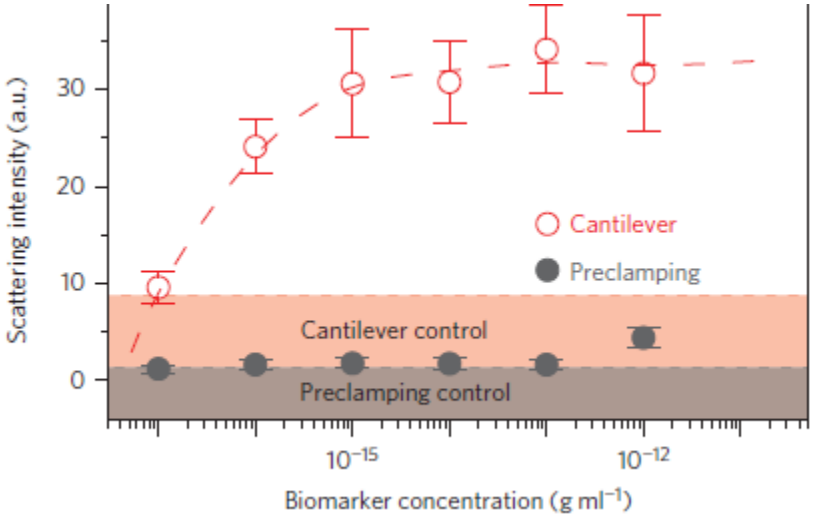


Different pathways for the generation of the dark-field signal in the cantilever via multiple internal reflections

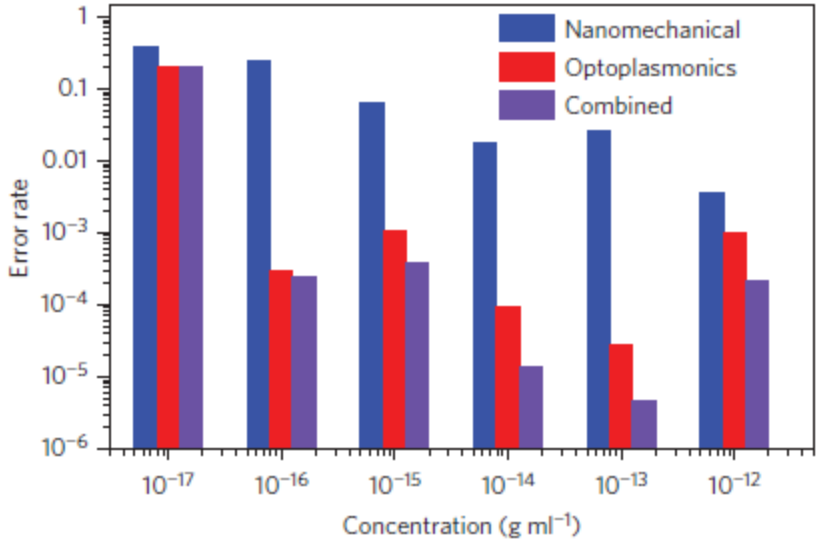


Dark-field optical images of the cantilever after the recognition step with the antibodies tethered to the nanoparticles

- Biomarker detection limit by an unsophisticated plasmonic readout in cantilevers



- Reliability of the optomechanoplasmonic device



Conclusion

- Silicon cantilevers can provide both plasmonic and nanomechanical transduction for sandwich bioassays labelled with gold nanoparticles.

- The use of two different transduction mechanisms in a single platform allows to determine the presence of a protein with extremely high statistical significance.

- The capabilities of the approach are illustrated with two cancer biomarkers:
  - ✓ the carcinoembryonic antigen (CEA, colon cncer) and
  - ✓ the prostate specific antigen,(prostate cancer)

- A detection limit of $\mathbf{1 \times 10^{-16} g\ ml{-}1}$ in serum is achieved with both biomarkers.

- These attributes suggest that our hybrid mechanical and optoplasmonic device could be useful in the development of technologies capable of early-cancer detection.

Thank you

Introduction to Nanotechnology-B

Student Project Proposal On

CANCER CHEMORADIATION THERAPEUTICS USING DRUG CONJUGATED FLUORESCENT GOLD NANOCLUSTERS

Stalin Karuppiah

TIGP Nano Program

ID: D03223121

NTU Chemistry

Cancer: Introduction and Statistics

***Cancer*, the most fatal disease threatening mankind throughout the world due to its heavy morbidity and mortality rate.**

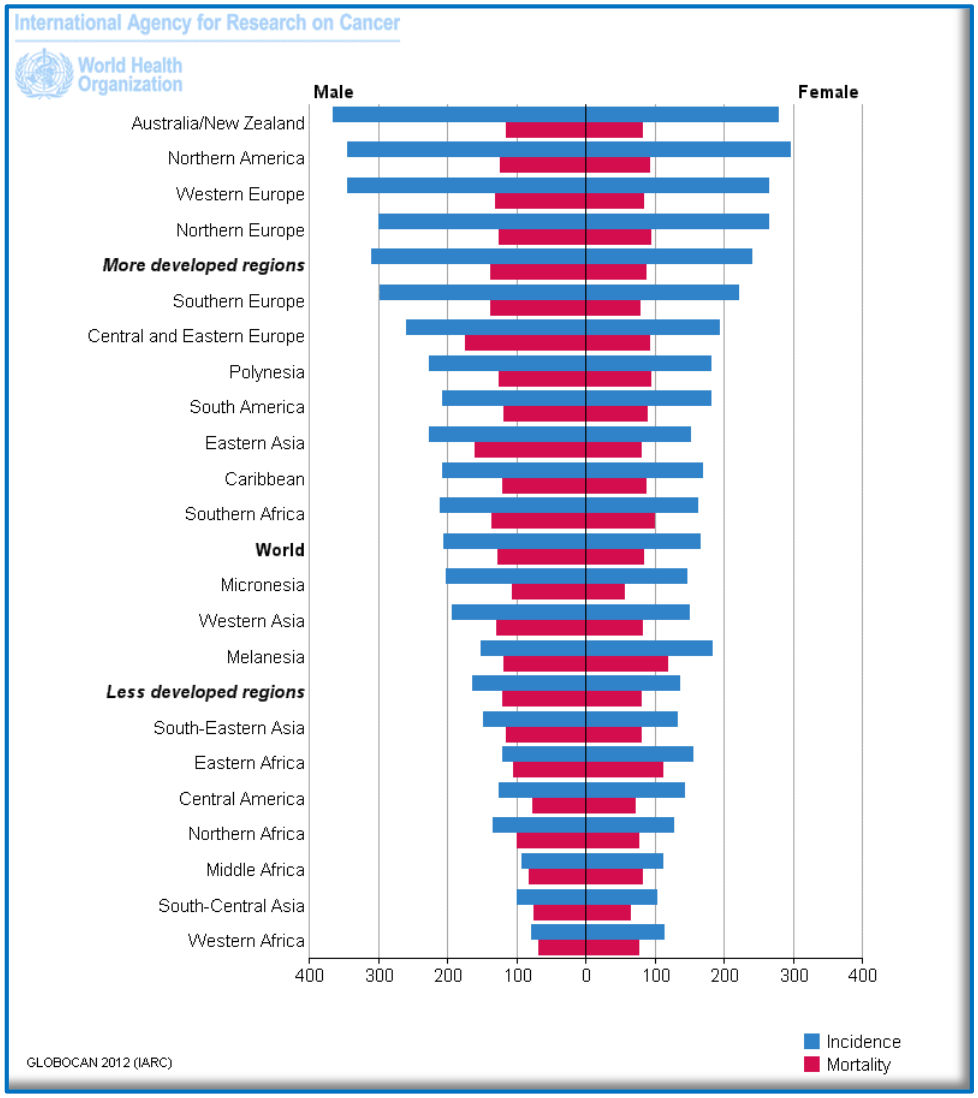


Fig. 1. Cancer- Worldwide data for incidence and mortality rates.

Source: International Agency for Research on Cancer, world Health Organization (WHO)

Key Culprit: ***Tumor***, can be of two types.

(i) Benign

These are not cancerous, can often be removed. Cells in benign tumors do not spread to the other parts of the body which render the reversal of this type of tumors after the treatment. i.e. Total curing rate is high

(ii) Malignant

- Are cancerous. Cells in this tumor grown out of control, Invade to the nearby tissues and spread to other parts of the body.
- Metastasis / Secondary cancer is possible.

Available Treatment

Diagnosis: One of the huge hurdles is to diagnose at early stage

Therapy

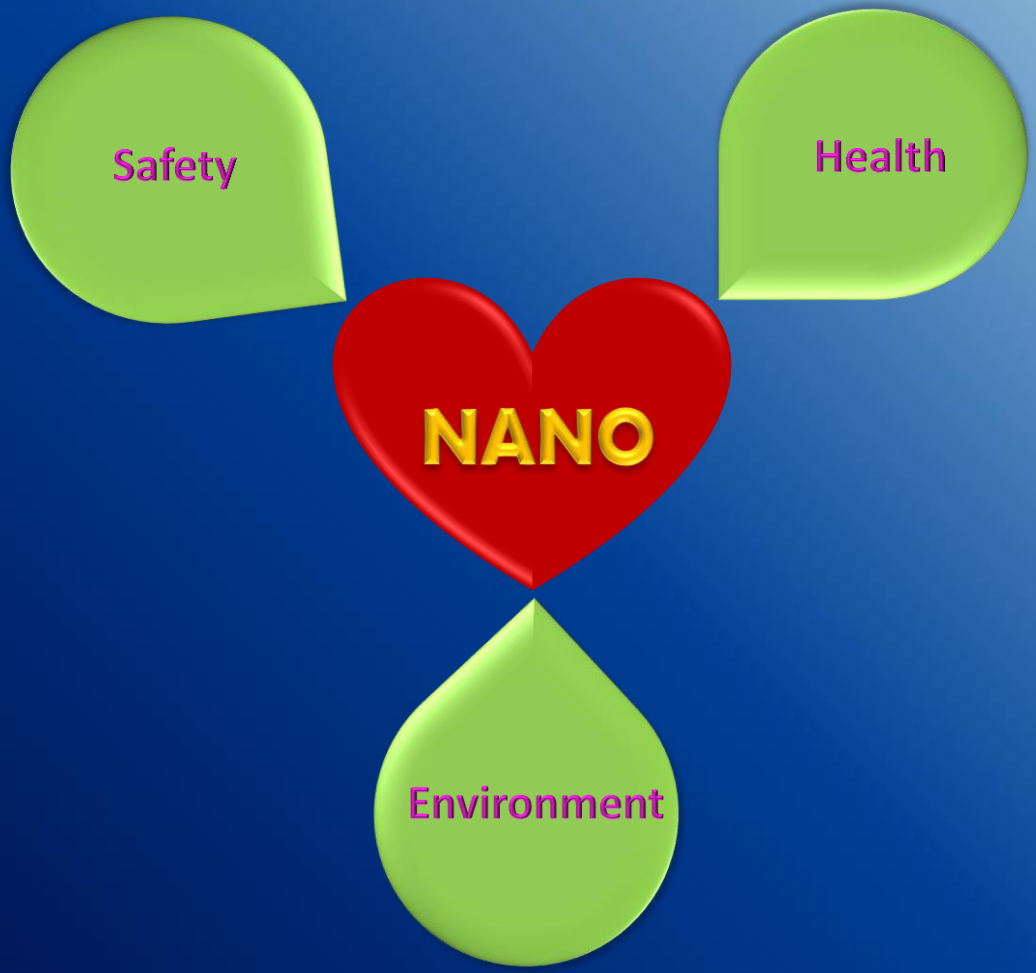
Various types of therapies have been used extensively which includes Photothermal, Photodynamic and Chemo therapies.
The proposed research exploits chemotherapy as well as radiation

A class of chemotherapeutic drugs have been tested, it is found that anthracyclins and plant alkaloids combats effectively against cancer cells. Also, Most of the anthracyclins are FDA approved (Federal Drug Administration), so their chemotherapeutic actions, radiosensitizing skills are renowned by preclinical and clinical trials. However, total administration of this drugs has certain hitches:

(i) its inefficiency due to the revelation of whole body to the chemotherapy toxicities that causes low tumor to non-tumor drug ratios and

(ii) Meager spatial distribution. So, chemoradiation therapy (CRT) by harmonizing chemotherapy with radiation could be potentially kills the cancer cells

How nanotechnology can be a betterment to the drug delivery....



The Proposal

Idea

Employing the Fluorescent Gold Nanoclusters bounded with proteins in two ways

- **Extract the fluorescence for cell imaging**
- **Bioconjugation techniques to bind drug and deliver to the targeted tumors**

Spark*: *Gold nanomaterials have been already proved its mastery in drug delivery for the several years

Nanoclusters are the quantum entities built by few number of atoms chemically bonded together. Such as M_x (M: Ag, Au, Mg etc.,) and 'x' is ranging from 10-50 atoms.

Advantages

- **Single excitation**
- **High quantum yield**
- **Narrow and Symmetric emission**
- **No photobleaching**
- **Ease of providing opportunity to both therapy & diagnostics**

Synthesis & Scheme

A solution of $HAuCl_4.xH_2O$- 5 mL

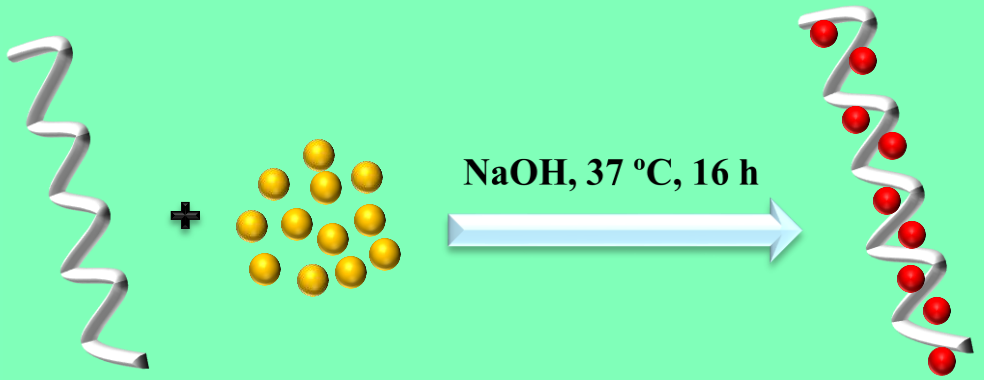
5 mL of protein solution

Stirring for 2 minutes at 37 °C

Add 0.5 mL of 1 M NaOH

37 °C for 12 h under vigorous stirring

Gold nanoclusters



Drugs : *Doxorubicin, Paclitaxel, Docetaxel*

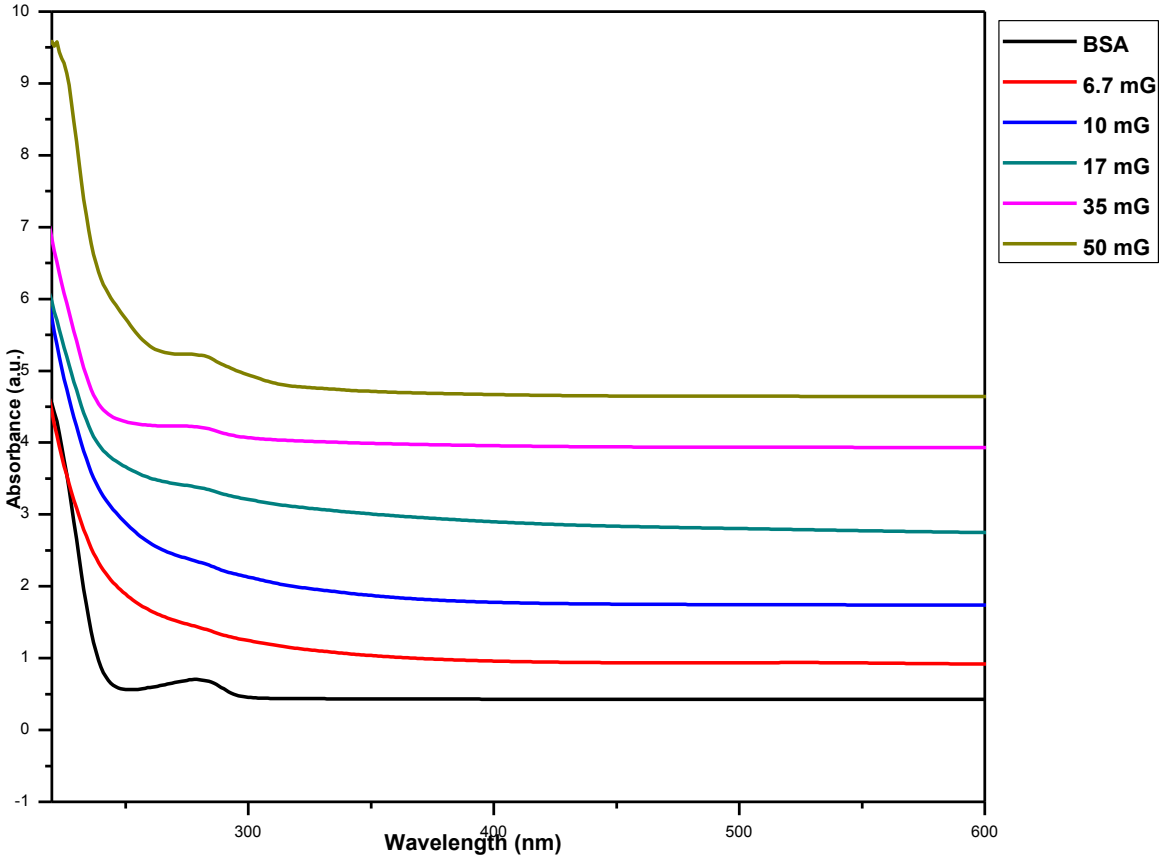
Bioconjugation of drugs: Strategies

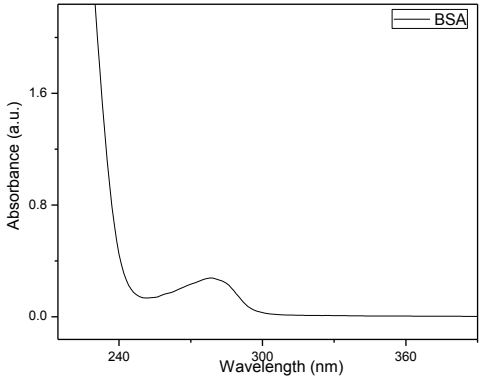
Amine binding sites are the key role players to conjugate the drug

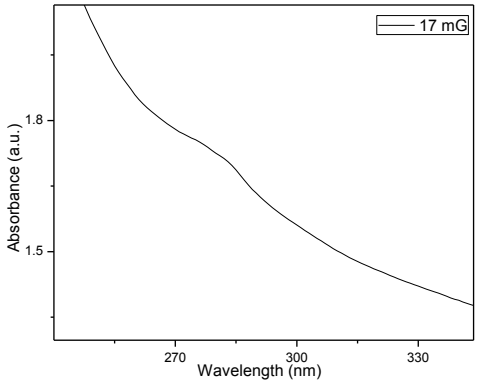
Proposed Technique : EDC-NHS Chemistry, Non-Covalent functionalization protocols

Results

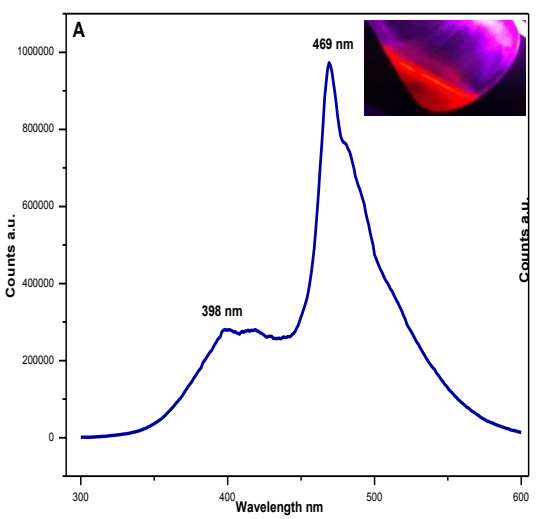
1. UV-Vis Spectroscopy

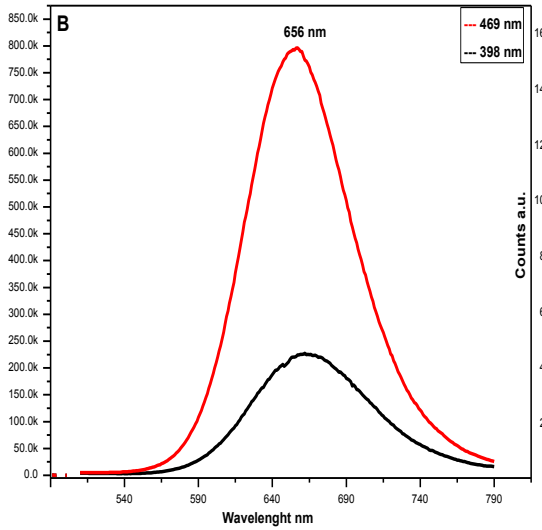


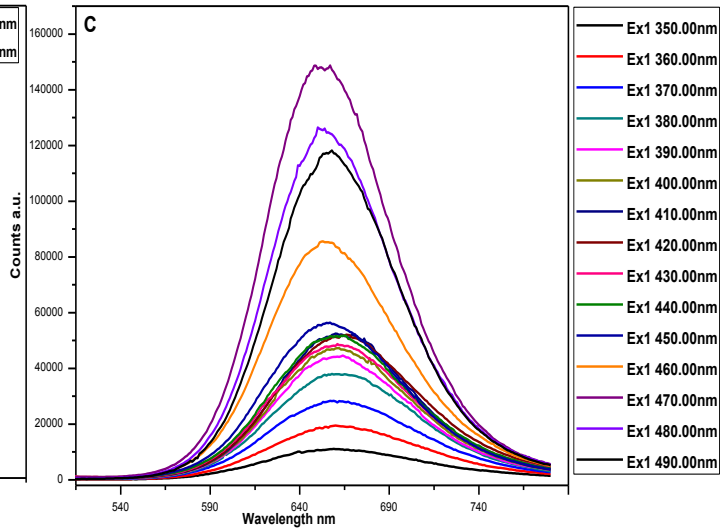




2. Fluorescence Spectroscopy

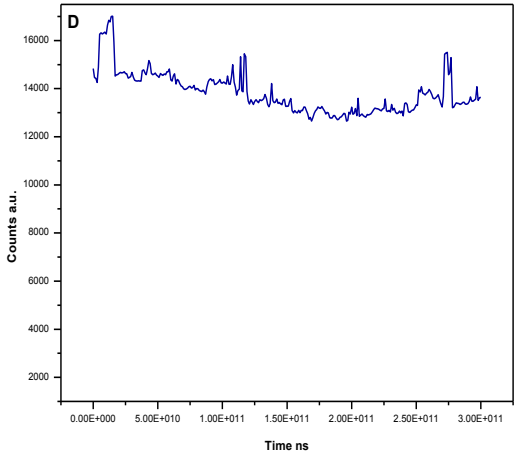


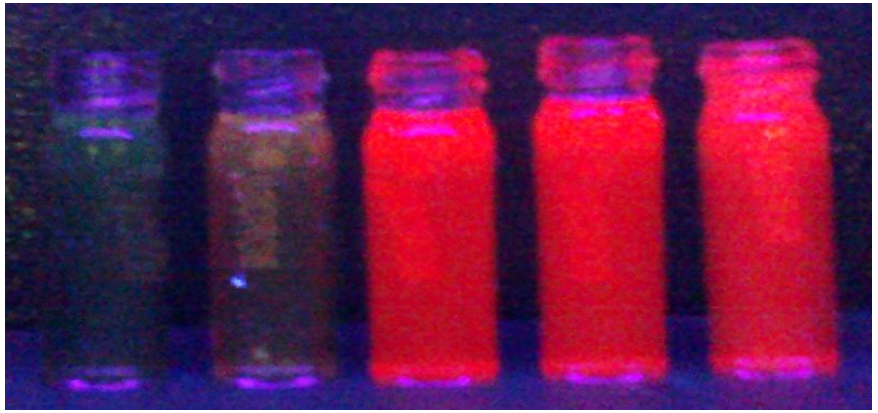




Photoluminescence spectroscopy of Au NCs A) Emission scan, B) Emission spectra at 398 nm and 469 nm illumination

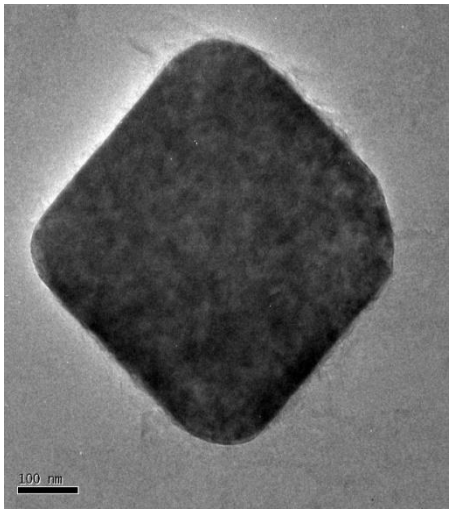
C) Contour plot starts from 350 nm to 490 nm of illumination

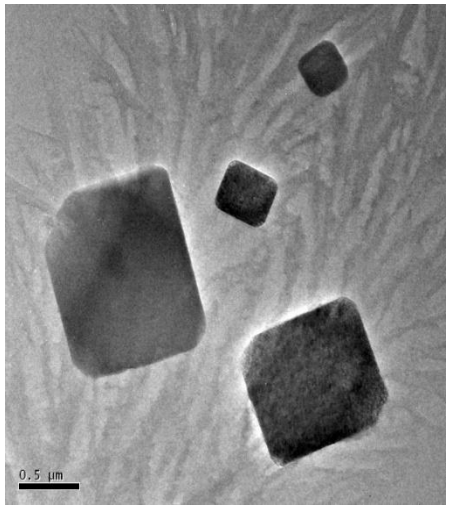


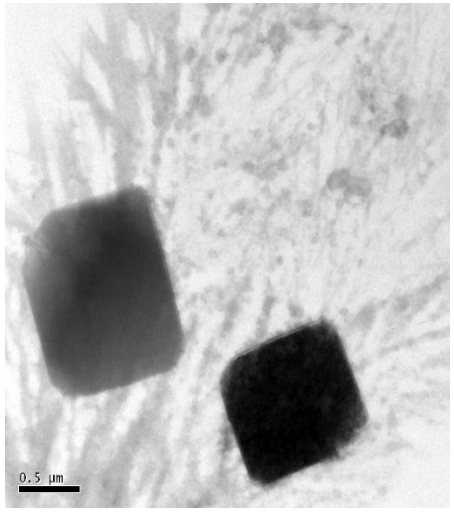


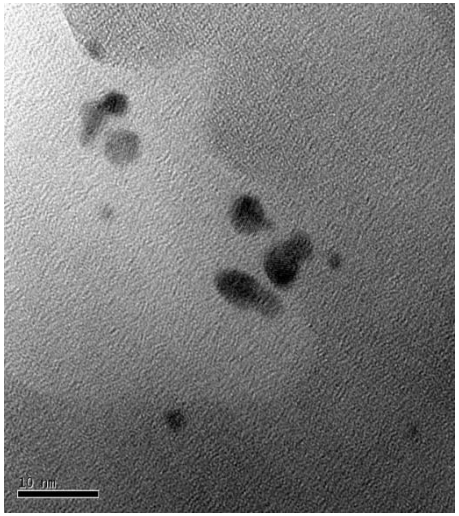
D) Kinetics scan to study photobleaching of Au NCs

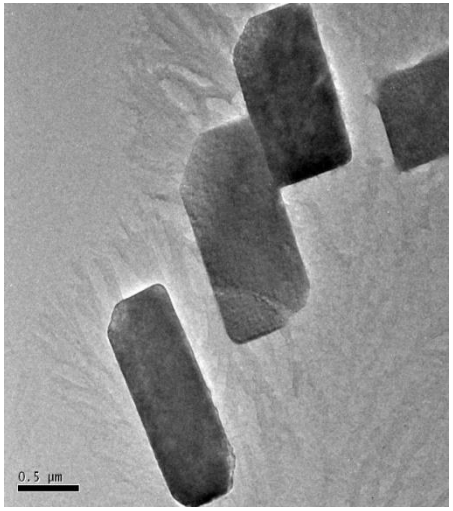
3. Transmission Electron Microscopy

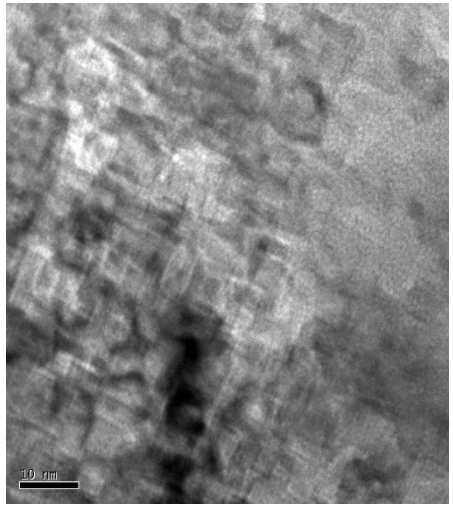


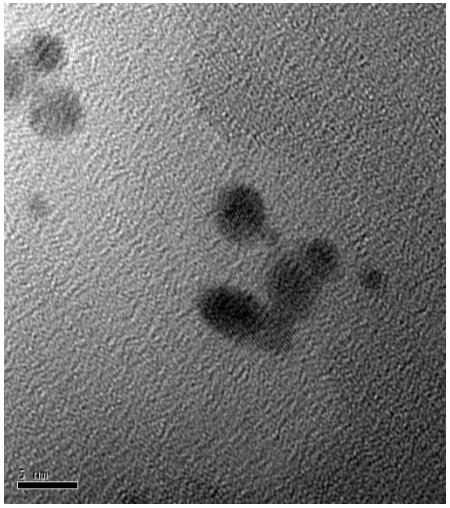


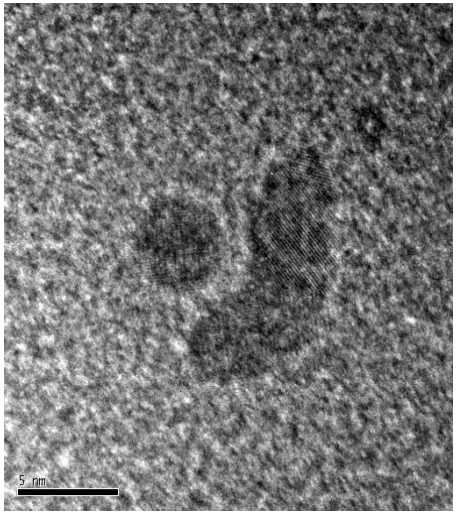












Gold Nanoclusters : Summary

Characterization	Status	Remarks
UV-Vis	Done	Distinct absorbance of protein at 280 nm was observed, it is noted that was disappeared at protein protected Au nanoclusters confirms the formation of Au NCs.
FT-IR	Done	The secondary structure has been analyzed by FTIR, which shows the presence of various amide, carbonyl and amine groups in protein and Au-protein assigned in figure. **Key observation**: In Au NCs, Broadening of H-Bonded amine group in protein at 3430 cm^{-1} and evolution of new absorption peak 876 cm^{-1}
TEM	Done	Nanoclusters were encapsulated inside the protein at which crystallizes as logs. i.e. Cuboid structure was observed.
Fluorescence studies	Done	Two different excitation wavelength has been observed. @398 nm and @469 nm. Ex at 469 gives high intensity emission at a peak intensity of 656 nm. Photobleaching studies revealed that there is no significant bleaching of fluorescence of NCs.
Femtosecond Laser	Yet to be done	

Problems need to be addressed : Future directions

1. ***in-vitro*** **and** ***in-vivo*** **toxicity evaluation for gold nanoclusters**
2. **Drug release kinetics of gold nanoclusters conjugated drug**
3. ***in-vitro*** **and** ***in-vivo*** **studies of developed nanocluster based drug delivery vehicle in cancer cells followed by radiation therapy**
4. **Need to try in-situ fluorescence imaging of cells during drug release by tracking the fluorescence of nanoclusters**


Thank You....

Introduction to Nanotechnology (B)

Student Project :

Optical Biosensor with Nanotechnology

Student: Yu-Da Chen ID: 101011867

Program: Nano Program, TIGP

Jan. 13, 2015

Outline

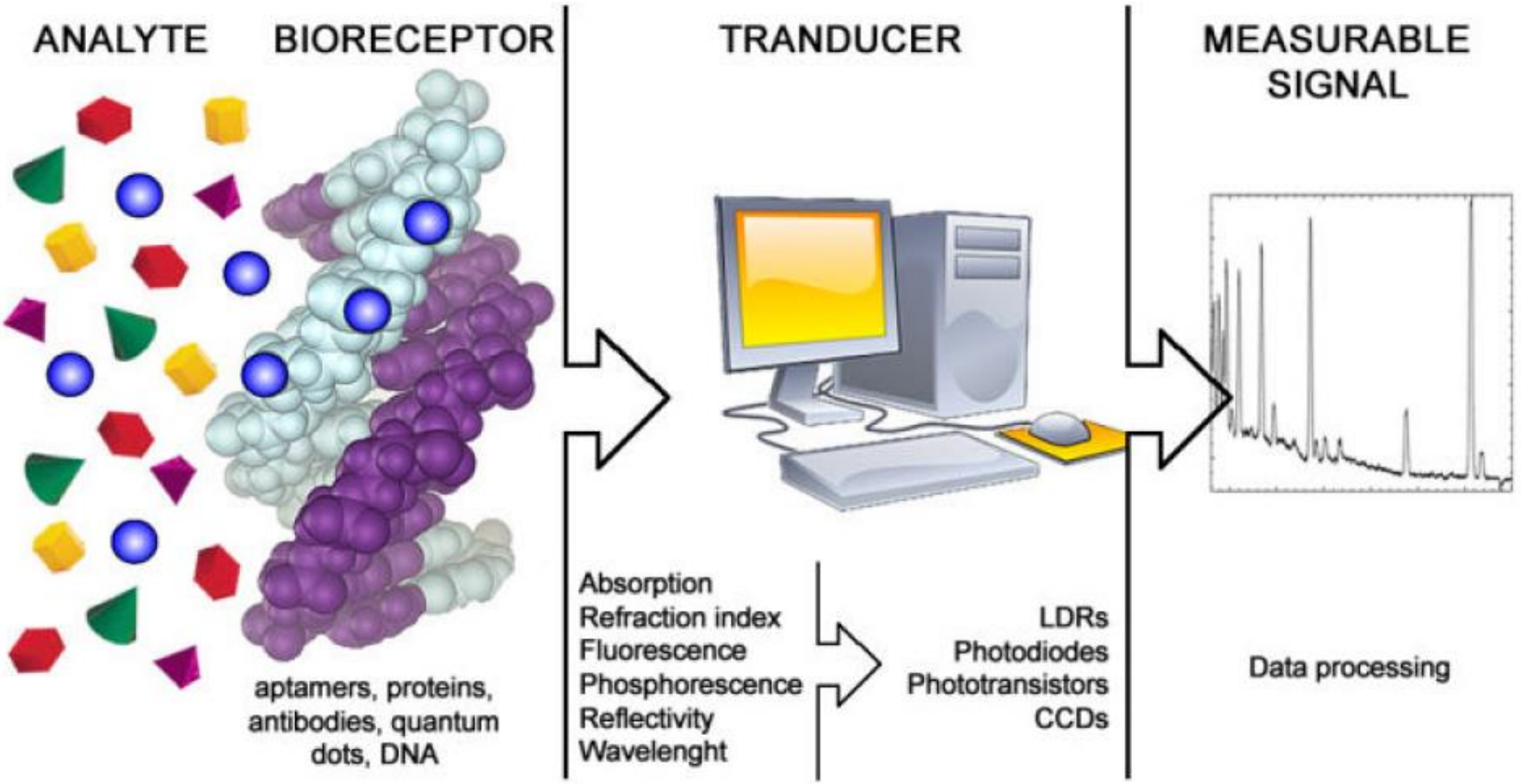
- Introduction
- Literature Review
- My Innovation
- Theory
- Fabrication
- Conclusion

Introduction

What is optical biosensor?

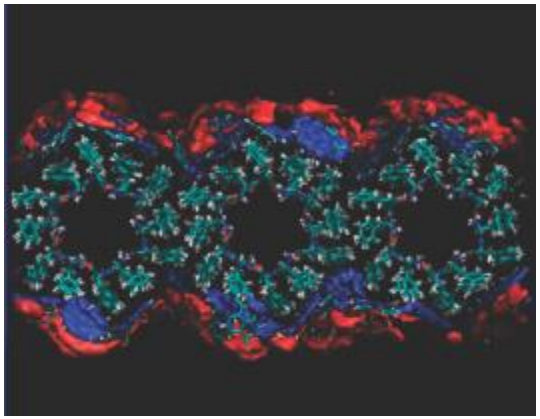
Detection of changes on absorption of UV/visible/infrared light when chemical reactions occur

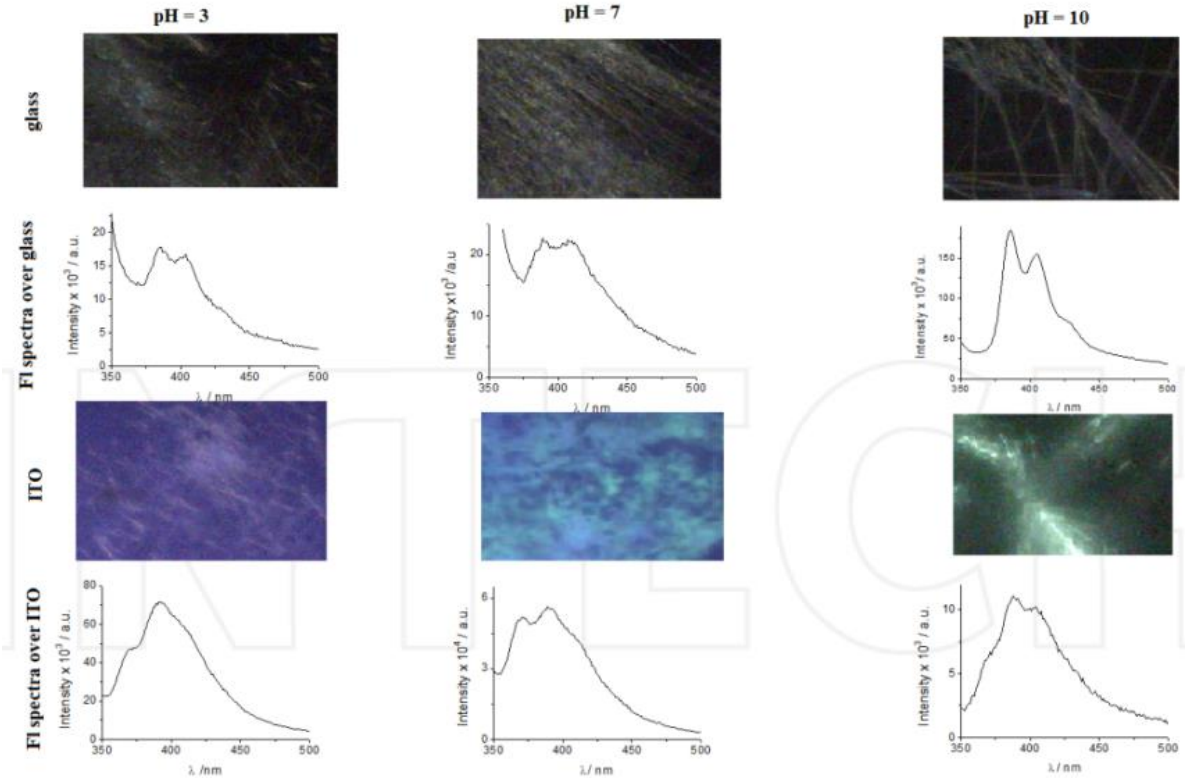
Introduction



Literature Review- Fluorescent biosensors

Using **fluorescence spectroscopy** techniques, the interaction of pyrenyl-1-carboxylic acid with diphenylalanine **nanotubes** and the effect of **pH** on the assembled nanostructures were studied.

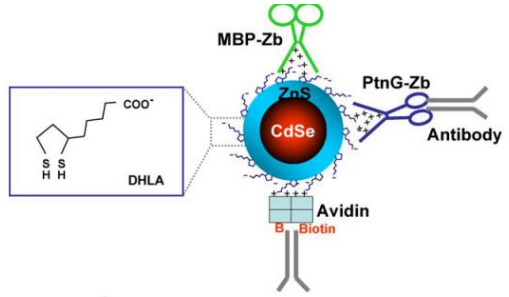


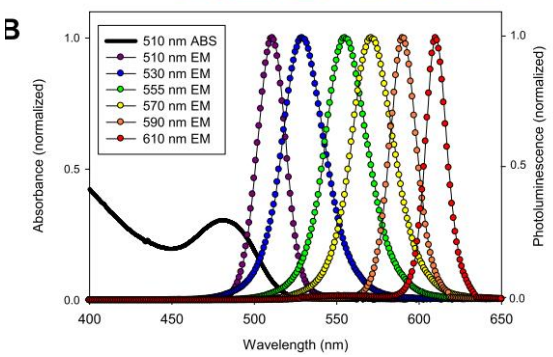


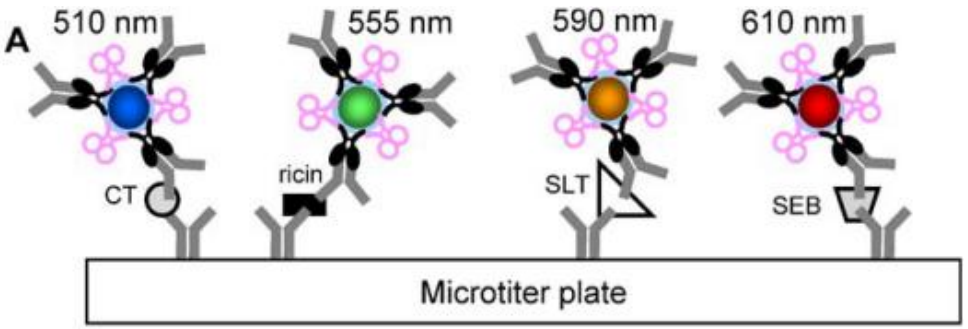
Ref: Martins TD, de Souza MI, Cunha BB, Takahashi PM, Ferreira FF, Souza JA, Fileti EE, Alves WA. Influence of pH and Pyrenyl on the Structural and Morphological Control of Peptide Nanotubes. Journal of Physical Chemistry C 2011; 115 7906–7913.

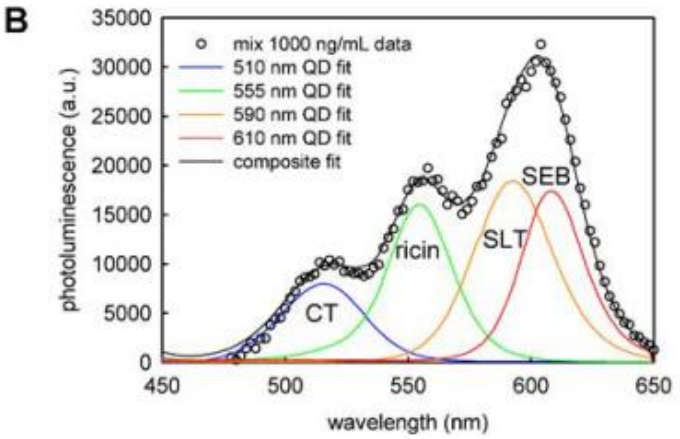
Literature Review- Quantum dots-based fluorescent biosensors

The characteristics of size-tunable luminescence and of **broad absorption spectra** make of **quantum dots** suitable for multi-color (or, as usually called multiplexed) immunoassays.





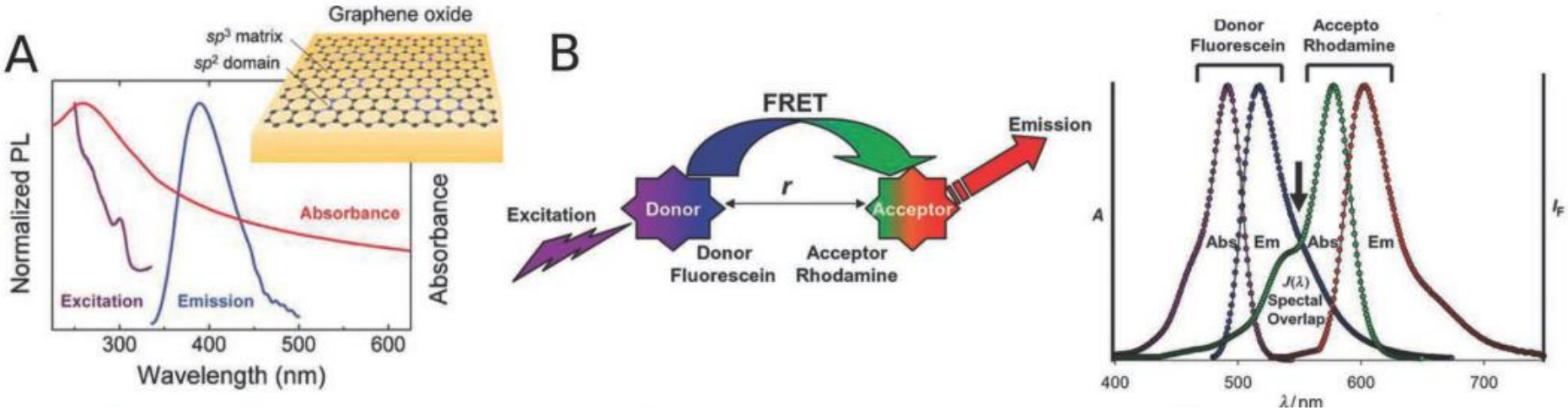




Ref: Goldman ER, Medintz IL, Mattoussi H. Luminescent Quantum Dots in Immunoassays. Analytical and Bioanalytical Chemistry 2006; 384 560-563.

Literature Review- graphene-based biosensors

Graphene oxide (GO) as a biosensing platform due to its ability of nanoassemble in wire form when in presence of biomolecules, its **processability in solution**. GO photoluminescence with **energy transfer donor/acceptor molecules** exposed in a planar surface and is even proposed as a universal highly efficient long-range quencher.



Ref: Morales-Narváez E, Merkoçi A.Graphene Oxide: Graphene Oxide as an Optical Biosensing Platform. Advanced Materials, 2012; 24(25) 3298–3308.

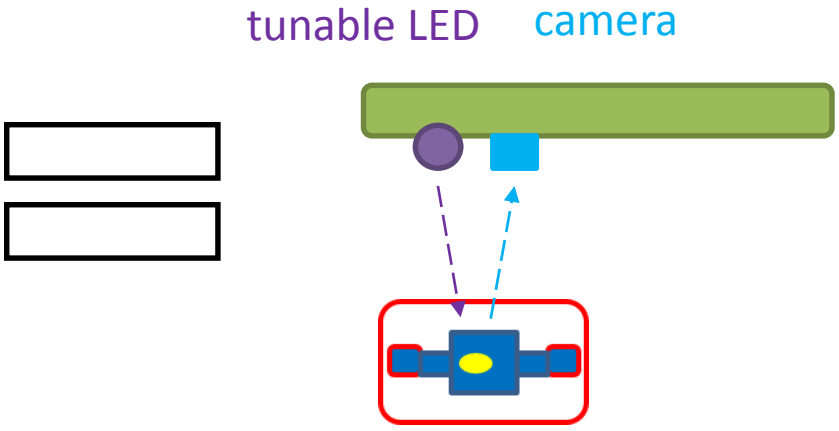
My Innovation- optical contrast biosensor

Cell phone with camera & tunable LED on backside

[TOP VIEW]

Microfluidic chip with biological cell

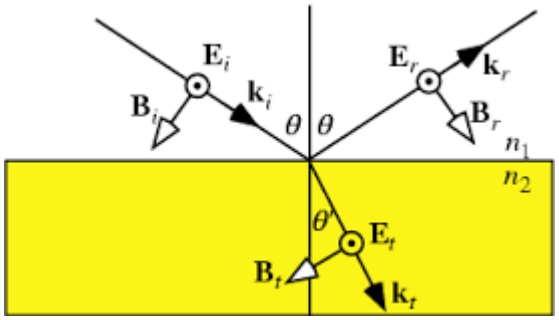
[TOP VIEW]

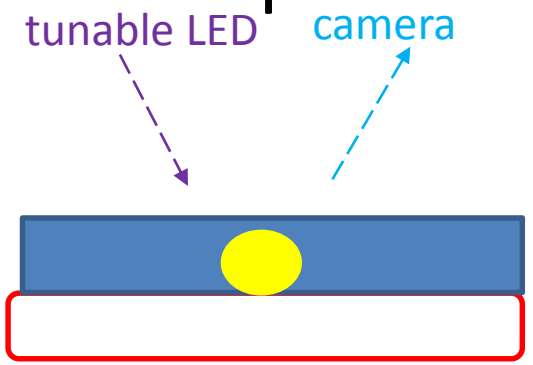


[SIDE VIEW]

Theory

- According Fresnel's equation in Optics:





i = 0 (air)
i = 1 (solution)
i = 2 (bio-cell)
i = 3 (PDMS)

$$I = \left| \frac{r_{01}e^{i(\Phi_1+\Phi_2)} + r_{12}e^{-i(\Phi_1-\Phi_2)} + r_{23}e^{-i(\Phi_1+\Phi_2)} + r_{01}r_{12}r_{23}e^{i(\Phi_1-\Phi_2)}}{e^{i(\Phi_1+\Phi_2)} + r_{01}r_{12}e^{-i(\Phi_1-\Phi_2)} + r_{01}r_{23}e^{-i(\Phi_1+\Phi_2)} + r_{12}r_{23}e^{i(\Phi_1-\Phi_2)}} \right|^2$$

$$r_{ij} = (n_i - n_j)/(n_i + n_j) \quad \text{and} \quad \Phi_i = 2\pi n_i d_i / \lambda$$

$where\ n = complex\ refrative\ index$

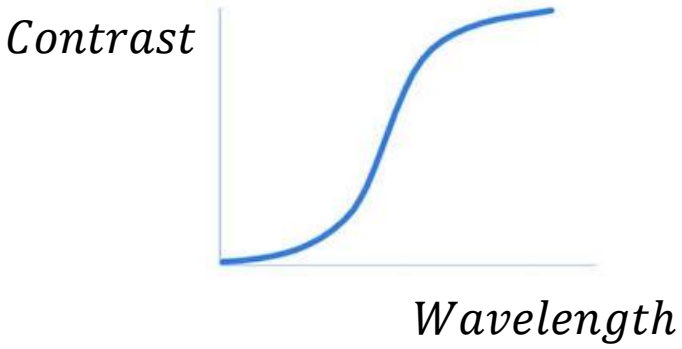
d = thickness

$\lambda = wavelength$

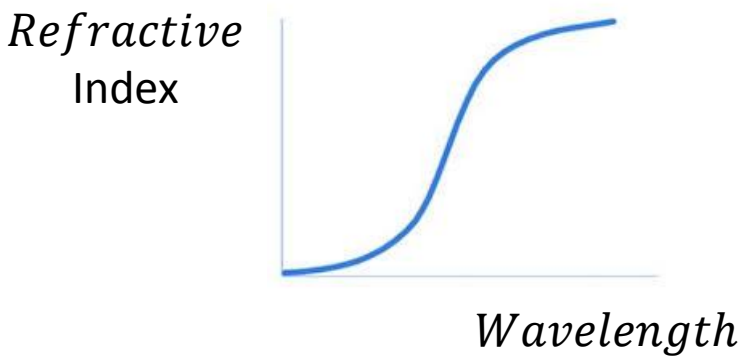
Theory

By $define$: $Contrast = \frac{I_{cell} - I_{PDMS}}{I_{cell} + I_{PDMS}}$

We can measure



Finally, get the refractive index curve of the biological cell by cellphone APPS calculation

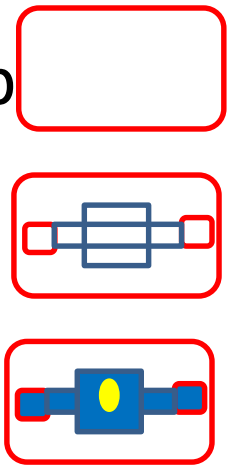


Fabrication

- Attach a tunable LED on cellphone:

- Make microfluidic chip by top down process:

1. make a pattern on Silicon wafer
2. Imprint the pattern from Si Wafer to PDMS chip
3. attach the inlet, outlet, channel PDMS
   by O2 plasma
4. Inlet the biological cell with solution



Conclusion

- Pros:
  - Faster than the setup of cellphone combined florescence biosensor
  - Potential real-time bio-imaging tracking

- Cons:
  - The environmental noise affects the accuracy

Thanks for your attention