Iceland Liechtenstein Norway grants
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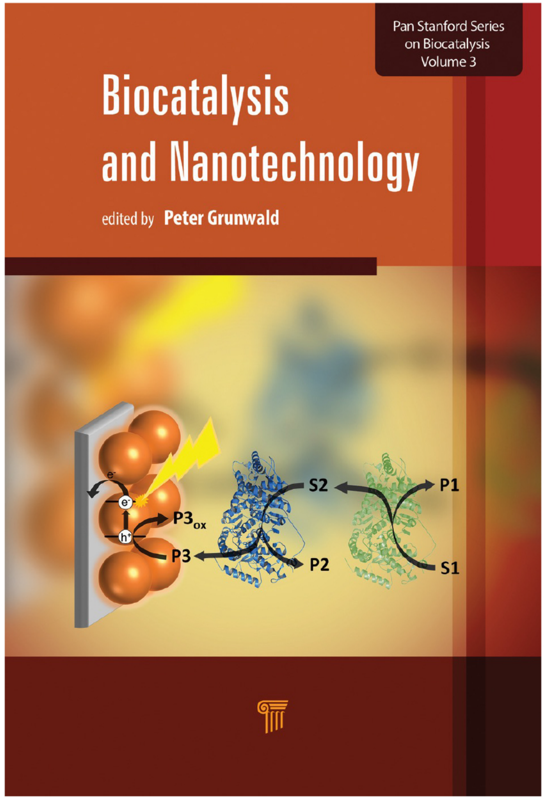
GREENCAM for tomorrow

UNIVERSITY OF BUCHAREST
VIRTUTE ET SAPIENTIA

NTNU
Norwegian University of Science and Technology

Biocatalysis and nanotechnology



5. Biological Strategies in Nanobiocatalyst Assembly
Ian Dominic F. Tabañag, and Shen-Long Tsai

6. Graphene-Based Nanobiocatalytic System
Michaela Patilaa , George Orfanakisa , Angeliki C. Polydera,
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7. Immobilization of Biocatalysts onto Nanosupports: Advantages for Green Technologies
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9. Potential Applications of Nanobiocatalysis for Industrial Biodiesel Production
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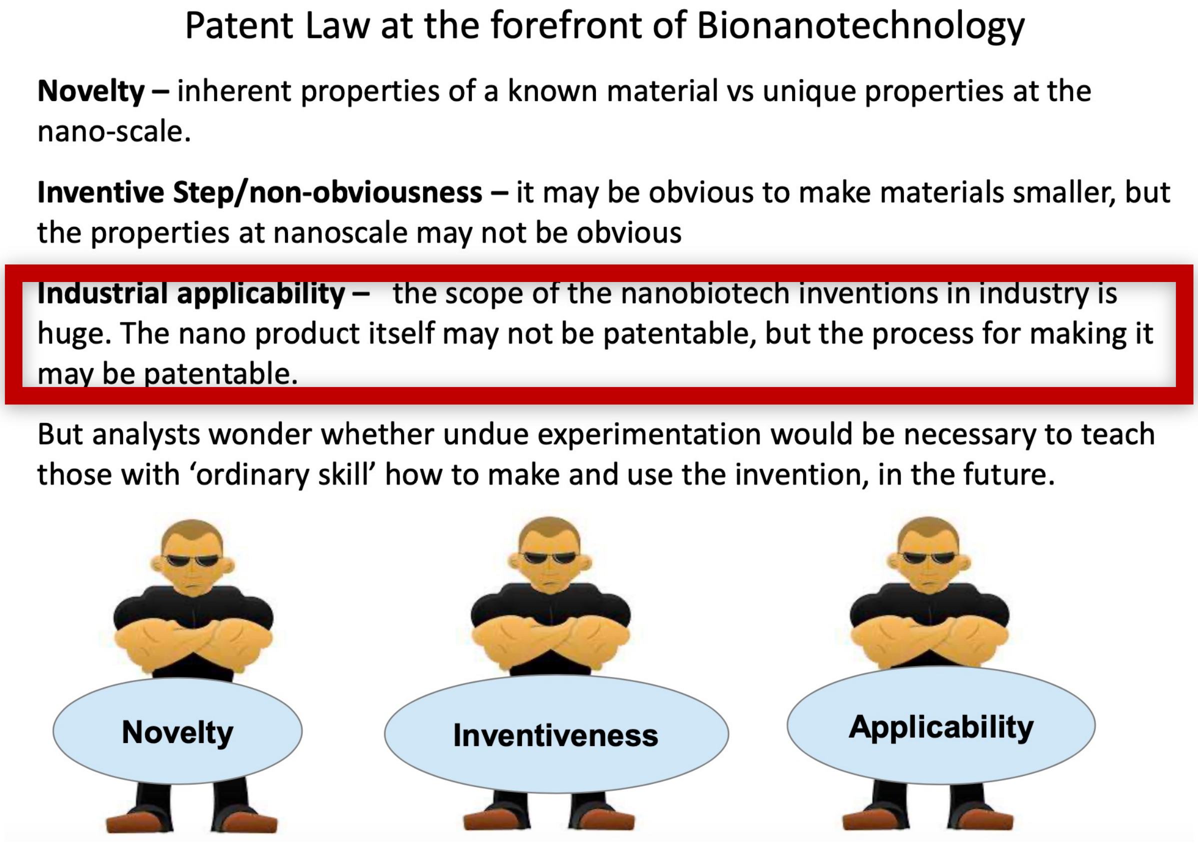
11. Recent Advances in Nanostructured Enzyme Catalysis for Chemical Synthesis in Organic Solvents
Zheng Liu, Jun Ge, Diannan Lu, Guoqiang Jiang, and Jianzhong Wu

Norwegian University of Science and Technology

CONFRONTING THE BIG 3



Patent Law at the forefront of Bionanotechnology

Novelty – inherent properties of a known material vs unique properties at the nano-scale.

Inventive Step/non-obviousness – it may be obvious to make materials smaller, but the properties at nanoscale may not be obvious

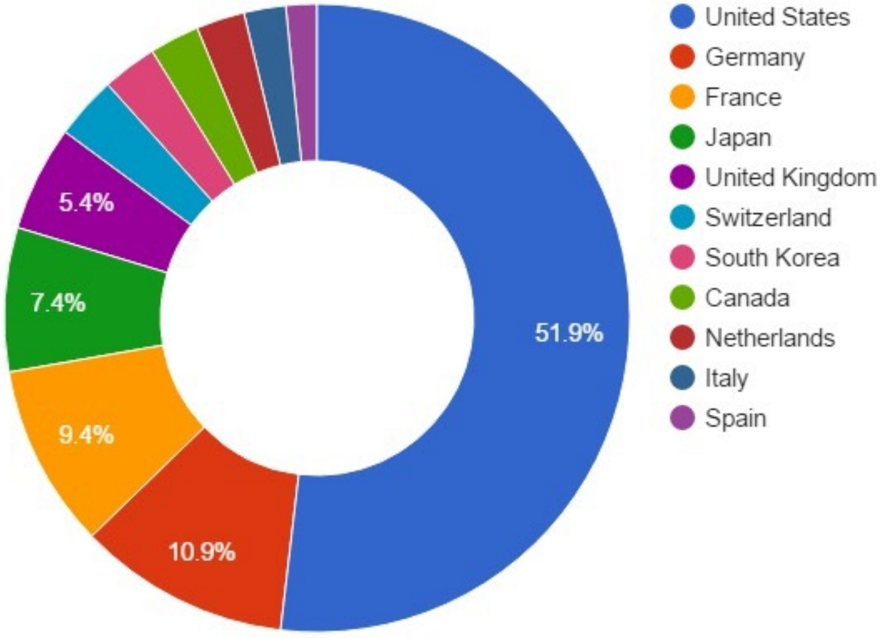
Industrial applicability – the scope of the nanobiotech inventions in industry is huge. The nano product itself may not be patentable, but the process for making it may be patentable.

But analysts wonder whether undue experimentation would be necessary to teach those with 'ordinary skill' how to make and use the invention, in the future.

Patents in Bionanotechnology

United States is leading the charge in bionanotechnology applications.

In part due to nanotechnologies having received **recognition** and **national funding** in the early 2000´s by the NNI. WIPO explains that US corporations are pushing many of these therapies forward:

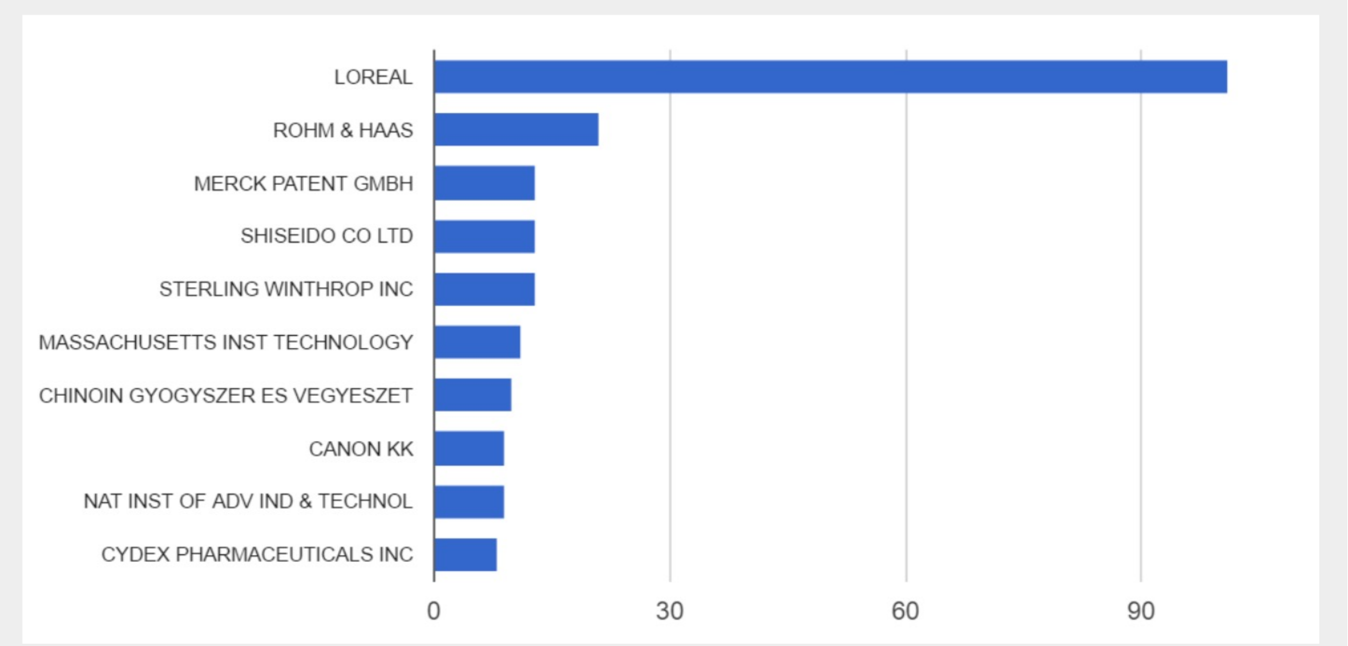


Bionanotechnology Patent Applications by Country

World Intellectual Property Organisation **WIPO**

“As of 2013, a few hundred nano-related medical therapies had been approved or had entered clinical trials in the United States”.

Patent filers of Japanese Applications



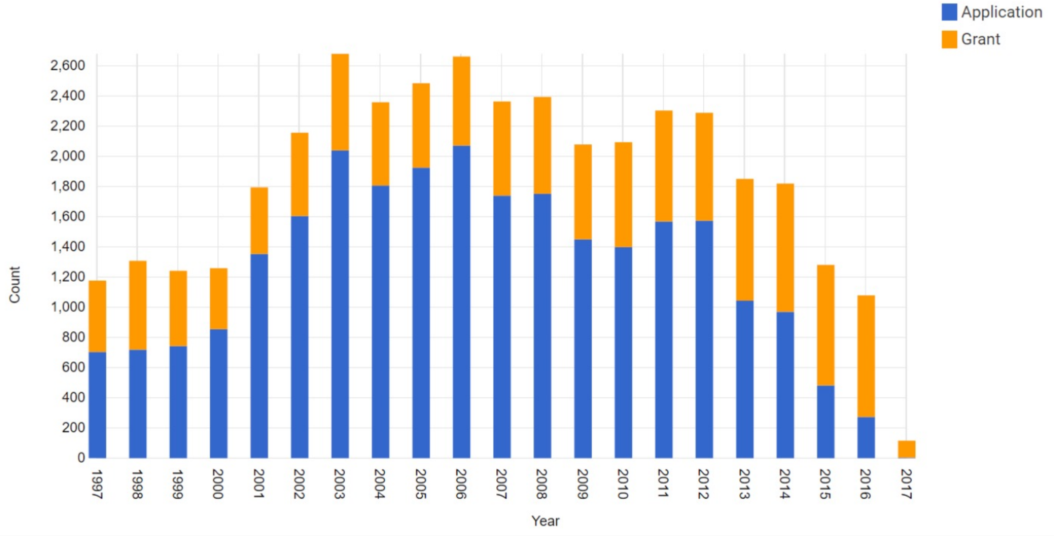
Source: ktMINE

China: World´s second largest economy- and largest in GDP

Out of over 49,000 patents and patent applications, Chinese entities own fewer than 1% of bionanotechnology applications and grants. It could be that China is focusing their current nanoscience efforts in the electronics and semiconductor space. But this data suggests China will face limitations to advance innovation in bionanotechnology applications.

Total patents WIPO

Compound annual Growth Rate (CAGR)

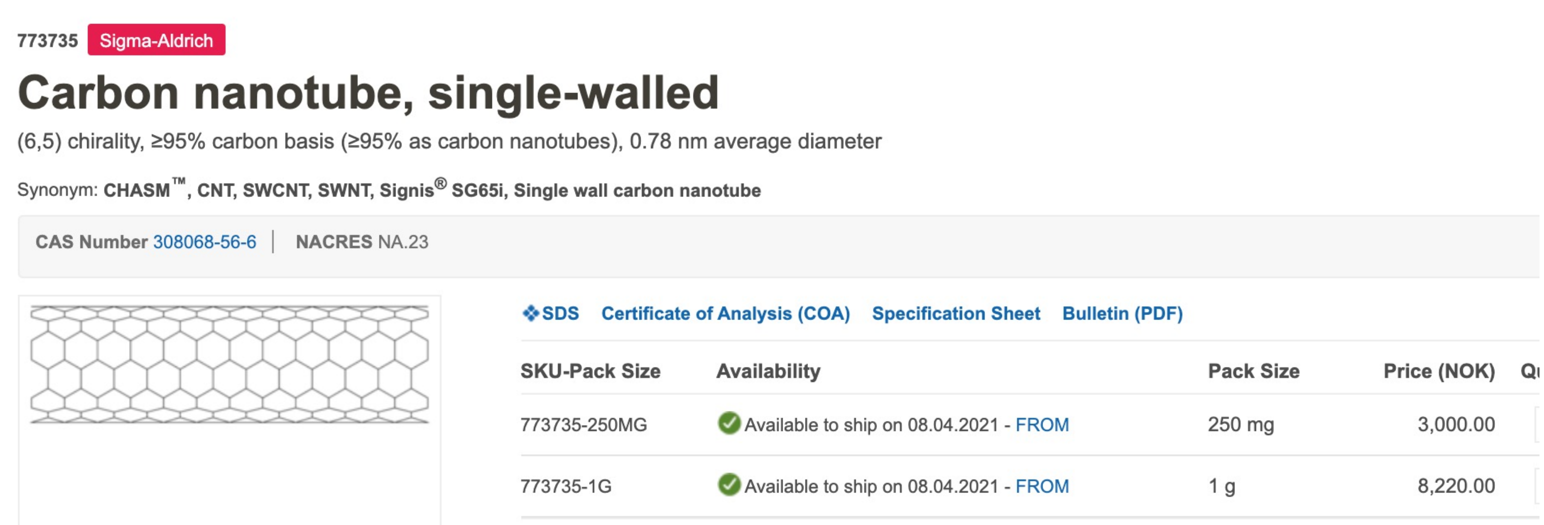


Source: ktMINE: CPC classifications A61, C12N AND B81, B82

“The global nanotechnology market is expected to grow at a CAGR of around 17% during the forecasted period of 2017-2024”. (Research and Markets)

Graphene oxide, nanotubes and enzyme prices

Amount of GO	Form	Amount of paste	Price (USD)
2 Kg	Aqueous acidic paste	10 Kg	3000
1 Kg	Aqueous acidic paste	5 Kg	1600
0			



773735 Sigma-Aldrich

Carbon nanotube, single-walled

(6,5) chirality, ≥95% carbon basis (≥95% as carbon nanotubes), 0.78 nm average diameter

Synonym: **CHASM™, CNT, SWCNT, SWNT, Signis® SG65i, Single wall carbon nanotube**

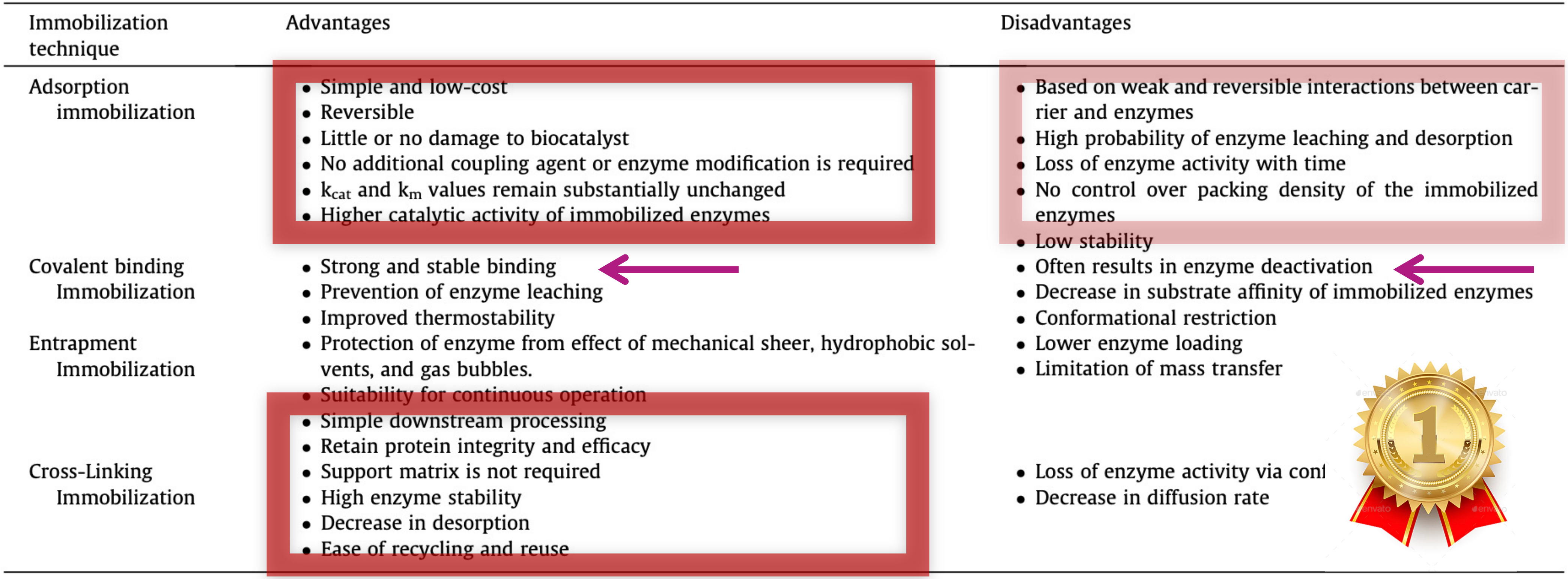
CAS Number 308068-56-6 | **NACRES** NA.23

SKU-Pack Size	Availability	Pack Size	Price (NOK)
773735-250MG	✓ Available to ship on 08.04.2021 - FROM	250 mg	3,000.00
773735-1G	✓ Available to ship on 08.04.2021 - FROM	1 g	8,220.00

300 EUR
822EUR/G

Novozym ® 435 market price > 1000 USD/KG

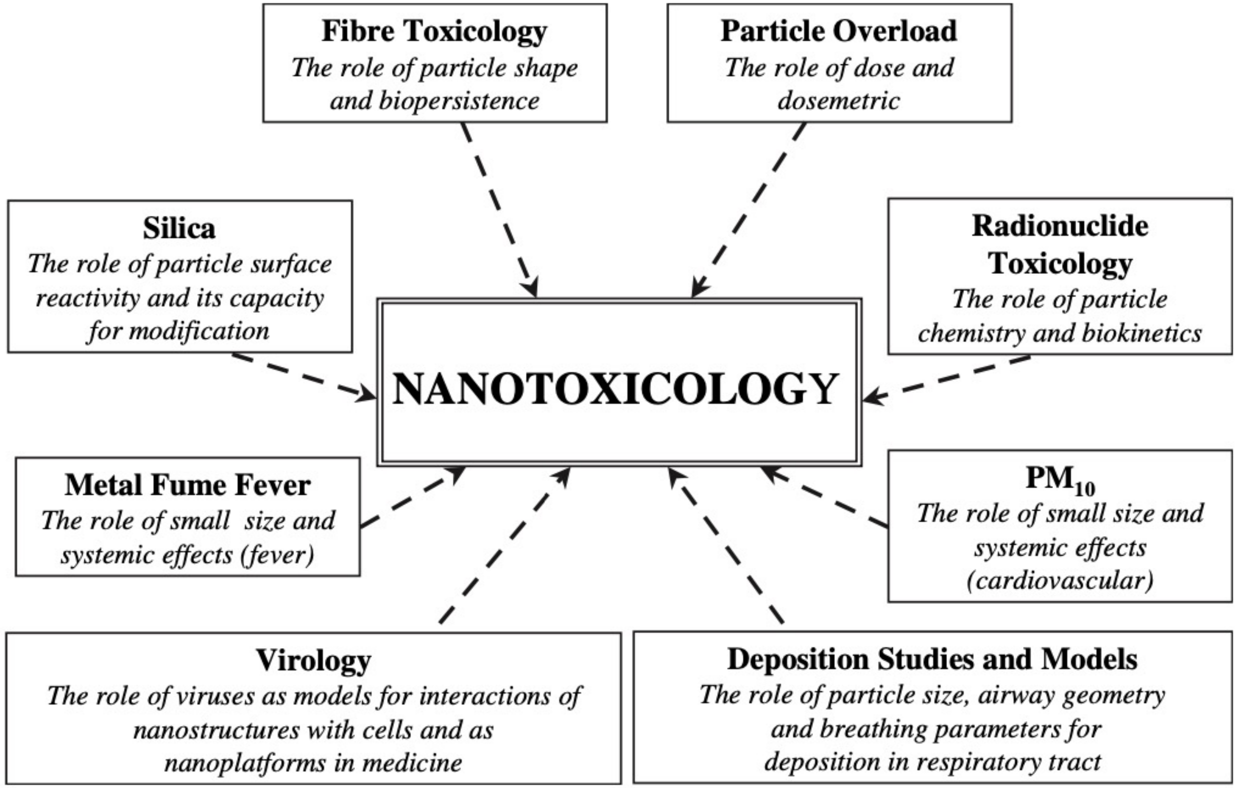
Nanobiocatalysis- a subarea of enzyme biotechnology
Advantages and disadvantages of various immobilization techniques



Immobilization technique	Advantages	Disadvantages
Adsorption immobilization	• Simple and low-cost • Reversible • Little or no damage to biocatalyst • No additional coupling agent or enzyme modification is required • k_{cat} and k_m values remain substantially unchanged • Higher catalytic activity of immobilized enzymes	• Based on weak and reversible interactions between carrier and enzymes • High probability of enzyme leaching and desorption • Loss of enzyme activity with time • No control over packing density of the immobilized enzymes • Low stability
Covalent binding Immobilization	• Strong and stable binding • Prevention of enzyme leaching • Improved thermostability	• Often results in enzyme deactivation • Decrease in substrate affinity of immobilized enzymes • Conformational restriction
Entrapment Immobilization	• Protection of enzyme from effect of mechanical sheer, hydrophobic solvents, and gas bubbles. • Suitability for continuous operation	• Lower enzyme loading • Limitation of mass transfer
Cross-Linking Immobilization	• Simple downstream processing • Retain protein integrity and efficacy • Support matrix is not required • High enzyme stability • Decrease in desorption • Ease of recycling and reuse	• Loss of enzyme activity via conf • Decrease in diffusion rate

Singh, N, Dhanya, BS, Verma, ML *Materials Science for Energy Technologies* **2020**, *3*, 808-824

Foundations of Nanotoxicology



Fibre Toxicology
The role of particle shape and biopersistence

Particle Overload
The role of dose and dosemetric

Silica
The role of particle surface reactivity and its capacity for modification

NANOTOXICOLOGY

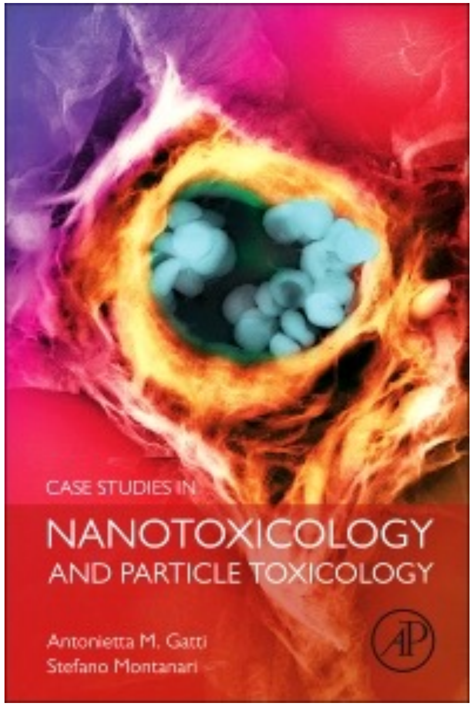
Radionuclide Toxicology
The role of particle chemistry and biokinetics

Metal Fume Fever
The role of small size and systemic effects (fever)

PM_{10}
The role of small size and systemic effects (cardiovascular)

Virology
The role of viruses as models for interactions of nanostructures with cells and as nanoplatforms in medicine

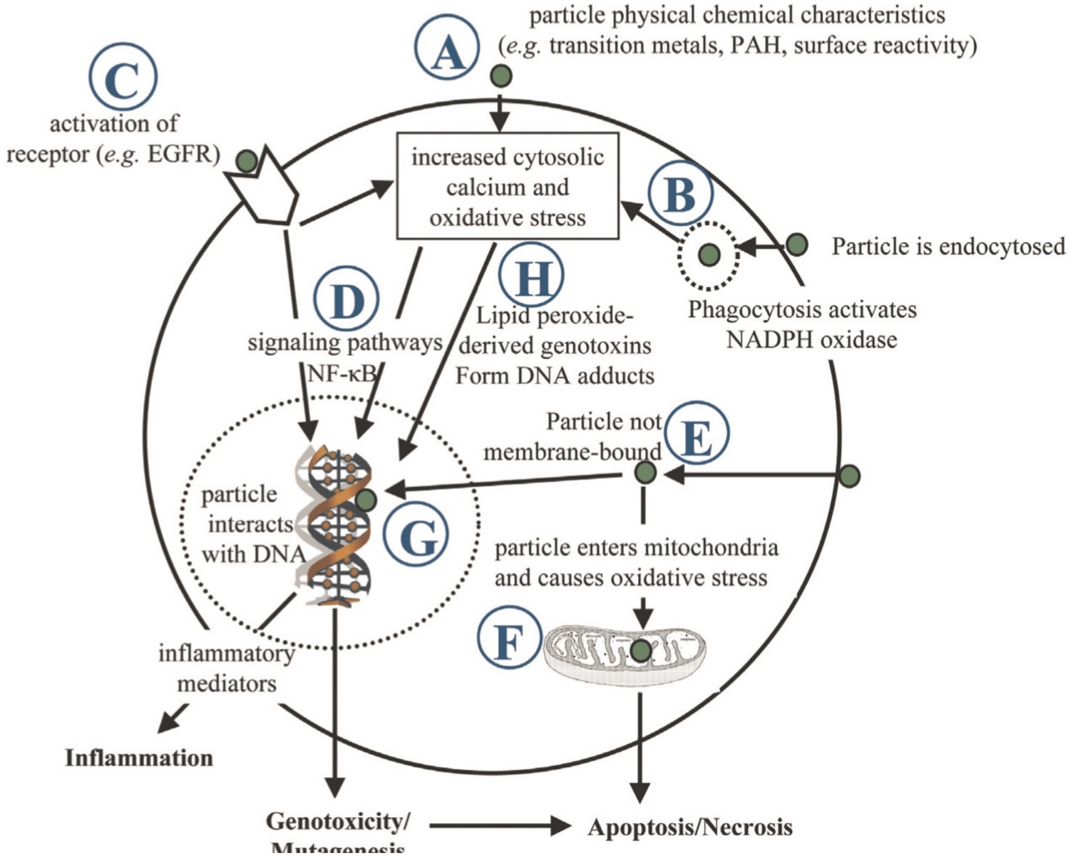
Deposition Studies and Models
The role of particle size, airway geometry and breathing parameters for deposition in respiratory tract



Oberdorster, G , Stone, V, Donaldson, K. *Nanotoxicology*, **2007**; *1* (1), 2-25

Hypothetical cellular interactions of NP

The cell

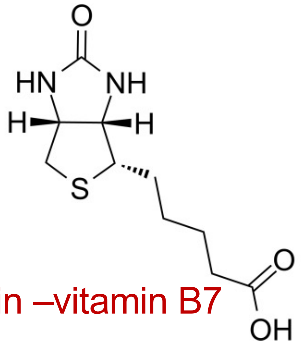


Oberdorster *et al*, 2007

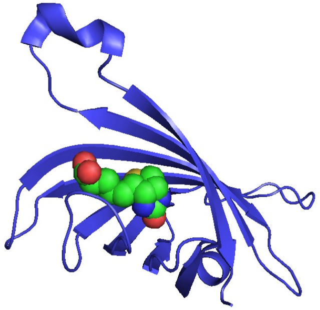
5. Biological Strategies in Nanobiocatalyst Assembly

Ian Dominic F. Tabañag, and Shen-Long Tsai

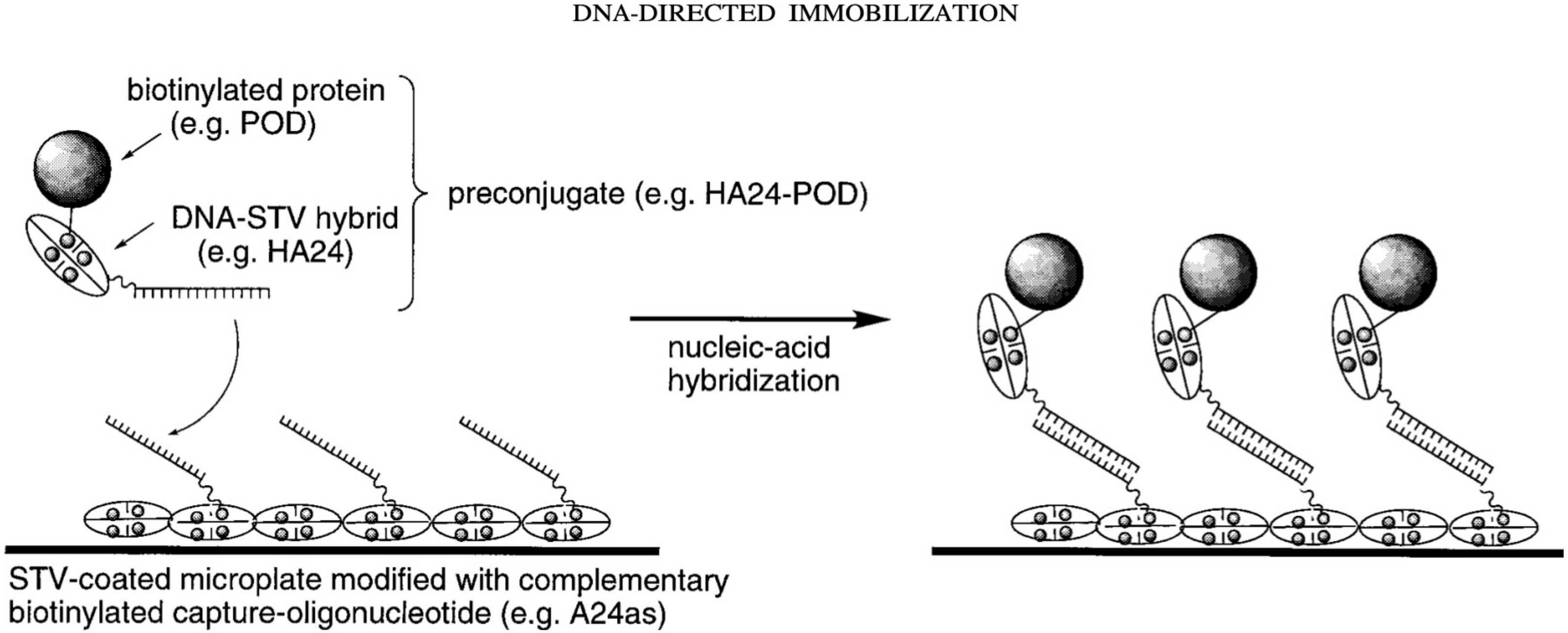
DDI Process



Biotin –vitamin B7



Streptavidin w biotin



Advantages: -Mild reversible technique

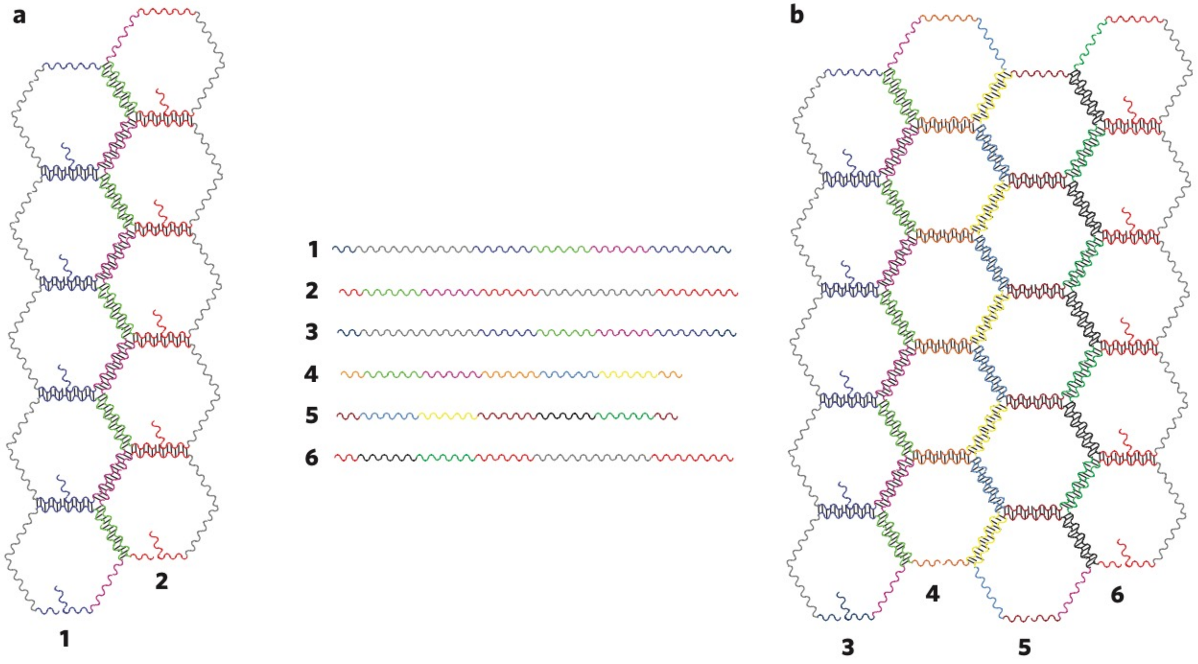
For biosensing and biomedical diagnostics, and fundamental studies in biology and medicine

Wilner, OI, Weizmann, Y, Gill, R, Lioubashevski, O, Freeman, R and Willner, I. *Nature Nanotechnology* **2009**, *4*, 249-254

Assembly of hexagon-like DNA strips and their structural imaging.

A: Two-hexagon DNA strip assembly

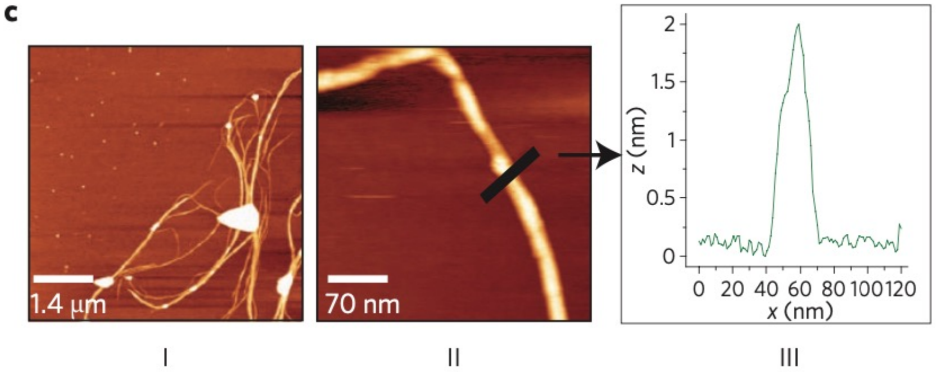
B: Four-hexagon DNA strip assembly.

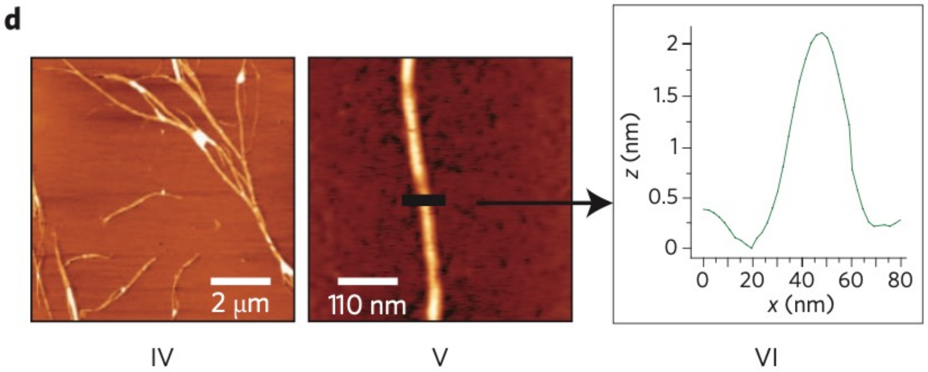


Wilner *et al*, 2009

Atomic Force Images

C: AFM images of the two-hexagon strip: (I) large-scale image that includes a collection of strips, (II) image of a single strip, and (III) cross-sectional analysis of a single strip.





D: AFM images of the four-hexagon strip: (IV) large-scale image that includes several strips, (V) image of a single strip, and (VI) cross- sectional analysis of a single strip.

Cascade enzyme reactions

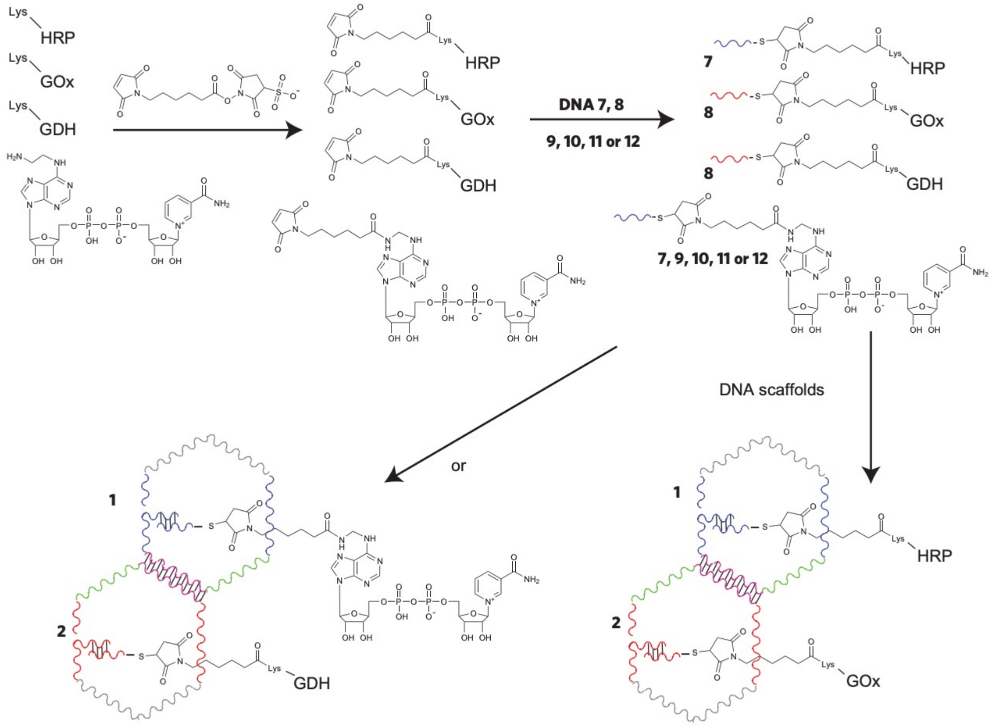
- two enzymes or a cofactor-enzyme pair are added to the scaffold
- shows that enzyme cascades or cofactor-mediated biocatalysis can proceed effectively
- similar processes are not observed in diffusion-controlled homogeneous mixtures of the same components.
- because relative position of the two enzymes or the cofactor-enzyme pair is determined by the topology of the DNA scaffold, it is possible to control the reactivity of the system through the design of the individual DNA strips.

Advantage: self-organization of complex multi-enzyme cascades.

Enzymes on 1D and 2D DNA scaffolds

Enzymes immobilised on:

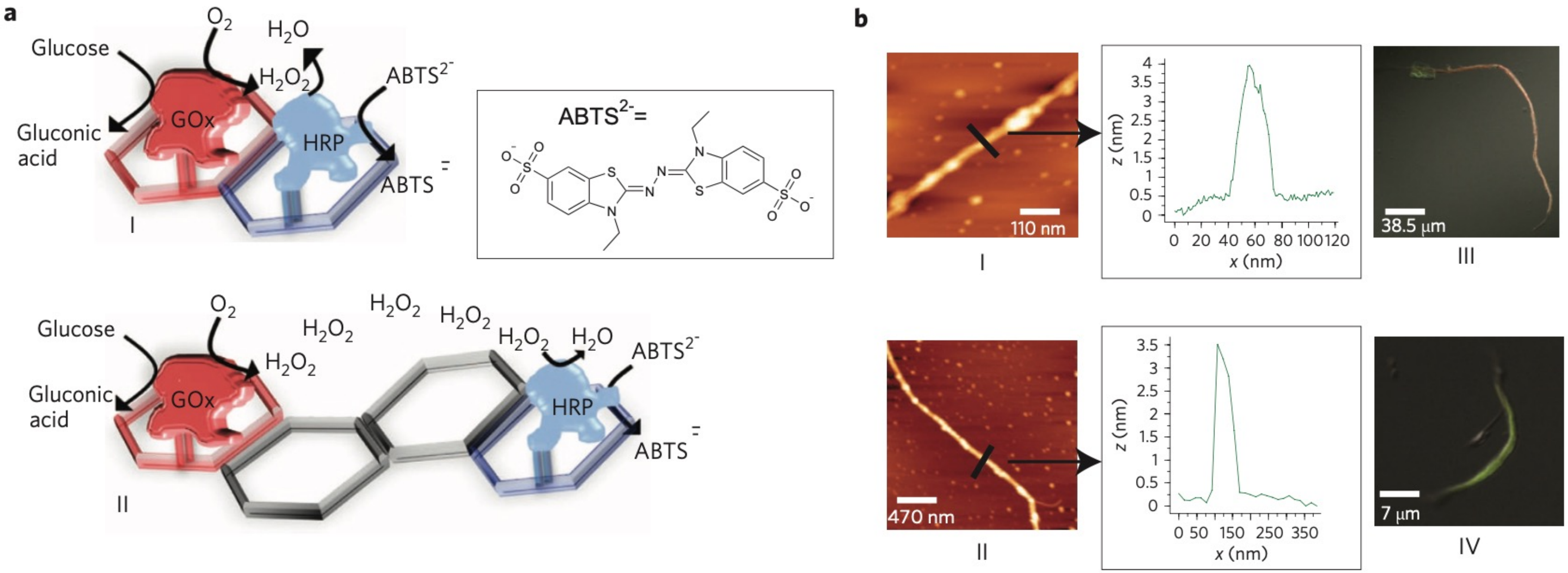
Enzymes:
Glucose oxidase (GOX) and horseradish peroxidase (HRP)
Glucose dehydrogenase (GDH)



Advantages:
-Increased flexibility and enzyme activity

Wilner *et al*, 2009

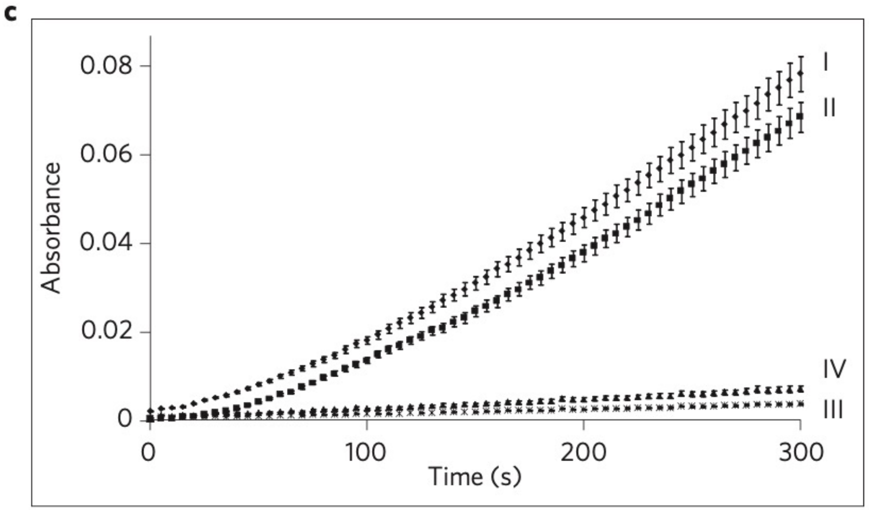
Assembly of enzyme cascades or cofactor–enzyme cascades on hexagon-like DNA scaffolds, their imaging and their functional characterization

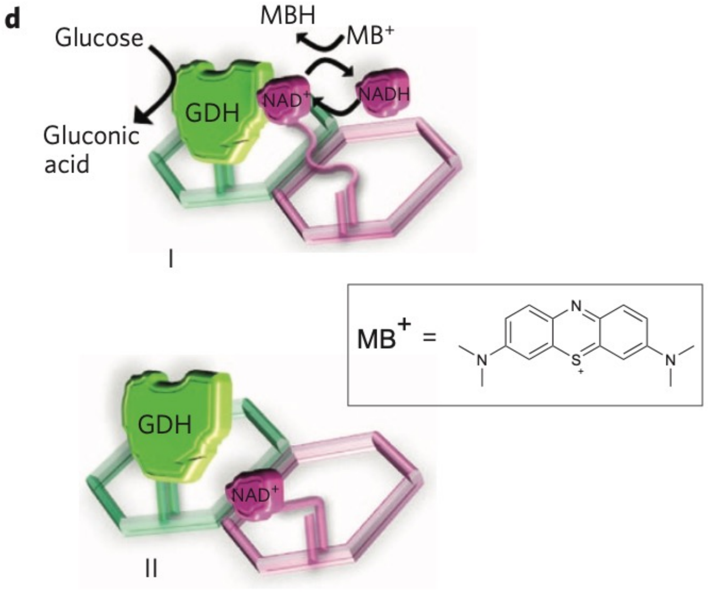


The primary enzyme GOx biocatalyses the oxidation of glucose to gluconic acid, with the concomitant formation of H_2O_2. The latter product acts as substrate for HRP, mediating the oxidation of 2´ 2´ azino-bis[3-ethylbenzthiazoline-6-sulphonic-acid], $ABTS^{2-}$, to the coloured product, $ABTS^{-}$

Wilner *et al*, 2009

C: Time-dependent absorbance changes as a result of the oxidation of $ABTS^{2-}$ by the GOx–HRP cascade in the presence of (I) the two-hexagon scaffold, (II) the four-hexagon scaffold, (III) in the absence of any DNA, and (IV) in the presence of foreign calf thymus DNA.

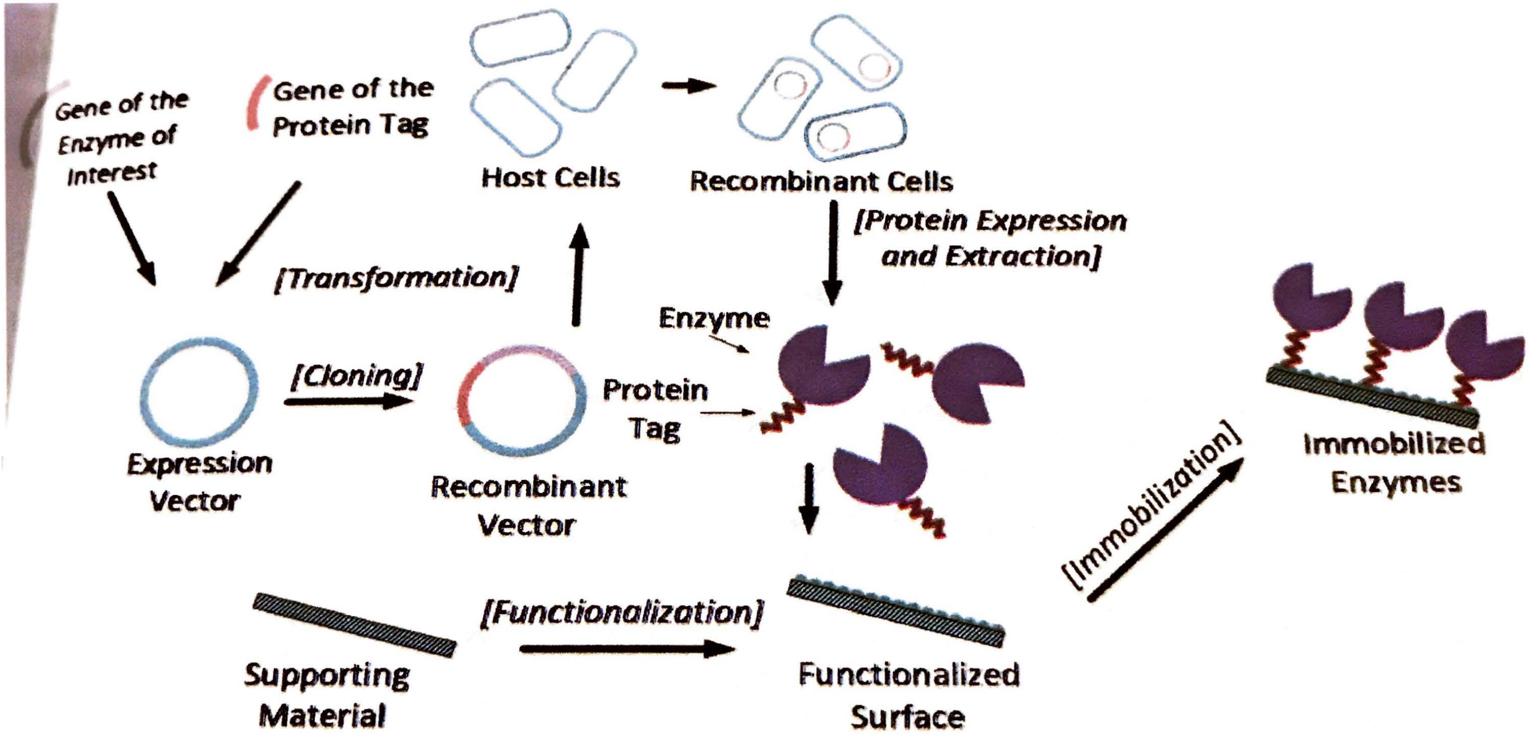




D: Assembly of the NAD^+/GDH system on the two-hexagon scaffold using different lengths of tethers linking the NAD^+ cofactor to the scaffold.

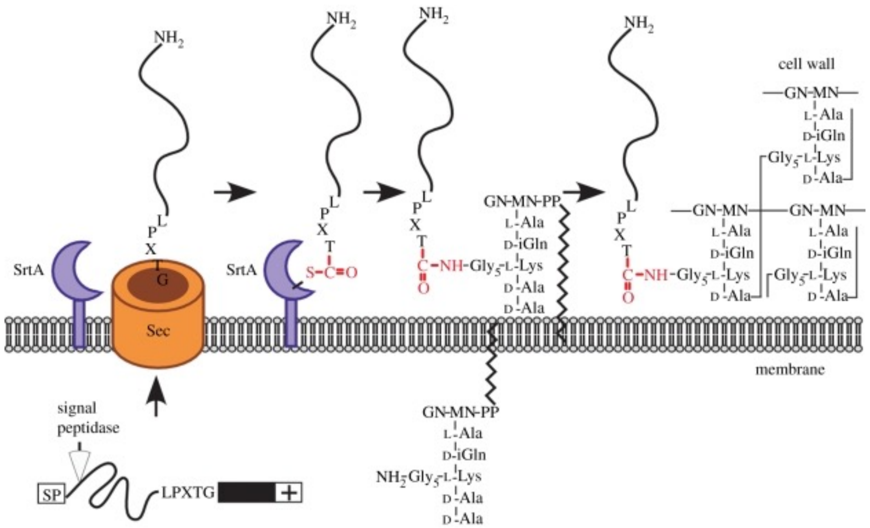
Wilner *et al*, 2009

Enzyme immobilisation *via* protein affinity tags



Advantage: No need for protein purification steps after protein expression and extraction of the transformed cells

Enzyme assisted covalent immobilisation



Sortase A-enzyme used by Gram-positive bacteria to anchor surface proteins to the cell wall between a C-terminal tag

Advantages for bionanocatalysis: Specific and mild-and no need for ligands.-conjugating enzyme instead

Mostly addition of tags to N and C terminals- HOWEVER: If these terminals are near active-the site active site may be blocked.

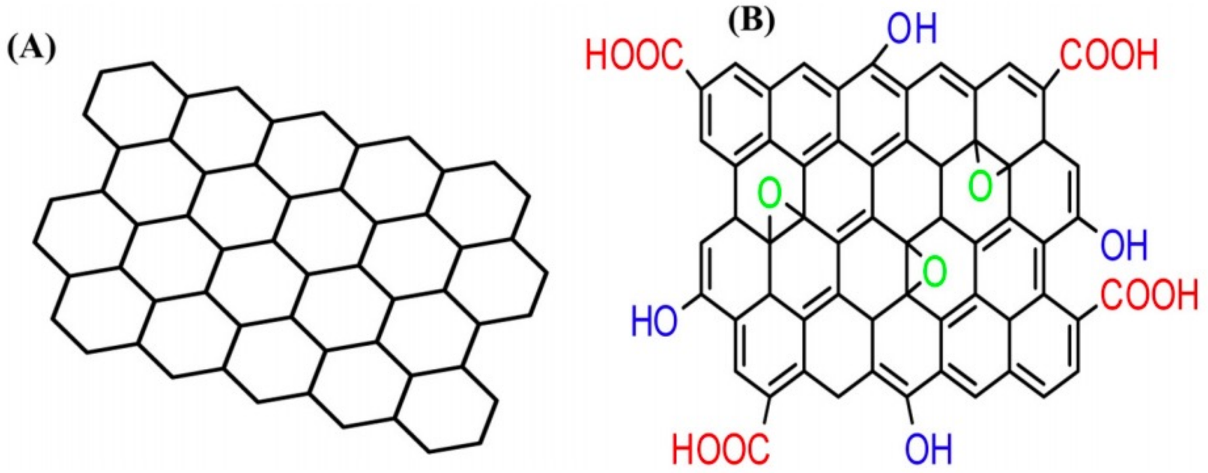
To avoid this: Must use unnatural amino acids (with unique functional groups) in the synthesis of proteins

Schneewind. O and Missiakas, DM *Phil. Trans. R. Soc. B* **2012**, *367*, 1123-1139
Parthasarathy, R, Subramanian, S, Boder, ET *Bioconjugate Chem*. **2007**, *18*, 469-476

6. Graphene-Based Nanobiocatalytic System (GBN´s)

Michaela Patilaa , George Orfanakisa , Angeliki C. Polydera, Ioannis V. Pavlidis, and Haralambos Stamatis

Application of graphene oxide (GO) for biomolecule immobilisation



Utilised for:

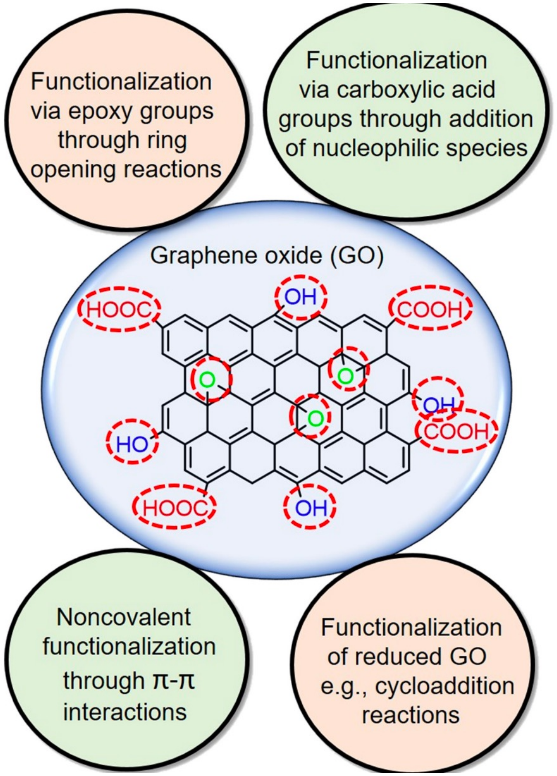
- Biofuel production
- Degradation of pollutants
- In situ protein digestion
- Biosensing

Adeel M. *et al. Int J of Biol Macromolecules* **2018**, *120*, 1430-1440

Graphene based nanomaterials as enzyme immobilisation supports

Strategies to immobilise enzymes onto graphene:

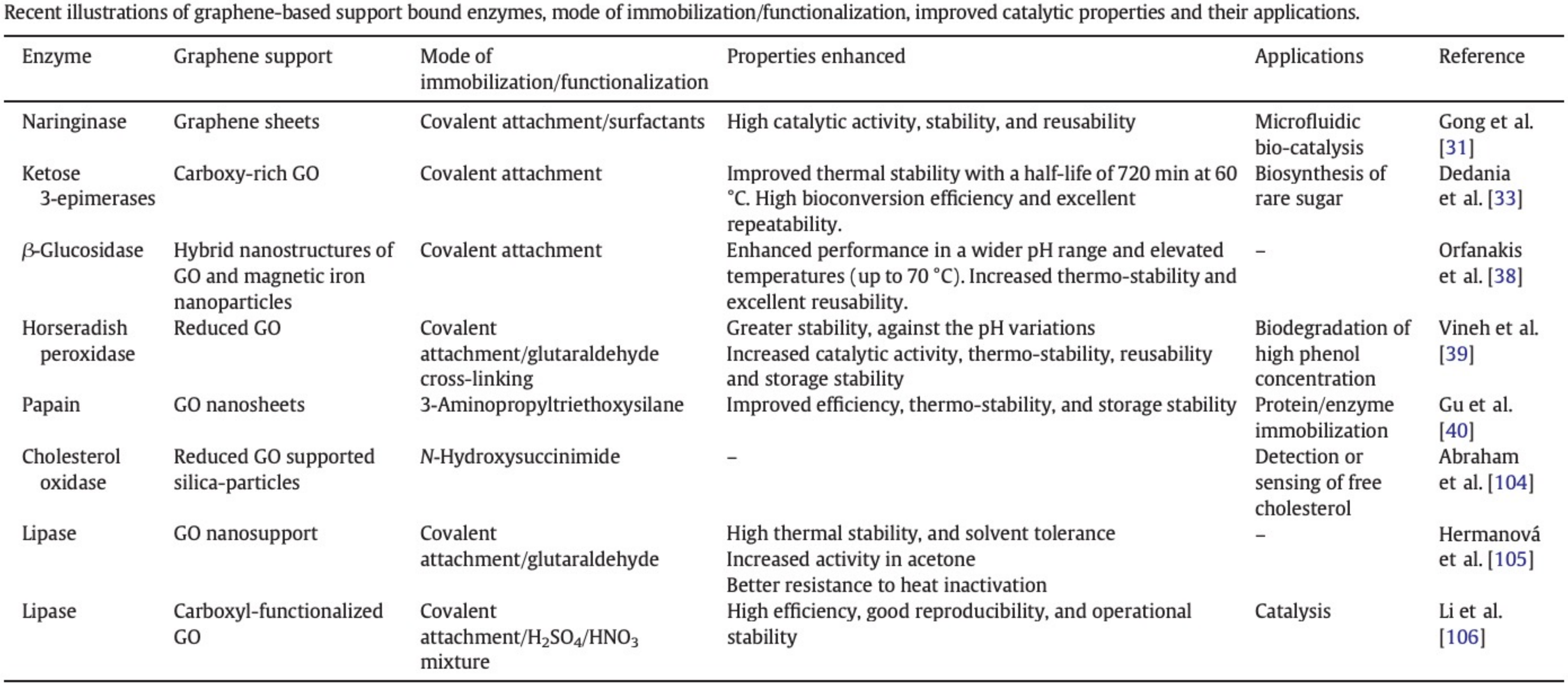
Physical adsorption, covalent attachment, site spesific affinity interactions, gluteraldehyde as linker



Advantages:
Surface chemistry of the nanomaterials affect the catalytic properties
and conformation of the enzymes

Adeel *et al*. **2018**

Graphene-based support bound enzymes, mode of immobilization/functionalization, improved catalytic properties and their applications

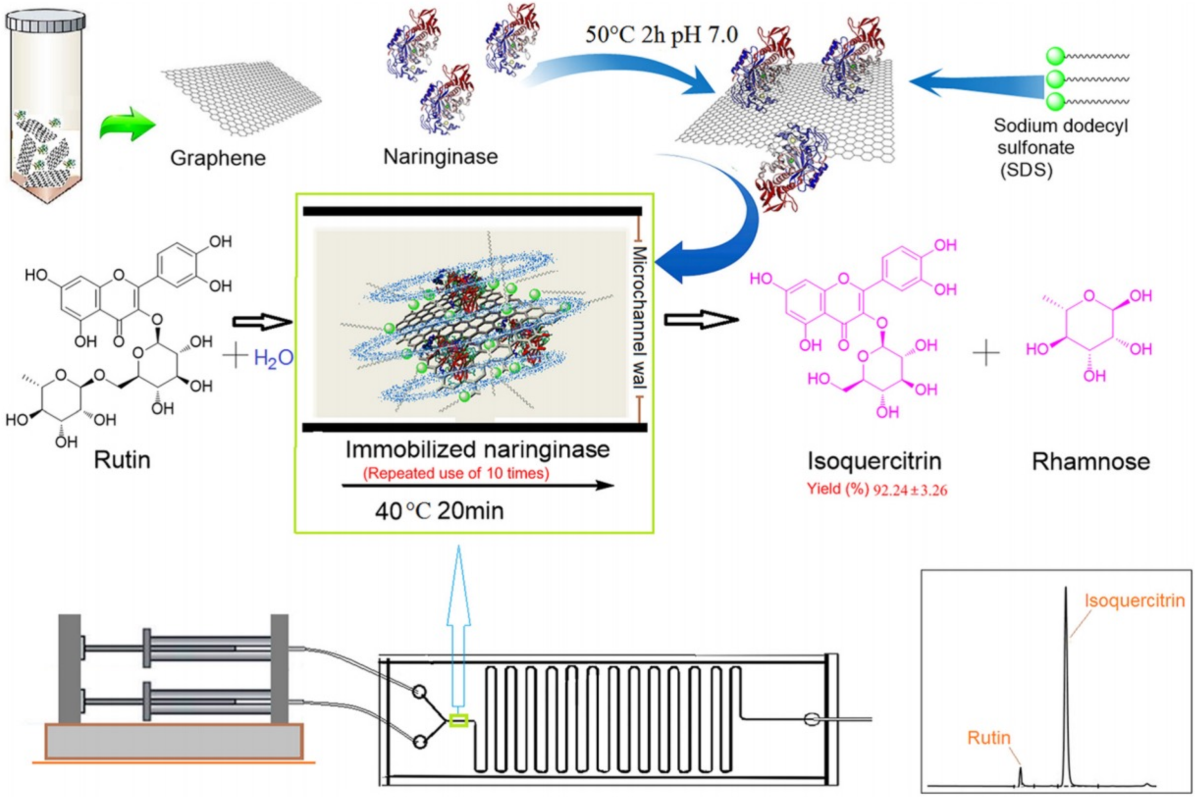


Recent illustrations of graphene-based support bound enzymes, mode of immobilization/functionalization, improved catalytic properties and their applications.

Enzyme	Graphene support	Mode of immobilization/functionalization	Properties enhanced	Applications	Reference
Naringinase	Graphene sheets	Covalent attachment/surfactants	High catalytic activity, stability, and reusability	Microfluidic bio-catalysis	Gong et al. [31]
Ketose 3-epimerases	Carboxy-rich GO	Covalent attachment	Improved thermal stability with a half-life of 720 min at 60 °C. High bioconversion efficiency and excellent repeatability.	Biosynthesis of rare sugar	Dedania et al. [33]
β-Glucosidase	Hybrid nanostructures of GO and magnetic iron nanoparticles	Covalent attachment	Enhanced performance in a wider pH range and elevated temperatures (up to 70 °C). Increased thermo-stability and excellent reusability.	–	Orfanakis et al. [38]
Horseradish peroxidase	Reduced GO	Covalent attachment/glutaraldehyde cross-linking	Greater stability, against the pH variations Increased catalytic activity, thermo-stability, reusability and storage stability	Biodegradation of high phenol concentration	Vineh et al. [39]
Papain	GO nanosheets	3-Aminopropyltriethoxysilane	Improved efficiency, thermo-stability, and storage stability	Protein/enzyme immobilization	Gu et al. [40]
Cholesterol oxidase	Reduced GO supported silica-particles	*N*-Hydroxysuccinimide	–	Detection or sensing of free cholesterol	Abraham et al. [104]
Lipase	GO nanosupport	Covalent attachment/glutaraldehyde	High thermal stability, and solvent tolerance Increased activity in acetone Better resistance to heat inactivation	–	Hermanová et al. [105]
Lipase	Carboxyl-functionalized GO	Covalent attachment/H_2SO_4/HNO_3 mixture	High efficiency, good reproducibility, and operational stability	Catalysis	Li et al. [106]

Adeel *et al*. **2018**

Biosynthesis diagram of isoquercitrin in a microchannel reactor with a fluid and unsinkable immobilized enzyme

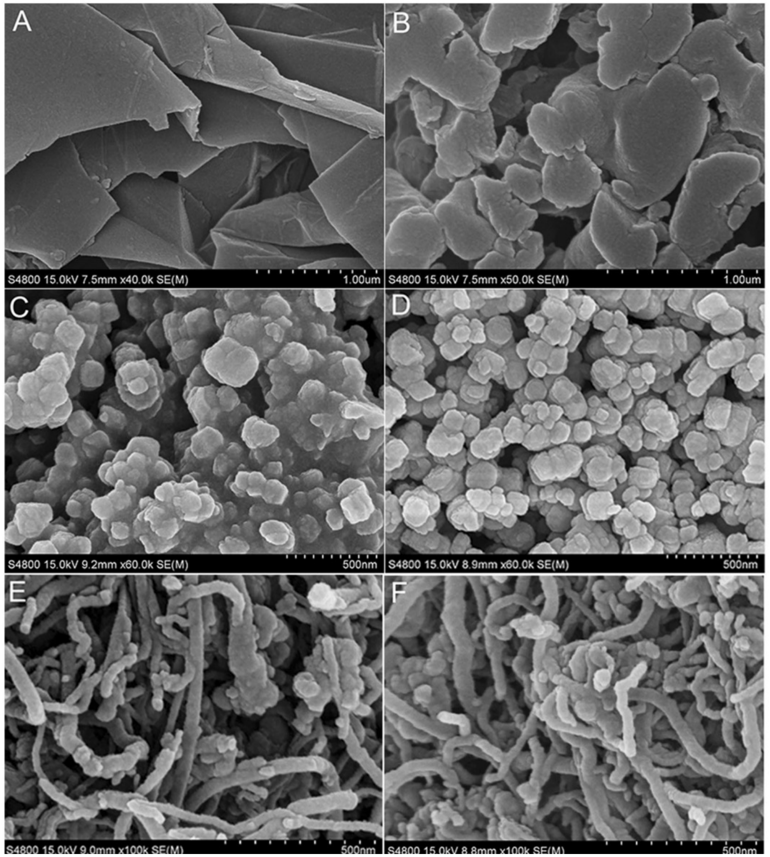


Gong, A, *et al, Scientific Reports* **2017**, *7*, 4309

SEM images:Graphene immobilisation

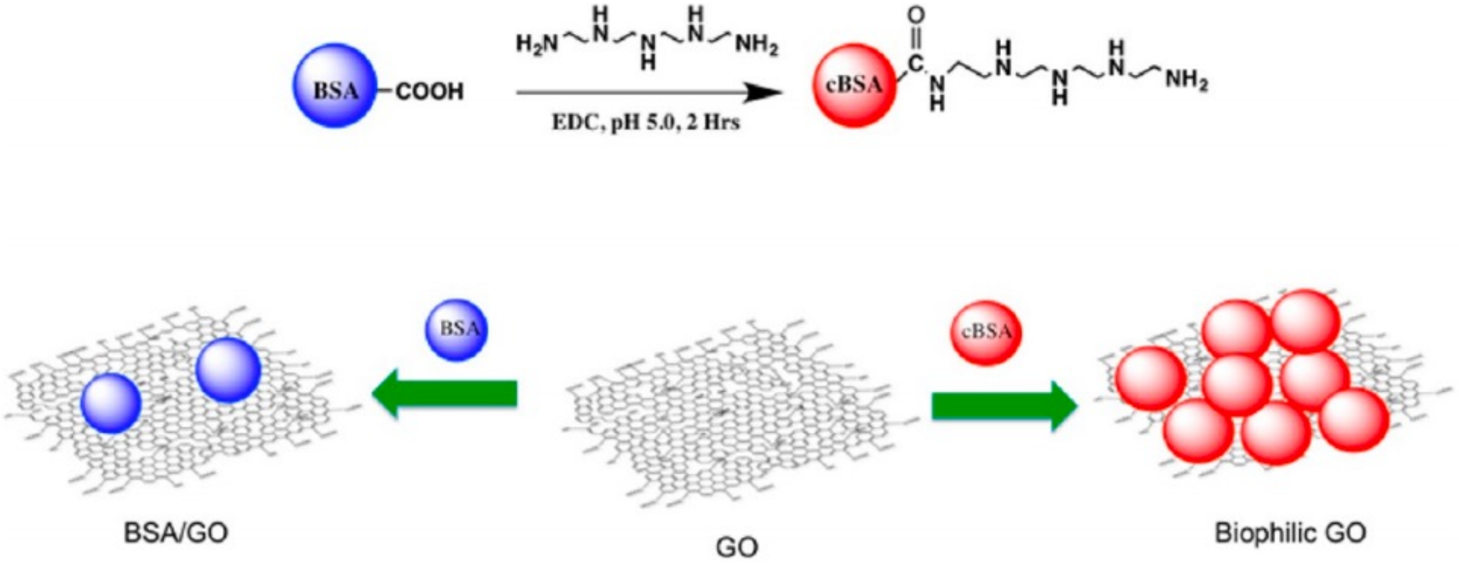
SEM photos of pure graphene (A and B), Fe_2O_3 (C and D) and
carbon nanotube nanoparticle (E and F)
before and after immobilizing.

Reaction condition:
enzyme solution (20 g/L) dissolved by disodium hydrogen phosphate-citrate buffer (pH 7); graphene nanoparticles mass (10mg) added in 2mL of enzyme solution, mixture stirred at 120 rpm in an incubator shaker for 3h, reaction temperatures 50 °C.



Cationisation of Bovine Serum Albumin (BSA)

Reaction by BSA side chains -COOH with tetraethylene-pentamine (TEPA) via carbodiimide coupling.

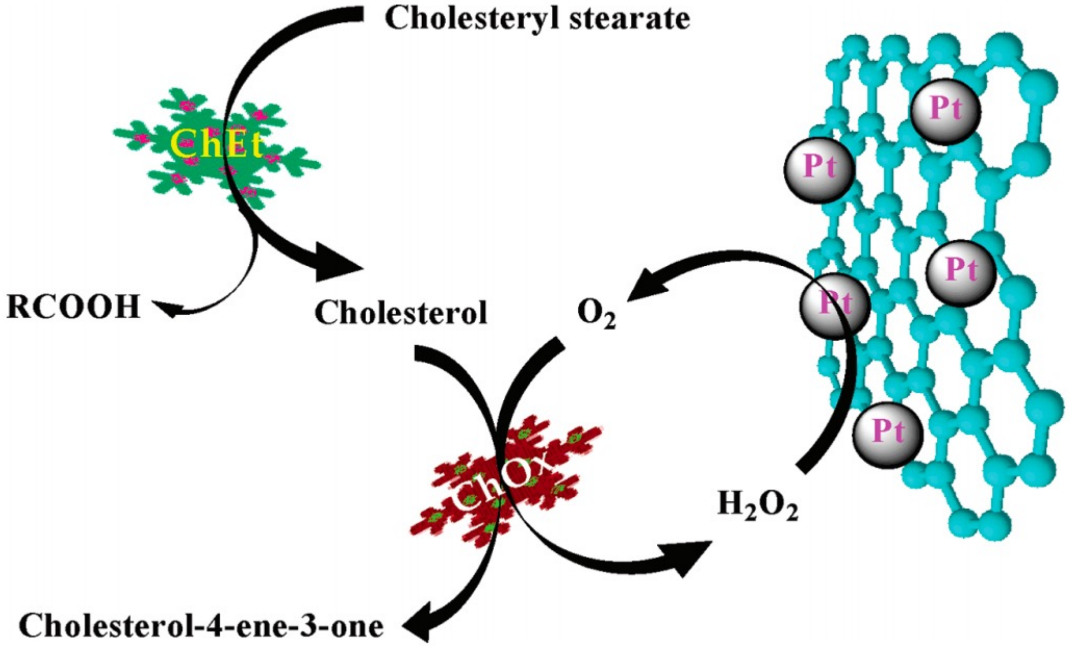


Drawback of physical adsorption: enzyme leakage.

Advantage: Covalent linking of enzyme to nanocatalyst.

Enzyme based biosensors

Biosensing of cholesterol ester with GNS-nPt-based biosensor



The enzyme ChEt hydrolyses the cholesterol ester to cholesterol and ChOx catalyzes the oxidation of cholesterol. The Pt nanoparticles on the surface of GNS can effectively sense the enzymatically generated H_2O_2

Dey, RS and Raj, CR *J. Phys. Chem. C*, **2010**,114 (49)

Graphene based enzymatic bioelectrodes and biofuel cells

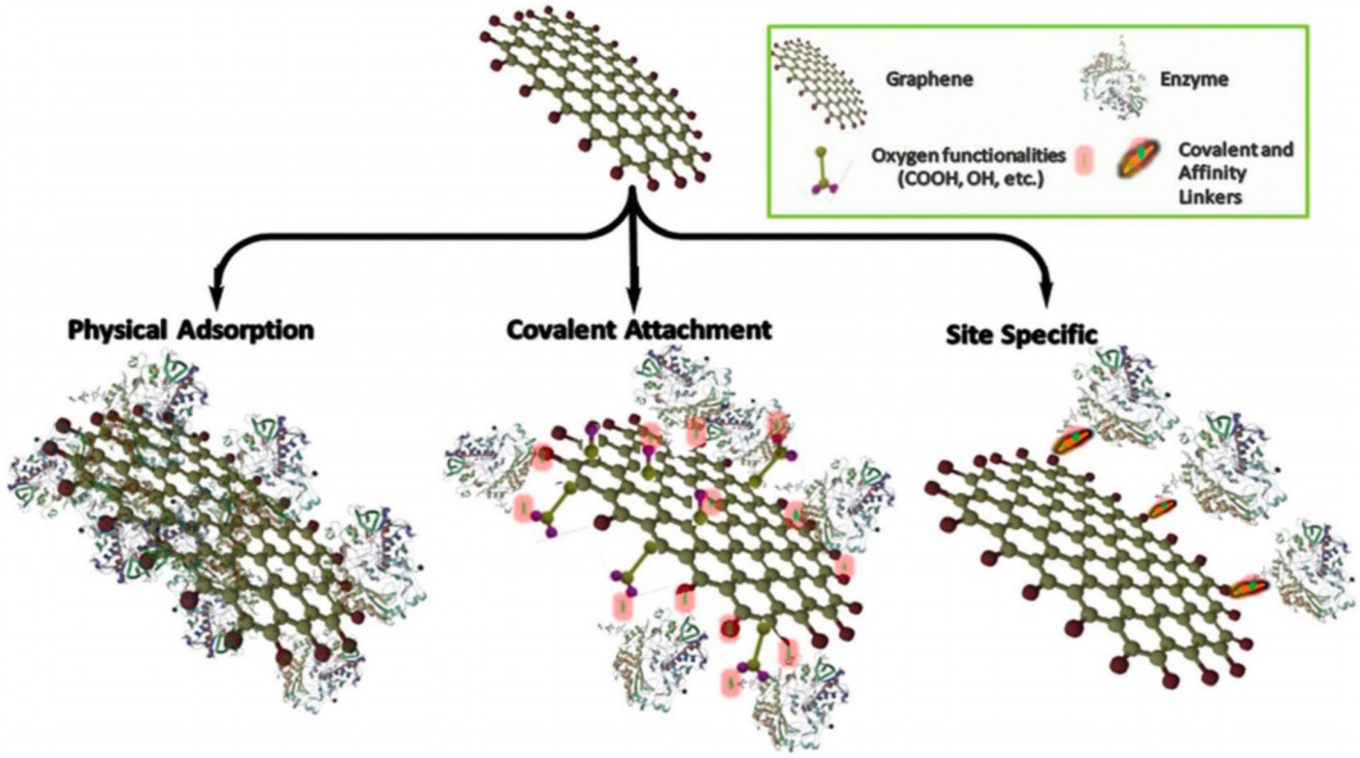


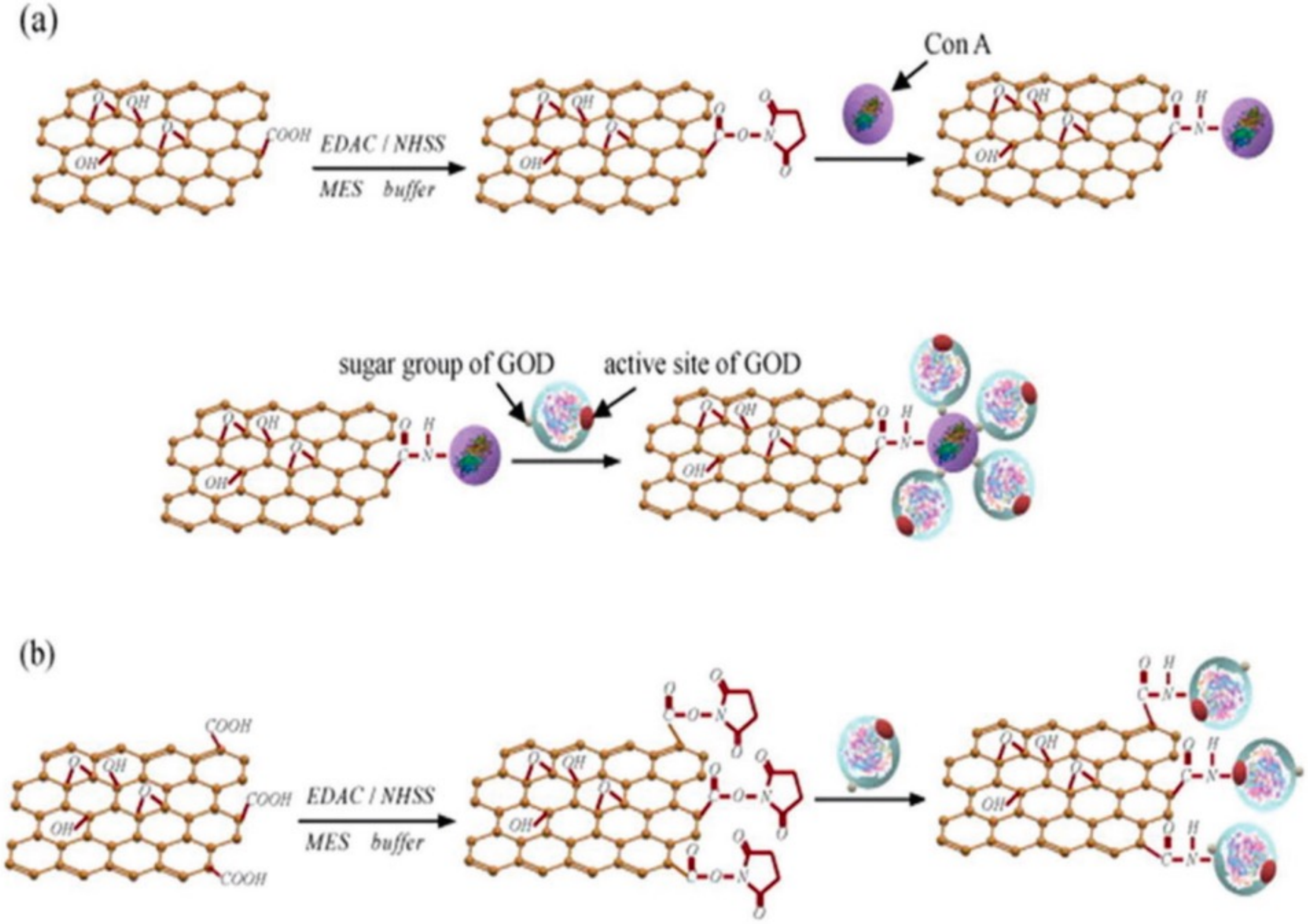
Illustration of enzyme immobilization methods onto graphene

Karimi *et al*, 2015

A: Comparison between site specific oriented (a)

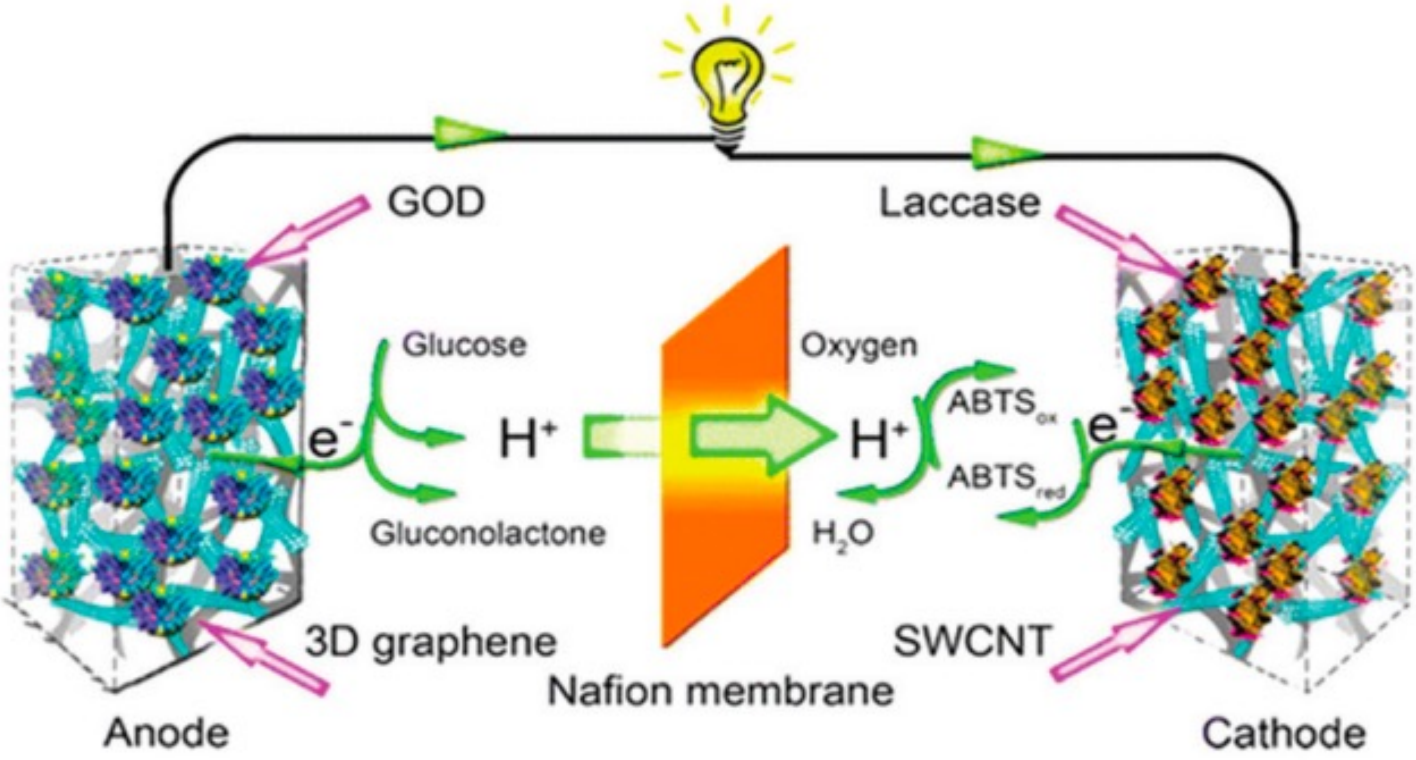
B: and random covalent immobilization of GOx on graphene *via* concanavalin A (Con A)

(GOD refers to GOx in the figure).



Zhou, LY, Jiang, Y J, Gao, J, Zhao, XQ, Ma L and Zhou, Q. L. *Biochem. Eng. J.*, **2012**, *69*, 28-31

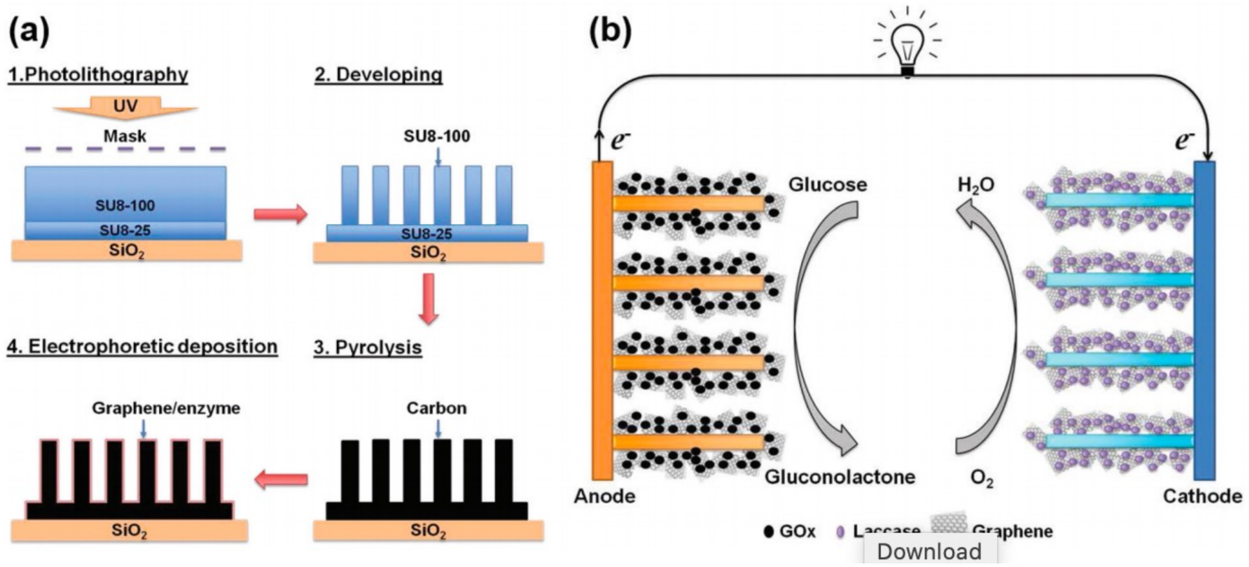
Enzymatic biofuel Cells (EBFC) based on 3D graphene-SWCNT hybrid electrodes.



Prasad, KP, Chen, Y and Chen, P*ACS Appl. Mater. Inter-faces*, **2014**, *6*, 3387-3393.

Carbon microelectromechanical systems C-MEMS

A: Fabrication of EBFC based on C-MEMS micropillar arrays.

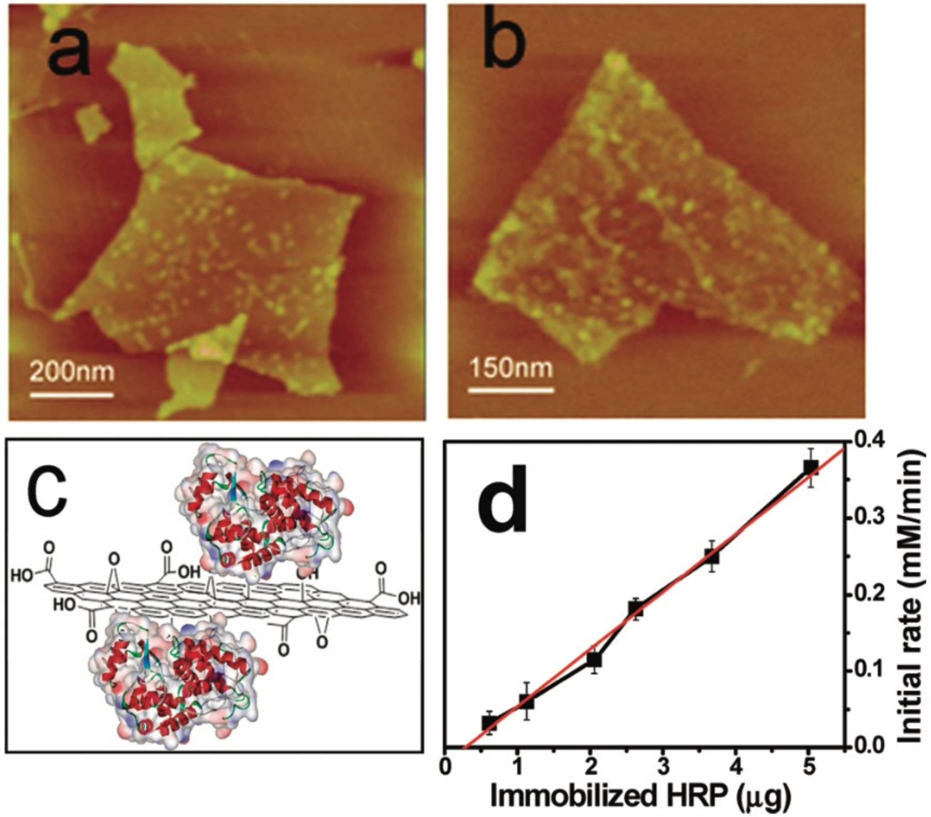


B: Illustration of the EBFC with graphene/enzyme-encrusted 3D carbon micropillar arrays (not to scale).

Song, Y, Chen C and Wang C *Nanoscale*, **2015**, *7*, 7084-7090

7. Immobilization of Biocatalysts onto Nanosupports: Advantages for Green Technologies

Alan S. Campbell, Andrew J. Maloney, Chenbo Dong, and Cerasela Z. Dinu

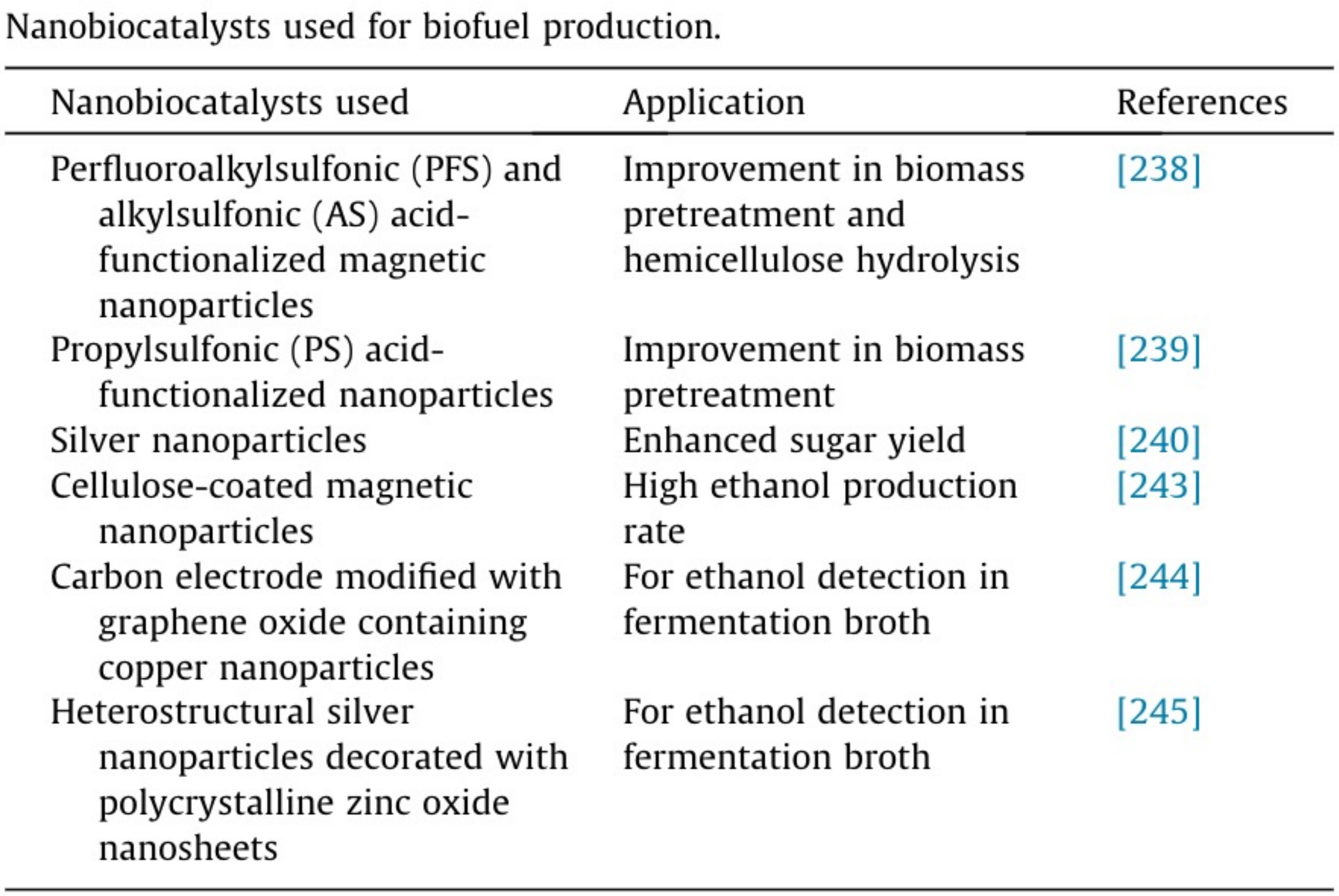


Tapping mode AFM images of the GO-bound HRP with
(a) lower and
(b) higher enzyme loadings acquired in a liquid cell.

(c) Schematic model of the GO-bound HRP.
(d) Initial reaction rates of GO-bound HRP versus HRP concentration.
GO Graphene Oxide

Zhang *et al*, *Langmuir* **2010**, *26* (9), 6083-6085

9. Potential Applications of Nanobiocatalysis for Industrial Biodiesel Production *Avinesh Byreddy and Munish Puri*



Nanobiocatalysts used for biofuel production.

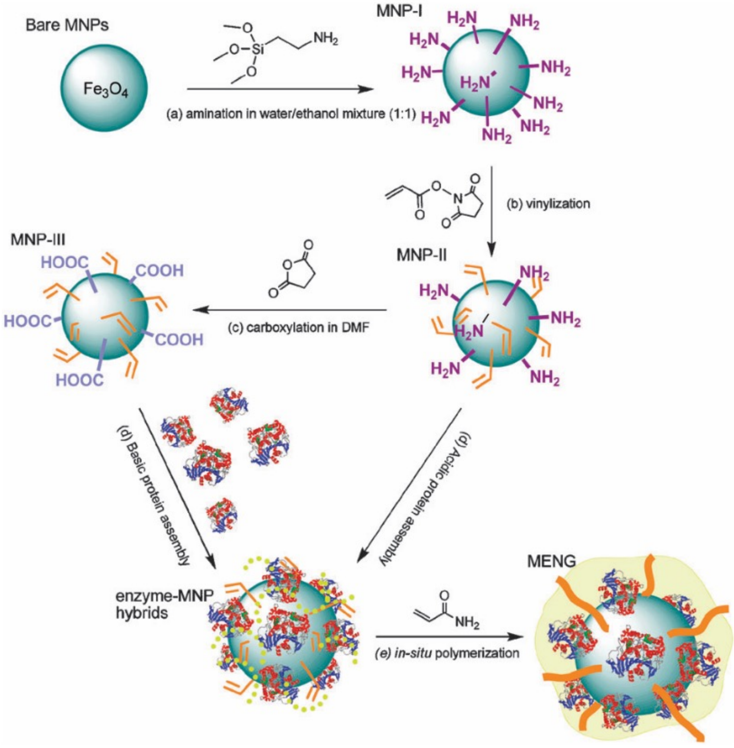
Nanobiocatalysts used	Application	References
Perfluoroalkylsulfonic (PFS) and alkylsulfonic (AS) acid-functionalized magnetic nanoparticles	Improvement in biomass pretreatment and hemicellulose hydrolysis	[238]
Propylsulfonic (PS) acid-functionalized nanoparticles	Improvement in biomass pretreatment	[239]
Silver nanoparticles	Enhanced sugar yield	[240]
Cellulose-coated magnetic nanoparticles	High ethanol production rate	[243]
Carbon electrode modified with graphene oxide containing copper nanoparticles	For ethanol detection in fermentation broth	[244]
Heterostructural silver nanoparticles decorated with polycrystalline zinc oxide nanosheets	For ethanol detection in fermentation broth	[245]

Singh *et al*, 2020

11. Recent Advances in Nanostructured Enzyme Catalysis for Chemical Synthesis in Organic Solvents

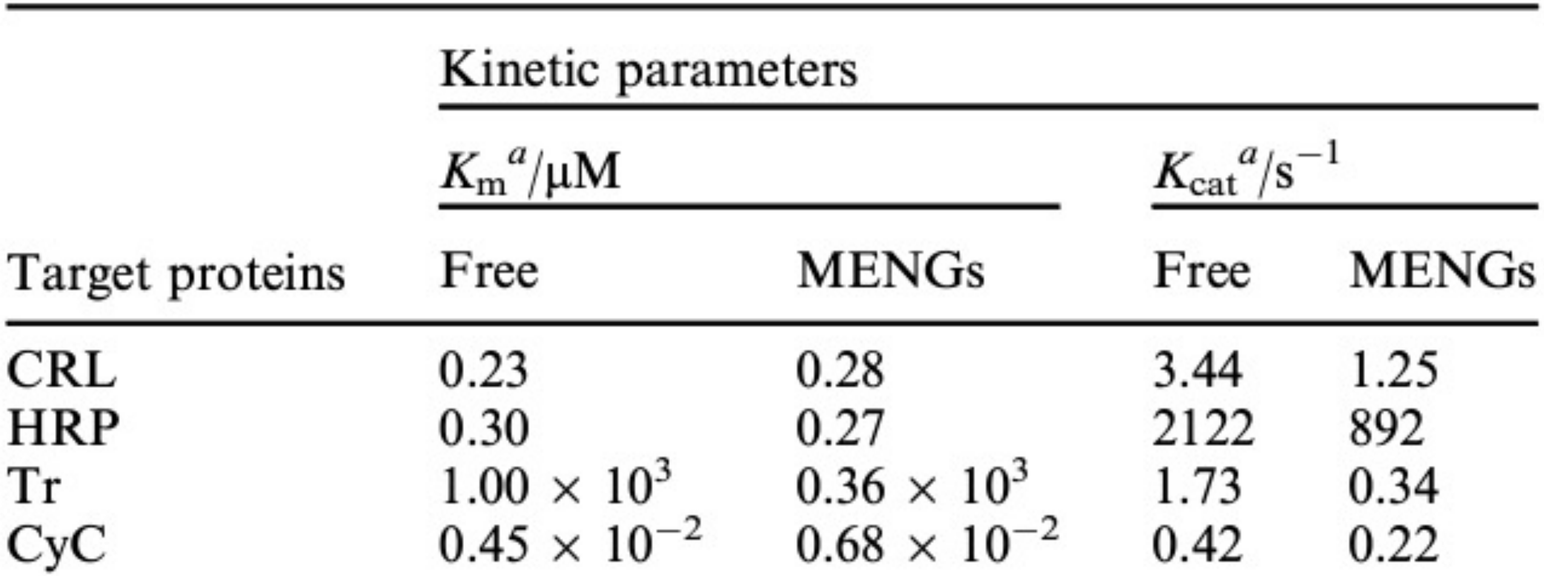
Zheng Liu, Jun Ge, Diannan Lu, Guoqiang Jiang, and Jianzhong Wu

Synthetic route of step-by-step fabrication of magnetic enzyme nanogels (MENG´s)



Lin *et al*, *Chem. Commun.*, **2012**, *48*, 3315-3317

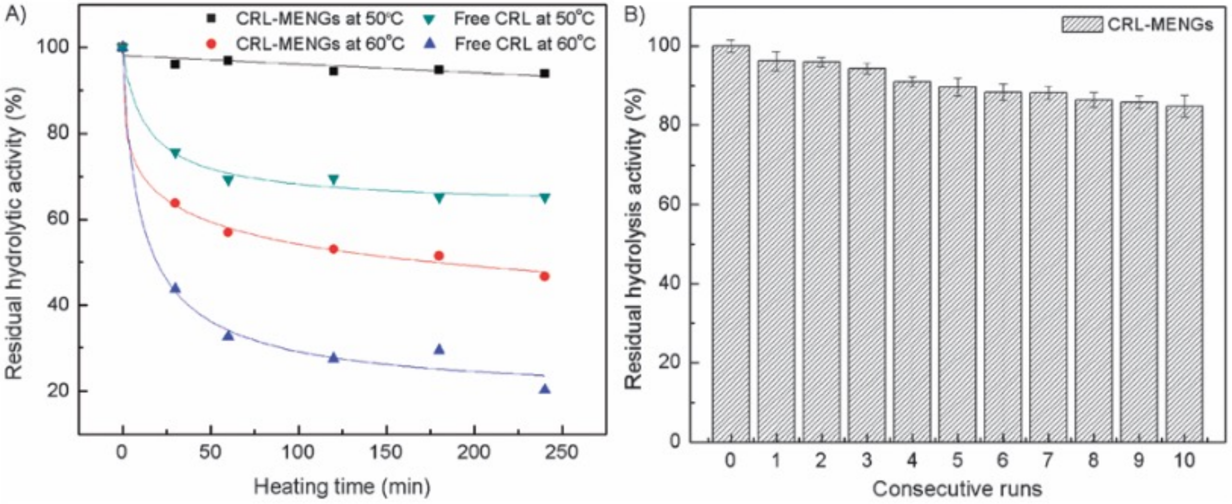
Kinetics parameters, Michaelis constant (K_m) and transformation efficiency (K_{cat}), of free enzymes and MENs



	Kinetic parameters			
	$K_m{}^a/\mu M$		$K_{cat}{}^a/s^{-1}$	
Target proteins	Free	MENGs	Free	MENGs
CRL	0.23	0.28	3.44	1.25
HRP	0.30	0.27	2122	892
Tr	1.00×10^3	0.36×10^3	1.73	0.34
CyC	0.45×10^{-2}	0.68×10^{-2}	0.42	0.22

After encapsulation within the magnetic polyacrylamide nanogel, the Kcat values of the MENGs decreased to 30–35% of the original values determined for their free counterparts. The slight increase in K_m and the decrease in K_{cat} values, except in the case of trypsin-MENGs, may be attributed to spatial hindrance in accessing the active site of the enzyme and additional mass-transport resistance by the polyacrylamide network

Thermal inactivation of enzyme activity Candida rugosa lipase-CRL



(A) Thermal inactivation kinetics of CRL in the free form and MENG´s at 50°C and 60°C

(B) recycling of the CRL-MENG´s in aqueous media, in which CRL-MENG´s were recovered by a bench magnet for 10 consecutive runs.

What’s next for bionanotechnology?

- Judging by the applicant countries of emerging nanotechnologies, we can continue to expect aggressive innovation from the above countries.

- However, whether or not China is planning to enter the fray has yet to be seen. They are certainly far behind in bionanotechnologies. In an area with a death of granted patents, it is crucial for large patenting venues to compare and determine the patentability of nanotechnologies moving forward.

- Bionanotechnology inventions will not only involve emerging methods of drug delivery, medical products, pharmaceuticals, but also the tools with which scientists study and even manufacture items at a nanoscale.

- It is not a question of if, but when, will bionanotechnologies disrupt a long-established industry with billions of dollars on the line.

https://www.ktmine.com/future-bionanotech-told-by-nanotechnology-patents/

DISCUSSION

- What can we as researchers bring to the table of bionanotechnology?
  - Continue the research in all areas
  - Inform the industry about the advantages-10-15 years scope
  - Inform the society about the advantages
  - Industry should look into bionanotechnology based cost effective processes