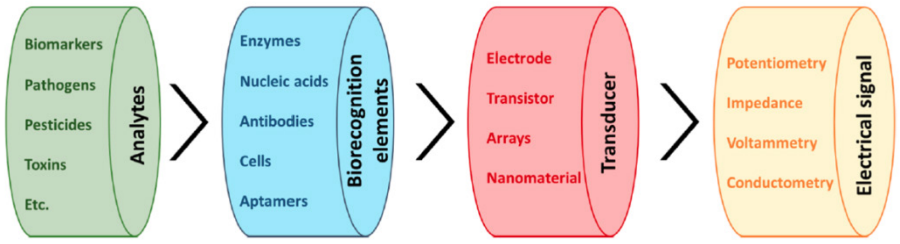
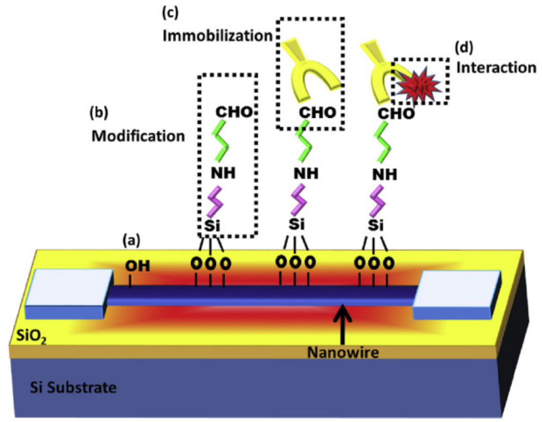
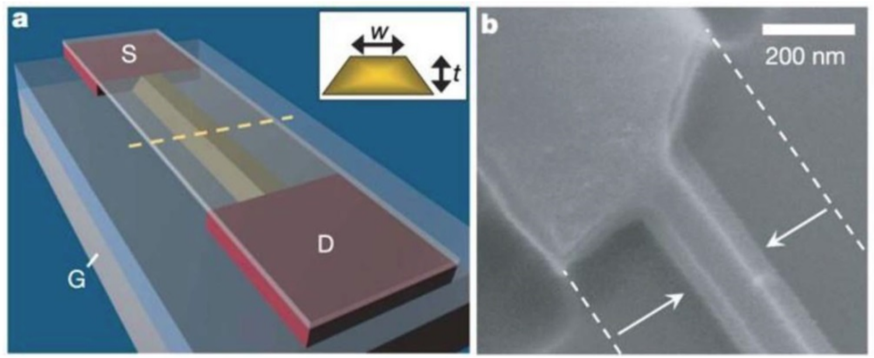
Introduction to BioMEMS & Medical Microdevices

Biosensors

Prof. Steven S. Saliterman, http://saliterman.umn.edu/







Topics

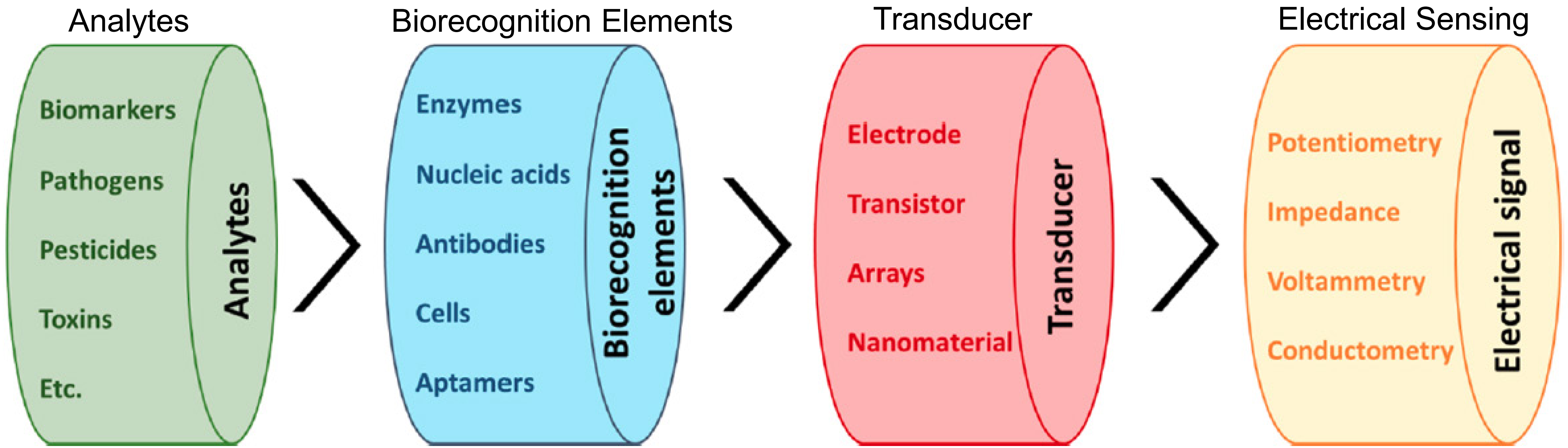
- What are biosensors and what is biosensing?
- Biological recognition elements.
- Transducers
  - Field effect transistors (FETs).
  - Biological and chemical FET transducers.
- Common sensing methods.
- Nanotechnology
  - Nanoparticles
  - Nanomaterials
- Immobilization techniques.
  - APTES-GA (3-aminopropyltriethoxysilane) method.
  - Ab, Enzyme, Biotin/Avidin, Aptamers

Biosensors – Some Definitions

- *"A device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds by electrical, thermal or optical signals."*
- A biological or biologically derived *sensitive recognition element* usually is *immobilized* on a *transducer* to measure one or more *analytes*.

Int. Union of Pure and Applied Chemistry
Syu YC, Hsu WE, Lin CT. Review-Field-Effect Transistor Biosensing: Devices and Clinical Applications. *Ecs Journal of Solid State Science and Technology*. 2018;7(7):Q3196-Q3207.
Luka G, Ahmadi A, Najjaran H, et al. Microfluidics Integrated Biosensors: A Leading Technology towards Lab-on-a-Chip and Sensing Applications. *Sensors*. 2015;15(12):30011-30031.

Biosensing…



Sample preparation – filtration, extraction, purification and enrichment.

Ideally retained in direct contact. The biomolecules change physical quantities, such as charge, mass or photons.

Measurement of current, potential, or field effect, realized through label-free or labeled approaches.

Wongkaew N, Simsek M, Griesche C, Baeumner AJ. Functional Nanomaterials and Nanostructures Enhancing Electrochemical Biosensors and Lab-on-a-Chip Performances: Recent Progress, Applications, and Future Perspective. *Chemical Reviews.* 2019;119(1):120-194.

Uses of Biosensors…

- Commonly combined with microfluidic systems for:
  - High throughput processing,
  - Enhanced transport for controlling the flow conditions,
  - Increased mixing rate of different reagents,
  - Reduced sample and reagent volumes (down to nanoliter), increase sensitivity of detection, and utilizing the same platform for both sample preparation and detection.
  - Portability, disposability, real-time detection, unprecedented accuracies, and simultaneous analysis of different analytes in a single device.

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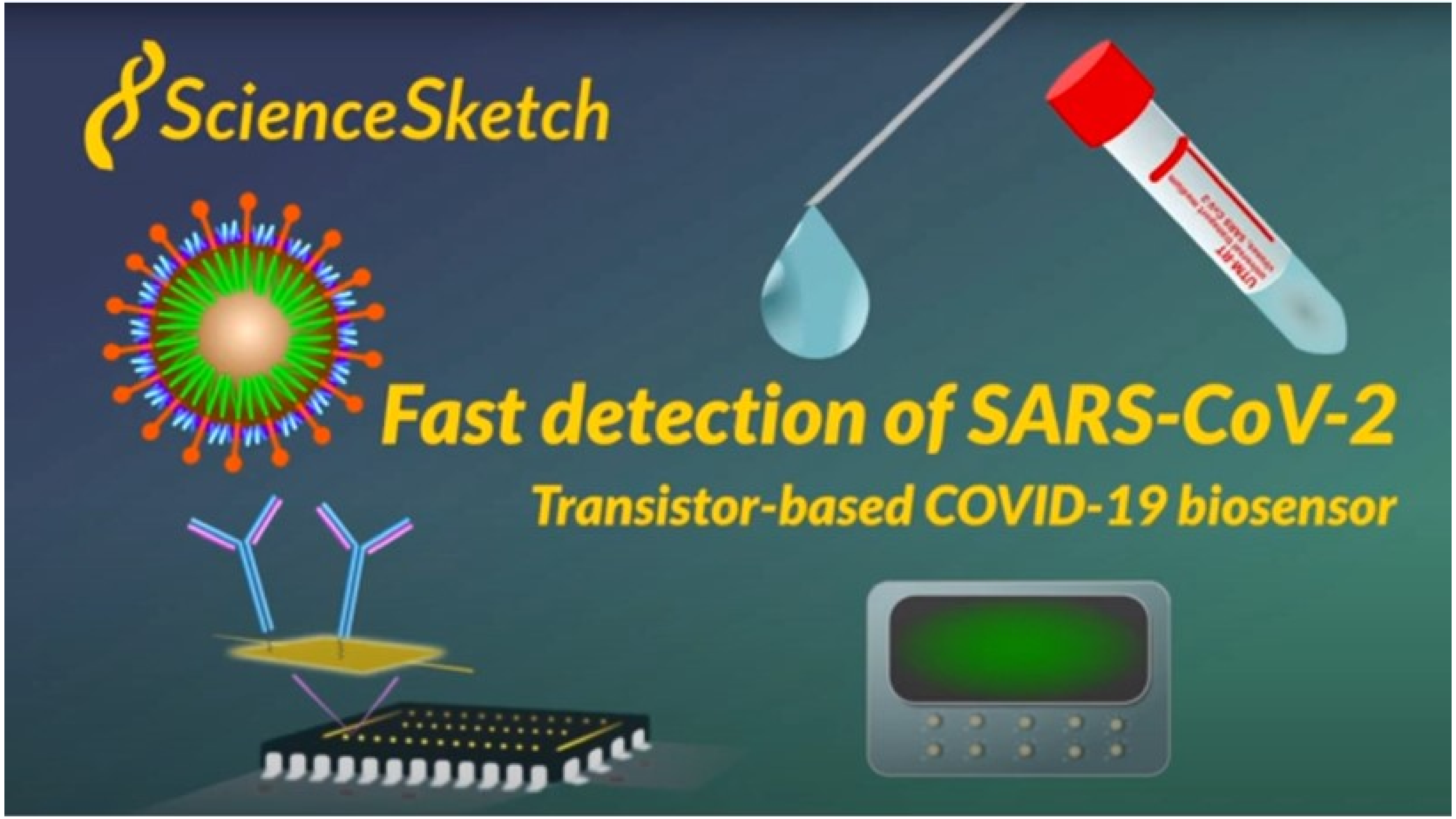
Luka G, Ahmadi A, Najjaran H, et al. Microfluidics Integrated Biosensors: A Leading Technology towards Lab-on-a-Chip and Sensing Applications. *Sensors.* 2015;15(12):30011-30031.

Example: COVID-19 Detection…



https://youtu.be/gmd5gSvZzV8

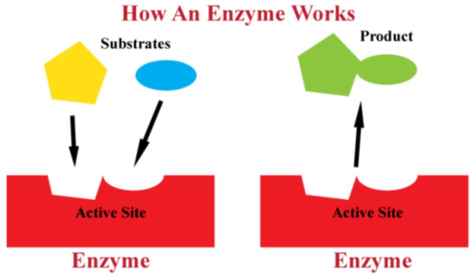
Optimization of Biosensors

- Selectivity – detect the analyte of interest.
- Sensitivity – minimum amount of analyte that can be detected.
- Linearity - better linearity means the higher the substrate concentration detection.
- Response Time – time to obtain 95% of the test result.
- Reproducibility – precision (repeatability) and accuracy (generating a mean value close the actual value).
- Stability – affected by the affinity of the bioreceptor and its degradation over time.

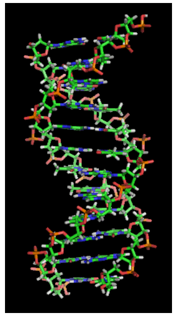
Naresh V, Lee N. A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*. Feb 2021;21(4)1109. doi:10.3390/s21041109

Four Biological Recognition Elements

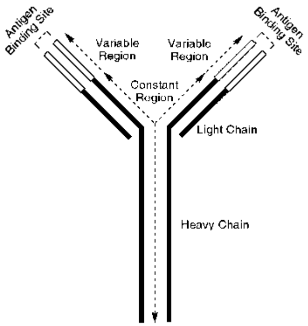
1. Enzymes
   - *Catalyst* for biochemical reactions that act upon *substrate* molecules producing a *product.*

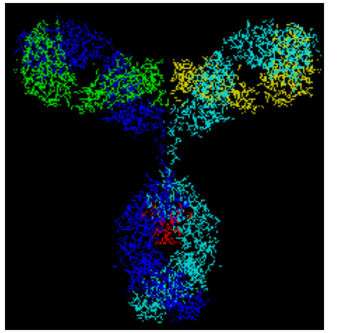


2. Nucleic acids
   - DNA, RNA – composed of nucleotides. Adenine, Thymine, Guanine, Cytosine (DNA) or Uracil (RNA). A-T, G-C or G-U.



3. Antibodies (Immunoglobulins)
   - Proteins produced in response to and *counteracting* a specific *antigen* (e.g., bacteria, virus, or foreign substance). IgG, IgM, IgA, IgE & IgD.





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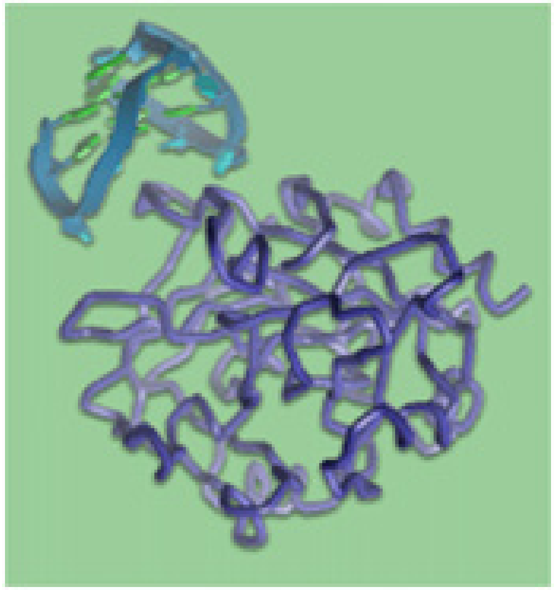
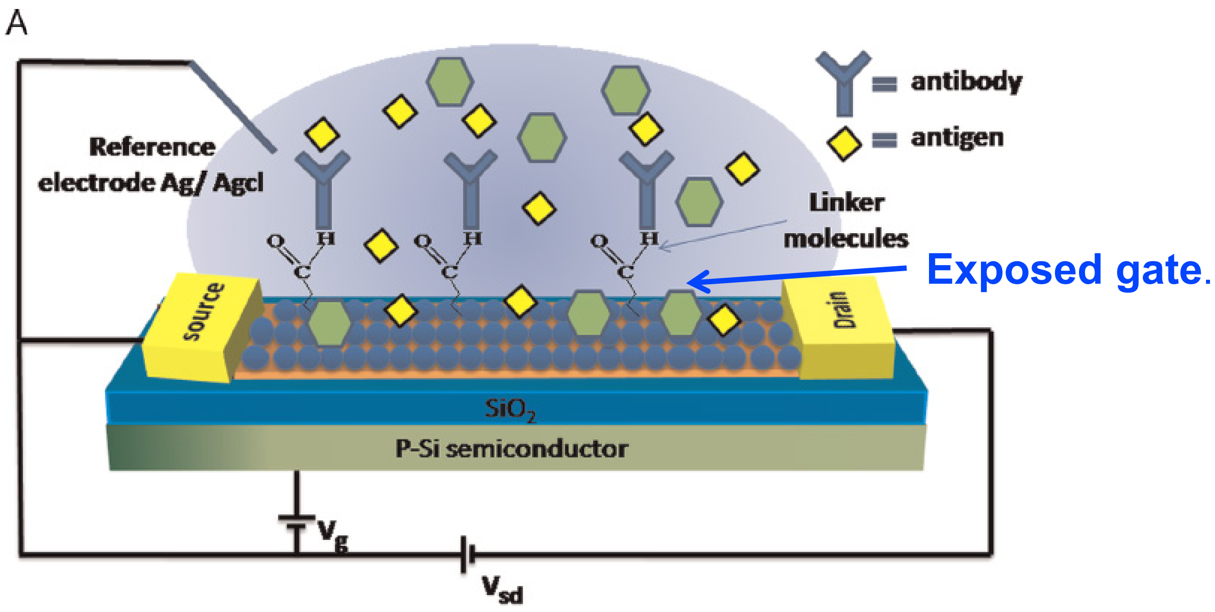
Enzyme illustration courtesy of Wilson, Z CK-12 Foundation
DNA courtesy of Wikimedia.org.
Antibody molecule courtesy of http://www.umass.edu/microbio/rasmol/padlan.htm.
Aptamer image Image courtesy of Archemix.

4. Aptamers

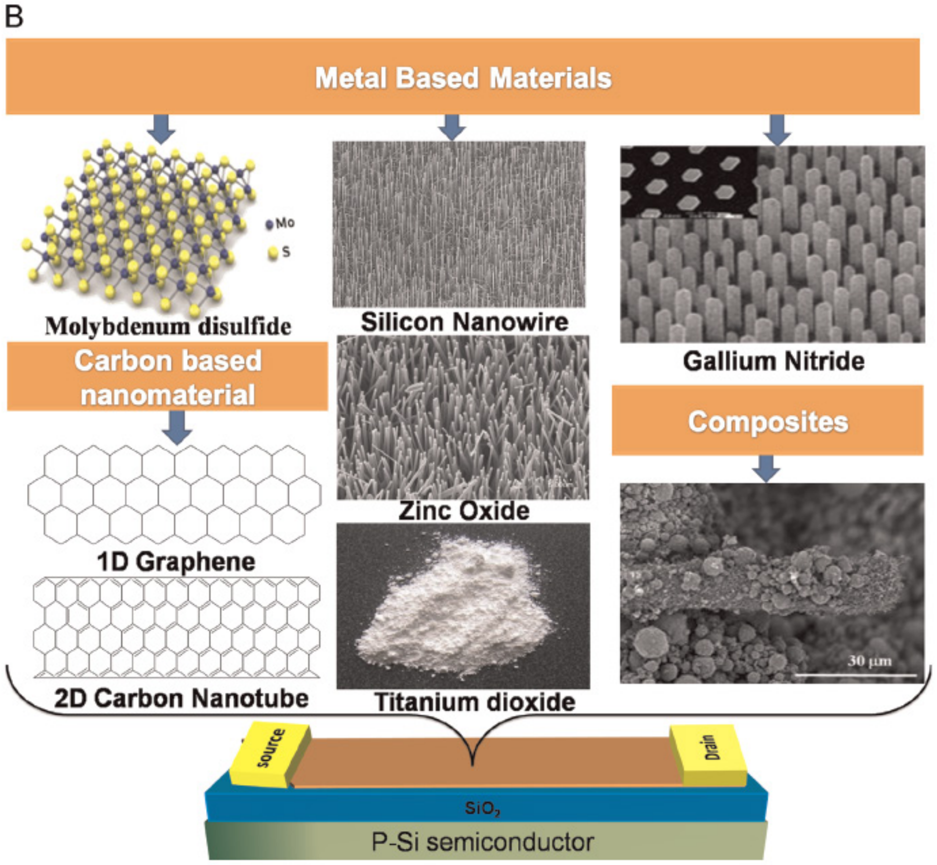
- These are *artificial nucleic acid ligands or peptide molecules* that can be generated against amino acids, drugs, proteins and other molecules. Function similar to antibodies.
- They are single-stranded DNA or RNA (ssDNA or ssRNA) molecules that *to bind to various molecular targets* such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms.
  - They bind because they fit their target, and by non-covalent interactions.
  - *Peptide aptamers* can bind cellular protein targets and exert biological effects, including interference with the normal protein interactions of their targeted molecules with other proteins.



Transducers – e.g., Field Effect Transistor



FET-based biosensor, based on complimentary metal oxide semiconductor (CMOS) technology.

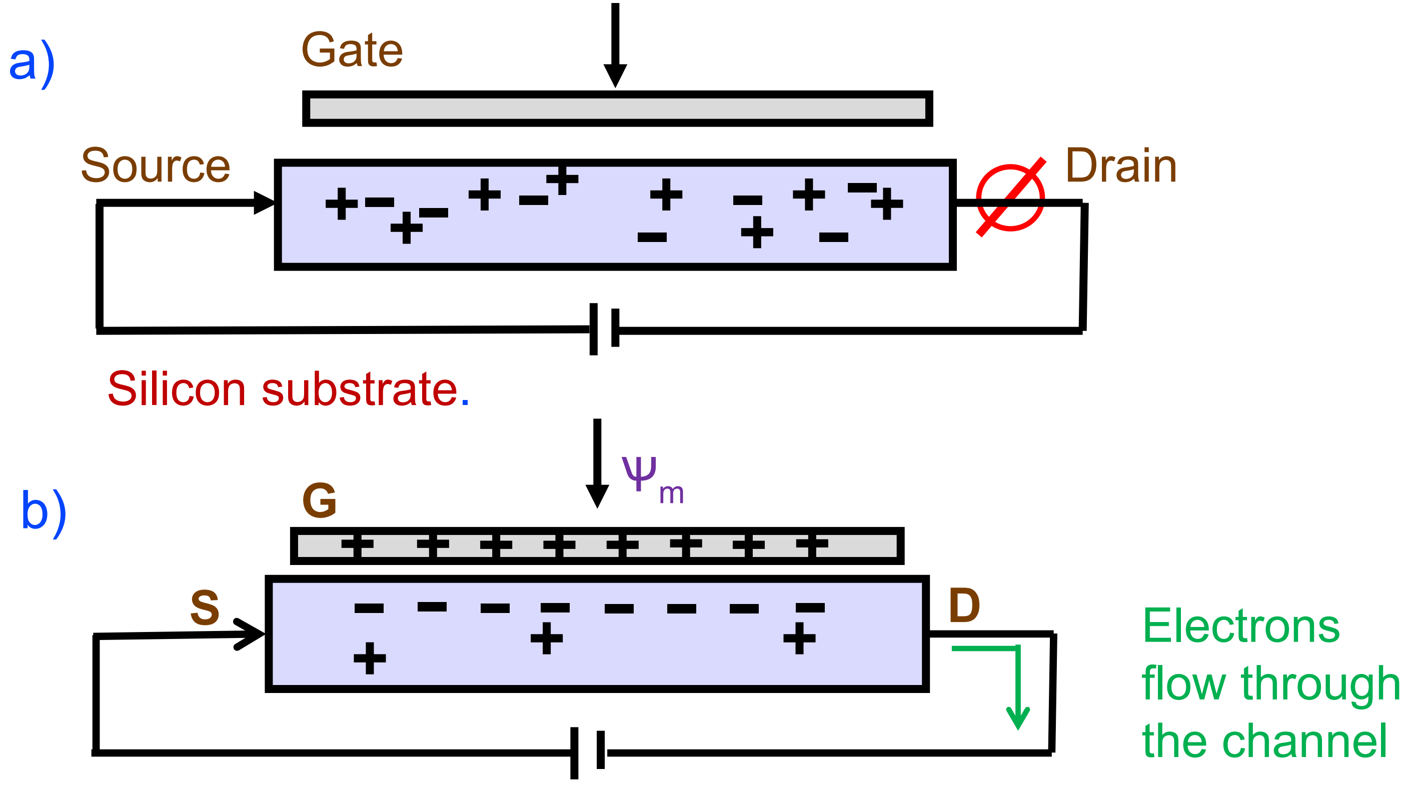


Different nanomaterials embedded onto the gated region of the FET-based biosensor.

Nehra A, Singh KP. Current trends in nanomaterial embedded field effect transistor-based biosensor. *Biosensors & Bioelectronics.* 2015;74:731-743.

FET Conceptually…

- When the gate metal potential (Ψ_m- psi) is changed, the electric field induces the "band bending" of the semiconductor channel accordingly.
- This results in channel carrier concentration changes, such as *inversion, depletion or accumulation (negative gate).*
- The gate potential can be given by other factors such as pH, ions or charge of biomolecules.



FET biosensors are typically "N-channel" – positive charge on gate attracts electrons, increasing conductance from source to drain.

Syu YC, Hsu WE, Lin CT. Review-Field-Effect Transistor Biosensing: Devices and Clinical Applications. *Ecs Journal of Solid State Science and Technology.* 2018;7(7):Q3196-Q3207

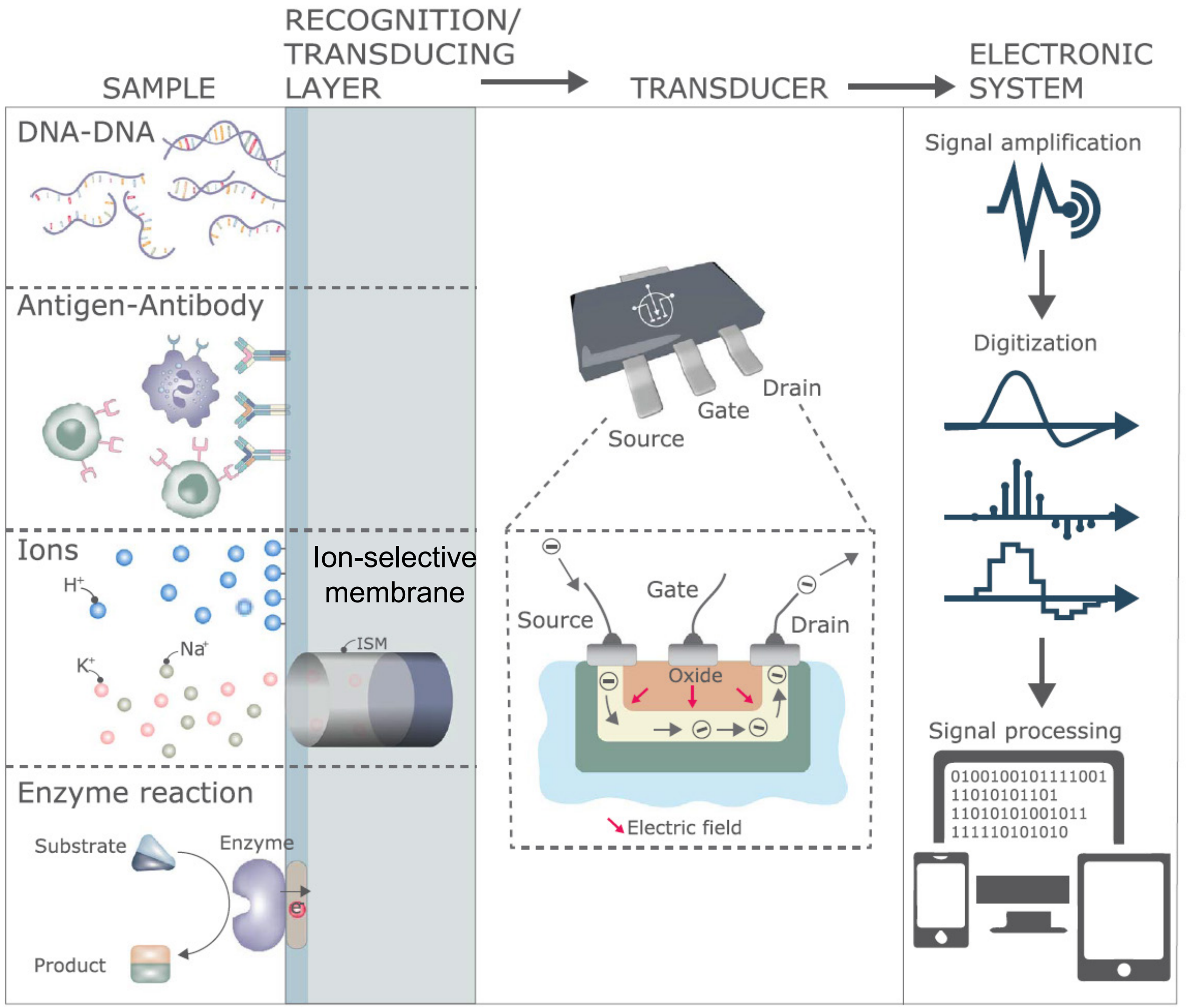
Types & Advantages of FET

- Types:
  - Ion selective FET (ISFET) – conventional and double gate.
  - Silicon nanowire biosensors.
  - Organic FET and graphene FET biosensors.
- Miniature, ultra sensitive and fast response time.
- Respond to electrostatic charges *and* potential changes.
  - Detection of nucleotides, amino acids, cells (e.g. bacterial and viruses).
- Arrays may allow parallel processing.
- Suitable for integration with other electronics.
- Excellent for point-of-service devices of the future.

Syu YC, Hsu WE, Lin CT. Review-Field-Effect Transistor Biosensing: Devices and Clinical Applications. *Ecs Journal of Solid State Science and Technology.* 2018;7(7):Q3196-Q3207

Biological & Chemical FET Sensor…

- The conventional ion-selective FET (ISFET) is comprised of a MOSFET *with the metal gate replaced by a dielectric layer as a sensing membrane.*
- This dielectric is normally silicon dioxide.



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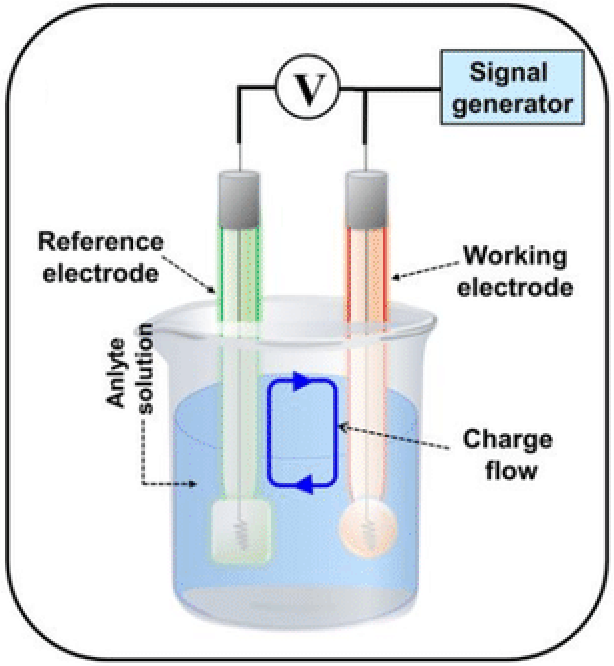
Kaisti M. Detection principles of biological and chemical FET sensors. *Biosensors & Bioelectronics.* 2017;98:437-448.

Electrochemical Detection – Several Examples

1) Potentiometry

- A potential difference between two half-cells with negligible current flowing. The cathode is the *indicator* and the anode the *reference electrode.*
  - The most prominent potentiometric sensors are *ion-selective electrodes* in which a membrane provides for the ion-selective response.
  - In most cases potentiometric sensors are chemosensors; however, when combined with a bioselective separation process they can also be assembled to be full biosensors.
  - In FETs the same principle is being applied through the measurement of ions present in the *gate electrode* area of the FET.



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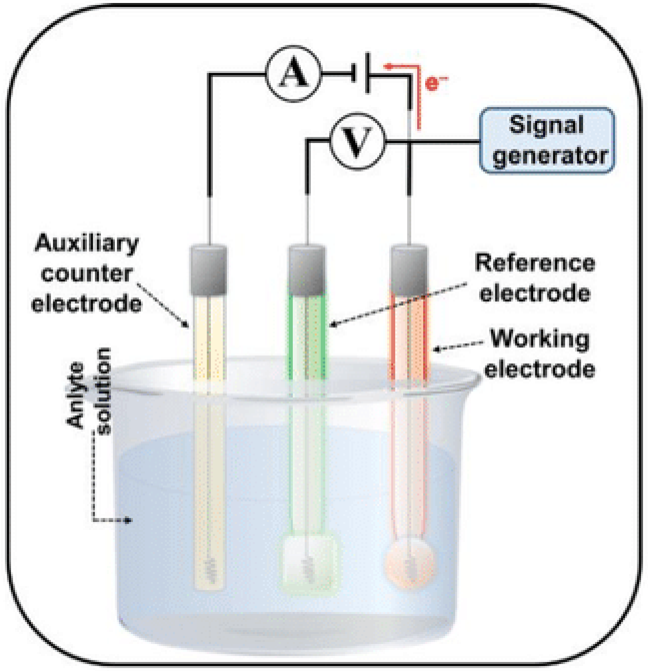
Curulli, A. Electrochemical Biosensors in Food Safety: Challenges and Perspectives, Molecules 26(10:2940 , 2021.

2) Voltammetry & Amperometry

- Application of a potential between a working electrode (WE) and reference electrode (RE). A current is flowing and measured between a counter electrode (CE) and the WE as a result of *reduction/oxidation* processes at the surface of the electrodes.
- In voltammetry, the applied potential to the electrode is varied over time to generate a current response.
- In amperometry, a constant potential is applied and the resulting steady-state current is measured, allowing for direct concentration determination of a specific analyte.



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Curulli, A. Electrochemical Biosensors in Food Safety: Challenges and Perspectives, Molecules 26(10:2940 , 2021.

3) Electrochemical Impedance Spectroscopy (EIS)

- Technique commonly used for investigating properties of nanomaterials.
- Real-time analysis
- Can be used to detect even the slightest change that occurs at the solution-electrode interface arising e.g., from virus activity.
- The EIS method is used to describe biosensors and binding events on *modified electrode surfaces*, such as antigen antibodies and DNA synthesis, as well to measure small molecules by monitoring changes in charge transfer impedance.
- See the Appendix for an example.

Wongkaew N, Simsek M, Griesche C, Baeumner AJ. Functional Nanomaterials and Nanostructures Enhancing Electrochemical Biosensors and Lab-on-a-Chip Performances: Recent Progress, Applications, and Future Perspective. *Chemical Reviews.* 2019;119(1):120-194.
Bigdeli IK, Yeganeh M, Shoushtari MT, Zadeh MK. Chapter 23 - Electrochemical impedance spectroscopy (EIS) for biosensing. In: Thomas S, Nguyen TA, Ahmadi M, Farmani A, Yasin G, eds. *Nanosensors for Smart Manufacturing*. Elsevier; 2021:533-554.

4) Conductometry

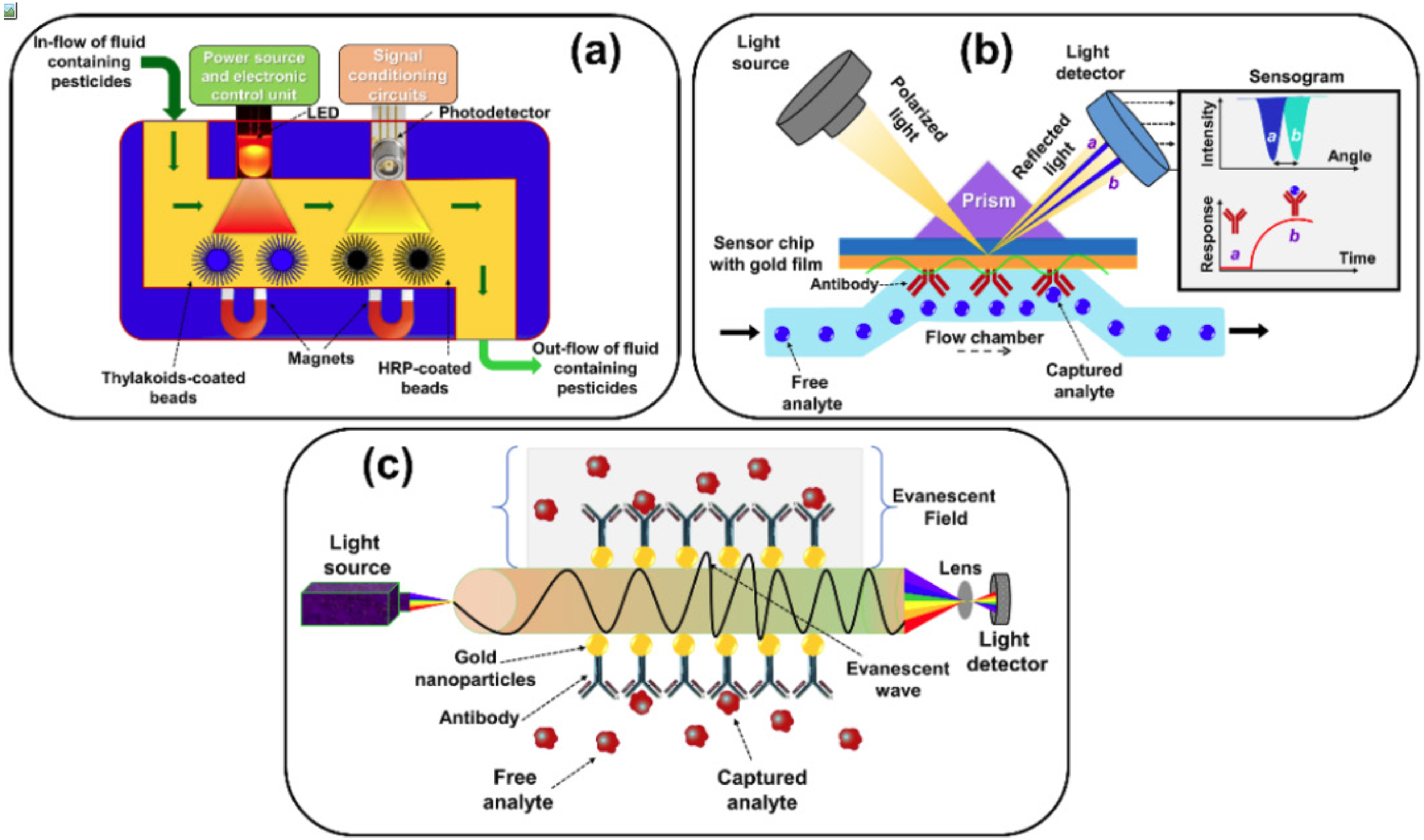
- Conductance is the inverse value of resistance measured in dc mode.
- The resulting sensors are often referred to as chemiresistors and typically serve to measure conductivity changes within the bulk of an electrochemical cell, for gas sensing or enzyme-based strategies.

Other Sensing Method Examples…

a) Chemiluminescence biosensor.

b) Surface plasmon resonance (SPR) biosensor.

c) Evanescent wave-based optical fiber biosensor.

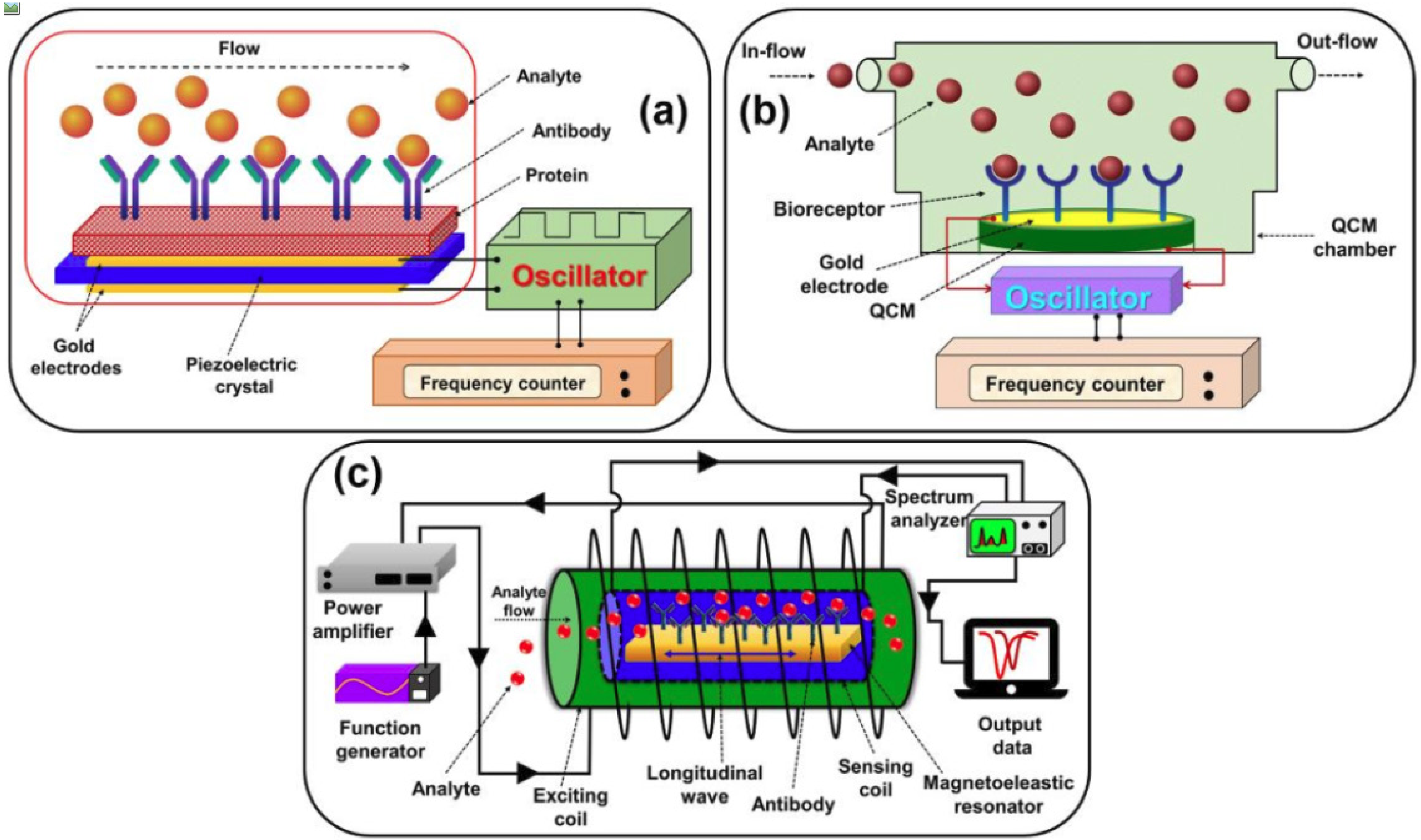


Naresh V, Lee N. A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*. Feb 2021;21(4)1109. doi:10.3390/s21041109

a) Piezoelectric-based biosensor.

b) Quartz crystal microbalance-based biosensor.

c) Magnetoelastic-based biosensor.

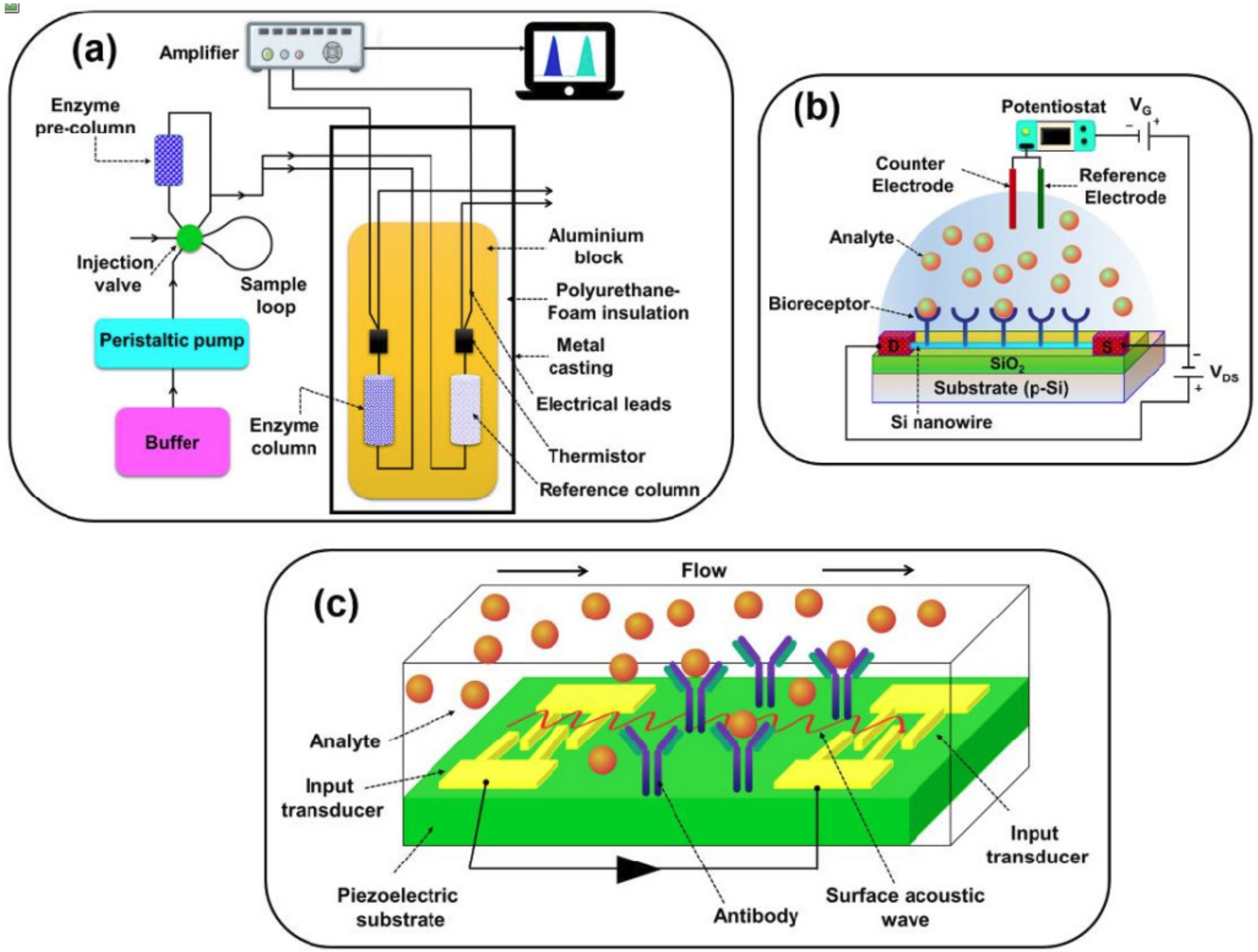


Naresh V, Lee N. A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*. Feb 2021;21(4)1109. doi:10.3390/s21041109

a) Enzyme thermistor-based biosensor.

b) Si nanowire-based field-effect transistor (FET) (D is drain and S is the source) biosensor. *(More later.)*

c) Surface acoustic wave (SAW)-based biosensor.



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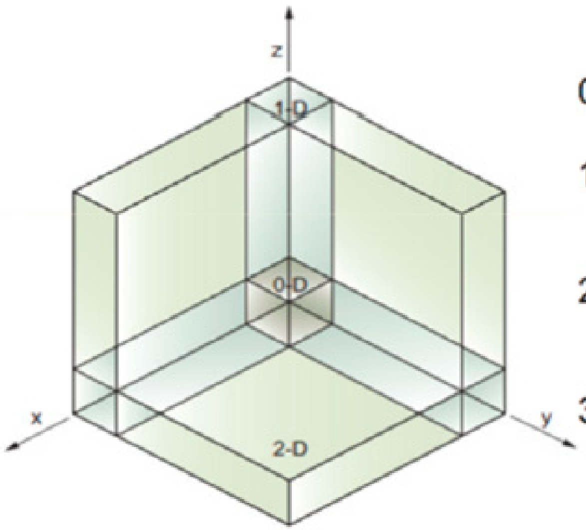
Nanotechnology

Classification of Nanomaterial *Dimensions*

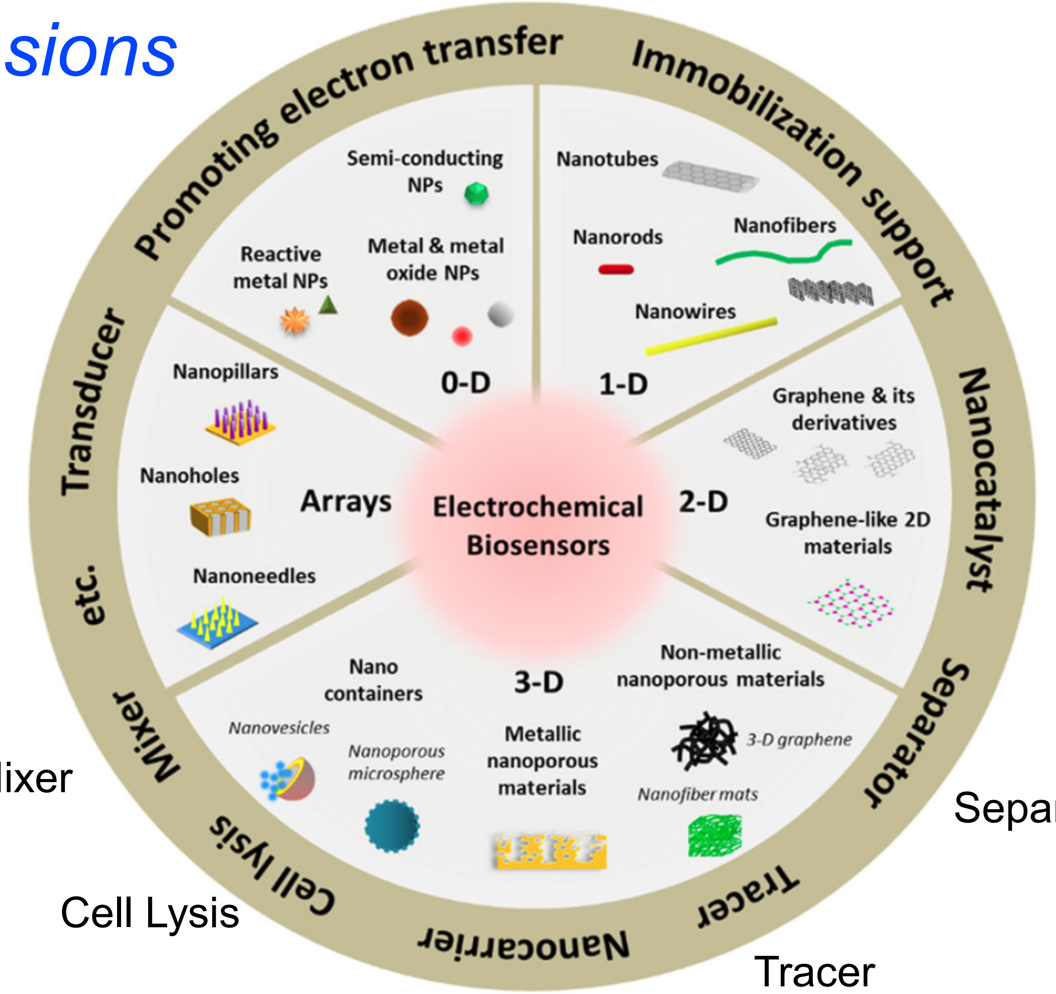


0-D: All dimensions at the nanoscale

1-D: Two dimensions at the nanoscale, one dimension at the macroscale

2-D: One dimension at the nanoscale, two dimensions at the macroscale

3-D: No dimensions at the nanoscale, all dimensions at the macroscale



Mixer

Cell Lysis

Nanocarrier

Tracer

Separator

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Schodek, D.; Ferreira, P.; Ashby, M. Nanomaterials, Nano-technologies and Design; Butterworth-Heinemann: 2009.

"Enzyme-Like" Activity of Nanoparticles…

- Nanomaterials like gold nanoparticles (AuNPs), Fe_3O_4, Pd, NiO, TiO_2 have *intrinsic enzyme-like activity*.
- Metals and metal oxides are well-known catalysts driving many catalytic reactions.
- They are also widely used in electrochemical biosensors enabling *nonenzymatic detection* of metabolites such as sugars and reactive oxygen species - enabling catalyst-enhanced signal amplification.

Nanoparticle fluid dispersions in the University of Minnesota Nano-Bio Lab.

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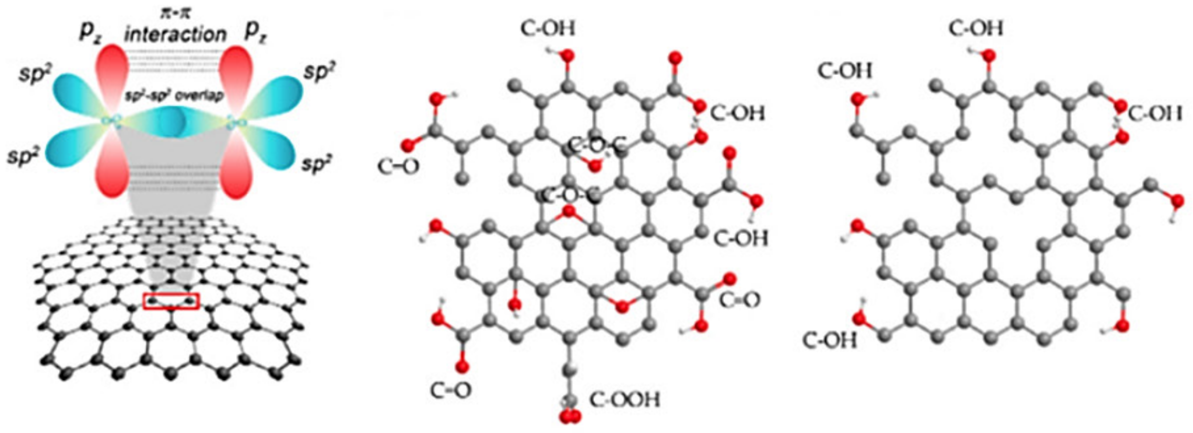
Wongkaew N, Simsek M, Griesche C, Baeumner AJ. Functional Nanomaterials and Nanostructures Enhancing Electrochemical Biosensors and Lab-on-a-Chip Performances: Recent Progress, Applications, and Future Perspective. *Chemical Reviews.* 2019;119(1):120-194.

Carbon Nanomaterials…

- Graphene, carbon nanotubes (CNTs), and carbon nanofibers (CNFs), have been extensively studied and applied in electrochemical sensors.

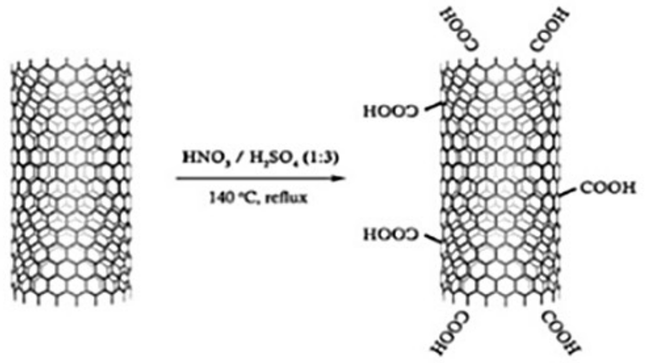


Graphene

Graphene oxide

Reduced graphene oxide

(1)



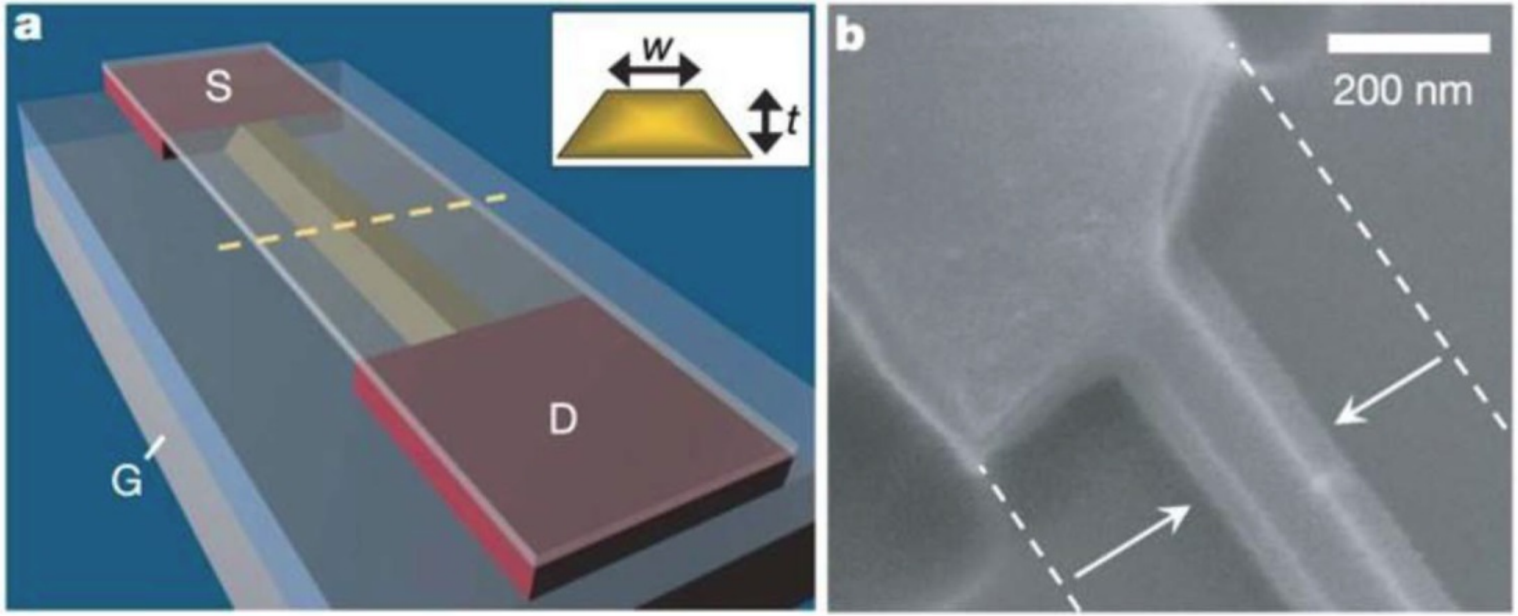
(2)

Carbon nanotube

Acid-treated carbon nanotube

Suvarnaphaet, P.; Pechprasarn, S. Graphene-Based Materials for Biosensors: A Review. Sensors 2017, 17, 2161. Bikiaris, D.; Vassiliou, A.; Chrissafis, K.; Paraskevopoulos, K. M.; Jannakoudakis, A.; Docoslis, A. Effect of Acid Treated Multi-Walled Carbon Nanotubes on the Mechanical, Permeability, Thermal Properties and Thermo-Oxidative Stability of Isotactic Polypropylene. Polym. Degrad. Stab. 2008, 93, 952−967.

Silicon Nanowire Biosensor…



a) An illustration of silicon nanowire; a wire-like channel connects to source and drain electrodes.

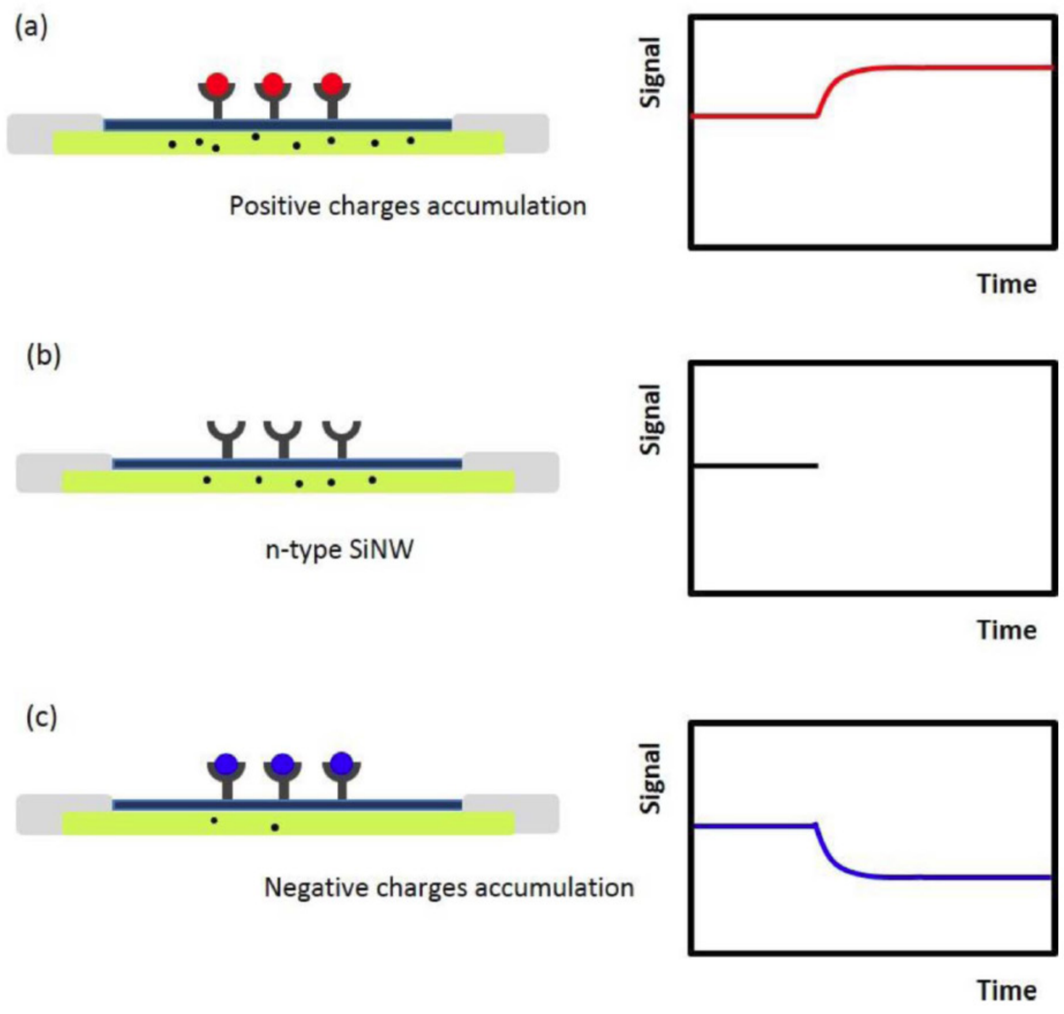
b) The SEM image of a silicon nanowire.

Eric Stern, James F. Klemic, David A. Routenberg, Pauline N. Wyrembak, Daniel B. Turner-Evans, Andrew D. Hamilton, David A. LaVan, Tarek M. Fahmy, and Mark A. Reed, Nature, 445, 519 (2007).
Syu YC, Hsu WE, Lin CT. Review-Field-Effect Transistor Biosensing: Devices and Clinical Applications. *Ecs Journal of Solid State Science and Technology.* 2018;7(7):Q3196-Q3207

a) Positive charges accumulate on the surface. The electrostatic attraction force to electron carriers results in higher conductance.

b) The original state of SiNW.

c) Negative charges accumulate on the surface. The electrostatic repulsion force to electron carriers results in lower conductance.



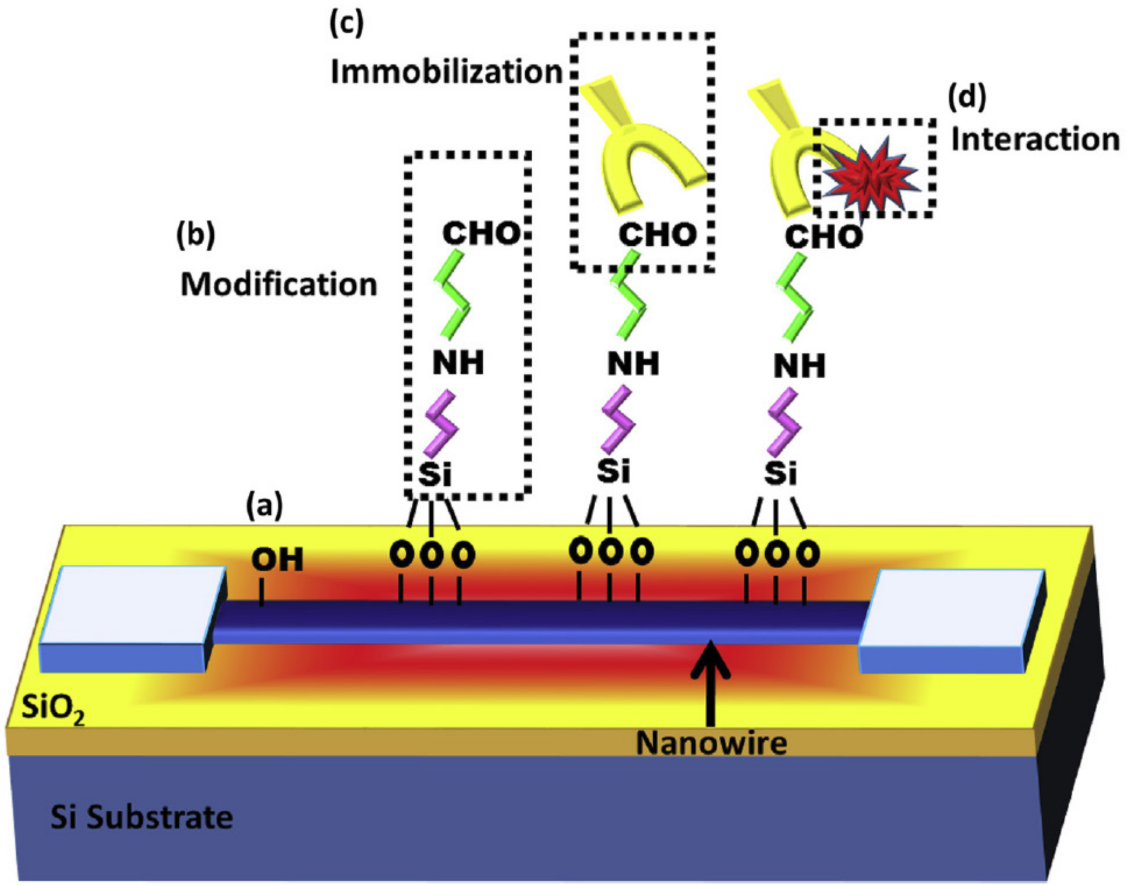
Biomolecule Detection…

a) Attachment of hydroxide groups on the surface.

b) Surface modification of the first linker from the silane group and the second linker from the carboxyl group.

c) Antibody immobilization.

d) Binding of antigen. Measurements occur between the source and drain.



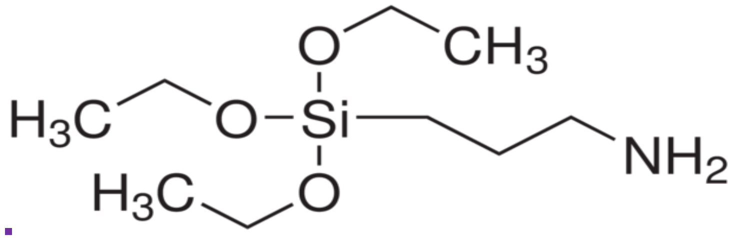
Adzhri R, Arshad KM, Gopinath SCB, et al. High-performance integrated field-effect transistor-based sensors. *Analytica Chimica Acta.* 2016;917:1-18.

Immobilization Methods

- The functionalization process required more complex process than immobilizing the probes only.
  - This is because the functional groups of biomolecules need cross-linkers to form covalent bonds with the sensing film (dielectric layer) of FET-based biosensors.
- The most commonly used cross-linking process for ISFET and SiNW (i.e., the oxide-based sensing dielectric) is the **APTES-GA** method. (**3-Aminopropyl-tri-ethoxysilane**).

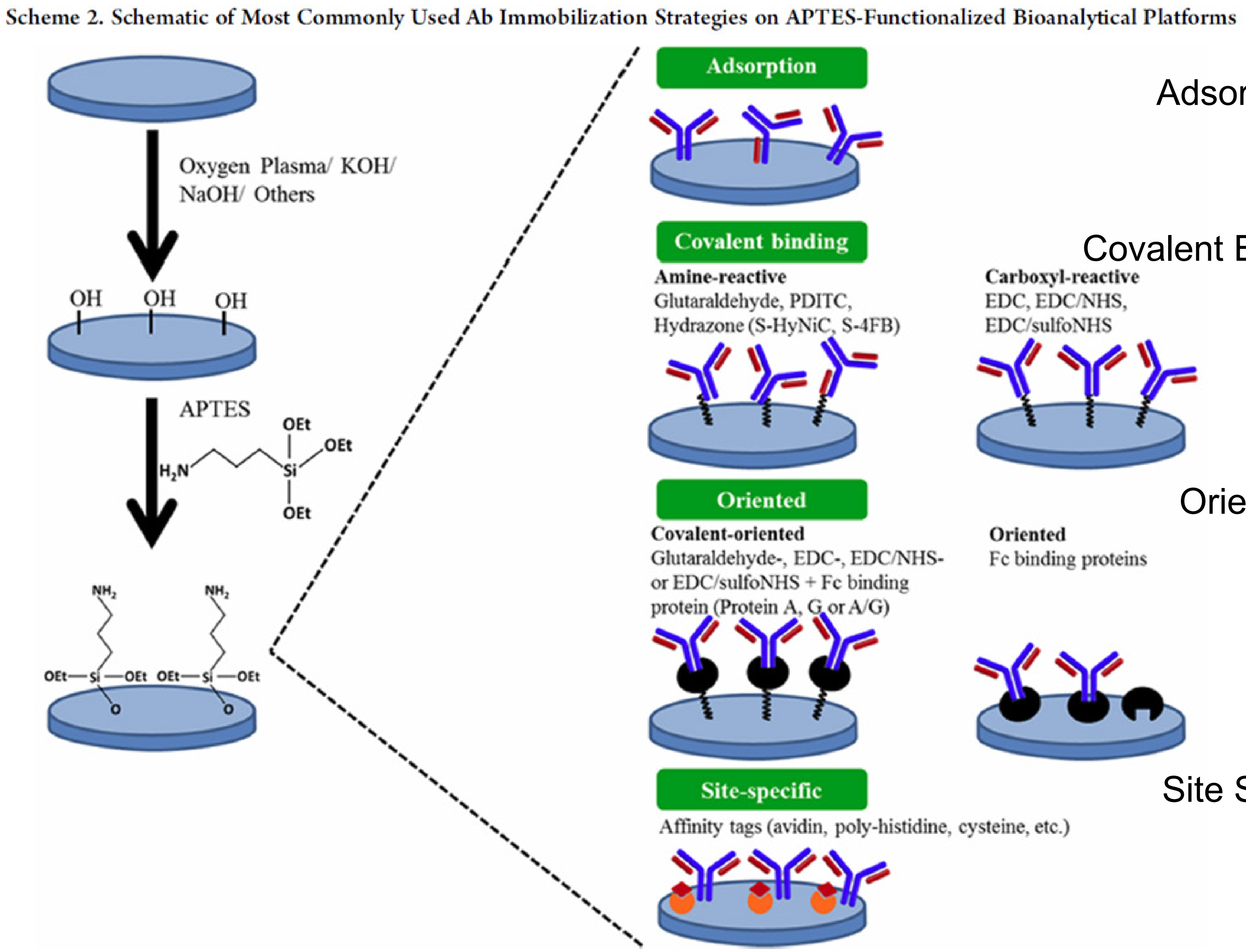
Syu YC, Hsu WE, Lin CT. Review-Field-Effect Transistor Biosensing: Devices and Clinical Applications. *Ecs Journal of Solid State Science and Technology.* 2018;7(7):Q3196-Q3207

APTES



- *APTES is a silane molecule*,
  - Bonds with sensor dielectric surface, such as SiO_2.
  - It can be deposited on solid materials, electrode materials, nanomaterials, and nanocomposites.
- This self-assembled organic monolayer forms on the sensor surface and provides a good platform for the second stage linkers by its amine group in the other end.
- The second stage cross-linker is *glutaraldehyde*, which is a bifunctional reagent connecting the APTES and bio-probe by imide bonds.

Antibody Immobilization



Adsorption

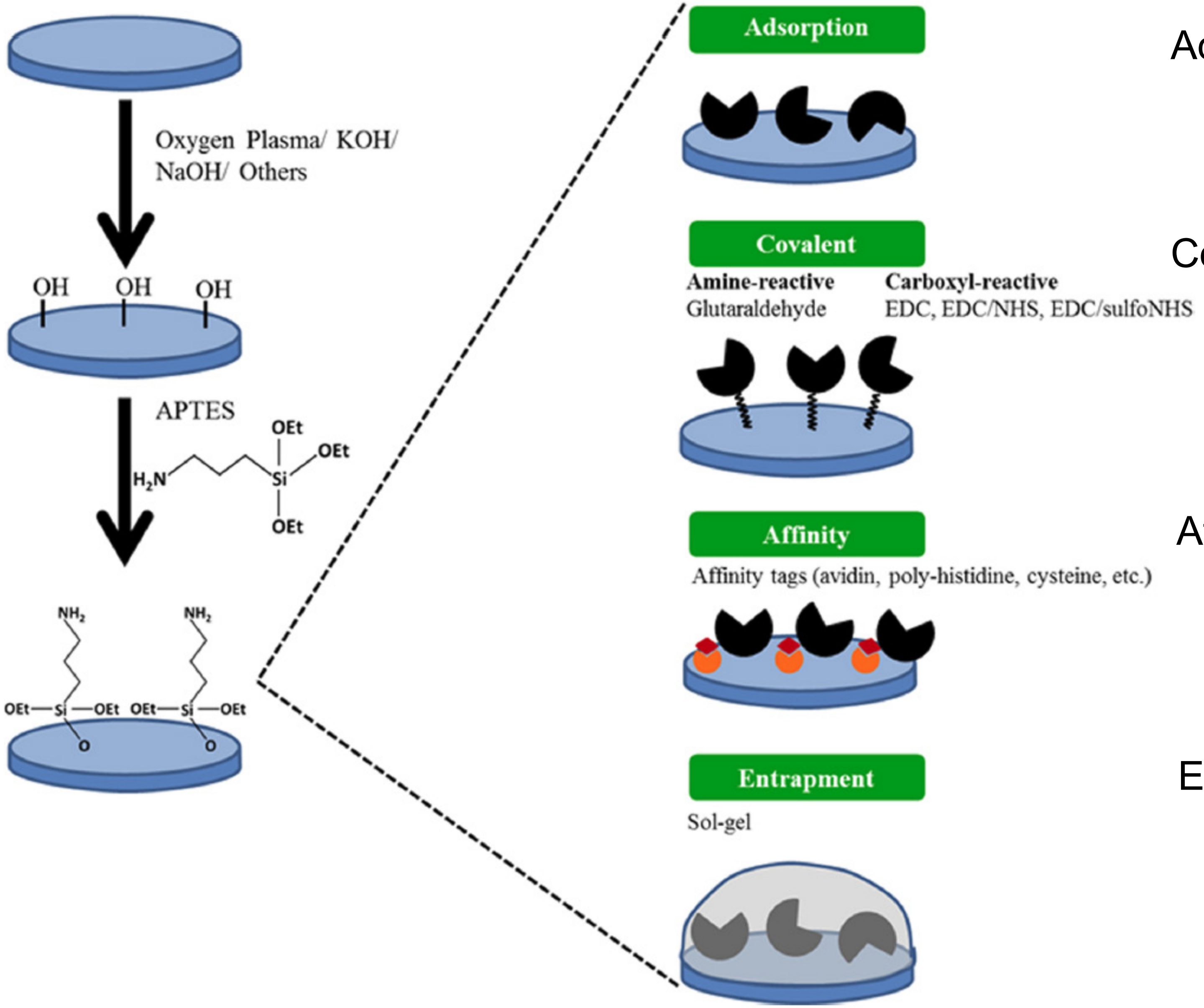
Covalent Bonding

Oriented

Site Specific

Vashist SK, Lam E, Hrapovic S, Male KB, Luong JHT. Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics. *Chemical Reviews.* 2014;114(21):11083-11130.

Enzyme Immobilization



Adsorption

Covalent

Affinity

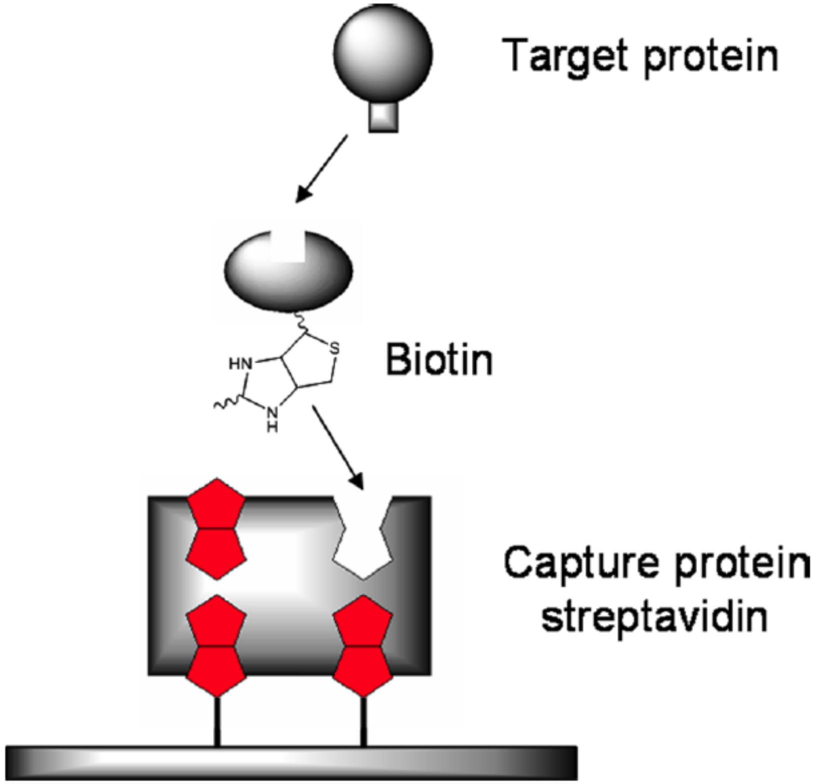
Entrapment

Vashist SK, Lam E, Hrapovic S, Male KB, Luong JHT. Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics. *Chemical Reviews.* 2014;114(21):11083-11130.

Protein Immobilization with Biotin/Avidin…

- The specific molecular interaction between the avidin and biotin pair is a well-known phenomenon and has been extensively advocated for biomolecule immobilization as the cubic shape avidin possesses four biotin binding sites.
- Avidin is covalently attached to APTES-surfaces by either GLD - mediated [*glutaraldehyde*], or EDC-mediated [*1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)*] reactions
- Avidin then acts as a biocompatible linker between APTES-surfaces and biotinylated biomolecules.

Scheme 5. Immobilization of a Protein by Affinity through a Streptavidin/Biotin Interaction



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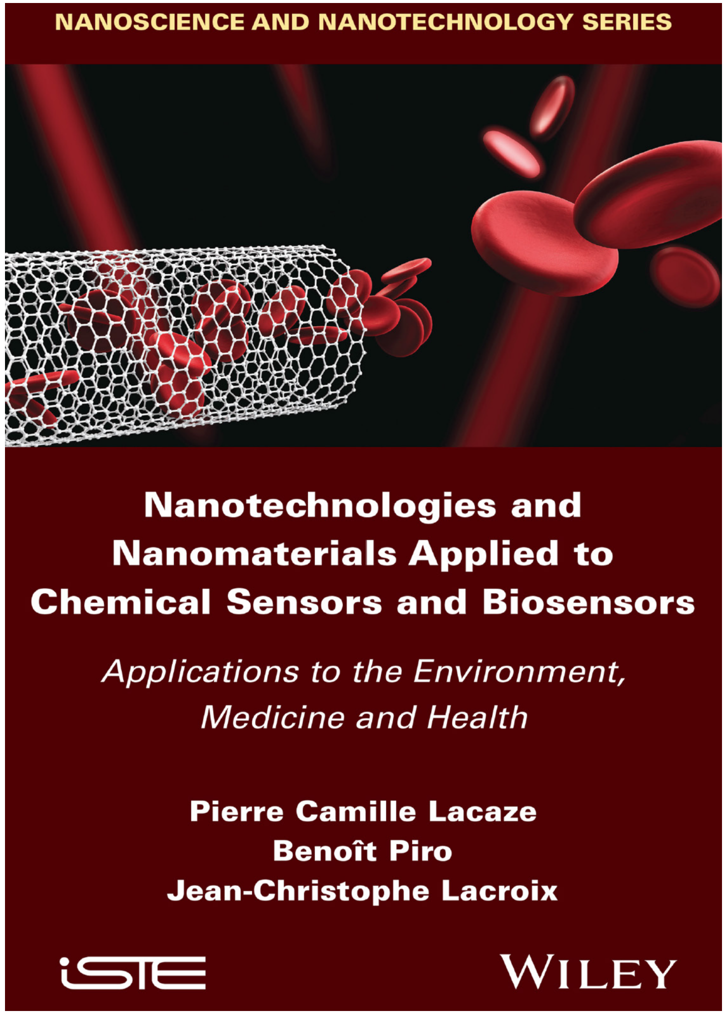
Vashist SK, Lam E, Hrapovic S, Male KB, Luong JHT. Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics. *Chemical Reviews.* 2014;114(21):11083-11130.

Recommended Reading



nature nanotechnology

REVIEW ARTICLE

https://doi.org/10.1038/s41565-021-01045-5

Check for updates

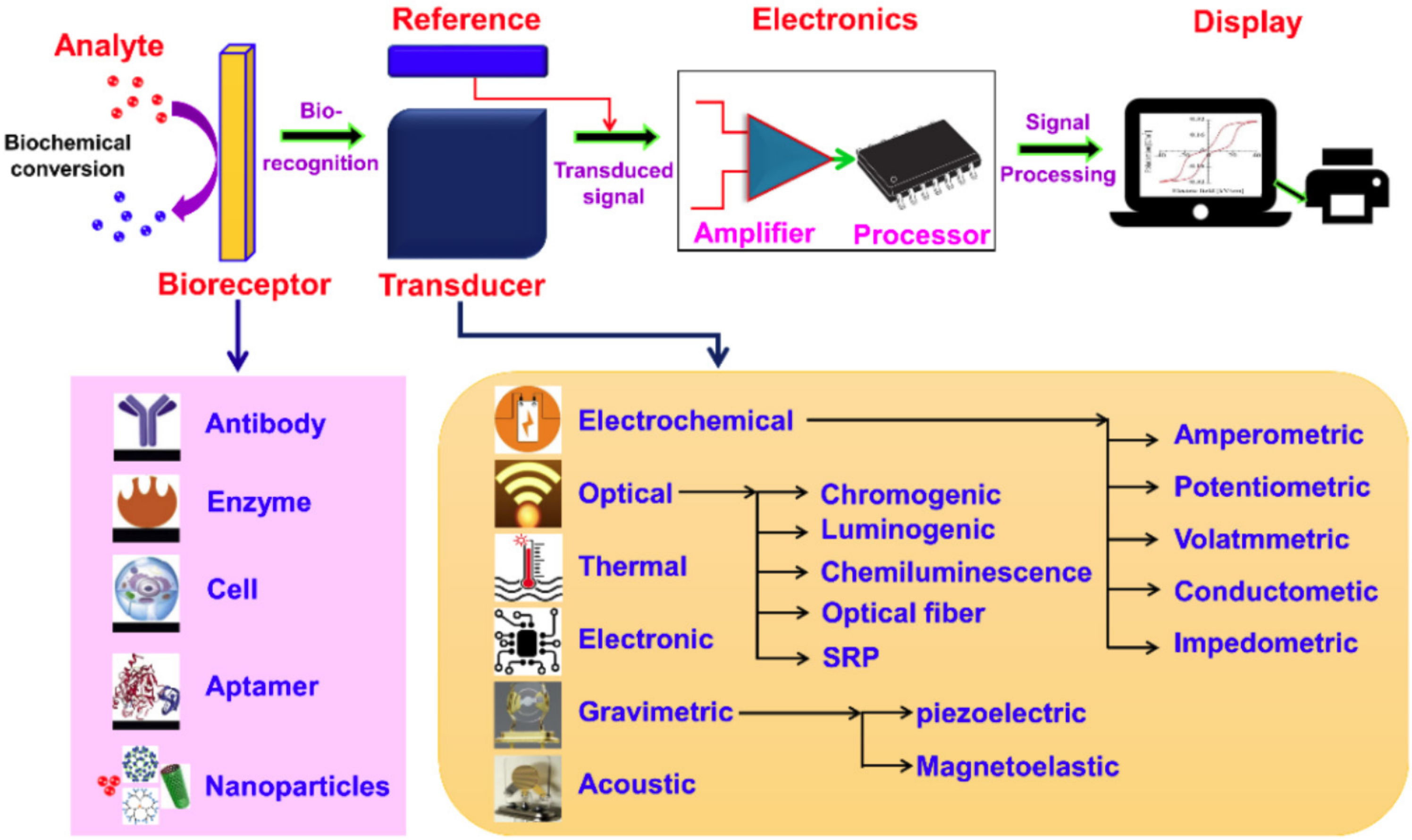
Advances and applications of nanophotonic biosensors

Hatice Altug[1], Sang-Hyun Oh[2], Stefan A. Maier[3,4] and Jiří Homola[5]

Nanophotonic devices, which control light in subwavelength volumes and enhance light–matter interactions, have opened up exciting prospects for biosensing. Numerous nanophotonic biosensors have emerged to address the limitations of the current bioanalytical methods in terms of sensitivity, throughput, ease-of-use and miniaturization. In this Review, we provide an overview of the recent developments of label-free nanophotonic biosensors using evanescent-field-based sensing with plasmon resonances in metals and Mie resonances in dielectrics. We highlight the prospects of achieving an improved sensor performance and added functionalities by leveraging nanostructures and on-chip and optoelectronic integration, as well as microfluidics, biochemistry and data science toolkits. We also discuss open challenges in nanophotonic biosensing, such as reducing the overall cost and handling of complex biological samples, and provide an outlook for future opportunities to improve these technologies and thereby increase their impact in terms of improving health and safety.

Key Points

- Know about analytes, bioreceptors, transducers, and required electronics.
- Understand the different transduction methods, including electrochemical, optical, thermal, electronic, gravimetric and acoustic techniques.
- If *antibodies*, *enzymes*, *cells*, and *aptamers* seem foreign, write a simple Google AI inquiry of each to learn more.



Naresh V, Lee N. A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*. Feb 2021;21(4)1109. doi:10.3390/s21041109

Key Points…

- Biosensors are combined with microfluidic systems for a number of reasons. What are these?
- Optimization of biosensors include selectivity, sensitivity, linearity, response time, reproducibility and stability.
- Field effect transistors can be useful transducers. What are they, how do they work internally, and how do they work with biological and chemical substances?
- What are four electrochemical detection methods? How does voltammetry differ from amperometry?
- Nanomaterials like gold nanoparticles (AuNPs), Fe_3O_4, Pd, NiO, TiO_2 have intrinsic enzyme-like activity.

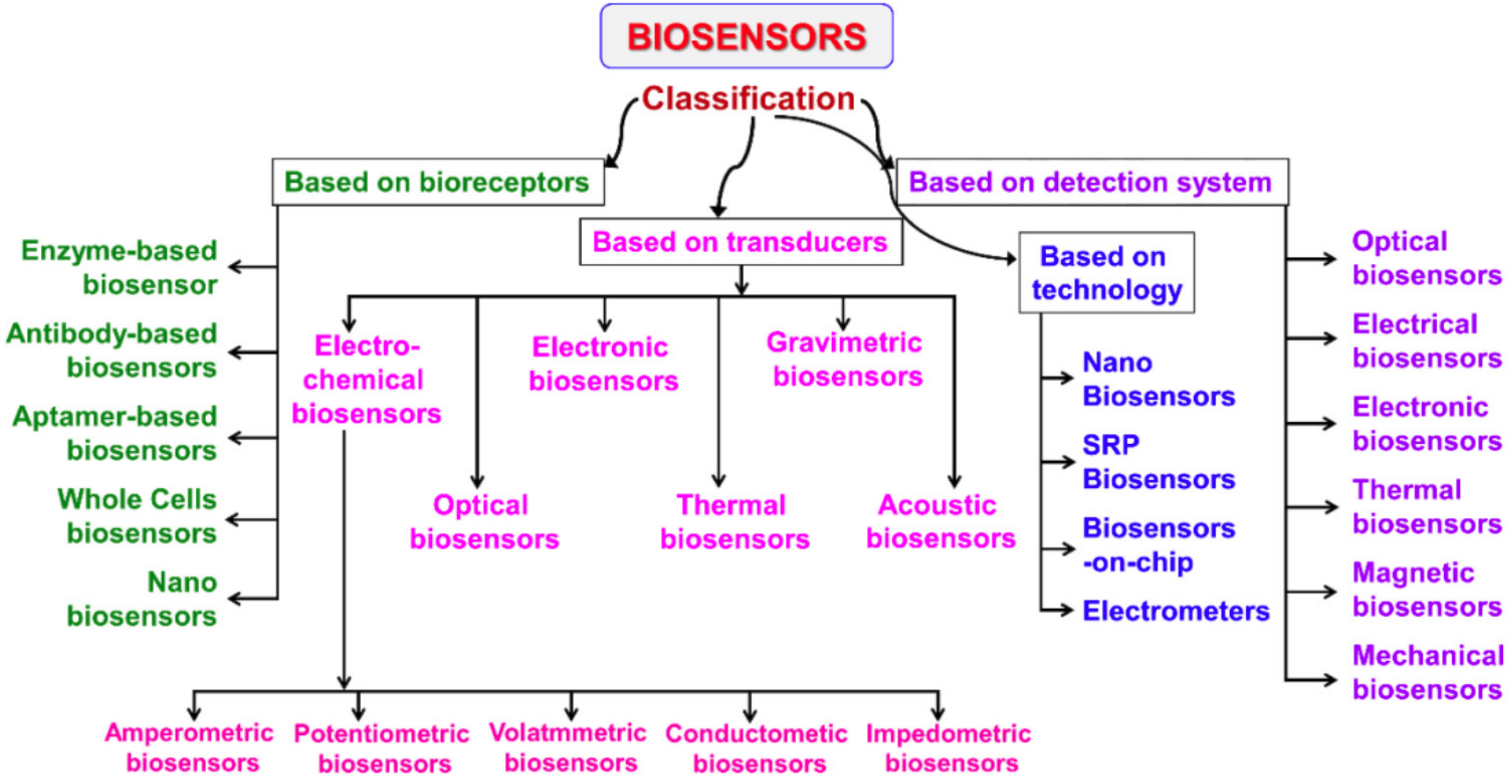
Key Points…

- How would you construct a silicon nanowire biosensor, with antibody immobilization for antigen binding?
- APTES is a silane molecule that bonds with sensor dielectric surfaces such as silicon oxide. The second stage cross-linker is glutaraldehyde, and this connects the APTES to a bio-probe by imide bonds. What steps would you take for antibody and enzyme immobilization?
- Immobilization of a protein by *affinity* through *streptavidin/biotin interaction* is possible. Avidin is covalently attached to APTES-surface and acts as a *biocompatible linker* between the surface and biotinylated biomolecules.

Appendix

- Biosensor classification schemes.
- Cyclic voltammetry setup.
- Example of Electrochemical Impedance Spectroscopy
- Bonding Modes for APTES on a Substrate Surface.

Various Biosensors Classifications



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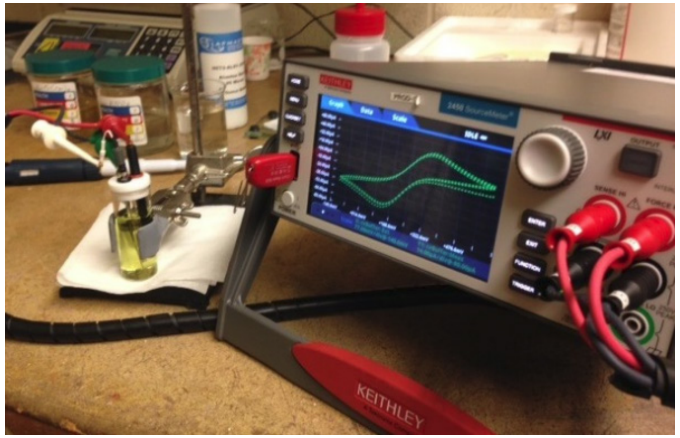
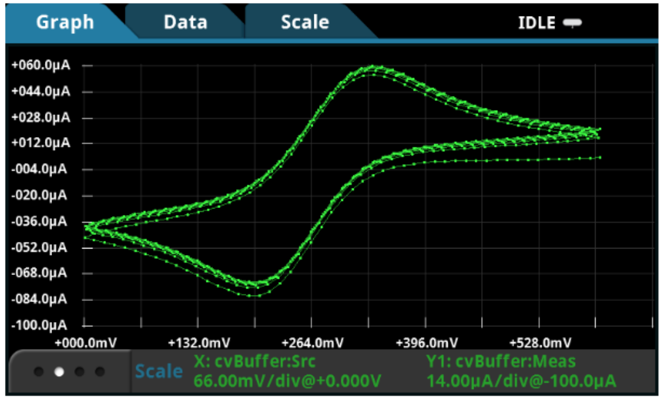
Simple Cyclic Voltammetry Setup

Keithley Model 2450-EC Source Meter (SMU)





Voltammogram from using a cyclic voltammetry *script*. (V vs. I)

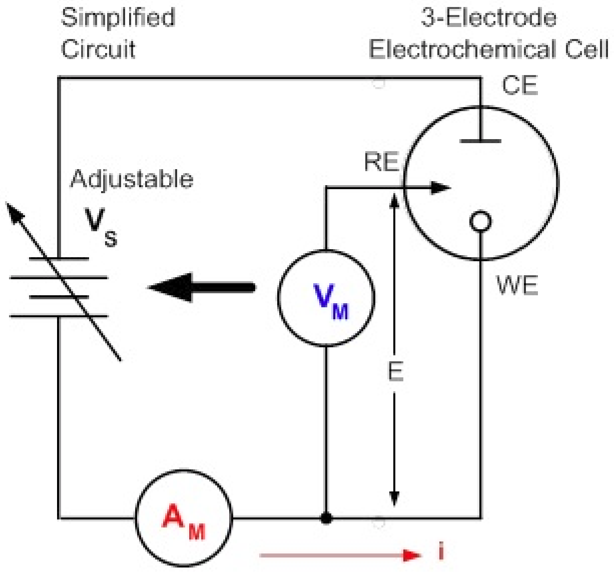


Figure 1. Simplified measurement circuit for performing cyclic voltammetry

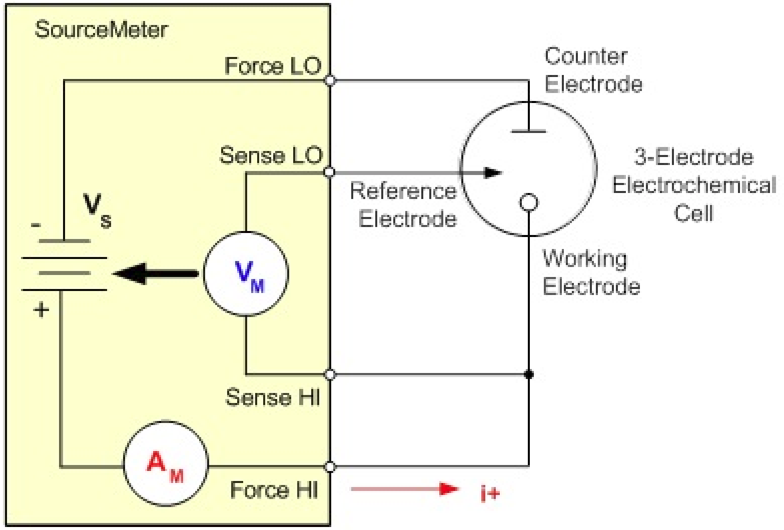
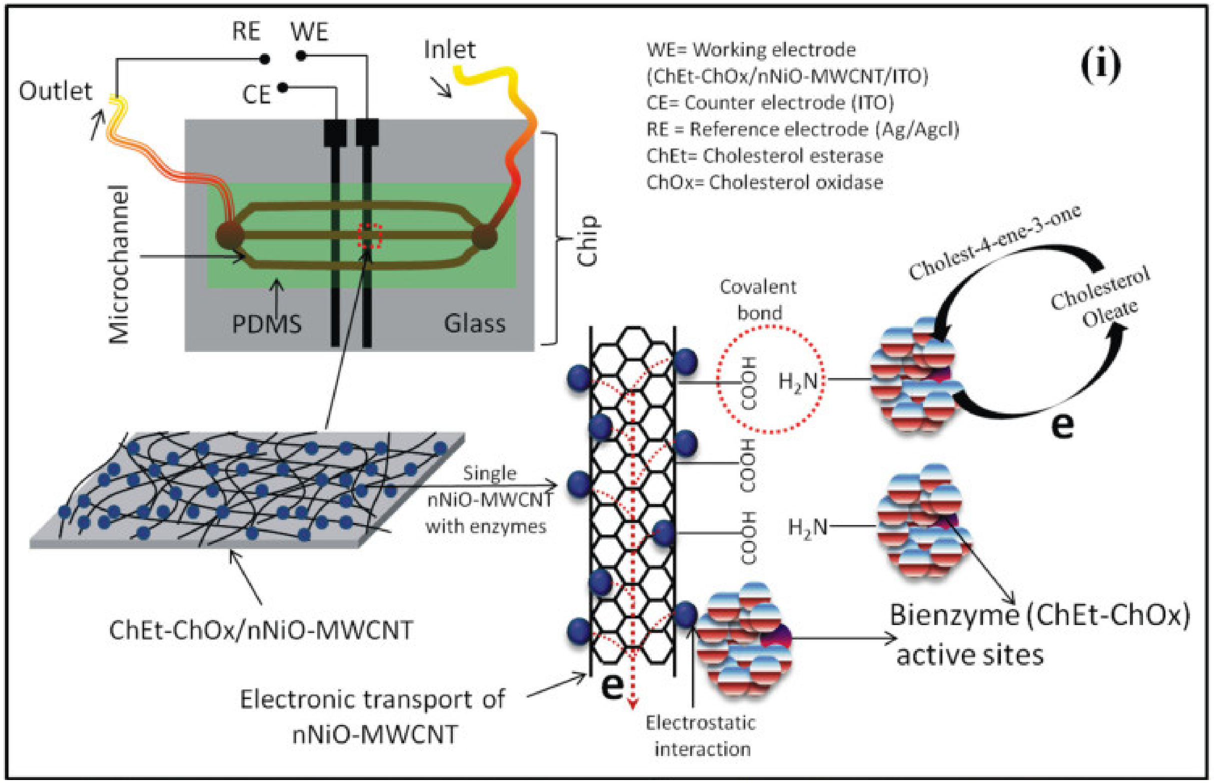


Figure 2. Connecting a Source Meter to an electrochemical cell for cyclic voltammetry

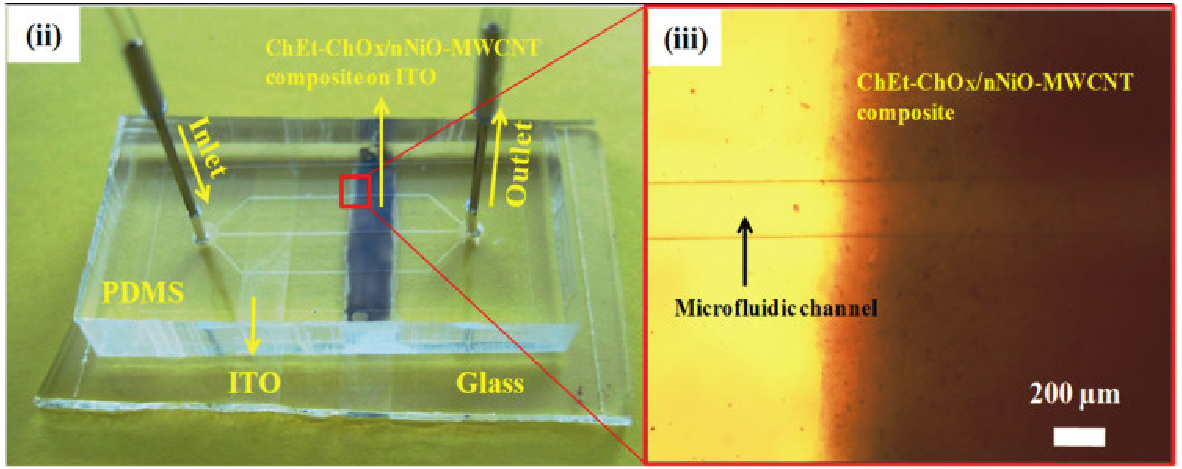
When an SMU is programmed to source voltage in the remote sense (4-wire) configuration, internal sensing provides a feedback voltage that is measured and compared to the programmed level. The voltage source is adjusted until the feedback voltage equals the programmed voltage level. Remote sensing compensates for the voltage drop in the test leads and analyte, ensuring that the programmed voltage level is delivered to the working electrode.

Tupta, MA. *Keithley Product Line at Tektronix,* https://tw.tek.com/blog/performing-cyclic-voltammetry

Example of Voltammetry Use…



Nickel oxide nanoparticles (nNiO) and multiwalled carbon nanotubes (MWCNT). ITO – Indium Tin Oxide WE and RE.



Bi-enzyme (cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) functionalized nanocomposite microfluidic-based biosensor developed for the detection of cholesterol .

Ali, M.A.; Srivastava, S.; Solanki, P.R.; Reddy, V.; Agrawal, V.V.; Kim, C.; John, R.; Malhotra, B.D. Highly efficient bienzyme functionalized nanocomposite-based microfluidics biosensor platform for biomedical application. Sci. Rep. 2013, 3, 2661.

Example of Electrochemical Impedance Spectroscopy

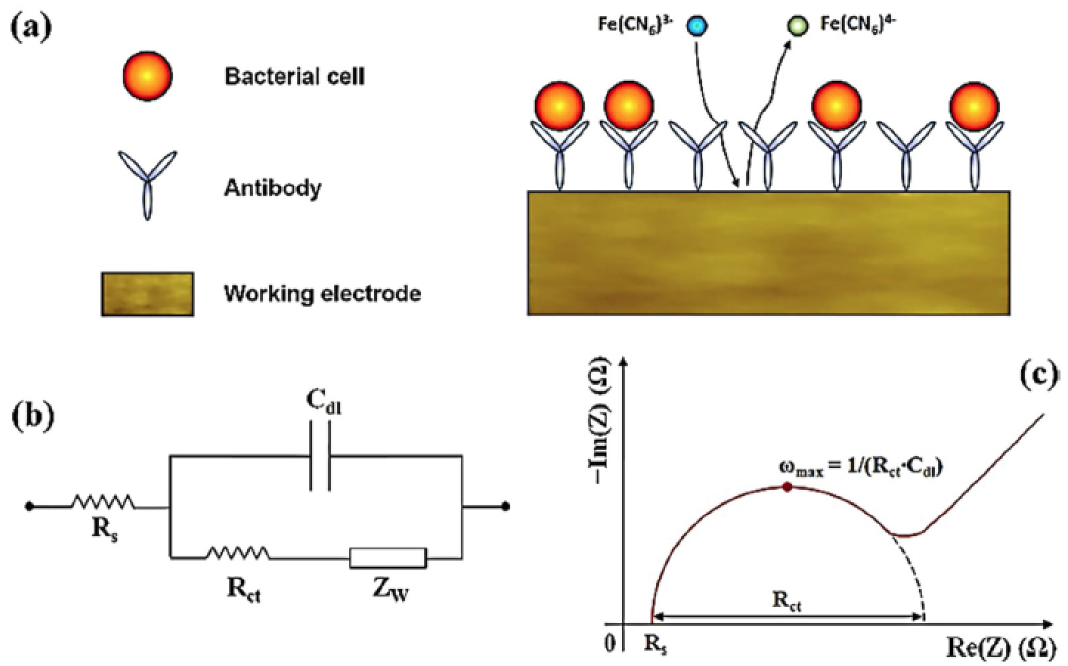


Fig. 23.1 (A) Modified working electrode with immobilized bioreceptor; (B) equivalent electrical circuit of the biosensor; (C) Nyquist plot of the equivalent circuit [1].

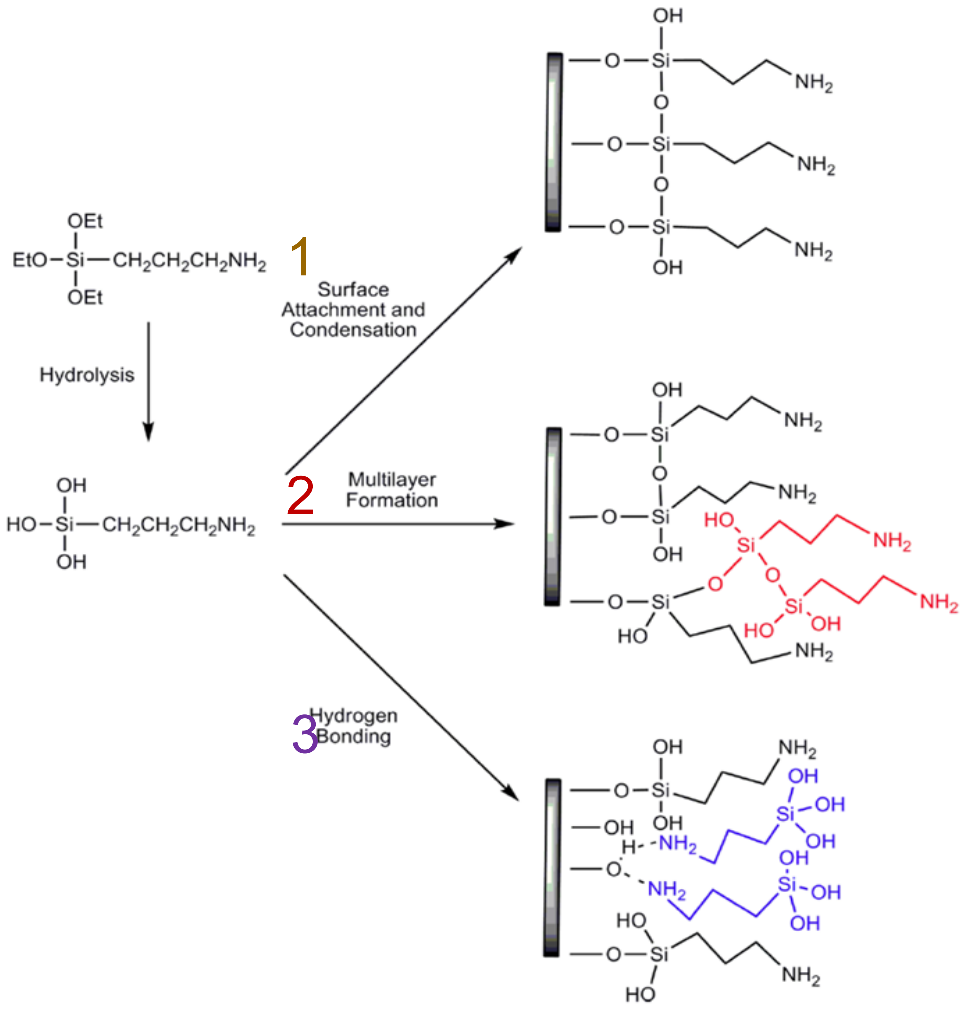
The equivalent circuit used to explain the behavior of the electrochemical impedance of a cell is schematically shown in Fig. 23.1B, where R_s is the electrical resistance of the electrolyte, C_{dl} the double-layer capacitance at the W_E electrolyte interface, and R_{ct} the charge transfer resistance due to the redox reaction of $[Fe\ (CN)\ _6]^{3-/4-}$ with the W_E. Finally, Z_W describes the Warburg impedance due to the diffusion process of reactants. The electrical circuit can be described using a Nyquist diagram, as shown in Fig. 23.1C. For low frequencies, the predominant effect is ion diffusion (Warburg impedance) in a straight line with a slope of about 45 degrees. At high frequencies, the graph is described by a semicircle with a diameter given by the load transfer resistance R_{ct}. This parameter is mostly used to evaluate the bacterial concentration, because binding bacterial cells to the target receptors at the W_E surface reduces the oxidation reaction and increases the R_{ct}. Sometimes, however, the double-layer capacitance C_{dl} is used instead [1].

C_{dl} = double-layer capacitance of the electrodes,
R_{sol} = resistance of the solution,
C_{de} = capacitance of the electrode,
Z_{cell} = impedance introduced by the bound nanoparticles,
R_{cell} & C_{cell} are the resistance and capacitance in parallel).

Bigdeli IK, Yeganeh M, Shoushtari MT, Zadeh MK. Chapter 23 - Electrochemical impedance spectroscopy (EIS) for biosensing. In: Thomas S, Nguyen TA, Ahmadi M, Farmani A, Yasin G, eds. *Nanosensors for Smart Manufacturing*. Elsevier; 2021:533-554.

APTES Bonding Options on a Substrate

1. Surface attachment and condensation lead to horizontal polymerization when a surface-bound APTES molecule forms siloxane with its neighboring surface-bound APTES.
2. In vertical polymerization, the surface-bound APTES reacts with a nearby APTES in solution.
3. Where the substrate possesses surface hydroxyl groups, the amine of APTES forms a hydrogen bond with the metal surface or becomes protonated by abstracting protons from the surface.



Vashist SK, Lam E, Hrapovic S, Male KB, Luong JHT. Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics. *Chemical Reviews.* 2014;114(21):11083-11130.

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Pre-Treatment with KOH and 0_2 Plasma…

Silanization occurs on any substrate, normally with chemically active hydroxyl groups for silane grafting.

Some substrates (e.g., silica, agarose, etc.) already have hydroxyl groups, while others require a pretreatment step using KOH/NaOH, acid, piranha solution, or plasma treatment to introduce hydroxyl groups.

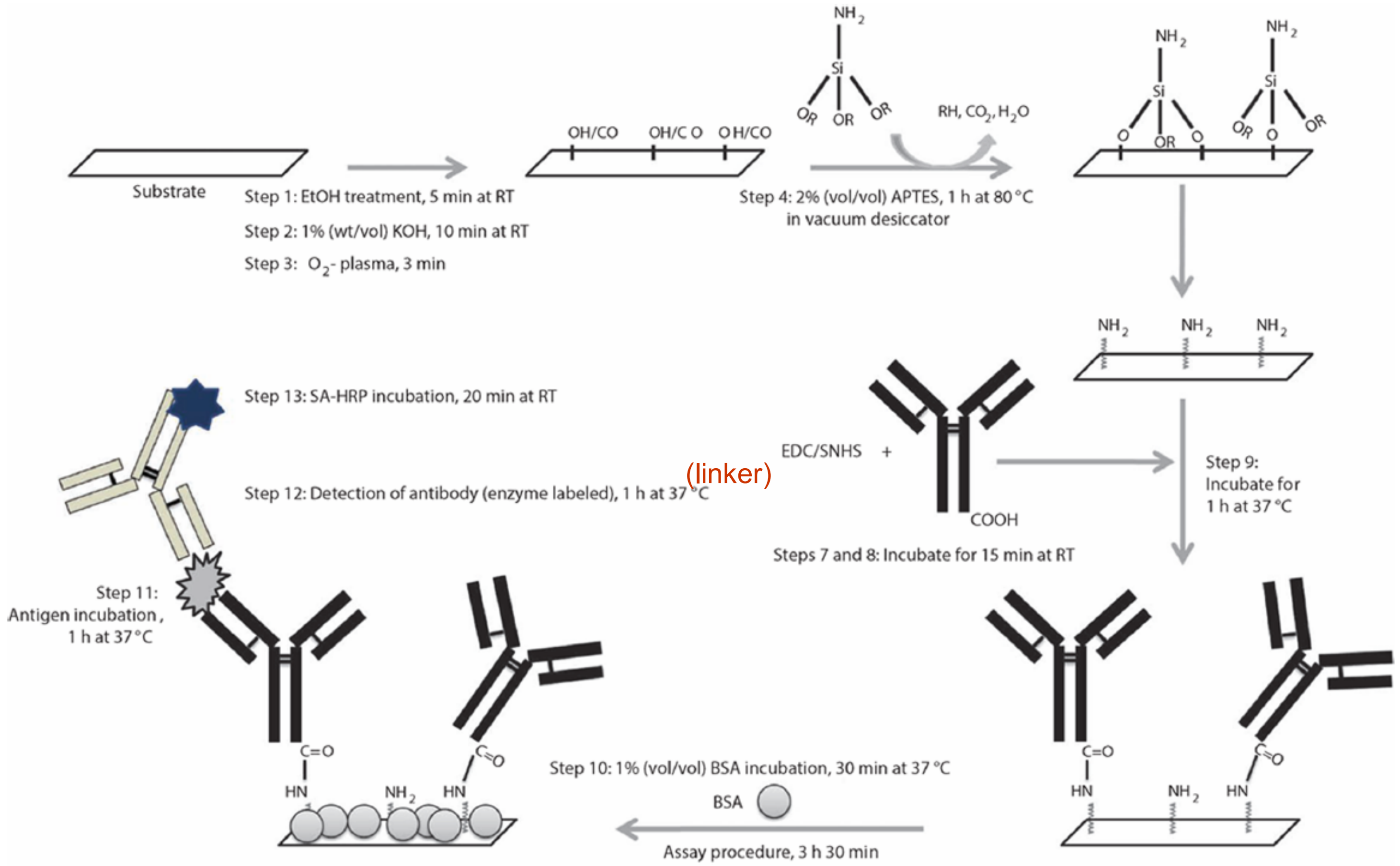

Figure 1. Treatment of a substrate with KOH and O_2 plasma prior to APTES functionalization as part of an APTES-based sandwich ELISA procedure. Reproduced with permission from ref 60. Copyright 2011 Nature America Inc.

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Dixit, C. K.; Vashist, S. K.; MacCraith, B. D.; O'Kennedy, R. Nat. Protoc. 2011, 6, 439.

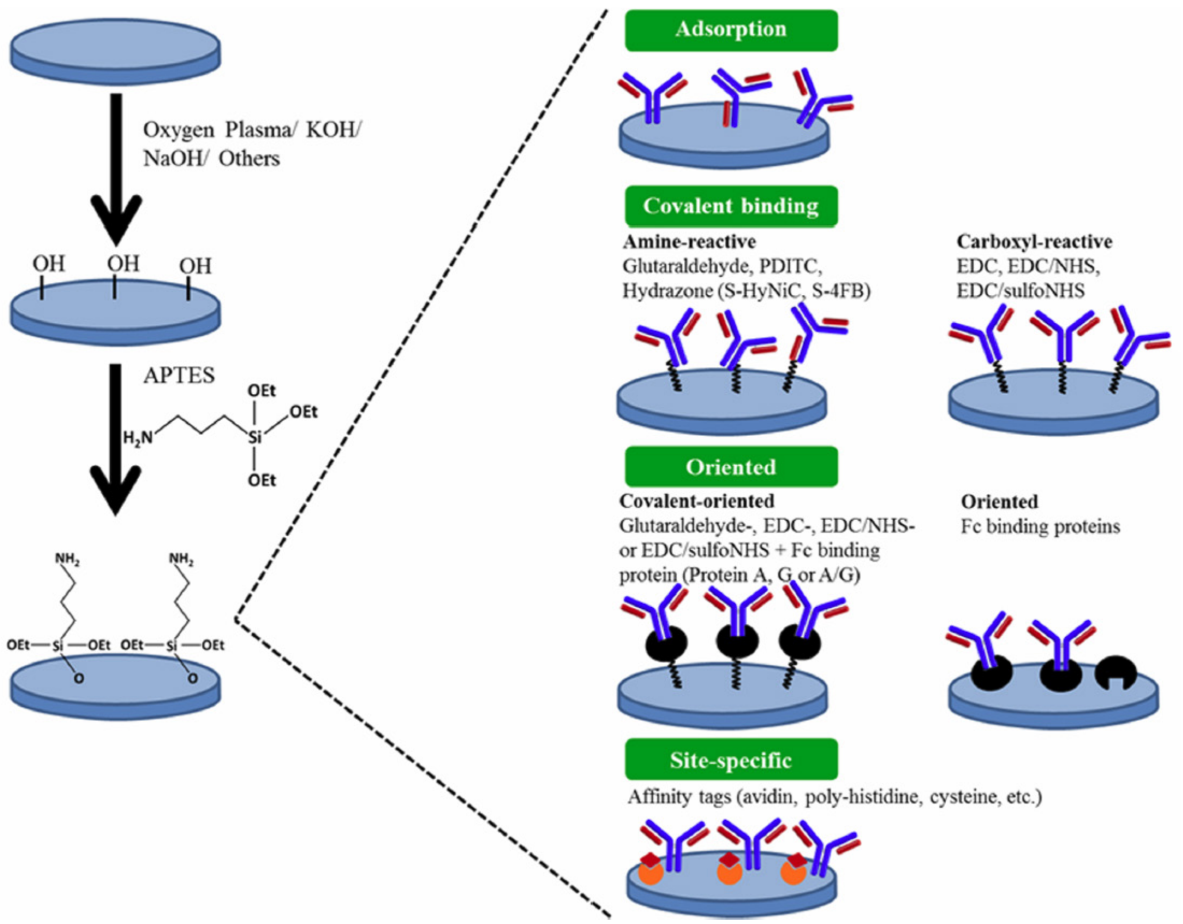
Specifics of Antibody Immobilization…

- The amine group of APTES enables the covalent bonding of biomolecules based on the use of a functional linker. GLD (glutaraldehyde), a *homo-bifunctional agent* (same reactive groups on each end of the crosslinker), cross-links the amino groups of APTES-functionalized surfaces to the biomolecule amino groups.

- Aldehyde groups form imine bonds with amine groups of lysine common to almost every protein or enzyme to form reversible Schiff bases.

- The most widely used *hetero-bifunctional* cross-linker combination is EDC and N-hydroxysuccinimide (NHS)/N-hydroxysulfosuccinimide (SNHS), where EDC of the EDC-NHS/SNHS complex first binds to the carboxyl-terminal of the Abs.

Scheme 2. Schematic of Most Commonly Used Ab Immobilization Strategies on APTES-Functionalized Bioanalytical Platforms



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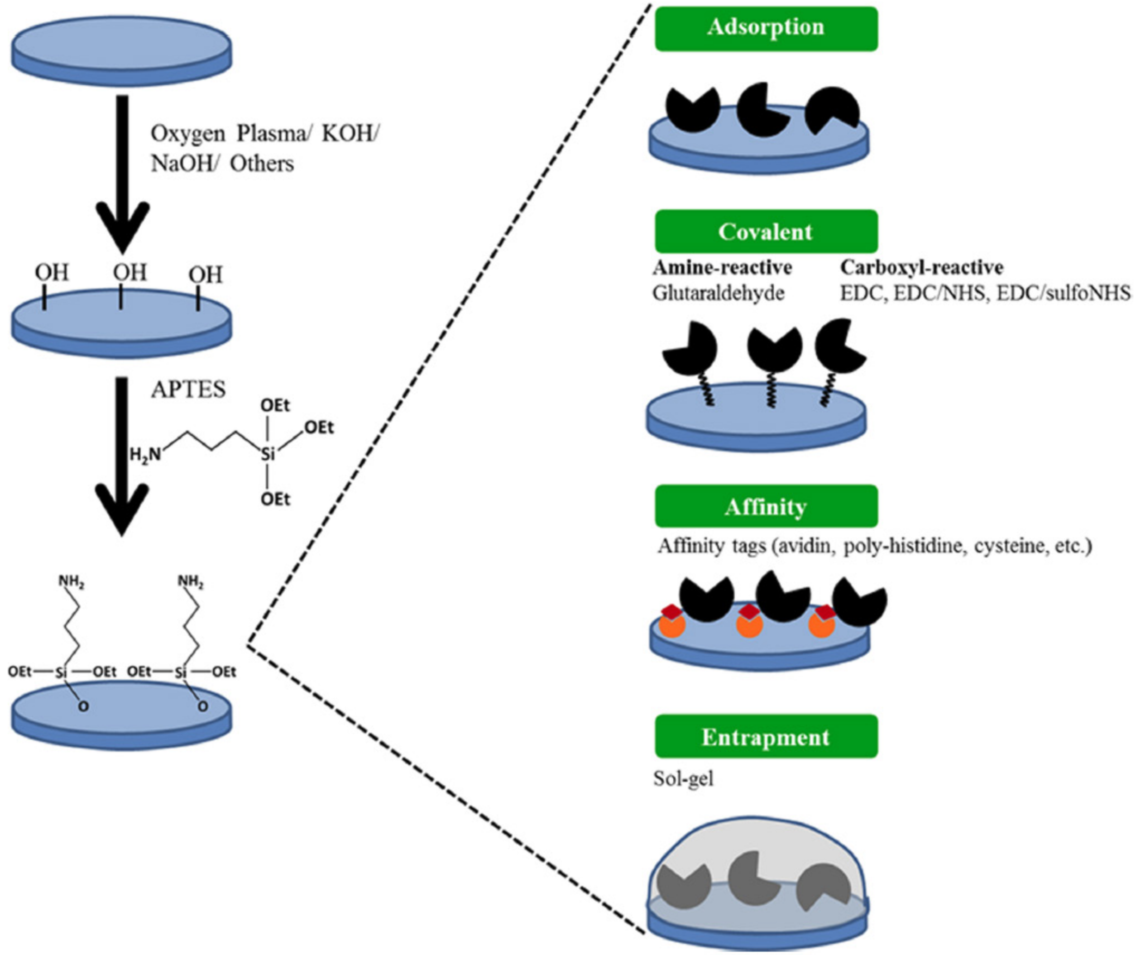
More on Enzyme Immobilization…

Most commonly used enzyme immobilization strategies on APTEs - functionalized bioanalytical platforms.



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