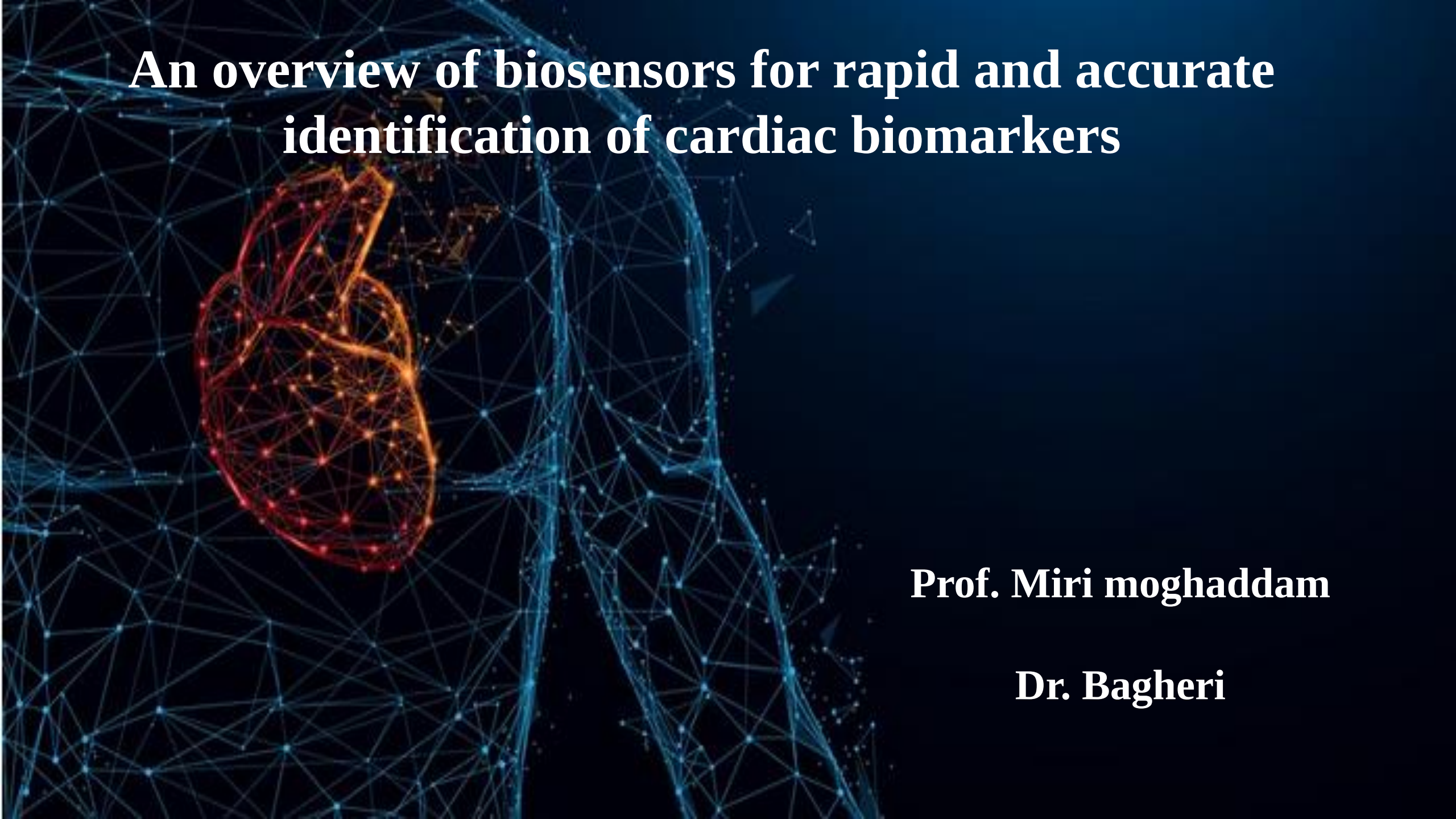
An overview of biosensors for rapid and accurate identification of cardiac biomarkers



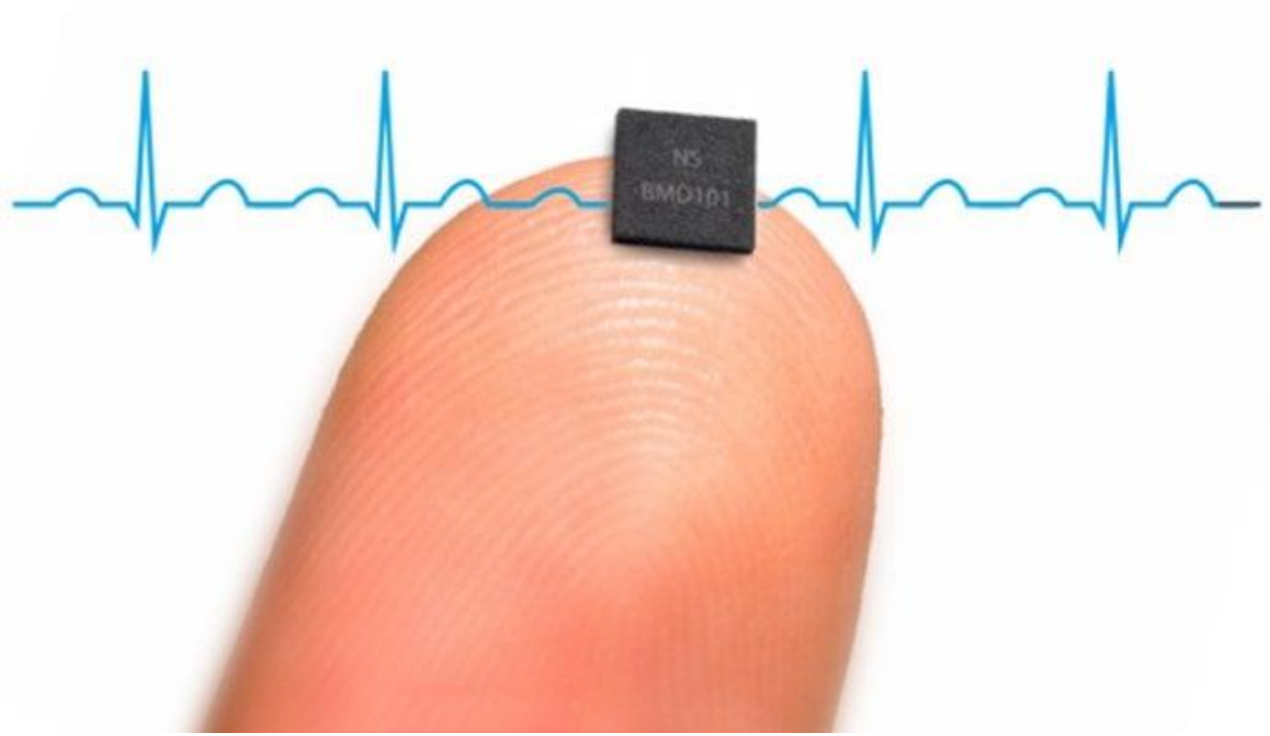
Prof. Miri moghaddam

Dr. Bagheri

- ❑ Introduction of cardiovascular diseases

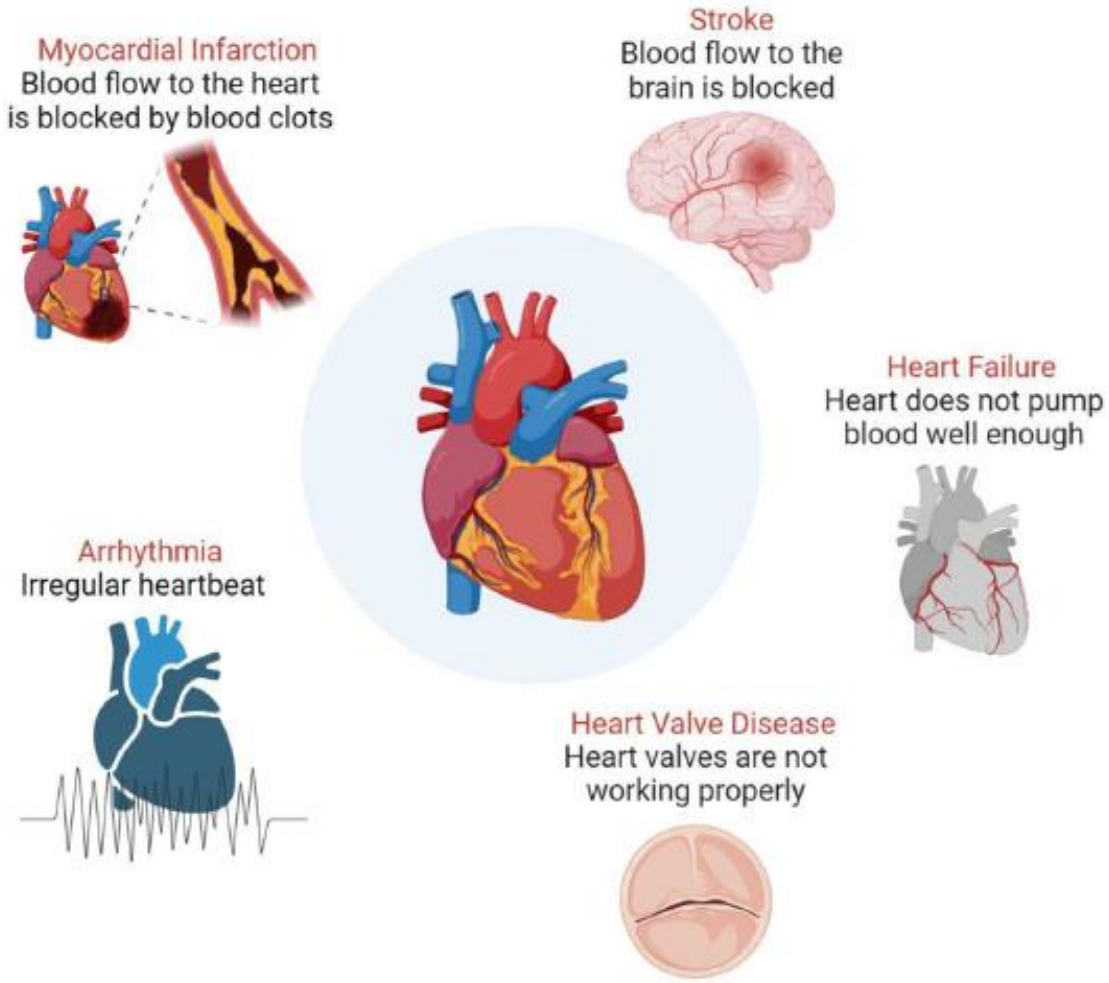
- ❑ Introduction of biosensor
- ❑ Introduction Basic components of Biosensor
- ❑ Types of Biosensor
- ❑ cardiac biomarkers
- ❑ Antibody based sensors
- ❑ aptamer based sensors
- ❑ Wearable devices
- ❑ Point of care Testing

- ❑ Signal based sensor



What are cardiovascular diseases?

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels.

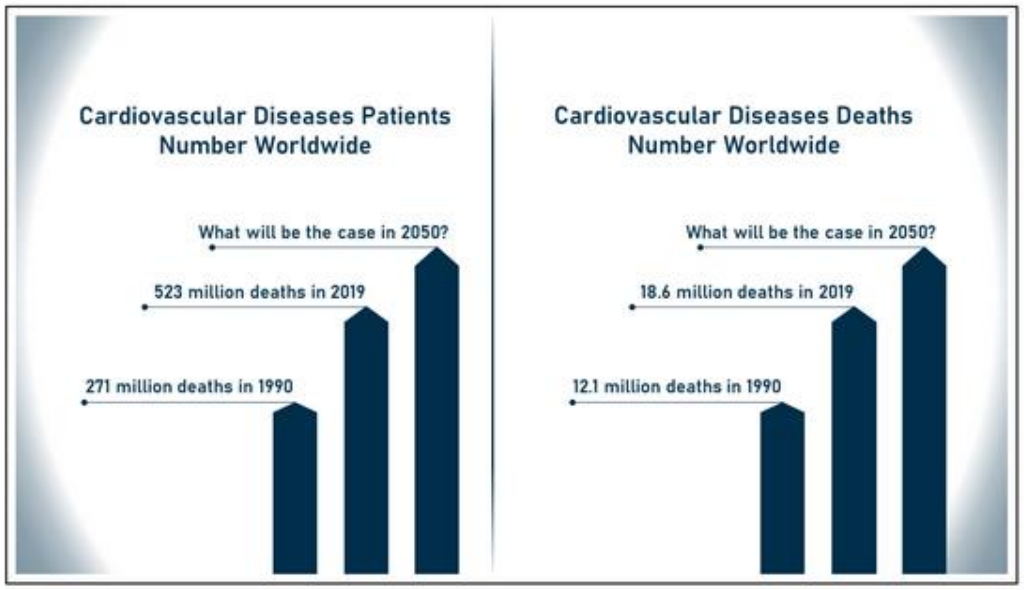


What is the importance of cardiovascular disease detection?

- According to reports from the Centers for Disease Control and Prevention (CDC), heart disease remains the leading cause of death in the United States, **ahead of cancer and COVID-19**.

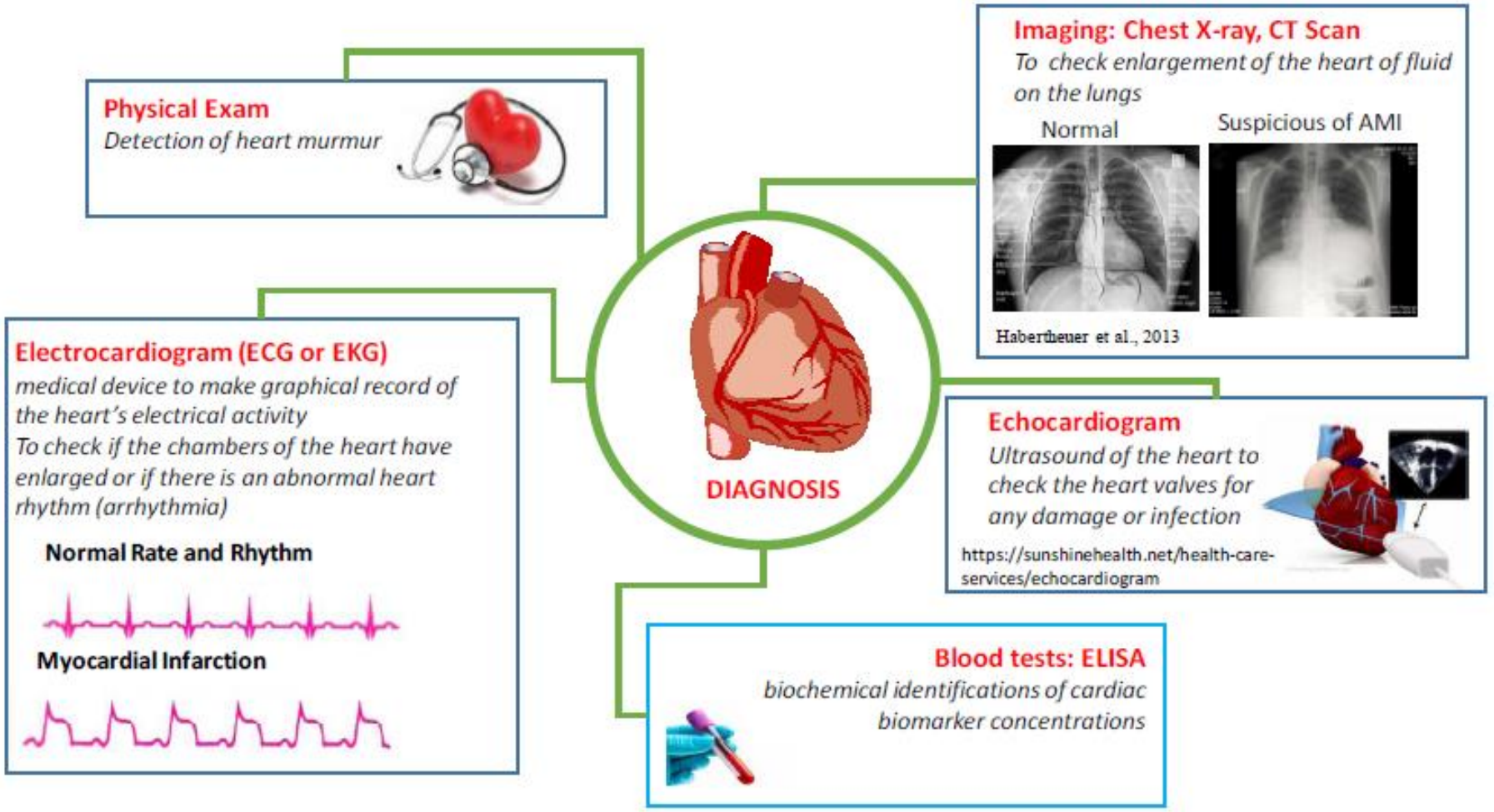
- The WHO report estimates that over 23.3 million people will die annually from cardiovascular diseases by 2030.

- The most important behavioural risk factors of heart disease and stroke are **genetic** factor (cholostrol), **environmental** factor(smoking , physical inactivity), **behavioral** factors (alcohol, stress) and **other disease**(thyroid disease, kidney disease), **age** factor.



Current diagnostic tools to detect cardiovascular diseases in clinical settings

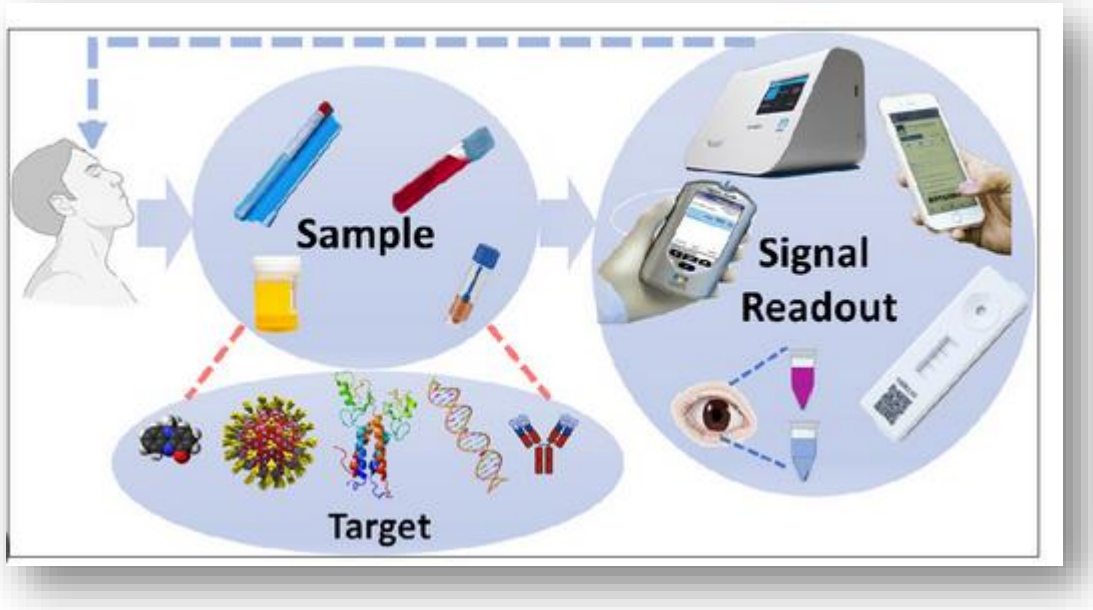
- **electrocardiograms (ECG**): not entirely reliable to diagnose cardiac vascular diseases
- **electrocardiography chest X-rays**: low sensitivity and specificity
- **Echocardiograms:** difficult to perform in an emergency
- **Enzyme-linked Immunosorbent Assay (ELISA)**



How can the burden of cardiovascular diseases be reduced?

Early detection of any of these diseases allows for better life-saving therapeutic intervention and can also reduce health care costs.

- ❑ Rapid
- ❑ Reliable
- ❑ Real-time analysis
- ❑ High selectivity
- ❑ High sensitivity
- ❑ Portable
- ❑ Flexible
- ❑ Small



What is Biosensor?

- ❑ Sample (Analyte or Substrate)
- ❑ Signal Processing Device
- ❑ Bio-recognition Element
- ❑ Transducer

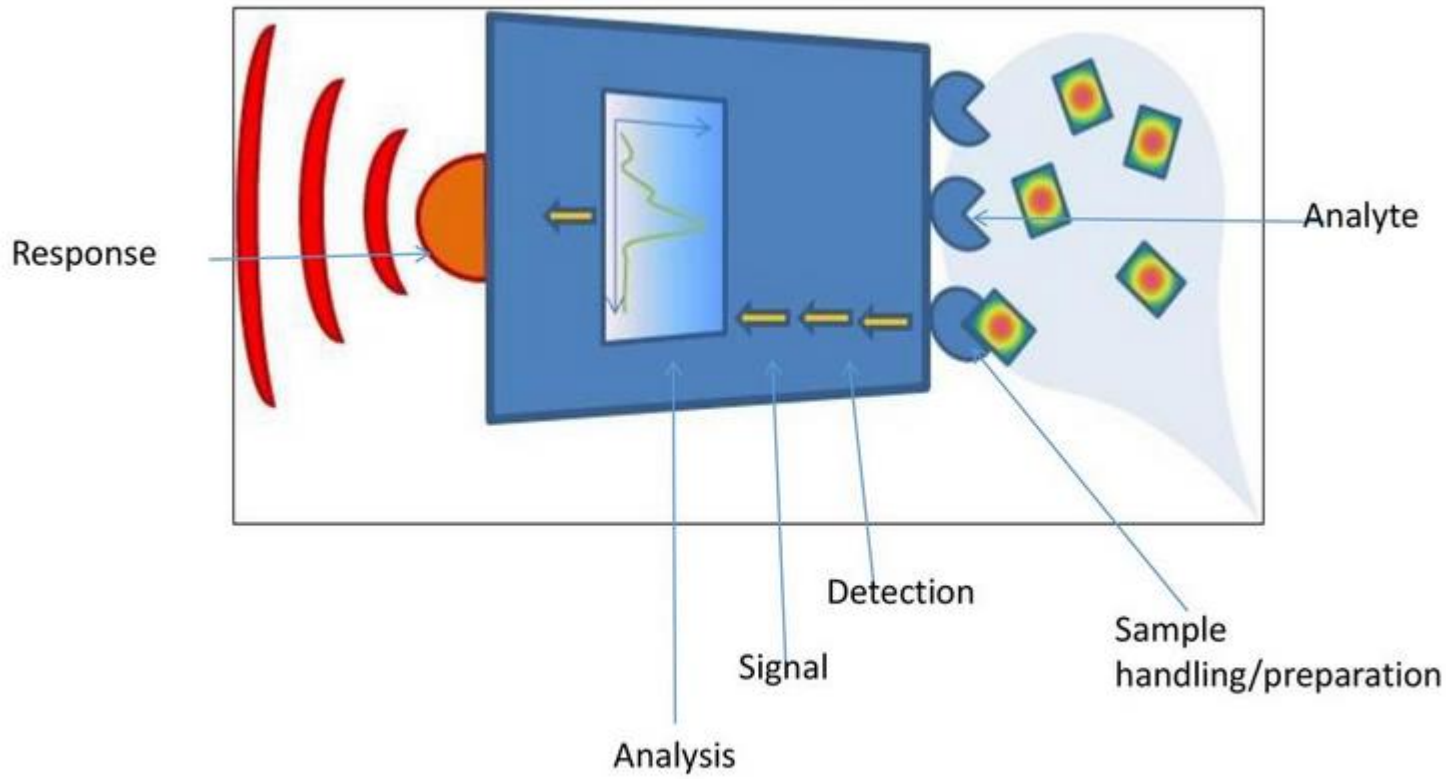
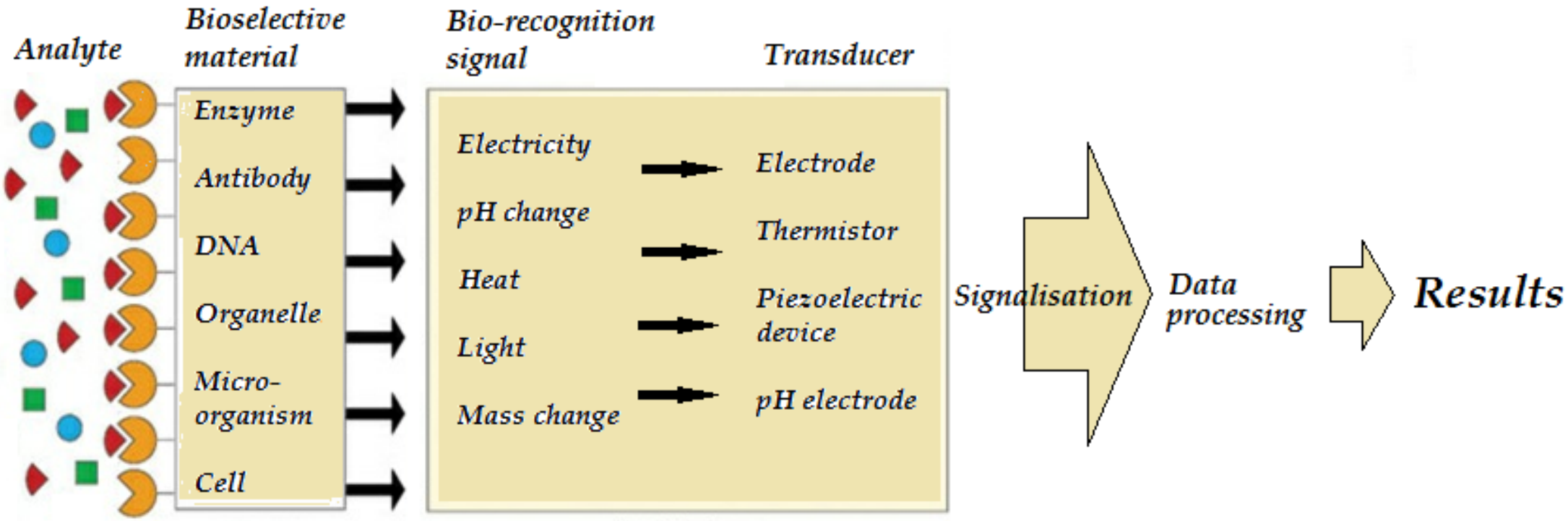
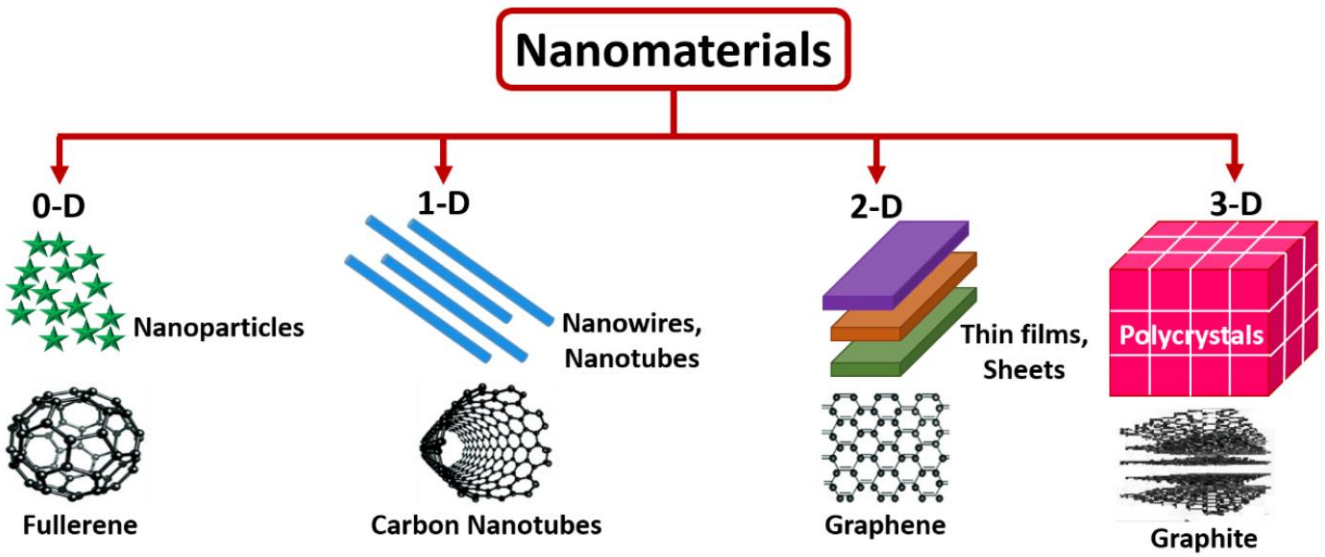
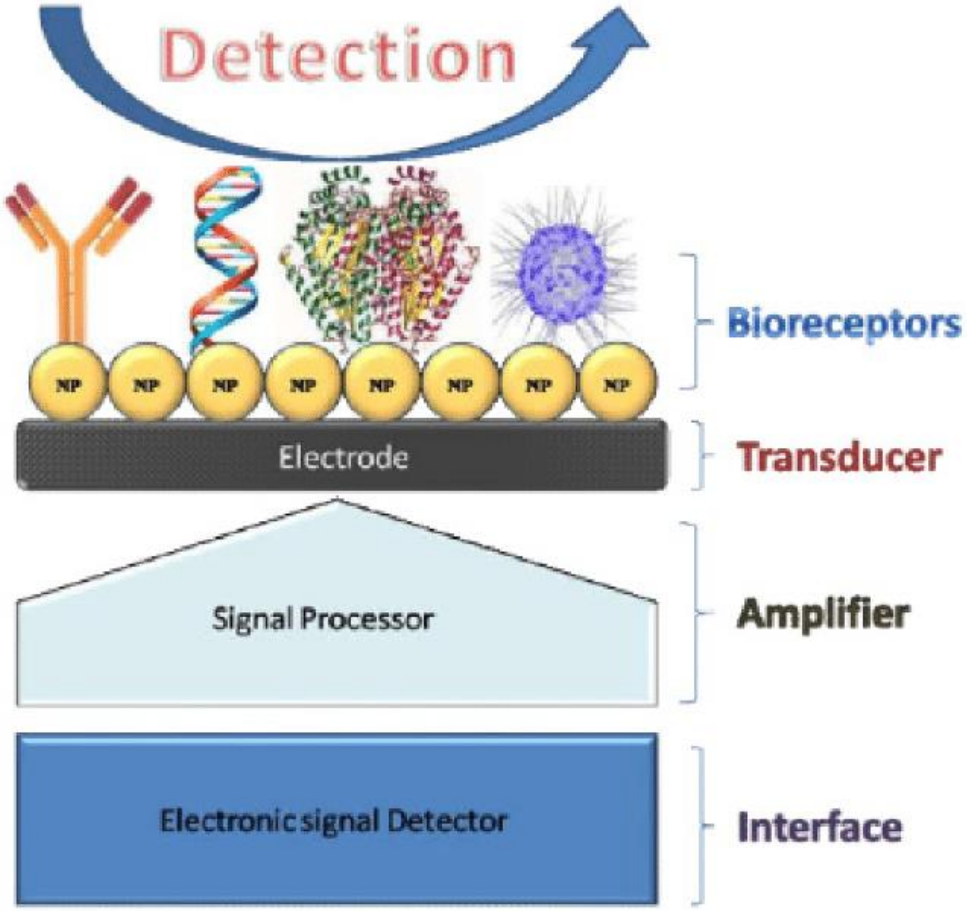


Diagram of a Biosensor

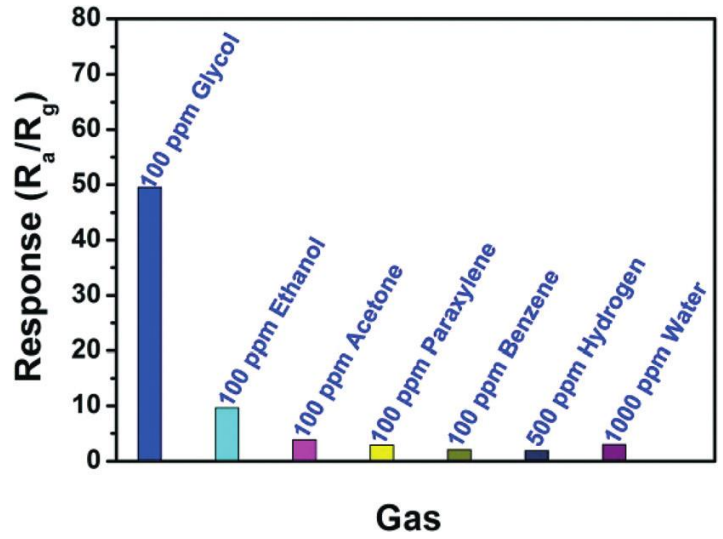


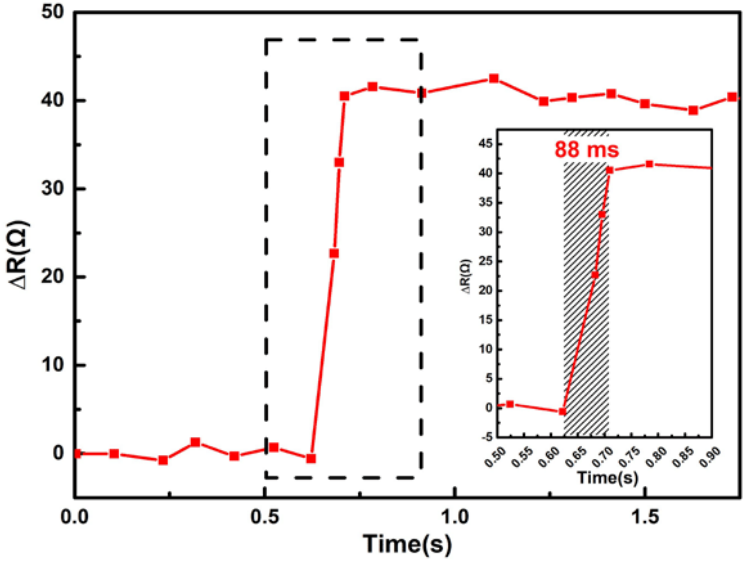
what is Nano biosensors?

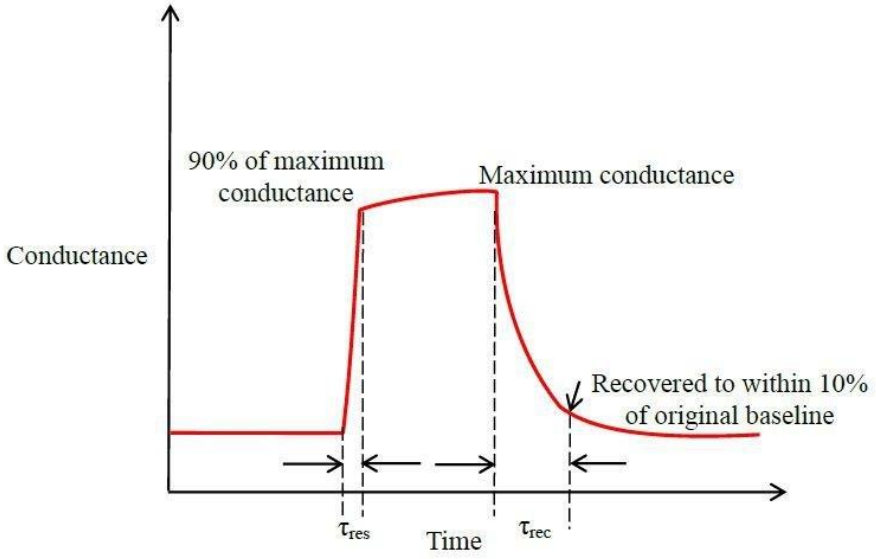


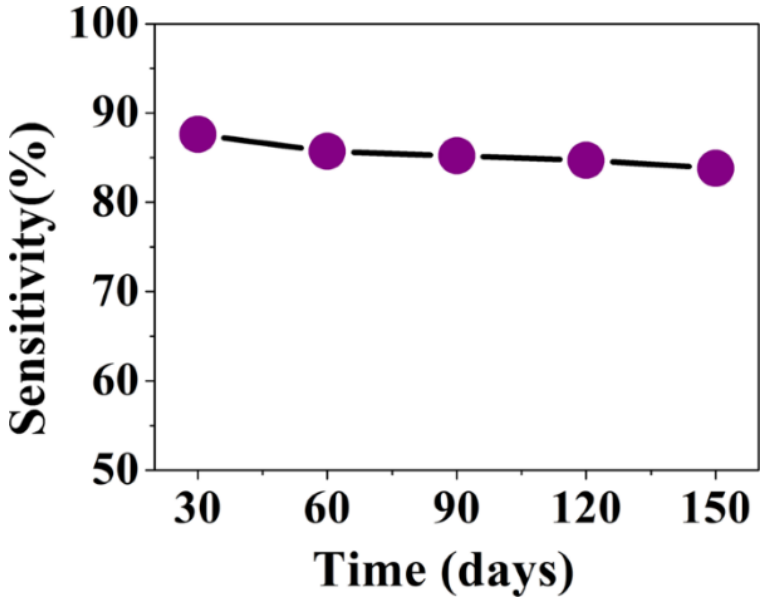


- ❑ Selectivity
- ❑ Sensitivity
- ❑ specificity
- ❑ Stability
- ❑ Response time
- ❑ Recovery time









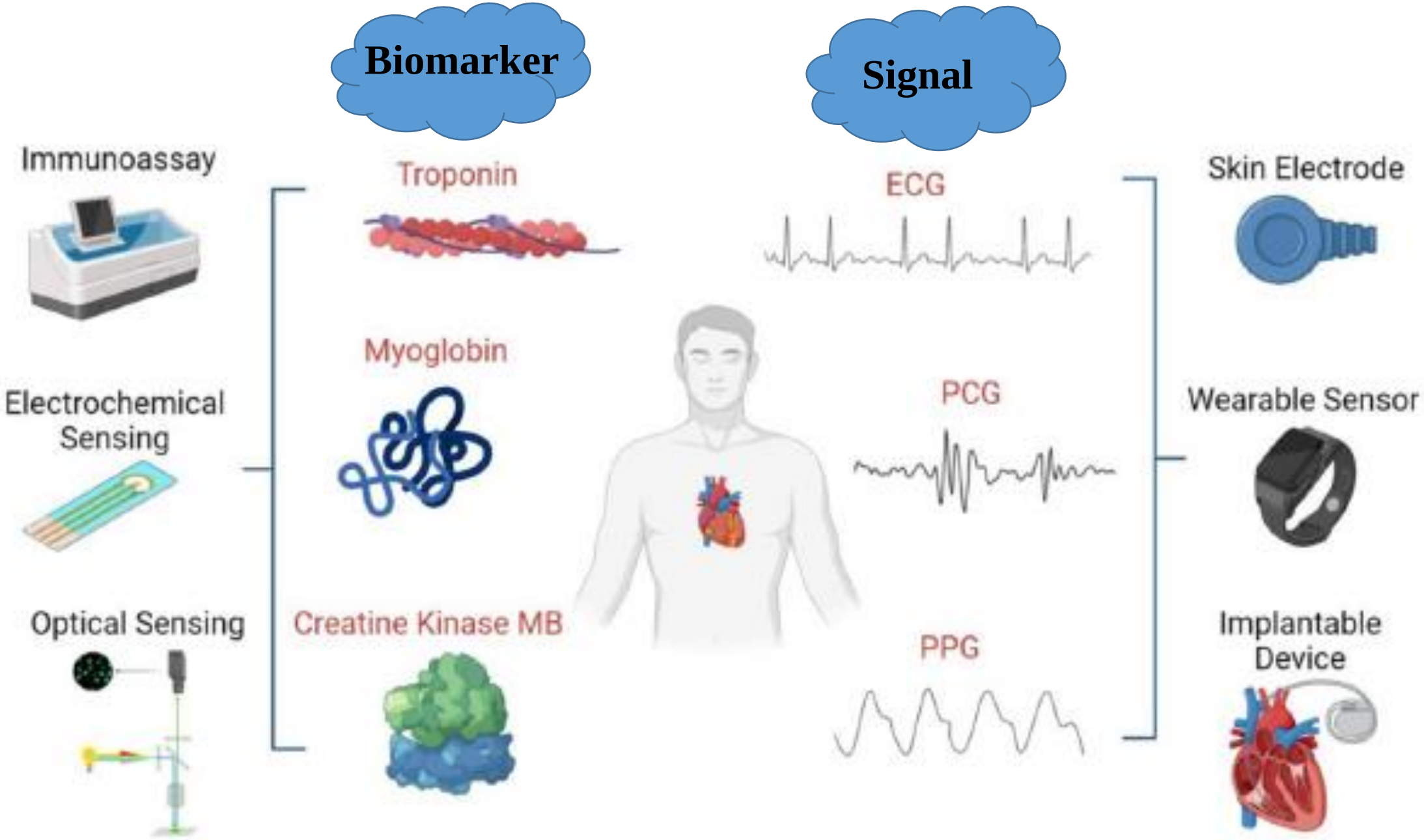
Classical of biosensor

Biomarker

- **Based on bioreceptor**: antibody-based biosensor, aptamer-based biosensor
- **Based on transducers**: electrochemical, optical, pressure
- **Lateral flow assay**
- **Microfluidic paper-based sensors**
- **biochip**

Signal

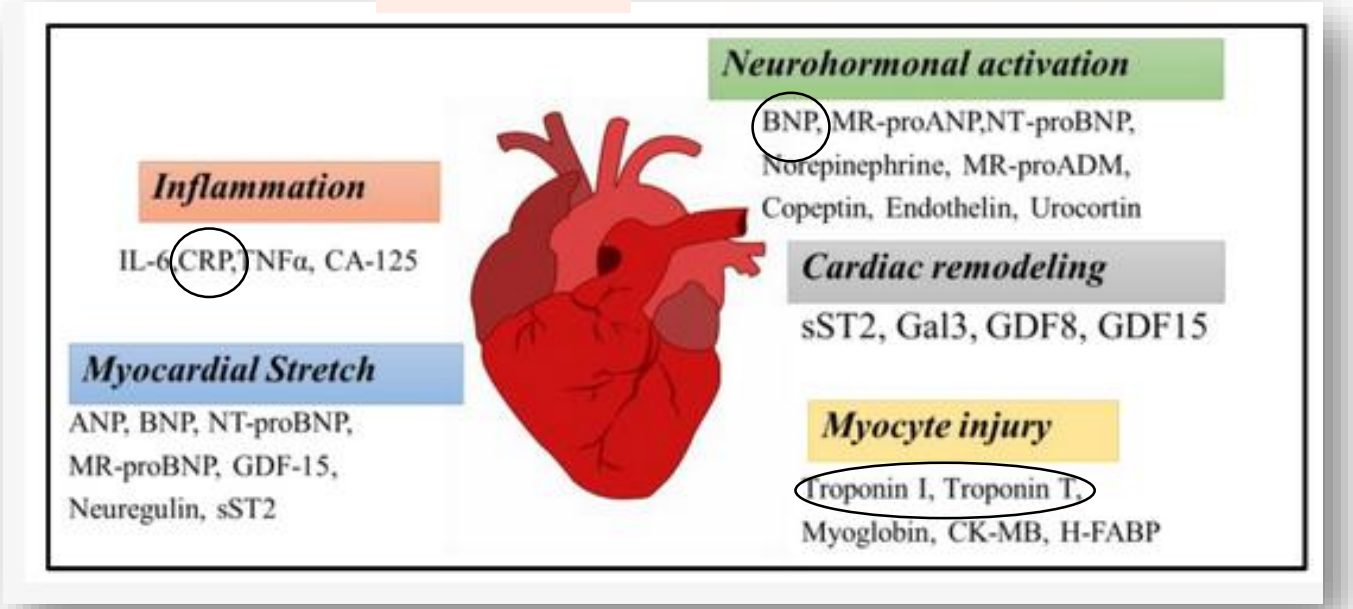
- Pulse wave monitoring
- PPG
- PCG
- ECG

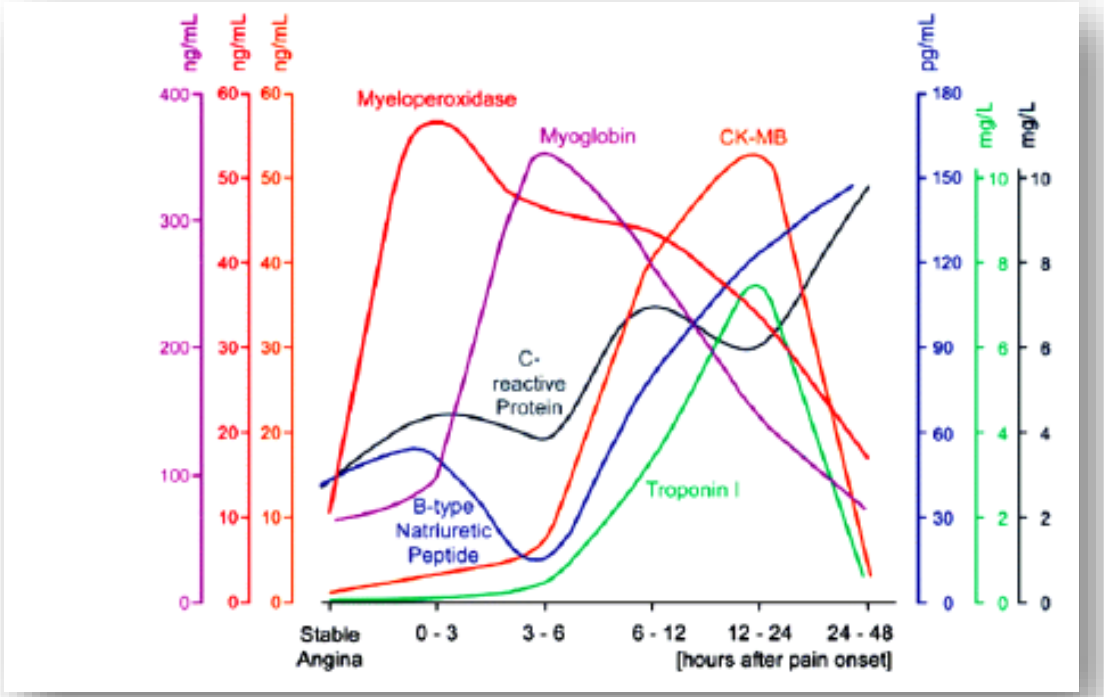

Biomarker
Signal
Immunoassay
Troponin
ECG
Skin Electrode
Myoglobin
Electrochemical Sensing
PCG
Wearable Sensor
Optical Sensing
Creatine Kinase MB
PPG
Implantable Device

What do you want to detect?

- Molecule
  Protein, peptide

Biomarker Release





POC tests

History

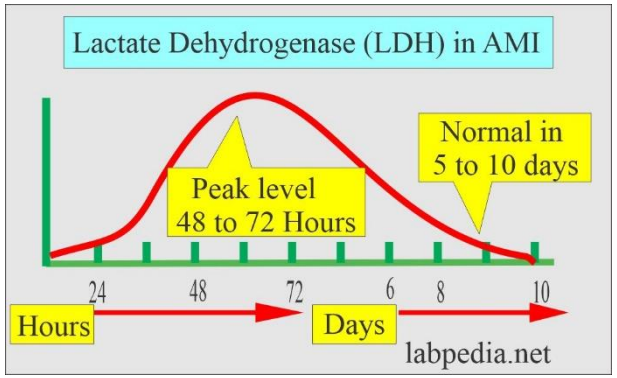
1960

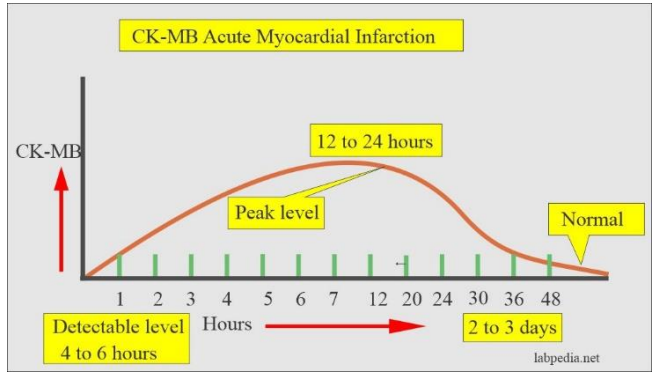
Aspartate Transaminase (AST) for acute myocardial infarction (AMI) detection

1970

Lactate dehydrogenase (LDH)

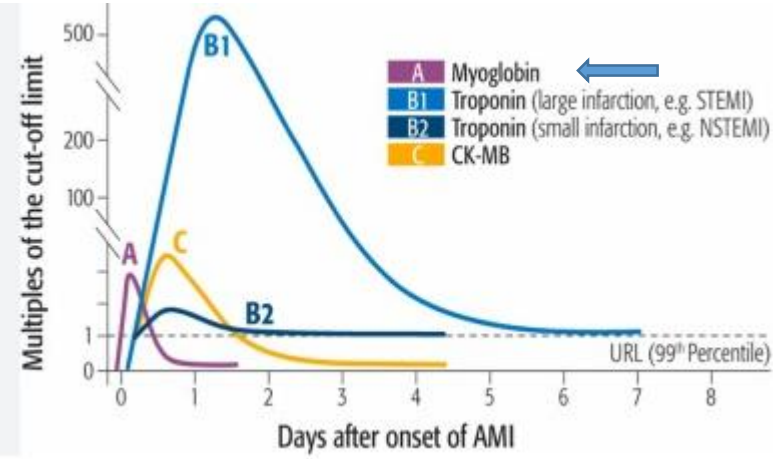
Creatine kinase (CK)





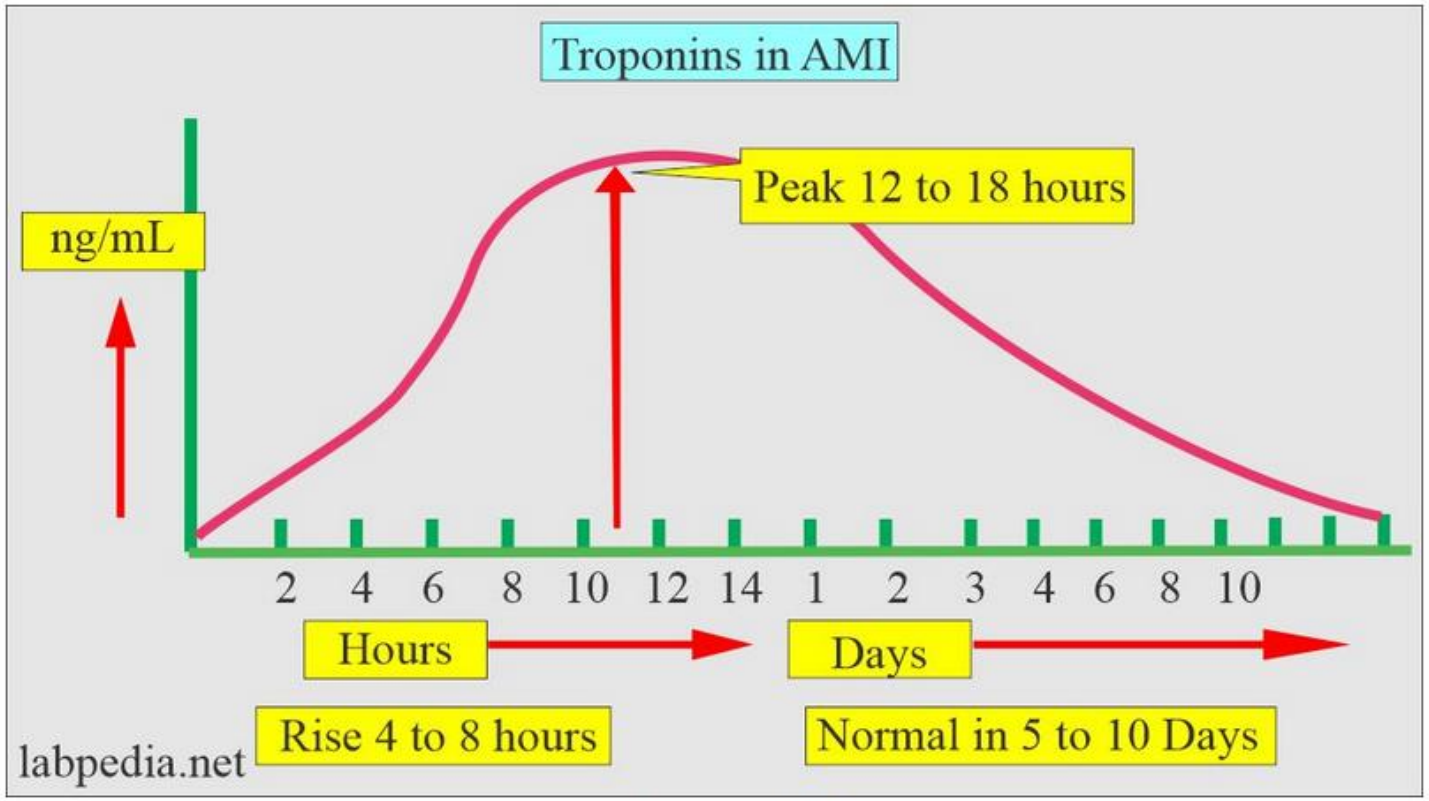
1978

Myoglobin



Cardiac troponins(cTn) biomarker

cTn sensing has become the golden standard myocardial infarction diagnosis, owing to its production only in the case of direct **damage of the myocardium**

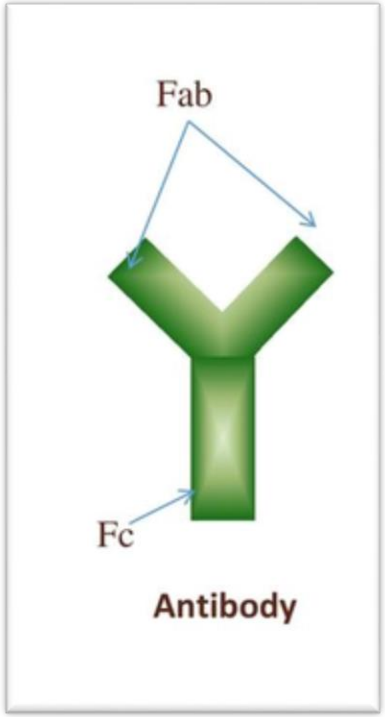


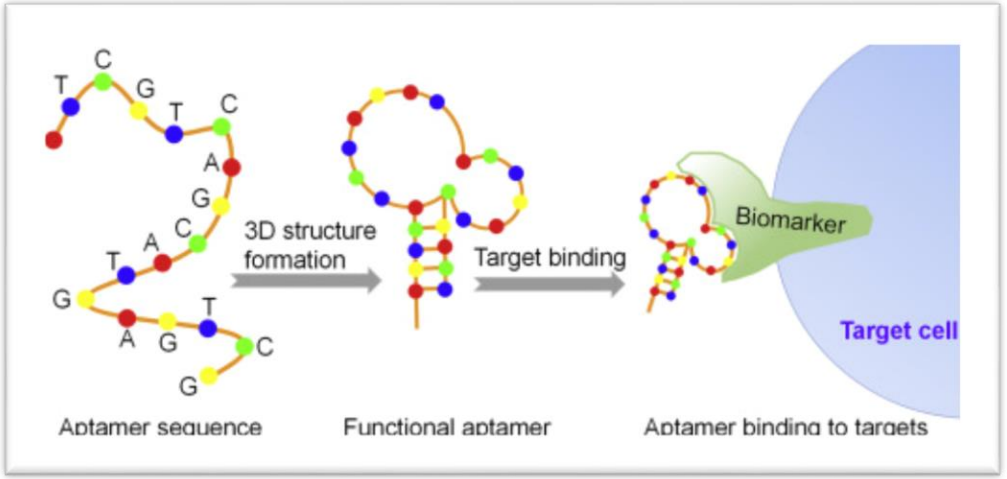
Troponins in AMI

A summary of primary clinically utilized cardiac biomarkers, highlighting their respective cut-off values.

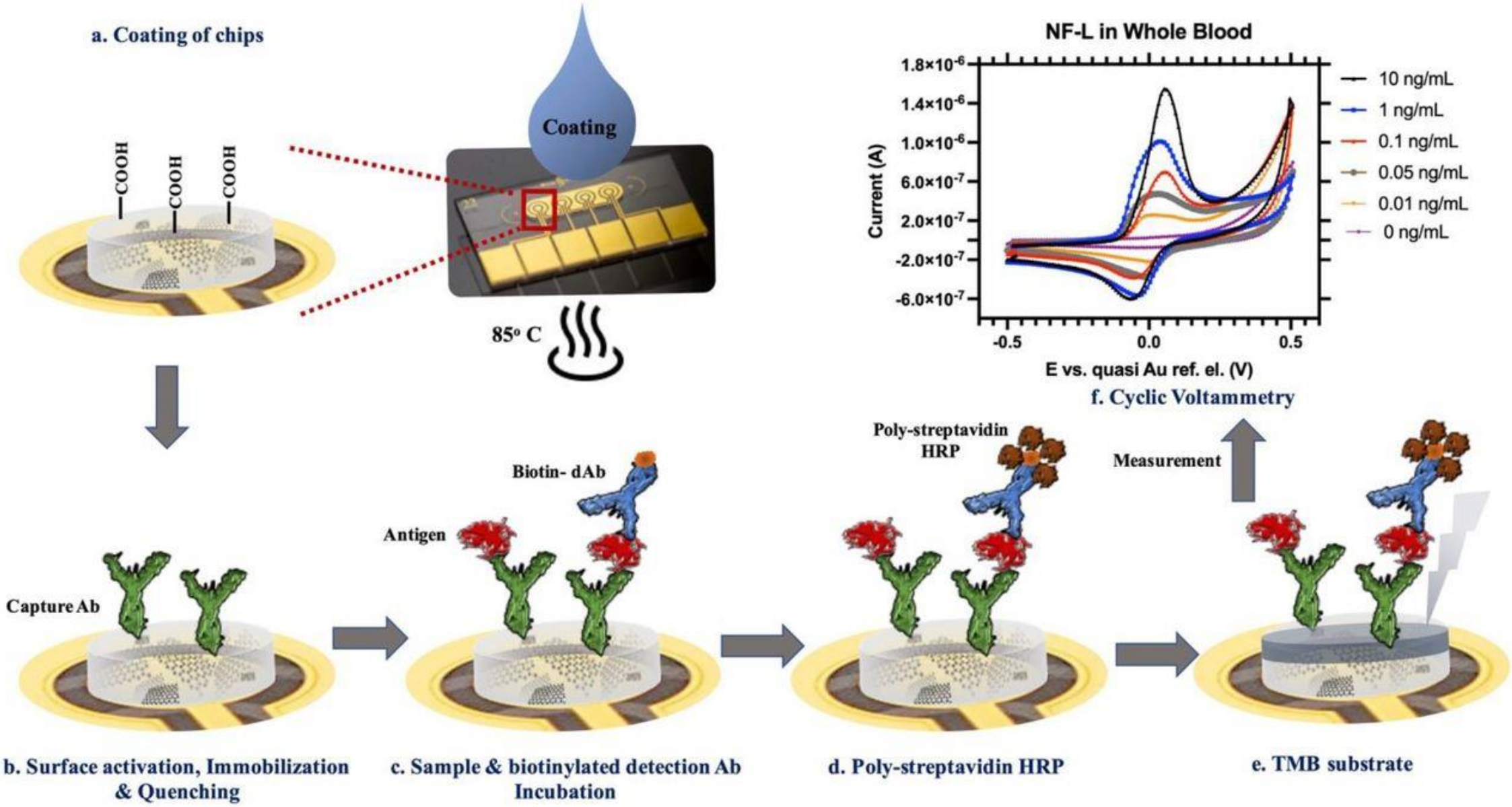
Cardiac biomarker	Type of cardiovascular diseases involved	Cut-off levels	Specificity (low, medium, high)	MW (kDa)	Initial elevation	Time to peak	Return to normal
Troponin I (cTnI)	Detection of acute myocardial infarction (AMI)	0.01–0.1 ng mL^{-1}	High	23.5	4–6 h	12–24 h	6–8 days
Troponin T (cTnT)	Detection of AMI	0.05–0.1 ng mL^{-1}	High	37	4–6 h	12–24 h	7–10 days
Myoglobin	Early detection of AMI	70–200 ng mL^{-1}	Low	18	1–3 h	6–12 h	24–48 days
C-reactive protein (CRP)	Early detection of inflammation/cardiac risk factor	$<10^3$ ng mL^{-1} low risk $1–3 \times 10^3$ ng mL^{-1} intermediate risk $>3–15 \times 10^3$ ng mL^{-1} high risk (no definitive)	High	125	ND	ND	ND
Creatine kinase MB subform (CK-MB)	Early detection of AMI	10 ng mL^{-1}	Medium	85	4–6 h	12–24 h	3–4 days
B-type natriuretic peptide (BNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload		High	3.4	ND	ND	ND
N-terminal pro-B-type natriuretic peptide (NT-proBNP)	Acute coronary syndromes/diagnosis of heart failure/ventricular overload	0.25–2 ng mL^{-1}	High	8.5	ND	ND	ND
Myeloperoxidase (MPO)	Detection of inflammation	Patients with elevated MPO levels >350 ng mL^{-1} stratification risk	Medium	150	ND	ND	ND
Heart fatty acid binding protein (H-FABP)	Myocardial necrosis	Patients with elevated H-FABP levels elevated $\geq$6 ng mL^{-1} stratification risk	Low	15	2–3 h	8–10 h	18–30 h
TNF-α	Inflammation/cardiac risk factor	<0.0036 ng mL^{-1} low risk $\geq$0.0036 ng mL^{-1} high risk	ND	ND	ND	ND	ND
Interlukin-6 (IL-6)	Inflammation/cardiac risk factor	Low < 0.0013 ng mL^{-1} Mid 0.00138–0.002 ng mL^{-1} High > 0.002 ng mL^{-1}	ND	ND	ND	ND	ND
Fibrinogen		Low $< 3.58 \times 10^6$ ng mL^{-1} Mid $3.58–4.20 \times 10^6$ ng mL^{-1} High $> 4.20 \times 10^6$ ng mL^{-1}	ND	ND	ND	ND	ND

Biological elements



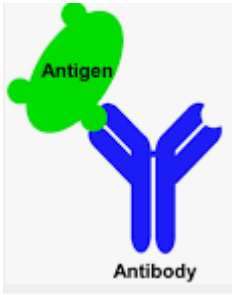


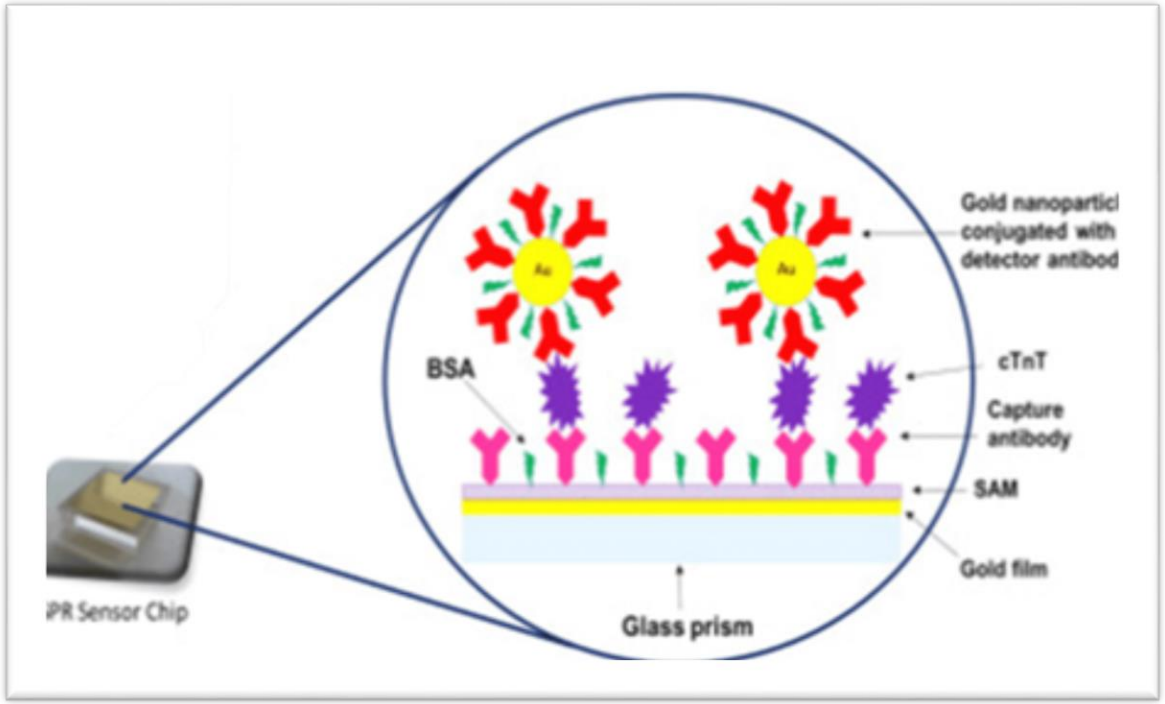
Antibody/antigen(Immunosensor):



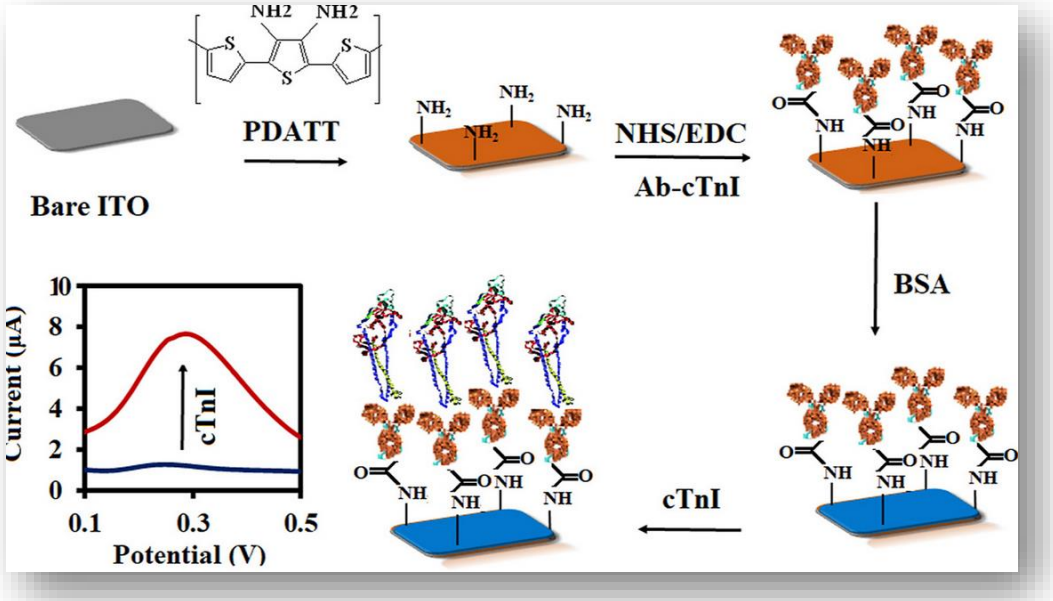
Antibody/antigen(Immunosensor):

- high specificity between an antibody and antigen

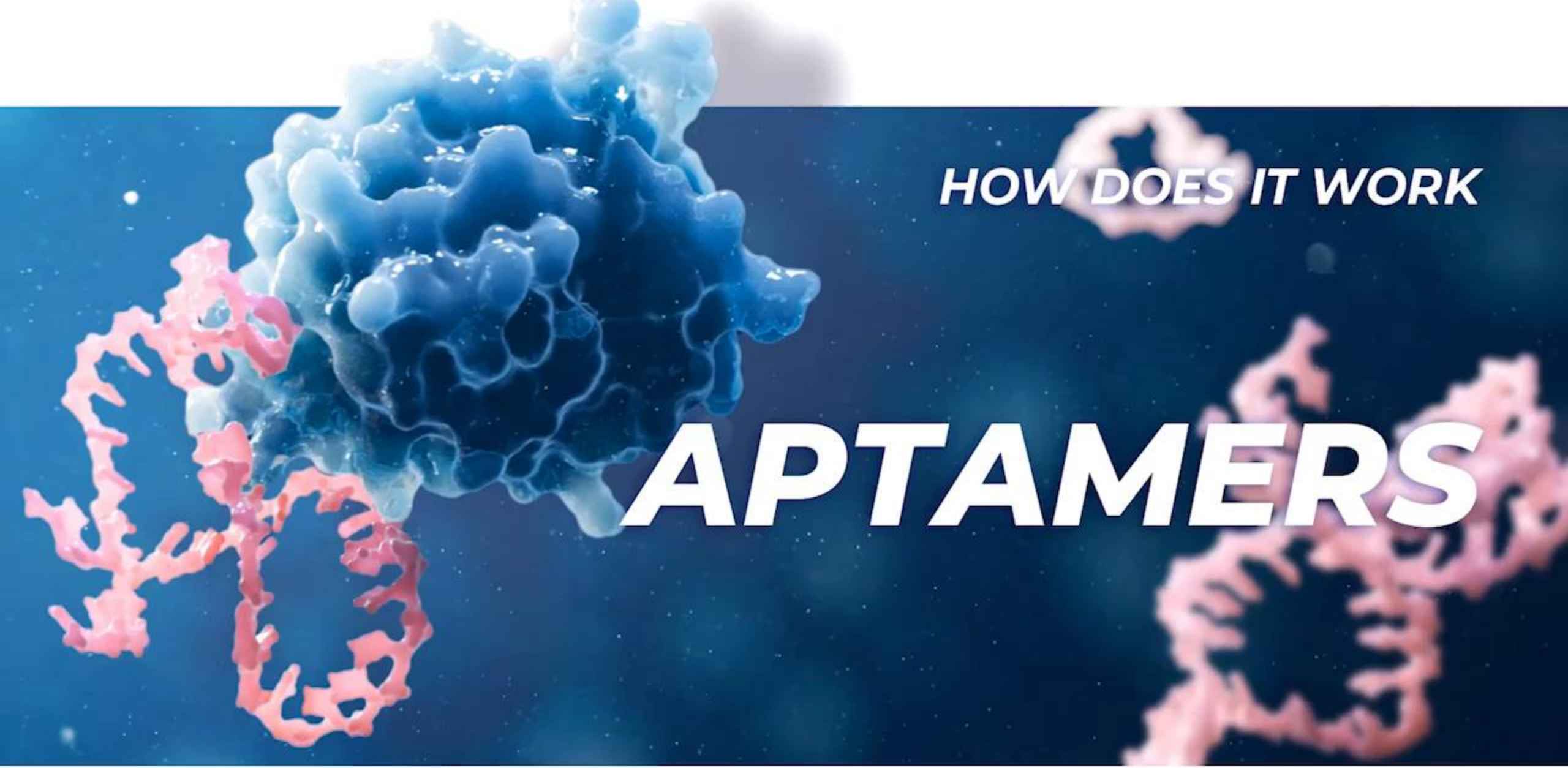




LOD: 100 ng/mL within 2 min



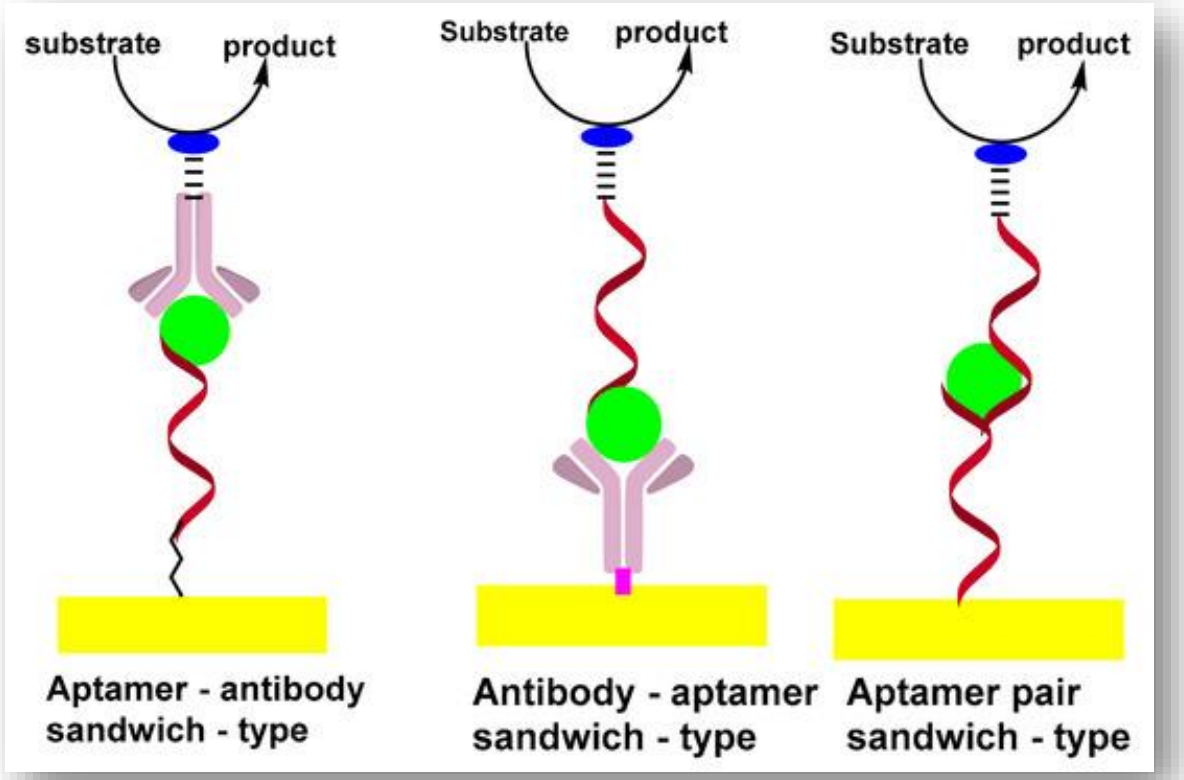
LOD: 0.01 ng/ mL

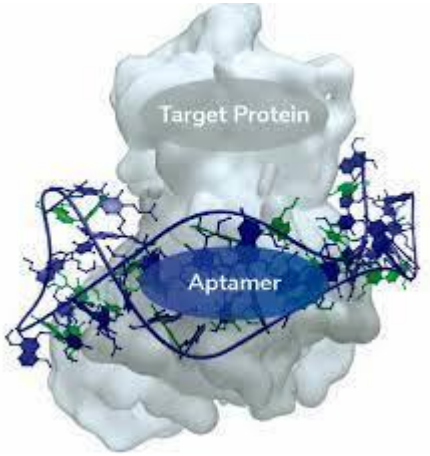


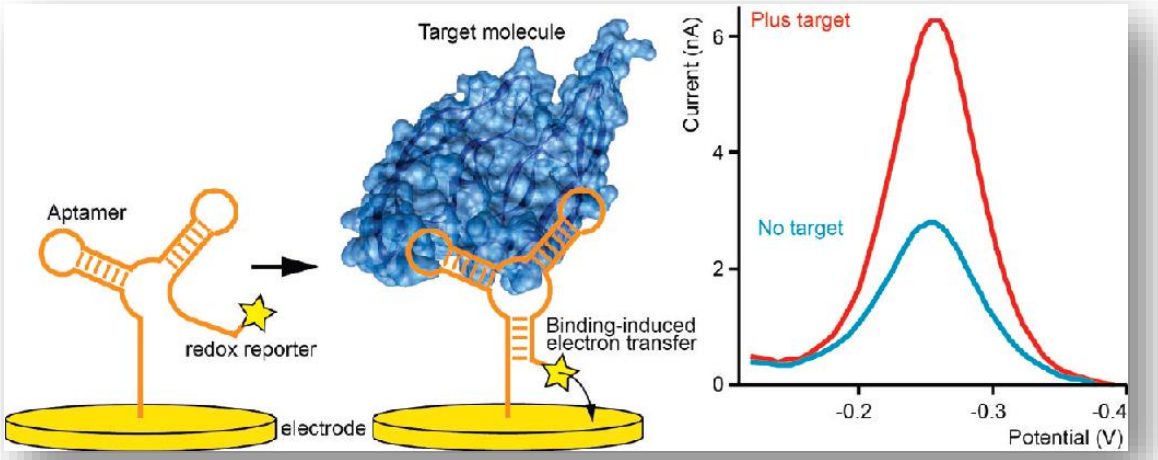
药明康德
WuXi AppTec

	Aptamer	Antibodies
Molecular weight	Small (~12–30 kDa)	Relatively big (~150–180 kDa)
Secondary structures	Various structures: hairpin, loop, G-quadruplex, *etc*	β-sheets
Generation time	Few hours to months	Several months (~six months)
Batches variations	Low	High
Immunogenicity	Low	High
Minimal target size	Targets small sizes ~60 Da	~600 Da
Targets	Wide range of targets	Immunogenic molecules
Shelf life	Long	Short
Allowed chemical modifications	Various modifications	Limited modifications
Nuclease degradation	Sensitive	Resistant
In vivo half-life	Short (~20 min)	Long (~one month)
Stability	Very stable	Sensitive to temperature and pH changes
Cost	Lower	Higher

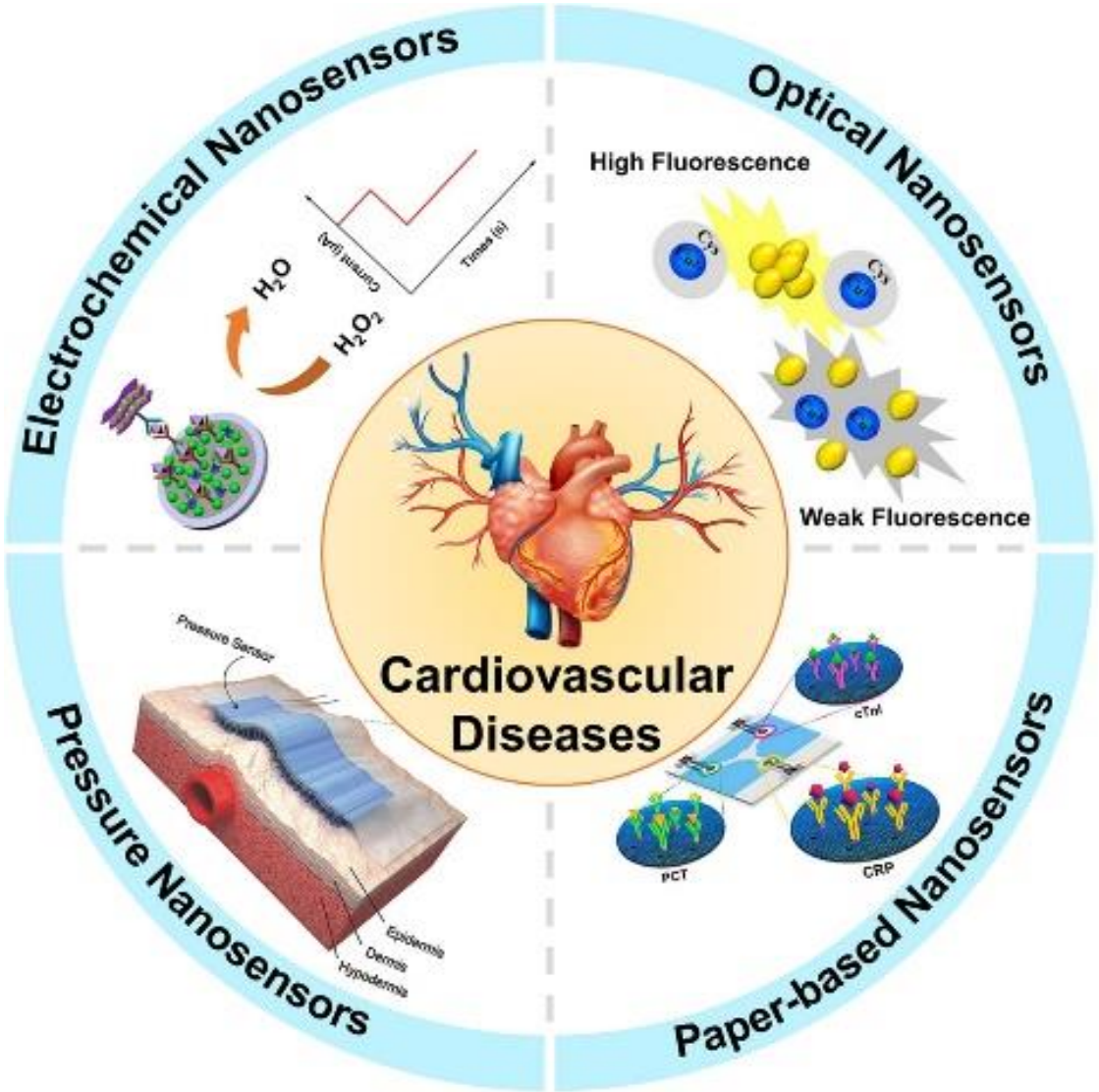
Aptasensor


substrate
product
Substrate
product
Substrate
product
Aptamer - antibody sandwich - type
Antibody - aptamer sandwich - type
Aptamer pair sandwich - type


Target Protein
Aptamer

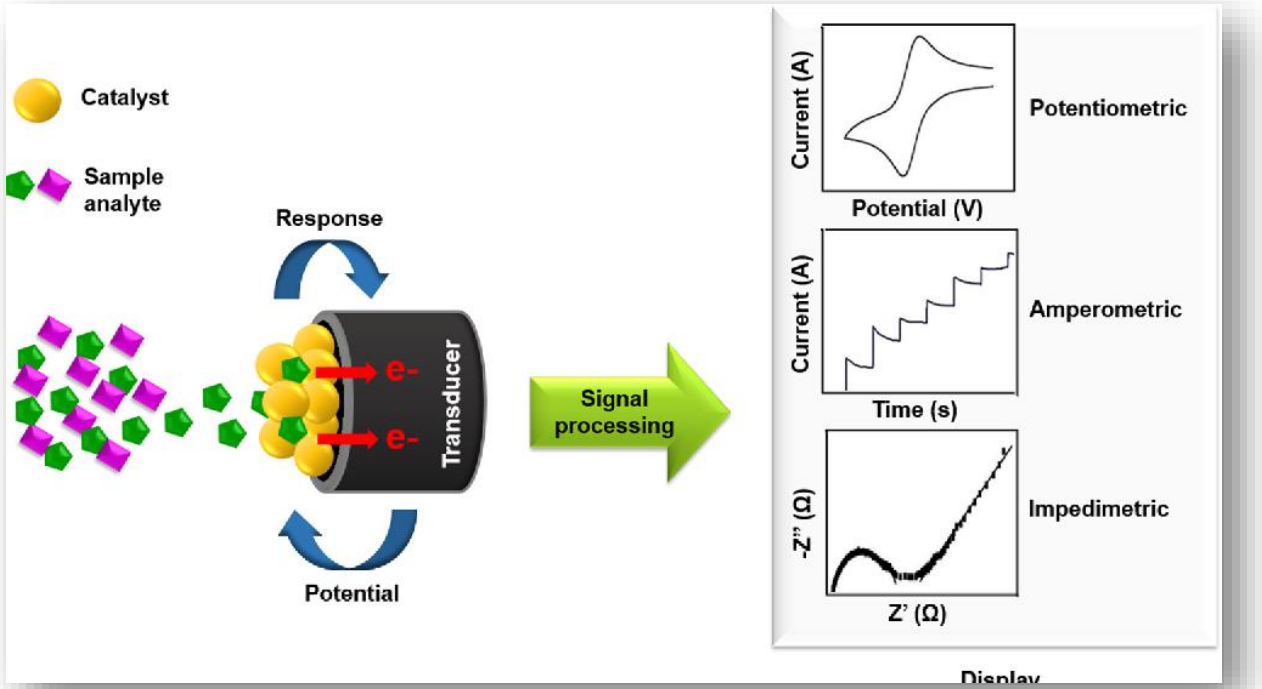

Target molecule
Aptamer
redox reporter
electrode
Binding-induced electron transfer
Plus target
No target
Current (nA)
6
4
2
0
-0.2
-0.3
-0.4
Potential (V)

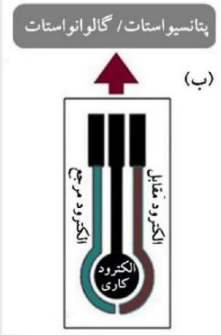
How to detect cardiac biomarkers?

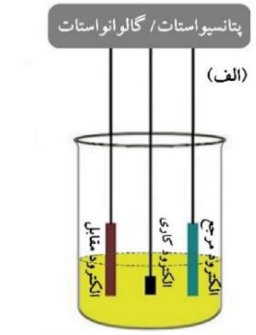


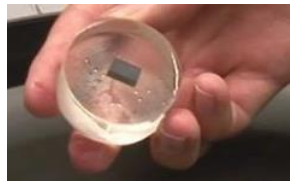
Electrochemical Nanobiosensor

- sensing mechanism: electrical and chemical
- Advantages: real time detection, high sensitivity, cheap Instrument
- Disadvantage: limited temperature range

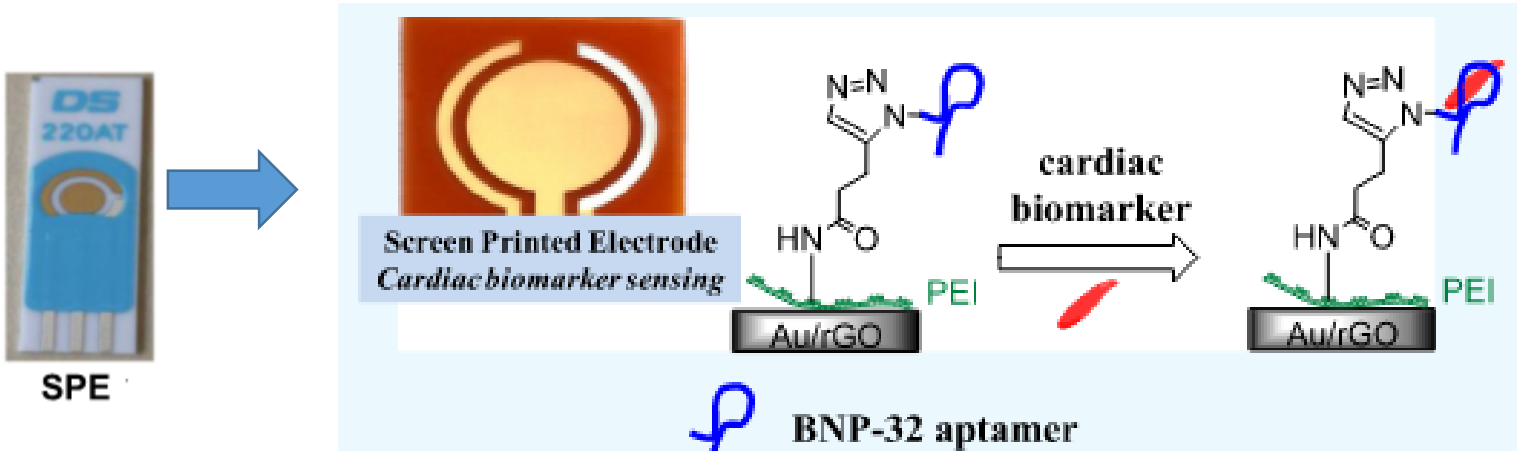


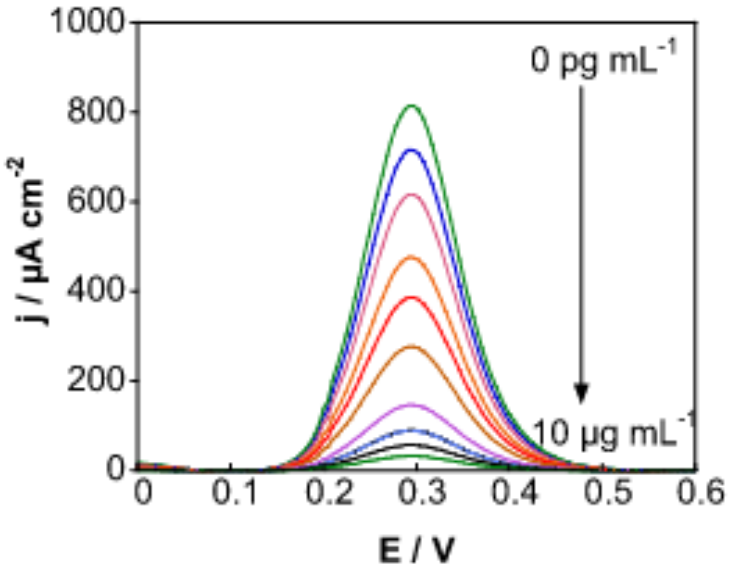


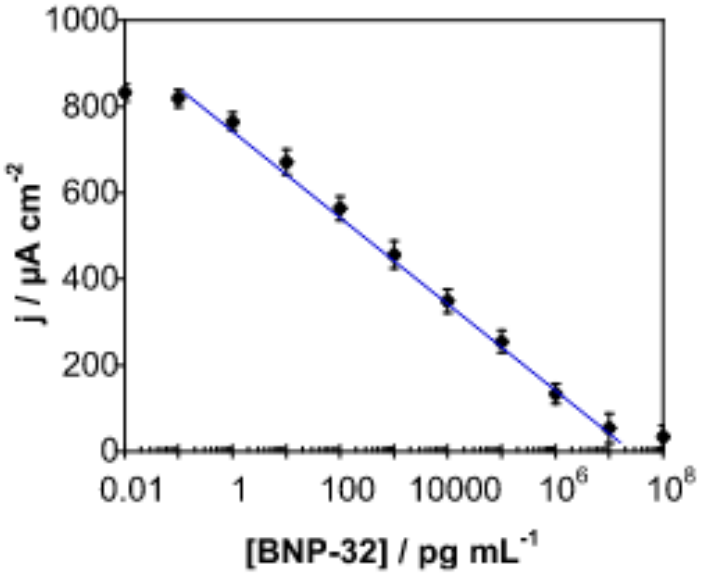


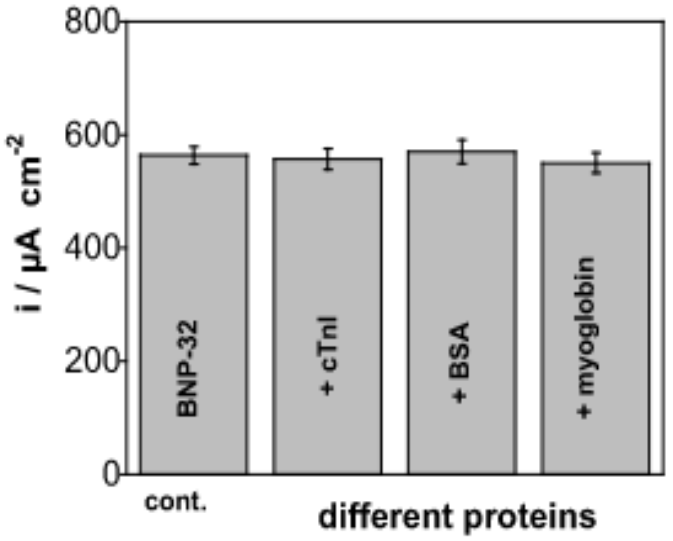


Electrochemical Nanobiosensor









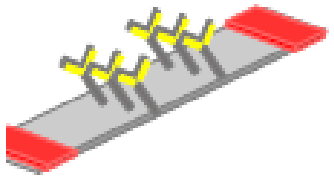
Optical Nanobiosensor

A. Colorimetric assay

Ag NPs enhancement

LOD: 10 pg ml^{-1} (cTnI)

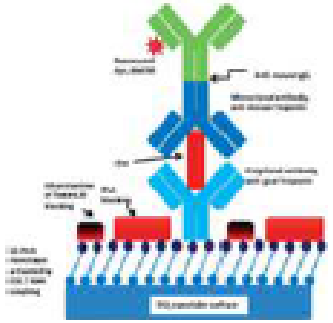
Wu et al Sens. Actuators B 147 (2010) 298–303.



CARDIAC AFFINITY BIOSENSORS

B. Fluorescence immunoassay

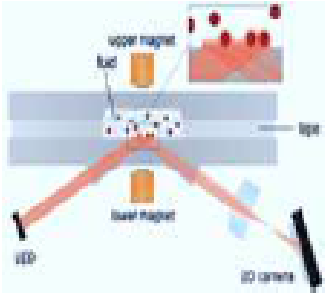
LOD: 0.1 pg ml^{-1} (cTnI)



P. Kar, Lab Chip 12 (2012) 821-828

E. Optomagnetic

LOD: 11 pg ml^{-1} (cTnI)

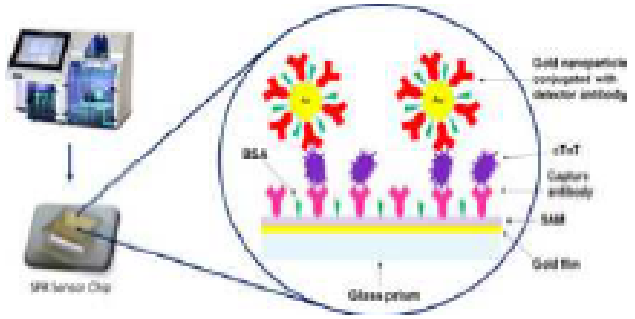


Bruls et al, Lab Chip, 9 (2009) 3504-3510.

C. Surface Plasmon Resonance

detection of cTnT using AuNP modified detector antibodies.

LOD: 500 pg mL^{-1} cTnT



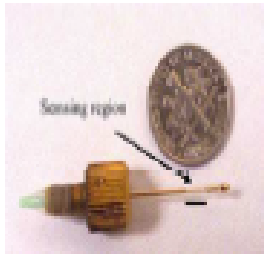
Pawula et al Talanta 146 (2016) s 823-830.

D. Fiber-optic-based SPR sensor

Antibodies attached to carboxymethylated dextran layer

LOD: 2.9 ng ml^{-1} (MG)

1.4 ng ml^{-1} (cTnI)



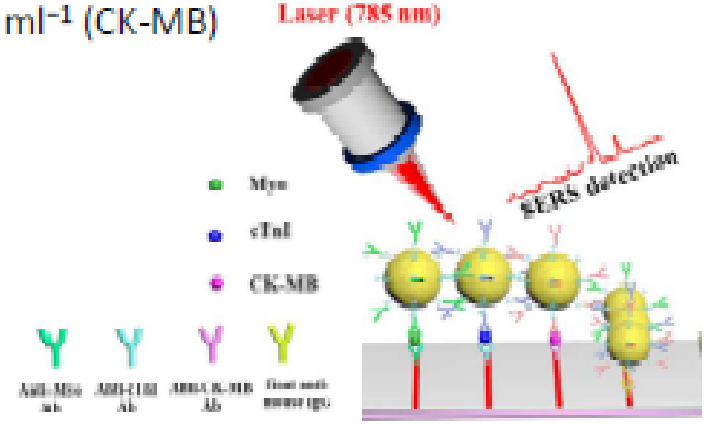
Masson et al Talanta 62 (2004) 865.

F. Surface Enhanced Raman Spectroscopy

Ag$^{\text{Nile blue A}}$@Au NPs modified detector antibodies

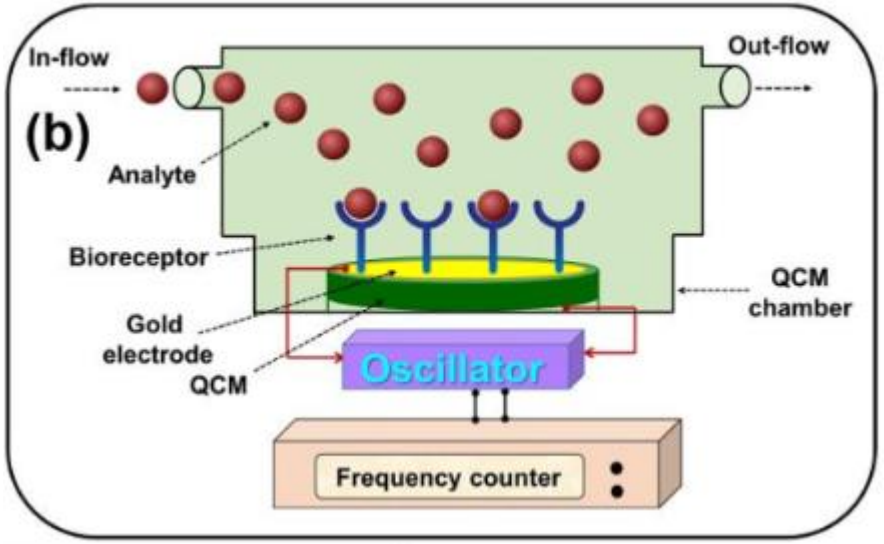
LOD: 1 ng ml^{-1} (MG); 0.8 ng ml^{-1} (cTnI);

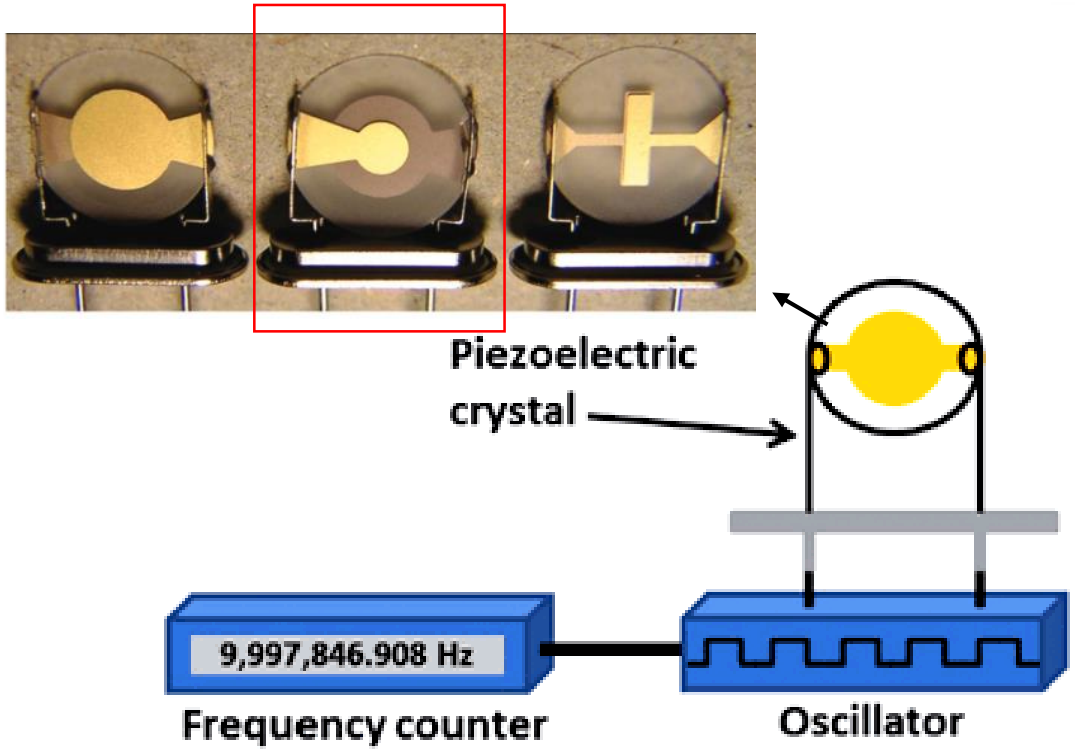
0.7 ng ml^{-1} (CK-MB)

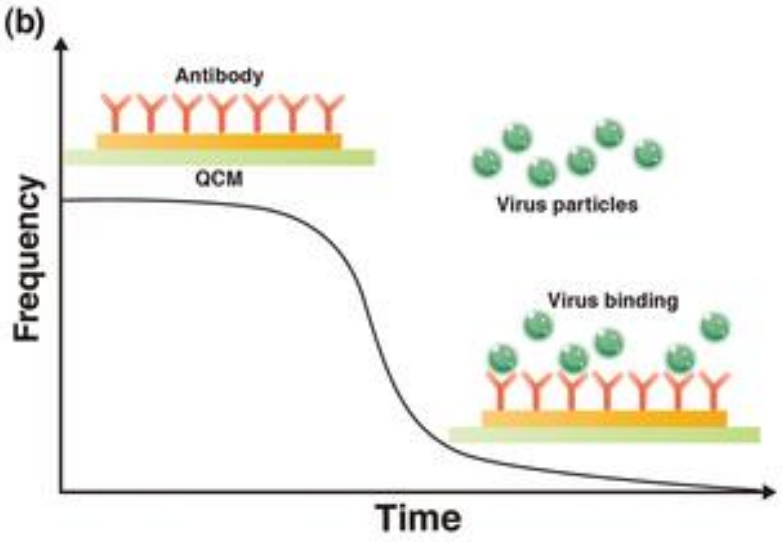


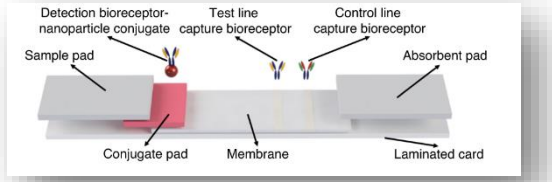
Zhang et al Biosens. Bioelectron. 106 (2018) 204.

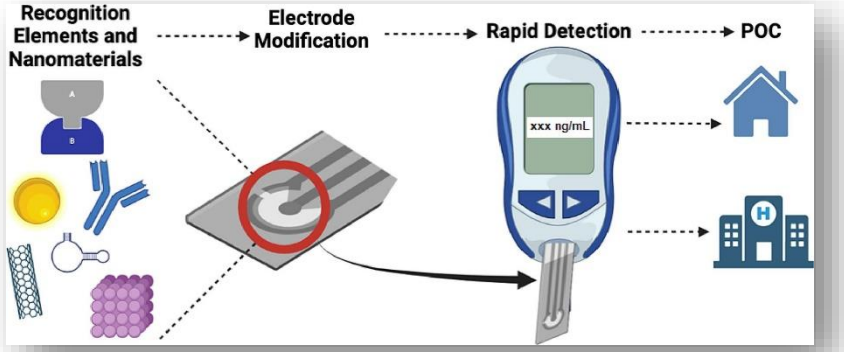
Pressure Nanobiosensor





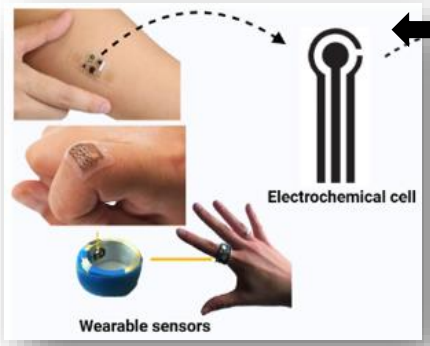


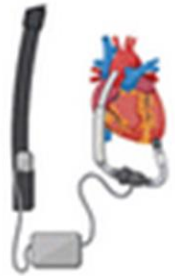




Point-of-care Testing

Wearable Devices



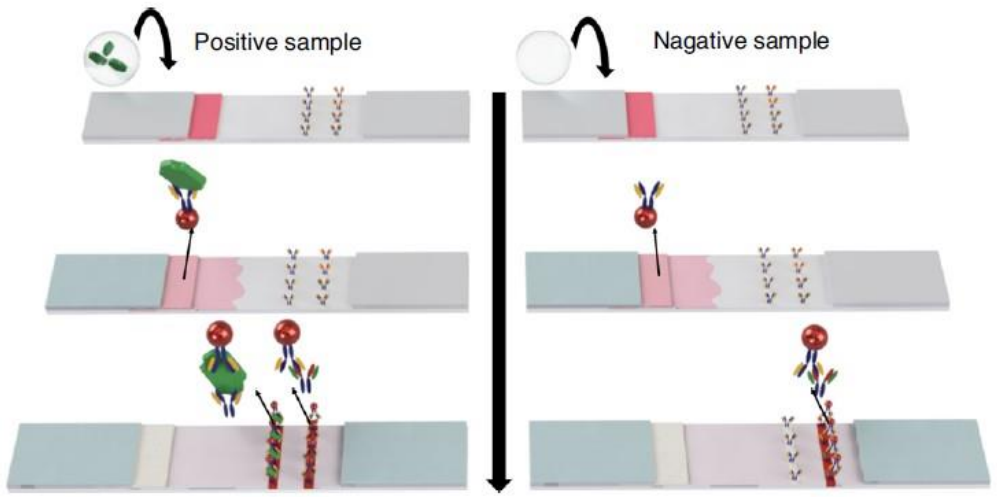


Implantable Devices

Cardiovascular Disease Biosensing

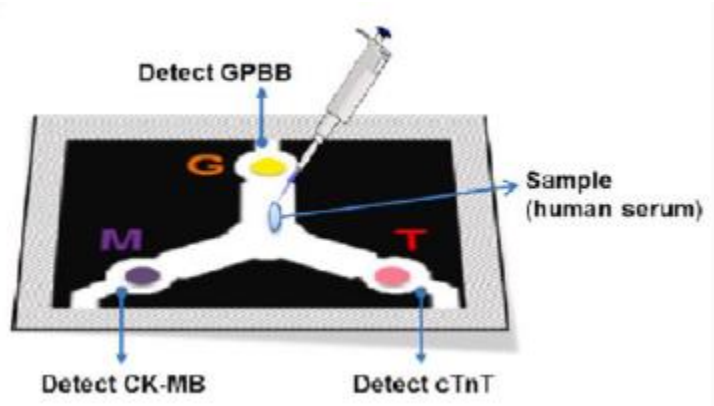
Lateral flow assay (LFA)

- Disadvantage: Limited space on lateral flow strips, a high number of required samples, reduced measurement sensitivity



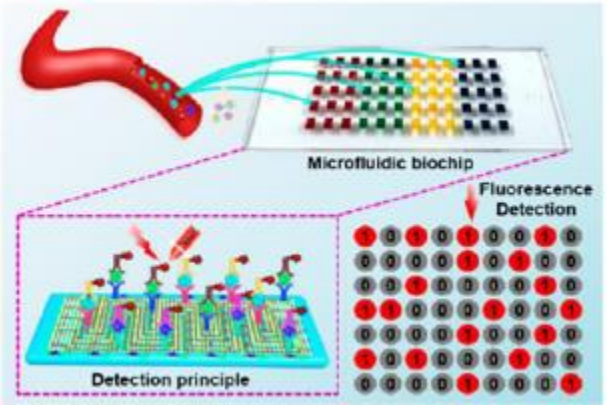
Microfluidic paper-based device (μPAD)

- ❑ Advantage: large sample size testing



Biochip array

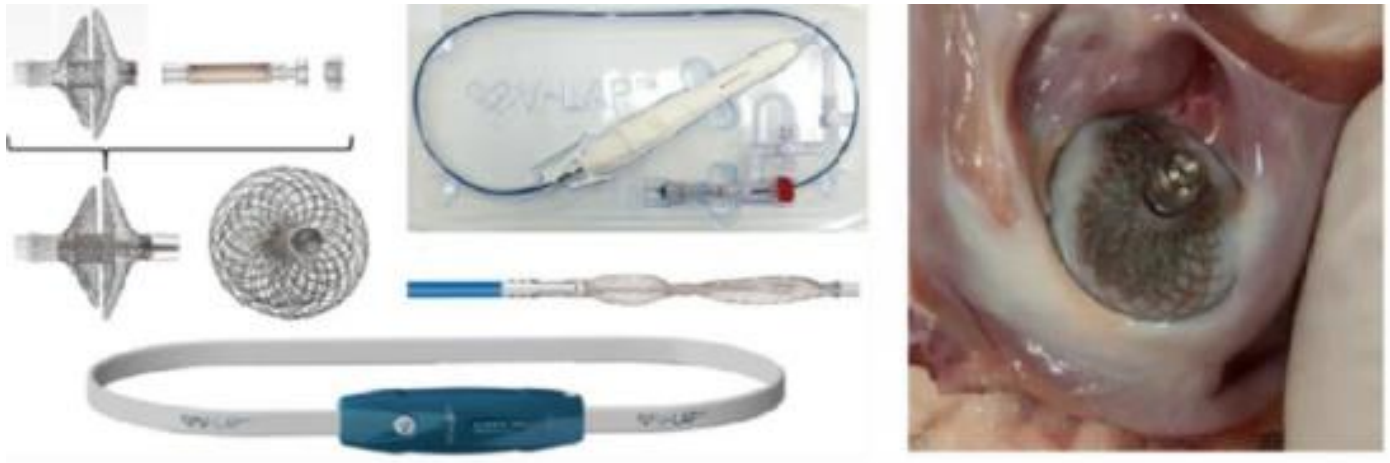
- ❑ Advantage: more suitable for large-scale clinical needs, large sample size testing,



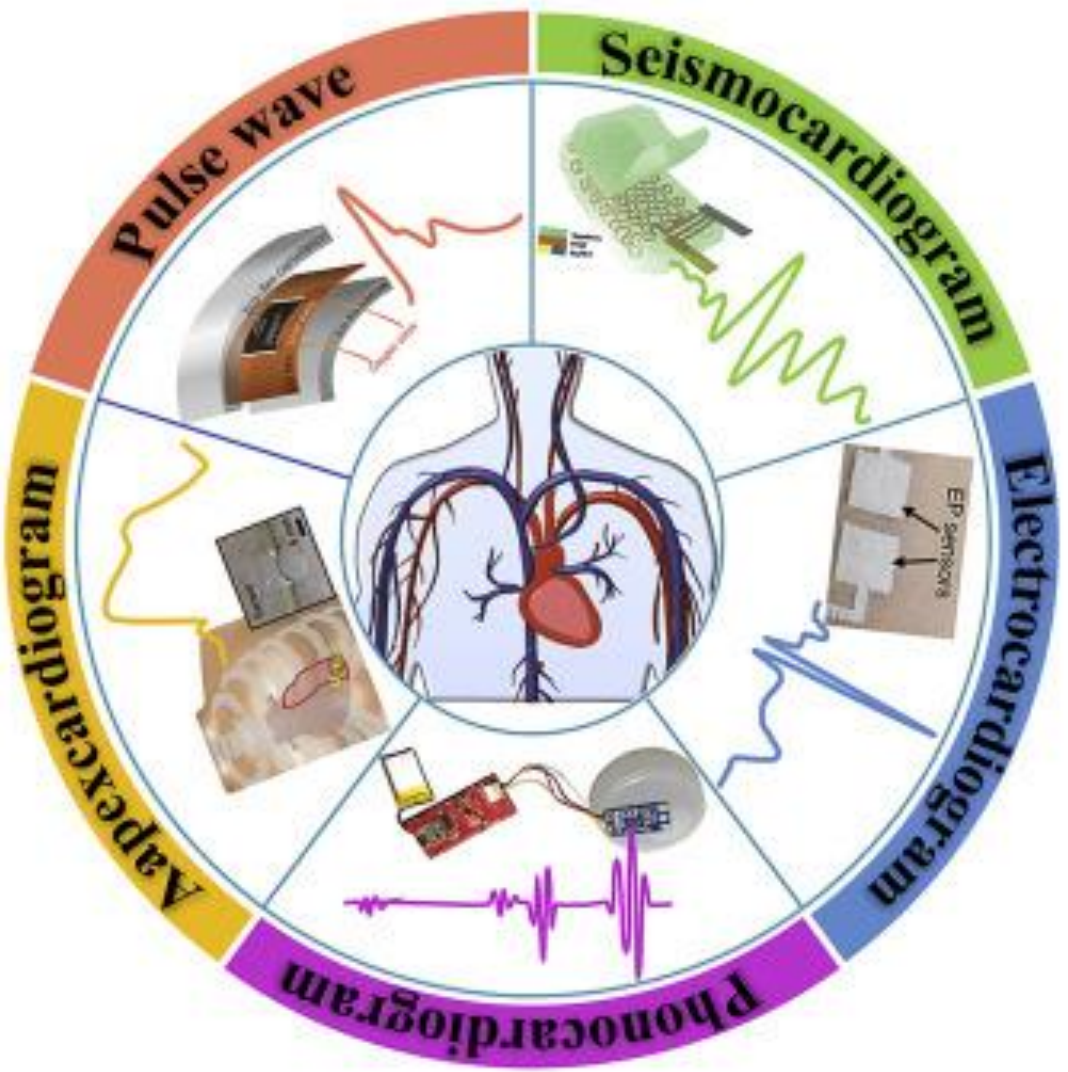
Implantable Cardiac Sensing Devices (CIED)

- ❑ sensing mechanism: mechanical and electrical and optical
- ❑ CIED can directly capture physiological parameters, convert them to electrical signals, and wirelessly transmit the data for display and processing.

- ❑ Advantages: particularly suitable for long-term postsurgical care; real-time monitoring
- ❑ disadvantage: risk for infection; invasiveness; limited device lifespan; need for power-harvesting feature



Signal sensor



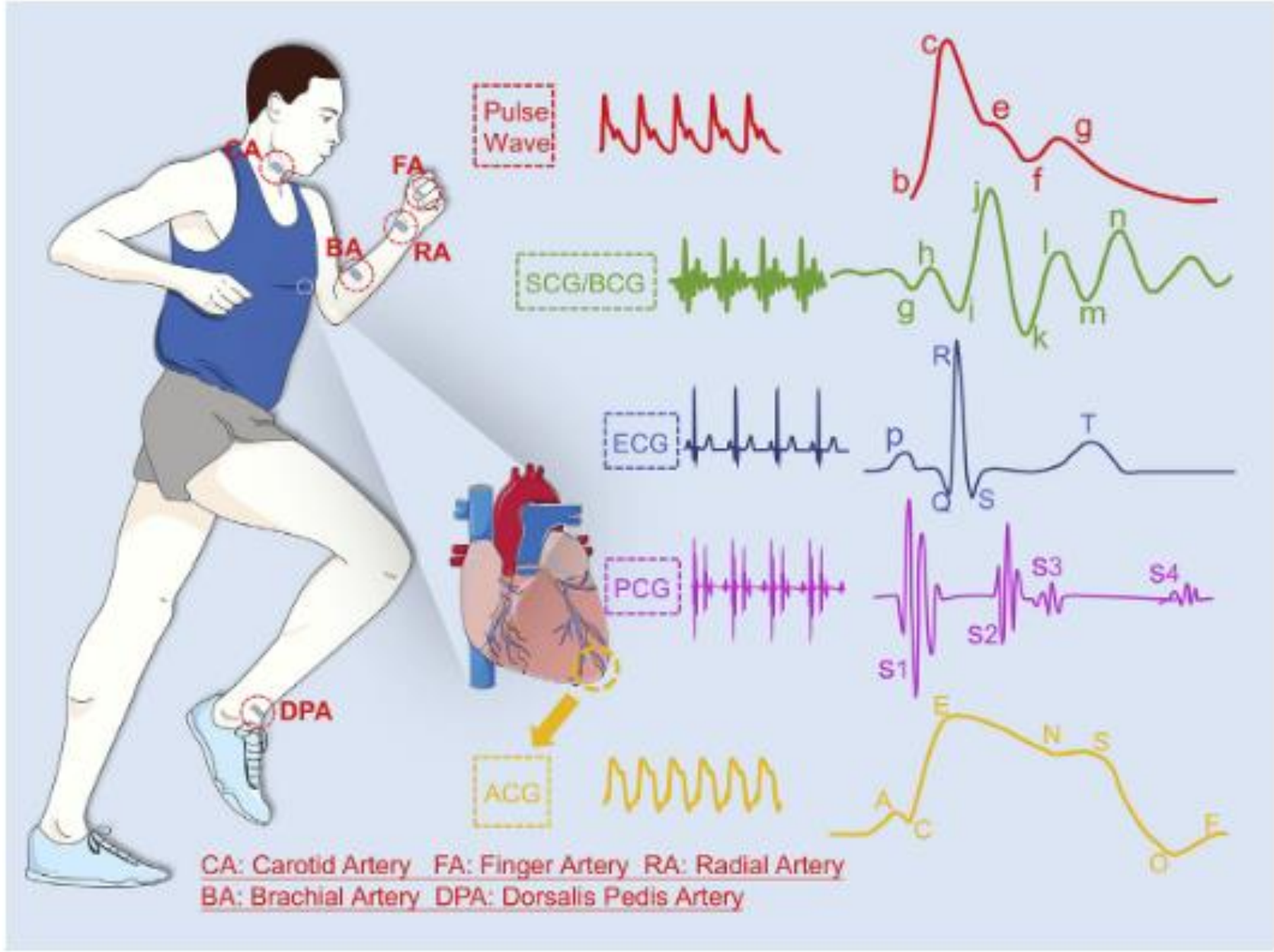


Figure 1. Schematic illustration of multiple physiological signals for the prevention of CVD

Physiological signals include pulse wave, SCG/BCG, ECG, PCG, and ACG.

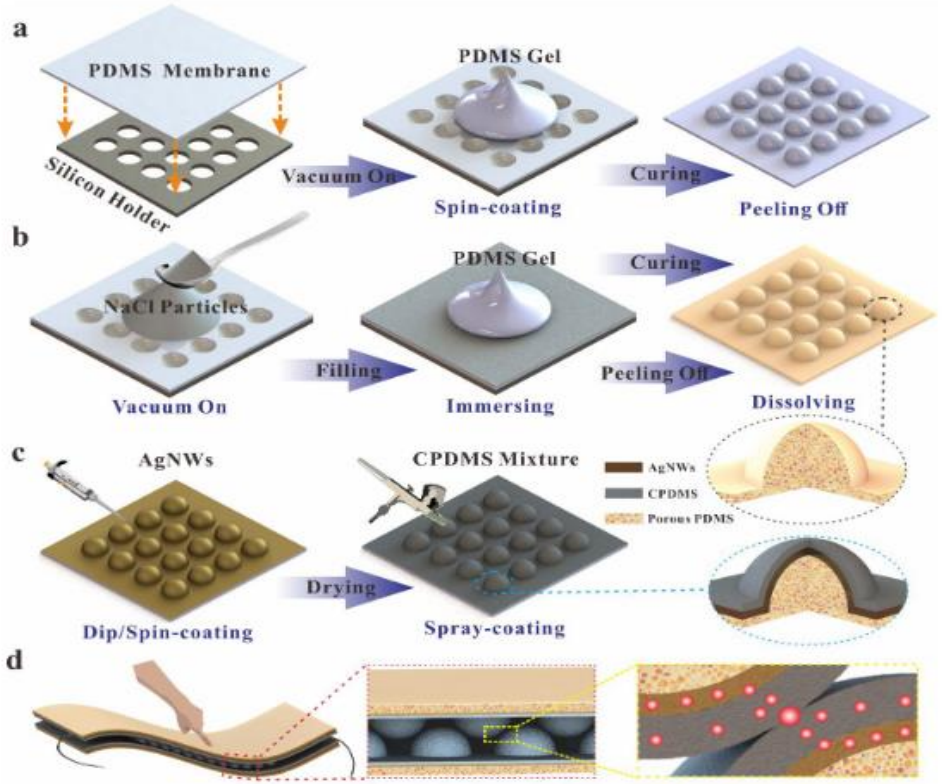
Pulse wave monitoring

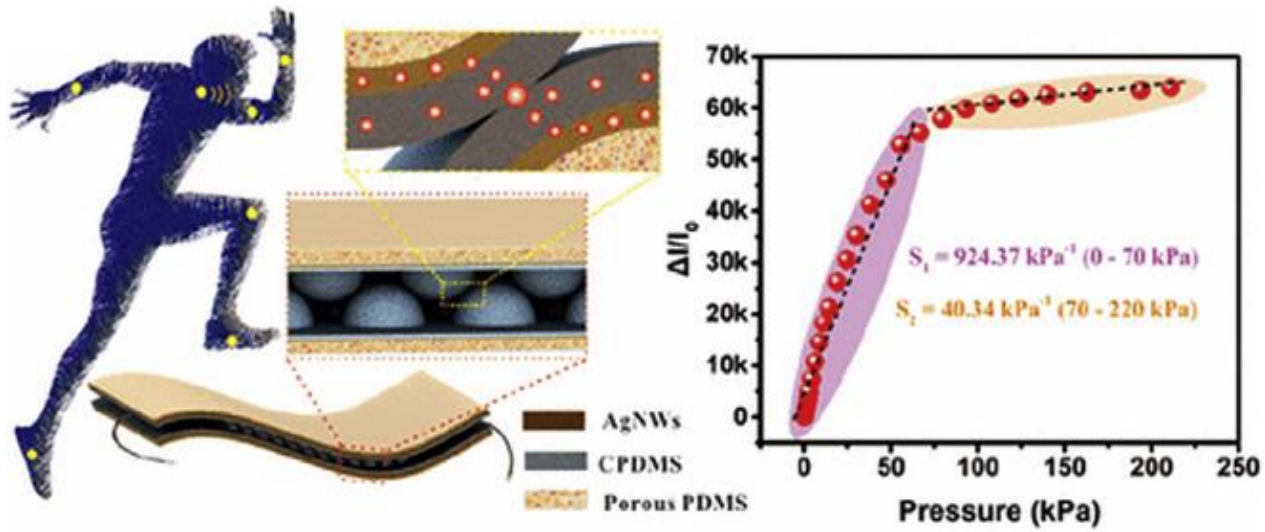
- Flexible piezoresistive sensor
- Flexible pressure sensor
- Flexible self-powered pressure sensor
- Flexible capacitive pressure sensor

Pulse wave monitoring: piezoresistive sensors

- Flexible piezoresistive sensors are used for pulse wave monitoring

- Ji et al constructed a solid microdome array dual conductive layer sensor using cyclohexane polydimethylsiloxane (CPDMS)/AgNWs.





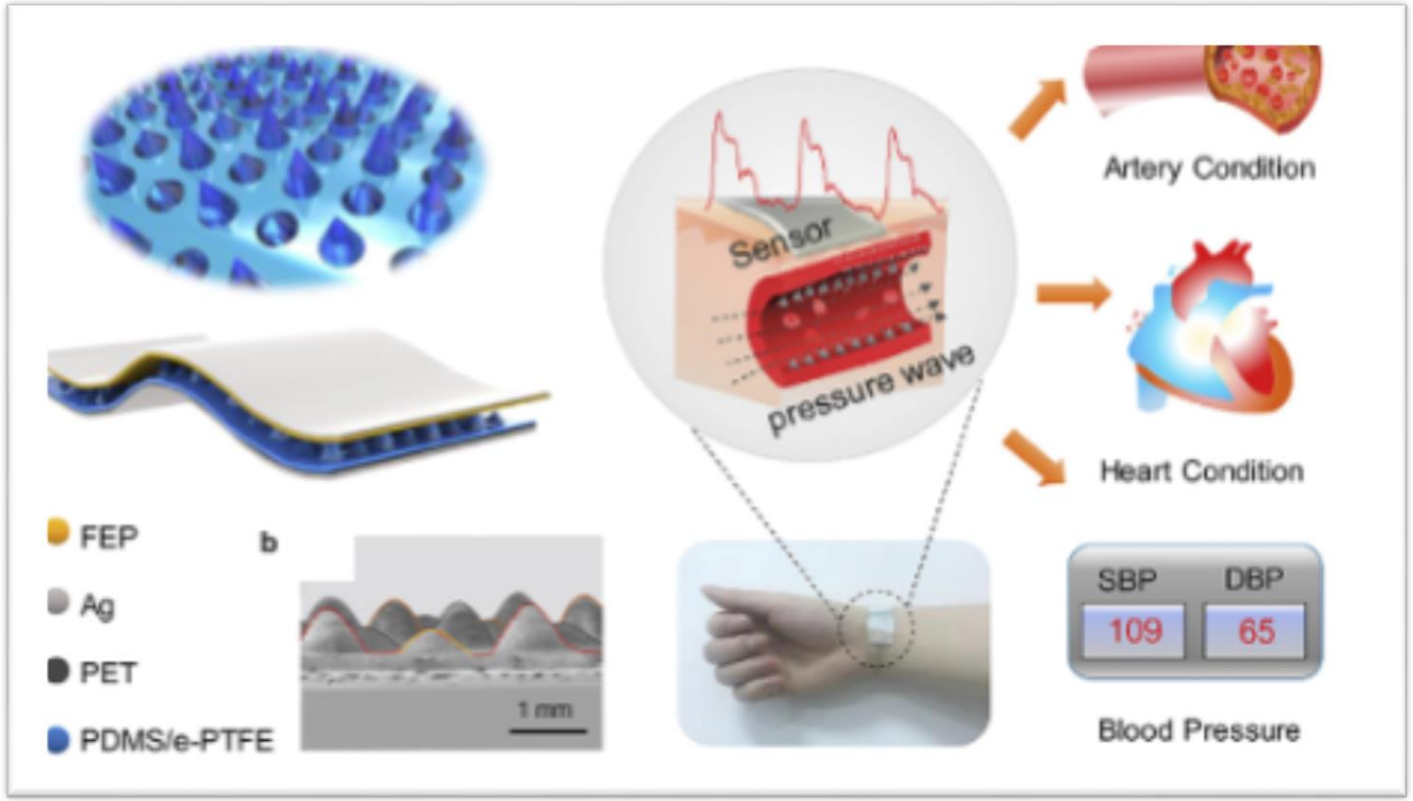
Pulse wave monitoring: self-powered pressure sensors

- Flexible **self-powered pressure**(do not require an external power supply) sensors are used for pulse wave monitoring

advantages : direct risk indication; self-monitoring; highly reproducible

Disadvantages: accuracy affected by body movement

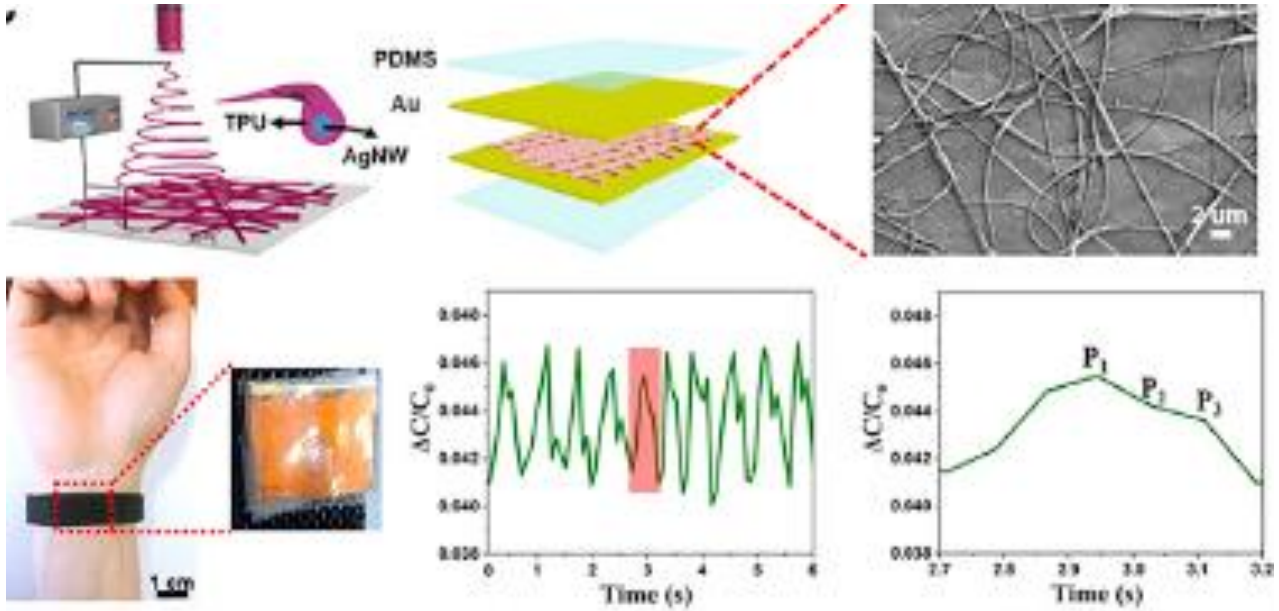
- Chen et al. reported an electrostatic nanogenerator-based self-powered pressure sensor based on hierarchical elastomer microstructures (HEM)



Pulse wave monitoring : capacitive pressure sensors

Flexible **capacitive pressure** sensors are used for pulse wave monitoring.

- The change of capacitance can be used to detect the change of pressure.
- rapid dynamic response, outstanding temperature insensitivity, low power consumption

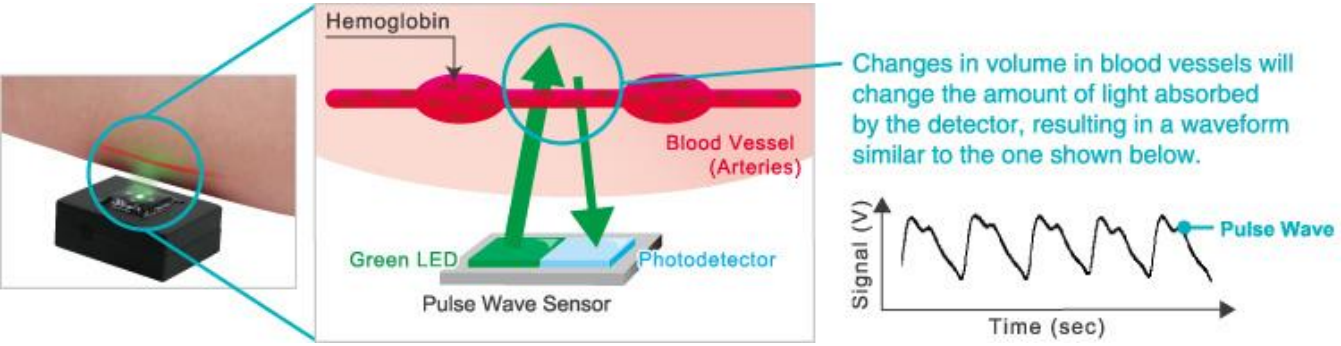


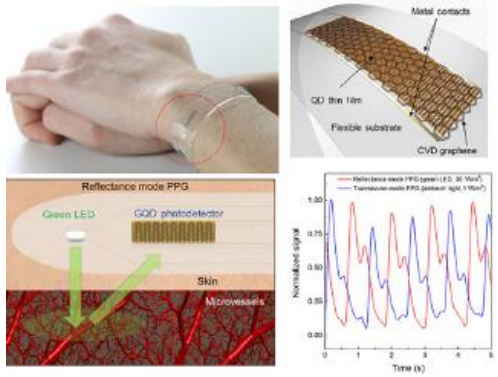
Flexible photoplethysmography (PPG)

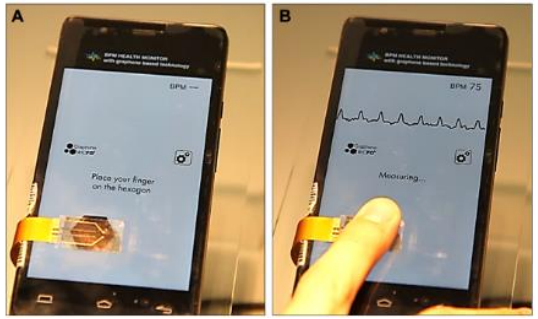
- ❑ sensing mechanism: optical
- ❑ measures changes in blood volume in the vessels by reflecting from the skin
- ❑ The flexible PPG method mainly starts from two aspects:

1. integrated chips on a flexible material substrate
2. flexible organic light-emitting materials and photosensitive materials

- ❑ Advantages: less expensive instrument; easy wearable device integration; no electrical interference
- ❑ disadvantages: need ECG signals as references; less suitable for heart rate variability measurement; long setting time







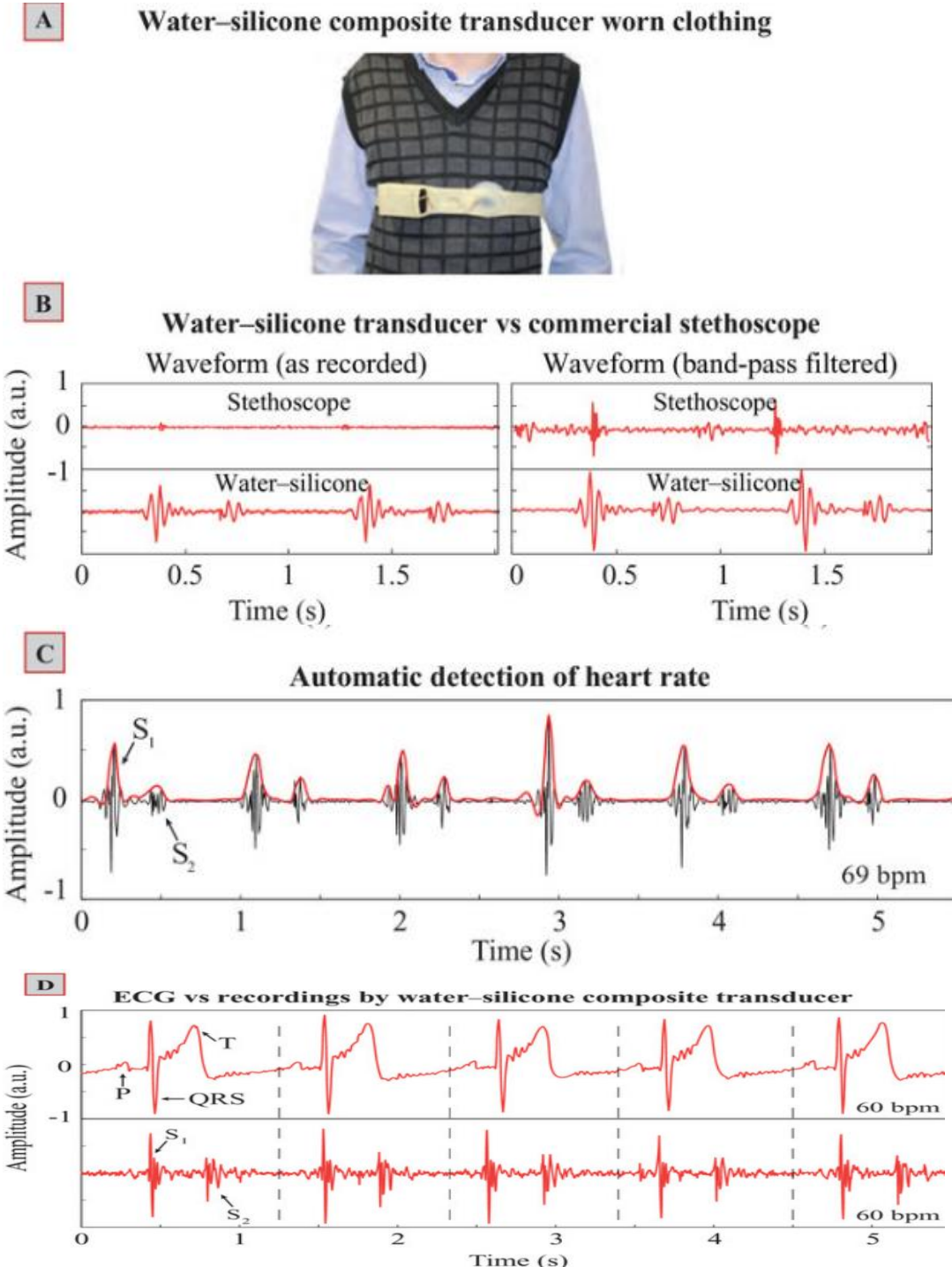
Flexible Phonocardiography (PCG)

- ❑ sensing mechanism: acoustic
- ❑ Cardiac auscultation (Heart sound signals) an provide valuable information about heart valve function and hemodynamics and yield many cardiac conditions such as **arrhythmias, valve disease, and heart failure.**

- ❑ Heart sound sensors are mainly produced from the direction of **miniaturization and flexibility**.
- ❑ Advantages :without the need for direct contact with the skin ,With the stretchable composite transducer, the recordings had a much better signal-to-noise profile, cheap instruments; no electrical interference

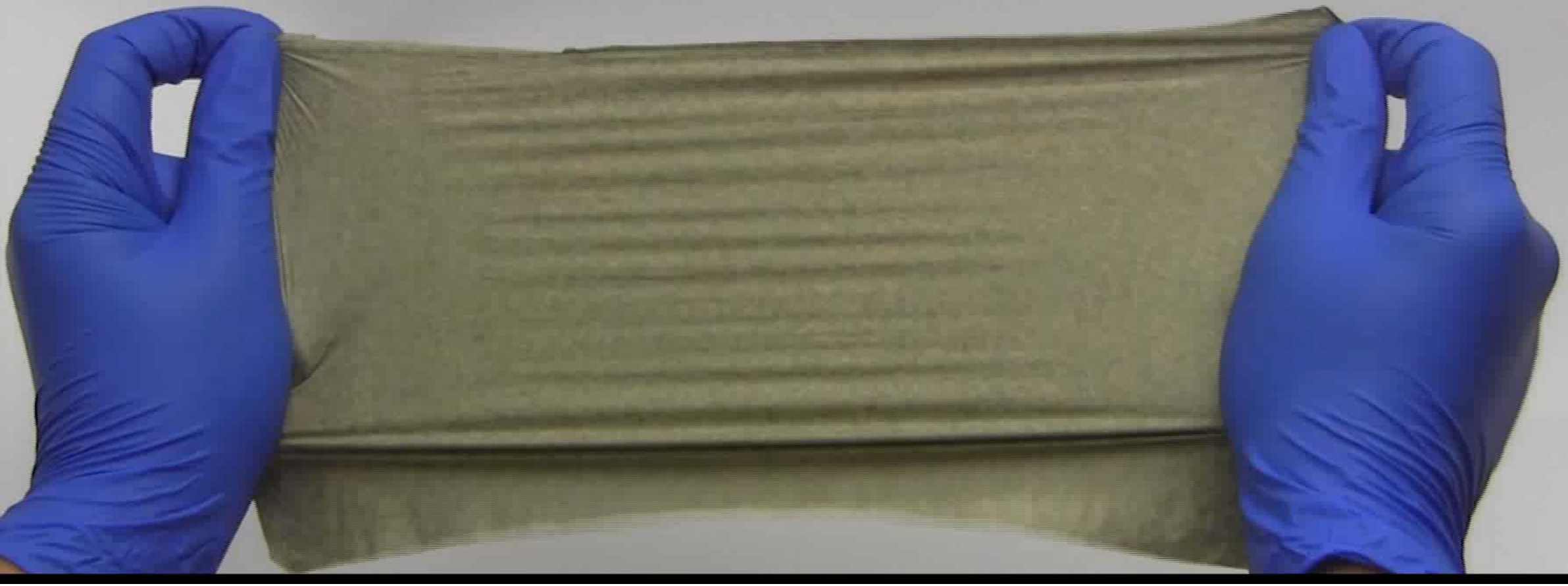
Disadvantages : bulky microphones: accuracy affected by background noises

- ❑ Filter: to remove unwanted signals, e.g., breathing sounds, ambient noise.



Simultaneous recording of ECG (using commercial electrodes attached directly on the skin) and PCG (recorded with the water–silicone composite transducer) signals showing functional agreement.

Stretching Test



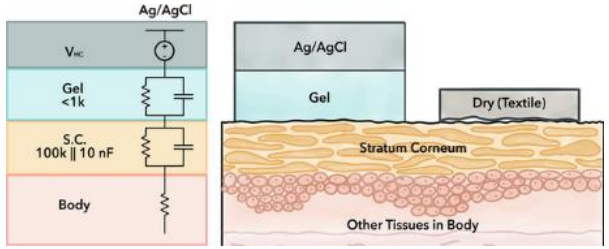
Flexible ECG

- ❑ sensing mechanism: electrical
- ❑ ECG signal is a typical electrophysiological (EP) signal that can reflect CVD and is most commonly used in clinical practice.

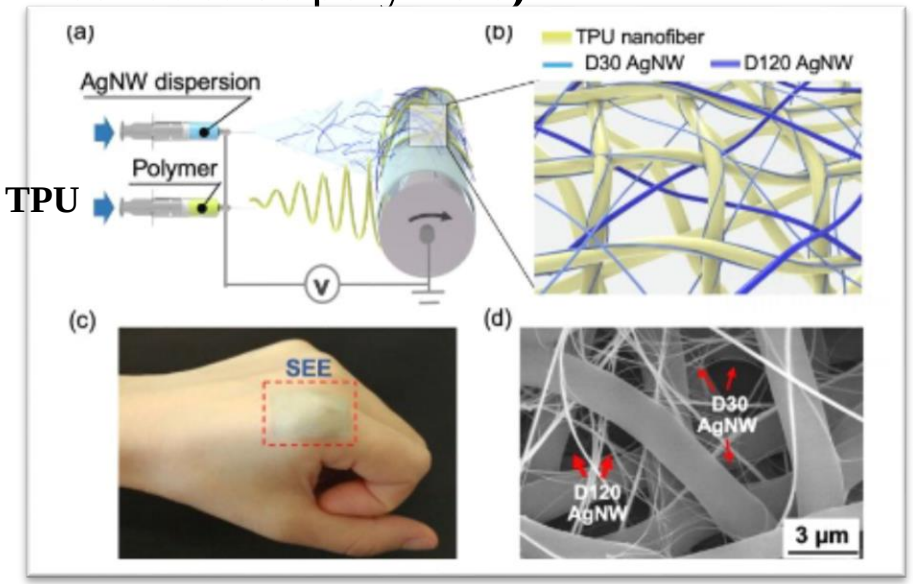
Advantages: more accurate and comprehensive information; short setting time

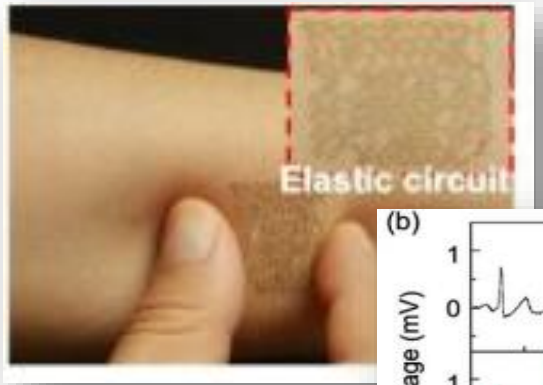
Disadventage: need for reliable electrodes attachment; electrical interference; accuracy affected by body movement

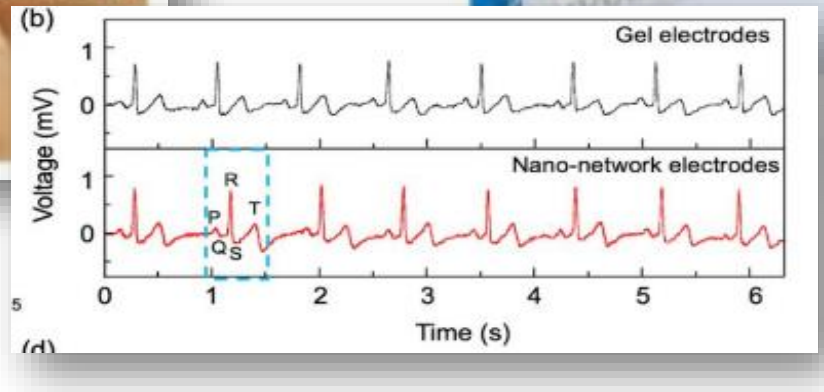
- ❑ Traditionally: using of **conductive gel** and **an Ag/AgCl electrode**.



- ❑ Recently: **flexible dry electrodes** to have a high signal-to-noise ratio, softness, stretchability and intimate contact with the skin **(**as Ag nanowires, Au, graphene, conductive polymers**)**







Cardiac biosensors: where are they on the market?

Device	Cardiac marker	Detection limit	Detection method
Dimension Vista (Siemens, Munich, Germany)	cTnI	15 pg mL^{-1}	Chemiluminescence
TROPT (Heidelberg, Germany)	cTnT	0.64 ng mL^{-1}	Colorimetry
AQT90 (Radiometer)	cTnI	0.010-50 ng mL^{-1} 0,0095 ng mL^{-1}	Fluorescence benchtop instrument
Elecsys (Roche, Basel, Switzerland)	cTnT	0.005 ng mL^{-1}	Electrochemiluminescence
ACS:180 (Bayer, Leverkusen, Germany)	cTnI	0.15 ng mL^{-1}	Chemiluminescence
Cobas h232 (Roche Diagnostics Ltd)	CK-MB Myoglobin cTnT NT-proBNP	1-40 ng mL^{-1} 30-700 ng mL^{-1} 50-2000 pg mL^{-1} 60-9000 pg mL^{-1}	Fluorescence Handheld device
i-STAT (Abbott Point of Care, Princeton, US)	cTnI CK-MB BNP	0.02 ng mL^{-1} 0.6 ng ml^{-1} 15 pg mL^{-1}	Electrochemical detection (amperometric) Handheld device
Cardiac Reader System (Roche)	CK-MB Myoglobin NT-proBNP cTnT	1-40 ng mL^{-1} 30-700 ng mL^{-1} 0.060-3 ng mL^{-1} 0.1 -3ng mL^{-1}	Fluorescence Benchtop POC

با تشکر از توجه شما