
In The Name of God

Design of Electrochemical biosensor for detection of S100B in Traumatic Brain Injury(TBI)

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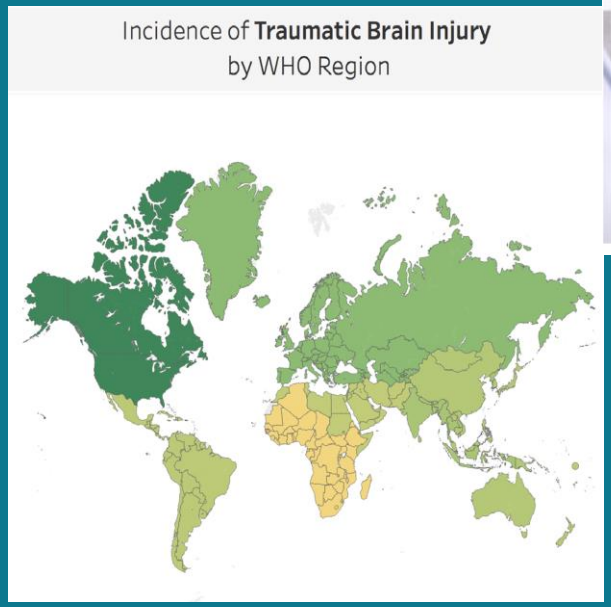
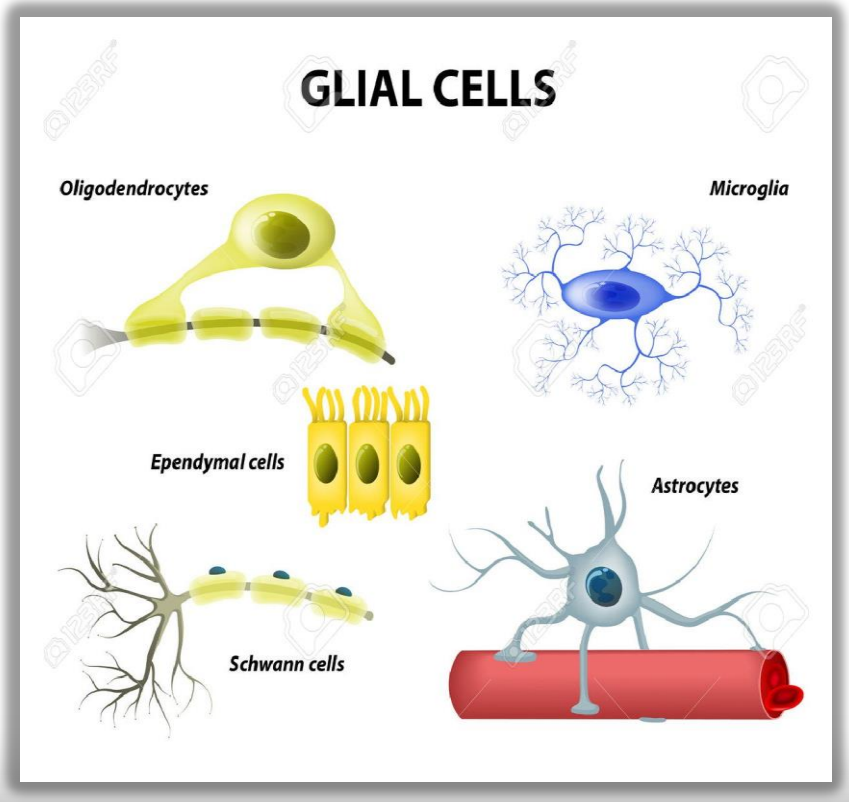
Traumatic brain injury

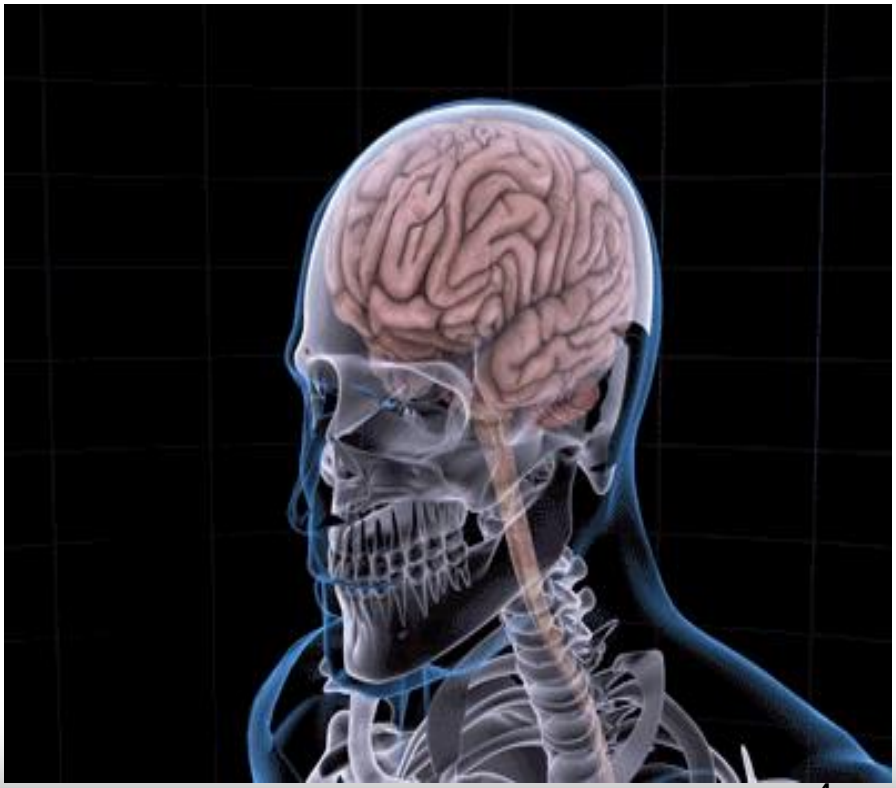
The sixth cause of death in the world

TBI contributes to 30.5% of all injury-related deaths in the USA



Traumatic Brain Injury (TBI)

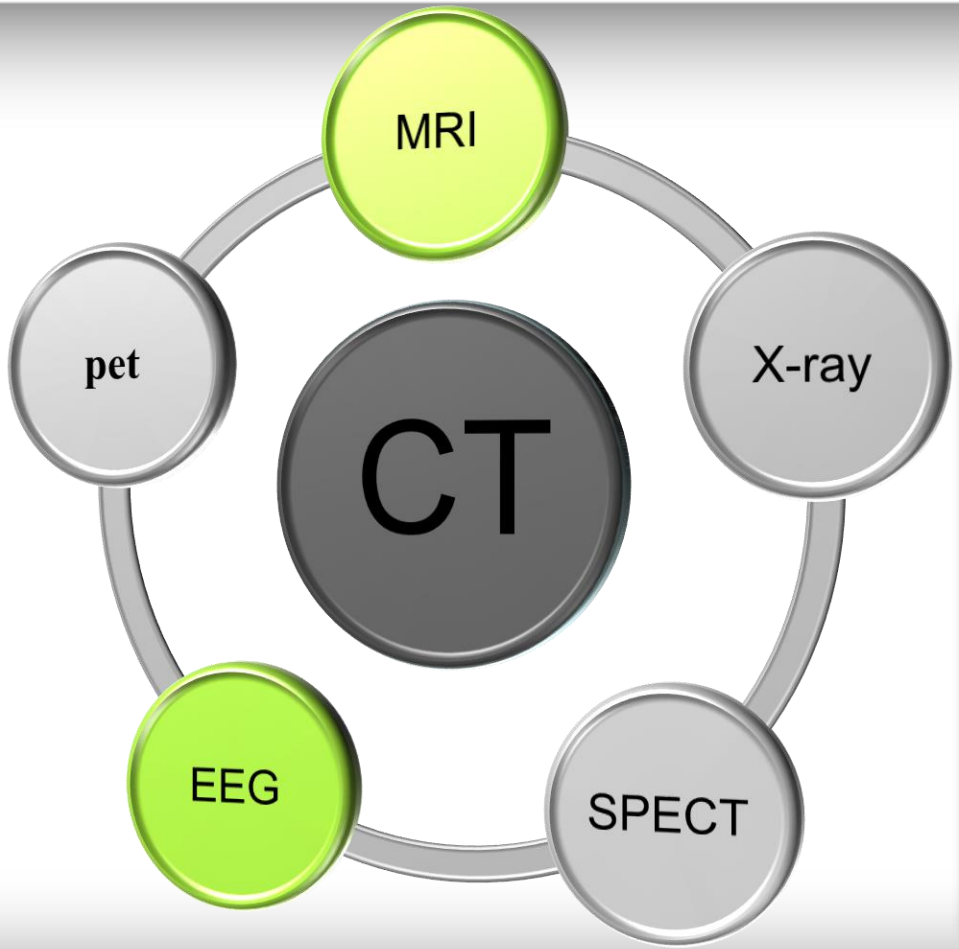




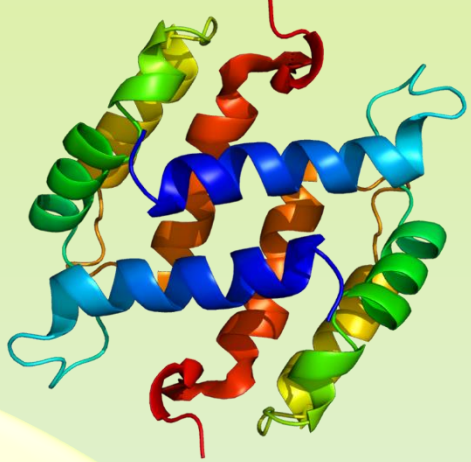
GSC:Glasgow Coma Scale

- GCS • Sever < 8
- GCS • Moderate 10-12
- GCS • Mild > 12

Diagnosis Traumatic Brain Injury

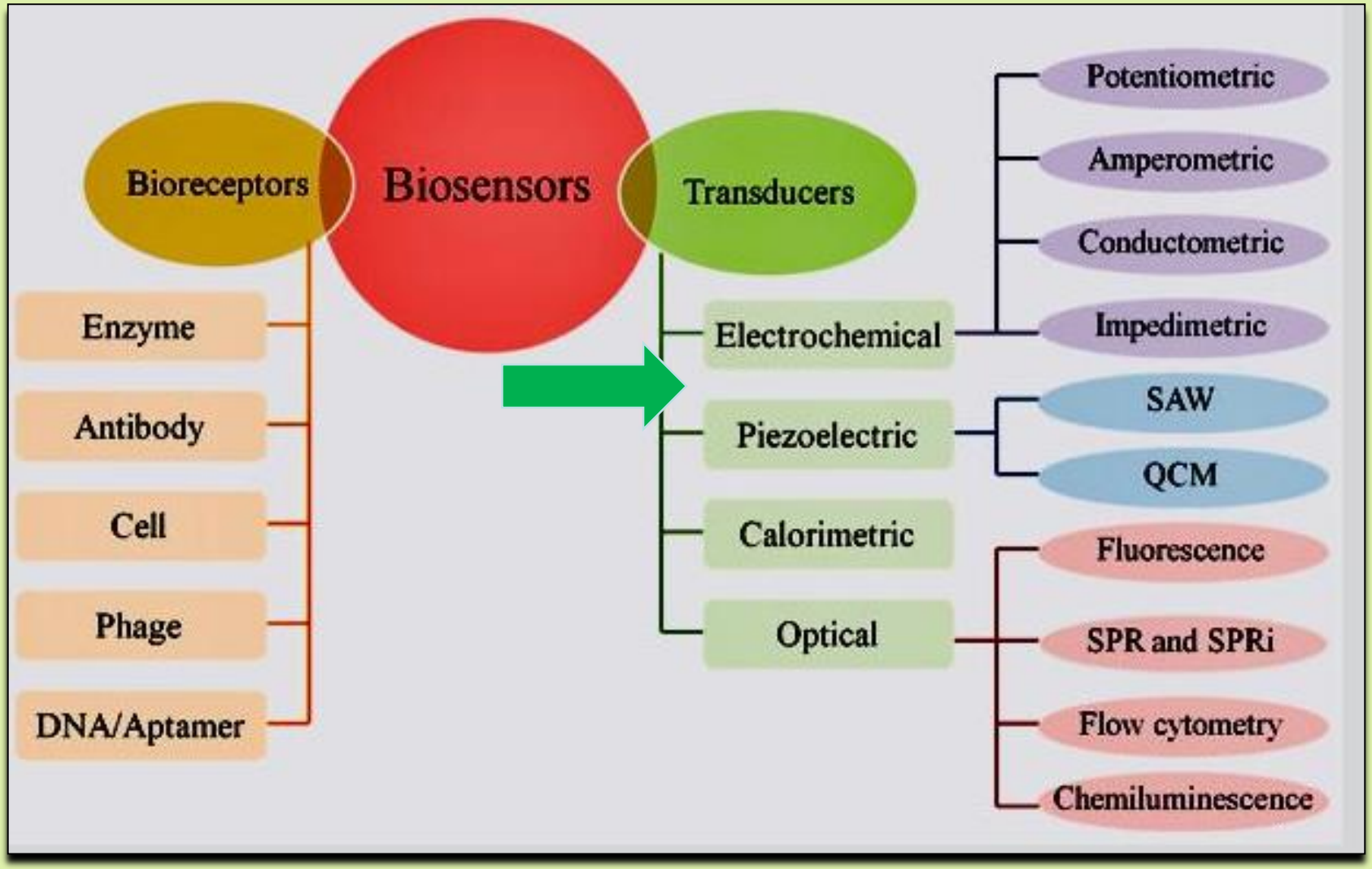


S100B



- Damage Associated Molecular Patterns(DAMP)
- S100B is glial-specific and is expressed primarily by astrocyte
- S100 calcium-binding protein B (S100B) is a protein of the S-100 protein family
- Balance calcium in body
- Gold standard measurement=Elisa kit

Structure of biosensors

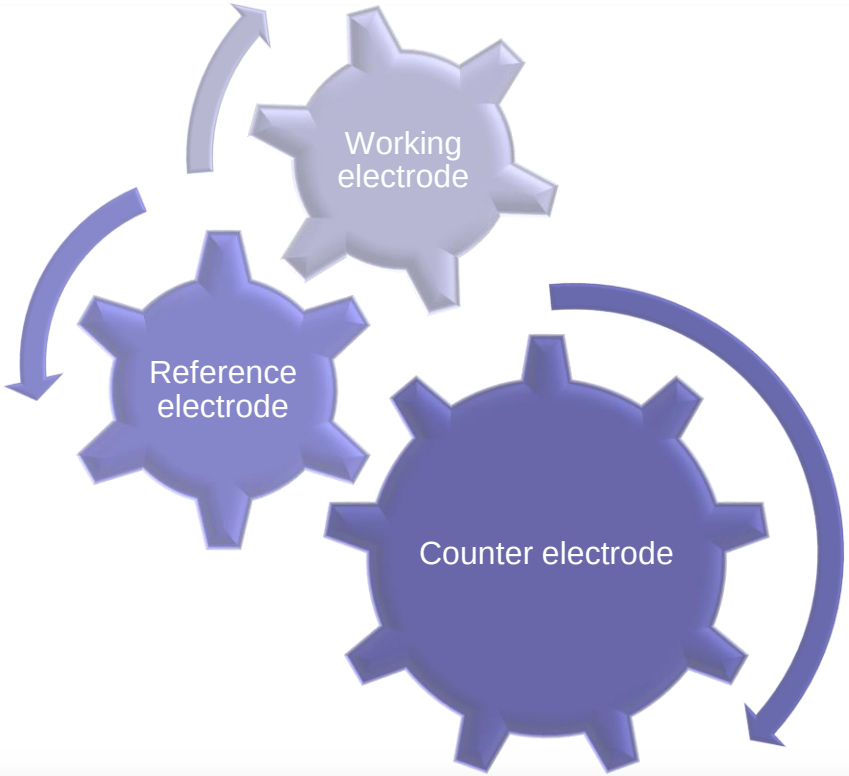


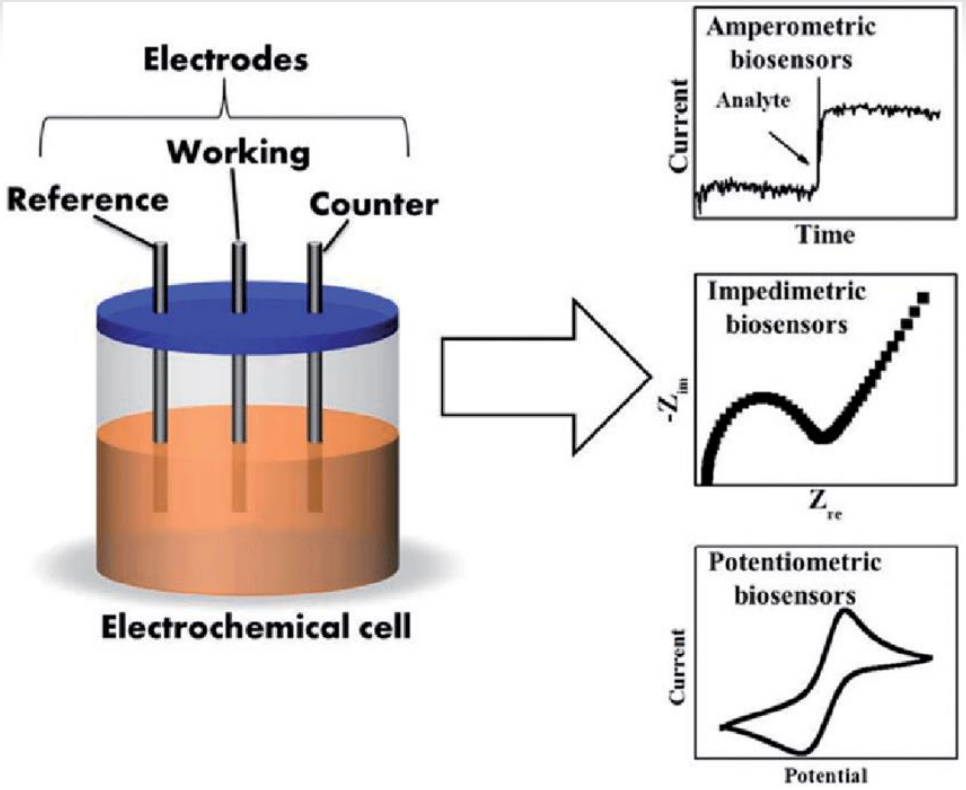
Advantages of Electrochemical biosensor

Sensitivity

- **Low cast**
- **LOD**
- **Very fast for detection**
- **Long term stability**
- **Real time analysis**
- **Selectivity**
- **Response time**
- **Repeatability**
- **Portability**
- **User friendly**

Electrochemical biosensor





Type Electrochemical immunosensors

1

- **labeled electrochemical immunosensors**

2

- **label-free electrochemical immunosensors**

Nanoparticles

Physical Property

Size

Roughness

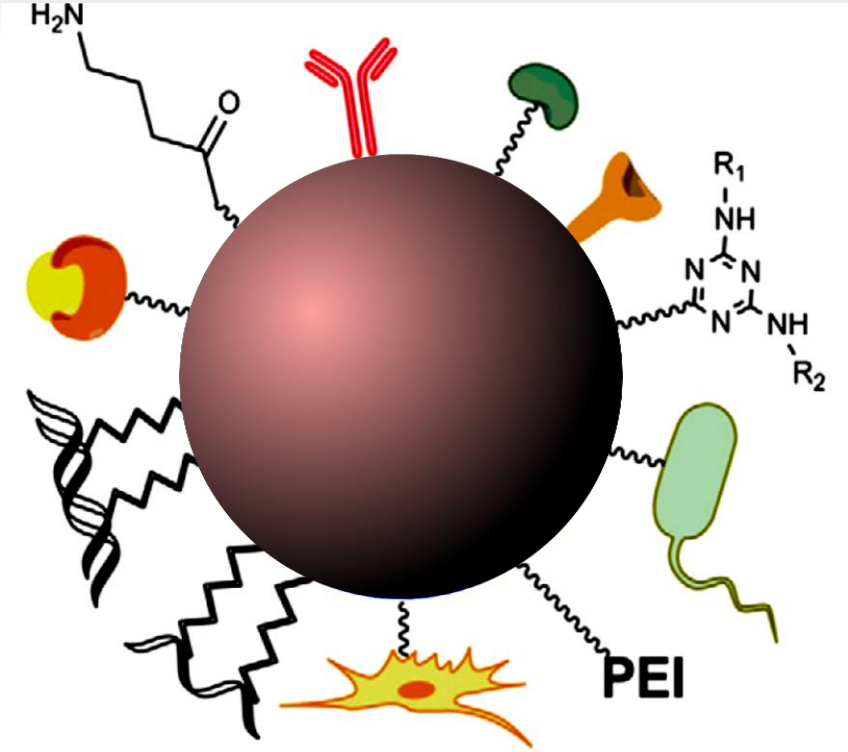
Porosity

Surface Chemistry

Surface Charge

Surface

Functionality



Biocompatibility

Toxicity

Dose

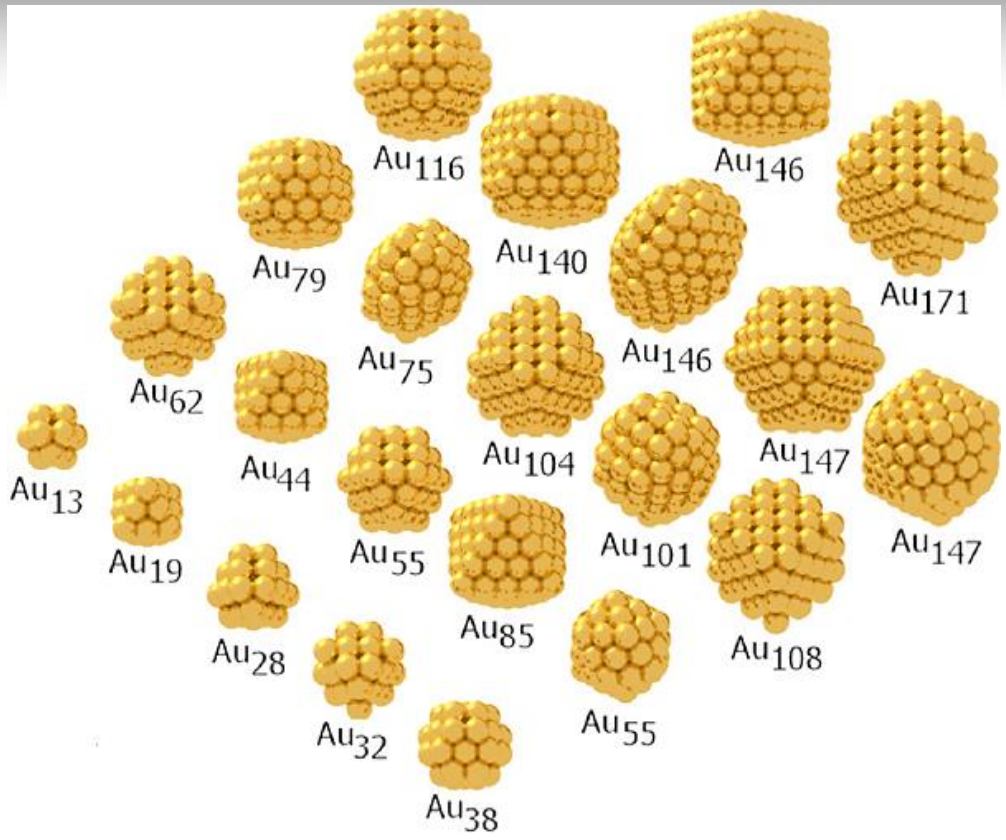
Rout of Administration

Metabolism

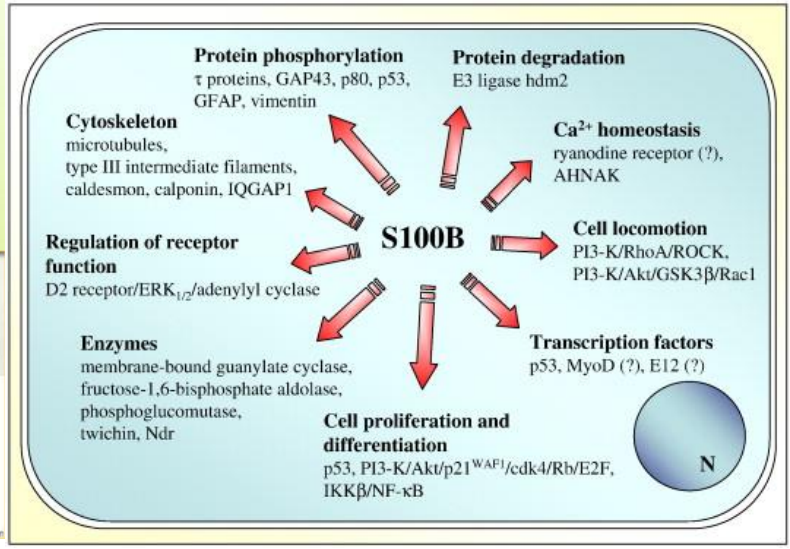
Clearance Rate

Gold Nanoparticles

- Anti-microbial Agent
- Therapeutics Delivery
- Photothermal Therapy
- Radiotherapy Dose Enhancer
- Diagnosis
- Theranostic
- Biological Imaging
- Biodetection And Biosensor
- Catalysts In Chemical Reactions



Recent researches

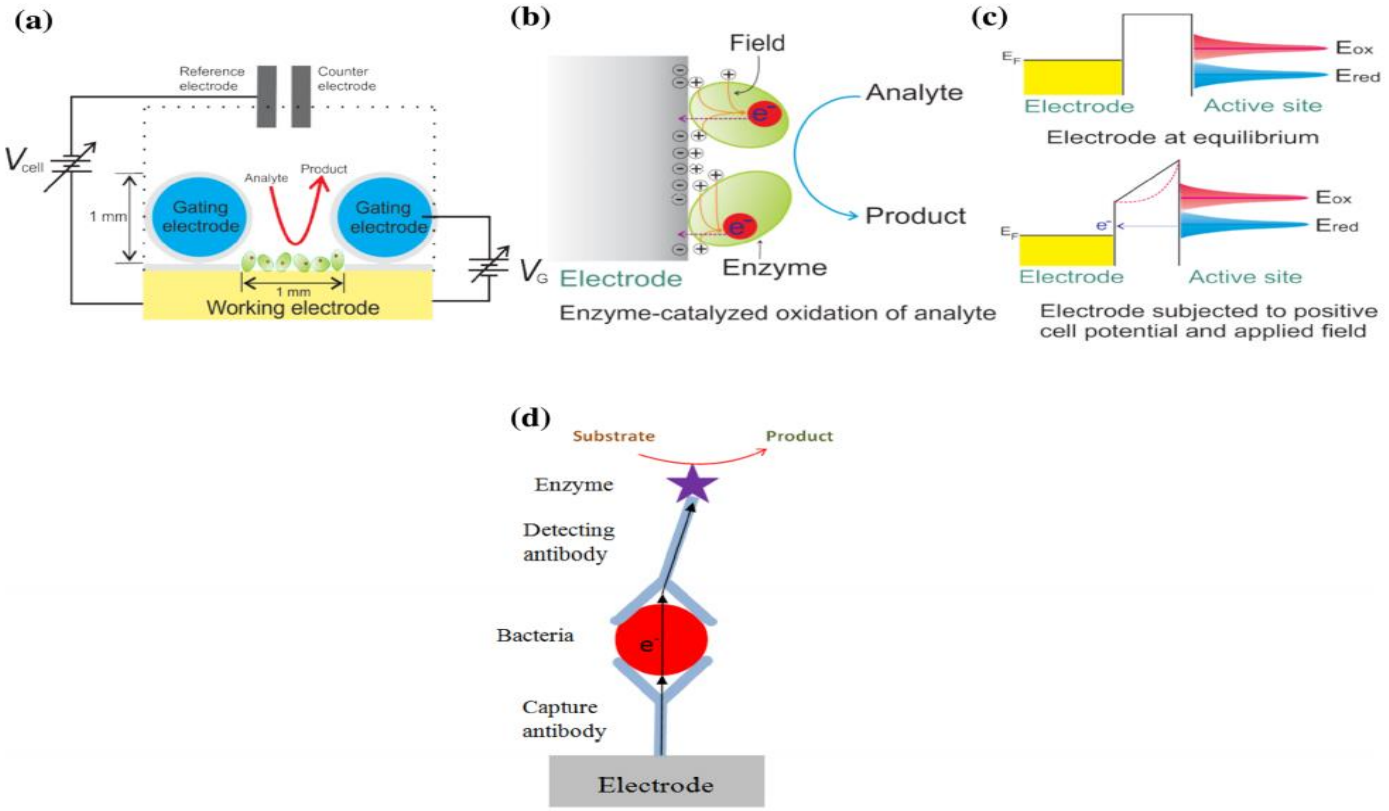


biomarker	transducer	bioreceptor	Label detection solution	Analysis time	sample	Low detection limit
S100B	Carbon SPE	SWCNTs-Nafion-GA/Ab1/T/HRP-Ab2	HRP Reagentless	60 min	HS	10 fg mL-1
S100B	Au DE	Recognition probe: MBs/Au/Ab/T	Label-free	30 min	Buffer Horse plasma	10 pM

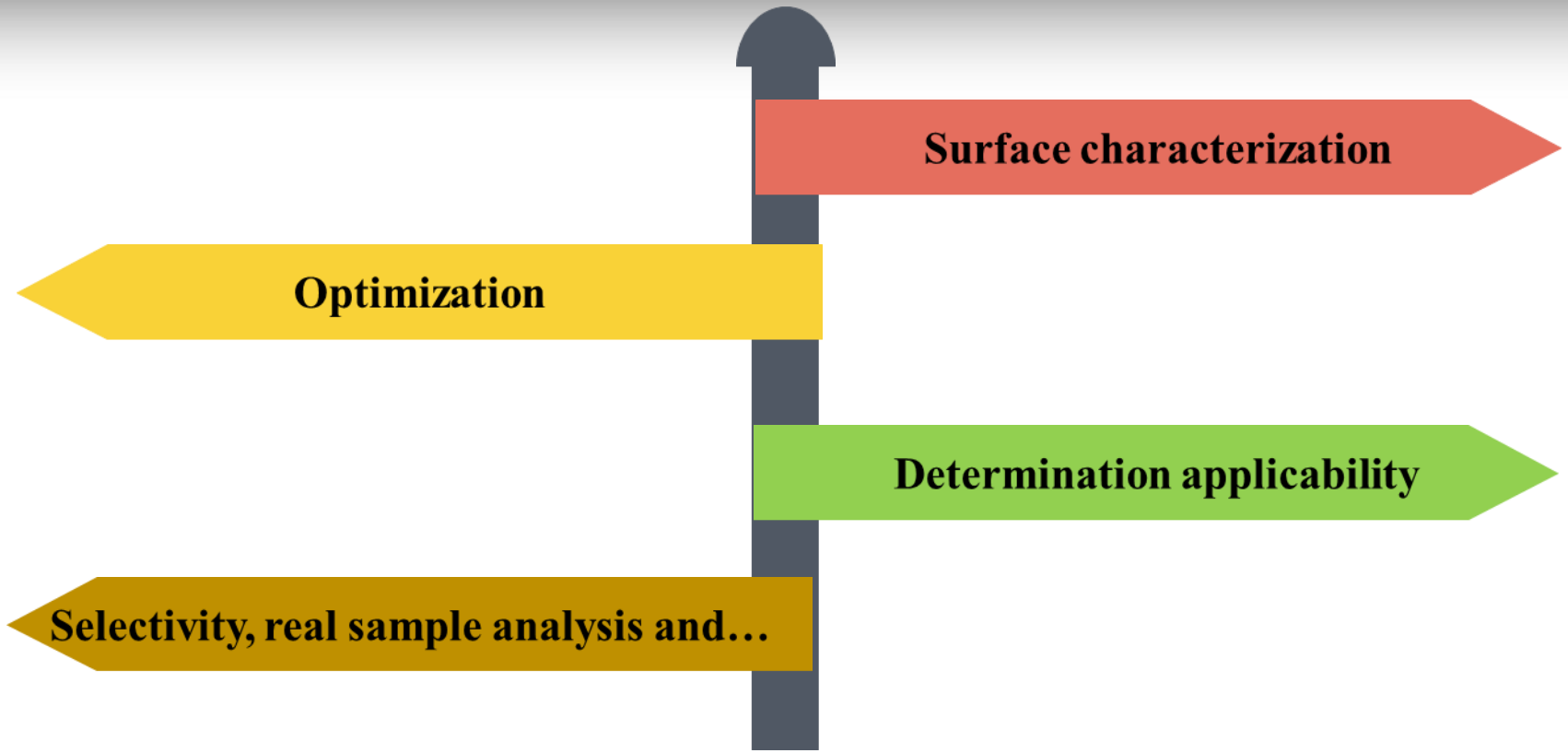
Detection of a Traumatic Brain Injury Biomarker

Anup S. Mathew[1] · Xuyang Shi[1] · Siu-Tung Yau[1,2]

biomarker	transducer	bioreceptor	Label detection solution	Analysis time	sample	Low detection limit
S100B	Carbon SPE	SWCNTs-Nafion-GA/Ab1/T/HRP-Ab2	HRP Reagentless	60 min	HS	10 fg mL-1



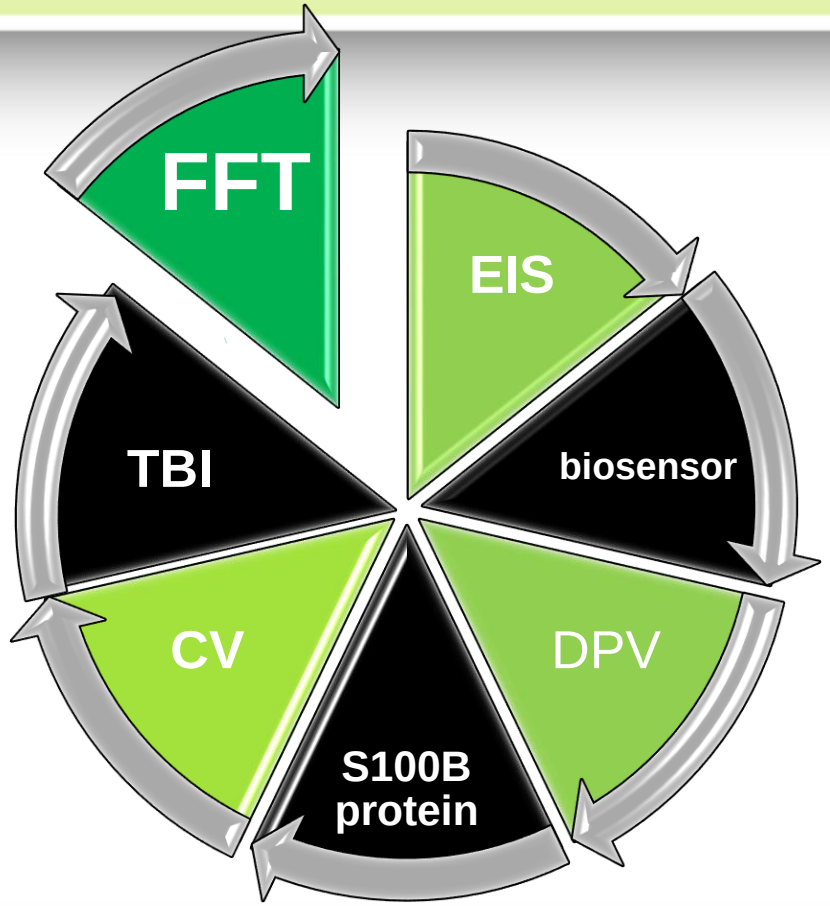
Design biosensor for detecting S100B



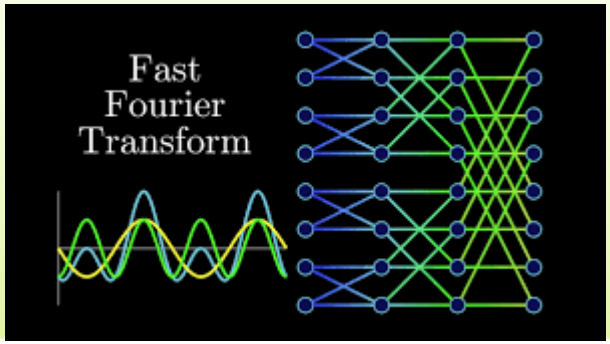
Design of electrochemical Immunosensor for detection of S100B

FFT:

A **fast Fourier transform** (**FFT**) is an algoritm that computes the discrete Fourier transform discrete Fourier transform(DFT) of a sequence, or its inverse (IDFT



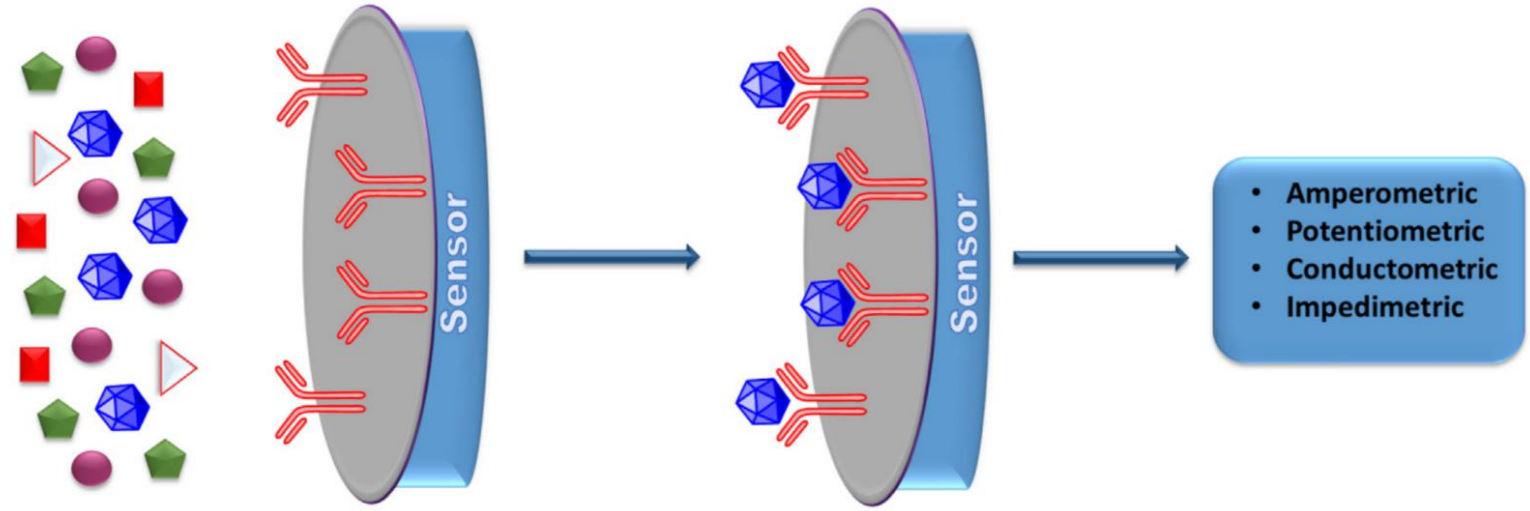
FFT



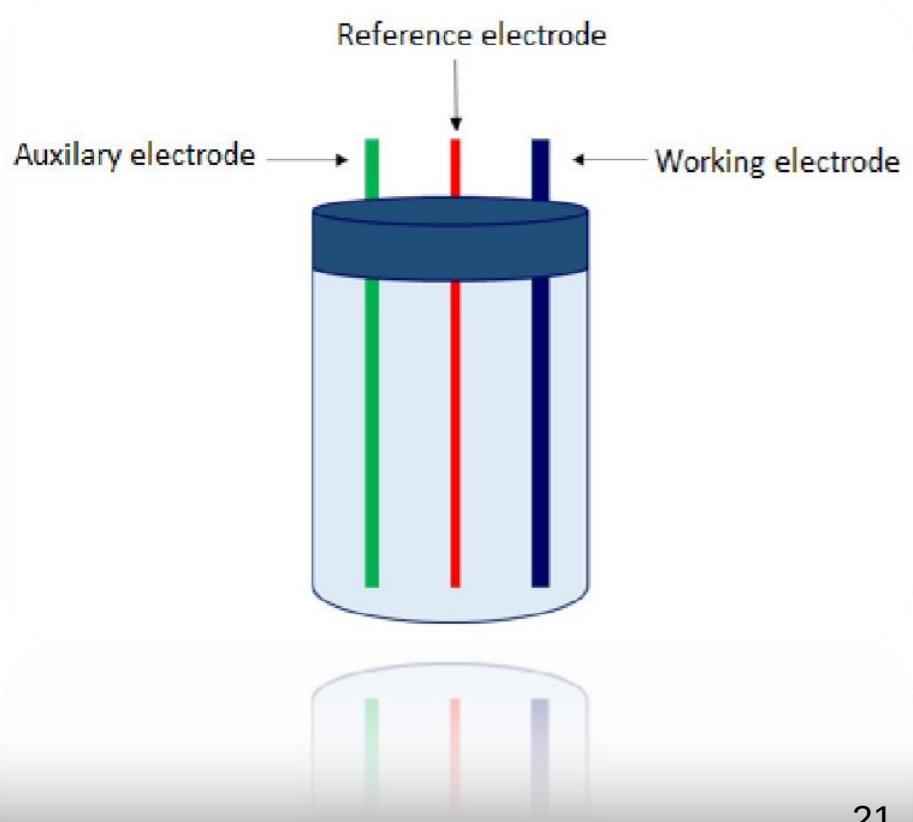
Design of Electrochemical immunosensor for detection of CPA

Candidatus Phytoplasma Aurantifolia

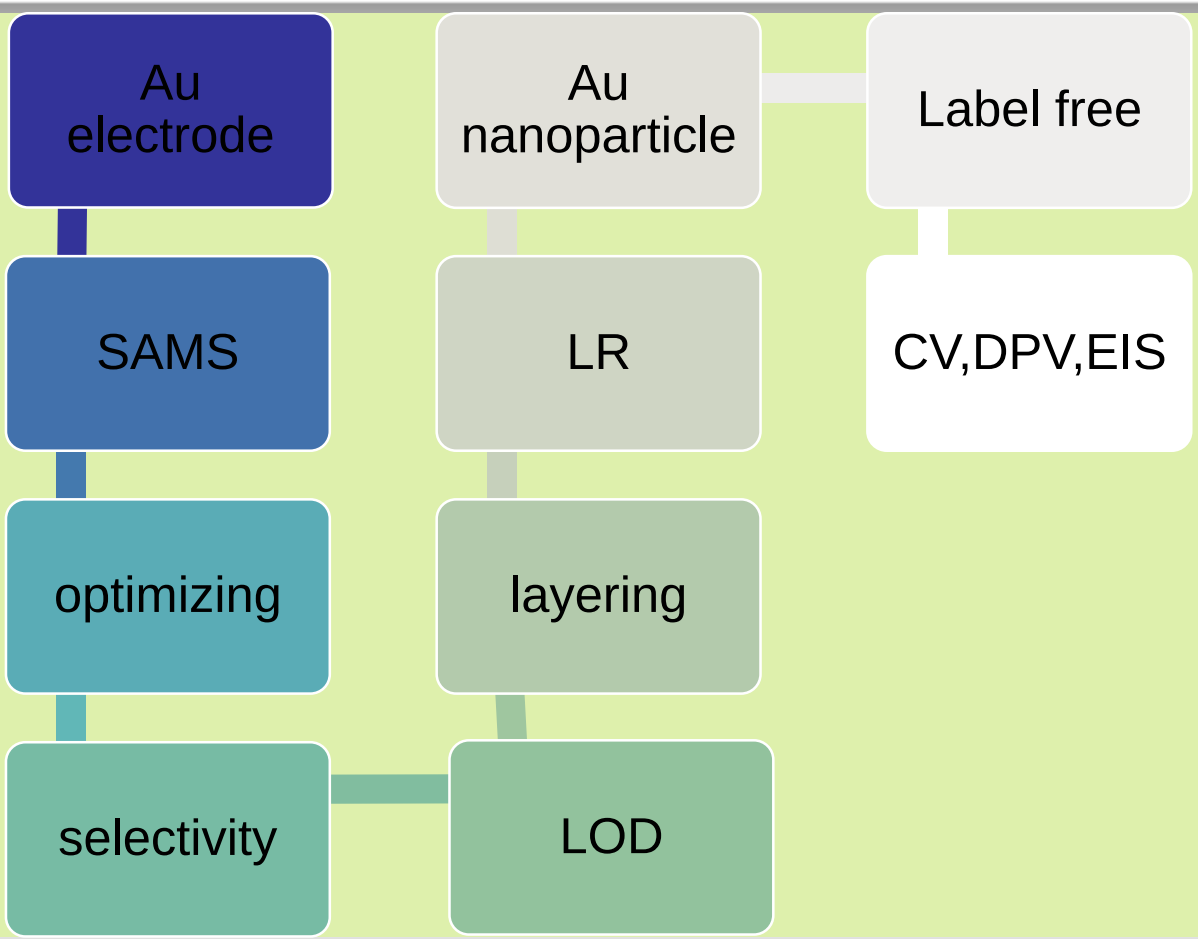
- CPA antigen
- CPA antibody



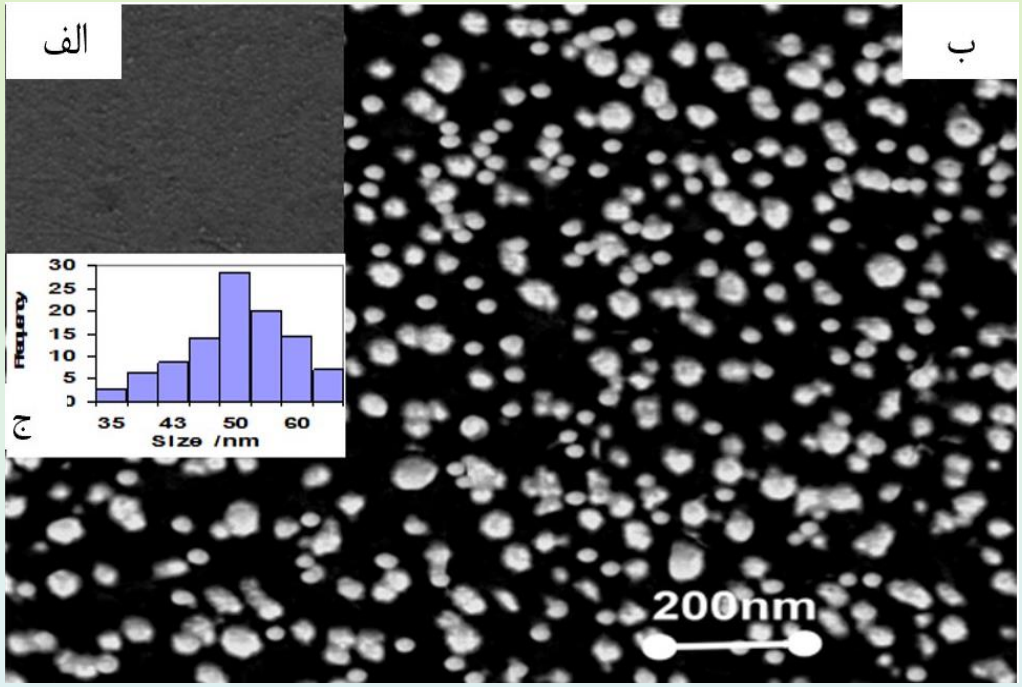
Apparatus



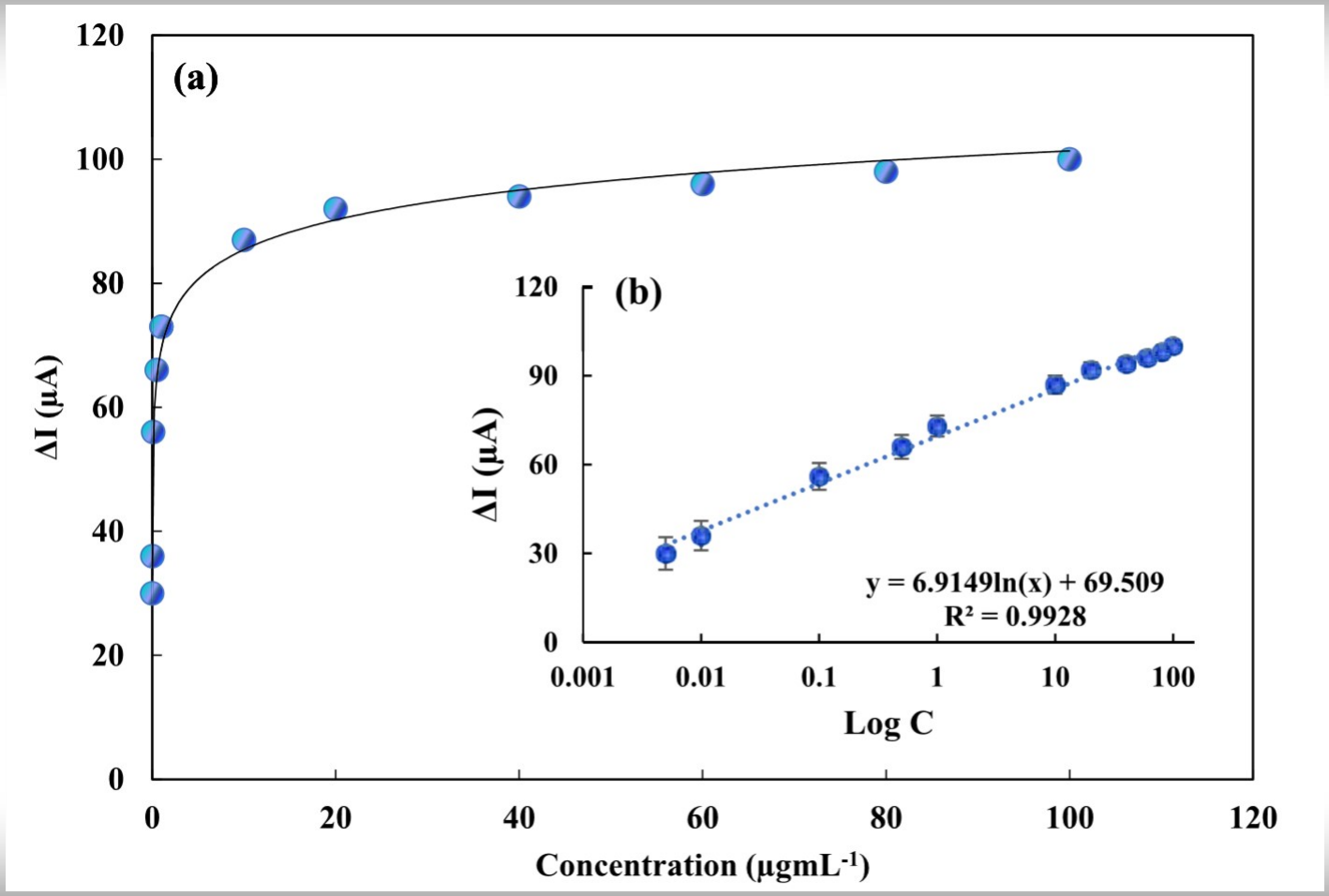
Design of electrochemical Immunosensor for detecting Candidatus Phytoplasma Aurantifolia



Characterization of Au/Au-NPs electrode



The calibration curve

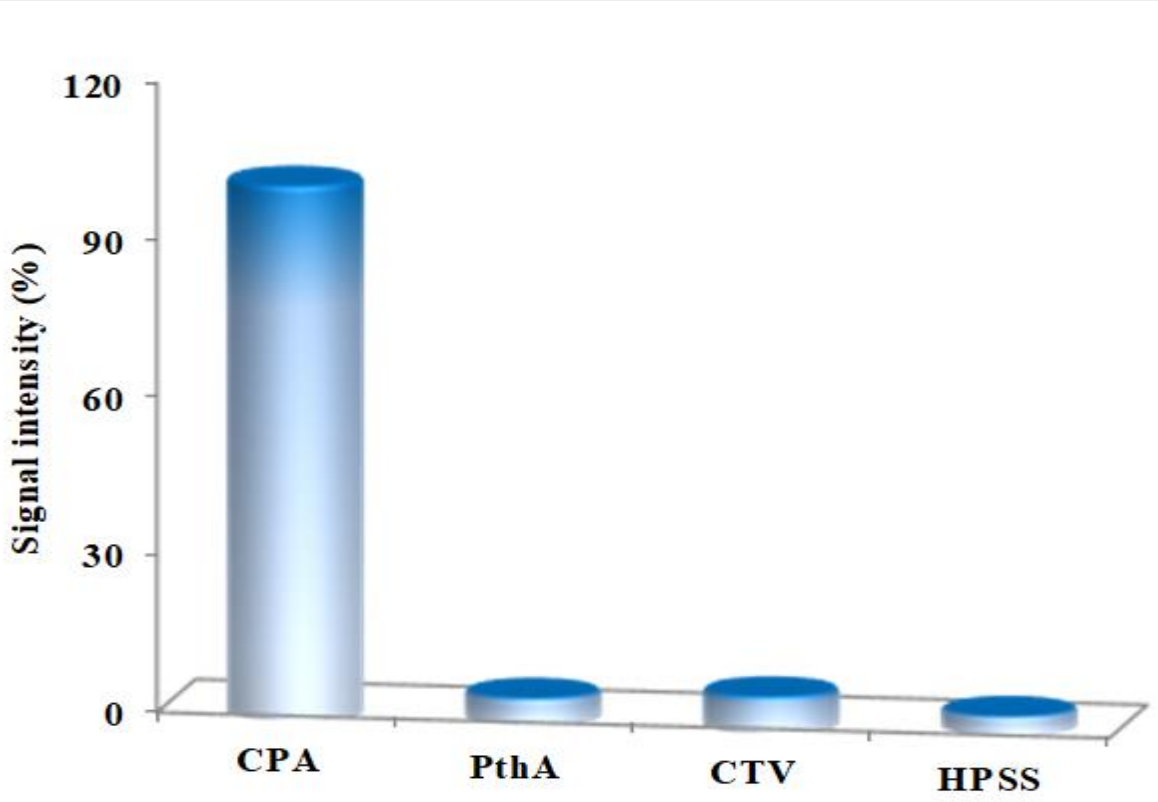


Result

Evaluation parameters	value
Limit of detection (LOD)	1.5 ng/ml
Linear dynamic rate	0.005-100ηm

Selectivity of the immunosensor

- CPA
- PTHA
- CTV
- HPSS



References:

- 1] Maduraiveeran G., Sasidharan M., Ganesan V., “Electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications” , Biosensors and Bioelectronics, Elsevier, 103, 113–129, 2018.
- [2] Haji-Hashemi H., Norouzi P., Safarnejad M. R., Ganjali M. R., “Label-free electrochemical immunosensor for direct detection of Citrus tristeza virus using modified gold electrode” , Sensors and Actuators B: Chemical, Elsevier, 244, 211–216, 2017.
- [3] Streicher W. W., Lopez M. M., Makhatadze G. I., “Modulation of quaternary structure of S100 proteins by calcium ions” , Biophysical chemistry, Elsevier, 151, 3, 181–186, 2010.
- [4] Serafín V., Torrente-Rodríguez R. M., González-Cortés A., de Frutos P. G., Sabaté M., Campuzano S. et al., “An electrochemical immunosensor for brain natriuretic peptide prepared with screen-printed carbon electrodes nanostructured with gold nanoparticles grafted through aryl diazonium salt chemistry” , Talanta, Elsevier, 179, 131–138, 2018.
- [5] Khetani S., Aburashed R., Singh A., Sen A., Sanati-Nezhad A., “Immunosensing of S100β biomarker for diagnosis of spinal cord injuries (SCI)” , Sensors and Actuators B: Chemical, Elsevier, 247, 163–169, 2017.
- [6] Ostendorp T., Diez J., Heizmann C. W., Fritz G., “The crystal structures of human S100B in the zinc- and calcium-loaded state at three pH values reveal zinc ligand swapping” , Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, Elsevier, 1813, 5, 1083–1091, 2011.
- [7] Kuo Y.-C., Lee C.-K., Lin C.-T., “Improving sensitivity of a miniaturized label-free electrochemical biosensor using zigzag electrodes” , Biosensors and Bioelectronics, Elsevier, 103, 130–137, 2018.
- [8] Kuo Y.-C., Lee C.-K., Lin C.-T., “Data on a new sensitivity-improved miniaturized label-free electrochemical biosensor” , Data in brief, Elsevier, 17, 1288–1294, 2018.
- [9] Kurzątkowska K., Jankowska A., Wysłouch-Cieszyńska A., Zhukova L., Puchalska M., Dehaen W. et al., “Voltammetric detection of the S100B protein using

References:

- his-tagged RAGE domain immobilized onto a gold electrode modified with a dipyrromethene–cu (II) complex and different diluents” , Journal of Electroanalytical Chemistry, Elsevier, 767, 76–83, 2016.
- [10] Barateiro A., Afonso V., Santos G., Cerqueira J. J., Brites D., van Horssen J. et al., “S100B as a potential biomarker and therapeutic target in multiple sclerosis” , Molecular neurobiology, Springer, 53, 6, 3976–3991, 2016.
- [11] Kleindienst A., Bullock M. R., “A critical analysis of the role of the neurotrophic protein S100B in acute brain injury” , Journal of neurotrauma, Mary Ann Liebert, Inc. 2 Madison Avenue Larchmont, NY 10538 USA, 23, 8, 1185–1200, 2006.
- [12] Hassani S., Akmal M. R., Salek-Maghsoudi A., Rahmani S., Ganjali M. R., Norouzi P. et al., “Novel label-free electrochemical aptasensor for determination of Diazinon using gold nanoparticles-modified screen-printed gold electrode” , Biosensors and Bioelectronics, Elsevier, 120, 122–128, 2018.
- [13] North S. H., Shriver-Lake L. C., Taitt C. R., Ligler F. S., “Rapid analytical methods for on-site triage for traumatic brain injury” , Annual review of analytical chemistry, Annual Reviews, 5, 35–56, 2012.
- [14] Zhang H., Wang G., Ding Y., Wang Z., Barraclough R., Rudland P. S. et al., “The crystal structure at 2 Å resolution of the Ca2+-binding protein S100P” , Journal of molecular biology, Elsevier, 325, 4, 785–794, 2003.
- [15] Alpdemir M., Özcan O., Alpdemir M. F., Şeneş M., Azak A., Duranay M. et al., “Serum Neuron Specific Enolase and S-100B Levels in Hemodialysis and Peritoneal Dialysis Patients” , 2019.

References:

- 16] Blennow K., Brody D. L., Kochanek P. M., Levin H., McKee A., Ribbers G. M. et al., “Traumatic brain injuries” , Nature reviews Disease primers, Nature Publishing Group, 2, 16084, 2016.
- [17] Mikuła E., Wysłouch-Cieszyńska A., Zhukova L., Puchalska M., Verwilst P., Dehaen W. et al., “Voltammetric detection of S100B protein using His-tagged receptor domains for advanced glycation end products (RAGE) immobilized onto a gold electrode surface” , Sensors, Multidisciplinary Digital Publishing Institute, 14, 6, 10650–10663, 2014.
- [18] Thelin E. P., Nelson D. W., Bellander B.-M., “A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury” , Acta neurochirurgica, Springer, 159, 2, 209–225, 2017.
- [19] Ji Y.-F., Huang H., Jiang F., Ni R.-Z., Xiao M.-B., “S100 family signaling network and related proteins in pancreatic cancer” , International journal of molecular medicine, Spandidos Publications, 33, 4, 769–776, 2014.
- [20] Yokobori S., Hosein K., Burks S., Sharma I., Gajavelli S., Bullock R., “Biomarkers for the clinical differential diagnosis in traumatic brain injury—a systematic review” , CNS neuroscience & therapeutics, Wiley Online Library, 19, 8, 556–565, 2013.
- [21] Zhang H., Qi S., Rao J., Li Q., Yin L., Lu Y., “Development of a rapid and high-performance chemiluminescence immunoassay based on magnetic particles for protein S100B in human serum” , Luminescence, Wiley Online Library, 28, 6, 927–932, 2013.

عنوان هزینه (مواد و وسایل مصرفی)	مبلغ
ELISA KIT (ABCAM)S100B	300,000,000
S100Bآنتی ژن	270,000,000
S100B آنتی بادی	260,000,000
گرافن	9,500,000
نانو مواد اکسیدی مانند اکسید لانتانیدها	20,000,000
نانو ذره طلا	22,000,000
الکترود طلا۲ عدد	32,000,000
الکترود پلاتین ۲	180,000,000
الکترود ر فرانس۲	27,000,000
Gold (III) chloride hydrate ($HAuCl_4 \cdot 4H_2O$, 99%)	9,500,000
حلال استون	8,000,000
الکترود کربنی۲	17,500,000
نافیون	5,500,000
3-Mercapto Propionic acid 99%	12,000,000
11-Mercapto Undecanoic acid 95%	18,000,000
bovine serum albumin	9,500,000
1-ethyl3-(3-dimethylaminopropyl) carbodiimide–HCl	16,000,000
N-Hydroxy Succinimide 98%	17,000,000
potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$)	10,000,000
potassium chloride	8,000,00
کیتوسان	8,000,000
مواد اصلاح کننده سطح الکترود , CNT MWCNTشامل نوژل و کربن اکنیو	30,000,000
HCl, NaOH,H2SO4	25,000,000
Screen print electrode Au and C	38,000,000
جمع	1,416,500,000

POC diagnostic device for mTBI

Presumed key product requirements specifications of a future sy

The number of biomarkers necessary to achieve sufficient diagnc ongoing clinical studies.[21,98–100]

Diagnostic sensitivity ≥ 95%	Number of biomarkers detected (multiplex multivariate analysis)
Diagnostic specificity ≥ 75%	Capillary whole blood (finger prick) sample volume ≤50 µL
Intra-assay %CV precision ≤10%	Linear range (*i.e.*, upper limit of quantification, ULOQ relative to L biomarker, *e.g.*, ≥ 1.1 ng mL^{-1} for GFAP
Inter-assay %CV precision ≤15%	Time-to-results ≤10 min
Reagent shelf life ≥6 months	Hands-on-time ≤5 min

Lower Detection Limit (LDL) 1/10 of the cutoff (CO) value to distinguish mTBI from physiologi

Biomarker (Target, T)	Technique Publication year and reference	Transducer (or FED architecture)	Surface modification/ Bioreceptor functionalization/ Assay format	Label Detection solution Substrate/redox probe/ mediator/catalyst/ signal enhancer/pH modulator etc.	Analysis time and Incubation parameters Volume/Target or Label/Time/Temperature/Flow rate/Other	Sample	Lower Detection Limit (1)	Range (2) Linear vs target concentration (c_T), if not stated otherwise (e.g., vs lgc_T)
S100β *S100β calcium-binding protein* *Continuation =>*	OSWV 2016[137]	Au DE	(DPM+NAC)/Cu^{2+}/His_6-RAGE VC1 or C2/T (a) (DPM+MBT)/Cu^{2+}/His_6-RAGE VC1 or C2/T (b)	Label-free	10 µL T Solutions deoxygenated	Buffer HP dil. 1:2	2.6 pM (a)4.9 pM (b) 0.9 pM (a)2.7 pM (b)	2.6-20 pM (a) 4.9-20 pM (b) 0.9-20 pM (a) 2.7-20 pM (b)
	DPV 2017[138]	Graphene SPE	Electrografted reduced FRGG/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4}$	T / 45 min, 4°C	Buffer HS (and CSF)	1 pg mL^{-1} 1 pg mL^{-1}	1 pg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)* 1 pg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)*
	EIS 2018[10]	Au IDE *(microfluidic ID-zigzag biochip)*	(4-ATP+cysteamine)/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	5 min (flow rate 25 µL min^{-1})	Buffer	10 ng mL^{-1}-	10 ng mL^{-1}-10 µg mL^{-1} *(vs lgC_T)*
	FED (FEED) 2018[139]	Carbon SPE	SWCNTs-Nafion-GA/Ab_1/T/HRP-Ab_2	HRP Reagentless	T / 60 min Ab_2 / 40 min	HS	10 fg mL^{-1}	10 fg mL^{-1}-10 ng mL^{-1}
	SWV LFA (with SERS) 2019[121]	FTO	AgNPs/Au/4-MBA/Ab/T	Label-free MuxT	T / 30 min	Buffer (pH 6.5) HS	10 pg mL^{-1} <1.8 ng mL^{-1} (1)	50 pg mL^{-1}-1 µg mL^{-1}
	EIS (SFI) 2019[46]	Au DE	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$ MuxT	Optimal Z-t measurement: 15 s	Buffer 5- 25 and 90% blood and plasma	2-5 pg mL^{-1} Recoveries; 14-67 pg mL^{-1} in 90% blood	0.1-2800 pg mL^{-1}
	PEC (CBP) 2019[140]	ITO	rGRO-AuNPs/3-ICT-sol-gel-film/ Ab/T/Ab/(EDC+NHS)/CdS-QDs	CdS-QDs AA	5 µL T / 30 (45 min Buffer; 20 µL T / 30 min (HS) 6 µL CdS-QDs / 30 min	Buffer HS	0.15 pg mL^{-1} <100 pg mL^{-1} (1)	0.25-10000 pg mL^{-1} *(vs lgC_T)*
	CSV (DPCSV) 2020[141]	Au DE	Recognition probe: MBs/Au/Ab/T	Label-free	50 µL T / 30 min	Buffer Horse plasma	10 pM <250 pM (1)	10 pM-100 nM *(non-linear)*
S100β *S100β calcium-binding protein*	DPV 2013[134]	Pencil graphite Microfluidic chip (PMMA)	WE (graphite): PMMA/-OH(NaOH)/-NH_2(PEI)/GA/Ab_1/T/Ab_2/ALP-IgG	ALP PAPP	20 µL T / 30 min, 37°C; 20 µL Ab_2 / 20 min, 37°C (flow rate 120 µL h^{-1})	Buffer	0.1 pg mL^{-1}	0.1-100 pg mL^{-1}
	SWV 2014[135]	Au DE	(Capture peptide+TCEP)/ (T+$CaCl_2$)/(signal peptide+Cu^{2+})	OPD; Cu^{2+} as catalyst for OPD oxidation	T / 2.5 h, 30°C	Buffer HS	0.1 nM <0.2 nM (1)	0.1-25.6 nM *(vs lgC_T)*
	OSWV 2014[136]	Au DE	(DPTA+NAC)/Cu^{2+}/His_6-RAGE VC1 or C2/T	Label-free	10 µL T / 30 min Nitrogen purging 15 min	Buffer HP dil.	0.52 pM 0.65 pM	1-20 pM

	reference		Assay format	enhancer/pH modulator etc.	Label/ Time/ Temperature/ Flo w rate/Other		(1)	(e.g., vs lgc_T)
	OSWV 2016[137]	Au DE	(DPM+NAC)/Cu^{2+}/His_6-RAGE VC1 or C2/T (a) (DPM+MBT)/Cu^{2+}/His_6-RAGE VC1 or C2/T (b)	Label-free	10 µL T Solutions deoxygenated	Buffer HP dil. 1:2	2.6 pM (a)4.9 pM (b) 0.9 pM (a)2.7 pM (b)	2.6-20 pM (a) 4.9-20 pM (b) 0.9-20 pM (a) 2.7-20 pM (b)
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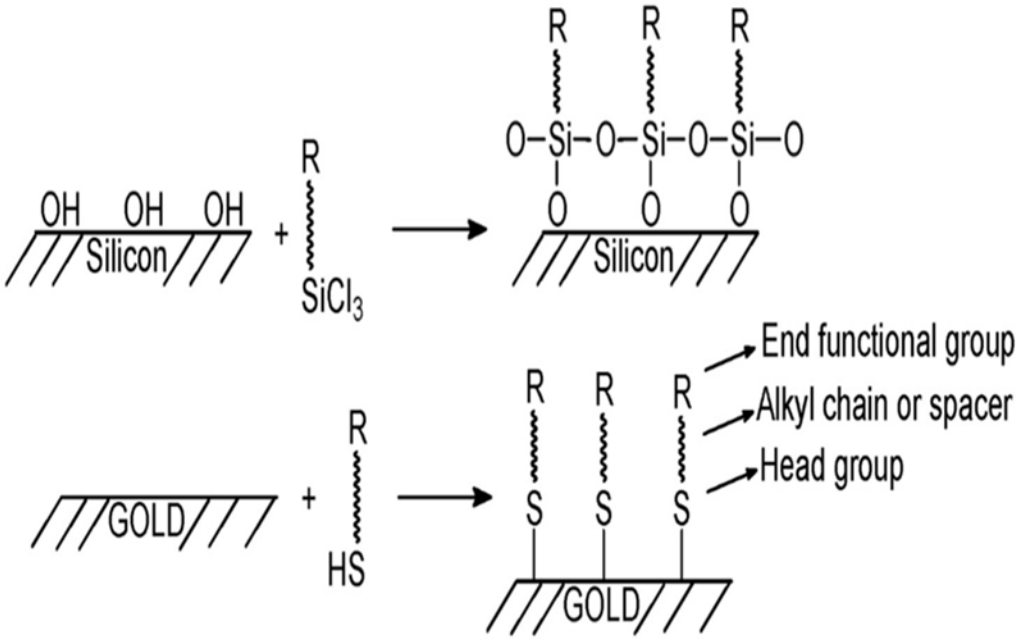
	EIS 2018[10]	Au IDE *(microfluidic ID-zigzag biochip)*	(4-ATP+cysteamine)/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	5 min (flow rate 25 µL min^{-1})	Buffer	10 ng mL^{-1}-	10 ng mL^{-1}-10 µg mL^{-1} *(vs lgC_T)*
S100β *S100β calcium-binding protein*	FED (FEED) 2018[139]	Carbon SPE	SWCNTs-Nafion-GA/Ab_1/T/HRP-Ab_2	HRP Reagentless	T / 60 min Ab_2 / 40 min	HS	10 fg mL^{-1}	10 fg mL^{-1}-10 ng mL^{-1}
	SWV LFA (with SERS) 2019[121]	FTO	AgNPs/Au/4-MBA/Ab/T	Label-free MuxT	T / 30 min	Buffer (pH 6.5) HS	10 pg mL^{-1} <1.8 ng mL^{-1} (1)	50 pg mL^{-1}-1 µg mL^{-1}
Continuation =>	EIS (SFI) 2019[46]	Au DE	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$ MuxT	Optimal Z-t measurement: 15 s	Buffer 5- 25 and 90% blood and plasma	2-5 pg mL^{-1} Recoveries; 14-67 pg mL^{-1} in 90% blood	0.1-2800 pg mL^{-1}
	PEC (CBP) 2019[140]	ITO	rGRO-AuNPs/3-ICT-sol-gel-film/ Ab/T/Ab/(EDC+NHS)/CdS-QDs	CdS-QDs AA	5 µL T / 30 (45 min Buffer; 20 µL T / 30 min (HS) 6 µL CdS-QDs / 30 min	Buffer HS	0.15 pg mL^{-1} <100 pg mL^{-1} (1)	0.25-10000 pg mL^{-1} *(vs lgC_T)*
	CSV (DPCSV) 2020[141]	Au DE	Recognition probe: MBs/Au/Ab/T	Label-free	50 µL T / 30 min	Buffer Horse plasma	10 pM <250 pM (1)	10 pM-100 nM *(non-linear)*

			solution			**limit**
S100B	Carbon SPE	SWCNTs-Nafion-GA/Ab1/T/HRP-Ab2	HRP Reagentless	60 min	HS	10 fg mL-1
S100B	Au DE	Recognition probe: MBs/Au/Ab/T	Label-free	30 min	Buffer Horse plasma	10 pM

SAMS



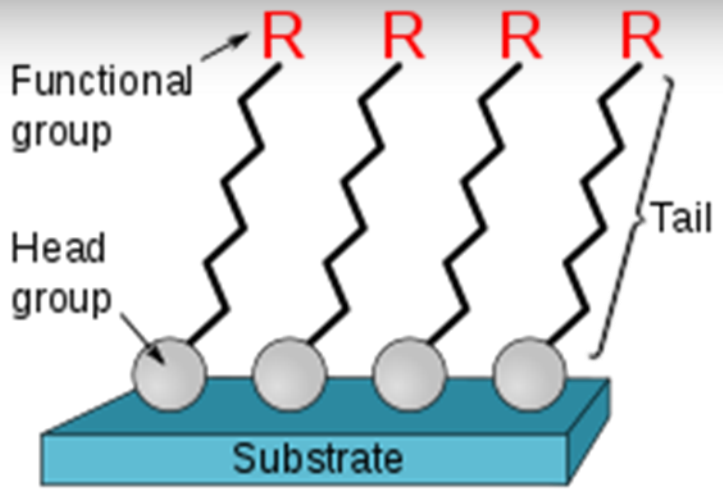


Table 2 Candidates for biochemical markers for traumatic brain injury (TBI)

Candidate TBI biomarker	Expression	Physiological characteristics	Timeline	Sample source	Normal serum levels (ng ml^{-1})	Diagnostic value	Comments
S100β (21 kDa)	Astrocytes, Schwann cells, adipocytes, chondrocytes, melanocytes	Gliosis, BBB compromise	Detection 24 h postinjury, peak levels after 48–72 h, serum half-life of 60–120 min	CSF serum	<0.05	>1.13 ng ml^{-1}; correlates with ↑ mortality, ↑ ICP, poor GCS and poor GOS, loss of BBB integrity	Marker for malignant melanoma, renal and/or intestinal ischemia, also observed increase in S100β in trauma patients with no head injury