Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Electrochemical Sensing of Blood Proteins for Mild Traumatic Brain Injury (mTBI) Diagnostics and Prognostics: Towards a Point-of-Care Application

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SI-1: Full list of published electrochemical strategies for the detection of blood protein biomarkers relevant to mTBI.

Publications related uniquely to a specific application other than blood analysis (e.g. measurements in saliva, sweat, urine, muscle-on-tissue designs etc.) or aimed specifically at electronics development have been omitted, with very few exceptions (detection of VCAM-1 in diluted urine[1], sequentially multiplexed amperometry for IL-6 detection[2], detection of CRP in synthetic urine using molybdenum-based electrode[3]). The search has been limited to scientific publications in peer-reviewed journals with one exception: a patent by Kumta et al.[4] has been included due to a very small amount of publications related to EC detection of UCH-L1. Research publications having accomplished multianalyte detection (a few biomarkers measured simultaneously or sequentially using the same sensing strategy) are denoted as 'MuxT' in *Column 5* ('*Label/Detection solution*'), label-free approaches are marked as 'Label-free' in the same column. To note, 'Label-free' in indicates the assays that include no additional incubation step(s) with the label/labelled antibody after the final incubation with the target analyte (T). That is, either no redox label is required or the redox-label has been already incorporated into the design of the sensor. NOTE: Information about biotin/streptavidin labelling as well as blocking steps (in vast majority of cases using bovine serum albumine) is omitted in *Column 4* ('*Surface modification/Bioreceptor functionalization/Assay format*').

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)
BDNF *Brain-derived neurotrophic factor*	CPA 2018[5]	Carbon SPE (DPI microfluidics, gap 19 µm)	AuNPs/pTTBA/(EDC+NHS)/Ab_1/T/Ab_2/(EDC+NHS)/TBO-pTTBPA/AuNPs/carbon SPE#2	TBO	T / 20 min, 35 °C	Buffer HS	0.015 ng mL^{-1} <0.1 ng mL^{-1} (1)	0.004–0.6 ng mL^{-1}
	DPV 2018[6]	Au np-wrinkled film *(electroless deposition)*	Cystamine/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min, 37 °C	Buffer HP	0.2 ng mL^{-1} <0.5 ng mL^{-1} (1)	0.1–2 ng mL^{-1}

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CRP *C-reactive protein* *(2018-2020)*	DPV 2018[7]	Graphene SPE	AuNPs/L-Cysteine/(EDC+NHS)/Ab_1/T/ Ab_2/(EDC+NHS)/AQ	AQ	2.5 µL T / 40 min 2.5 µL AQ-Ab_2 / 40 min	Buffer HS	1.5 ng mL^{-1} <20.7 µg mL^{-1} (1)	0.01-150 µg mL^{-1}
	SWV 2019[8]	GCE	PDANS/Ab_1/T/BSA-Ab_2-$Cu_3(PO4)_2$-NPs *(nanoflowers)*	BSA-Ab_2-$Cu_3(PO4)_2$-NPs, Na_2MoO_4; SWV in 0.5 M H_2SO_4	6 µL T / 1 h, 37 °C 6 µL Na_2MoO_4 / 1 h, 37 °C	Buffer HS	1.26 pg mL^{-1} <0.3 µg mL^{-1} (1)	5 pg mL^{-1}-1 ng mL^{-1} *(vs lgC_T)*
	EIS µPAD 2019[9]	Carbon SPE	CS/GA/CDP-choline/T	Label-free $[Fe(CN)_6]^{3-/4-}$	30 µL T / 10 min	Buffer HP	0.001 mg L^{-1} <0.1 mg L^{-1} (1) (est. Fig. 5 [9])	0.005-500 mg L^{-1} *(vs lgC_T)*
	EIS 2018[10]	Au IDEs microfab. *(microfluidic ID-zigzag biochip)*	(4-ATP+cysteamine)/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$ /MuxT	T / 5 min (flow rate 25 µL min^{-1})	Buffer	<5.9 pM (1)	5.9 pM-58.9 nM *(vs lgC_T)*
	SWV EIS 2019[11]	Au *(highly ordered wire arrays, microfab.)*	MPA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	20 µL T / 30 min	Buffer HS dil. 1:100	2.25 fg mL^{-1} (SWV) 3 fg mL^{-1} (EIS) 4.5 fg mL^{-1} (SWV)	5-220 fg mL^{-1} (SWV) 7-215 fg mL^{-1} (EIS) 12-166 fg mL^{-1} (SWV)
	Capacitive (impedance derived) 2019[12]	Au *(microfabrication)*	11-FcC/GRO/CBMA/(EDC+NHS)/Ab/T	Label-free	n/a	Buffer ($TBAClO_4$ in CAN and H_2O) HS	18.3 pM 99 pM	50-50'000 pM *(vs lgC_T)* 5'000-500'000 pM *(vs lgC_T)*
	EIS 2018[13]	Au DE	Fc-Peptide/(EDC+NHS)/Apt/T	Label-free	T / 30 min	Buffer ($TBAClO_4$ in ACN and H_2O, 1:4 v/v)	7.2 pM	10-5000 pM *(vs lgC_T)*
	DPV 2019[14]	CPE	CPE-IL/ZnO-MPC*/(EDC+NHS)/Ab/T *MPC obtained via MOF	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 60 min, 37 °C	Buffer HS dil. 1:500	5 pg mL^{-1} <10 ng mL^{-1} (1)	0.01-1000 ng mL^{-1} *(vs lgC_T)*
	DPV 2019[15]	CPE	Zr-tdc-IL (MOF)/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 60 min, 37 °C	Buffer	0.2 ng mL^{-1}	Two linear ranges : (I) 0.5-50 ng mL^{-1} (II) 50-600 ng mL^{-1}
	Conducto-Metry 2018[16]	CuPT-PPy nanowire mesh	NIPAAm-AM/Apt/CRP Polymer	Label-free	T / 15 min, PBS/ 4 min Detection at 95% relative humidity, RT	Buffer HS dil.	9.03 × 10^{-17} g mL^{-1} (7.85·10^{-19} M) <700 ng mL^{-1}(1) (est. Fig. 6[16])	Non-linear *(vs lgC_T)* signal increase up to ca. 10^{-8} M
	EIS/DPV 2018[17]	GCE	PEI-Fc/Ab/T	Label-free EIS redox probe: $[Fe(CN)_6]^{3-/4-}$ DPV repox probe: Fc (incorporated)	20 µL T / 2 h, 4 °C	Buffer Rat plasma dil.1:1000	2.5 ng mL^{-1}(EIS) 0.5 ng mL^{-1} (DPV) <400 µg mL^{-1}(1)	1-50 000 ng mL^{-1}

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CRP *C-reactive protein* *(2018-2020)* *Continuation =>*	FED (DG-ISFET) 2018[18]	Sensing area:high-K HfO_2	Sensing area: (a) H_2O_2(-OH)/APTES/GA/Ab_1/T/ GOx-Ab_2 [Real-time H-ELISA] (b)'Extended gate': Off-chip enzymatic reaction in a 96-well ELISA plate. [End-point H-ELISA]	GOx Glucose+$FeSO_4$ (+H_2O_2) MuxT	T / 2 h; Ab_2 / 1 h avidin-GOx / 30 min 10 min proton detection Standard ELISA, Glucose+$FeSO_4$ / 15 min, 37 °C HS: 1 µL used for dilution, theoretically needed: 10 nL	Buffer HS dil. 1:10000	25 pg mL^{-1} (real-time H-ELISA, (a)) 12.5 pg mL^{-1} (endpoint H-ELISA, (b)) <0.1 mg L^{-1} (1) (est. Fig. 5c[18])	0-200 pg mL^{-1} *(non-linear range)*
	SWV 2018[19]	Au DE	(TCEP)/HS-Apt	Label-free MeB	Complete assay: 40 min	Buffer HS dil. 1:10	1 pM n/a	1-100 pM n/a
	SWV 2018[20]	Au DE	NH_2-Ni-MOF(c)/AuNSs/Ab/T/Apt/(ssDNA$_1$/ MeB-DNA$_2$ duplex)	MeB Exo III enzyme	ssDNA$_1$ / 2h *to open MeB-hpDNA and form the duplex T / 1h Exo III / 2h, 37°C	Buffer HS dil. 1:10	0.029 pg mL^{-1} <0.01 ng mL^{-1} (1)	0.1 pg mL^{-1}–100 ng mL^{-1} *(vs lgC_T)*
	EIS µPAD (origami PAD) 2019[21]	Carbon-graphene SPE	AuNPs/L-cysteine/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	5 µL T / 50 min, 4 °C	Buffer HS, different dilutions	15 ng mL^{-1} <5 µg mL^{-1} (1) (dilution 1:4)	0.05-100 µg mL^{-1} *(vs lgC_T)*
	DPV µPAD 2019[22]	Carbon SPE	AuNPs/PMPC-SH/T	Label-free $[Fe(CN)_6]^{3-/4-}$ Ca^{2+}	100 µL Ca^{2+} / 10 min 100 µL T / 1 h 100 µL $[Fe(CN)_6]^{3-/4-}$ / 10 min Complete assay: 1.5 h	Buffer (pH 6) HS dil.	1.55 ng mL^{-1} <0.1 ng mL^{-1} (1)	5-5000 ng mL^{-1} *(vs lgC_T)*
	CPA 2018[23]	Carbon SPE	AuNPs/L-cysteine/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	30 µL T / 30 min	Buffer HS dil. 1:10	17 ng mL^{-1} <0.932 mg L^{-1} (1)	0.047-23.6 µg mL^{-1}
	EIS 2018[3]	Mo	(EDC+NHS)/Ab/T	Label-free	30 µL T / 5 min	Synthetic urine	100 pg mL^{-1}	0.1-1000 ng mL^{-1} *(vs lgC_T, non-linear part incl.)*
	Capacitive (impedance derived) 2020[24]	Graphene nanoplate SPE	PANI-PA/Ab/T	Label-free; Reagentless	T / 10 min	Buffer FBS, dil. 1:100	0.5 µg mL^{-1} Tested in 2 µg mL^{-1}	145
	EIS (SFI) 2019[25]	ZnO-CuO composite nano-surface	Ab/T	Label-free	40 µL T / 10 min	Buffer	<1 ng mL^{-1} (1)	n/a, ca. from < 1 ng mL^{-1} to 10 ng mL^{-1} *(vs lgC_T)*, (est. Fig. 7[25])
	EIS (SFI) 2019[26]	Au IDEs microfab. *(wave-shaped microel. array)*	DTSP/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 10 min	Buffer HS dil. 1:100	0.025 ng mL^{-1} 0.23 ng mL^{-1}	0.01-10000 ng mL^{-1} *(vs lgC_T)* 0.01-10000 ng mL^{-1} *(vs lgC_T)*
	EIS (SFI) 2019[27]	Au DE	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	100 µL (T+$[Fe(CN)_6]^{3-/4-}$)	Buffer* *Rabbit blood, dil. 1:10 only BNP-target	3 µg mL^{-1}	Up to 10 µg mL^{-1} shown *(vs lgC_T)*
	EIS/CV 2020[28]	Carbon film	MWCNTs (multiple-bent)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 1 h, 4 °C	Buffer	40 pM (EIS) (~4.5 µg mL^{-1}) similar (CV)	10-1000 ng mL^{-1} (EIS) 10-1000 ng mL^{-1} (CV)

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CRP *C-reactive protein* *(2018-2020)* *Continuation =>*	EIS 2020[29]	Au DE	MUA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min	Buffer	3.7 pg mL^{-1} (32 fM)	200-5000 ng mL^{-1} *(vs lgC_T)*
	Dielectric voltammetry 2019[30]	Silica, dielectric-gapped nanosurface	APTES/GA/Ab/[T-(EDC+NHS)-MHDA-GNRs]	Label-free	GNRs with MHDA/ 5 min (EDC+NHS) / 10 min; T / 15 min	Buffer HS dil. 1:1000	10 fM 10 fM	10 fM-1 nM *(vs lgC_T)*
	FED (FET) 2019[31]	Microfluidic chip Drive unit: Si/GaN/AlGaN Sensing area (separated gate): Si/GaN/Au	Sensing area: Thiolated Apt/T	Label-free MuxT	4 µL T / 5 min	Purified T (4% BSA) HS/HP	0.14 µg mL^{-1} <3 µg mL^{-1} (1)	0.1-50 µg mL^{-1} *(vs lgC_T)*
	EIS 2020[32]	Au DE	CTA/pHEMA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 2 h ; T / 15 min*: * dil. FBS, higher T concentrations	Buffer FBS undil. or dil. 1:10, 1:20	7.02 pg mL^{-1} (62 fM) <0.2 µg mL^{-1} (1)	0.2-31.5 µg mL^{-1}
	CPA 2019[33]	Carbon SPE *(dual probe)*	MBs/Ab_1/T/Ab_2-HRP	HRP, H_2O_2 MuxT	50 µL T / 5 min 50 µL Ab_2 / 5 min	Buffer HP dil.	8 ng mL^{-1} <1.7 µg mL^{-1} (1)	0.01-5 µg mL^{-1} *(working range, sigmoid vs lgC_T)*
	RPS (3) 2019[34]	Nanocarriers: SPBs	Peptide-Apt/Non-binding DNA/T	Label-free	MBs with Apt (or DNA) / 30 min; T / 1 h	Buffer	n/a	Low µM range: ca. 0.5-2.5 µM *(estimated from Fig. 4[34])*
	FED (FET) 2019[35]	Si/SiO_2/CeO_2	Ab/T	Label-free	20 µL T / 30 min	Buffer HS	0.1 µg mL^{-1} <1 µg mL^{-1} (1)	0.1-2.5 µg mL^{-1} (working range, not linear)
	PEC 2019[36]	GCE	PNS-777 MOF/AuNPs/Capture strand/ HT/Primer/Padlock probe+dNTPs/T4 ligase+Th-T/phi29 polymerase	Zr-based MOF (PNS-777) as photoactive material Th-T H_2O_2	T: MBs+(EDC+NHS) / 1 h; MBs with amino Apt / 1 h; Primer / 2 h, 37 °C; 50 µL T / 30 min, 25 °C;	Buffer HS dil. 1:50	16 fM <100 fM (1)	50 fM–50 nM (vs lgC_T)
	PEC (CBP) 2019[37]	ITO	NiS/pCOFs/AgNPs/Apt/T	Label-free pCOFs (as photoactive material) H_2O_2	10 µL Apt / 30 min, 37 °C	Buffer HS dil. 1:10	0.1 ng mL^{-1} <20 ng mL^{-1} (1)	0.5-100 ng mL^{-1} (3.5 pM-710 pM)
	CPA 2020[38]	Carbon SPE *(8 multiplexed units)*	MBs/Ab_1/T/Ab_2-HRP	HRP, H_2O_2, HQ	MBs with Ab_1 / 15 min T / 5 min Complete assay (after Ab_1 immobilization): 15 min	Buffer Whole blood dil. 1:10 HP dil. 1:10	1.5 ng mL^{-1} <1 µg mL^{-1} (1) ~2 µg mL^{-1} (1)	0.005-1 µg mL^{-1} (vs lgC_T, non-linear)
	EIS non-farad. 2020[39]	nano-ZnO and ZnO/CuO nitrocellulose membrane	Ab/T	Label-free	40 µL T / 10 min	Buffer	2.5 ng mL^{-1} (nano-ZnO) 16 ng mL^{-1} (nano-ZnO)	0.1-15 ng mL^{-1} (vs lgC_T, non-linear)
	CPA 2020[40]	Carbon SPE *(microfluidic)*	rGRO/Ni/PtNPs micromotors/Ab_1/T/Ab_2-HRP	HRP, H_2O_2, HQ	10 µL T / 5 min	Buffer HP HS	0.8 µg mL^{-1} <3 µg mL^{-1} (1) <41 µg mL^{-1} (1)	2–100 µg mL^{-1} (vs lgC_T)
	SWV 2020[41]	Carbon SPE	Aryldiazonium/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 1h	Buffer	~0.1 ng mL^{-1} (1)	0.01–10 ng mL^{-1}

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CRP *C-reactive protein (2018-2020)* *Continuation =>*	EIS 2020[42]	Au	Fc-Peptide/(EDC+NHS)/Ab	Label-free	Redox-tagged peptide / 16h; (EDC+NHS) / 30 min; Ab / 1h; T / 30 min	Buffer	240 pM Peptide 2 300 pM Peptide 3	0.5-10 nM *(non-linear)*
	CPA 2020[43]	GCE	Chitosan/AuNPs/IL-MoS_2/T/Ab-Ir NPs-GRO-DN	Ab-Ir NPs-GRO-DN H_2O_2	10 µL Ab / overnight T / 60 min at 37 °C	Buffer HS dil. 1:1000	3.3 pg mL^{-1} <5 ng mL^{-1} (1)	0.01–100 ng mL^{-1}
GFAP *Glial fibrillary acidic protein*	EIS 2013[44]	Au MDEA (a) Au MECS (b) *(microfabrication)*	DTSP/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	15 µL T / 30 min (a) 60 µL T / 30 min (b)	Buffer	1 pg mL^{-1}	1 pg mL^{-1}–100 ng mL^{-1}
	FED (OFET) 2014[45]	Si/SiO_2/(*w/wo* Pentacene *or* 8-3 NTCDI)/CYTOP/C44H90/ NHS-PS-*block* PAA	(EDC+NHS)/Ab/T	Label-free	T / 30 min	Buffer	1 ng mL^{-1}	0.8-400 ng mL^{-1} *(vs lgc_T, strictly not linear)*
	DPV 2017[46]	Carbon SPE	MIP-MWCNTs: (MWCNTs+AIBN+ DMAA+AEDP+EGDMA[GFAP])/ agarose film/(SDS+HCl)/EDTA	Label-free $[Fe(CN)_6]^{3-/4-}$	50 µL T / T accumulation (prior to DPV): 2 min at constant E	Buffer HS	0.04 µg mL^{-1} <0.9 µg mL^{-1} (1)	0.2-10 µg mL^{-1}
	FED (OFET) 2017[47]	Drive:Si/SiO_2/Pentacene/Au Sensing: Si	Sensing: (PS-MA+PEG)/Ab/T	Label-free	Drain current almost constant after 30 min	Buffer	1 ng mL^{-1}	0.5-100 ng mL^{-1}
	EIS 2018[48]	Graphene SPE	NaOH(-OH)/PEI/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	50 µL T / 30 min	Buffer HS	1 pg mL^{-1} 1 pg mL^{-1}	1 pg mL^{-1}–100 ng mL^{-1} *(vs lgC_T)*
	EIS (SFI) 2019[49]	Au DE	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$ MuxT	Z-t measurement: 3 s	Buffer 5-25 and 90% rat blood and plasma	2-5 pg mL^{-1} Recov: 14-67 pg mL^{-1} in 90% blood	0.1-2800 pg mL^{-1}
GM-CSF *Granulocyte-macrophage colony-stimulating factor*	CPA 1999[50]	Carbon SPE	EDC/Ab/(free + ALP-labelled T) *(competitive assay)*	ALP PAPP	T / 30 min Complete assay: 35 min	Buffer	0.1 µg mL^{-1}	1.1-30 µg mL^{-1}
	RPS (3) 2005[51]	Pairs of microfabricated Pt electrodes (3)	Probe: Latex colloid/ Ab_1/T/Ab_2	Label-free MuxT	(T+Ab_2) / 20 min	Buffer	<84 ng mL^{-1} (1)	n/a
h-FABP *Heart-fatty acidic binding protein*	CPA 1996[52]	Pt *(Clark type oxygen probe)*	Immunosandwich on nitrocellulose: CDI/Ab_1/T/Ab_2-GOx	GOx Glucose Measurement at pH 5.5	100 µL T / 10 min, 37°C 100 µL Ab_2-GOx / 10 min, 37°C	Buffer HP dil. 1:10	5 ng mL^{-1} n/a	5-80 ng mL^{-1} Clinical range
	CPA 1997[53]	Graphite SPE	Ab_1/T/Ab_2-ALP	ALP PAPP (pH 9.6)	Complete assay: 20 min	HP	10 ng mL^{-1} (Fig. 5-7[53])	10-350 ng mL^{-1} *(not linear in the whole range)*

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h-FABP *Heart-fatty acidic binding protein* *Continuation =>*	CPA 2002[54]	Carbon SPE	Ab_1/T/Ab_2-ALP	ALP PAPP	150 µL (T+ Ab_2-ALP)/ 45 min, 37°C Complete assay: 50 min	Buffer Whole blood Blood dil. 1:10	1 ng mL^{-1} 4 ng mL^{-1} 1 ng mL^{-1}	4-250 ng mL^{-1} *(vs lgC_T)* 10-250 ng mL^{-1} *(vs lgC_T)* 4-250 ng mL^{-1} *(vs lgC_T)*
	SWV 2012[55]	GCE	GRONRs/(EDC+NHS)/Ab_1/T/Ab_2/GA/TiP-Zn^{2+}-probe	TiP-Zn^{2+}-probe MuxT	20 µL T / 60 min; 20 µL Ab_2-TiP-Zn^{2+} probe / 60 min	Buffer HS	3 fg mL^{-1} <1.7 µg mL^{-1} (1)	0.05 pg mL^{-1} –50 ng mL^{-1} *(vs lgC_T)*
	EIS 2012[56]	Au *(microfabrication)*	MUA/(EDC+NHS)/Ab/T *[mSAM]* (MUA+MCOH)/(EDC+NHS)/Ab/T *[hSAM]*	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min, 37°C	Buffer HS	117 pg mL^{-1} *[mSAM]* 524 pg mL^{-1} *[hSAM]* *Similar*	98 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)* *Similar, with decreased sensitivity*
	Capacitive 2015[57]	Au IDEs *(microfabrication)*	MUA/(EDC+NHS)/Ab/T *[mSAM]* (MUA+MPOH)/(EDC+NHS)/Ab/T *[hSAM]*	Label-free $[Fe(CN)_6]^{3-/4-}$	Microfluidic platform: 50 µL T / 30 min	Buffer	0.836 ng mL^{-1} *[mSAM]* 0.968 ng mL^{-1} *[hSAM]*	98 pg mL^{-1}–100 ng mL^{-1} *(vs lgC_T)*
	ASV (DPASV) 2017[58]	GCE	CD-GS/Ab_1/T/Ab_2-ZnO-MWCNTs/CdS	ZnO-MWCNTs/CdS pH 5 prior to DPV MuxT	6 µL T /1 h, 37°C; 6 µL Ab_2-ZnO-MWCNTs / 40 min, 37°C; 8 µL [$Cd(NO_3)_2$ + TAA] / 15 min, 37°C	Buffer HS dil. 1:10	0.3 fg mL^{-1} <5 pg mL^{-1} (1)	1.3 fg mL^{-1}-130 ng mL^{-1} *(vs lgC_T)*
IL-6 *Interleukin 6* (2018-2020)	EIS 2018[59]	PPy-NWs layer	PPyPAC/(EDC+NHS)/Ab/T	Label-free	T / 30 min	Buffer	0.36 pg mL^{-1}	21
	DPV 2018[60]	GCE	AMCs/CTIL/Ab_1/T/(OAMs+APTES)/ACP/(EDC+NHS)/Ab_2-HRP	Ab_2-HRP/(EDC+NHS)/ACP /(OAMs+APTES) 1-NPP, H_2O_2	T / 30 min, 4 °C Ab_2-HRP/ACP/OAMs / 40 min, 4 °C	Buffer HS	0.32 fg mL^{-1} <10 pg mL^{-1} (1)	10 fg mL^{-1}-90 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[61]	GCE	CP|PPC/(EDC+NHS)/Ab_1/T/Ab_2-GRO-NB	NB MuxT	T / 30 min Ab_2-GO-NB / 30 min	Buffer Mouse serum	5 pg mL^{-1} <50 pg mL^{-1} (1)	5–200 pg mL^{-1}
	CPA (Bead-based ELISA) 2018[2]	Au *(microfabrication)* Microfluidic multiplexed assay	WE: CT$(PEG)_{12}$/ (EDC+NHS)/Ab_1 Recognition probe on MBs: Ab_2-HRP	HRP TMB, H_2O_2 Suggested for MuxT	T with 10 µL bead solution / 30 min; MB-T mixture kept in each channel at the sensor / 10 min	Buffer Calf serum dil. 1:4	2.6 pg mL^{-1} 5 pg mL^{-1}	linear between ca. 40 and 1000 pg mL^{-1} *(vs lgC_T)* *(estimated from Fig. 6[2])*
	FED (OECT) 2018[62]	Drive: Kapton/PEDOT:PSS Sensing: Au wire	Sensing area: EG_6COOH/(EDC+NHS)/Ab/T rc-membrane: GA/protein G/glycine/Ab/T	Label-free	Rc. preconc.: 1 mL of T/ 2h; T release in detection buffer: 30 min (100 µL) Gate with T / 1 h	Buffer	220 pg mL^{-1}	n/a

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IL-6 *Interleukin 6* (2018-2020) *Continuation =>*	EIS 2019[63]	Graphite SPE Magneto-immunosensors	Recognition probe on MBs: Protein G/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	10 µL (T+MBs, suspension 1:1) /30 min, 20ºC	Buffer HS dil. 1:100	0.3 pg mL^{-1} <100 pg mL^{-1} (1)	1 pg mL^{-1}–1 µg mL^{-1} *(linear at low concentrations only)*
	EIS 2019[64]	GCE	pABA/(EDC+NHS)/pATP/AuNPs/Apt/T	Label-free $[Fe(CN)_6]^{3-/4-}$	15 µL T / 60 min	Buffer HS dil. 1:1	1.66 pg mL^{-1} <2 pg mL^{-1} (1)	5 pg mL^{-1} –100 ng mL^{-1} *(vs lgC_T)*
	FED (GFET) 2019[65]	Si/SiO2/Graphene	PASE/Apt	Label-free	T / 10 min	Buffer	2.78 pg mL^{-1} (139 fM)	1.5 pM-100 nM (non-linear)
	DPV 2019[66]	Au *(needle microelectrode)*	Sulfo-LC-SPDP/DTT/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 2.5 min	Buffer HS	<20 pg mL^{-1} <100 pg mL^{-1} (1)	0-80 pg mL^{-1} *(linear)* 80-100 pg mL^{-1} *(non-linear)*
	EIS 2020[67]	ITO	PPy-NHS/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:10	10.2 fg mL^{-1} <0.6 pg mL^{-1} (1)	0.03-22.5 pg mL^{-1}
	EIS 2020[68]	ITO	PPCE/IL-6 receptor/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min	Buffer HS dil. 1:10	6 fg mL^{-1} <0.9 pg mL^{-1} (1)	0.02-16 pg mL^{-1}
	EIS 2021[69]	ITO	AB/epoxy-substituted-PPy/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:5	3.2 fg mL^{-1} <1 pg mL^{-1} (1)	0.01–50 pg mL^{-1}
IL-8 *Interleukin 8* (2018-2020)	EIS (SFI) 2018[70]	ITO	Star polymer SPGMA-Super P® carbon black-PVDF composite/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:200	3.3 fg mL^{-1} <26 pg mL^{-1} (1)	0.01-3 pg mL^{-1}
	EIS 2018[71]	ITO	NH_4OH:H_2O_2:H_2O/PHA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min	Buffer HS dil. 1:50	6 fg mL^{-1} <26 pg mL^{-1} (1)	0.02-3 pg mL^{-1}
	SWV 2019[72]	Carbon SPE	PEI-AuNPs/GA/Ab_1/T/PEI-AuNPs-Ab_2-Ag^+	PEI-AuNPs-Ab_2-Ag^+ MuxT	2 µL T / 40 min; 2 µL PEI-AuNPs-Ab_2-Ag^+ / 40 min	Buffer (pH 4.5) HS (pH 4.5)	1 fg mL^{-1} <2.5 pg mL^{-1} (1)	0.5-100 pg mL^{-1} *(vs lgC_T)* 2.5–50 pg mL^{-1}
	ASV (LSASV) 2018[73]	Carbon/MWCNTs AJPE	Ab_1/T/Ab_2-ALP/Ag^0	$AgNO_3$, AA; Stv-ALP as catalyst for Ag^+ reduction	T / 2 h, Ab_2 / 2 h 10 s constant E before LSV	Buffer	0.3 ng mL^{-1}	1.25-10 ng mL^{-1}
	DPV 2020[74]	ITO	β-Ag_2MoO_4/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 10 min	Buffer	90 pg mL^{-1}	1 fg mL^{-1}-40 ng mL^{-1} *(non-linear/two linear ranges)*
	ASV (SWASV) 2020[75]	GCE *(Hg film-modified)*	MBs/(EDC+NHS)/Ab/TCEP treated T	TCEP-treated T/ Maleimide-mod.DNA QDs	50 µL MBs/DNA-QD+250 µL HNO_3 (RT) / 1h; N_2 15 min	Buffer HS dil. 1:10	3.36 fg mL^{-1} <5 fg mL^{-1} (1)	5-5000 fg mL^{-1} *(vs lgC_T)*
IL-10 *Interleukin 10*	CV 2007[76,77]	SiO_2 nanowires Microfluidic chip	APTMS/Ab_1/T/ALP-Ab_2	ALP/pNPP/MuxT	3 µL T / 2 h; Ab_2 / 2h; Stv-ALP / 30 min<; 30 µL pNPP / 20 min, RT	Buffer Lung serum	~ ag mL^{-1} 1 pg mL^{-1} (1)	n/a
	SWV 2012[78]	Au DE	(FRGG+TBAP/MeCN)/(EDC+NHS)/Fc-Ab/T	Label-free MuxT	T / 15 min Complete assay: 20 min	Buffer	<1 pg mL^{-1} (1)	0.001-50 ng mL^{-1} *(vs lgC_T)*

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IL-10 *Interleukin 10* *Continuation =>*	EIS 2012[79]	HfO_2	TESUD/Ab/T	Label-free	T / 30 min, 4°C Total volume: 10 mL	Buffer	0.1 pg mL^{-1}	0.1-20 pg mL^{-1} *(vs lgC_T)*
	EIS 2015[80]	Al_2O_3	APTES/MWCNTs/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	50 µL T / 30 min, 4°C	Buffer	<0.5 pg mL^{-1} (1)	0.5-500 pg mL^{-1} *(vs lgC_T)*
	EIS 2016[81]	Au *(microfabrication)* Microfluidic chip	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min, 4°C	Buffer	n/a	1–15 pg mL^{-1} *(non-linear)*
	EIS 2017[82]	Au *(microfabrication)*	CMA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	T / 30 min, 4°C 15 min for detection	Buffer	0.3 pg mL^{-1}	1–15 pg mL^{-1}
	EIS 2020[83]	Graphene ID AJPE	(EDC+NHS)/T	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	100 µL T / 30 min Complete assay: 33 min	BIS dil. 1:1000	46 pg mL^{-1}	0.1-2 g mL^{-1} *(vs lgC_T)*
	EIS 2020[84]	$Si/SiO_2/Si_3N_4$-(Spy-PPy)	CMA/(EDC+NHS)/Ab/T	Label-free	T / 30 min, 4°C	Buffer	0.347 pg mL^{-1}	1-10 pg mL^{-1}
MMP-2 *Metallo-proteinase-2*	DPV 2013[85]	GCE	Au-NGR/Ab_1/T/HRP-Ab_2-(GA)-PDA-GRO	HRP Thi+H_2O_2	10 µL T / 1 h, 37°C; 50 µL HRP-Ab_2/PDA-GRO/ 50 min, 37°C	Buffer HS	0.11 pg mL^{-1} <0.4 ng mL^{-1} (1)	0.0005-50 ng mL^{-1} *(vs lgC_T)*
	DPV 2013[86]	Au DE	Thiolated DNA/MCH/Collagen-like Pept (5) (target-induced degradation)	Label-free $[Fe(CN)_6]^{3-/4-}$ (5) APMA for T activation; Captopril for modulating T	(T+APMA) / overnight, 37°C WE with activ. T / 2 h, 37°C Captopril / 30 min, 37°C	Buffer	0.1 µg mL^{-1}	0.1-1 µg mL^{-1}
	ASV (SWASV) 2013[87]	Au thin film *(PDMS-AuNPs composite)*	Pept-SH/AuNPs-DNA-(EDC)-$CdSe_{0.5}Te_{0.5}$QDs (target-induced cleavage)	$CdSe_{0.5}Te_{0.5}$QDs; HNO_3; Bi^{3+} prior to SWV (pH 5.2)	100 µL T / 2h, 37°C 200 µL HNO_3 / 2h	Buffer (pH 5.2) HS dil. 1:10	0.63 pg mL^{-1} <1.7 ng mL^{-1} (1)	1-500 pg mL^{-1}
	FED (FET) 2013[88]	SiO_2	APTES/GA/FN (target-induced degradation)	Label-free $CaCl_2$	Measurement 3 h after addition of (T+$CaCl_2$)	Buffer	<150 ng mL^{-1} (1)	Only 150 ng mL^{-1} executed
	FED (FET) 2013[89,90]	SiNWs *(zigzag structure)*	TESBA/peptide)[89] TESBA/peptide/DNA/AuNPs[90] (target-induced cleavage)	Label-free	T / conductance change registered after 20 s[89] and 13 s[90]	Buffer	ca. 1 pM[89] ca. 0.1 pM[90]	1 pM-100 nM *(vs lgC_T)*[89] 100 fM-10 nM *(vs lgC_T)* [90]
	PEC (CBP) 2014[91]	TiO_2-NTs	CdS:Mn/CdTe-QDs/Ab_1/ T/Ab_2@SiO_2	Ab_2@SiO_2 label; TiO_2-NTs/ CdS:Mn/CdTe-QDs	20 µL T / 1 h, 37°C 20 µL Ab_2@SiO_2 / 1 h, 37°C	Buffer	3.6 fg mL^{-1}	10 fg mL^{-1}-500 pg mL^{-1} *(vs lgC_T)*
	DPV 2015[92]	Au DE	9-MN/Fc-Pept (target-induced cleavage)	Label-free APMA for T activation	T in TCNB buffer; 20 µL T with APMA/ 1 h, 37°C / WE with activated T / 1 h, RT	Buffer HS dil. 1:10	0.3 ng mL^{-1} <13.7 ng mL^{-1} (1)	1-200 ng mL^{-1} *(vs lgC_T)*
	DPV 2015[93]	GCE	Au/(ssDNA$_1$-pPtNPs-Pept-SH)/(ssDNA$_1$+ ssDNA$_2$+Thi) (target-induced cleavage)	Label-free H_2O_2	T / 2 h, 37°C	Buffer HS dil. 1:10	0.32 pg mL^{-1} <0.1 pg mL^{-1} (1)	1 pg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)*

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MMP-2 *Metallo-proteinase-2* *Continuation =>*	EIS 2015[94]	Au *(microfabrication)* Microfluidic chip	Pept-SH (target-induced cleavage)	Label-free/MuxT (MMP-2, MMP-7)	10 µL peptide P5 / 30 min; 20 µL T / 30 min; Whole assay: 67,5 min	Buffer	0.5 pg mL^{-1}	0.1–400 ng mL^{-1} *(non-linear, impedance reduction vs c_T)*
	DPV 2016[95]	GCE	Au/Pept-SH/Stv-Thi-Pt-Pd-mhCeO_2NS-NPrs (target-induced cleavage)	Thi-Pt-Pd-mhCeO_2NS-NPrs H_2O_2	20 µL T / 2 h, 37°C 20 µL NPrs/ 1 h, 37°C	Buffer HS dil. 1:10	0.078 pg mL^{-1} <1 ng mL^{-1} (1)	0.1 pg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)*
	DPV 2016[96]	GCE	GCE: Au/CB[7]/ released MeB Probe: MBs/(EDC+NHS)/Pept-SH/AuNPs-DNA_1 (target-induced cleavage)	MeB-DNA_2+Exo III	20 µL (Probe+T)/ 40 min, 37°C (Cleaved-Pept/AuNPs-DNA_1 + MeB-DNA_2) / 60 min, 37°C; Exo III / 60 min, 37°C; deact. / 20 min, 80°C	Buffer HS dil. 1:20	0.15 pg mL^{-1} <0.1 ng mL^{-1} (1)	0.5 pg mL^{-1}-50 ng mL^{-1} *(vs lgC_T)*
	DPV 2017[97]	GCE	Au/Fc-Pept; Probe: (CB[7]-PtNPs with Fc-HRP)/(CB[7]-PtNPs with Fc-GOx) (target-induced cleavage)	(CB[7]-PtNPs with Fc-HRP)/(CB[7]-PtNPs with Fc-GOx) H_2O_2, Glucose (4)	T / 50 min, 37°C CB[7]-PtNPs, Fc-HRP, CB[7]-PtNPs and Fc-GOx / 30 min	Buffer HS dil. 1:50	0.03 pg mL^{-1} <1 pg mL^{-1} (1)	0.1 pg mL^{-1}-20 ng mL^{-1} *(vs lgC_T)*
	CPA 2017[98]	ITO	K-GS@CS@$C_9H_{14}NBF_4$/GA/Ab/T/GA/ $ssDNA_3$/$ssDNA_1$@HRPAuNPs@$ssDNA_2$@Thi	$ssDNA_1$@HRP-AuNPs@ $ssDNA_2$@Thi H_2O_2	6 µL T	Buffer HS	35 fg mL^{-1} <1 pg mL^{-1} (1)	100 fg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[99]	GCE	Au-rGRO-pMeB/Pept-SH/(EDC+NHS)/ PtNPs-amFc-BSA (target-induced cleavage)	Label-free	60 µL T / 3 h	Buffer HS dil. 1:1000	<0.01 ng mL^{-1} <0.5 ng mL^{-1} (1)	0.01-10 ng mL^{-1} *(vs lgC_T)*
	SWV 2019[100]	GCE	PANI gel/AuNPs/Pept-SH/CS-AuNPs-Pb(II)/ Na-tartrate gel (target-induced cleavage)	Label-free $[Fe(CN)_6]^{3-/4-}$	20 µL T / 60 min, 37°C Buffer / 45 min, 37°C prior to SWV	Buffer HS	0.4 pg mL^{-1} <110 ng mL^{-1} (1)	1 pg mL^{-1}-1 µg mL^{-1} *(vs lgC_T)*
	PEC (CBP) 2020[101]	ITO	Fe_3O_4@SiO_2/(EDC+NHS)/Ab_1/T/Ab_2/ TiO_2-AgNPs	TiO_2-Ag NPs/Ab_2	100 µL T with Fe_3O_4@SiO_2-Ab_1 / 60 min, 37°C; mag. separation with TiO_2-Ag NPs-Ab_2 / 60 min, 37°C	Buffer HS	0.34 fg mL^{-1} <350 pg mL^{-1} (1)	1 fg mL^{-1}-100 pg mL^{-1} *(vs lgC_T)*
MT3 *Metallo-Thionein*	DPV 2013[102]	GCE	$K_3[Fe(CN)_6]$-CS-GA/C-dots+Nafion/Ab/T	Label-free	T / 60 min, 37°C	Buffer HS	2.5 pg mL^{-1} <170 pg mL^{-1} (1)	5 pg mL^{-1}– 20 ng mL^{-1}
NCAM *Neuron cell adhesion molecule*	DPV 2020[103]	GCE	MIP (pABA + PolySia®)	$[Fe(CN)_6]^{3-/4-}$ *p*-ABA	T in *p*-ABA solution (buffer, pH 9.0) / 60 min	Buffer HS dil. 1:1000	"Probe-type" 4.74 ng mL^{-1}; "Sandwich" 0.47 ng mL^{-1} In SI, n/a	"Probe-type": 10-150 ng mL^{-1} *(vs lgC_T);* "Sandwich" 1-1000 ng mL^{-1} *(vs lgC_T)*

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NFL *Neuro-filament ligt*	EIS 2020[104]	Au *(microfabrication)*	MAC/GMA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	n/a	KCl	5.21 ng L^{-1}	1-50 µg L^{-1}
	PEC (no bias) 2020[105]	Pt NWs on FTO (biocathode)	(MUA+MCH)/Ab Photoanode: FTO/$BiVO_4$-FeOOH	Label-free	60 µL T / 1 h, RT	Buffer HP dil. 1:10	38.2 fg mL^{-1} n/a	0.1-1000 pg mL^{-1} *(vs lgC_T)*
NGB *Neuroglobin*	CV 2020[106]	Au DE	np-Au/MCH/TMSE/T (a) np-Au/CPT/(EDC+NHS)/T (b)	H_2O_2 and Cyt *c* as redox partners/substrates	n/a	Buffer	*Qualitative study: strategy for exploring the molecular basis of NGB coupled with electron transfer*	
NRGN *Neurogranin*	CPA 2000[107]	GCE	Pd@POAP (electro-catalytic oxidation of T by NO)	NO (physiological level)	Detection: 1-2 min	Buffer	sub-µM	sub-µM -10 µM range
NSE *Neuron-specific enolase* (2018-2020)	DPV 2018[108]	GCE	PC/AuNA/ $MVIMBF_4$/ MIP-Poly(DPIMBr)/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 15 min	Buffer HS dil. 1:100	2.6 pg mL^{-1} <7.7 ng mL^{-1} (1)	ng mL^{-1}
	DPV 2018[109]	Graphite SPE	GR nanosheets/PpPD/ AuNPs/Ab/T	Label-free AA	T / 60 min	Buffer HS	0.3 ng mL^{-1} <11 ng mL^{-1} (1)	1-1000 ng mL^{-1}
	DPV 2018[110]	Au *(3D-SiCPCC-modified)*	3DM rGRO-PANI/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	10 µL T / 40 min, 37°C	Buffer HS dil.	0.1 pg mL^{-1} <0.5 ng mL^{-1} (1)	0.5 pg mL^{-1}-10 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[111]	GCE	CS-Fc/AuPd-MWCNTs/GA/Ab/T	Label-free H_2O_2	20 µL T / 50 min, 37°C	Buffer HS	0.48 pg mL^{-1} <1 ng mL^{-1} (1)	1 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[112]	Carbon SPE	pTMB-Au|Pd-SA-AuNPs-Ca^{2+} hydrogel/Ab/T	Label-free H_2O_2 / MuxT	10 µL T / 45 min, 37°C	Buffer (pH 6.5) HS	2.3 pg mL^{-1} <1.7 ng mL^{-1} (1)	0.01-200 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[113]	GCE	PANI hydrogel/AuNPs/Ab_1/T/Ab_2-AuNPs-Thi-rGRO-Hem	Ab_2-AuNPs-Thi-rGRO-Hem/ H_2O_2	80 µL T / 45 min, 37°C ; 40 µL Ab_2-AuNPs-Thi-rGRO-Hem / 37°C	Buffer HS	0.026 pg mL^{-1} <1 ng mL^{-1} (1)	0.1 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[114]	GCE	Alginate/PANI/hydrogel/GA/Ab/T/ [Nanogel/Cu@AuNPs]	Probe: Cu@AuNPs	200 µL probe with T / 1 h 60 µL T + 20 µL probe free T / 1 h	Buffer (pH 5.5) HS	4.6 pg mL^{-1} <3.3 ng mL^{-1} (1)	0.01-100 ng mL^{-1} *(vs lgC_T)*
	SWV 2018[115]	GCE	PPy-polyThi-hydrogel with GOx/AuNPs/Ab/T	Label-free; H_2O_2 Glucose; GOx doping	T / 50 min	Buffer HS	0.65 pg mL^{-1} <5.5 ng mL^{-1} (1)	1 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	DPV µPAD 2019[116]	Carbon Ink	PB-PEDOT-AuNPs/SH-Apt/T	Label-free MuxT	20 µL T / 1 h	Buffer HS	10 pg mL^{-1} <1.25 ng mL^{-1} (1)	0.05-500 ng mL^{-1}

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NSE *Neuron-specific enolase* (2018-2020) *Continuation =>*	DPV 2019[117]	GCE	AuNPs/Ab_1/T/TB/WP6@ PdPt PCONs/Ab_2	TB/WP6@PdPt PCONs/Ab_2 $[Fe(CN)_6]^{3-/4-}$ H_2O_2	GCE/AuNPs/Ab_1 with T / 1 h TB/WP6@PdPt PCONs/Ab_2 / 1 h, Complete assay: 2 h	Buffer HS	95 fg mL^{-1} <3.9 ng mL^{-1} (1)	300 fg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	SWV 2019[118]	Au wires Au QCM chips	HME+MIP-Scopoletin/T CME/MIP-Scopoletin/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min	Buffer	1 ng mL^{-1} *(CME)* 0.25 ng mL^{-1} *(HME)*	1-64 ng mL^{-1} (CME) *(non-linear)* 0.25-64 ng mL^{-1}(HME) *(non-linear)*
	SWV 2019[119]	GCE	GCE: AuNPs/(TCEP+Thiolated DNA)/MCH Sandwiched immunocomplex: Ab_2-PtCu/T/Ab_1-MBs	PtCu-nanoprobe $[Fe(CN)_6]^{3-/4-}$ H_2O_2, I^-	MBs-Ab_1 with T / 40 min, 37°C; with PtCu-Ab_2 / 50 min, 37°C; NaJ and H_2O_2 / 1 h, 37°C; GCE/AuNPs/ Thiolated DNA/MCH/ 50 min, 37°C	Buffer HS	52.14 fg mL^{-1} <1.1 ng mL^{-1} (1)	100 fg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	SWV 2019[120]	GCE	GCE: MWCNTs/Fe^{3+}-alginate hydrogel Sandwiched immunocomplex: Ab_2-GOx-SiO_2-GOx/T/Ab_1-MBs	GOx-SiO_2-GOx immunoprobe $[Fe(CN)_6]^{3-/4-}$/Glucose	MBs-Ab_1 with T (30 µL) / 40 min, 37°C MBs-Ab_1/T with Ab_2-GOx-SiO_2-GOx / 40 min, 37°C	Buffer HS	0.447 pg mL^{-1} <50 pg mL^{-1} (1)	1 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	SWV LFA (with SERS) 2019[121]	FTO	AgNPs/Au/NBA/Ab/T	Label-free MuxT	T / 30 min	Buffer (pH 6.5) HS	40 pg mL^{-1} <1.4 ng mL^{-1} (1)	50 pg mL^{-1}-1 µg mL^{-1}
	EIS 2019[122]	ITO	P(ThiPh-gMAm)/GA/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:10000	6.1 fg mL^{-1} <0.55 pg mL^{-1} (1)	0.02-4 pg 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: 13 s	Buffer 5-25 and 90% rat blood and plasma	2-5 pg mL^{-1} Recoveries: 14-67 pg mL^{-1} in 90% blood	0.1-2800 pg mL^{-1}
	EIS (SFI) 2019[123]	Au DE	MHDA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$ /MuxT	T+$[Fe(CN)_6]^{3-/4-}$: 100 µL	Buffer	3.95 pg mL^{-1}	1-25000 pg mL^{-1}
	ASV (LSASV) 2019[124]	GCE	3D-GRS/CS/GA/Ab_1/T/Ab_2-OMCSi-AuNPs/ 3D-GRS/AgNPs	OMCSi-AuNPs/3D-GRS/AgNPs	T / 45 min, 25°C 10 µL Ab_2-OMCSi-AuNPs / 40 min, RT 20 µL Ag enhancer / 6 min, 25°C	Buffer HS	0.008 pg mL^{-1} <27 pg mL^{-1} (1)	0.02 pg mL^{-1}-35 ng mL^{-1}

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NSE *Neuron-specific enolase* (2018-2020) *Continuation =>*	Poten. 2019[125]	pH electrode *(commercial)*	Immunoassay immobilization on PS-microplates: Ab_1/T/Ab_2-GOx-LS	GOx-LS; Triton X-100 (to release GOx), Glucose	(T + Ab_2-GOx-LS) [50+50 µL/well]/ 35 min; Glucose / 10 min	Buffer HS	8.9 pg mL^{-1} <0.5 ng mL^{-1} (1)	0.01-100 ng mL^{-1} *(dynamic linear range: pH vs lgC_T)*
	PEC (CBP) 2019[126]	ITO	$NiWO_4$-NStr/Ab/T	Label-free Uric acid	PEC measurement: 150 s with 20 s light on/off cycles	Buffer HS	0.12 ng mL^{-1} <10.7 ng mL^{-1} (1)	75-723 ng mL^{-1} *(vs lgC_T)*
	DPV 2020[127]	GCE	Au@MOFs/(EDC+NHS)/ Ab_1/T/Ab_2-Au@Pd^Pt NCbs/MnO_2 UNs	MnO_2 UNs/Au@ Pd^Pt NCbs label* HQ, H_2O_2	6 µL MnO_2 UNs/Au@ Pd^Pt NCbs-Ab_2 / 1 h	Buffer HS	4.17 fg mL^{-1} <0.7 ng mL^{-1} (1)	10 fg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
	DPV 2020[128]	GCE	Fc-g-Au@Pd-P(BBY)/(TCEP+Apt_1)/T/ Apt_2/AuPt NAs/Thi/rGRO	Thi and Fc as signal probes	Fc-g-Au@Pd-P(BBY)/(TCEP+Apt_1) with T / 60 min, 37°C, Apt_2/AuPt NAs/Thi/rGRO / 60 min, 37°C	Buffer HS dil.	30 fg mL^{-1} n/a	100 fg mL^{-1}-50 ng mL^{-1} *(vs lgC_T)*
	SWV 2020[129]	Au wires	AuNPs-MIPs (epitope-mediated)	Label-free $[Fe(CN)_6]^{3-/4-}$	2 mL T / 15 min	HS dil. 1:2	25 /200 pg mL^{-1} *(w/wo Au NPs)*	25-4000 / 50-500 pg mL^{-1} *(w/wo Au NPs) (non-linear)*
	EIS 2020[130]	ITO	P(Pyr-Epx)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 30 min	Buffer HS dil. 1:10000	6.1 fg mL^{-1} <1.2 pg mL^{-1} (1)	0.02-7.5 pg mL^{-1}
	EIS 2020[131]	ITO	Str($PGMA)_3$/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:10000	9.1 fg mL^{-1} <1.2 pg mL^{-1} (1)	0.03-6 pg mL^{-1}
	EIS 2020[132]	Au DE	Zr-TAPP/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 50 min	Buffer HS dil. 1:500	7.1 fg mL^{-1} <10 fg mL^{-1} (1)	10 fg mL^{-1}-2 ng mL^{-1} *(vs lgC_T)*
	CPA 2021[133]	GCE	AuPt NSNs/Ab_1/T/Ab_2/ Au-Cu_xO@CeO_2	Au-Cu_xO@CeO_2/Ab_2 H_2O_2	6 µL T / 40 min, RT 6 µL Ab_2 / 50 min, RT	Buffer HS 1:10	31.3 fg mL^{-1} <1.5 ng mL^{-1} (1)	50 fg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)*
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

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)*
Tau protein(s) / T-Tau *Total tau (P- + non-phosphor.)*	EIS 2014[142]	Au DE	Lip-NHS/Tau-protein/T	Label-free $[Fe(CN)_6]^{3-/4-}$	5 µL T / 2h	Buffer	0.2 µM	0.1-1.0 µM 2N4R (tau441)
	DPV 2017[143]	Carbon SPE	GRO/(EDC+NHS)(+DMAP)/pPG/GA/Ab_1/T/ Ab_2/(PbS+MUA)/(EDC+NHS)/pPG	PbS-NCs-probe; HNO_3 for NCs ionization/ MuxT	1 mL in a cell (Buffer); 10 µL drop-casted (HS); 15 min with HNO_3	Buffer HS dil. 1:100	0.15 nM <0.5 nM (1)	0.15-250 nM *(non-linear)*
	EIS 2017[144]	Au *(microfabrication)*	DTSSP/Protein G/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 25 min	Buffer HS	0.03 pM 0.01 pM	0.01 pM-10 nM 0.01 pM-10 nM 2N4R (tau441)

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)
Tau protein(s) / T-Tau *Total tau (P- + non-phosphor.)* *Continuation =>*	DPV 2018[145]	Au *(microfabrication)*	(SATA+Ab)/Thiolated pGluA/T (a) (SATA+Ab)/T (b)	Label-free $[Fe(CN)_6]^{3-/4-}$/MuxT	20 µL T / 30 min	Buffer (pH 6.2)	0.968 pM (a) 9.68 pM (b)	0.968-454 pM (a,b) *(vs lgC_T)*
	DPV 2018[146]	Au DE	MPA/(EDC+NHS)/Ab/T/AuNPs-SH-Apt	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 45 min	Buffer HS dil. 1:100	0.42 pM <1.5 pM (1)	0.5-100 pM 1N3R (tau381)
	EIS 2018[4]	Multiarray of vertically aligned Pt wires	Cysteamine/GA/Ab and(or) Apt/T	Label-free/$[Fe(CN)_6]^{3-/4-}$ Suggested for MuxT	2 µL T / 5 min	Buffer	0.001 pg mL^{-1}	0.001-10 pg mL^{-1}
	EIS 2018[147]	Au DE	Lipoic acid/(EDC+NHS)/Ab/n-butylamine/hexanethiol/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 1h	Buffer	nM range	nM to µM range 2N4R (tau441)
	DPV 2019[148]	GCE	CGR/Thi/AuNPs/Apt/T	Label-free	20 µL T / 30 min	Buffer HS dil. 1:100	0.7 pM <1 pM (1)	1-100 pM 1N3R (tau381)
	DPV 2020[149]	Au	MWCNTs/rGRO/CS/Ab/T/AuNPs	Au NP $[Fe(CN)_6]^{3-/4-}$	T with AuNPs / 4 h, 4°C; T-AuNPs conjugate with WE / 30 min, 4°C	Buffer HS	0.46 fM <1.5 fM (2)	0.5-80 fM (vs lgC_T) 2N4R (tau441)
	SWV 2020[150]	GCE	SL-rGRO@PTSA/Cu^{2+}/ (EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	6 µL T / 30 min, 4°C	Buffer HS dil. 1:1000	75 fM <2.5 pM (1)	0.08-80 pM (vs lgC_T) 2N4R (tau441)
	SWV 2020[151]	Au *(mini pilar-based sensor)*	Au nanodendrites/Ab/T	Label-free $Ru(NH_3)_6^{3+}$/MuxT	10 µL Ab / 4h at RT 10 µL T / 4h at RT	Buffer HS	$7.14\ 10^{-11}$ mg mL^{-1} n/a	10^{-10}-10^{-7} mg mL^{-1} (vs lgC_T)
	EIS 2020[152]	PET-ITO	rGRO/Au NP/11-MUA/(EDC+NHS)/Ab/T	Label free $[Fe(CN)_6]^{3-/4-}$	T / 60 min, dark	Buffer HS	0.091 pg mL^{-1} <10 pg mL^{-1} (1)	1-500 pg mL^{-1} 2N4R (tau441)
	FED (FET) 2020[153]	Sensing : Glass/Ti/Au *(microfluidic chamber)*	Sensing area: Au/COOH-EG_8-thiol/PEG/(EDC+NHS)/Ab/T	Label-free	Complete assay: 30 min	Buffer (CSF)	1 pM (~10 pM)	1 pM-10 nM *(Fig. 2)* [153] (vs lgC_T)
	CPA 2020[154,155]	Carbon SPE[154] Dual SPCE[155]	pABA/(EDC+NHSS)/3D-Au-PAMAM/GA/Ab_1/T/Ab_2-HRP	Ab_2/HRP HQ/H_2O_2 MuxT	T / 1h Ab_2 / 60 min	Buffer HP	1.7 pg mL^{-1} [154] 2.3 pg mL^{-1} [155] (~pg mL^{-1}) (1)[154,155]	6-5000 pg mL^{-1} [154] 8-5000 pg mL^{-1} [155] 2N4R (tau441)

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)
Tau protein(s) / T-Tau *Total tau (P- + non-phosphor.)* *Continuation =>*	ELA-PEC 2021[156]	Carbon paste electrode	AuNPs-$MoSe_2$/MCH/Apt/T/Ab/Protein G-AP	Protein G-AP AAP, $Mg(NO_3)_2$	35 µL T / 30 min, 37°C; 35 µL Ab / 60 min, 37°C; 35 µL Protein G-AP / 60 min, 37°C; [AAP+$Mg(NO_3)_2$] / 60 min, 37°C	Buffer HS dil. 1:100	0.3 fM <0.5 pM (1)	0.5 fM-1.0 nM (vs lgC_T) 1N3R (tau381)
	DPV 2017[157]	Au *(microfabrication)*	MPA/(EDC+NHS)/Ab/T	Label-free $[Fe(CN)_6]^{3-/4-}$	T / 3h	Buffer HS	1000 pg mL^{-1} 1000 pg mL^{-1}	1000-100000 pg mL^{-1} 1000-100000 pg mL^{-1} T-Tau
	PEC 2020[158]	FTO	Mo:$BiVO_4$/FeOOH/Ab_1/T/Ab_2-HRP	Ab_2-HRP DAB	70 µL T / 1h, RT 30 µL Ab_2 / 1h DAB / 10 min	Buffer HP dil. 1:10	1.59 fM ~fM (1)	~fM to >10^4 fM *(vs lgC_T)* (Buffer, HP, *Fig. 4*)[158] T-Tau
	FED (GFET) 2020[159]	Si/SiO_2	APMES/rGRO/PBASE/Ab/T	Label-free MuxT	20 µL T / 30 min	Buffer HP dil. 1:10	n/a 1 pg mL^{-1} (HP)	100 fg mL^{-1}-1 ng mL^{-1} *(vs lgC_T)* 100 fg mL^{-1}-10 ng mL^{-1} *(HP, vs lgC_T)* T-Tau
UCH-L1 *Ubiquitin C-terminal hydrolase*	EIS 2018[4]	Multiarray of vertically aligned Pt	Cysteamine/GA/Ab and(or) Apt/T	Label-free $[Fe(CN)_6]^{3-/4-}$ Suggested for MuxT	The suggested array has been patented for the detection of UCH-L1, GFAP and tau-proteins. However, the array has been tested in detail for tau-protein detection only.			
	SWV 2019[160]	Graphene SPE	pNE/Ab (a) pDE/Ab (b)	Label-free $[Fe(CN)_6]^{3-/4-}$	50 µL T / 30 min	Buffer HS	1.91(a) 0.70 (b) pg mL^{-1} 1.68 (a) 0.63 (b) pg mL^{-1}	0.1 pg mL^{-1}-100 ng mL^{-1} *(vs lgC_T)* (a) 1 pg mL^{-1}- 100 ng mL^{-1} *(vs lgC_T)* (b)
VCAM-1 Vascular cell adhesion protein 1	EIS (6) non-farad. 2017[1]	Au microelectrode	DTSP/Ab/T or DTSP/Ab_1/T/Ab_2	Label-free	50-100 µL T / 15 min	Buffer Urine dil. 1:5000	8 fg mL^{-1} <500 µg mL^{-1} (1)	8 fg mL^{-1}- 800 pg mL^{-1} (vs lgC_T)

(1) Lowest reported LDL using EC detection methods; '<x' corresponds to the lowest concentration analyzed within the working range of the sensor (employing standard addition method and/or a reference material/method for validation, with a decent recovery), actual LDL being possibly lower than the indicated value. Redox couple ($[Fe(CN)_6]^{3-}/^{4-}$) indicated if used. **(2)** The upper limit of the range indicated often presents the maximum concentration explored but not the upper detection limit. Please consult original paper for details. **(3)** Increase in diameter of a sub-micron latex colloid upon binding to an unlabelled specific antibody results in changes in pore resistance. Particles passing through the pore displace the conducting fluid in that pore. **(4)** Enzyme cascade amplification: GOx catalyses glucose to gluconic acid with concomitant formation of H_2O_2 for accelerating the redox reaction of Fc in the presence of HRP and PtNPs. **(5)** Collagen in the complex is being degraded by MMP-2. The inhibition effect of captopril to MMP-2 can be revealed by the electrochemical signal. With the increase of MMP-2 concentration more collagen molecules will be digested, thus a larger amount of electrochemical probe $[Fe(CN)_6]^{3-/4-}$ can get closer to the electrode leading to an increase of the electrochemical signal. **(6)** Application for the target detection in urine has been exceptionally noted here, due to the fact that no other publications have been found on the electrochemical detection of VCAM-1 biomarker. For **Column 7 'Sample'**: Dilution factor ('dil.', if indicated) corresponds to the primary dilution of the sample to be analyzed and does not account for the further dilution steps implied by the suggested protocol (mixing with the redox probe/mediator/labelling solution/signal enhancer/detection buffer/etc.). pH of the (detection) buffer is indicated, if significantly different from clinical ranges in blood samples (ca. 7.5). **ABBREVIATIONS:** see last Page. For more detailed information on EC strategies for the detection of CRP biomarker readers should refer to the review articles by Bakirhan et al.[161], Sohrabi et al.[162], Dhara and Mahapatra[163] and Chen et al.[164].

As of December 2020 no EC detection strategies have been found on the following biomarkers: **BMX** (*bone marrow tyrosine kinase on chromosome X*), **CKBB** (*creatine kinase B type*), **ICAM-1** (*Intracellular adhesion molecule-1*), **MDA-LDL** (*malondialdehyde modified low density lipoprotein*), **NFM** (*neurofilament medium*), **Nogo-A** (*neurite outgrowth inhibitor protein*), **pNF-H (NF-H)** *((phosphorylated) neurofilament heavy protein*), **E-selectin** (*E-selectin*), **SNTF** (*calpain-derived αII-spectrin N-terminal fragment*) and **Ub** (*ubiquitin*).

SI-2: Summary of Key Observations and Outstanding Challenges

Summary of Key Observations and Outstanding Challenges

A total number of **127 publications** on **EC** techniques and protocols for **19 different mTBI protein biomarkers** were compiled (**Table 3 and SI-1**).

- **Techniques**

EIS (35 entries) followed by **DPV** (29) and **SWV** (23) were the most frequent **EC** methods employed for determining **mTBI** relevant blood proteins concentrations.

- **Assay performance**

99 publications report measurement data obtained in complex matrix (e.g., **HS**, **HP**, etc.), but the vast majority did so under significantly diluted sample conditions and/or compromised analytical performance characteristics. Sample dilution may be a feasible approach to reduce **NSB** (see **SI-3**), but this brings up additional requirements to sample preparation (e.g., microfluidic cartridge design) or operator usability aspects, the latter not being ideal for POC diagnostic testing. While reproducibility of results is indicated in many of the publications, only few have determined accuracy and precision data with real/clinical samples (e.g., goal of CV < 6% in laboratory medicine), with multiple reagent/sensor lots, with a statistically significant patient sample number and by systematically comparing performances against a reference method. In this context the question comes up to which degree the impressive detection limits (**LDL**) reported can be confirmed in real-world situations to reliably differentiate brain injured from healthy individuals based on physiological cutoffs (**CO**).

- **Diagnostic Specificity / Multiplexing**

It is primarily an **mTBI** biomarker discovery and validation rather than sensor development task to improve the diagnostic specificity (i.e., reduce the number of false positives). However, since no single protein biomarker provides sufficient specificity, the right combination (e.g., 5-plex?) may do so in the future. Therefore, enabling a multiprotein detection modality is likely to be crucial, especially for a POC diagnostic application. **EC** sensors seem technologically apt for (simultaneous) multiple protein **mTBI** biomarker target detection - in 26 publications authors report data on multiprotein detection within a single assay (**MuxT**). However, very limited information is provided in terms of multi-analyte panels (comprising various protein mixtures in complex matrix representing physiological situations) used to challenge sensor performances.

- **Sample Volume**

In many referenced publications sample volumes of 50 µL and less were used, which – being a design constraint in the context of **mTBI** POC diagnostic applications – is compatible with **EC** sensing.

- **Time-to-results**

Most of the reported **EC** sensor measurement times exceed acceptable time-to-results (< 15 min) requirements for POC diagnostic applications. It is conceivable that in the future, optimized assay and shortened incubation conditions will still be compatible with good assay performance, but this requires likely a significant R&D effort.

- **Manufacturability and Costs of Goods Produced (COGP)**

As pointed out in **Figure 8**, the small sample and reagent consumption anticipated as well as the low costs of the materials and fabrication make EC sensors attractive candidates for a future POC device for **mTBI** diagnostics. The main challenges, however, may be the difficulties and costs associated with electrode-bioreceptor functionalization (for multiple **mTBI** protein target analytes) and limited sensor stability and thus short shelf-life.

SI-3: Antifouling approaches in EC sensing

The process of non-specific binding (NSB) is a complex phenomenon that is extremely sensitive to the properties of both the sensing surface (e.g., heterogeneity, topography, functional groups, surface potential) and the protein(s) to be adsorbed (e.g., size, chemical and 3D structure, charges, apolar properties), as well as the sample media. The interaction between the surface and the protein defines its conformation and is strongly affected by the ionic strength and the pH value of the sample, specifically by the composition of the solution adjacent to the electrode.[165] Integration of antifouling materials reducing NSB is crucial in order to enable reliable detection in a complex matrix and is typically achieved via one or more of the following mechanisms: (i) formation of a hydration layer, i.e. increasing the hydrophilicity of the sensing surface resulting in decreased adhesion of biofoulant; (ii) steric repulsion, e.g. via integration of polymers sterically preventing the foulants from reaching the electrode surface; (iii) electrostatic repulsion via attachment of molecules with anionic and/or cationic moieties (e.g. zwitterionic materials); (iv) optimized surface topography (altering the surface roughness on nanoscale level).[166]

Among most commonly applied strategies is the immobilization of 'blocking' proteins, e.g. avidin, streptavidin, neutravidin, casein or (most frequently) bovine serum albumin (BSA).[165,166] This and other methods based on physical adsorption provide a relatively inexpensive and fast solution, however possess a few disadvantages, such as non-uniformity of the adsorbed layer and reversibility of the adsorption process. The latter is governed by the weak intermolecular interactions and is sensitive towards the experimental conditions (solvent polarity, ionic strength, temperature, pH).[166] Furthermore, most protein blockers have a high lot-to-lot variability and cross-reactivity, alter original surface properties, and, as some studies have reported, e.g. a BSA layer does not always efficiently prevent protein adsorption.[167] An NSB suppressing layer can be obtained or optimized using some other physical approaches to surface modification or combinations thereof: mechanical coatings (polymer films), integration of nanoporous structures (e.g. carbon nanotubes, graphene-based materials, metallic nanoparticles) and/or superhydrophobic surfaces.[165,166]

Chemical approaches present a more robust antifouling strategy for EC biosensing in comparison to physical approaches discussed above and are often accomplished via formation of SAMs containing antifouling moieties such as polyethylene glycol (PEG), oligo(ethylene glycol), zwitterionic peptide-based molecules or polymers.[166,167] Furthermore, the thiolated alcohol compounds, such as e.g. 6-mercaptohexanol or 11-mercaptoundecanol, are often applied to gold surfaces in order to 'block' empty spots and stabilize the SAM conformation. However, the relatively poor stability of SAMs, narrow choice of transducer substrates (mainly applied to gold, less frequently to silver, copper and platinum) and grafting molecule types (mainly thiolated compounds) limit the application of SAMs for NSB reduction in EC sensing.[166]

As an alternative to SAMs, polymer brushes can be tethered on substrates using different grafting methods. Unlike SAMs, this strategy is not limited to gold surfaces and has been applied to numerous substrates such as carbon, ITO, graphene etc.[24,166] A typical example would be electrodeposition of PEDOT or PANI films, with or without additional doping with PEG (or grafting of PEG) on a carbon- or graphite-based substrate.[24,168,169] While grafting of non-conductive antifouling reagents with the long chains (such as PEG) directly onto transducer surface often results in the loss of sensitivity due high impedance of the polymeric layer, incorporation of PEG with conductive soft polymers such as PEDOT and PANI is one way to resolve this issue.[166,169] However, in many cases CP layers have been shown to suffer from low mechanical and complex media stability.[166,170,171] In another promising strategy the polymeric brushes are formed via reduction of diazonium salts providing a rapid single-step approach to polymeric brush immobilization. This approach ensures a low energy barrier for the injection of electrons at the contact between the metal and organic molecule, along with the improved stability due to covalent character of the formed bond.[166,172]

Despite the large number of strategies suggested in the literature for NSB reduction, hardly any of them are sufficient to completely overcome this problem in view of POC diagnostic applications in biological matrices. Further efforts are needed in this field in order to establish an effective combination of antifouling materials with surface modification strategies and to better understand the synergetic effect of the complex media and the antifouling probes on the properties of the biorecognition element.[166,167]

ABBREVIATIONS for SI

µPAD: microfluidic paper-based analytical device; **1-NPP:** 1-naphthyl phosphate; **11-FcC:** 11-ferrocenyl-undecanethiol; **3D-GRS:** porous three-dimensional graphene-starch architecture; **3DM:** three dimensionally macroporous; **3D-SiCPCC:** three dimensional silica close-packed colloidal crystal; **3-ICT:** (3-Isocyanatopropyl)triethoxysilane; **4-ATP:** 4-aminothiophenol; **4-MBA:** 4-mercaptobenzoic acid; **9-MN:** 9-mercapto-nonanol; **AA:** ascorbic acid; **AAP:** ascorbic acid 2-phosphate; **Ab:** antibody; **AB:** acetylene black; **ACN:** acetonitrile; **ACP:** acid phosphatase; **AEDP:** monomer, 2-acrylamidoethyl dihydrogen phosphate; **AIBN:** 2,2'-azoisobutyronitrile; **AJPE:** aerosol jet printed electrode; **AM:** acrylamide; **AMCs:** TiO2 (anatase) mesocages, here: Ru(bpy)32+@AMCs composite for dual response [DVP and ECL, Ru(bpy)32+: ruthenium (II) tris(bipyridine)]; **ALP:** alkaline phosphatase; **amFc:** aminoferrocene; **APMA:** 4-aminophenylmercuric acetate; **Apt:** (oligonucleotide) aptamer for the target (T); **APTES:** 3-aminopropyl triethoxysilane; **APTMS:** 3-aminopropyl trimethoxysilane; **AQ:** anthraquinone; **ASV:** anodic stripping voltammetry; **Au@Pd-P(BBY):** coreshell Au nanoparticles @Pd nanoclusters-poly(bismarck brown Y); **AuNA:** gold nanoarray; **AuNPs:** gold nanoparticles; **AuNSs:** gold nanostars; **AuPt NAs:** hierarchical AuPt nanoassemblies; **Av:** avidin; **BIS:** bovine implant serum; **BNP:** B-type natriuretic peptide; **BSA:** bovine serum albumin; **$C_9H_{14}NBF_4$:** 1-butylpyridine tetrafluoroborate; **CB[7]:** cucurbit[7] uril; **CBMA:** [2-carboxy*N*,*N*-dimethyl-*N*-(2'-methacryloyloxyethyl) ethanaminium inner salt], zwitterionic monomer; **CBP:** constant bias potential; **CD-GS:** β-cyclodextrin-graphene sheets; **CDI:** carbonyldiimidazole; **CDP-choline:** cytidine diphosphate-choline (cytidine 5'-diphosphocholine sodium salt dihydrate); **CGR:** carboxyl graphene; **CMA:** 4-carboxymethyl aryl diazonium; **CME:** cysteine-modified epitope; **CNTs:** carbon nanotubes; **COF:** covalent organic framework; **CP:** conductive polymer; **CPA:** constant potential amperometry; **CPE:** carbon paste electrode; **CPT:** 5-carboxy-1-pentanethiol; **CP|PPC:** mixed layers of 4-carboxylic phenyl and 4-aminophenyl phosphorylcholine; **CS:** chitosan; **CSV:** cathodic stripping voltammetry; **CT(PEG)$_{12}$:** carboxy-PEG12-thiol; **CTIL:** carboxyl-terminated ionic liquid; **CuPT:** copper phthalocyanine-3,4',4'',4'''-tatrasulfonic acid tetrasodium salt (as dopant counterion); **Cyt c:** ferric cytochrome c; **DAB:** diaminobenzene; **DG:** dual-gated (transistors); **DG-ISFET:** dual gated ion-sensitive field effect transistor; **dil.:** diluted; **DMAA:** monomer, dimethylacrylamide; **DMAP:** 4-(dimethylamino)pyridine; **DN:** 1,5-diaminonaphtalene; **dNTPs:** deoxyribonucleoside triphosphate; **DPASV:** differential pulse anodic stripping voltammetry; **DPI:** dual probe immunosensor; **DPIMBr:** 1,3-di(3-N-pyrrol-propyl)imidazolium bromine ionic liquid; **DPM:** dipyrromethene; **DPTA:** thiol derivative of pentetic acid; **DTSP:** dithiobis (succinimidyl propionate); **DTSSP:** 3,3'-dithiobis(sulfosuccinimidyl propionate); **DPV:** differential pulse voltammetry; **EC:** electrochemical; **EDC:** 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; **EDTA:** ethylene diamine tetraacetic acid disodium salt; **EG$_6$COOH:** (11-mercaptoundecyl)hexa(ethylene glycol) acetic acid terminated; **EGDMA:** ethylene glycol dimethylacryalte; **ELISA:** enzyme-linked immunosorbent assay; **Exo III:** exonuclease III; **FBS:** fetal bovine serum; **Fc:** ferrocene; **Fc-g-Au@Pd-P(BBY):** ferrocene grafted Au@Pd-P(BBY); **FED:** field-effect based detection (voltage controlled current amplification); **FN:** fibronectin; **FRGG:** p-Nitrobenzene diazonium tetrafluoroborate (Fast Red GG salt); **fUb:** free ubiquitin; **GA:** glutaraldehyde; **GCE:** glassy carbon electrode; **GNRs:** gold nanorods; **GOx:** glucose oxidase; **GOx-LS:** glucose oxidase loaded liposomes; **GRO:** graphene oxide; **GRONRs:** graphene oxide nanoribons; **H-ELISA:** proton-ELISA; **Hem:** hemin; **His:** histidine; **HME:** histidine-modified epitope; **HP:** human plasma; **hpDNA:** hairpin DNA; **HRP:** horseradish peroxidase; **HS:** human serum; **HT:** hexanethiol; **HQ:** hydroquinone; **hSAM:** homogenous ordered self-assembled monolayer; **IDE:** interdigitated electrode; **IL:** ionic liquid; **ITO:** indium tin oxide; **K-GS:** K-modified graphene; **Lip-NHS:** N-hydroxysuccinimide ester; **LSASV:** linear sweep ASV; **MAC:** N-methacyloyl-L-cysteine; **MBs:** magnetic beads; **MBT:** 4-mercaptobutanol; **MCH:** mercaptohexanol; **MDEA:** microdisc electrode array; **MeB:** methylene blue; **MeCN:** acetonitrile; **MECS:** macroelectrode with a comb structure; **mhCeO$_2$NS:** mesoporous-hollow ceria nanospheres; **MHDA:** mercaptohexadecanoic acid; **microel.:** microelectrode(s); **microfab.:** microfabricated **MOF(c):** metal-organic framework type *c* (particle size 300 nm); **MOF:** metal-organic framework; **MPA:** 3-mercaptopropionic acid; **MPC:** porous carbon matrix; **MPOH:** 3-mercaptopropanol; **mSAM:** mixed self-assembled monolayer; **mtUb:** multiubiquitin chains; **MUA:** 11-Mercaptoundecanoic acid; **MuxT:** multiple protein biomarker targets detected within the same immunoassay; **MVIMBF$_4$:** 1-(3-mercapto-propyl)-3-vinyl-imidazolium tetrafluoroborate ionic liquid; **MWCNTs:** multiwalled carbon nanotubes; **NAC:** N-acetylcysteamine; **NBA:** Nile blue A; **NCbs:** nanocubes; **NCs:** nanocrystals; **NGR:** nitrogen-doped graphene; **NHS:** N-hydroxysuccinimide; **NIPAAm:** N-Isopropylacrylamide; **NiWO4-NStr:** saw-blade-like NiWO4 nanostructures; **NPrs:** nanoprobes; **NSNs:** nanoblock spherical nanoarchitectonics; **NSs:** nanospheres; **NTCDI:** naphthalenetetracarboxylic diimide; **NTs:** nanotubes; **NWs:** nanowires; **OAMs:** octahedral anatase TiO2 mesocrystals; **OECT:** organic electrochemical transistor; **OFET:** organic field effect transistor; **OMCSi-AuNPs:** gold nanoparticle incorporated ordered mesoporous carbon-silica; **OPD:** o-phenylenediamine; **OSWV**: Osteryoung square-wave voltammetry; **pABA:** p-aminobenzoic acid; **PAD:** microfluidic paper-based device; **PAMAM:** poly(amido)amine; **PANI:** polyaniline; **PANI-PA:** phytic acid-doped polyaniline; **PAPP:** 4-aminophenylphosphate; **PASE:** pyrenebutanoic acid succinimidyl ester; **pATP:** poly-aminothiophenol; **PB:** Prussian blue; **PBASE:** 1-pyrenebutyric acid N-hydroxysuccinimide ester; **PB-PEDOT-AuNPs:** Prussian blue poly(3,4-ethylenedioxythiophene)-AuNPs; **PBS:** phosphate buffer saline; **PC:** porous polycarbonate membrane; **pCOF:** porphyrinic covalent organic framework; **PDA:** polydopamine; **PDANS:** polydopamine nanospheres; **PEC:** photoelectrochemical (detection); **PEDOT:** poly(3,4-ethylenedioxythiophene); **PEI:** poly(ethyleneimine); **PEG:** polyethylene glycol; **Pept-SH:** thiolated peptide; **pGluA:** poly-glutamic acid; **PHA:** 6-phosphonohexanoic acid; **pHEMA:** poly(2-hydroxethyl methacrylate); **pMeB:** poly(methylene blue); **PMMA:** poly(methyl methacrylate); **PMPC-SH:** thiol-terminated poly(2-methacryloyloxyethyl phosphorylcholine); **pNE:** polynorepinephrine; **pNPP:** p-nitrophenyl phosphate; **PPCE:** conjugated polypyrrole polymer containing epoxy active side groups; **pPG:** amine functionalized 1st generation trimethylolpropane tris[poly(propyleneglycol)] dendrimers; **PpPD:** poly(p-phenylenediamine); **pPtNPs:** porous platinum nanoparticles; **PPy:** polypyrrole; **PPy-NWs:** polypyrrole-nanowires; **PPyPAC:** polypyrrole electrodes modified by electrodeposition of diazonium salts using 4-aminophenylacetic acid (4APAC); **preconc.:** preconcentration; **Protein G-AP:** protein G labeled with alkaline phosphatase; **PS:** polystyrene; **PS-MA:** polystyrene-co-methacrylic acid; **PSS:** polystyrene sulfonate; **pTMB:** poly (3,3',5,5'-tetramethylbenzidine); **PTSA:** 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt; **pTTBA:** (2,2:5,2-

terthiophene-3-(p-benzoic acid)); **pTTBPA:** 4′-([2,2’:5′,2''-terthiophen]-3’-yl)-[1,1′-biphenyl]-4-carboxylic acid; **PVDF:** polyvinylidene fluoride; **P(Pyr-Epx):** epoxy-substituted-polypyrrole; **P(ThiPh-gMAm):** poly(thiophene)-graft-poly(methacrylamide) polymer; **QCM:** quartz crystal microbalance; **RAGE:** receptor domains for advanced glycation end products (three extracellular immunoglobulin domains: V, C1, C2); **rc:** regenerated cellulose; **rGRO:** reduced graphene oxide; **RPS:** resistive pulse sensing; **QDs:** quantum dots; **SA-AuNPs:** sodium alginate-Au nanoparticles; **SAM(s):** self-assembled monolayer(s); **SATA:** N-succinimidyl S-acetylthioacetate; **SDS:** sodium-dodecyl sulphate; **SERS:** surface enhanced Raman spectroscopy; **SFI:** single frequency impedance; **SH:** thiol group; **SH-Apt:** thiolated aptamer for the target (T); **SPBs:** superparamagnetic beads; **SPE:** screen-printed electrode; **SPGMA:** four-armed star shaped poly (glycidyl methacrylate); **SPy:** pyrrole-silane; **ssDNA:** single-stranded DNA; **Str(PGMA)$_3$:** tri-armed star poly(glycidyl methacrylate); **Stv:** streptavidin; **SWASV:** square wave ASV; **SWCNTs:** single wall carbon nanotubes; **SWV:** square wave voltammetry; **T:** target; **TAA:** thioacetamide; **TAPP:** tetra(4-aminophenyl) porphyrin; **TB:** toluidine blue; **TBAClO$_4$:** tetrabutylammonium perchlorate; **TBAP:** tetrabutylammonium perchlorate; **TBO:** toluidine blue O; **TCEP:** tris (2-carboxyethyl) phosphine hydrochloride; **TESBA:** 3-(triethoxysilyl)butyl aldehyde; **TESUD:** 11-(triethoxysilyl) undecanal; **Thi:** thionine; **ThiPh:** thiophene; **Th-T:** Thioflavine-T; **TMB:** 3,3’,5,5’-tetramethylbenzidine; **TMSE:** 1,2-bis (trimethoxysilyl)ethane; **undil.:** undiluted; **UNs:** ultrathin nanosheets; **WP6@PdPt PCONs:** water-soluble pillar[6]arene functionalized PdPt porous core-shell octahedral nanodendrites; **ZnO-MPC:** ZnO/porous carbon matrix composite; **Zr-tdc:** Zr(IV)-organic framework with 2,5-thiophenedicarboxylate ligand.

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