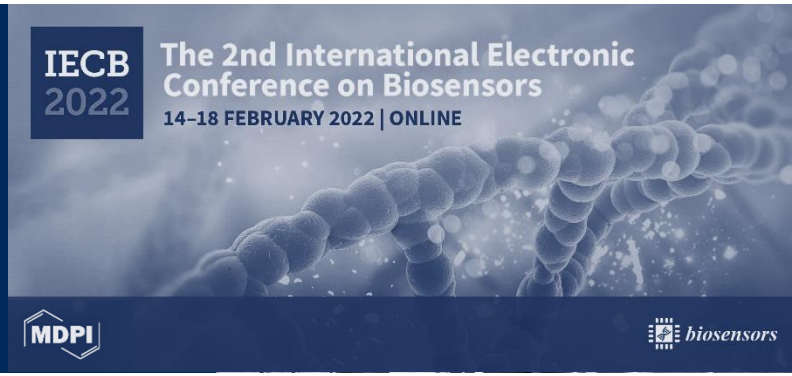
A precise electrochemical point-of-care testing device for early diagnosis of ovarian cancer

Soha Ahmadi, Ph.D.

Professor Michael Thompson

Department of Chemistry

University of Toronto



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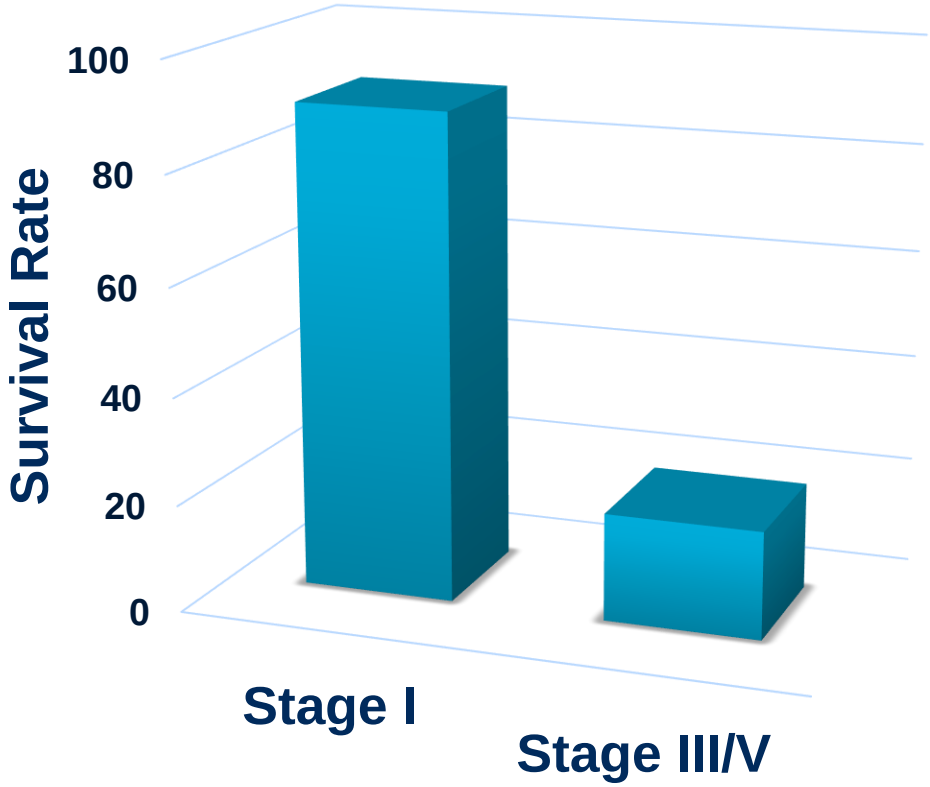
Ovarian Cancer: Silent Killer

150000 deaths worldwide of nearly **300000** new cases each year.

Only **20%** of patients are diagnosed at stages I /II when the treatment is more effective:

- Very few or no specific symptoms
- No mass screening techniques



F. Reif, The World Ovarian Cancer Coalition; ***2018****; pp 1–39*

Sharma et al. Enzyme Microb. Technol. ***2016****, 89, 15-30*

Cancer Biomarkers

- A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process.

Can be used for:

- **Screening and/or early diagnosis**
- **Prognosis**
- **Longitudinal treatment/recurrence monitoring**

Ovarian Cancer Biomarkers

Cancer antigen-125 (CA125):

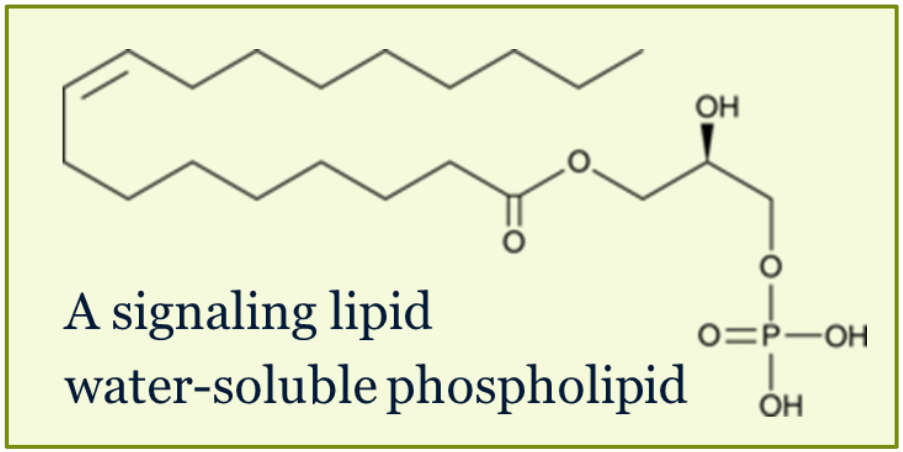
- Only biomarker that are clinically used
- Has prognostic value during treatment
- Sensitivity <60% in the early stages
- False-positive and false-negative results

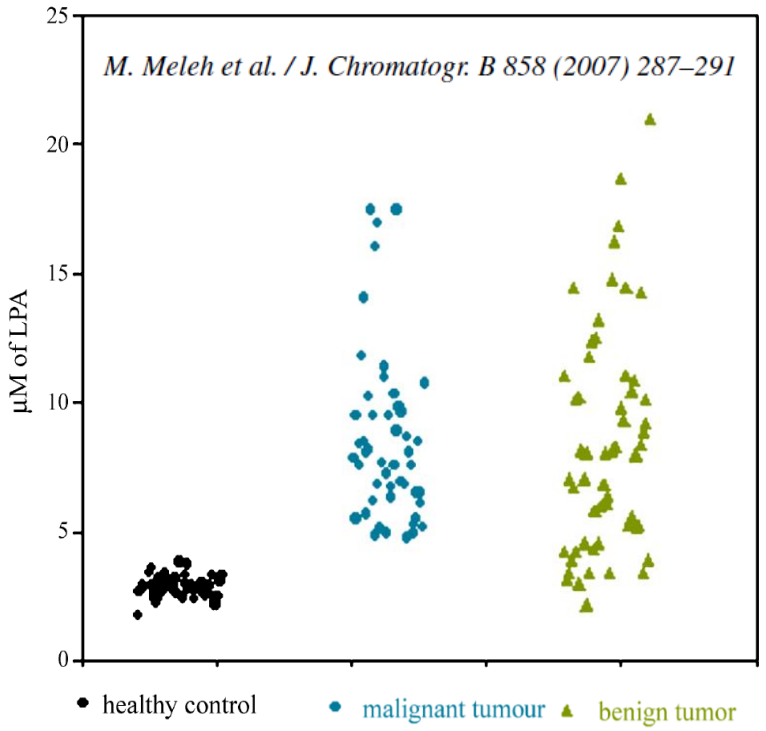
Source	Screening Method	No. Analyzed		Ovarian Cancer Deaths, No. (%)		Ovarian Cancer Mortality per 10 000 Person-Years	
		Screening Group	Control Group	Intervention	Control	Intervention	Control
UKCTOCS,[31] 2016	CA-125 ROCA	50 624	101 299	160 (0.32)	358 (0.35)	2.9	3.3
	TVU	50 623	101 299	163 (0.32)	358 (0.35)	3.0	3.3
PLCO,[21] 2011	CA-125 + TVU	34 253	34 304	118 (0.34)	100 (0.29)	3.1	2.6
UK Pilot,[33] 1999[e]	CA-125	10 958	10 977	9 (0.08)	18 (0.16)	NR	NR

Wang et al. J. Obstet. Gynecol. Res. ***2019****, 45 (5), 1006–1011*
Sharma et al. Enzyme Microb. Technol. ***2016****, 89, 15-30*

Lysophosphatidic acid (LPA):
Ovarian Cancer (OC) biomarker

- The basal serum level of 0-5 μM
- At stage I of OC increases to 5-50 μM
- Potential OC biomarker
- Potential target for OC therapy





Feng et al. J. Inflamm. (United Kingdom) 2020, 17 (1), 1–5.

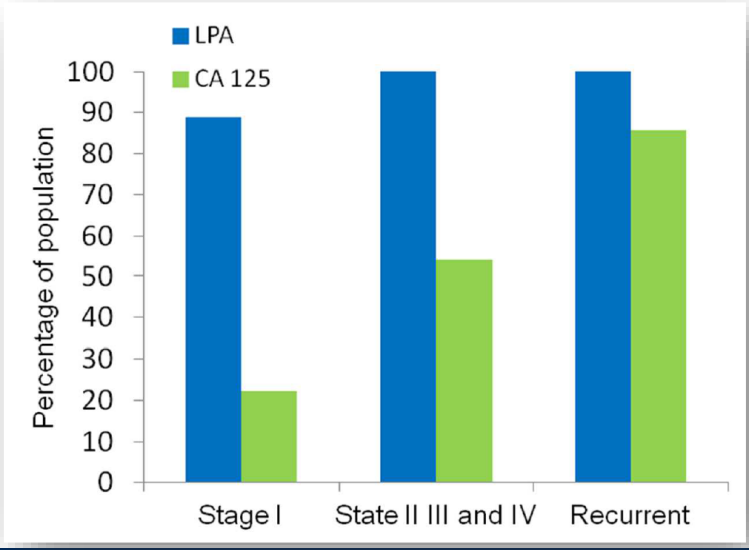
Kobayashi et al. Biomarkers Prev. 2012, 21 (11), 1902–1912

OC biomarker: LPA vs CA125

	Cut-off	SE (%)	SP (%)
CA125	>35 U/mL	82.2	67.3
	>65 U/mL	75.6	86.6
CA19-9	>40 U/mL	35.6	81.1
CA15-3	>32 U/mL	57.1	93.9
CA72-4	>3.8 U/mL	70.7	91.8
CEA	>3 ng/mL nonsmoke, >5 ng/mL smoker	16	93
HE4	>70 pmol/L	72.9	95
LPA	1.3 μmol/L	98	90
IAP	482 μg/mL	93.3	91
HP-α	65 μg/mL	64	90
OVX-1	7.2 μ/mL	70	95
Methothelin	–	60	98

Kobayashi et al. Cancer Epidemiol Biomarkers Prev; 21(11 2012

Xu et al. JAMA. 1998;280(8):719–723.



J. Wang (2015). Dissertations and Theses. 10.15760/etd.2233

LPA Detection: Standard analytical methods

- Time consuming
- Expensive
- Require highly skilled technicians
- Unsuitable for widescale screening

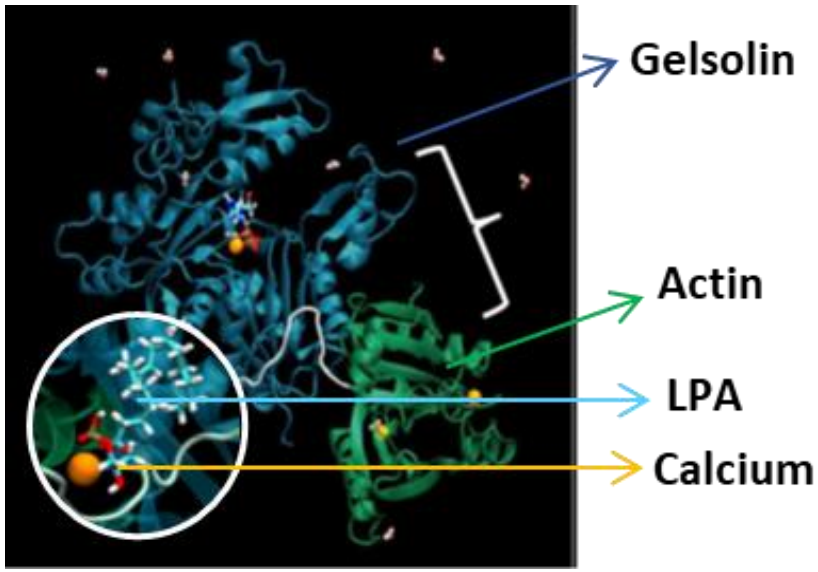
Method	Lipid extraction	LPA molecular species	References
Radioenzymatic	+	–	Saulnier-Blache et al. [32]
Fluorimetric	+	–	Aoki et al. [28] Morita et al. [57]
Colorimetric	–	–	Kishimoto et al. [44]
DIFA	–	–	Chen et al. [59,60]
ELISA	–	–	
CE	+	+	Chen and Xu [49,54]
GC/MS	+	+	Tokumura et al. [61] Sugiura et al. [34] Bese et al. [22]
TLC/MS	+	–/+	Xiao et al. [37,48] Sutphen et al. [62]
LC/MS	+	+	Baker et al. [30,53]
LC/MS/MS	+	+	Meleh et al. [52] Shan et al. [39]
MALDI–TOF	+	+	Scherer et al. [33] Bollinger et al. [63] Zhao and Xu [51] Tanaka et al. [65] Morishige et al. [45]

Jesionowska et al. Anal. Biochem. 2014 453, 38–43

LPA Detection: Gelsolin-Actin System

Gelsolin:

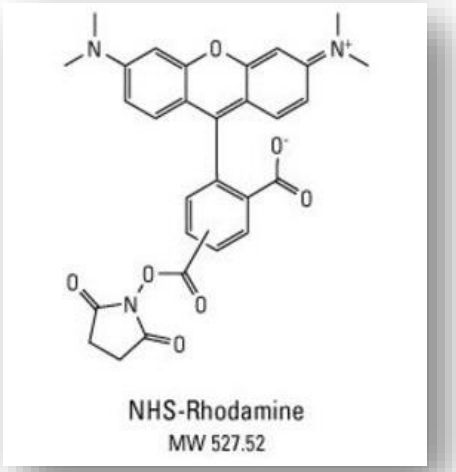
- Actin-binding protein
- Activity is stimulated by Ca^{2+}
- LPA regulates the Gelsolin-Actin binding



Thompson Group, unpublished

LPA Detection: Fluorescence Technique Gelsolin-Actin System

- Rapid
- Easy to perform
- Low cost,
- **LOD: 5 µM (**LPA in whole serum)



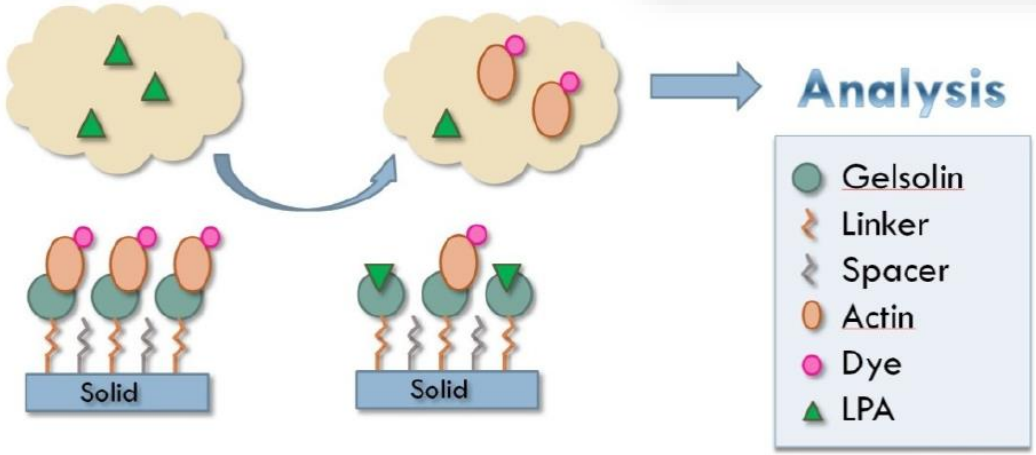


Figure 1. Principle behind actin–gelsolin chemistry for detection of LPA in patient samples. Solid support used in these experiments was silica gel.

B. De La Franier and M. Thompson, Biosensors, 2020, 10, 13
US Patent:15/572,295

LPA Detection: Fluorescence Technique Gelsolin-Actin System

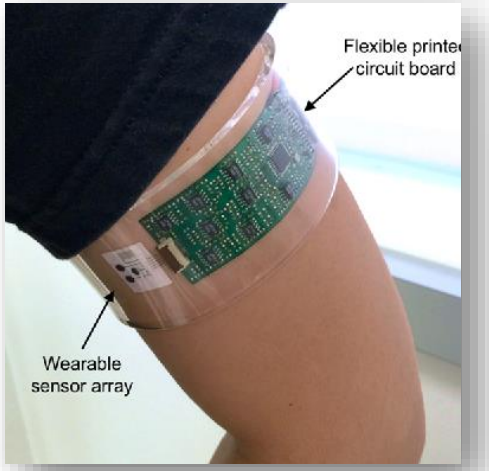
Limitations:

- The limit of quantification is not low enough for early-stage ovarian cancer.
- Needs benchtop instrument and sample preparation.
- Can not be used to fabricate small compact POCT devices.

De La Franier and Thompson, Biosensors, 2020, 10, 13
US Patent:15/572,295

Electrochemical techniques: POCT device

- Sensitive
- Selective,
- Cost-effective
- Can be miniaturized to use as a POCT or a wearable sensor



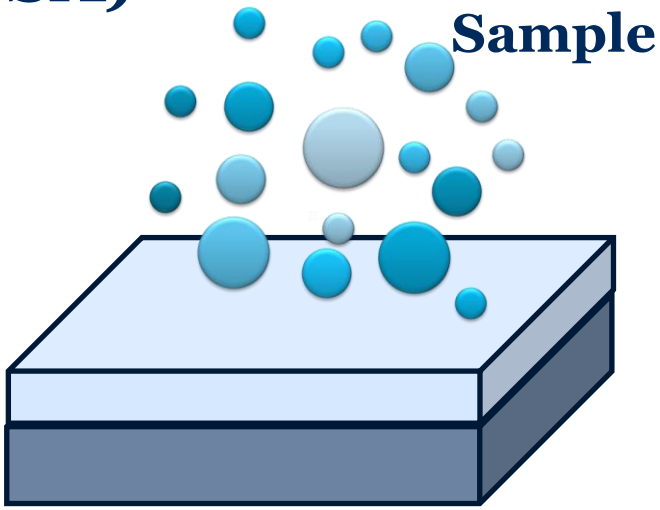
ACS Nano,2016 10(7),7216-2

Electrochemical Biosensors:

Challenges

The main challenge is contacting artificial materials with biological fluids such as blood and serum:

- **Bio-incompatibility**
- **Non-specific adsorption (NSA)**

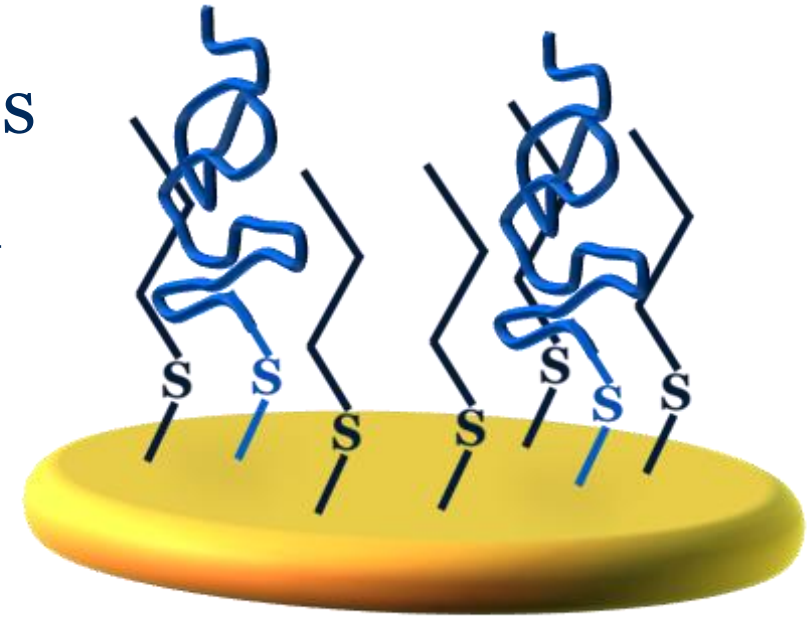


Electrochemical Biosensors: Challenges

Gold electrode:

Sulfur chemistry a double-edged sword:

- Self-assembly monolayer (SAM) for biofunctionalization.
- **NSA**: Any biomolecules with a thiol group such as cysteine-contain proteins.



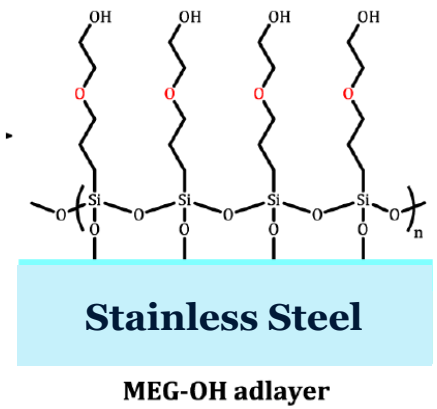
Medical-grade stainless steel

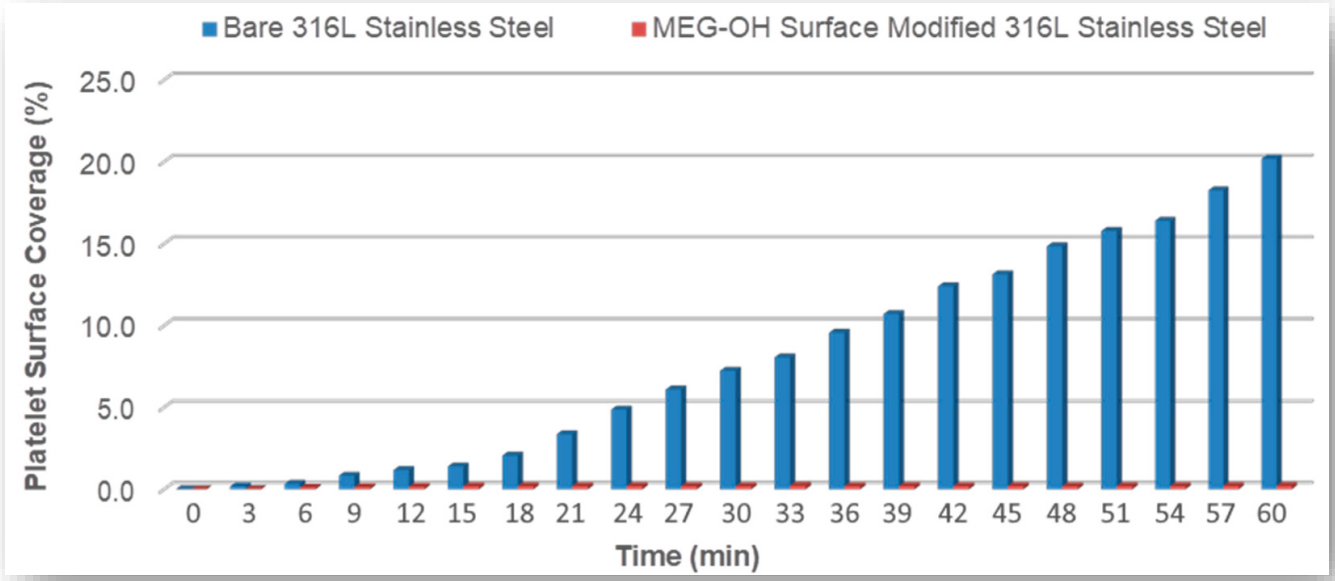
A non-conventional material for fabricating working electrode:

- **Biocompatibility**: used in the fabrication of many biomedical devices
- **Antifouling properties:** Silane-based interfacial chemistry

Silane-based chemistry: Anti-fouling surface

Silane monoethylene glycol (MEG-OH):

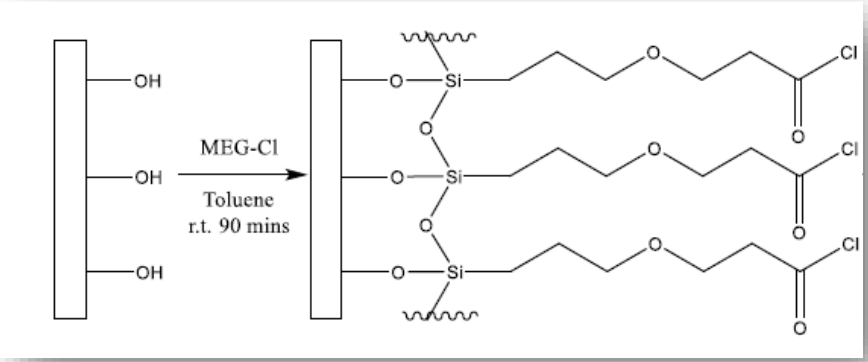


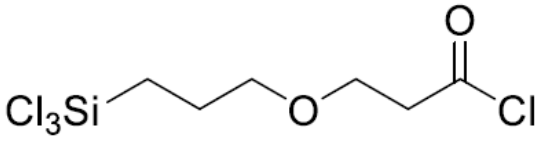


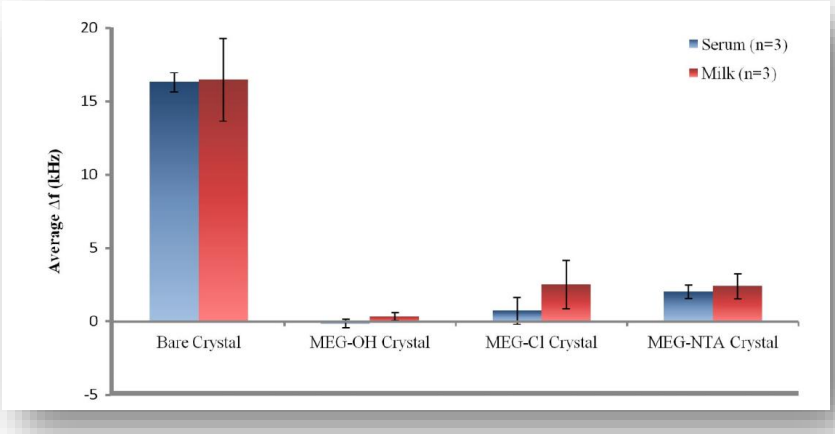
Real-time platelet surface percentage coverage on bare and MEG-OH-modified 316L stainless steel

Silane-based chemistry:
Anti-fouling surface

3-(3-(trichlorosilyl)propoxy)propanoyl chloride (MEG-Cl)



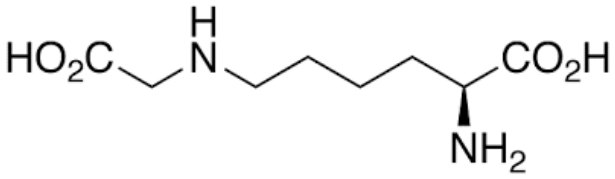


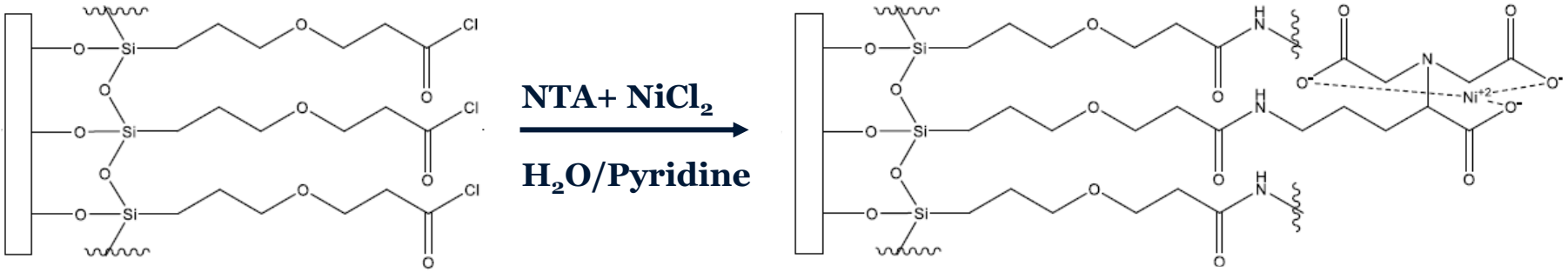


Thompson Group, Applied Surface Science 414 (2017) 435–441
Thompson Group, Biosensors 2020, 10, 20

MEG-Cl as a linker:
immobilizing His-tag proteins

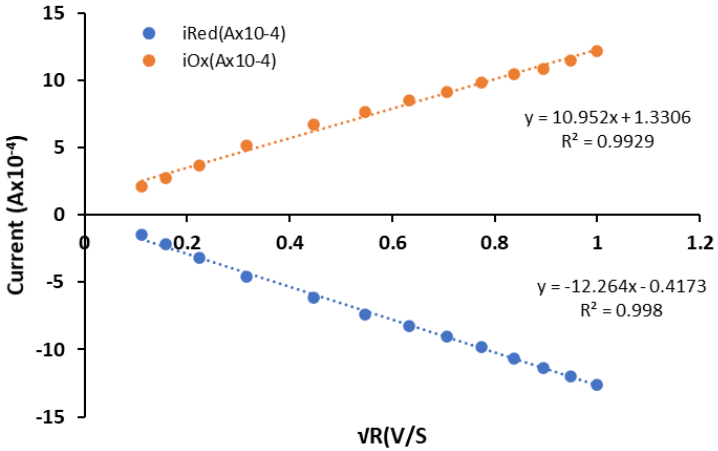
Nα,Nα-bis(carboxymethyl)-L-lysine (NTA) + $NiCl_2$



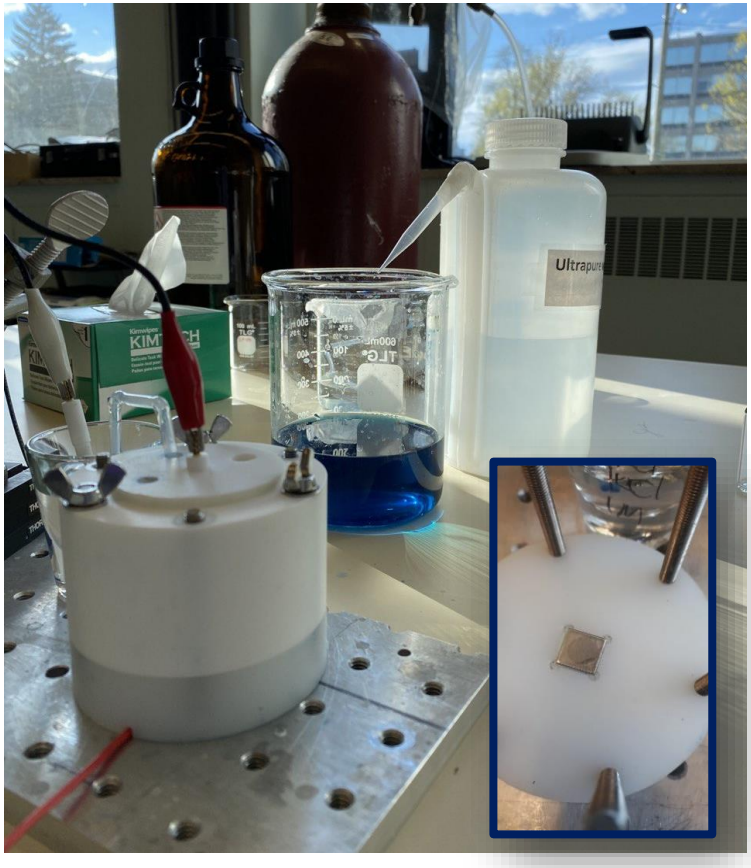


Electrochemical Setup

- Working electrode: Stainless Steel Plate (1x1x0.1 cm)
- Auxiliary electrode: Pt wire
- Reference electrode: Ag/AgCl
- Redox prob 10 mM $[Fe(CN)_6]^{3-/4-}$
- Supporting Electrolyte: 0.1 M KCl



CV scan rate plot of bare Stainless Steel



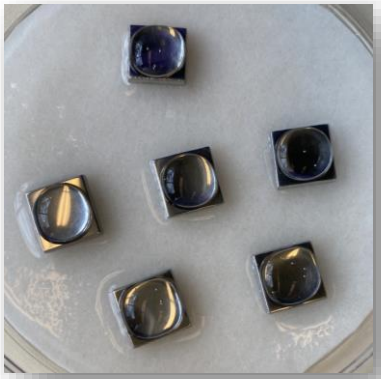
Thompson Group, Unpublished

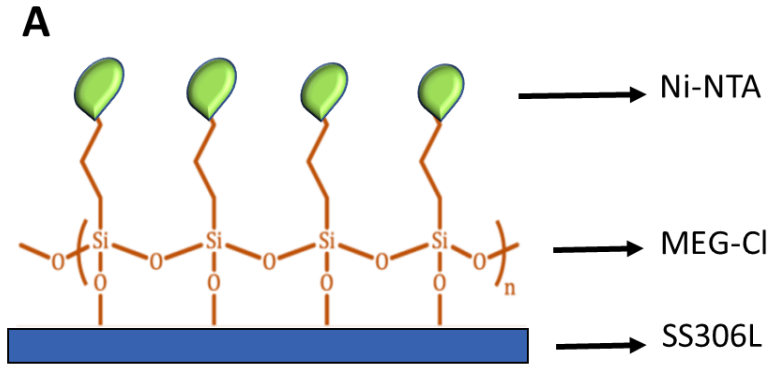
Surface Modification:
Electrochemical Characterization

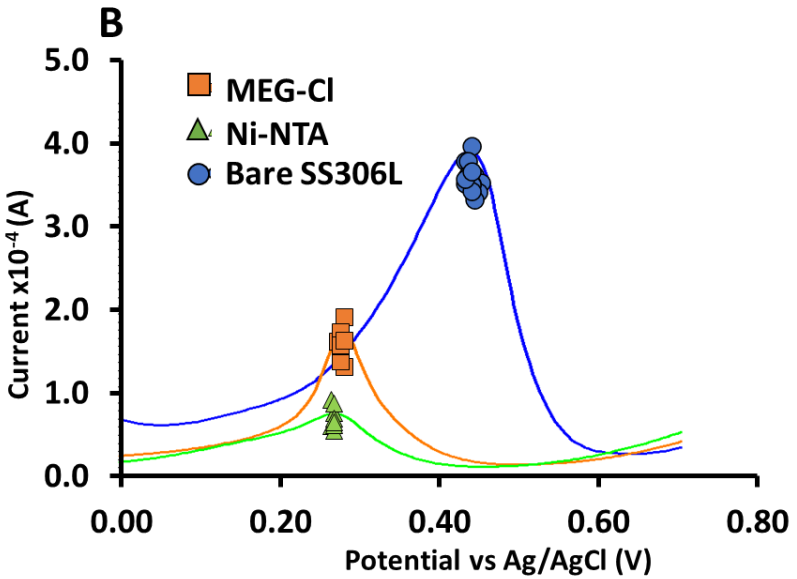
Working electrode:

- H2O/EtOH (over night/RT)
- MEG-Cl (90 min/ RT)
- Ni-NTA (24 h/ RT)

Square Wave Voltammetry (SWV)



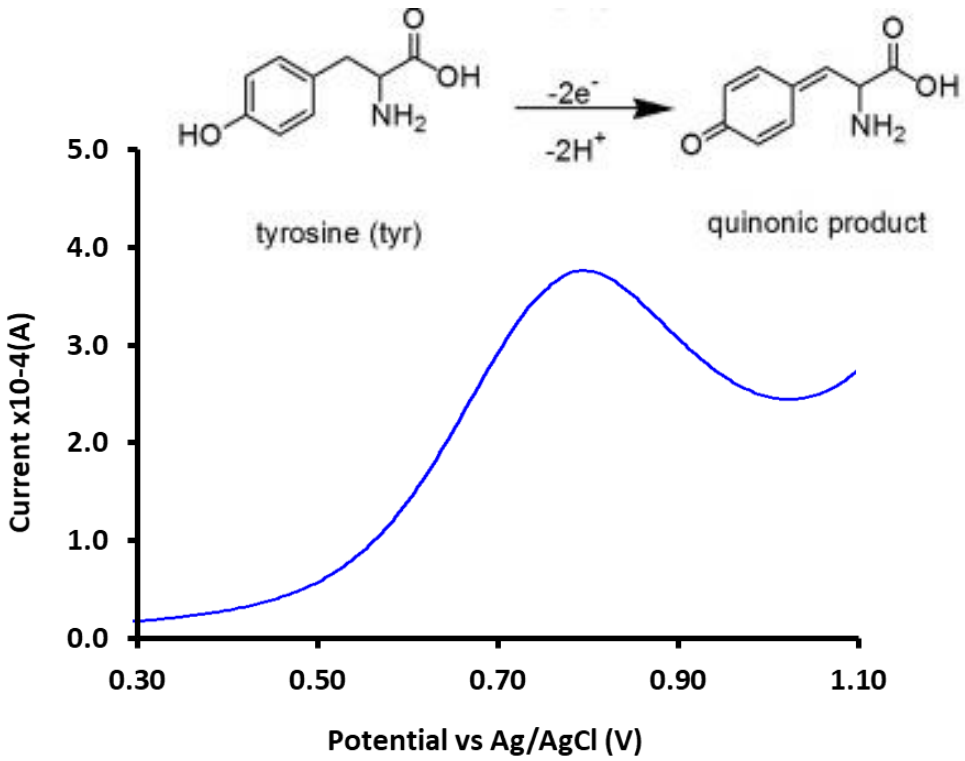


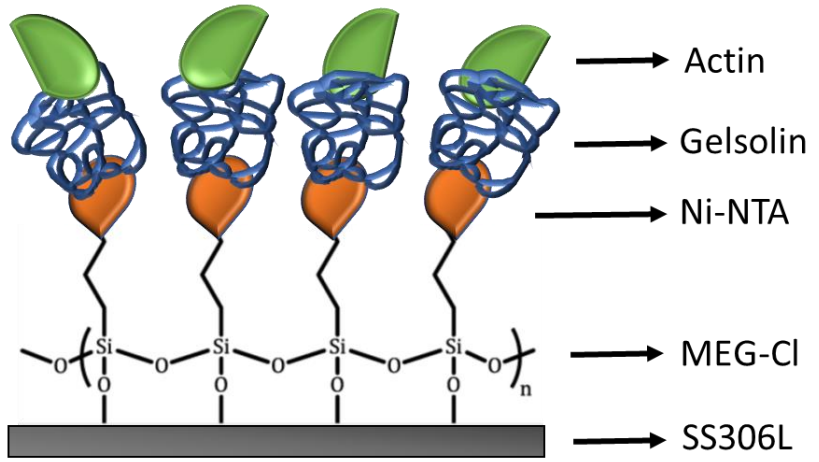


Thompson Group, Unpublished

Biorecognition Surface:
Gelsolin-Actin system

- Gelsolin-Actin (60 min, RT)
- SWV, Phosphate Buffer, pH 7.4

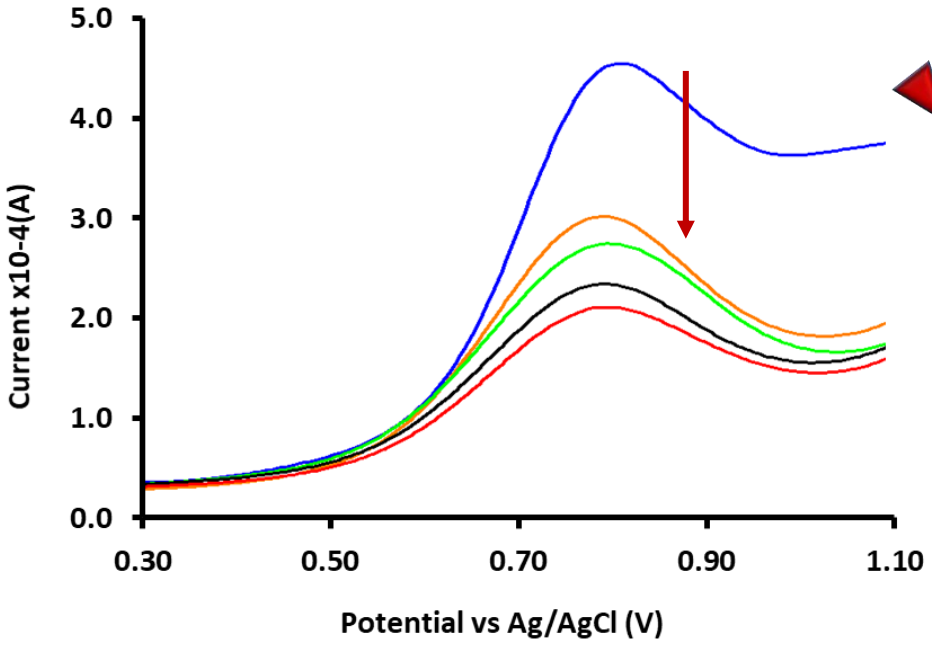


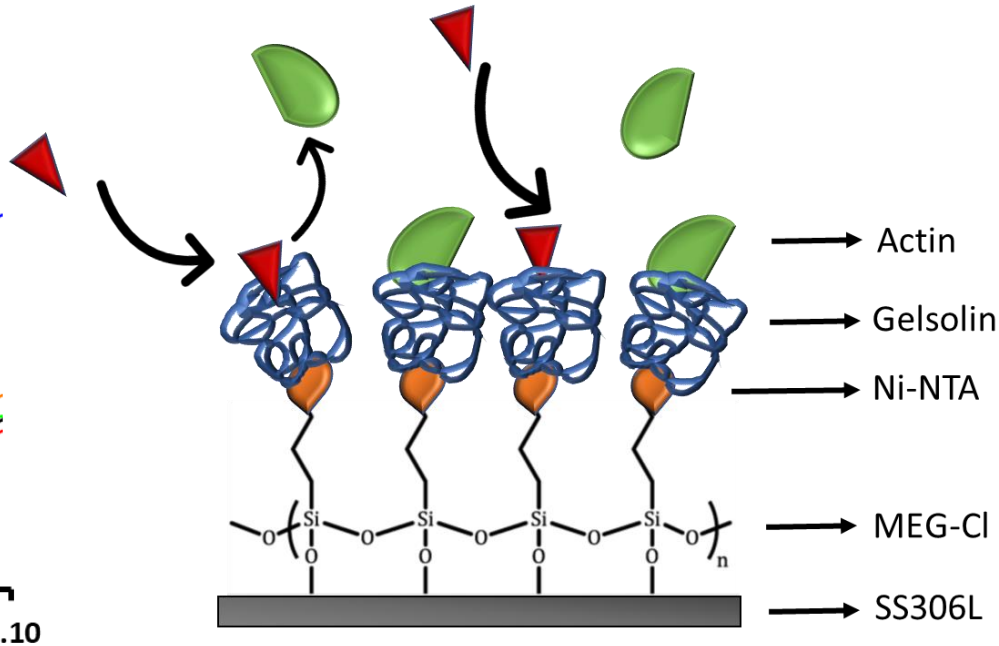


Thompson Group, Unpublished

Electrochemical Biosensor:
LPA Detection

- LPA, phosphate buffer (1 h, RT)





Thompson Group, Unpublished

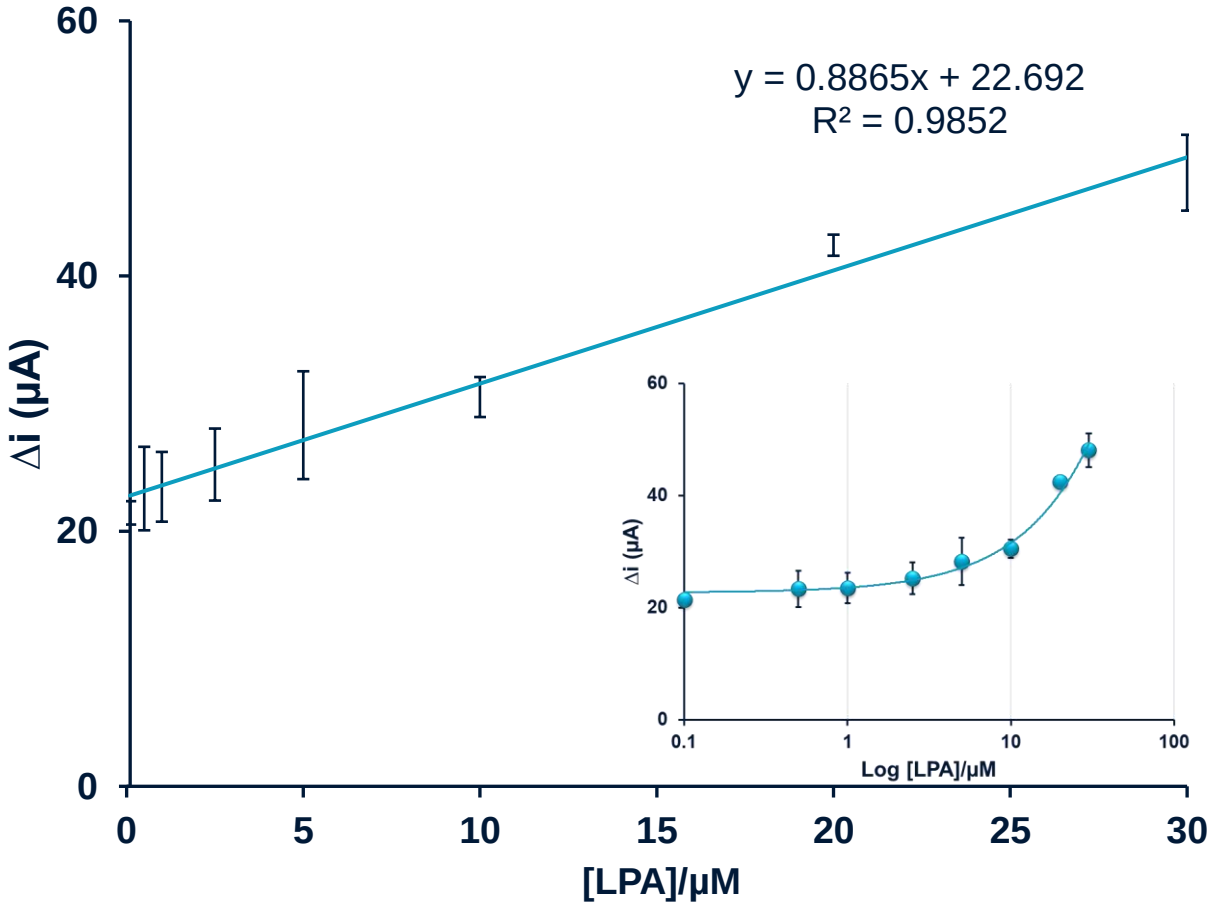
Electrochemical Biosensor:
LPA Detection

Confidence Level of 98%:

LOD: 2.6 µM

LOQ:7.9 µM

Standard Error: 0.71

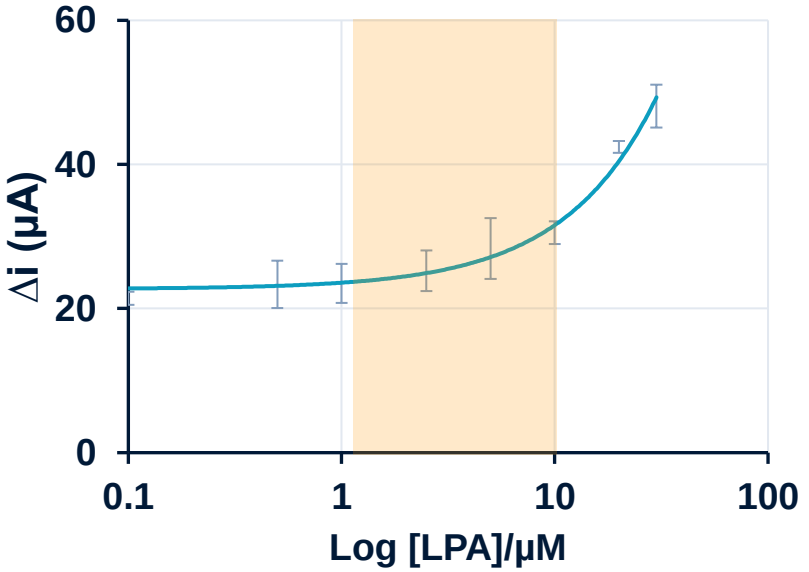


Thompson Group, Unpublished

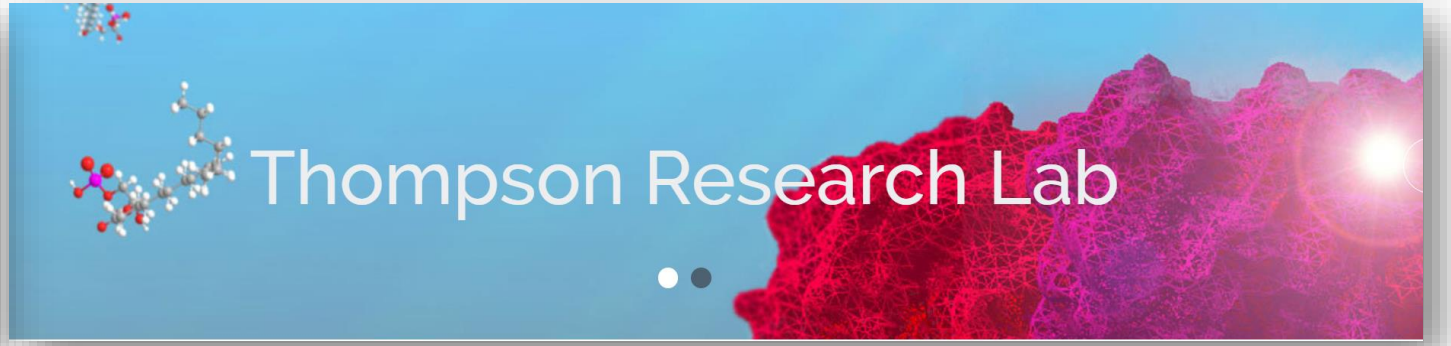
Conclusion and Future work

Proof-of Concept of a POCT device for LPA detection:

- Using a non-traditional material for working electrode: i) reduce the NSA; ii)reduce the cost of fabrication, iii) larger surface area, enhance the sensitivity.
- The LOD of 2.6 μM is enough for screening but needs to be improved for the plasma samples.
- The LOQ of 7.9 μM is within the pathogenic level of LPA, but needs to be improved for the plasma samples.



Acknowledgment



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