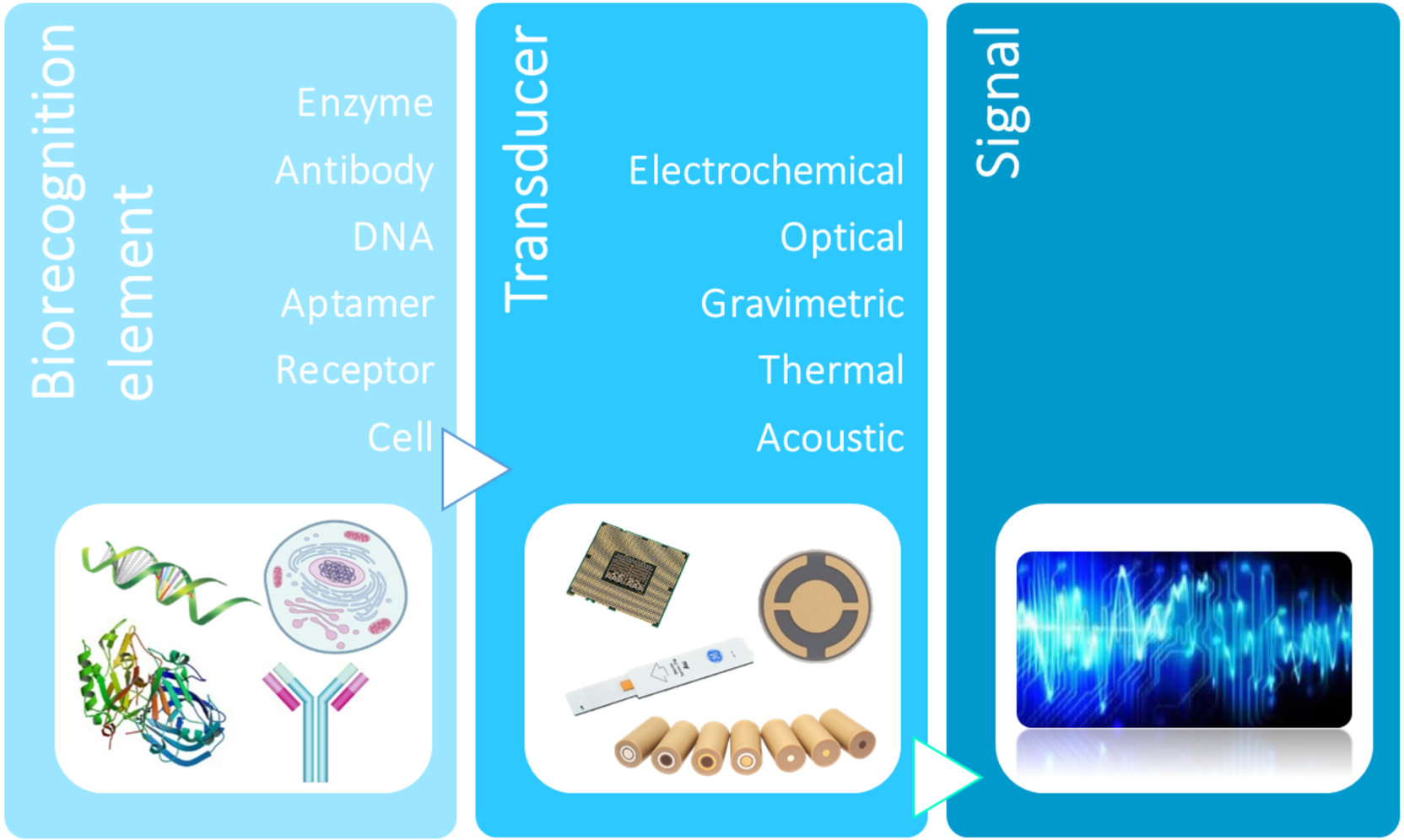
Bioanalytical tools for the challenging screening and quantification of marine toxins

Mònica Campàs

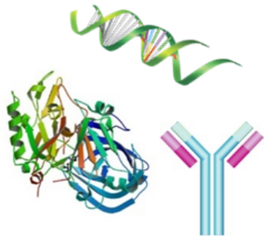
Marine and Continental Waters Program

Institute
of Agrifood Research
and Technology

The principle of biosensors

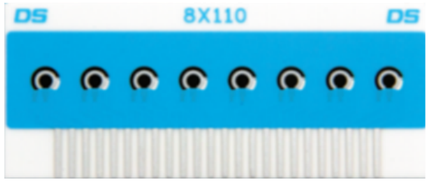


Developing and validating the biosensors



Biorecognition molecule

Electrode



Colorimetric assays

Electrochemical biosensors

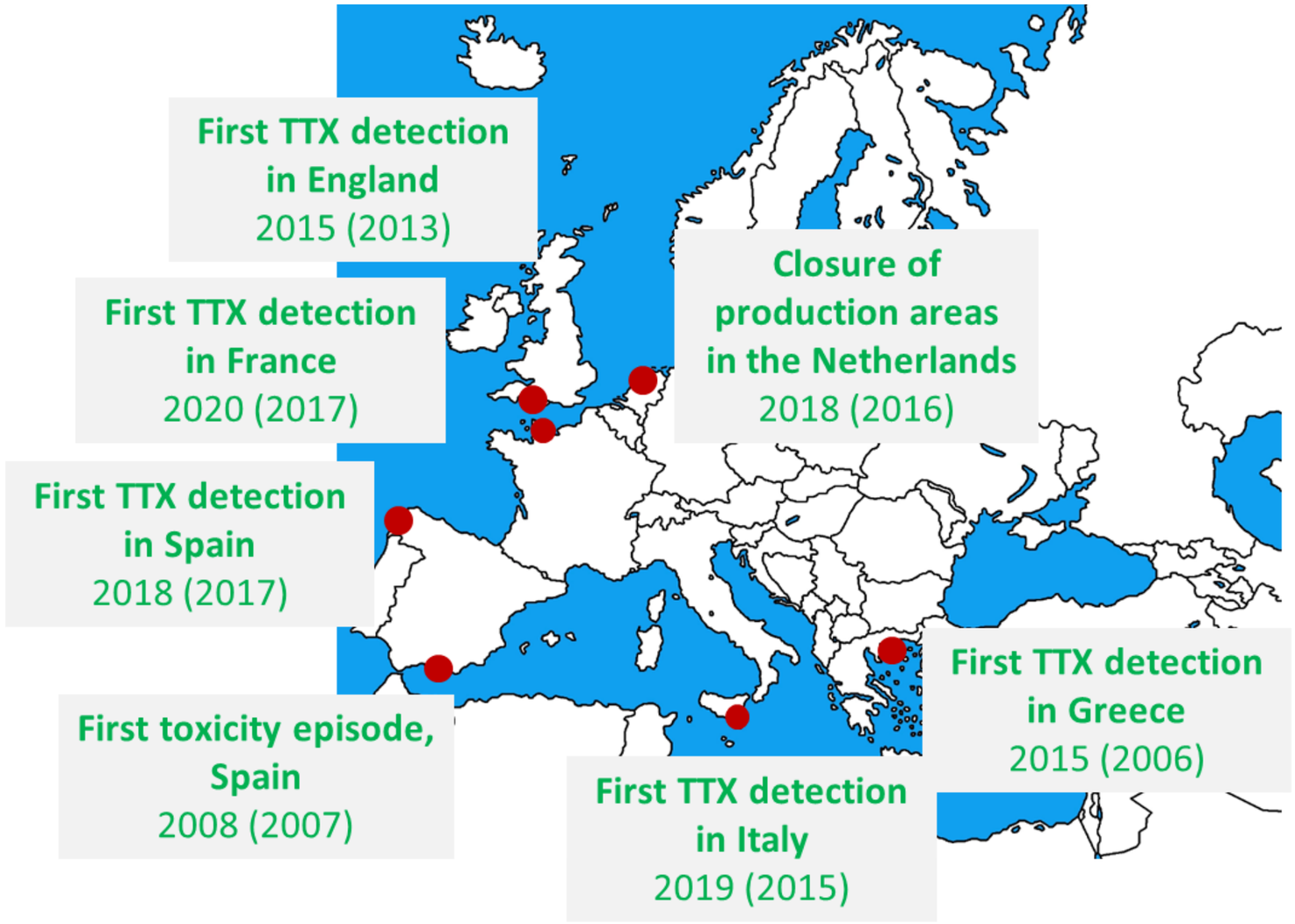
Analysis of samples and comparison with other techniques

Tetrodotoxins in puffer fish

Since 2005:
Egypt
Israel
Turkey
Cyprus
Spain!

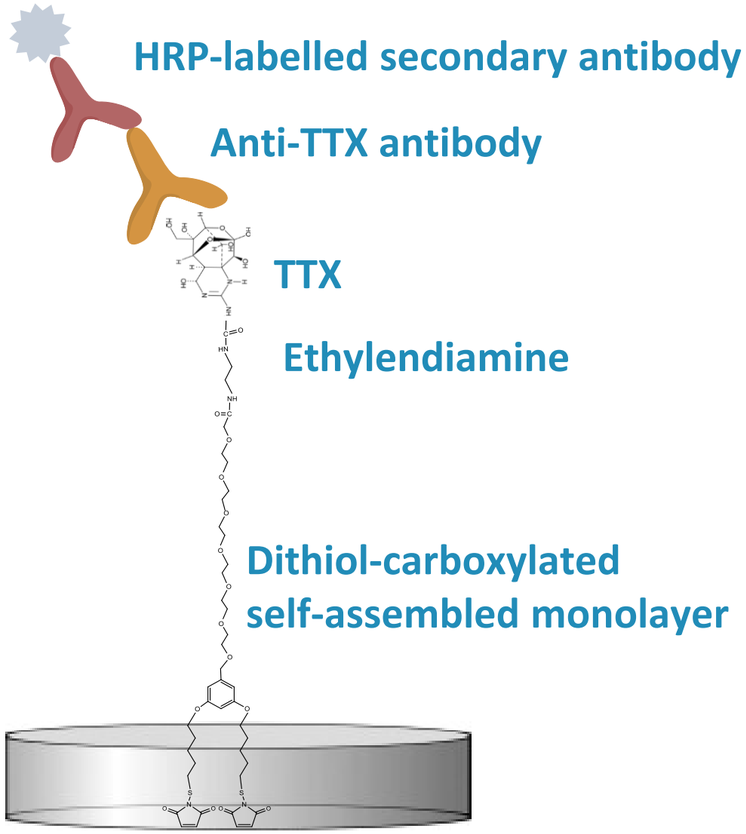
Lagocephalus sceleratus

Tetrodotoxins in shellfish

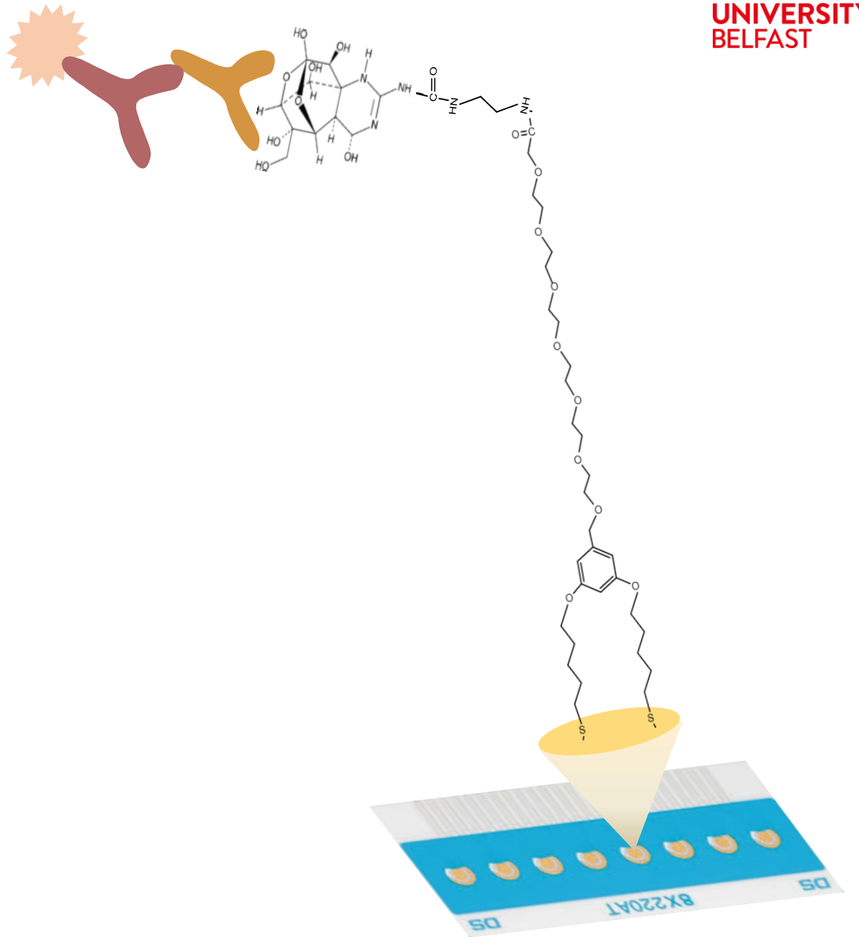


Colorimetric assays and electrochemical biosensors

On plates

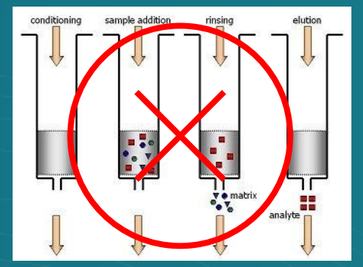


On electrodes

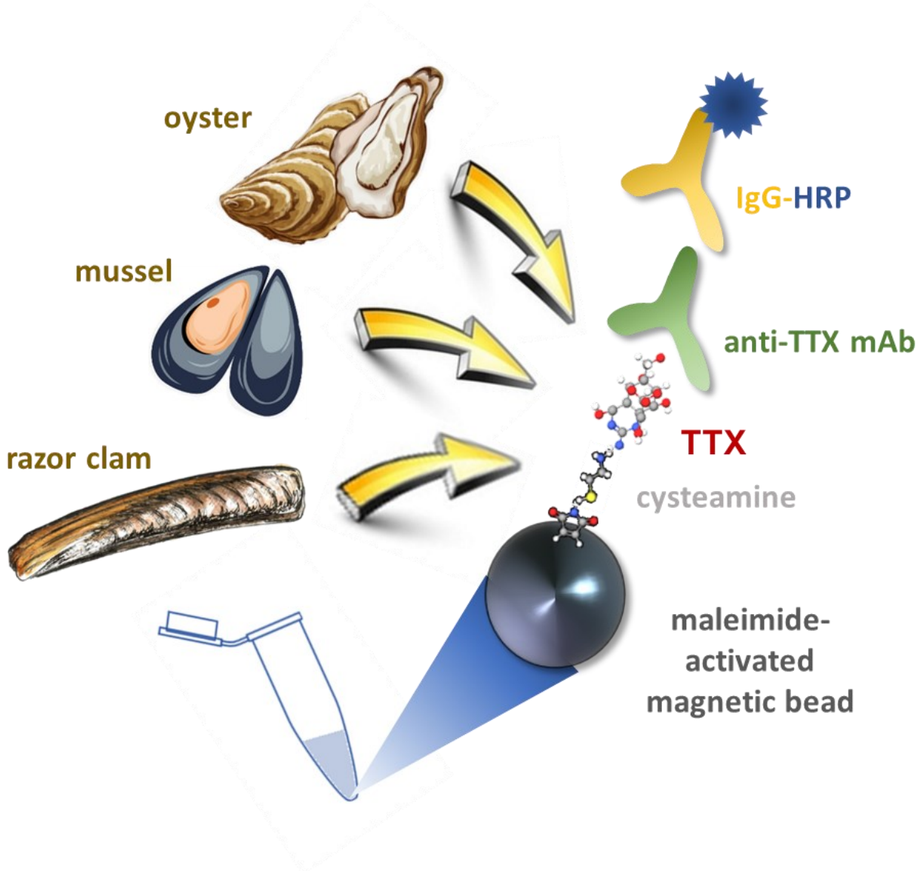


QUEEN'S UNIVERSITY BELFAST

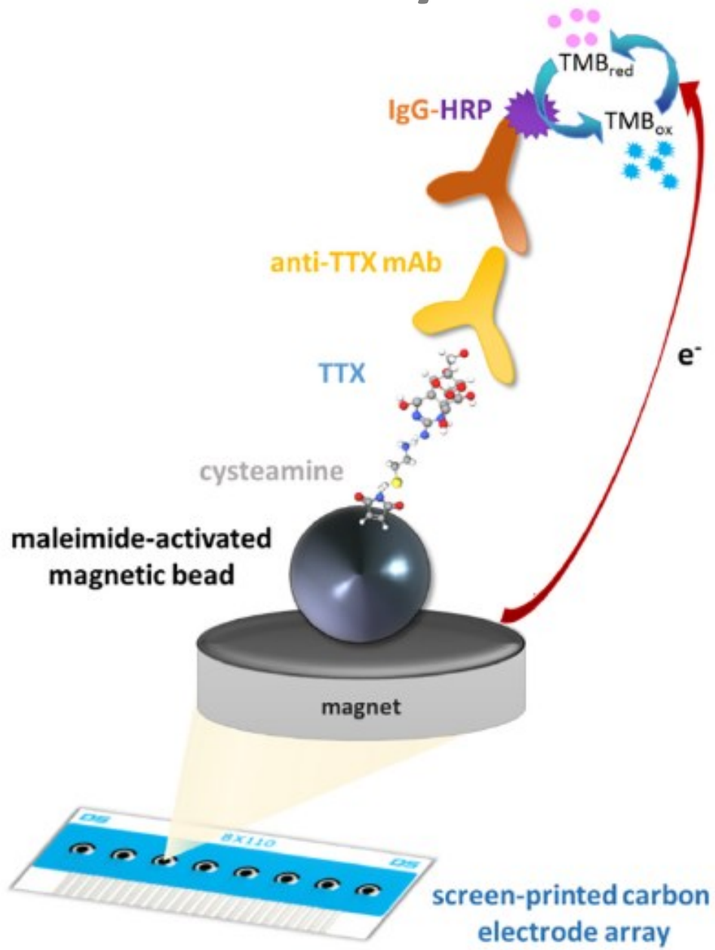
Use of magnetic beads as supports

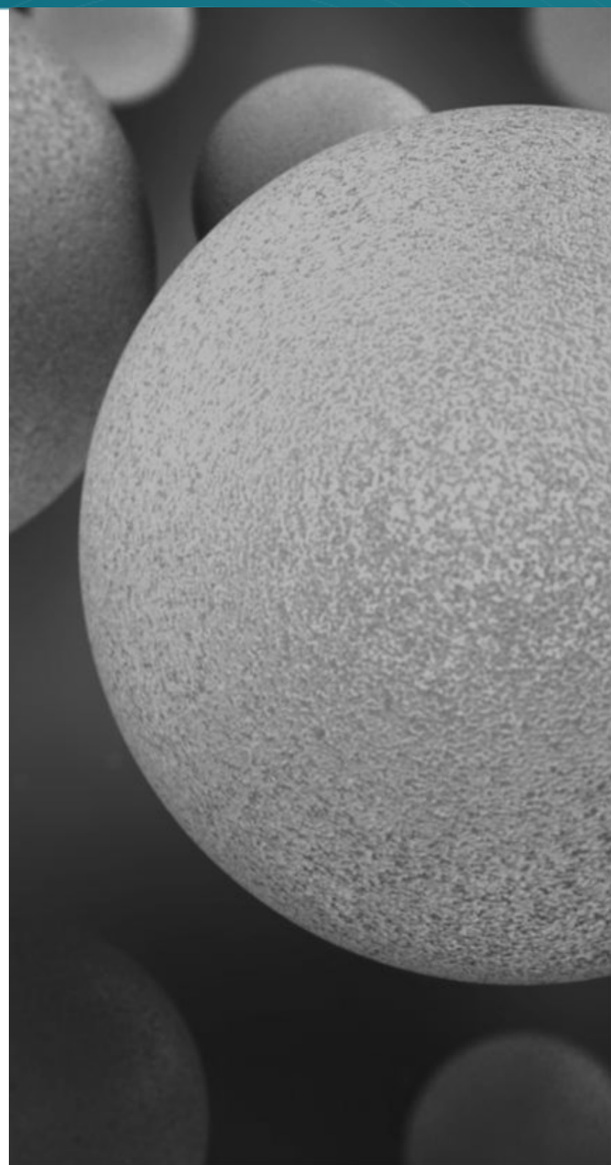


Colorimetry



Electrochemistry





Analysis of samples with immunosensing tools

Puffer fish from Spain

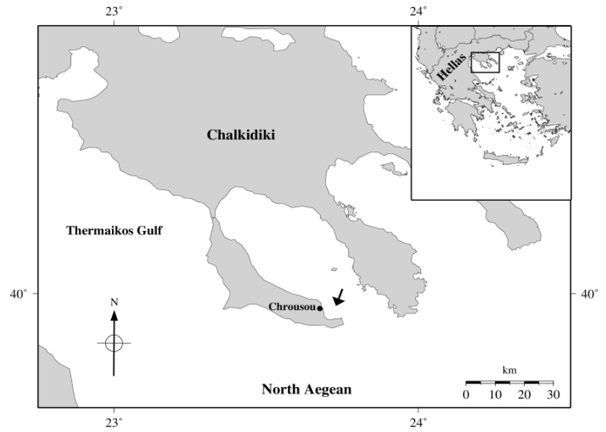
2014 2015 2016

Toxic *L. sceleratus*

Table 2
TTX equivalent contents (mg TTX equiv./kg tissue) in *L. sceleratus* by LC-MS/MS, LC-HRMS and mELISA.

	Σ LC-MS/MS	Σ LC-HRMS	mELISA
Gonads	25.95	25.22	33.55
Liver	3.08	5.36	28.30
Skin	1.65	2.08	3.50
Muscle	1.01	0.98	2.53

Puffer fish juveniles from Greece

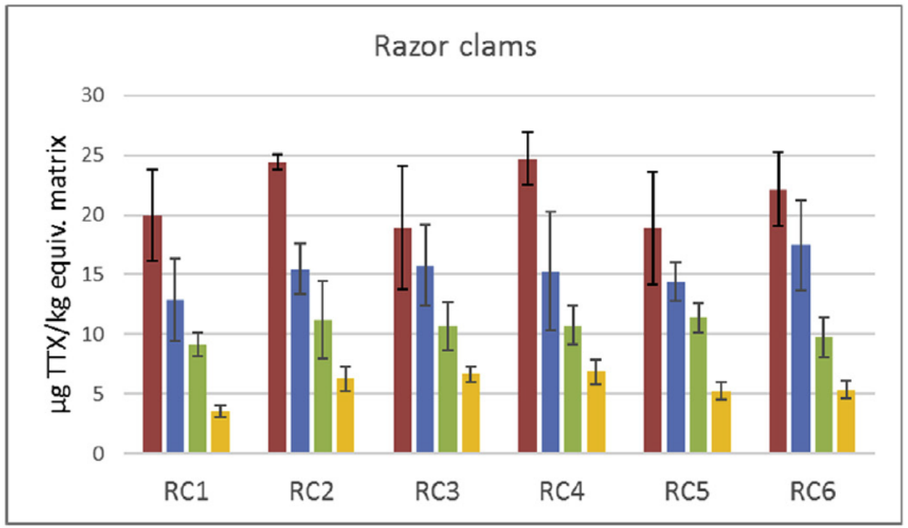


TTX contents (μg TTX/kg tissue)

	Electrochemical immunosensing tool	LC-HRMS	mELISA
M#1	2878	2077	2327
M#2	1395	478	1520
S#1	2588	1239	2773
S#2	2780	1188	3175
O#2	2882	733	10834

Analysis of samples with immunosensing tools

Shellfish from The Netherlands



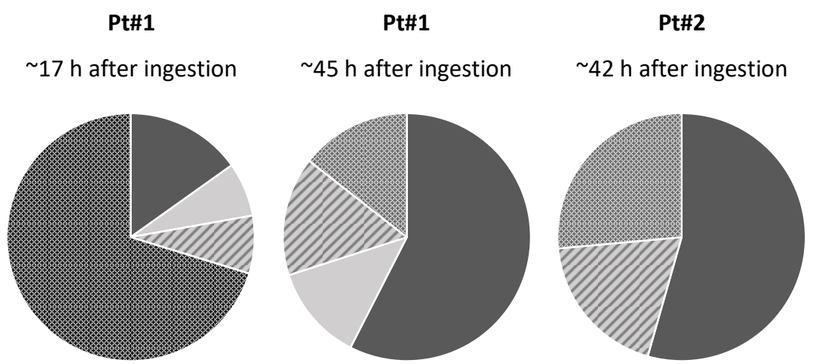
LOD (EC_{80}) = 1 µg/kg (EFSA: 44 µg/kg)

WAGENINGEN UNIVERSITY & RESEARCH

Also for oysters and mussels

Human body fluids from New Caledonia

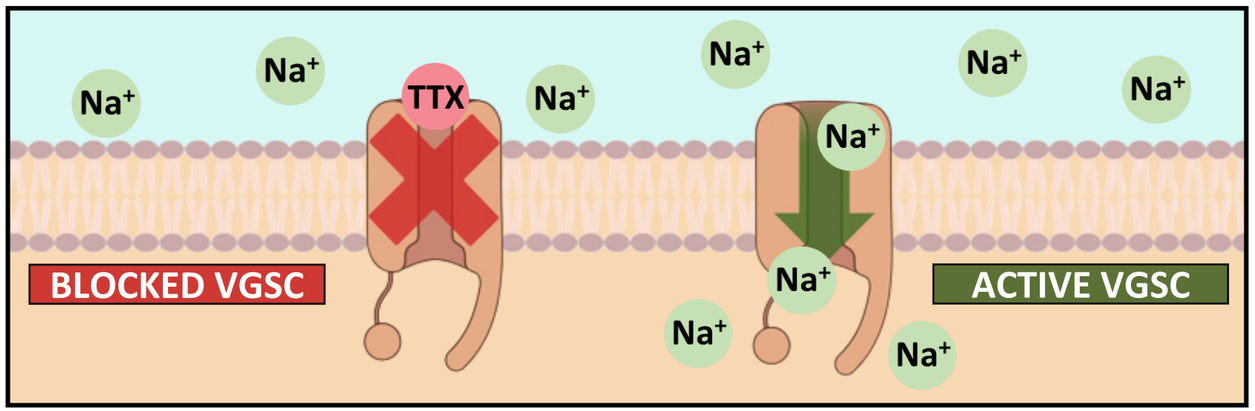
		TTXs (ng/mL)						
		LC-MS/MS						mELISA
	Hours after ingestion	TTX	4-*epi*TTX	4,9-anhydro TTX	5,6,11-trideoxy TTX	TTX equiv. (applying TEFs)	TTX equiv. (applying CRFs)	TTX equiv.
Pt#1	17	217.4	101.2	108.3	1008.2	246.3	217.4–318.6	211.1
Pt#1	45	683.9	149.0	184.7	171.6	712.6	683.9–833.9	561.4
Pt#2	42	65.4	-	23.0	31.8	66.2	65.4	32.0



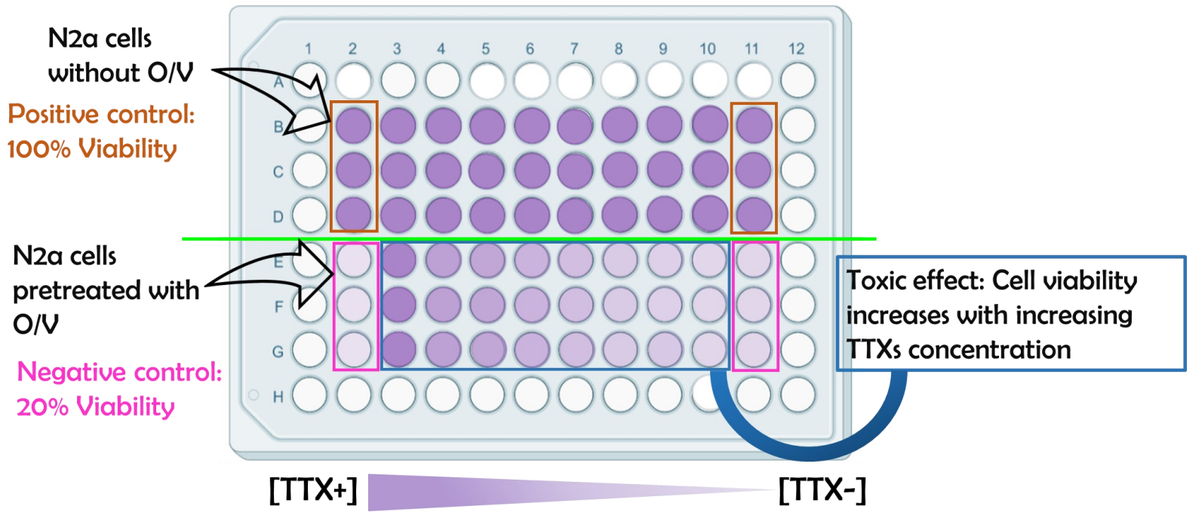
CENTRE HOSPITALIER TERRITORIAL GASTON-BOURRET NOUVELLE-CALÉDONIE

Cell-based assay (CBA)

Assay development and analysis of puffer fish

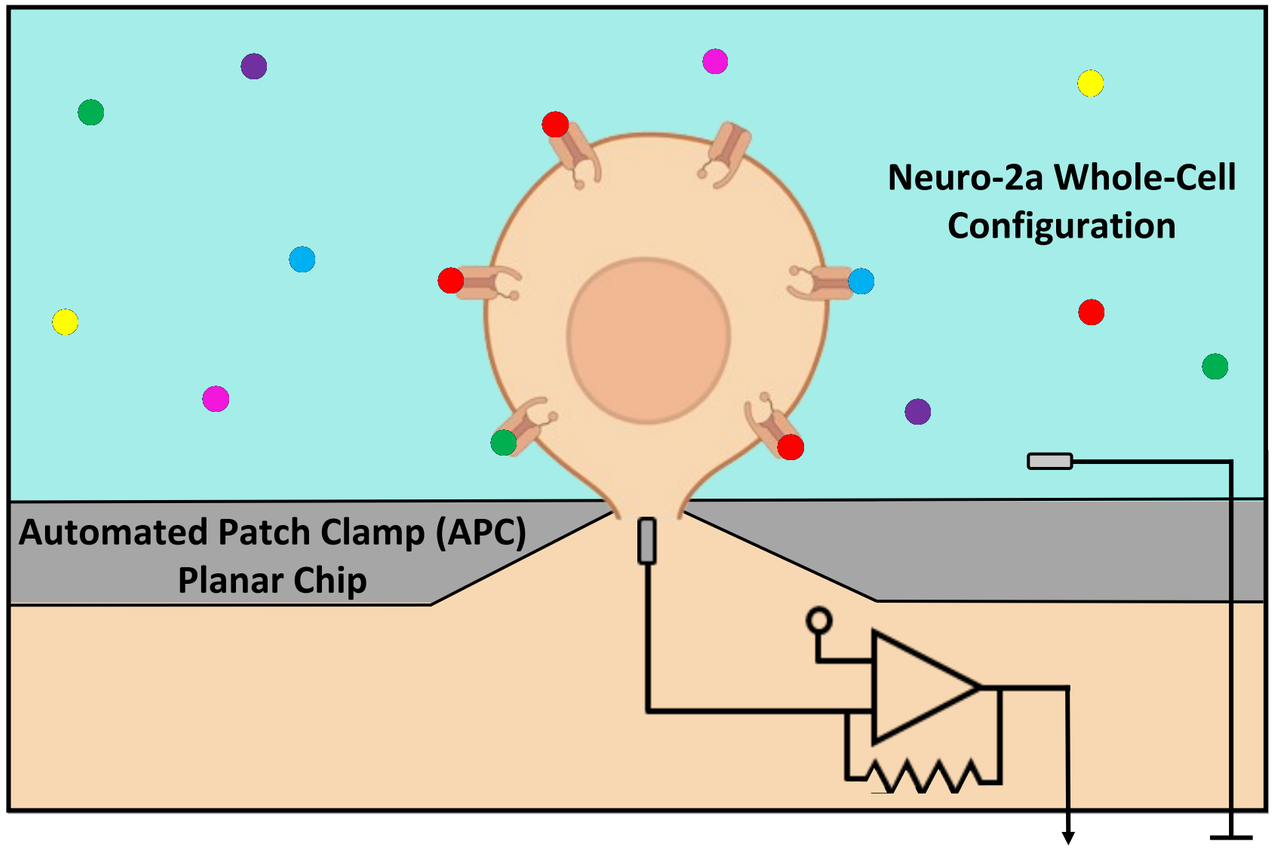


O/V Combinations (mM)	Cell Viability (%)	IC_{50} (ng/mL)	Saturation Plateau (%)
0.5/0.05	28 ± 2	8.65	57 ± 1
0.45/0.045	29 ± 2	3.55	63 ± 2
0.4/0.04	21 ± 2	1.07	78 ± 3
0.35/0.035	20 ± 2	1.67	72 ± 3
0.3/0.03	50 ± 2	2.69	68 ± 2
0.2/0.1	38 ± 1	2.37	86 ± 4
0.125/0.2	20 ± 1	1.65	102 ± 3



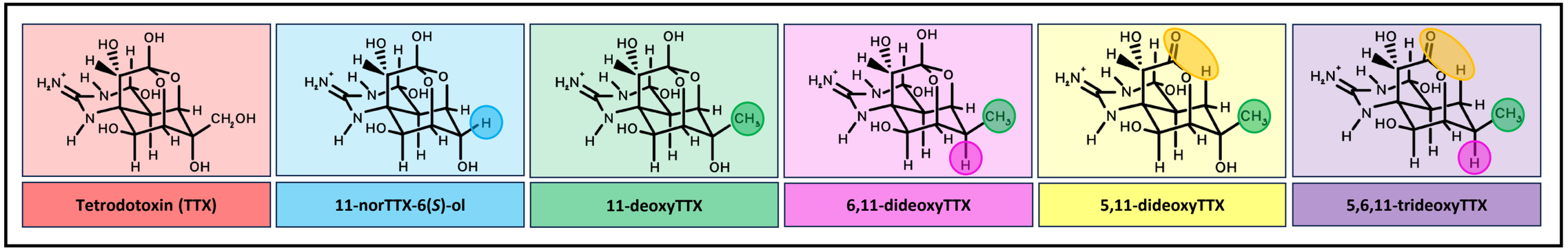
Automated patch clamp (APC)

Assay development and analysis of puffer fish

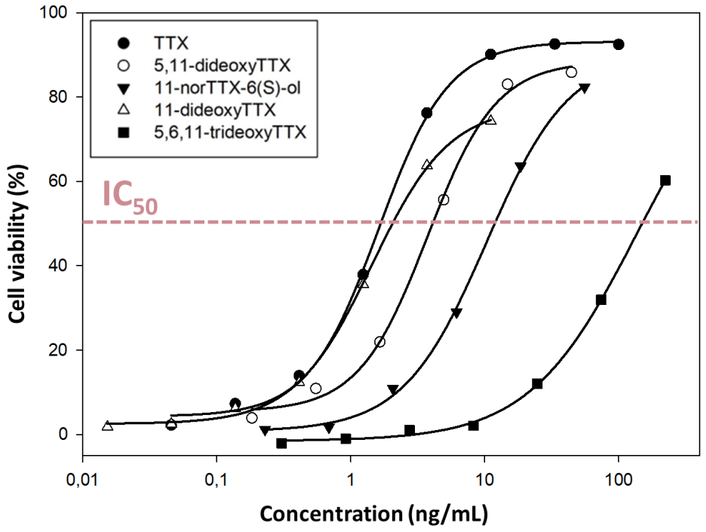


UNIVERSITAT ROVIRA i VIRGILI

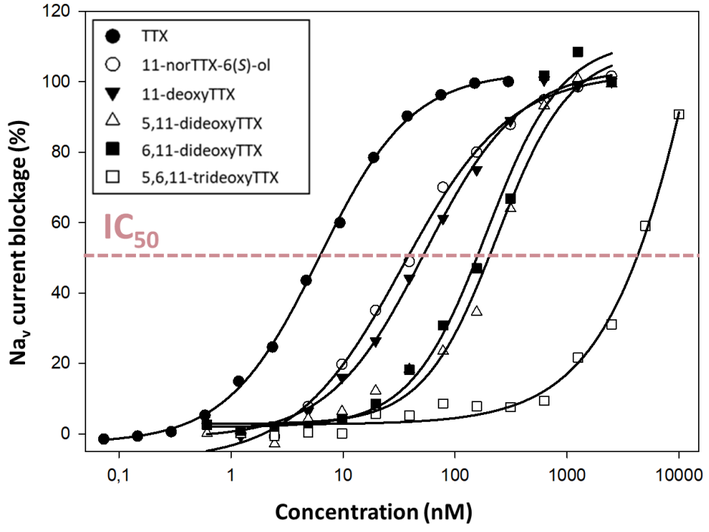
Toxicity equivalency factors (TEFs) *vs.* cross-reactivity factors (CRFs)



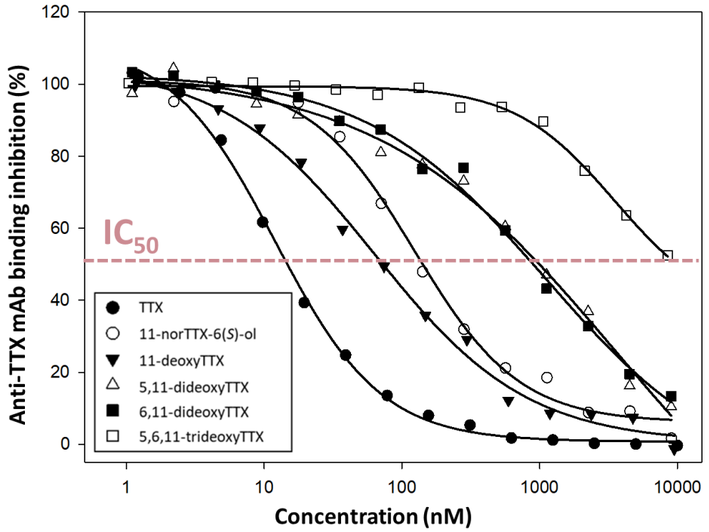
CBA



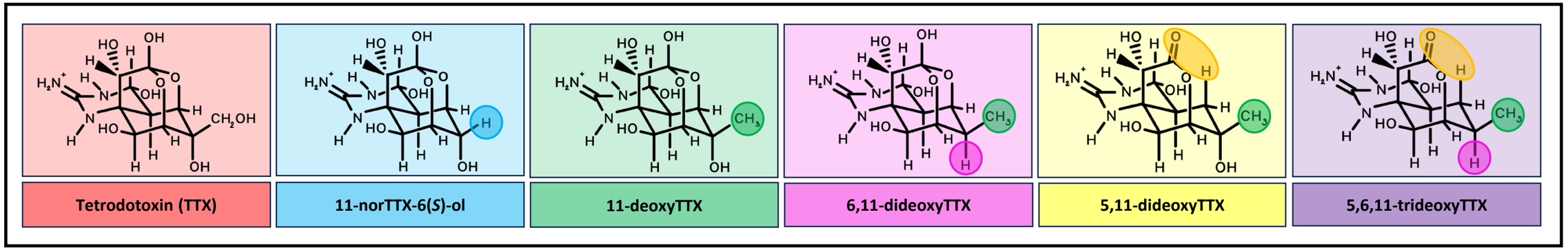
APC



Immunoassay



Toxicity equivalency factors (TEFs) *vs.* cross-reactivity factors (CRFs)



CBA

TEFs	
11-norTTX-6(*S*)-ol	0.404
11-deoxyTTX	0.139
5,11-dideoxyTTX	0.750
6,11-dideoxyTTX	NA
5,6,11-trideoxyTTX	0.011

APC

TEFs	
11-norTTX-6(*S*)-ol	0.238
11-deoxyTTX	0.107
5,11-dideoxyTTX	0.027
6,11-dideoxyTTX	0.035
5,6,11-trideoxyTTX	0.001

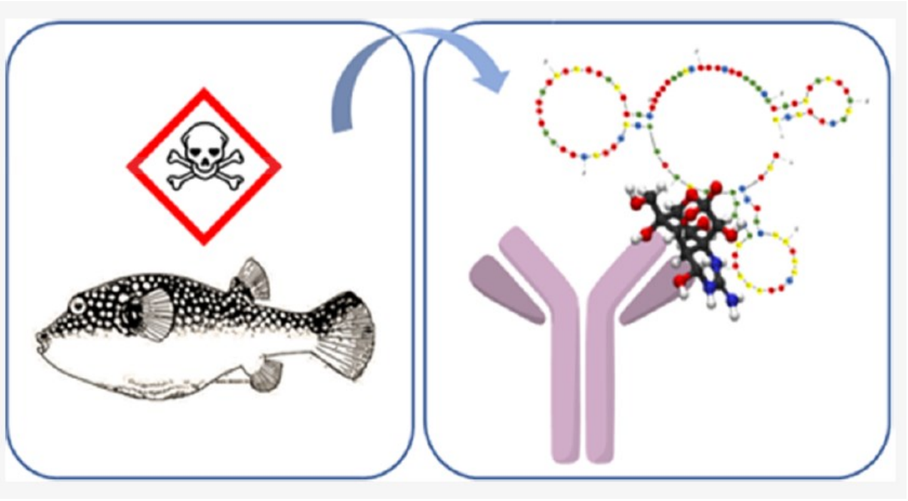
Immunoassay

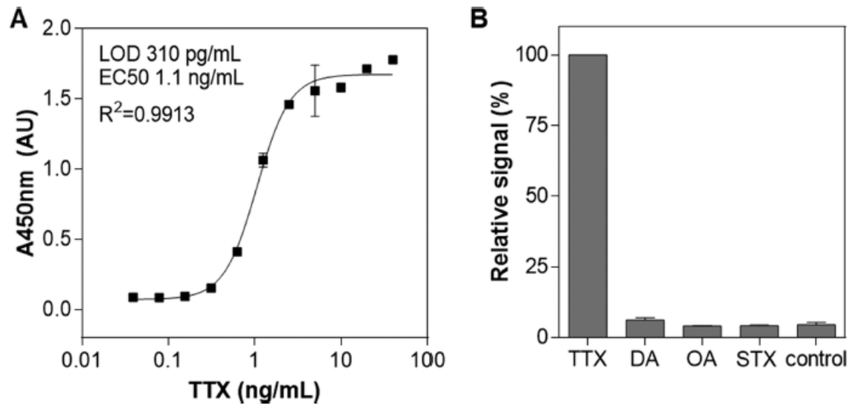
CRFs	
11-norTTX-6(*S*)-ol	0.103
11-deoxyTTX	0.202
5,11-dideoxyTTX	0.015
6,11-dideoxyTTX	0.016
5,6,11-trideoxyTTX	0.001

Aptamer/antibody-based assay

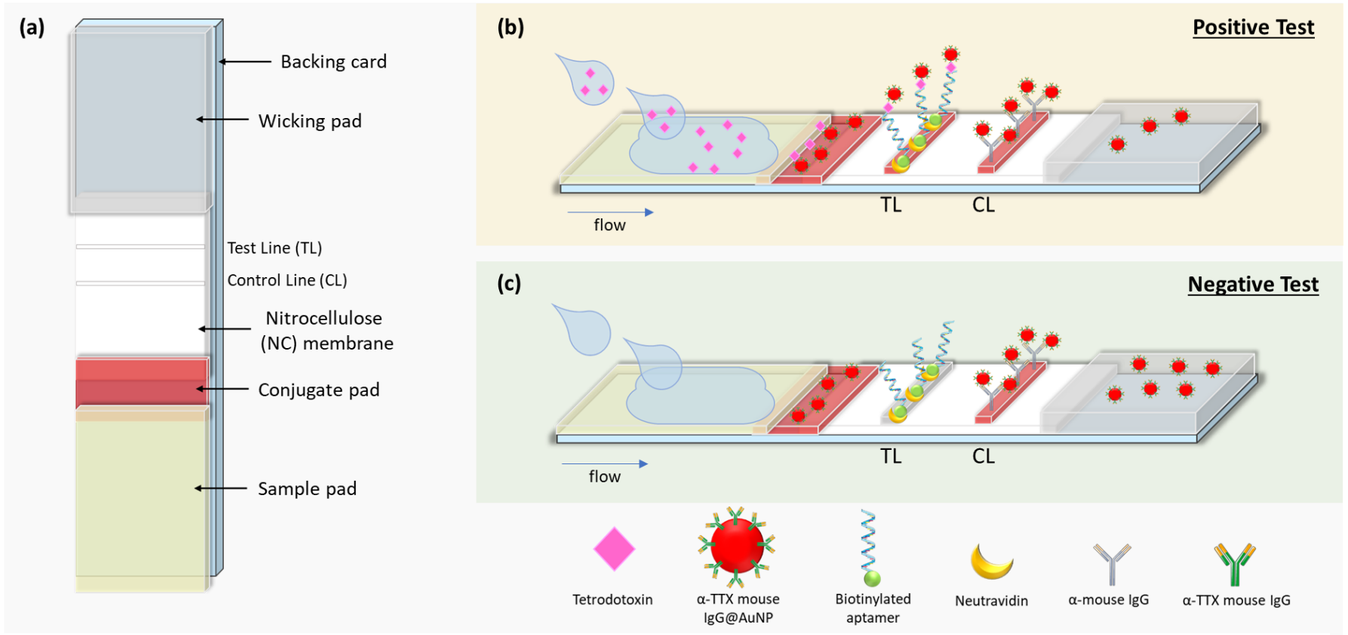
UNIVERSITAT ROVIRA i VIRGILI

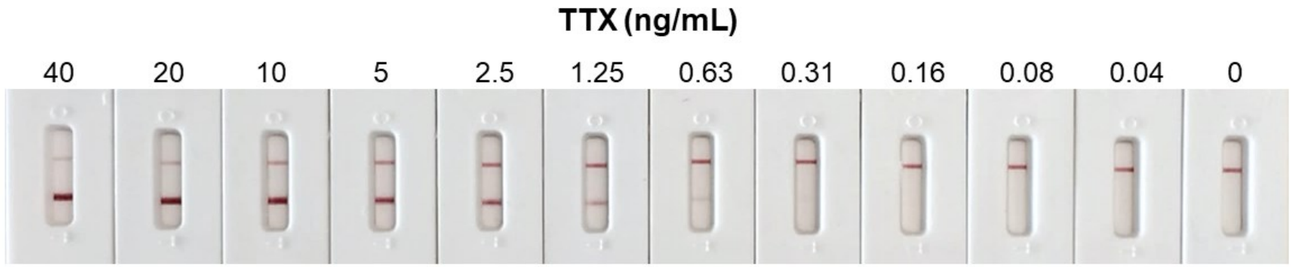
Colorimetric (plates)





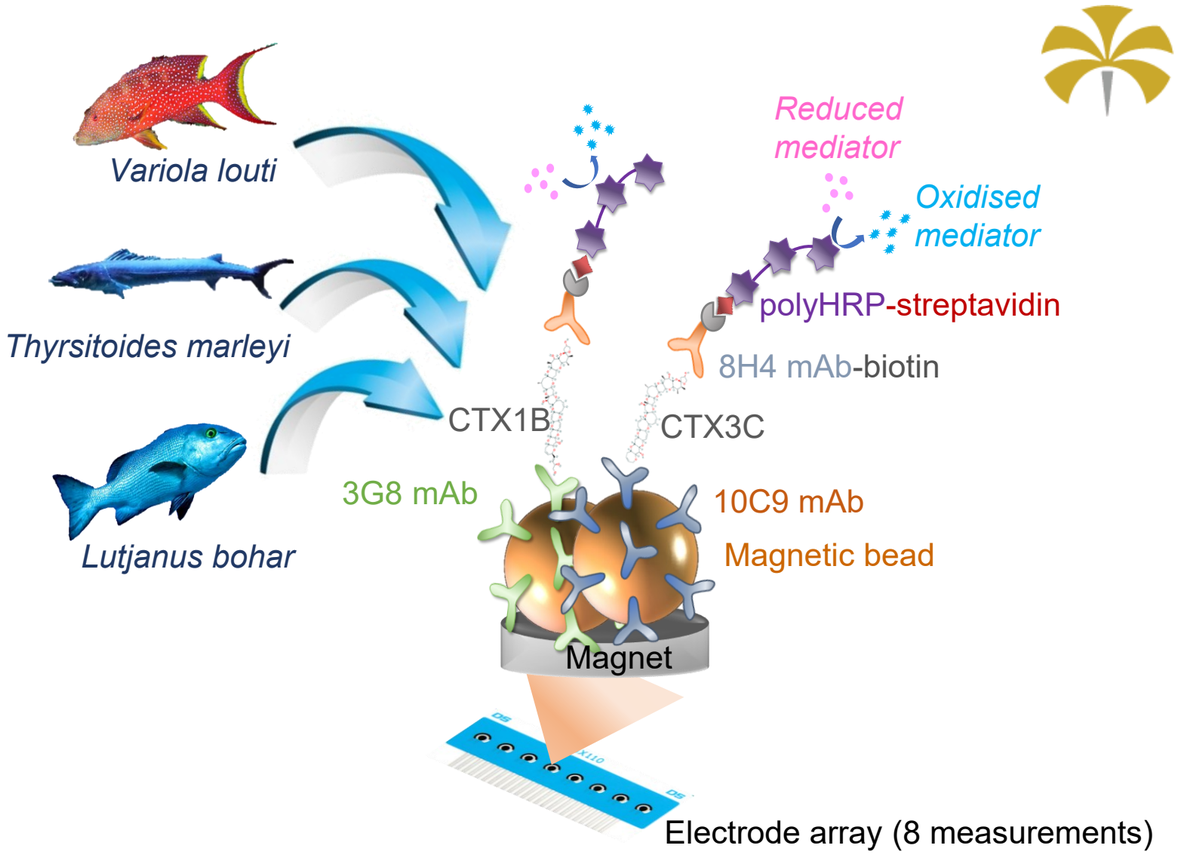
Visual & colorimetric (LFA)





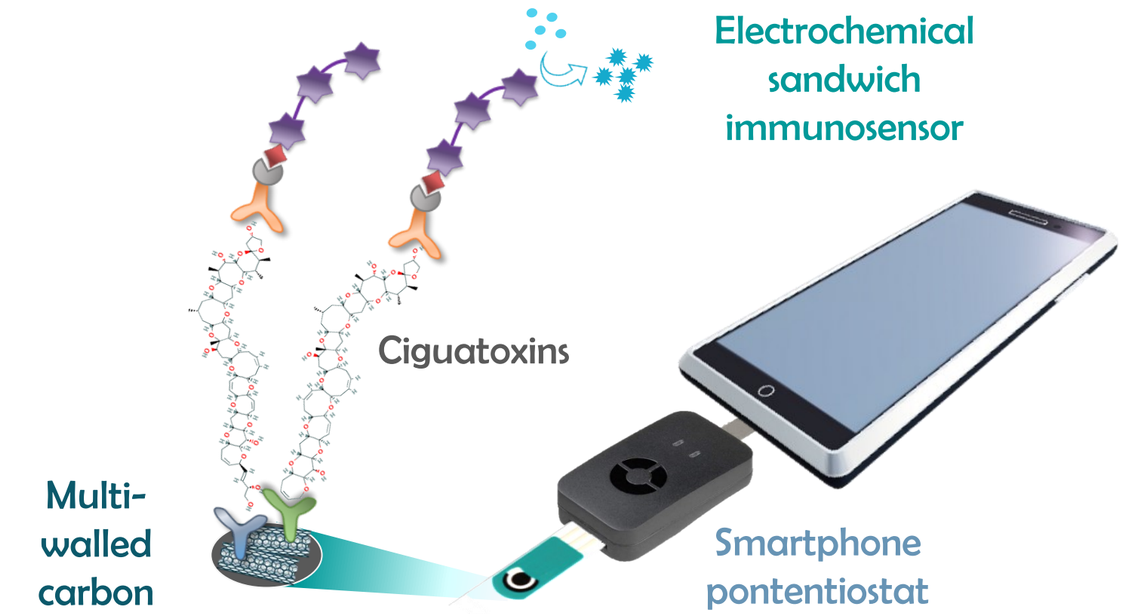
Colorimetric assays and electrochemical biosensors

With magnetic beads on electrodes



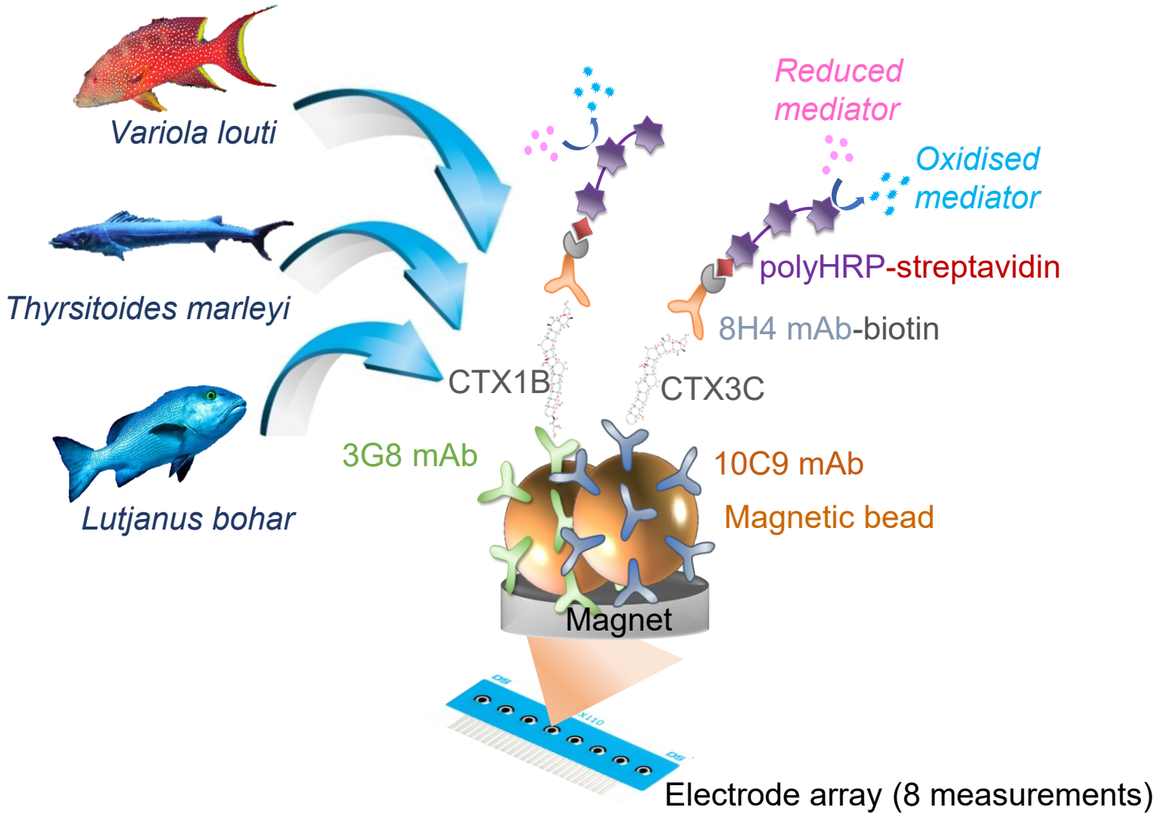
Osaka Metropolitan University

Portable potentiostat and smartphone



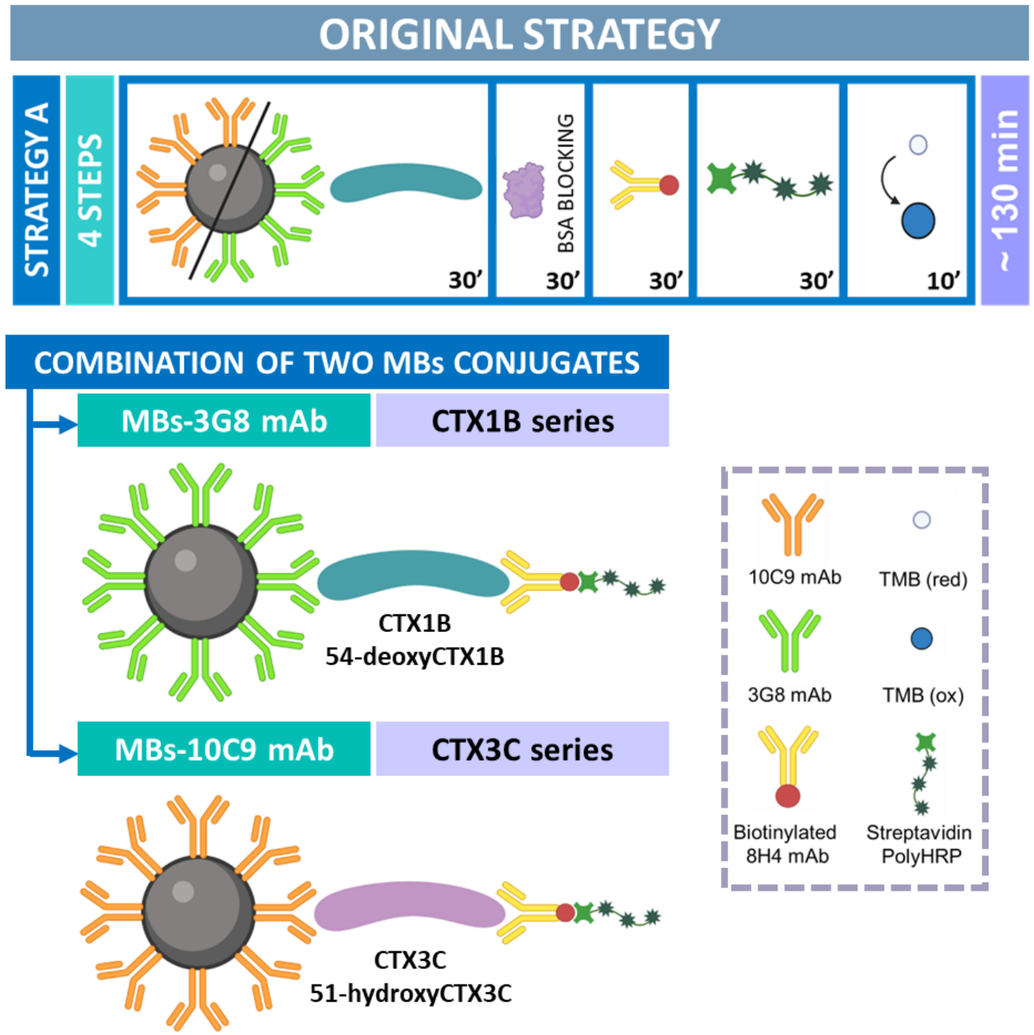
Colorimetric assays and electrochemical biosensors

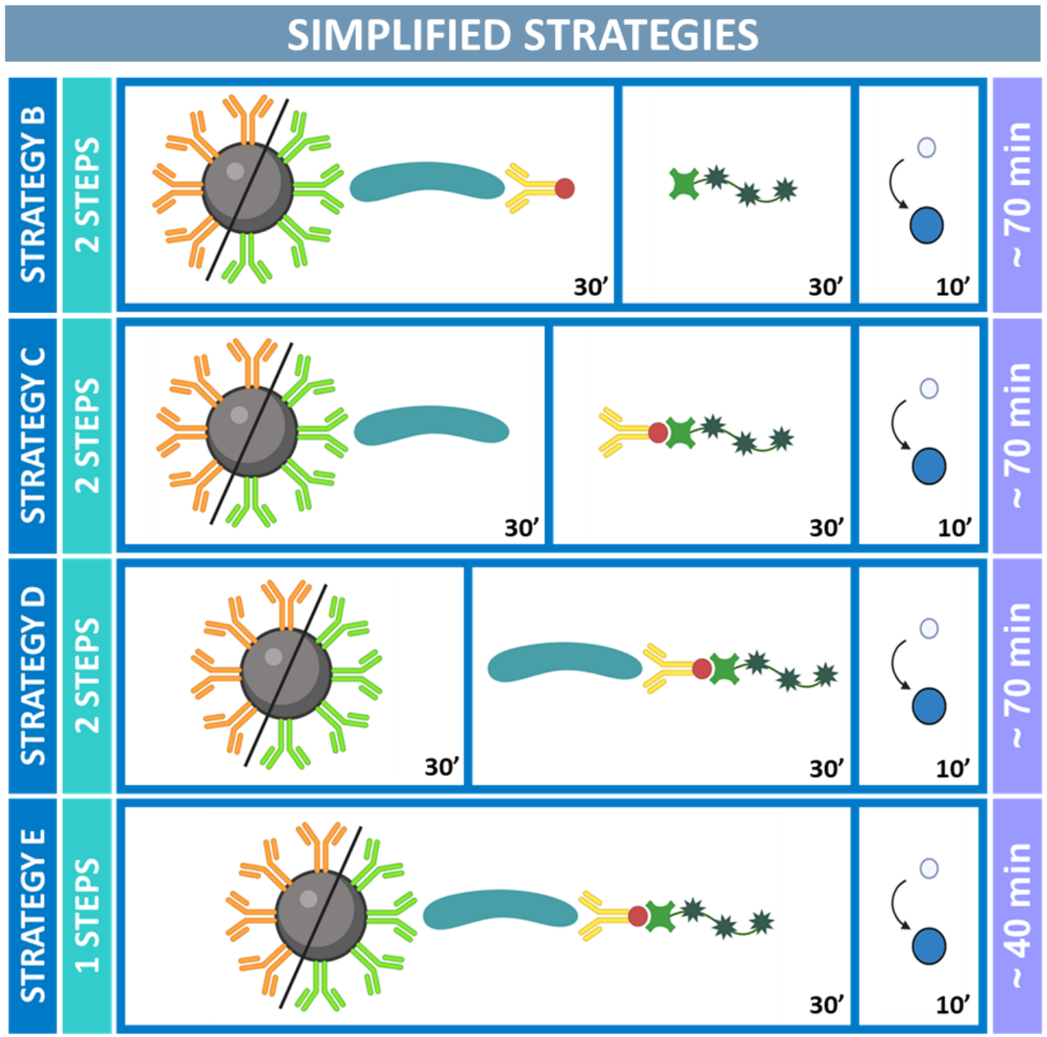
With magnetic beads on electrodes



Portable potentiostat and smartphone

Simplified colorimetric assay





2 steps!
40 min!
FDA level!
Stable!

Analysis of samples with immunosensing tools

Fish from La Réunion, Maurice, Japan and Fiji

Lutjanus bohar

Thyrsitoides marleyi

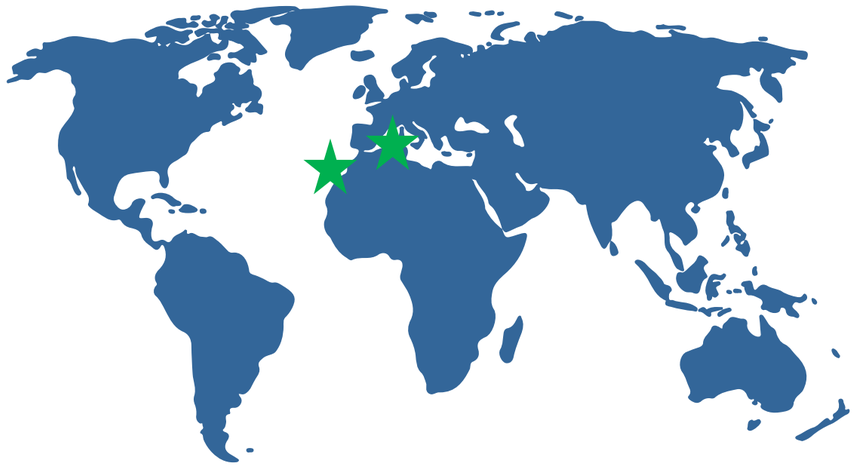
Variola louti

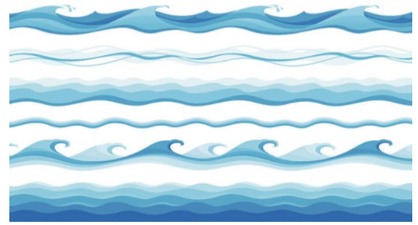
CiTEB
CENTRE TECHNIQUE de RECHERCHE et de VALORISATION des MILIEUX AQUATIQUES

NIHS 国立医薬品食品衛生研究所
National Institute of Health Sciences

Microalgae from the Canary and Balearic Islands

Toxins were extracted from only 20,000 cells



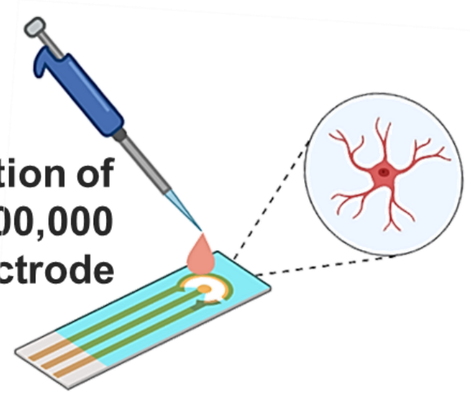


Toxins were also detected in field seawater samples

Cell-based biosensors for CTXs/TTXs

Resuspended cells

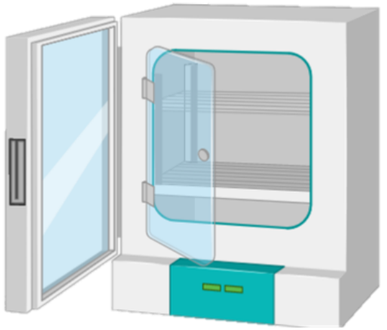
Addition of 100,000 cells/electrode



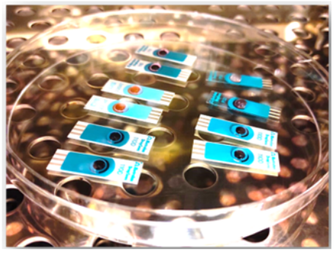
Incubation

37 ºC

2 h



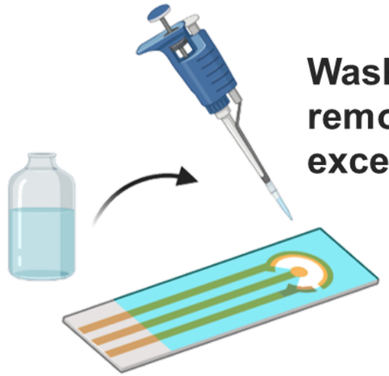
Wash to remove unbound cells



37 ºC

25 min

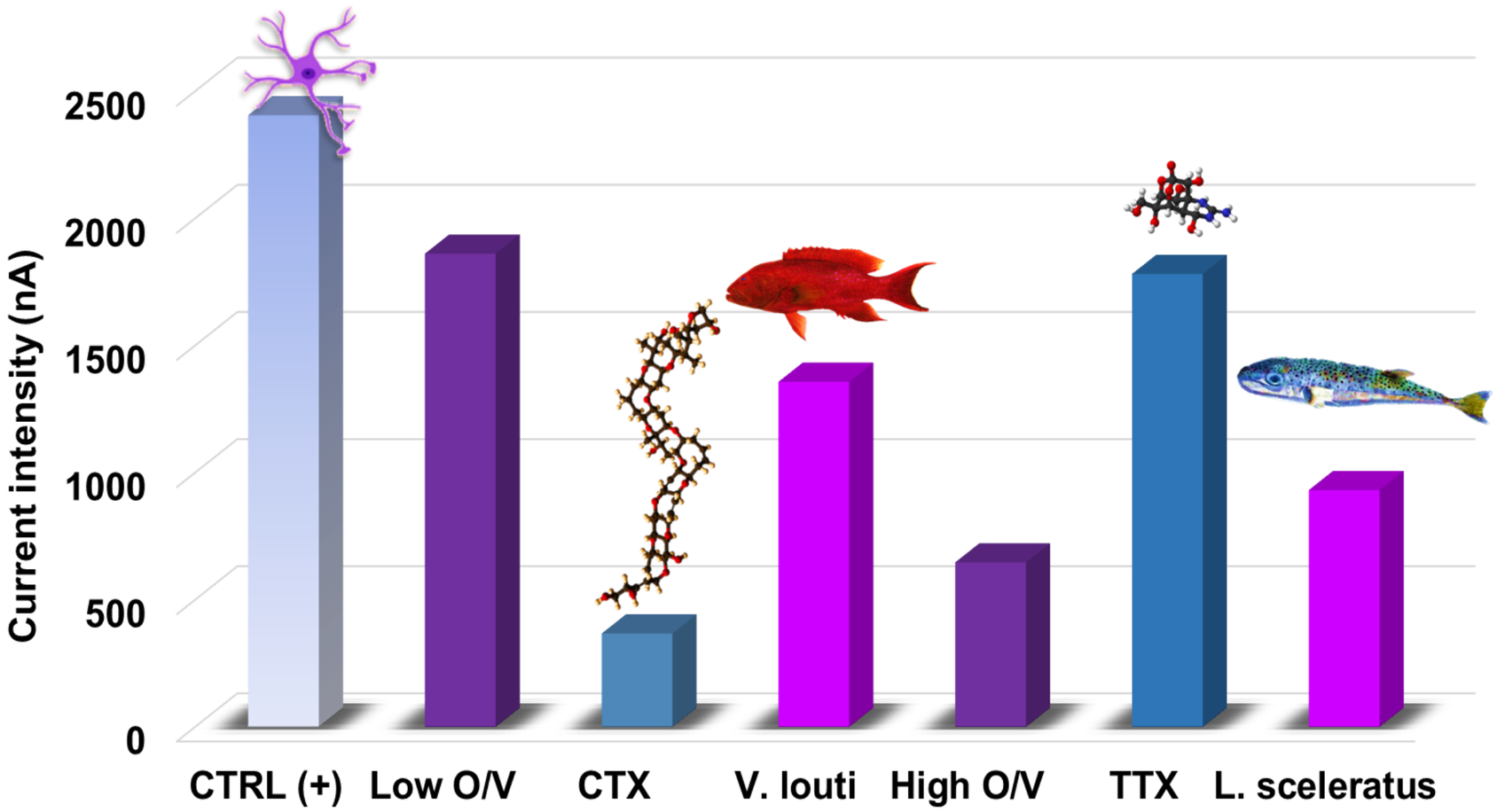
Incubation with MTT



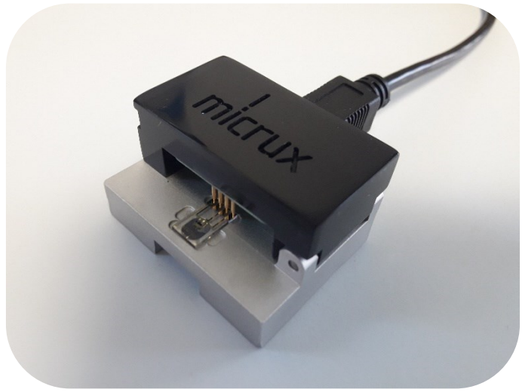
Wash to remove the excess of MTT

Cyclic voltammetry and chronoamperometry

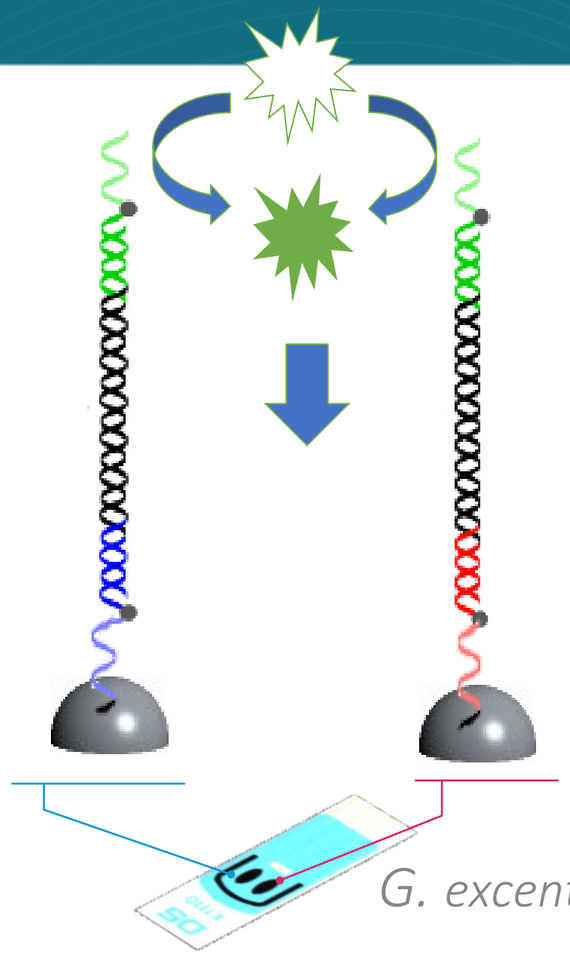
Analysis of samples with cell-based biosensors



Trying to improve reproducibility

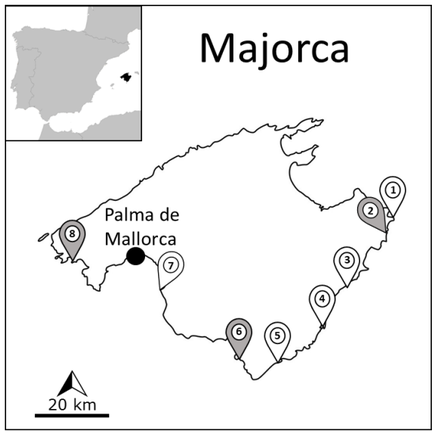


Detecting the toxin producers with DNA sensors and dipsticks



G. excentricus

G. australes



First report of *G. excentricus* in the Balearic Islands

G. australes
G. excentricus

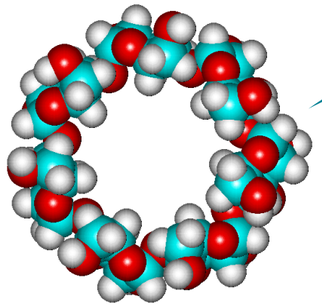
Control line
Test lines

G. australes	+	-	+	-
G. excentricus	-	+	+	-

UNIVERSITAT ROVIRA I VIRGILI

Capture of toxins in seawater

Cyclodextrin polymers for toxin capture



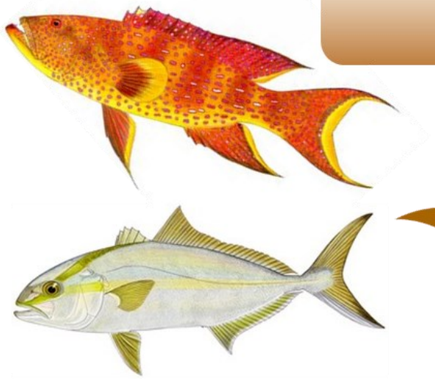
Universitat Rovira i Virgili

Università degli Studi di Napoli Federico II

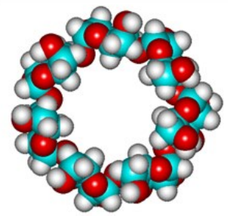
Capture of toxins in extracts

Cyclodextrin polymers for sample clean-up and pre-concentration

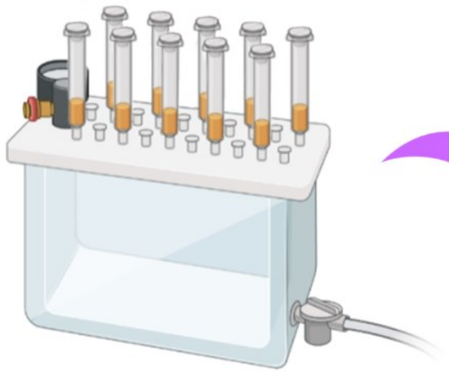
CTXs in fish



2. Fish flesh extract

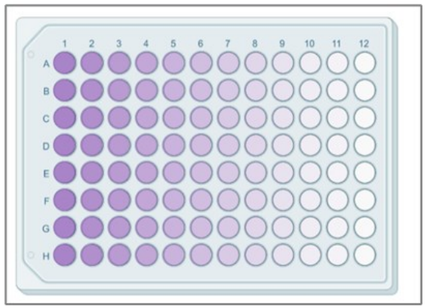


1. Cyclodextrin polymer



3. Clean-up

4. Cell-based assay



WAGENINGEN UNIVERSITY & RESEARCH

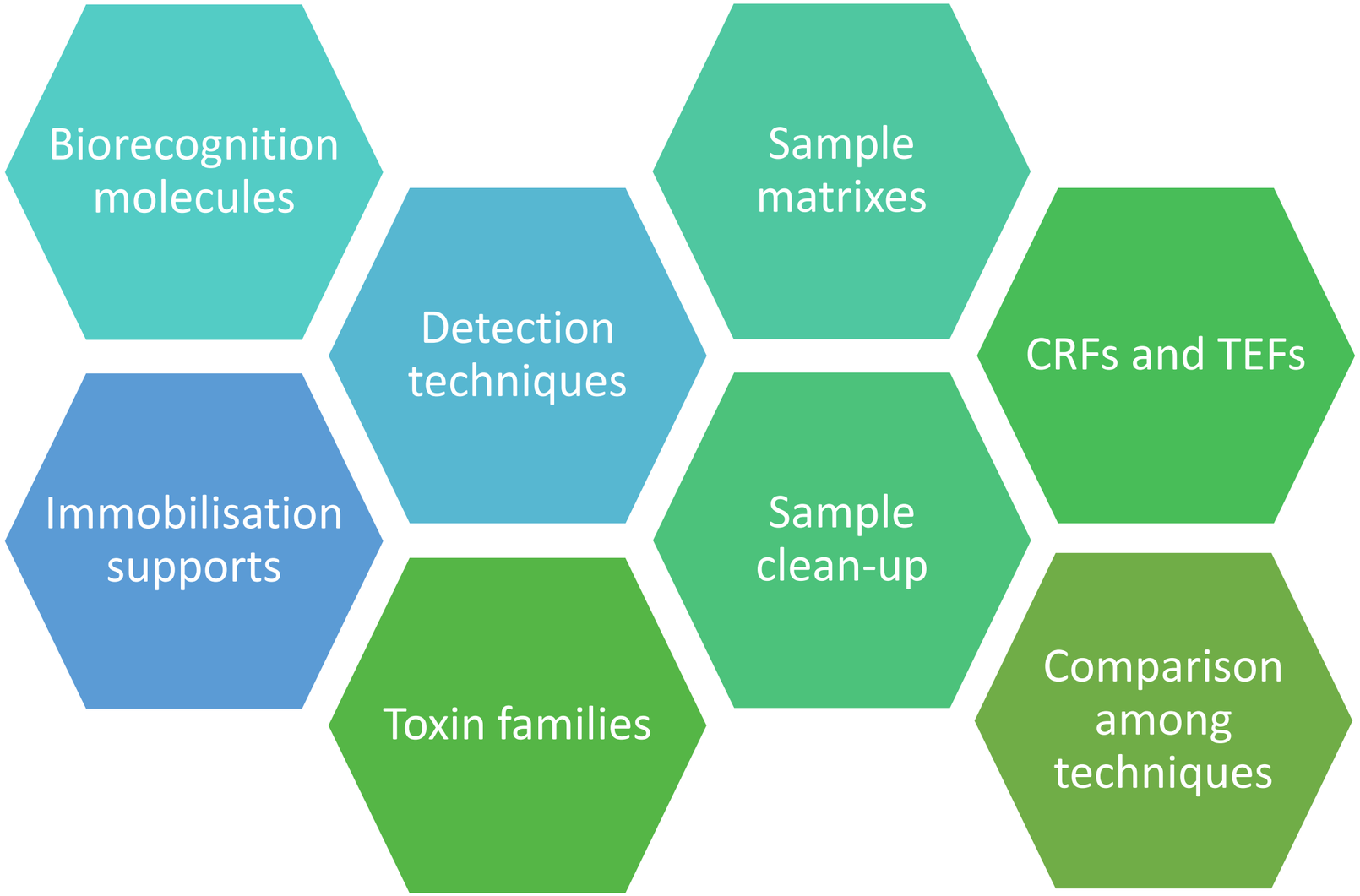
TTXs in shellfish

UNIVERSITAT ROVIRA I VIRGILI

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

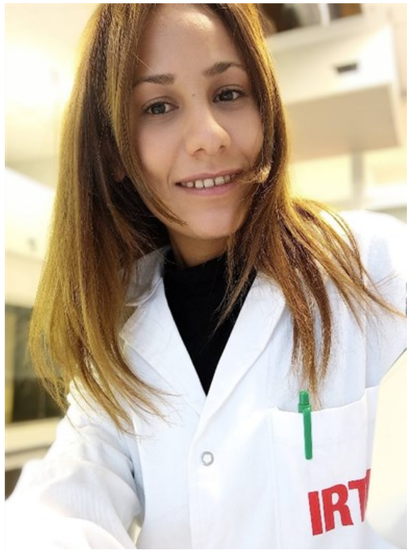
GAMB & 44-Me-GAMB in microalgae

Summarising



Thanks

Laia Greta Sandra Anna



Mounira

Jaume

Ulises