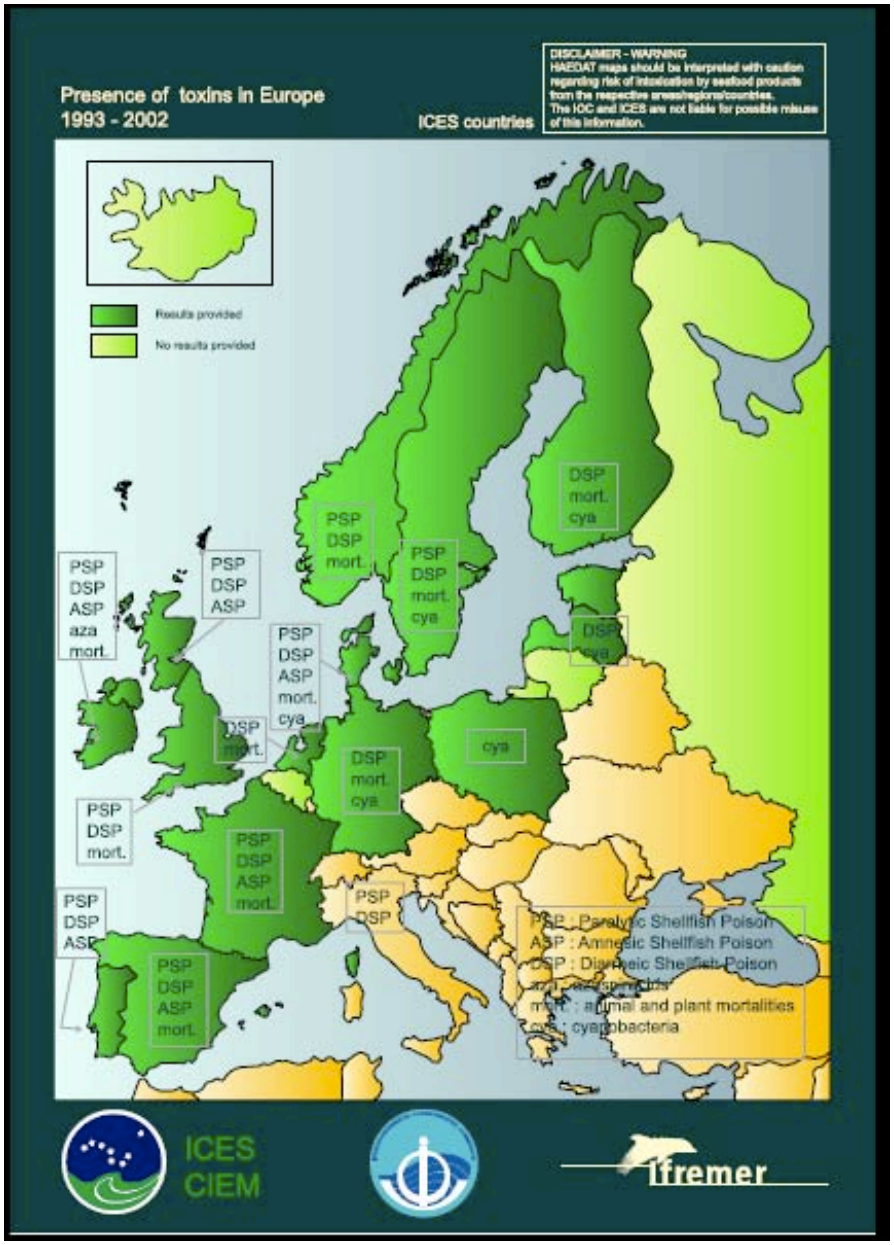
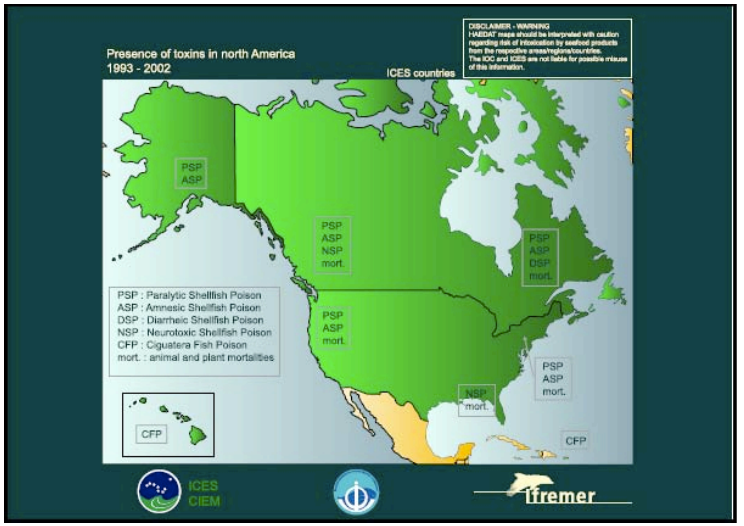
Università di Modena e Reggio Emilia, Modena, Italy

Mirella Bellocci

A CELL-BASED ASSAY FOR THE DETECTION OF A POTENT ALGAL NEUROTOXIN IN BIOLOGICAL MATRICES

Alicante October 2008





Harmful Algal Blooms

increse in frequency and geographic distribution

New toxins

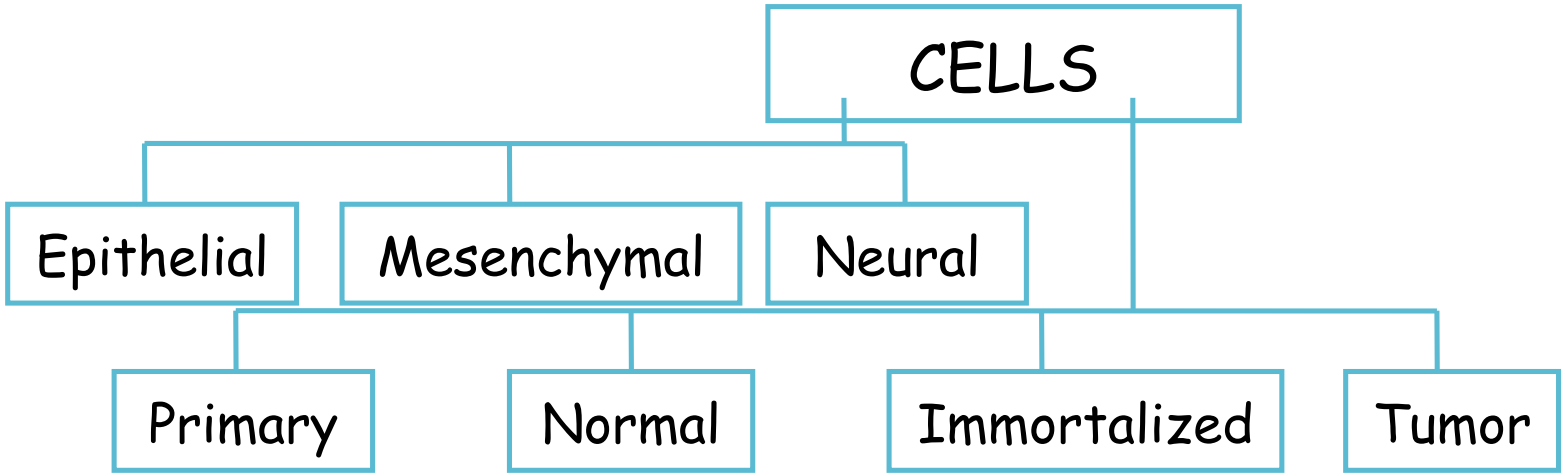
continuously isolate and characterize

To protect public health

- ✓Routine monitoring programmes of algal populations at sea and seafood toxicity

- ✓Studies of molecular mechanism of action of different marine biotoxins

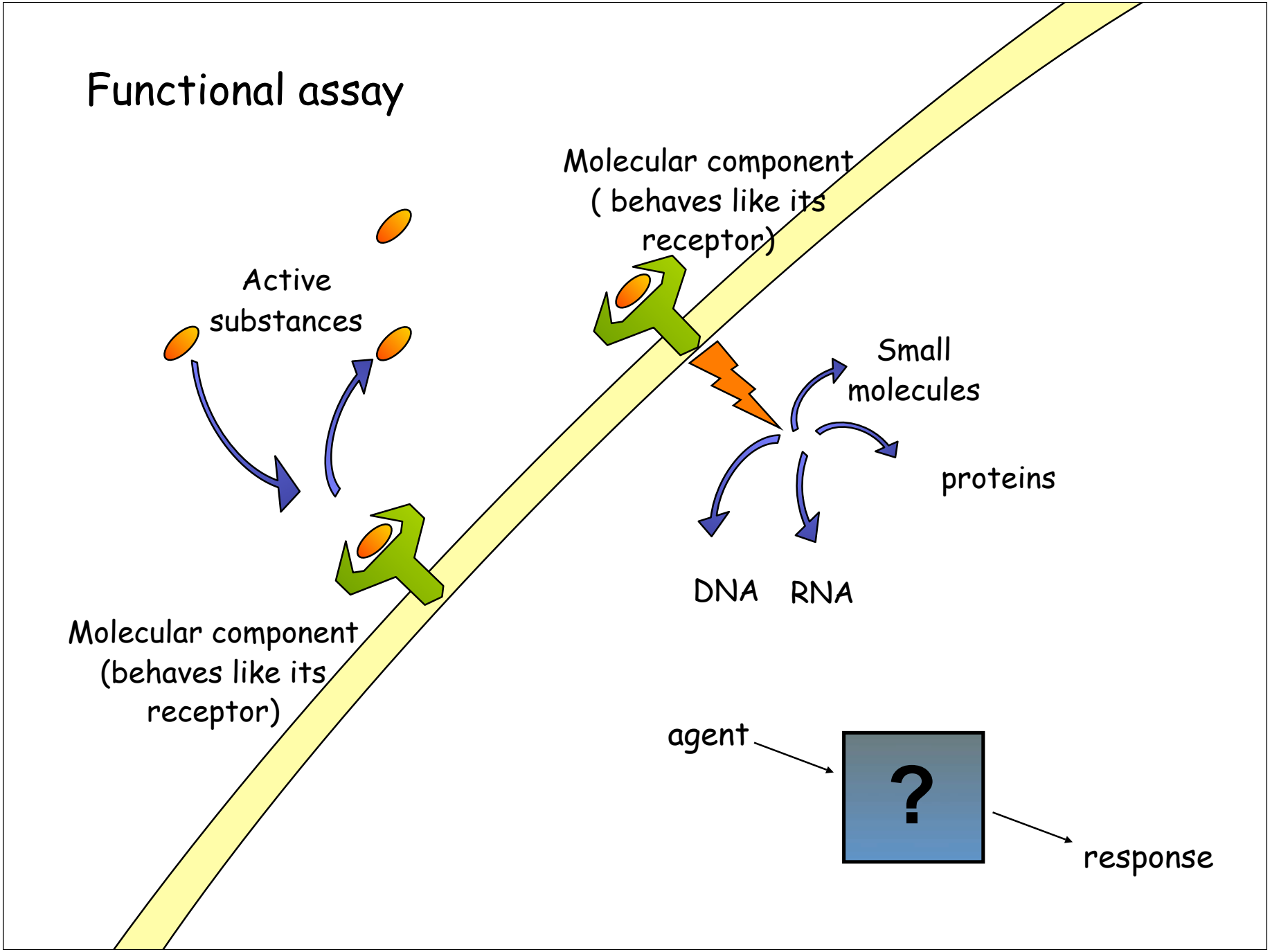
Cultured cells are our model systems



Cultured cells in algal biotoxin monitoring aimed at:

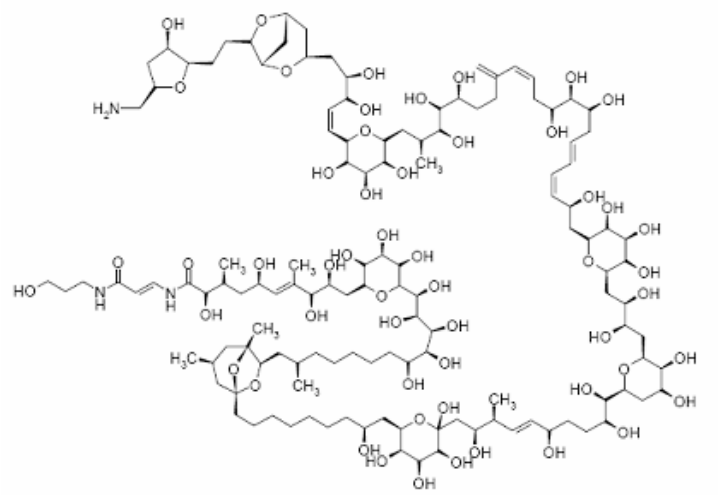
✓obtaining a biologically-oriented detection

✓substitute the use of lab animals

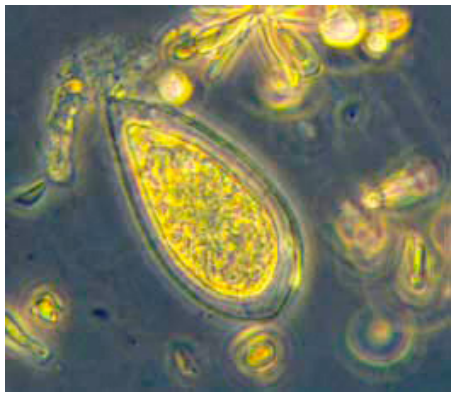

Functional assay
Molecular component
(behaves like its
receptor)
Active
substances
Small
molecules
proteins
DNA
RNA
Molecular component
(behaves like its
receptor)
agent
?
response

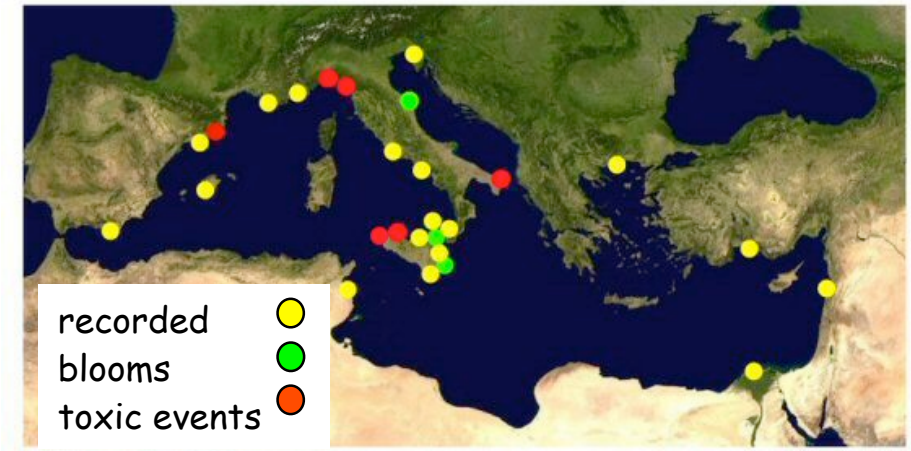
Functional assay in monitoring…

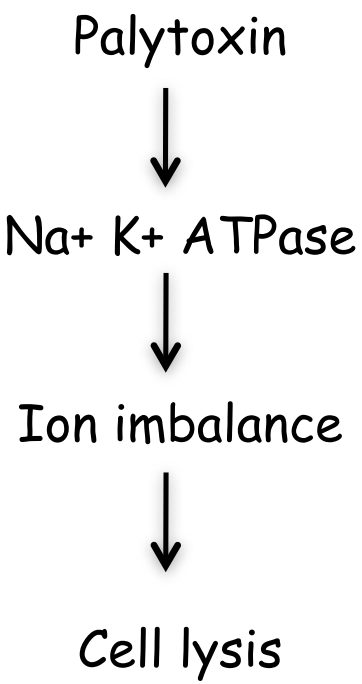
Palytoxin

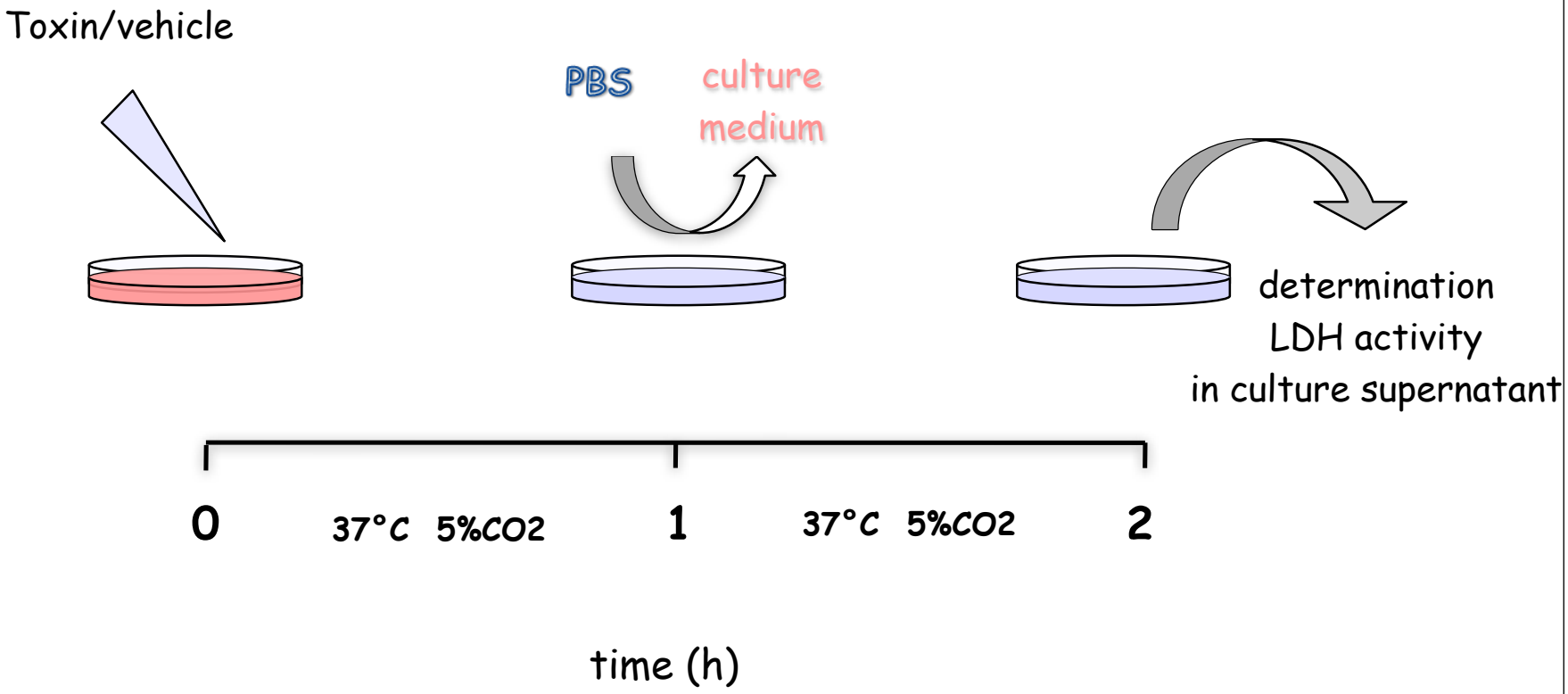


Osteopsis ovata

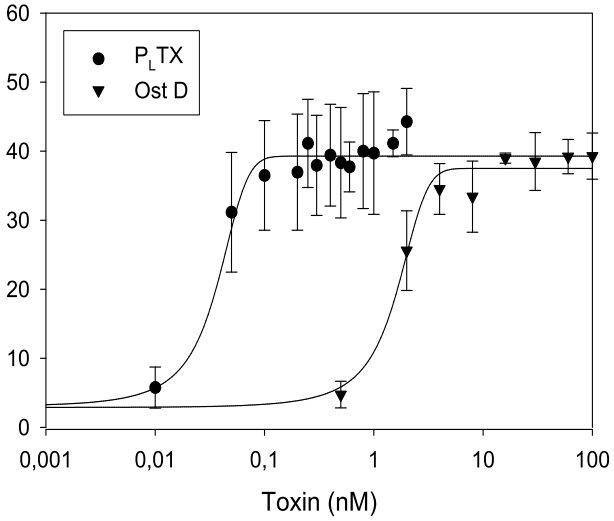





Palytoxin
Na+ K+ ATPase
Ion imbalance
Cell lysis

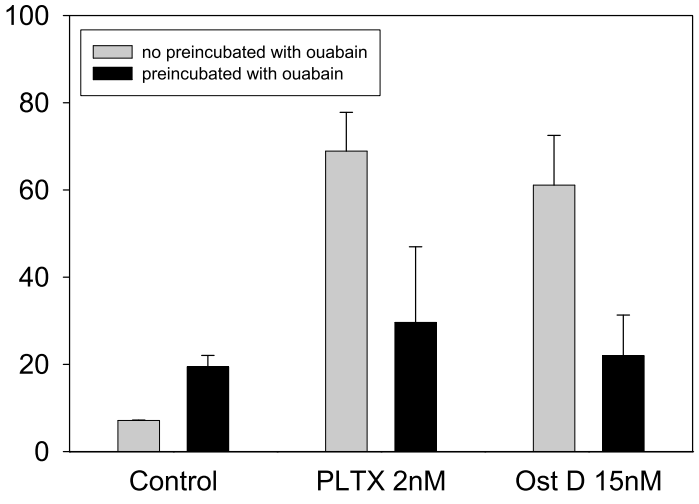

Toxin/vehicle
PBS
culture
medium
determination
LDH activity
in culture supernatant
0
37°C 5%CO2
1
37°C 5%CO2
2
time (h)

sensitive



LOD 50 pg P_LTX/ ml culture medium

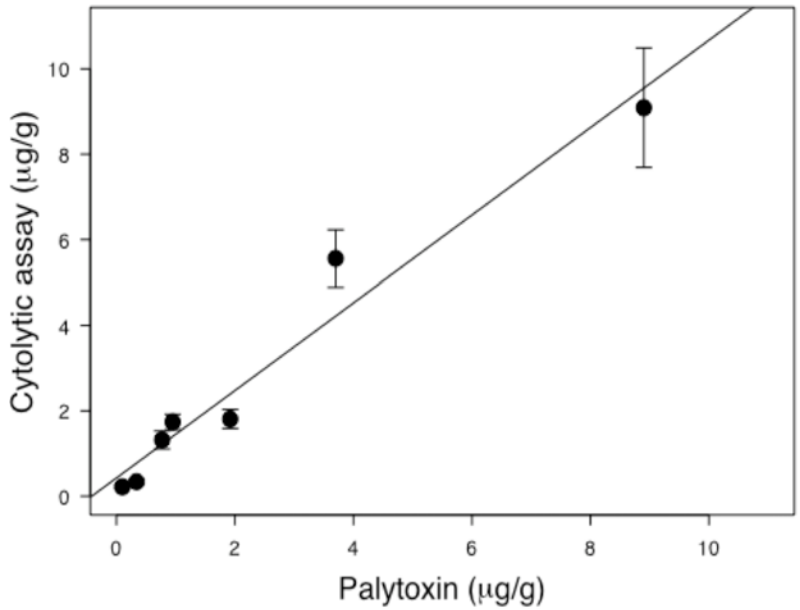
specific



selective

Treatment	LDH activity (mAU/min)
Control	7.9 ± 2.0
Palytoxin (2 nM)	88.2 ± 14.0
Ostreocin D (15 nM)	79.1 ± 14.6
Maitotoxin (10 nM)	2.7 ± 1.5
Tetrodotoxin (100 nM)	5.7 ± 2.6
Yessotoxin (1 μM)	6.2 ± 3.9

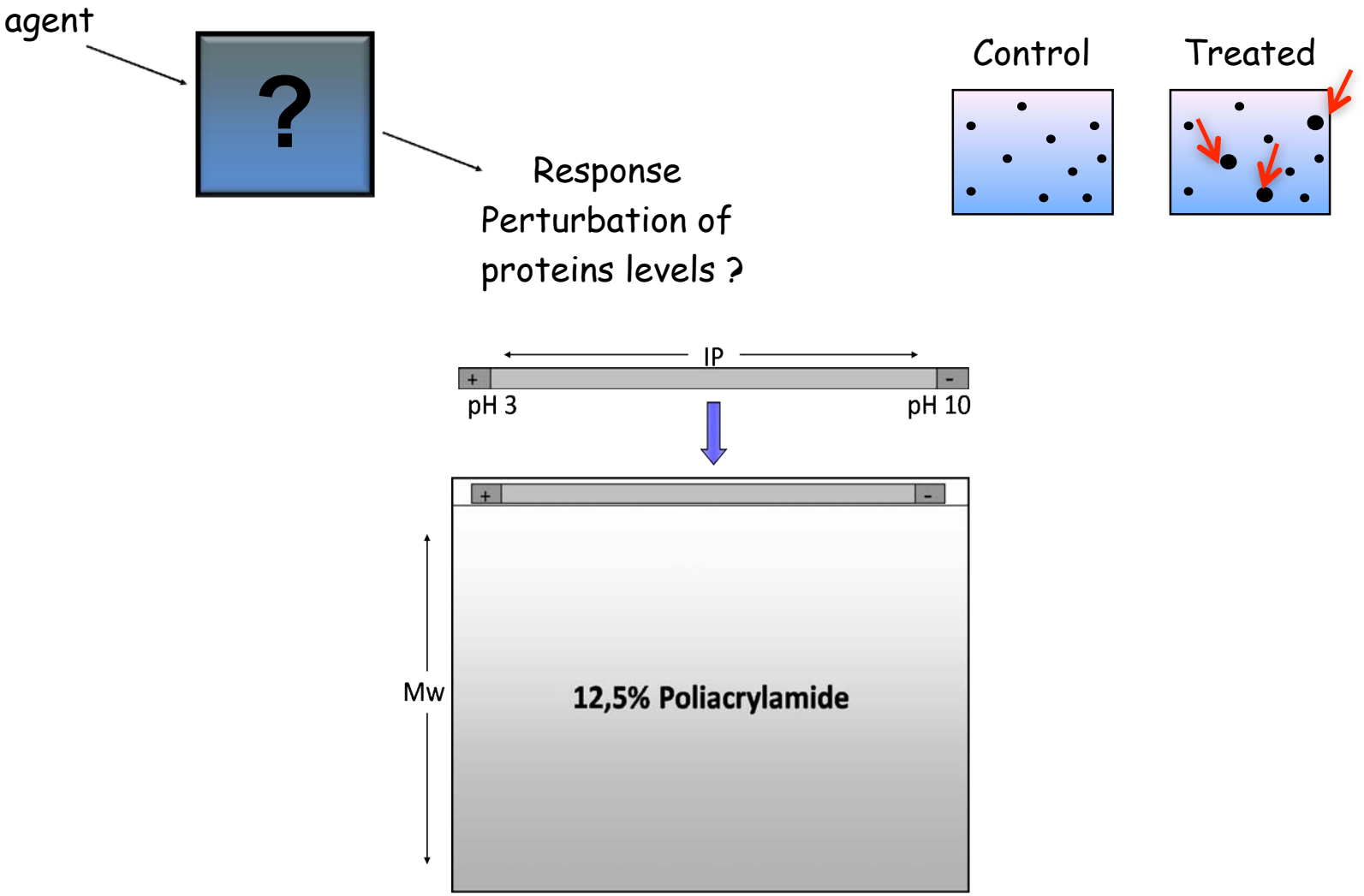
Spiked mussels extract

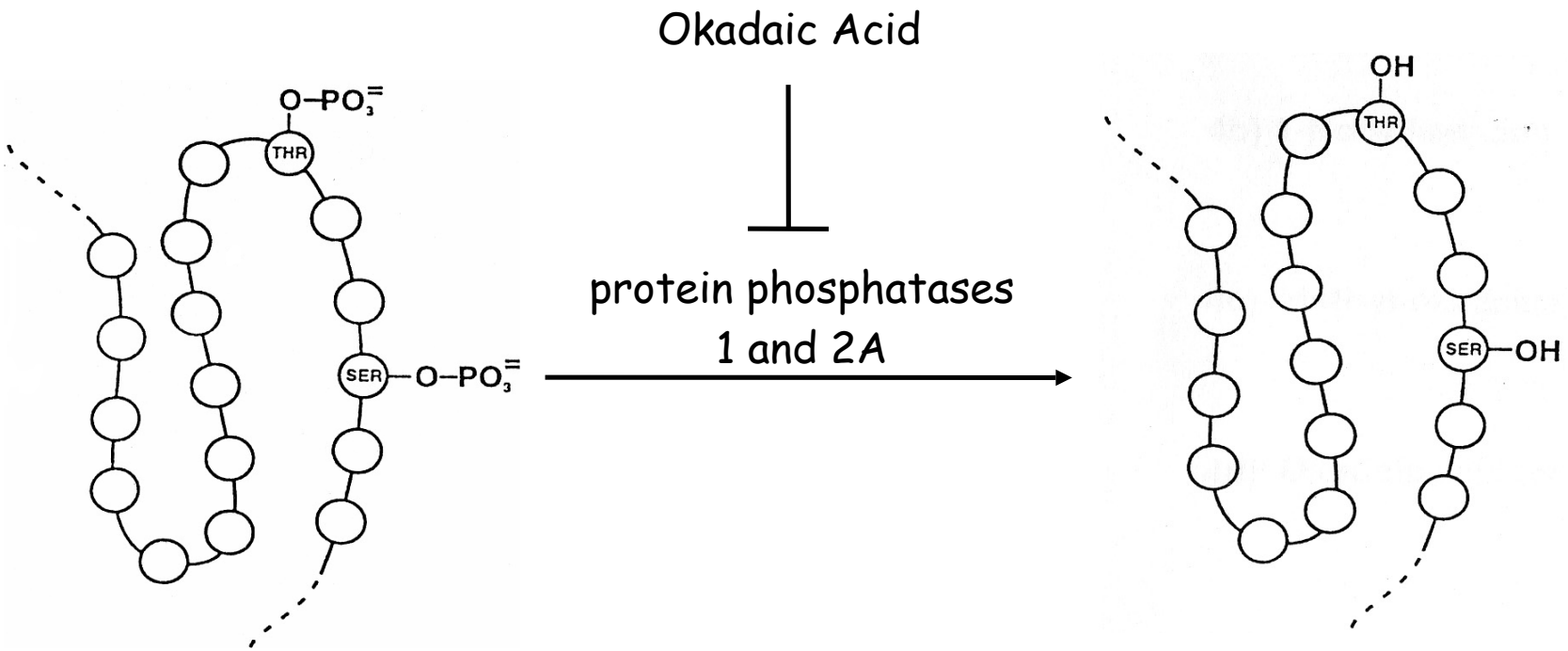


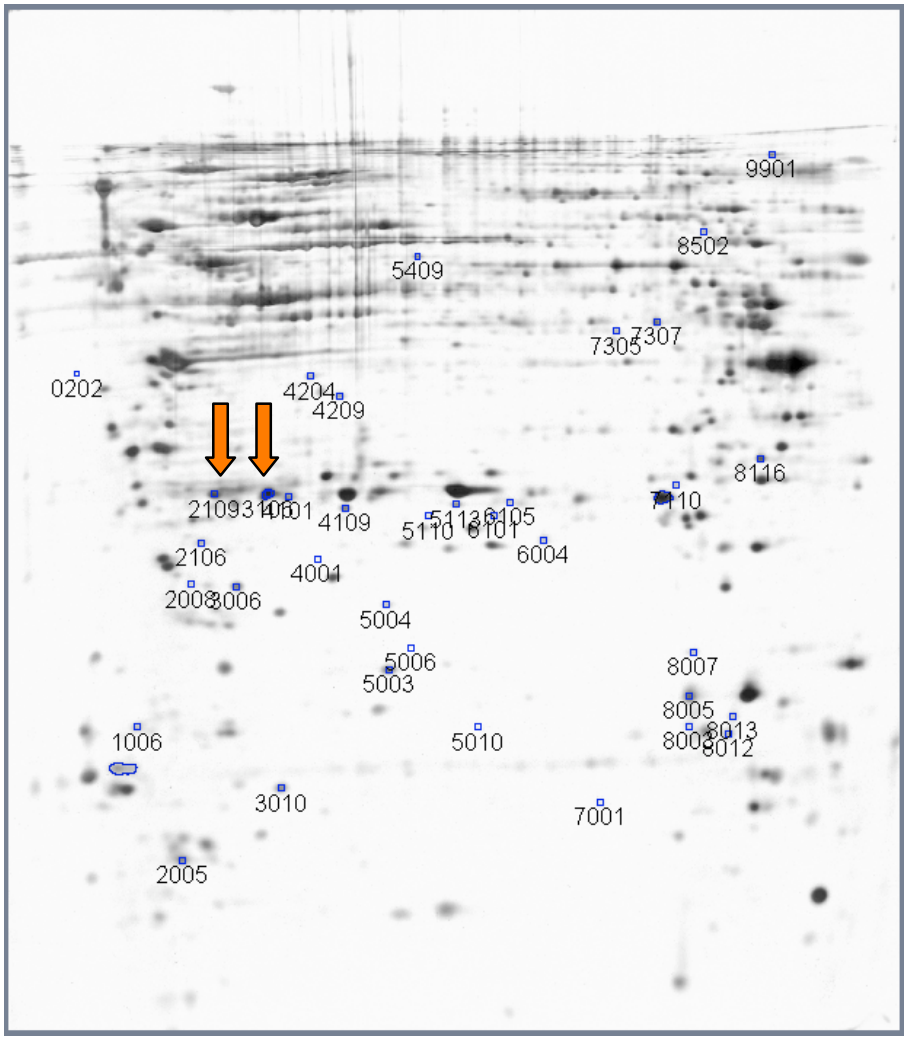
Naturally contaminated materials

Material	Palytoxin content		
Algal samples	pg/cell		
	Cytolytic assay	LC-MS	≈
6440/2	0.32	0.44	
6448/2	0.08	n.d.	↓
6450/2	0.06	0.37	
6452/2	0.31	0.57	
6942/2	0.11	0.54	
Mussels	µg/g		
	Cytolytic assay	LC-MS	≈
2162/07 (digestive gland)	0.16	0.12	
2162/07 (whole shellfish)	0.01	n.d.	
Sea Urchin	µg/g		↑
	Cytolytic assay	LC-MS	

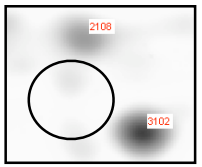
Proteomics in the study of molecular bases of biotoxin effects in cultured cells

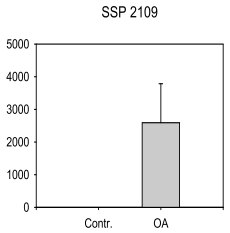



Okadaic Acid
protein phosphatases
1 and 2A
O–PO3=
THR
SER
O–PO3=
OH
THR
SER
OH



Control **OA 50 nM**





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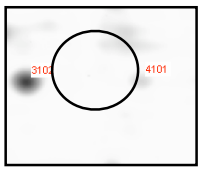

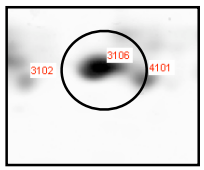

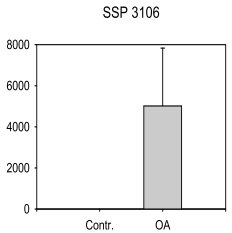

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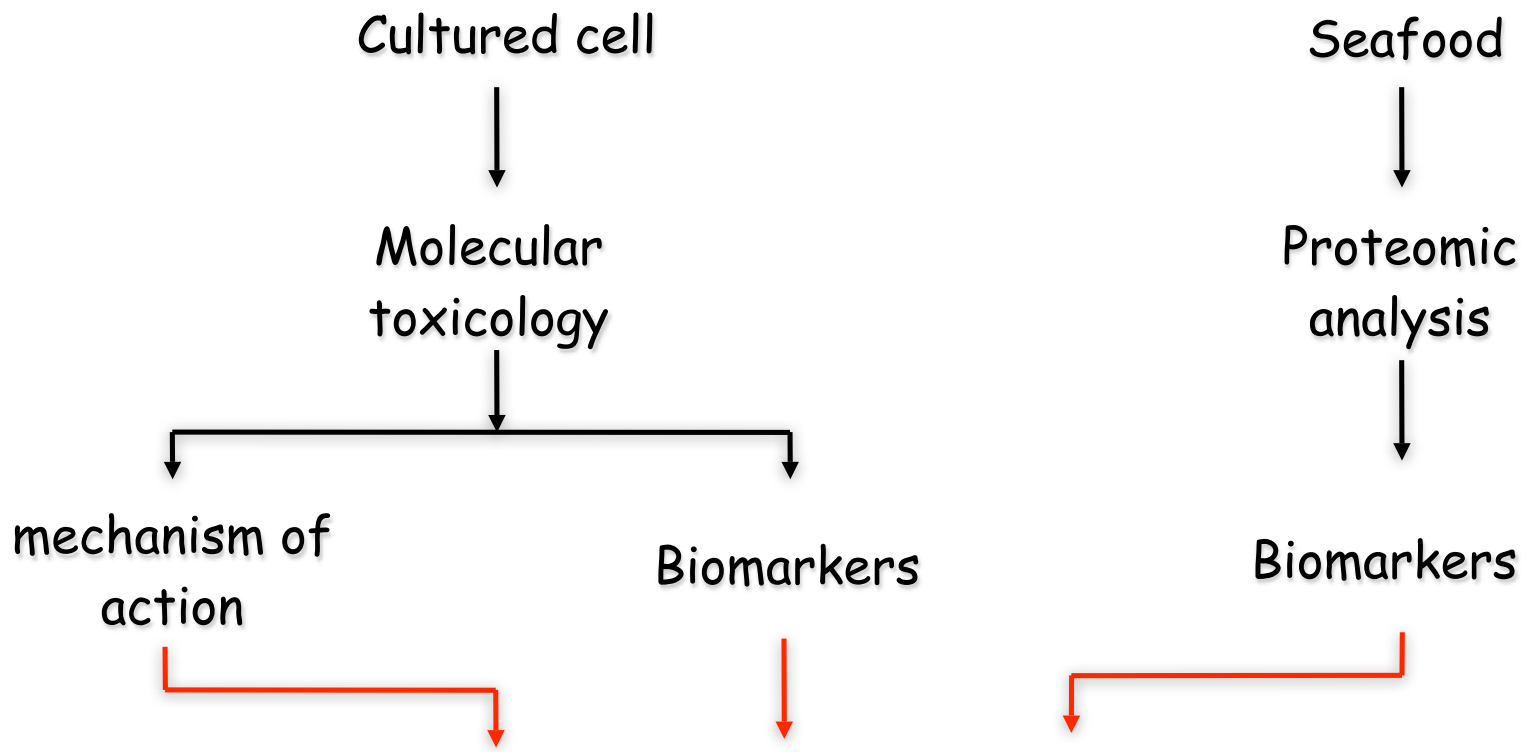

Substitution of animals in several areas of lab testing and lab research