Toxin Detection with a Microarray

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14th Harmful Algal Bloom Conference
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Queen's University Belfast

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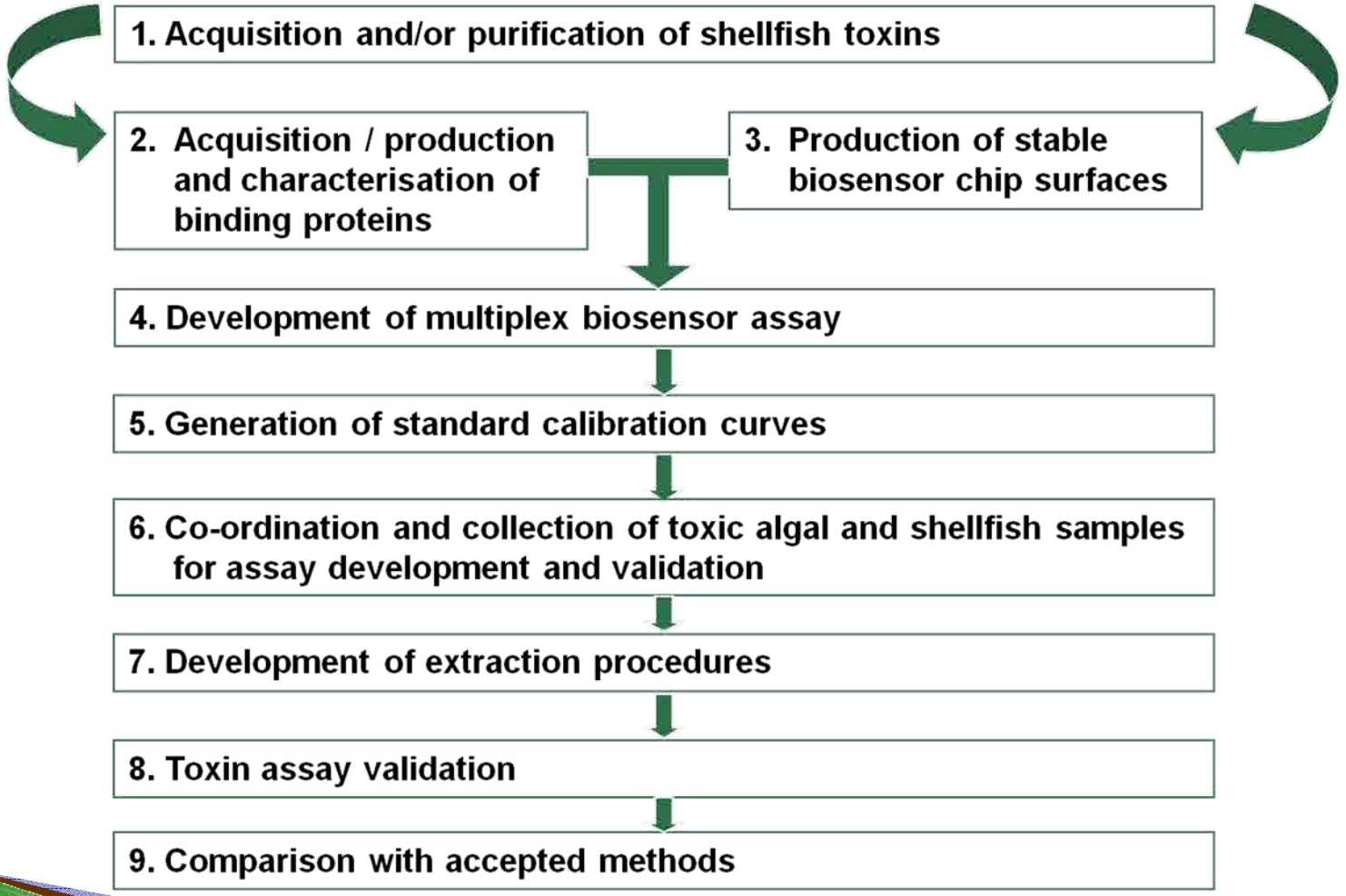
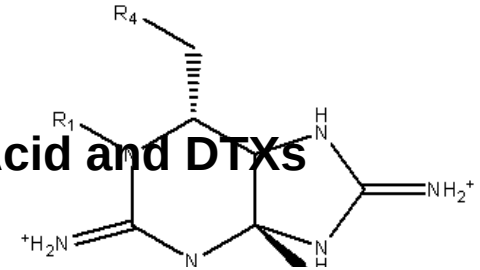
The Overall Plan

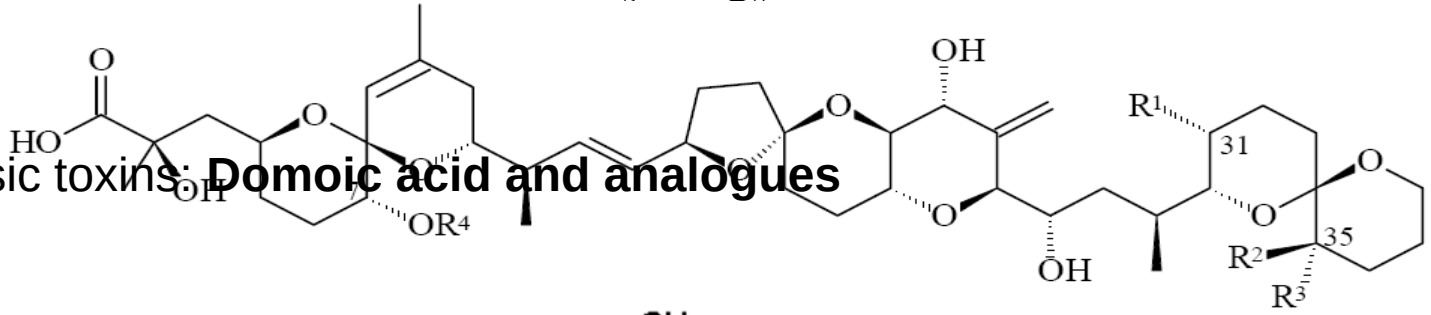


1. Acquisition and/or purification of shellfish toxins

- Paralytic toxins: **Saxitoxin and analogues**

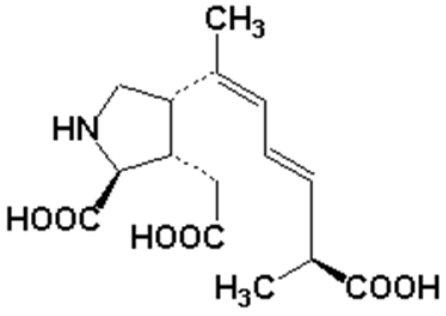


- Lipophilic toxins: **Okadaic Acid and DTXs**



- Amnesic toxins: **Domoic acid and analogues**

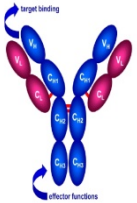
OA	
DTX1	
DTX2	
DTX3 (acy forms of O DTX1 and	



	R^4
	H
	H
	H
H_3	fatty acid

2. Production and characterisation of binding proteins

- Antibodies – polyclonal and monoclonal



  - PSP toxins

Campbell *et al*., 2007. An assessment of specific binding proteins suitable for the detection of paralytic shellfish poisons (PSP) using optical biosensor technology. Analytical Chemistry, 79 (15) 5906-5914

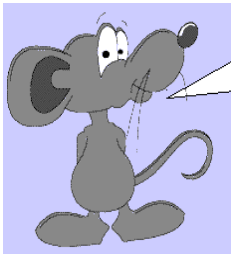
  - Okadaic acid and DTXs

Llamas *et al*., 2007. Development of a novel immunobiosensor method for the rapid detection of okadaic acid contamination in shellfish extracts. *Anal. Bioanal. Chem.*, 389: 581-587.

Stewart *et al*., 2009. Development of a monoclonal antibody binding okadaic acid and dinophysistoxins-1, -2 in proportion to their toxicity equivalence factors." Stewart et al., Toxicon, 54 (4) 491-498

  - Domoic Acid

Traynor *et al*., 2006. Immunobiosensor detection of domoic acid as a screening test in bivalve mollusks: Comparison with LC based analysis. JAOAC. 89, 868-872

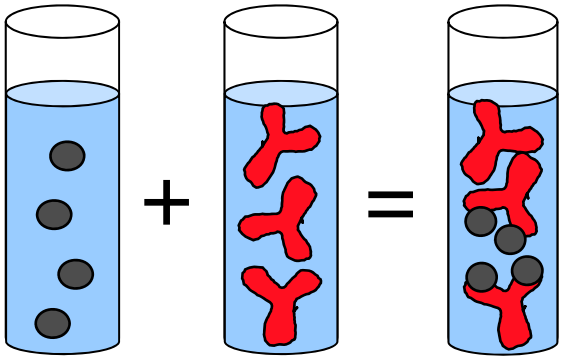


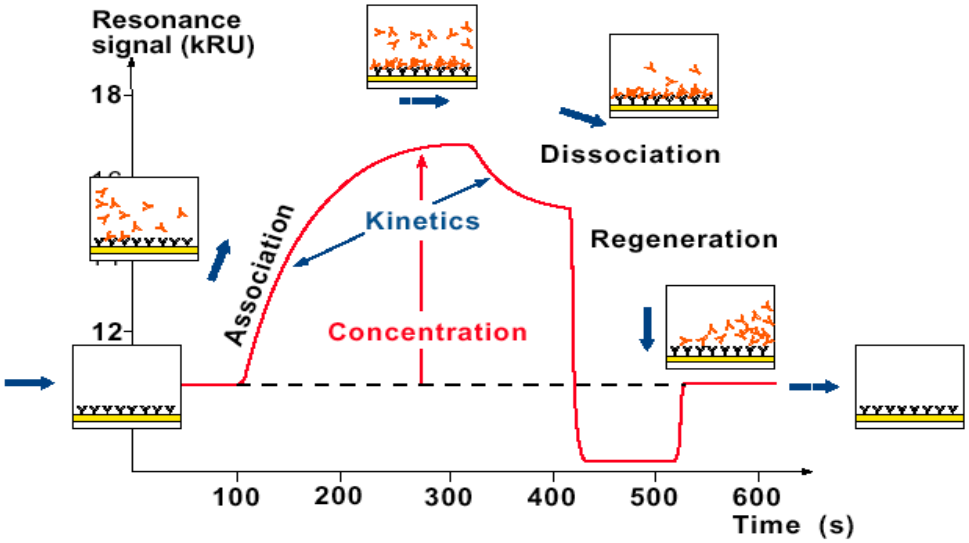
3Rs

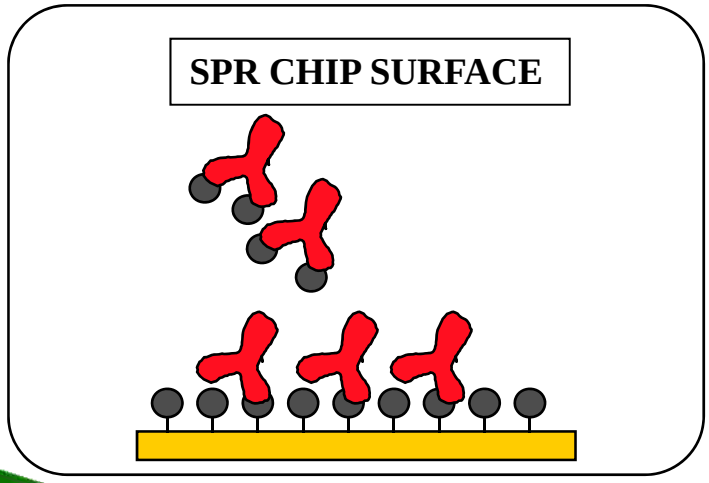
Reduce, replace, refine
EU Directive 86/609

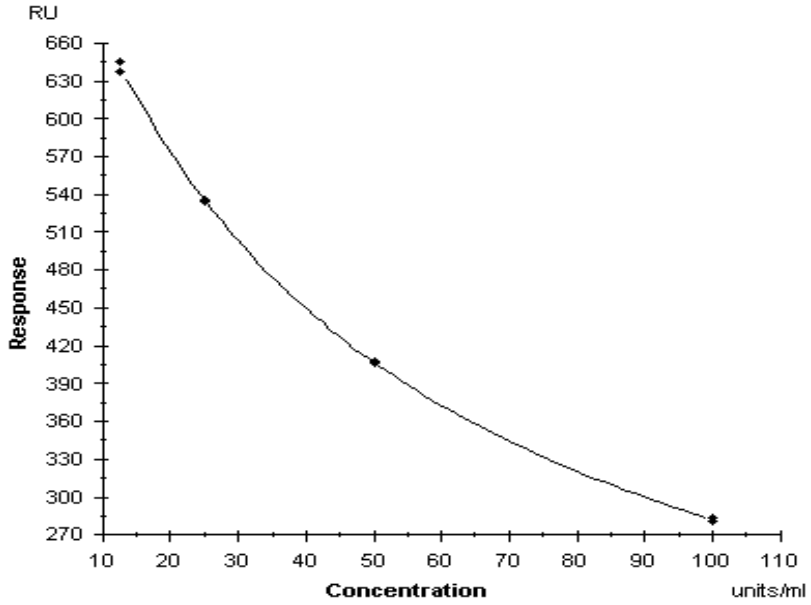
4. Development of biosensor assay – inhibition format

- Toxin Antibody



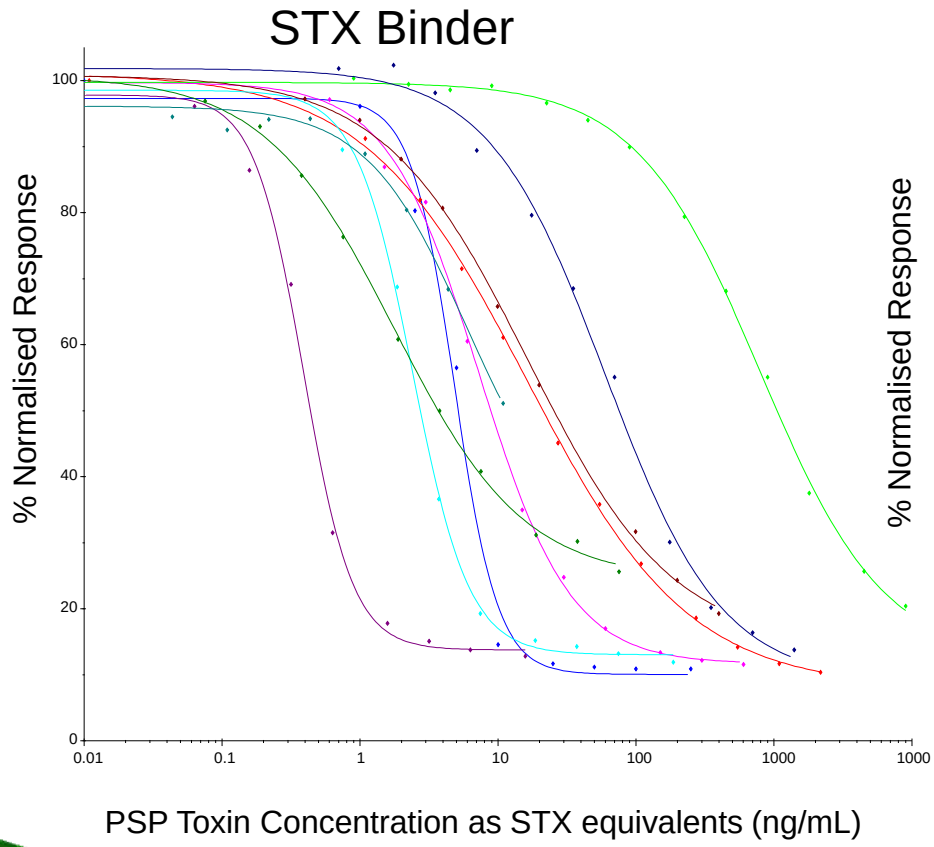


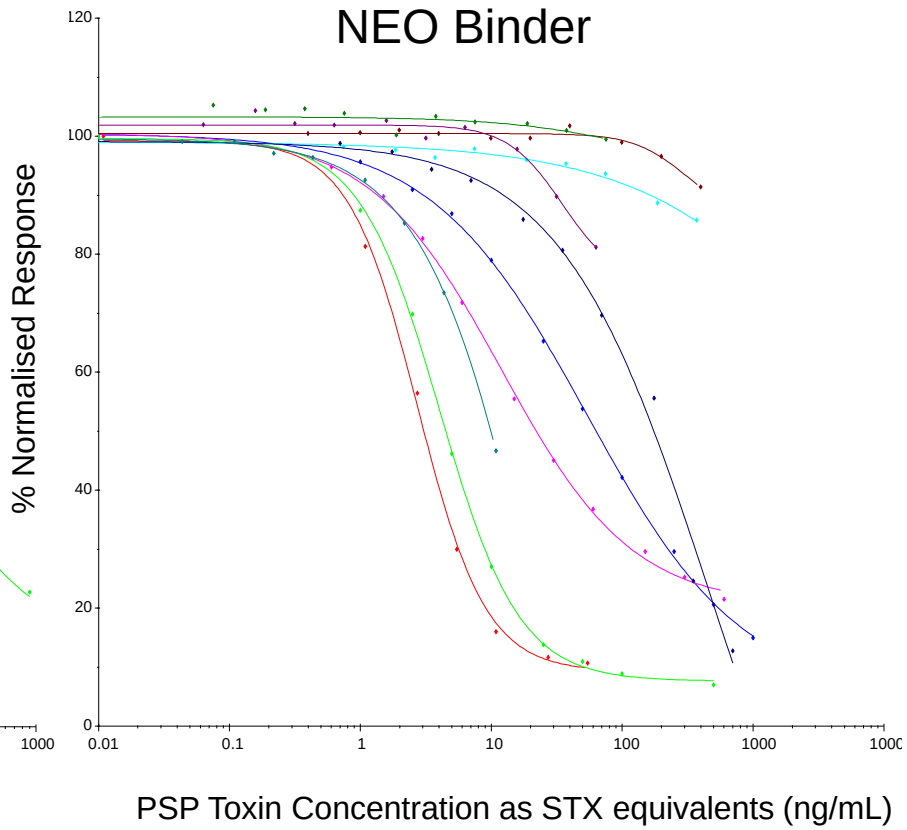




Antibodies for PSP toxins

- Saxitoxin Dihydrochloride
- Neosaxitoxin
- Gonyautoxin 2/3
- Gonyautoxin 1/4
- Decarbamoyl Saxitoxin
- Decarbamoyl Neosaxitoxin
- Decarbamoyl Gonyautoxin 2/3
- Gonyautoxin 5
- C1/C2
- C3/C4





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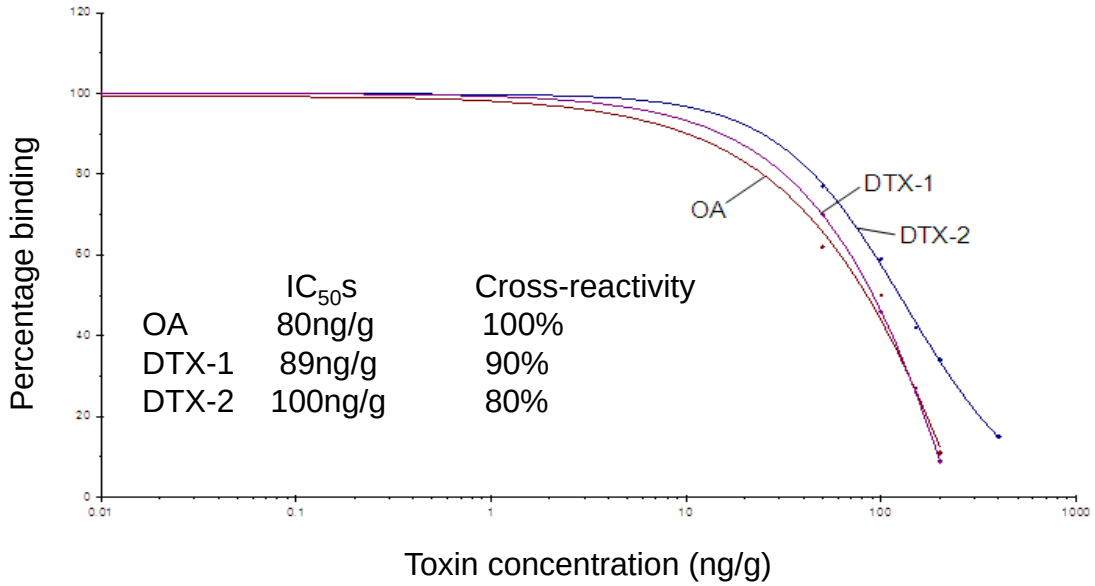
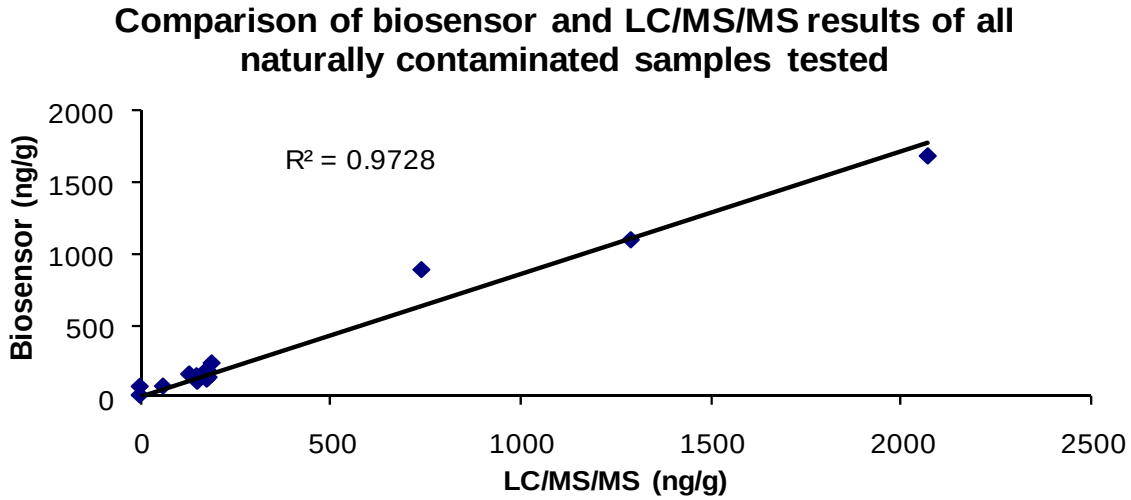
Antibody for Okadaic acid and DTXs





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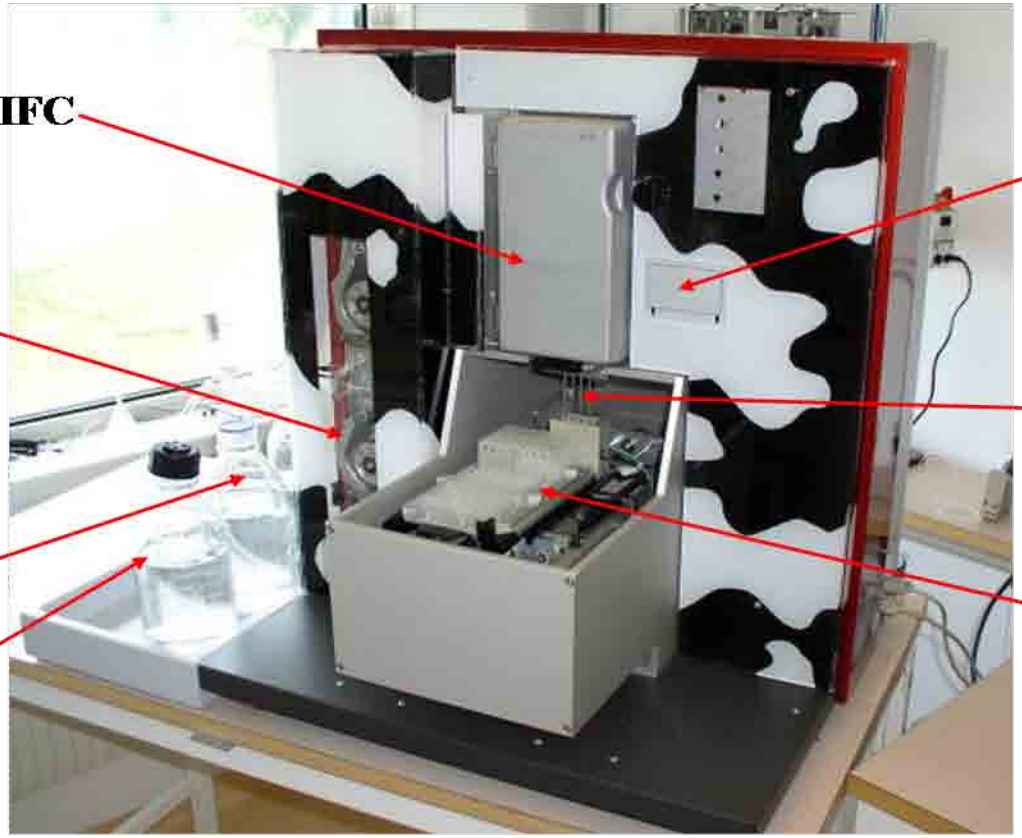
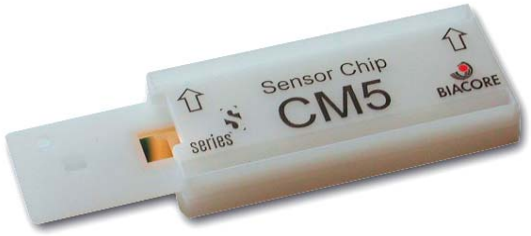
Technology Transfer to Prototype Multiplex biosensor

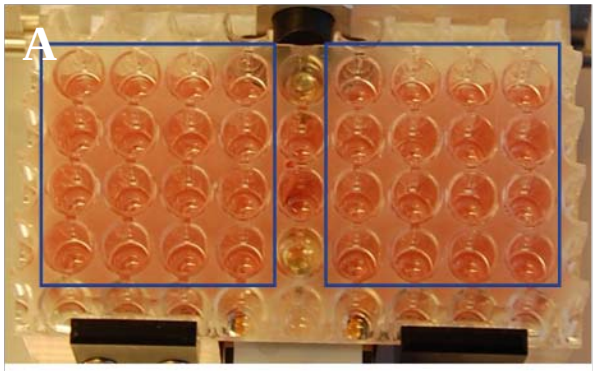
Framework 6 Project BioCop www.biocop.org

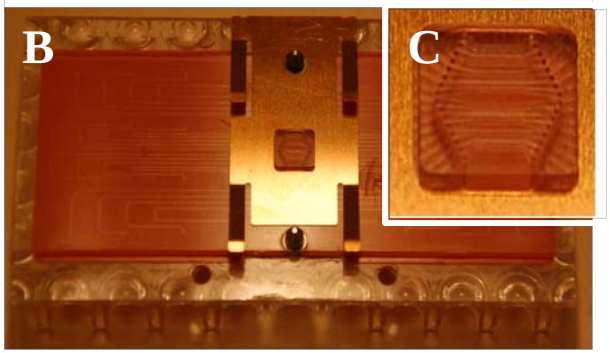


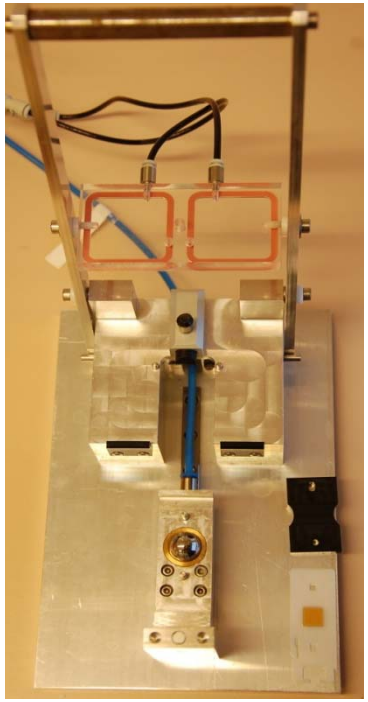
Analyse up to 16 analytes
4 x 4 design

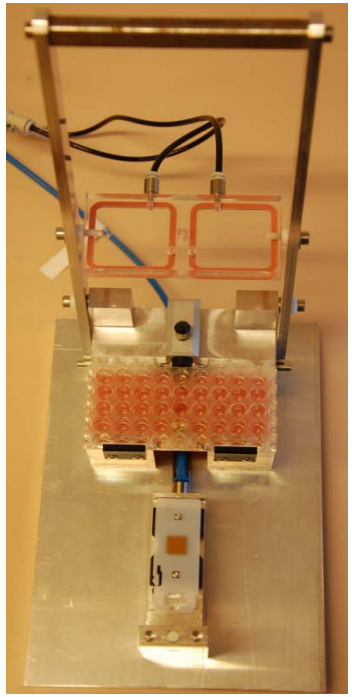
Production of biosensor multiplex chip surfaces and assay design

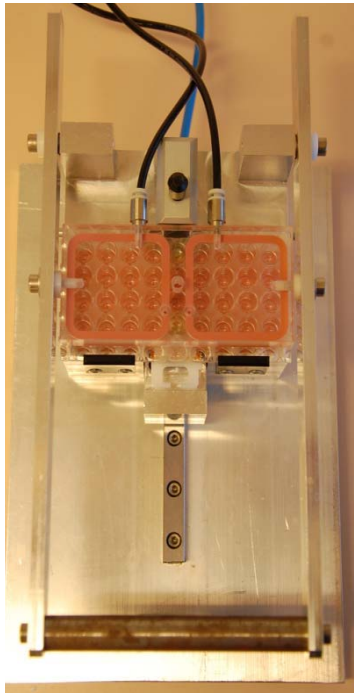












Chip Surface Preparation

- Wash
- Activation
- Amine linker
- Deactivation
- Immobilisation of toxin
- Wash and go

Production of biosensor multiplex chip surfaces and assay design

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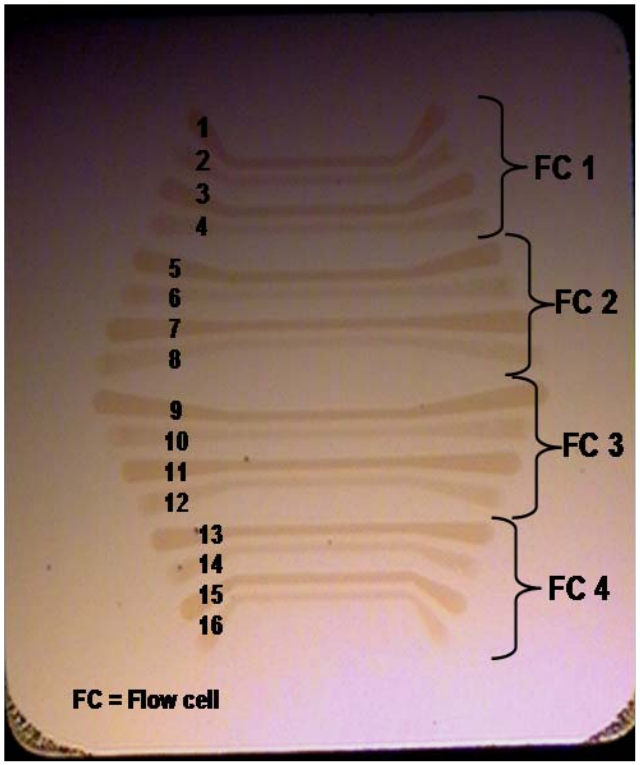

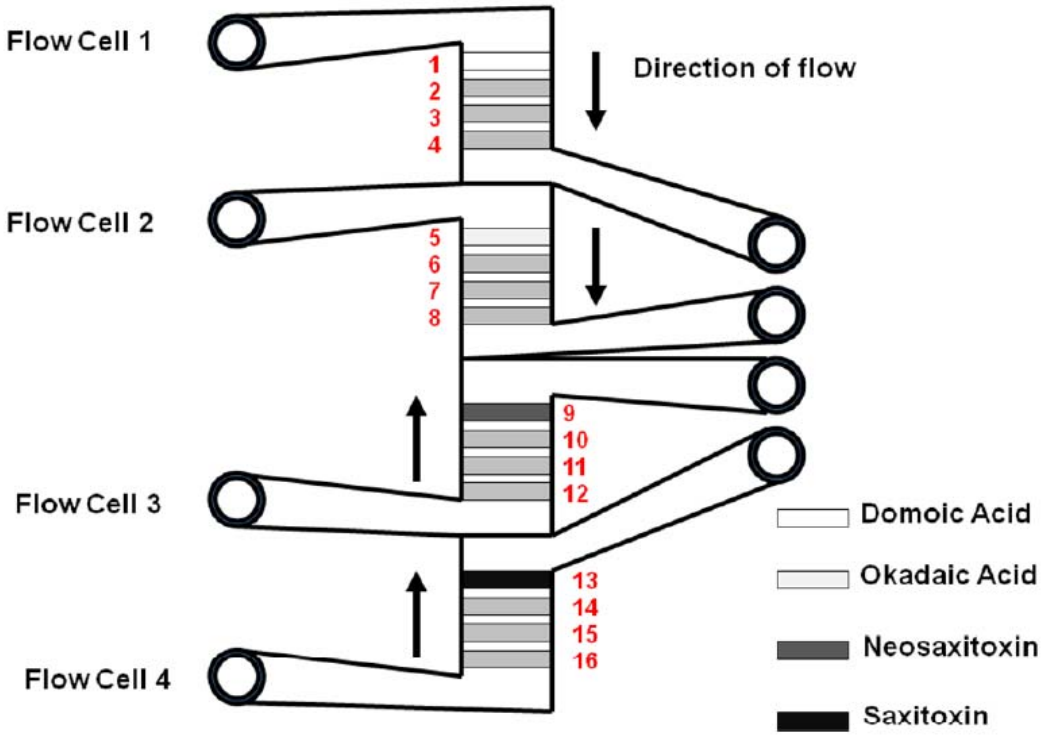

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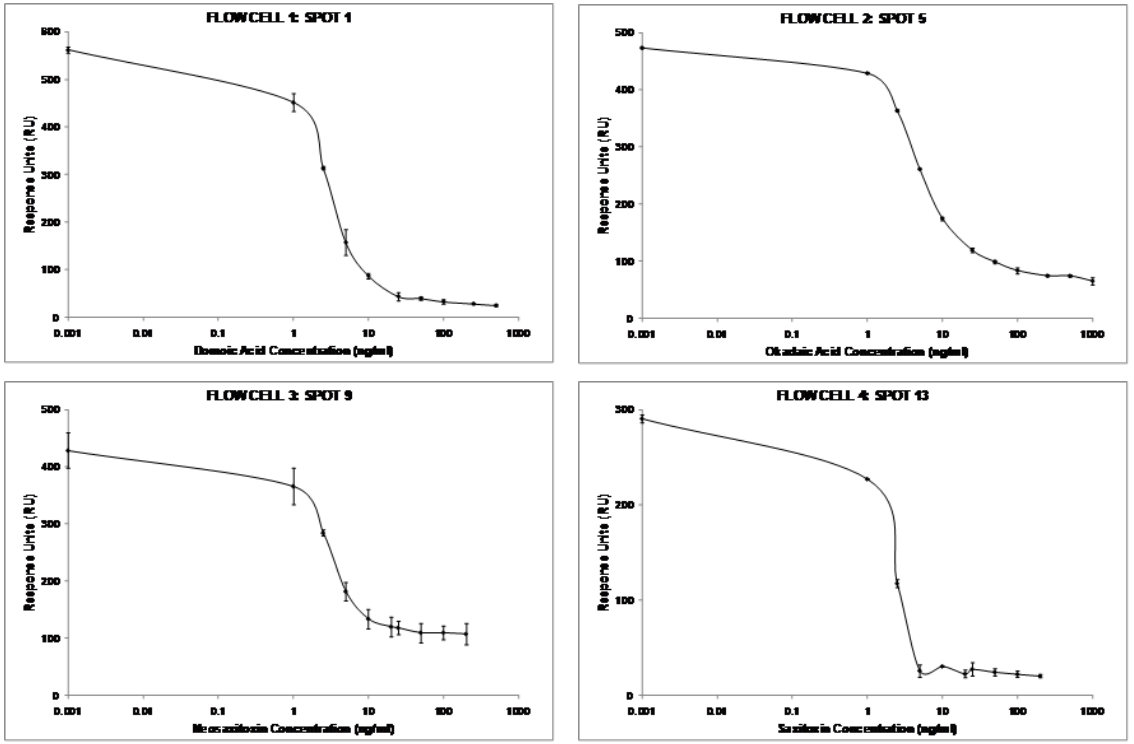
Buffer Calibration Curves

Biosensor parameters:
Antibody dilution
Mix ratio to sample 1:1 ratio
Flow rate: 20 µL/min
Contact times: 3min



Toxin	Antibody	Titre	IC_{50} (ng/ml)	$IC_{20} – IC_{80}$ (ng/ml)	Regeneration Solution
Domoic Acid	DA-Ab	1/200	2.6	1.0 – 6.4	75mM sodium hydroxide
Okadaic Acid	OA-Ab	1/4000	4.9	1.7 -14.4	180mM sodium hydroxide with 15% acetonitrile
Neosaxitoxin	NEO-Ab	1/25	2.6	1.1 – 6.0	100mM Hydrochloric acid
Saxitoxin	STX-Ab	1/1000	1.9	1.0 – 3.7	50mM Hydrochloric acid

Shellfish Toxicity Analysis

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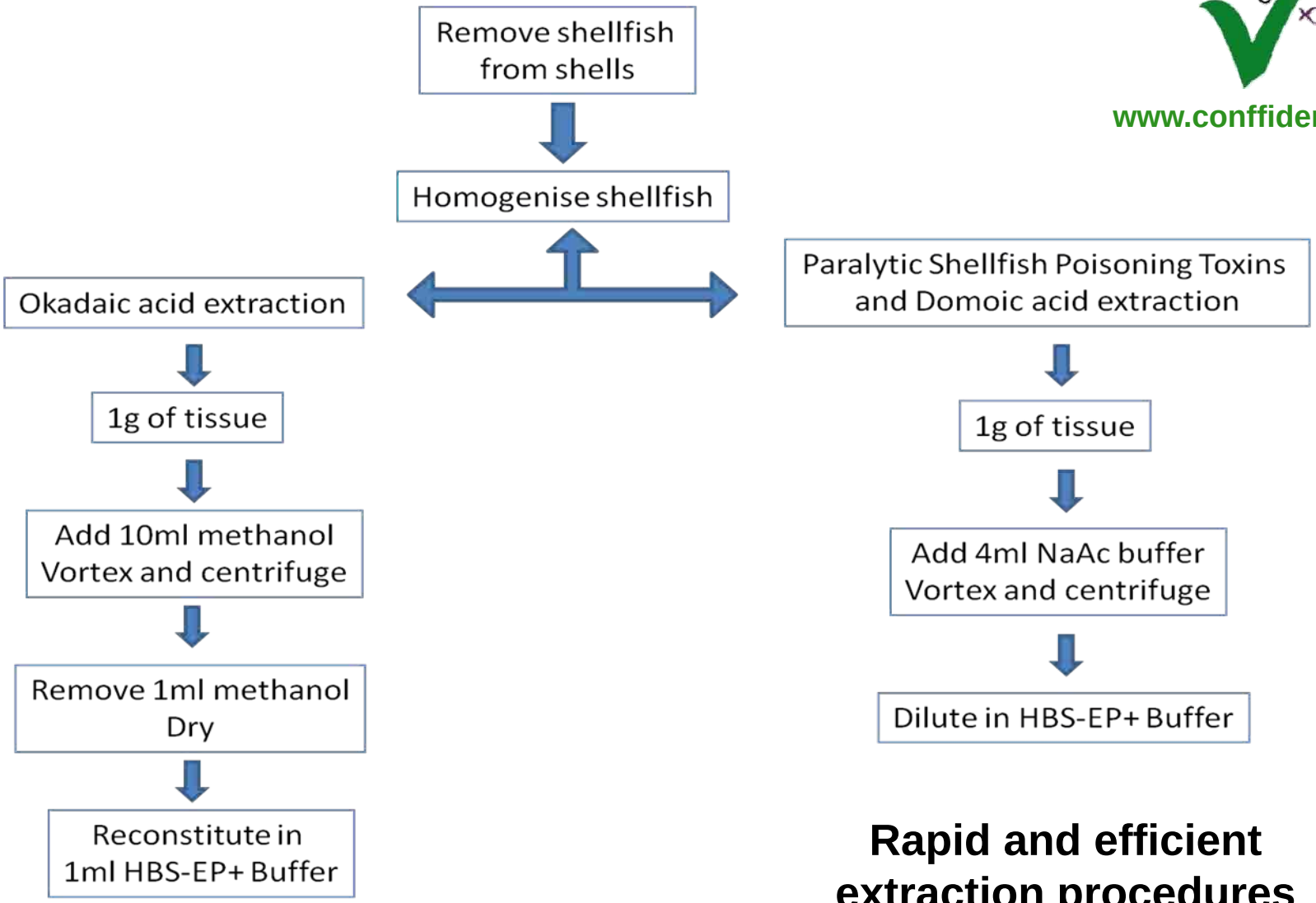

Rapid and efficient extraction procedures

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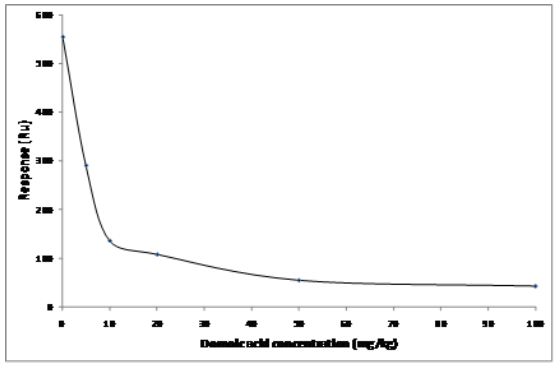
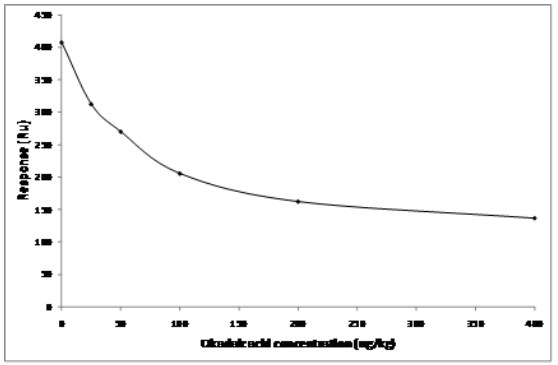
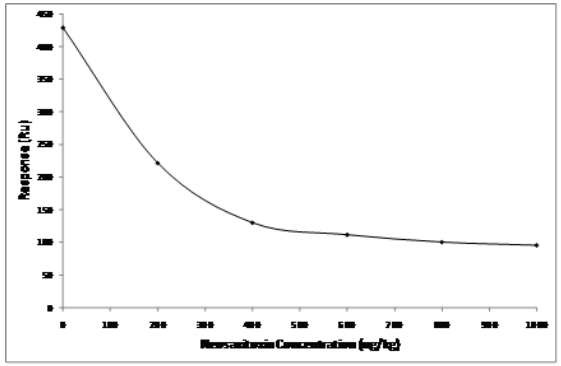
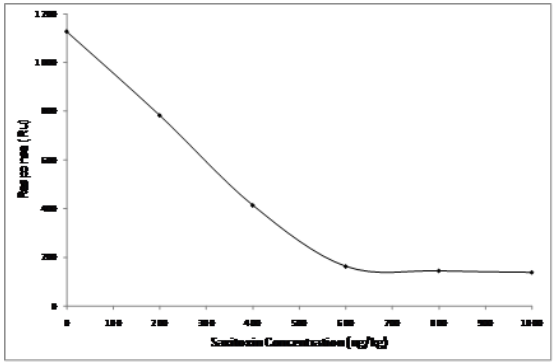
Shellfish (mussels) Calibration Curves









Toxin	Regulatory Limit (µg/kg)	Antibody	Titre	IC_{50} (µg/kg)	$IC_{20} - IC_{80}$ (µg/kg)	Regeneration Solution
Domoic Acid	20000	DA-Ab	1/200	4800	1900-9700	75mM sodium hydroxide
Okadaic Acid	160	OA-Ab	1/4000	48.7	14.3-134.0	180mM sodium hydroxide with 15% acetonitrile
Neosaxitoxin	800 STXeqs	NEO-Ab	1/25	160	64-330	100mM Hydrochloric acid
Saxitoxin	800	STX-Ab	1/1000	281	115-461	50mM Hydrochloric acid

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Detection levels for seawater algal samples

- Domoic Acid: 300 ng /L seawater
- Okadaic Acid: levels of pg/L seawater
- Paralytic Shellfish Poisoning toxins: levels of pg/L seawater

Unlike the shellfish samples whereby matrix effects may be a problem the difficulties with algal seawater samples is the sensitivity required.

Biosensor parameters to be adapted to improve the sensitivity

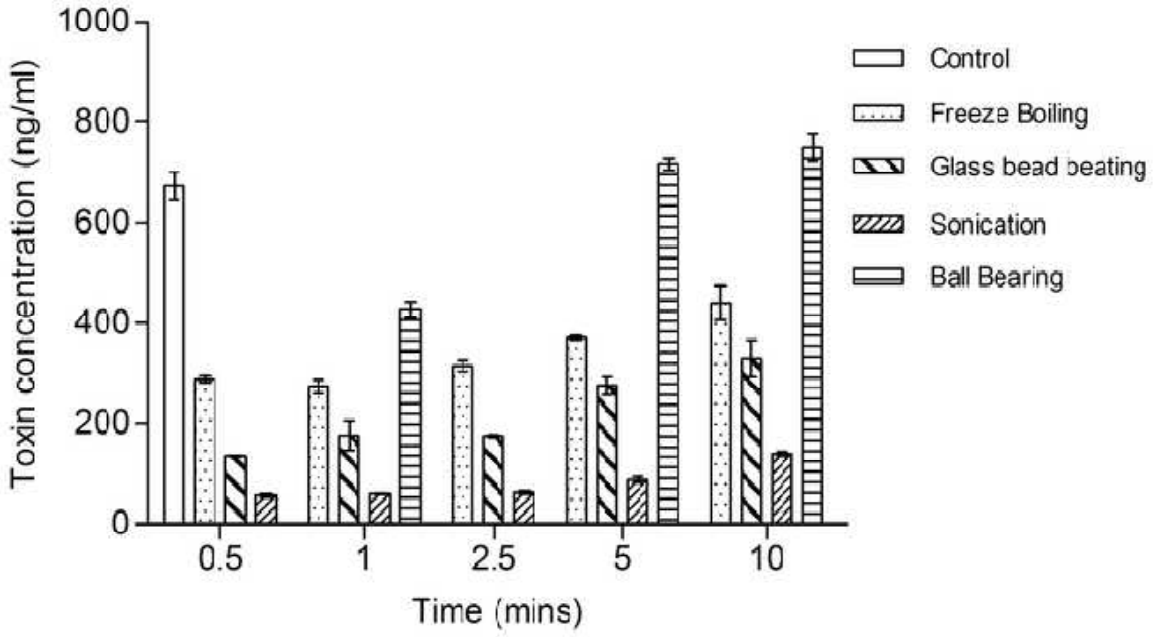
- Antibody dilution
- Mix ratio to sample
- Flow rate
- Contact time
- Response signal amplification

Sample Preparation of Algal (Seawater) Samples

Five different physical extraction methods:

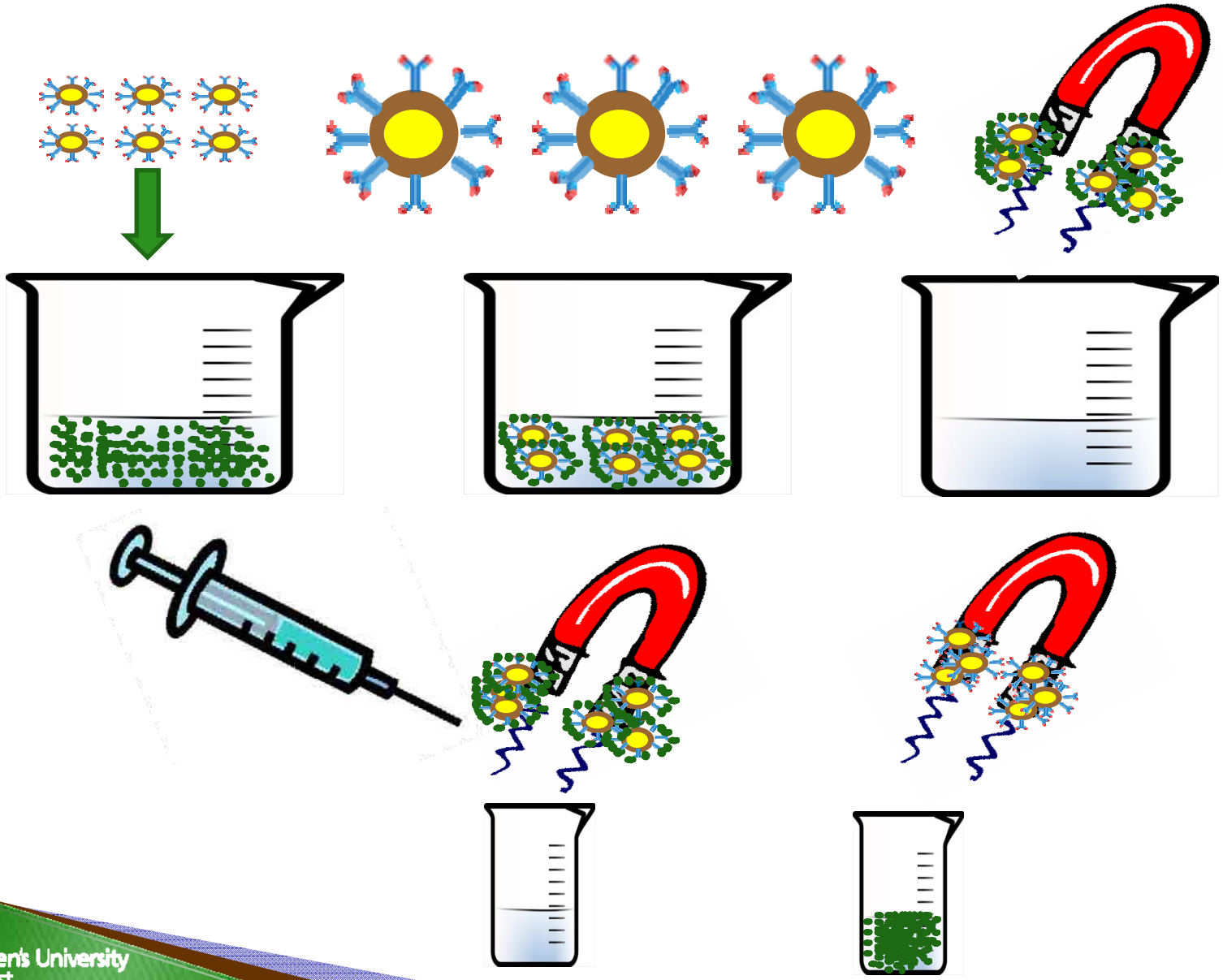
- Freeze-thawing method
- Freeze-boiling method
- Steel ball bearing beating method
- Glass bead beating method
- Ultrasonication water bath method

Toxic strain of *A. tamarense* at day 22 for PSP toxins



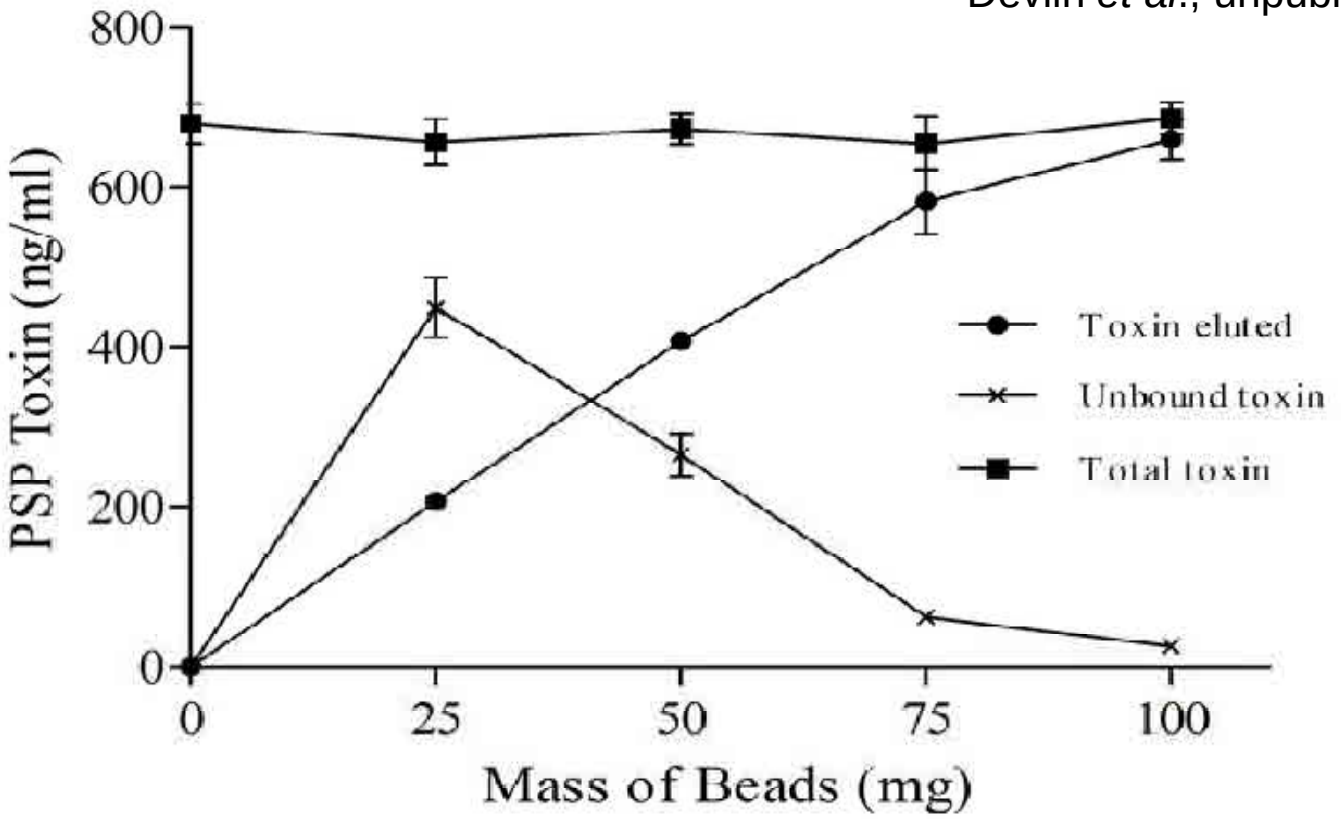
Devlin *et al*., unpublished data

Toxin capture using immunomagnetic particles



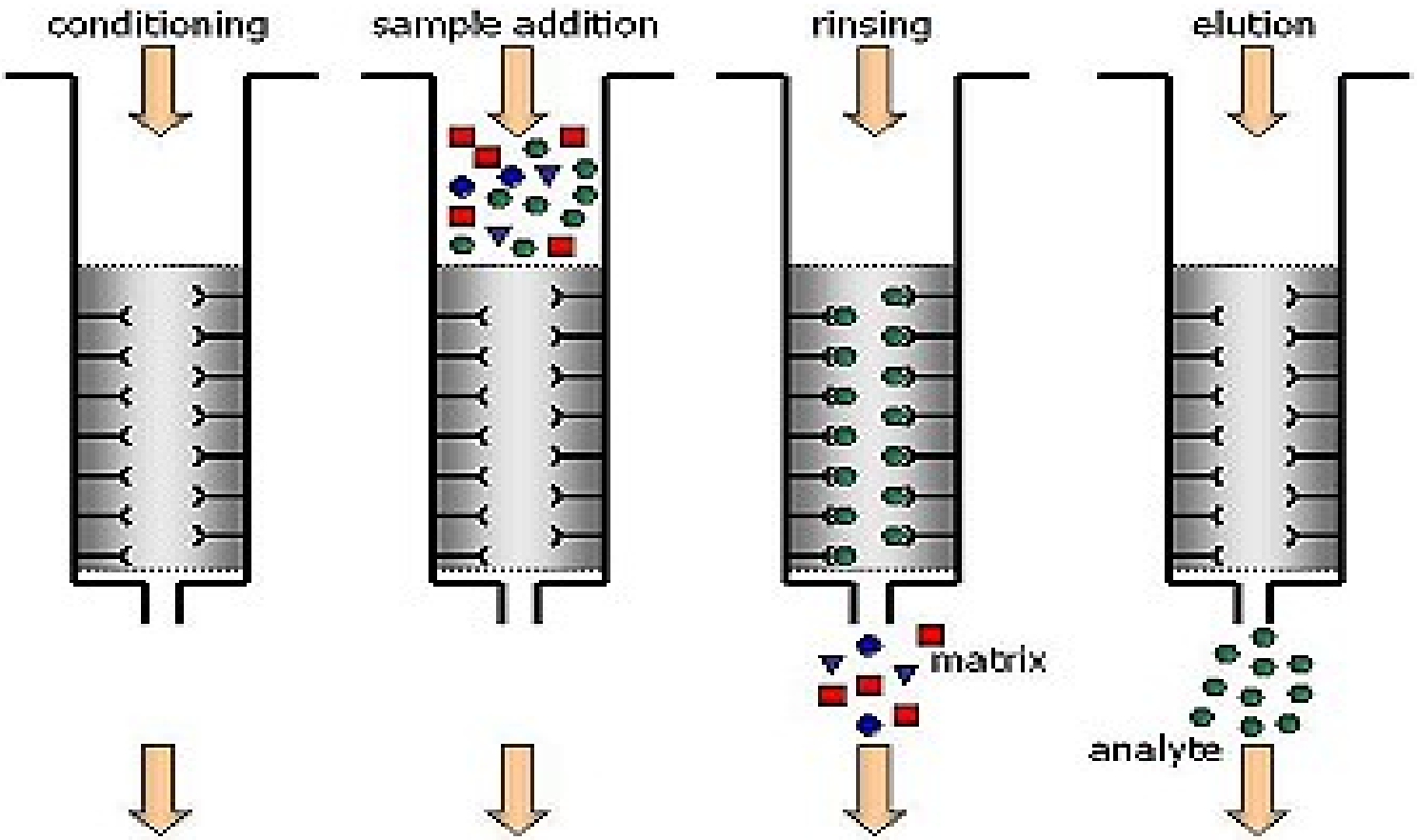
Magnetic particle evaluation for PSP toxins

Devlin *et al*., unpublished data



Increasing amount of particles increased toxin capture of PSP toxins

Toxin capture using immunoaffinity column purification



Summary

- Utilisation of prototype multiplex SPR instrument
  - Up to 16 toxins in 4 channels x 4 spots format
- Production of multiplex chips for four toxins
  - One toxin per channel
- Analysis of shellfish samples for regulatory monitoring
- Analysis of algal samples for toxin correlation

Any questions or comments??

http://www.qub.ac.uk/sites/ASSET2011/