About OMICS Group

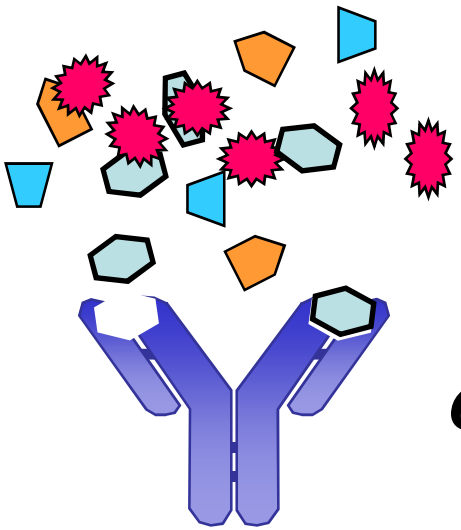
OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bangalore and Mumbai.

Development of immunoassays for small molecules using phage displayed peptides



Dr. Ting Xu

College of Resources and Environmental Sciences
China Agricultural University, Beijing, China

Outline

I. General information about phage display

II. Simultaneous development of both competitive and noncompetitive phage-based immunoassays for BDE-47

III. Selection of phage displayed peptides for the detection of insecticide imidacloprid

IV. Summary

Part I

General information about phage display

Research Background

Food safety

- Melamine
- Sudan reds
- Antibiotic residues
- Pesticide residues

Environmental pollution

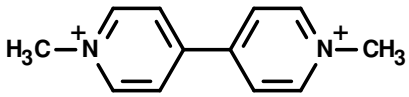
- Water eutrophication
- Soil contaminated with heavy metals
- New pollutants (BFRs)

Steps to Assay Development

Target selection

Immunizing hapten design

Competitive assay hapten design



Synthesis



Immunogens

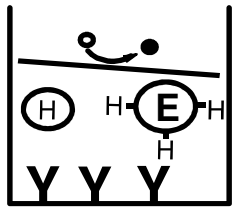
Coating antigens, Enzyme tracers

Covalent coupling to proteins



Animal immunizations





Serum collections

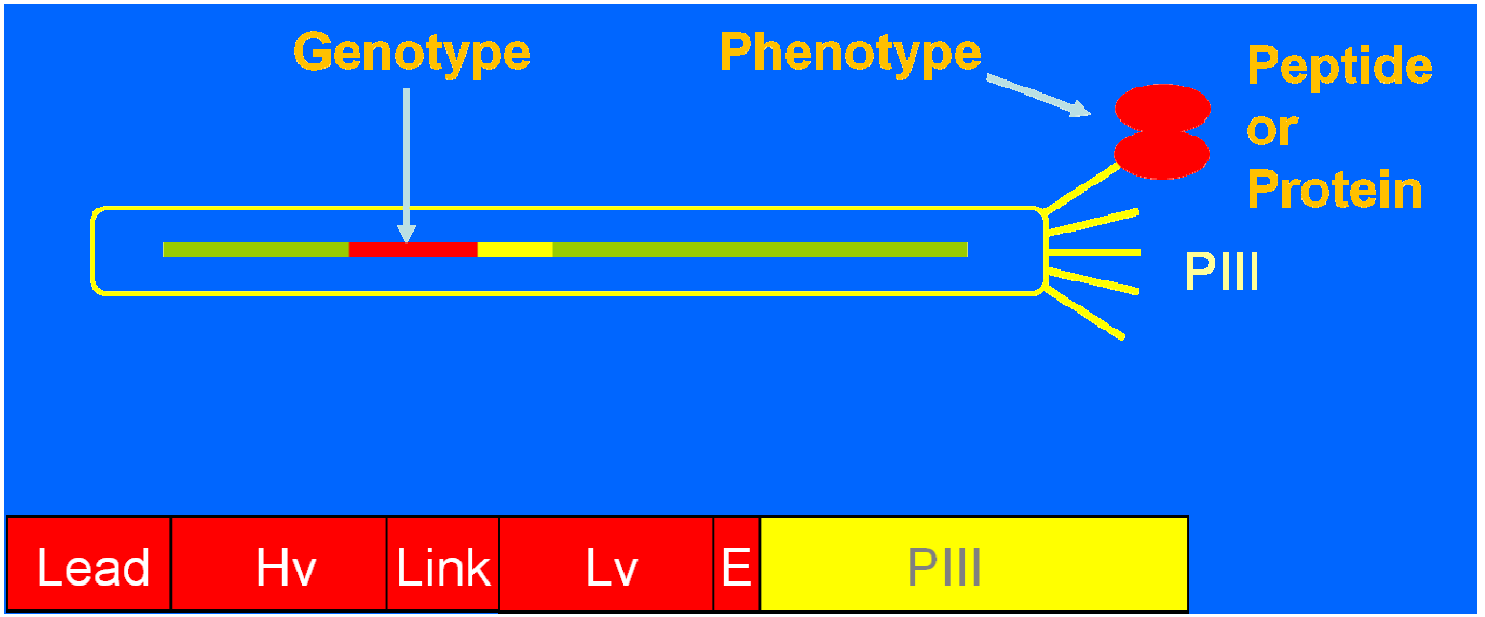
Screening of sera and Competitive assays

Optimization of the Immunoassay

Long assay development time

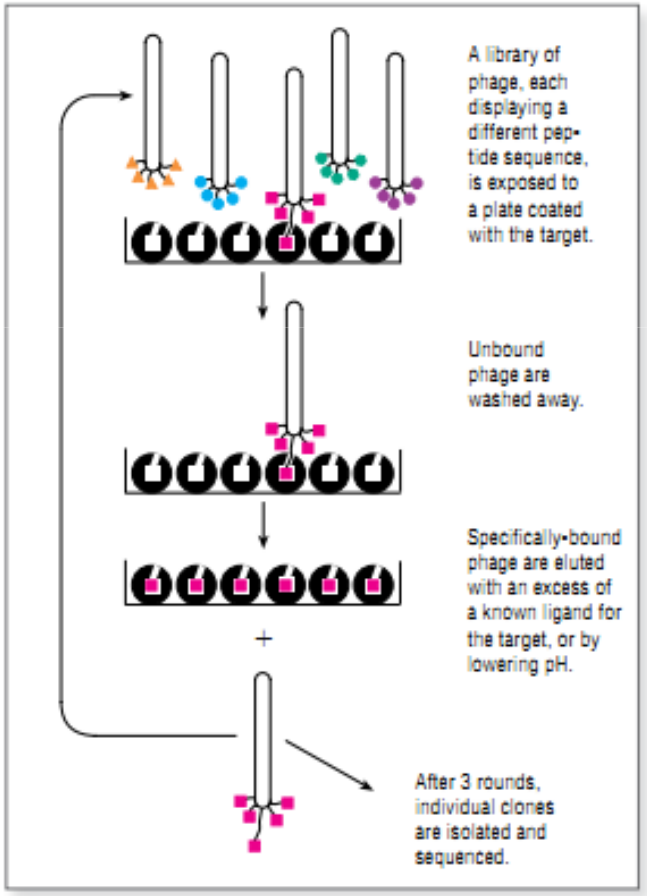
What is Phage Display?

An *in vitro* selection technique in which a peptide or protein is genetically fused to a coat protein of a bacteriophage, resulting in display of the fused peptide or protein on the exterior of the phage virion, while the DNA encoding the fusion resides within the virion.



Why Phage Display?

- Easy availability of library source
- Well established selection procedures (biopanning) can be easily incorporated in most laboratories
- Huge diversity of phage-displayed peptide library (approximately $1x10^9$ complexity/ml) increase possibility of selecting peptides with various binding affinity
- Phages are very stable and resistant to degrad ation by organic solvents, proteases, and heat



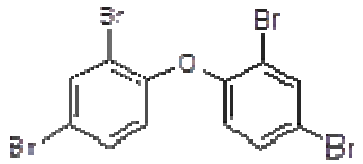
Panning with a pentavalent peptide library displayed on pIII.

Part II

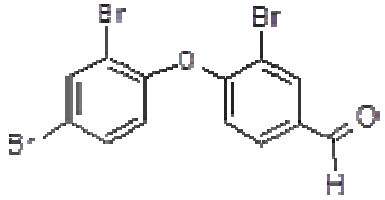
Simultaneous development of both competitive and noncompetitive immunoassays for BDE47 using phage-displayed peptides

Production of antibody for Brominated Flame Retardants

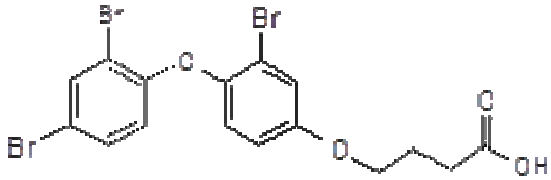
2,2',4,4'-tetrabromodiphenyl ether (BDE-47)



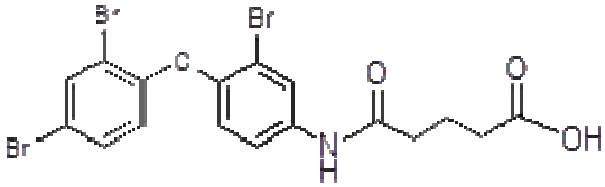
BDE-47



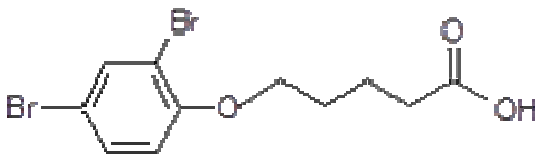
B1



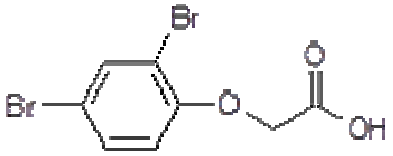
B2



B3

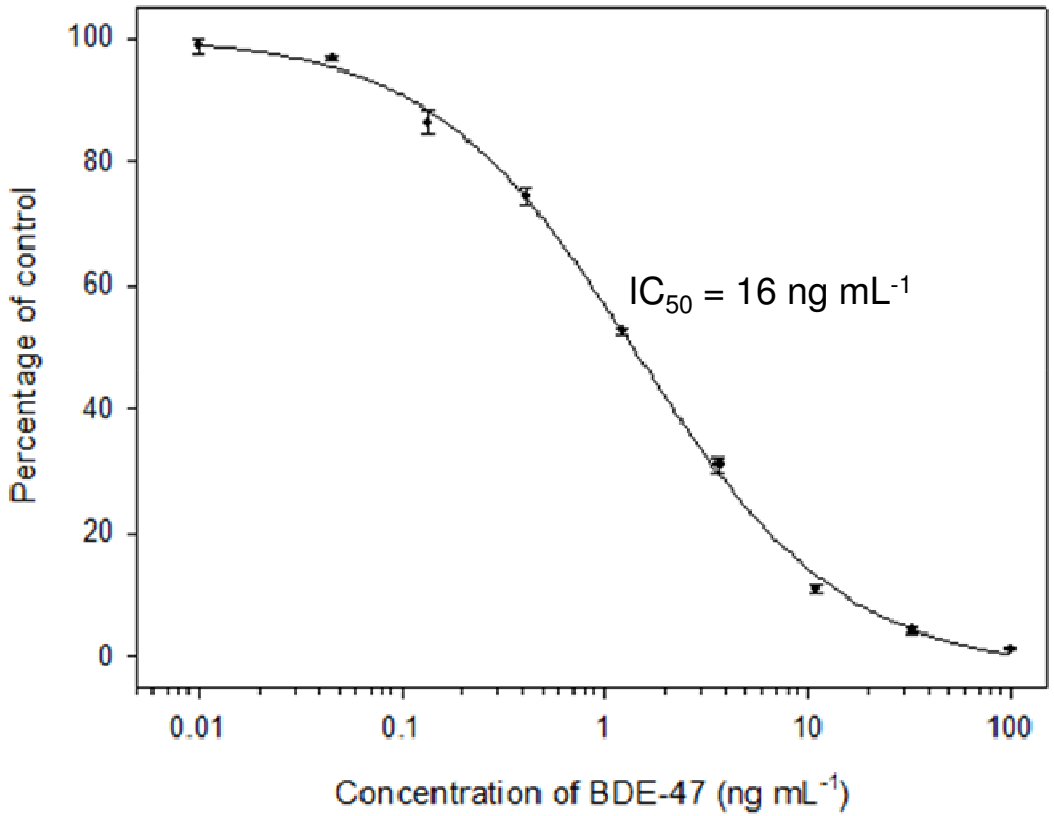


B4

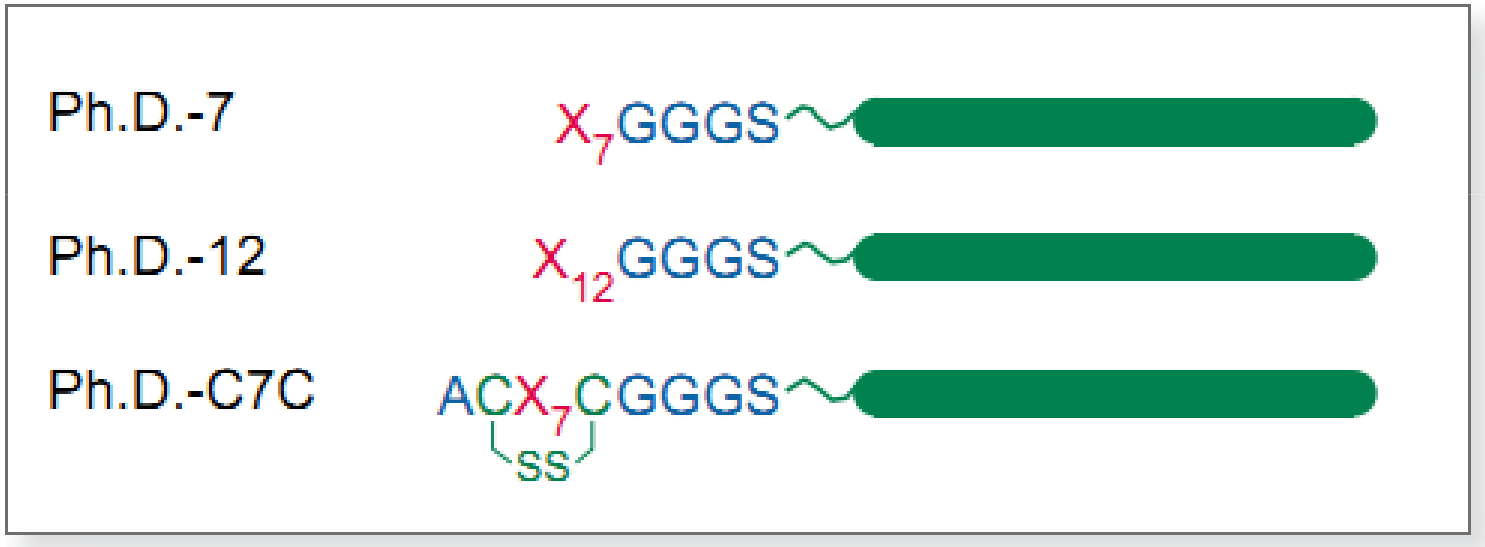


B5

IC_{50} value of the ELISA baed on Mab 1H2

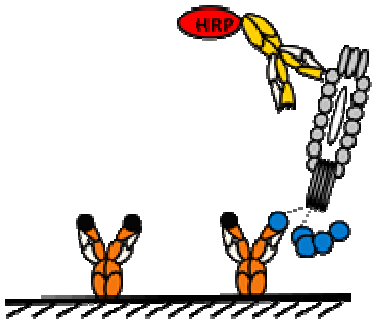


Phage display libraries

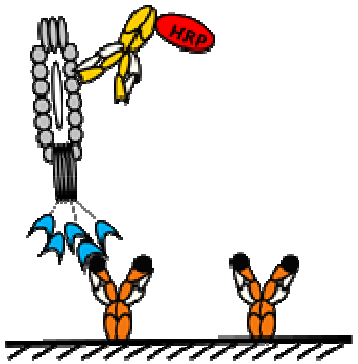


(New England Biolabs, Ltd)

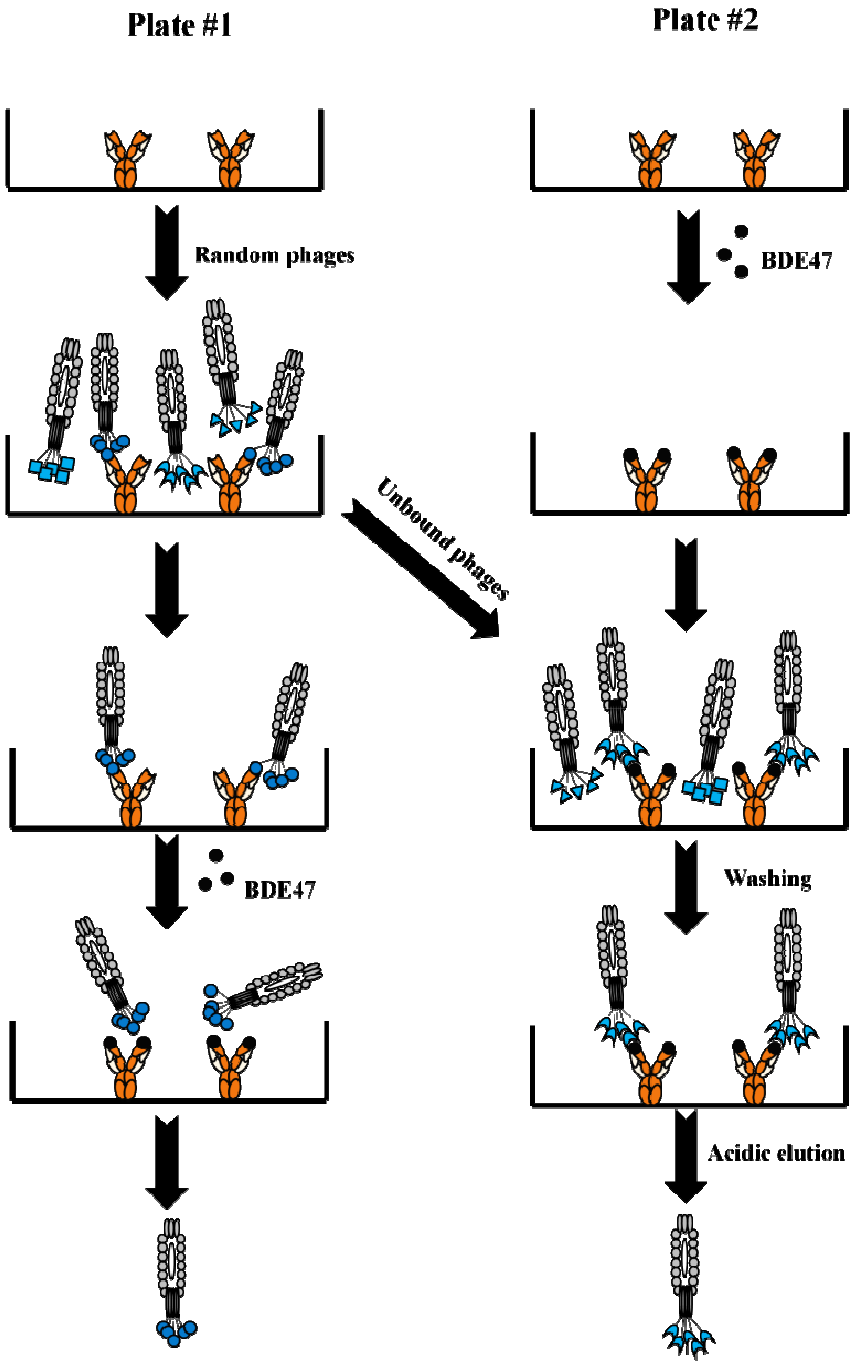
Biopanning



Competitive phage ELISA



Noncompetitive phage ELISA



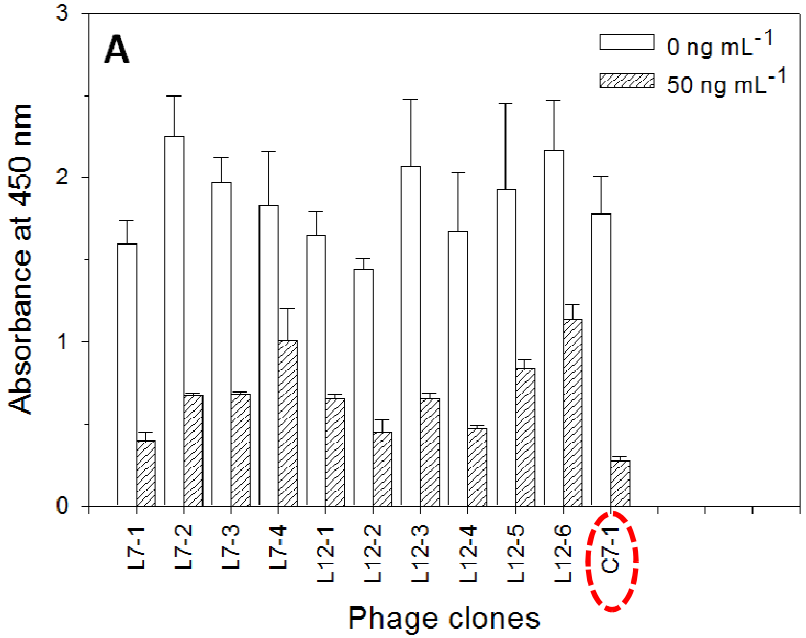
binding to free Mab

binding to immunocomplex

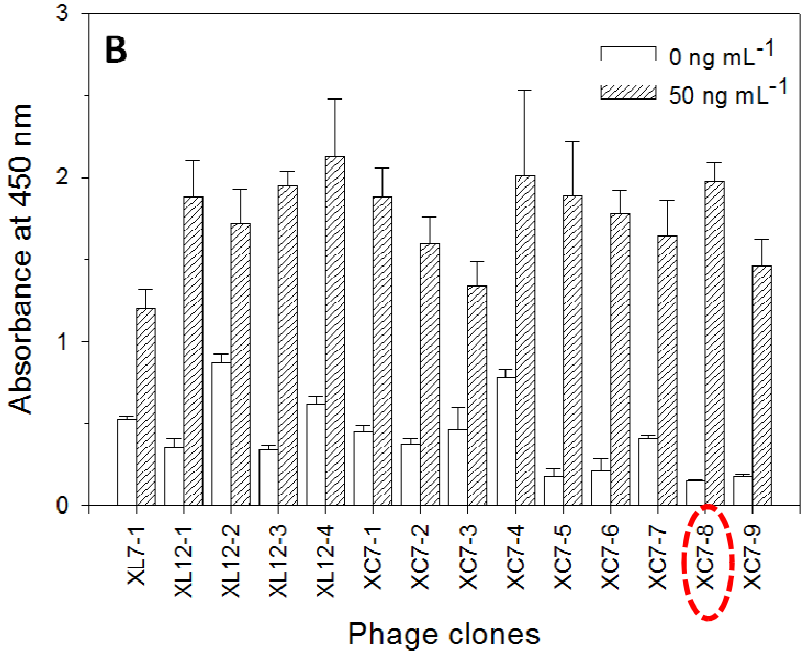
Titer of selected phage binding to BDE47-free Mab or Mab-BDE47 immunocomplex after each round of biopanning

Round	Output phage titer (pfu)					
	Binding to BDE47-free Mab			Binding to Mab-BDE47 immunocomplex		
	Ph.D.-C7C	Ph.D.-7	Ph.D.-12	Ph.D.-C7C	Ph.D.-7	Ph.D.-12
1st panning	2.8×10^5	3.6×10^5	5.0×10^5	2.4×10^5	4.2×10^5	1.2×10^5
2nd panning	1.6×10^6	2.4×10^6	4.0×10^6	6.8×10^6	8.0×10^6	6.0×10^6
3rd panning	7.0×10^8	7.5×10^7	5.0×10^7	8.0×10^8	2.4×10^7	1.7×10^7

Jia et al., *Anal Bioanal Chem* (2013) 405:9579–9583

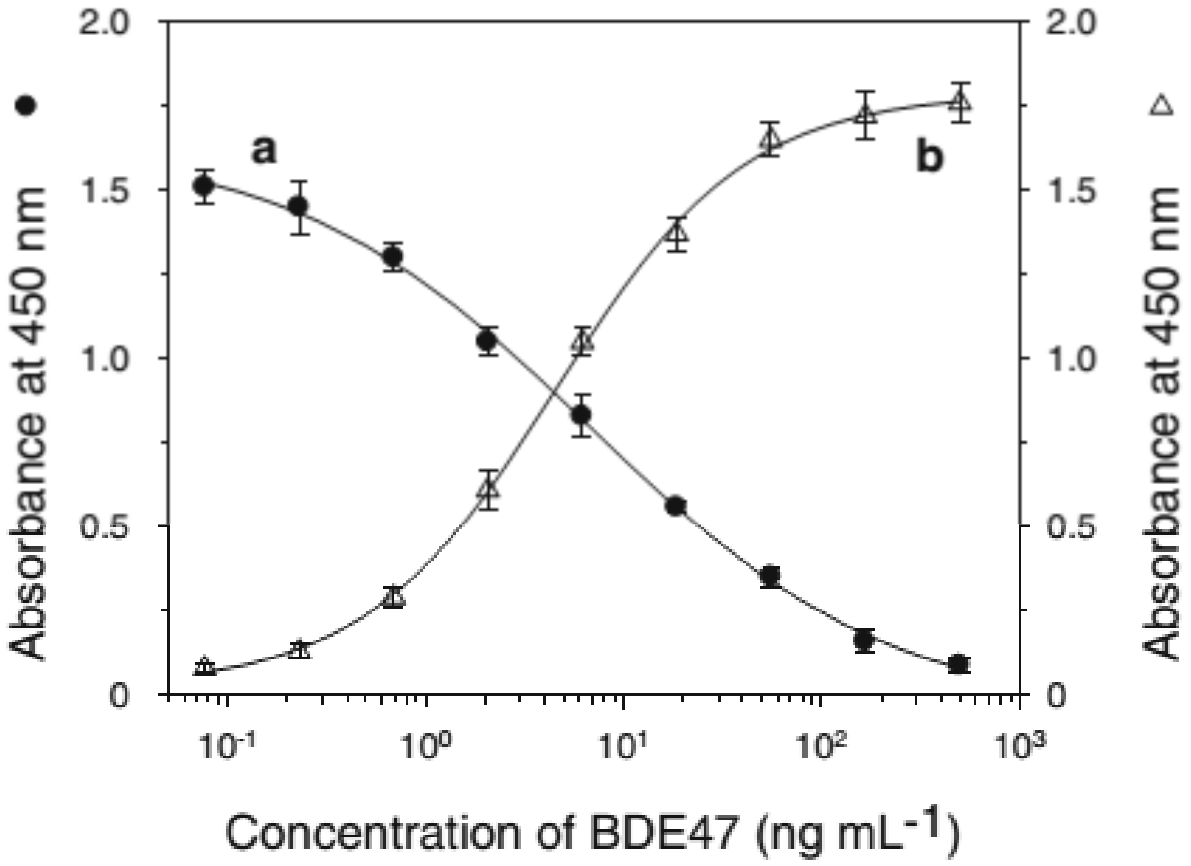


Positive phage clones specific to BDE47-free Mab



Positive phage clones specific to Mab-BDE47 immunocomplex

Standard Curves



Competitive assay (a): IC_{50} = 6.8 ng/mL
Non-competitive assay (b): EC_{50} = 4.2 ng/mL

Jia et al., *Anal Bioanal Chem* (2013) 405:9579–9583

Cross-reactivity of the phage ELISAs with compounds structurally related to BDE47

Compounds	Cross reactivity (%)	
	Competitive phage ELISA	Noncompetitive phage ELISA
BDE28	0.6	6.5
BDE47	100	100
BDE99	0.8	2.5
BDE100	0.3	1.3
BDE153	<0.1	<0.1
BDE154	<0.1	<0.1
BDE183	<0.1	<0.1
BDE209	<0.1	<0.1

Accuracies and precisions of phage ELISAs for BDE47 in the fortified samples

Fortification level ($ng\ g^{-1}$ dw)	Competitive phage ELISA			Noncompetitive phage ELISA		
	Average recovery (%)[a]	RSD_r (%)[b]	RSD_R (%)[c]	Average recovery (%)[a]	RSD_r (%)[b]	RSD_R (%)[c]
Sewage sludge						
5	107	4.5	5.5	97	6	7
20	96	7	12	103	3.5	9
100	118	6	10	120	6	11
Yellow croaker (*Pseudosciaena crocea*)						
5	113	10	14	109	10	15
20	124	11	16	116	9	14
100	103	6.7	9	112	8	10

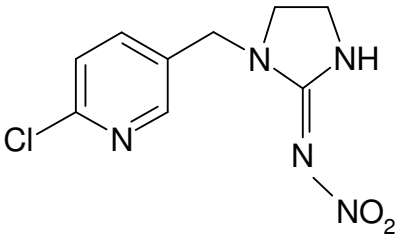
Concentrations of BDE47 in real world samples determined by phage ELISAs and GC/ITMS

Sample No.	BDE47 concentration [ng g^{-1} dw (lw)], n=3		
	Competitive phage ELISA	Noncompetitive phage ELISA	GC/ITMS
Sewage sludge			
#1	7.7	8.4	6.8
#2	4.6	4.9	5.4
#3	2.3	2.1	2.9
#4	5.7	5.2	5.0
#5	58	64	52
#6	28	30	24
#7	34	36	34
#8	11	13	9
Yellow croaker			
#9	12	17	14
#10	7.8	8.5	7.3
#11	28	23	13
#12	5.4	6	6.5
#13	25	23	27
#14	23	32	24
#15	8.4	11	7.9
#16	21	18	8.8
#17	1.8	2.2	1.5
#18	3.4	4	2.8

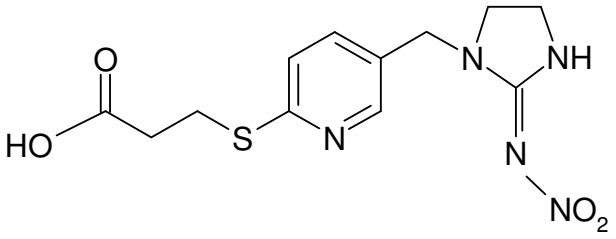
Part III

Selection of phage displayed peptides for the detection of insecticide imidacloprid in soil and water

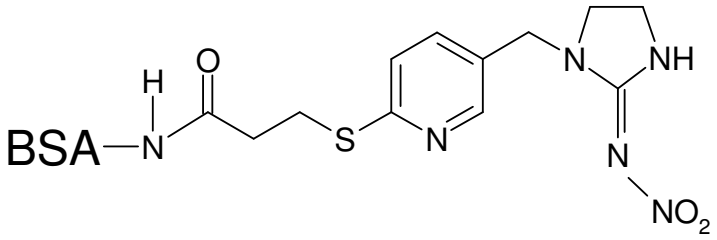
Preparation of coating antigen



Imidacloprid



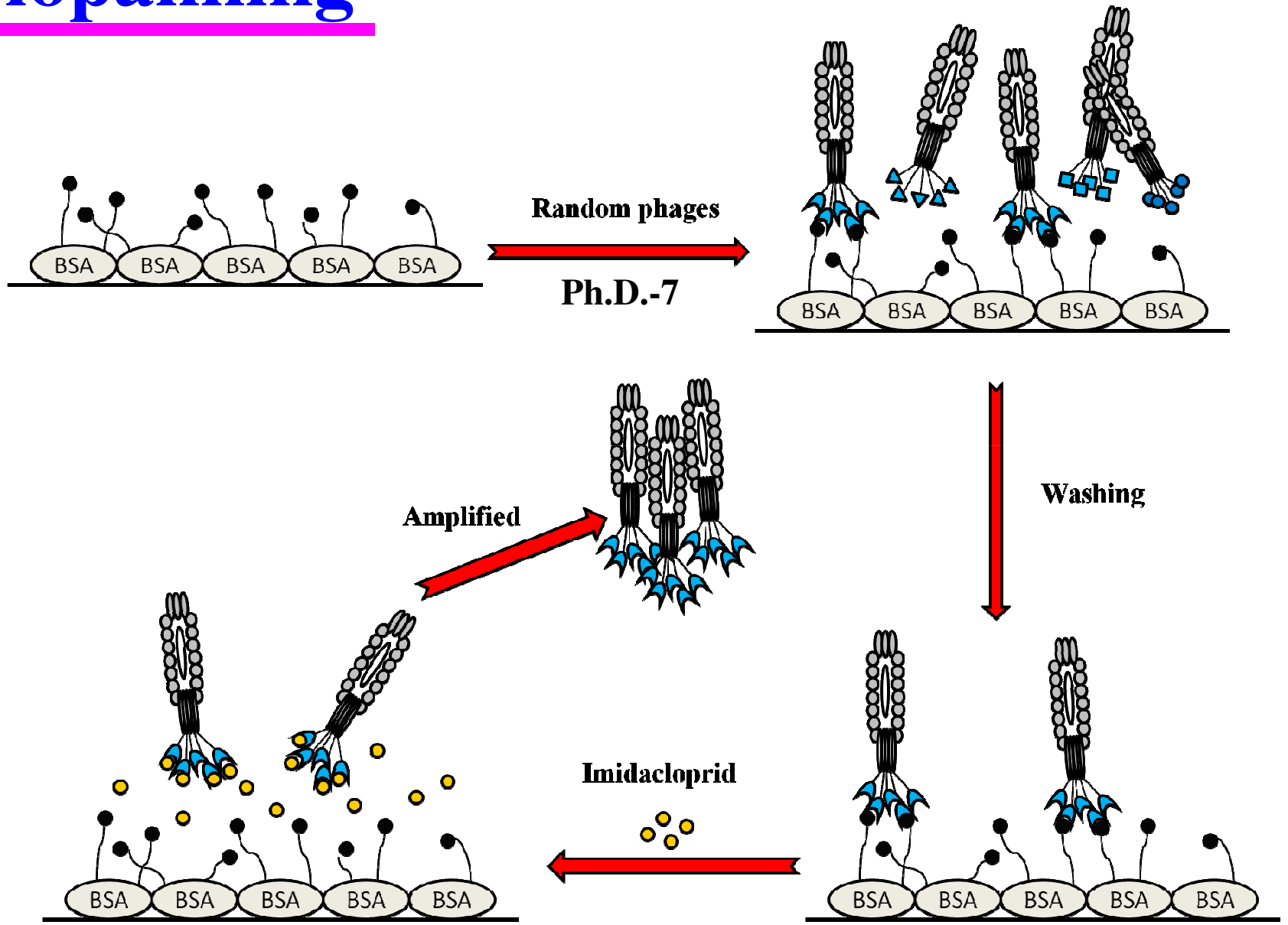
Hapten



Coating antigen

Li and Li, *J. Agric. Food Chem.* 2000, 48, 3378−3382

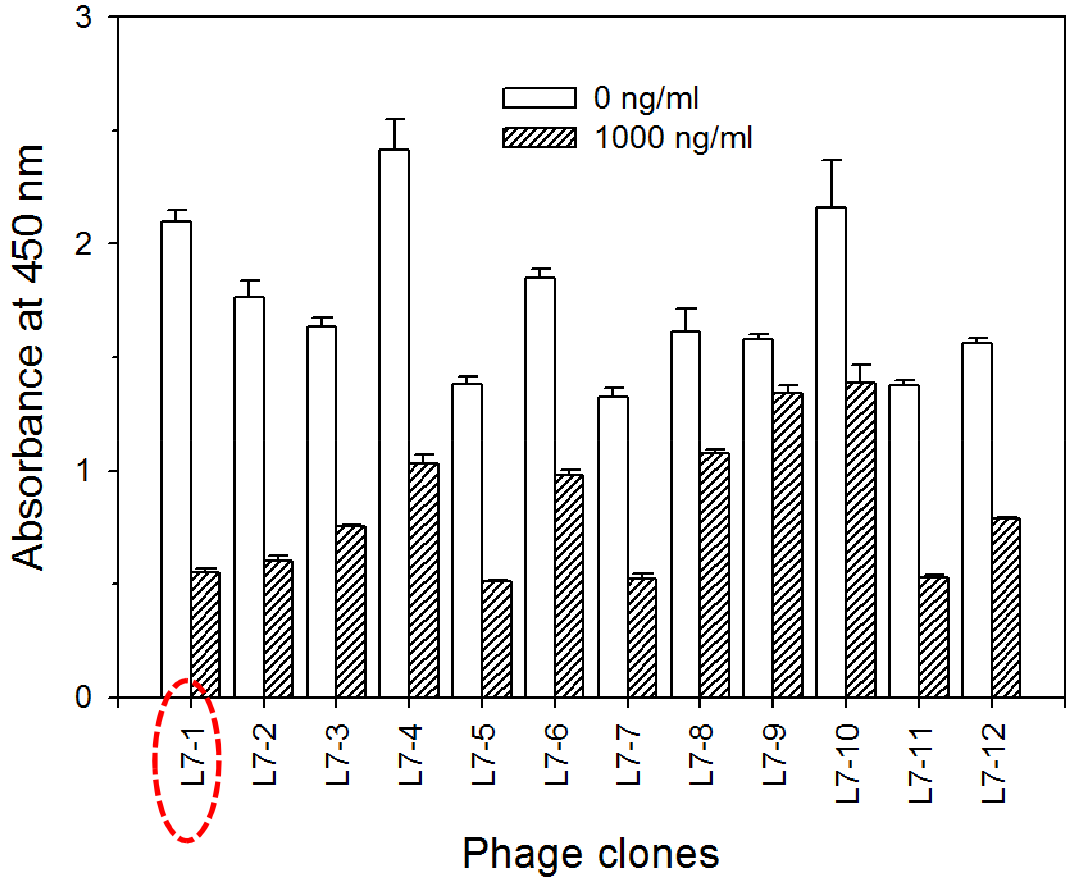
Biopanning


BSA
BSA
BSA
BSA
BSA
Random phages
Ph.D.-7
BSA
BSA
BSA
BSA
BSA
Washing
BSA
BSA
BSA
BSA
BSA
Imidacloprid
Amplified

Recovery of phage clones bound to coating antigen in rounds of biopanning

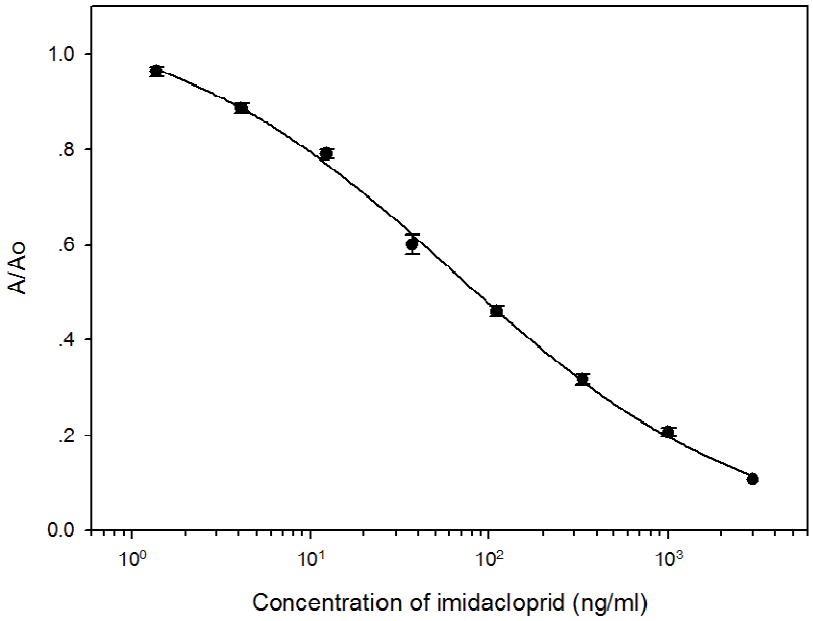
Round	Input phage titer (pfu)	Output phage titer (pfu)	Recovery (%)
1st panning	5.4×10^{11}	3.2×10^{4}	5.9×10^{-6}
2nd panning	5.2×10^{11}	2.4×10^{5}	4.4×10^{-5}
3rd panning	4.6×10^{11}	3.5×10^{6}	6.7×10^{-4}
4th panning	4.7×10^{11}	6.5×10^{7}	1.4×10^{-2}

Positive Clones

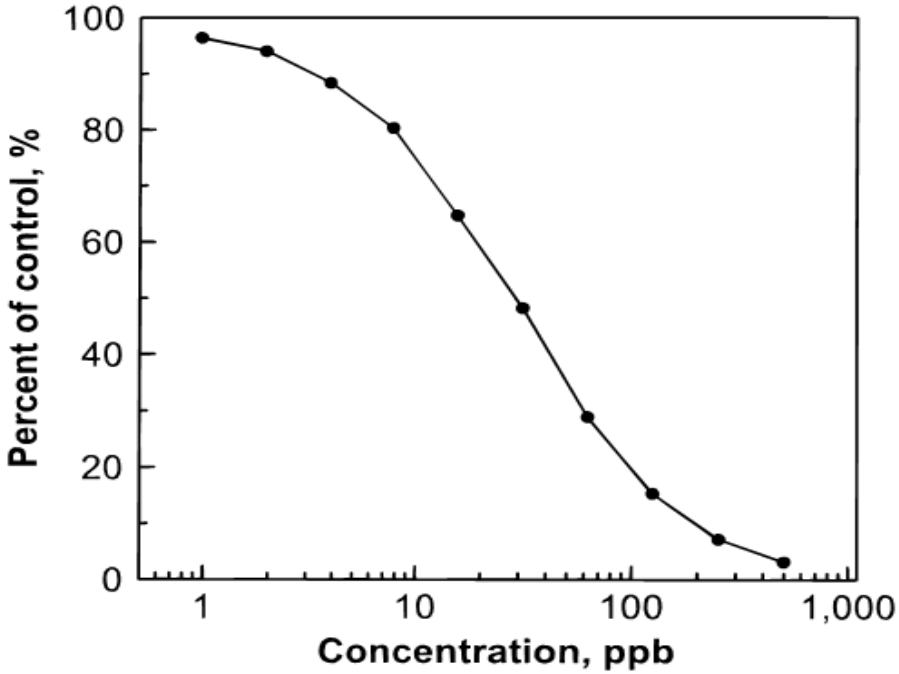


L7-1 sequence: **AKELSTW**

Standard Curves



Phage-based ELISA
(IC_{50} = 63.5 ng/mL)



Antibody-based ELISA
(IC_{50} = 35 ng/mL)

Li and Li, *J. Agric. Food Chem.* 2000, 48, 3378–3382

Cross-reactivity of the phage ELISA with compounds structurally related to imidacloprid

Compounds	IC_{50} (ng/ml)	Cross reactivity (%)
Imidacloprid	63.5	100
Thiamethoxam	15275	0.42
Acetamiprid	28342	0.22
Clothianidin	>60000	<0.1
Dinotefuran	>60000	<0.1
6-Chloronicotinic acid	>60000	<0.1

Accuracy of phage ELISA for imidacloprid in the fortified samples

Fortification Conc. (ppb)	Determined Conc. (ppb), n=3	Average recovery (%)
Water		
50	41.2±1.6	82
200	187±3.3	94
500	436±4.5	87
Soil		
10	10.6±0.8	106
50	48.6±2.5	97
200	225±6.4	113

Part IV

Summary

- Phage display technique offers an attractive means of developing alternate immunoassay formats that may enhance assay performance by increasing sensitivity and specificity.

- The phage particles could be used as final assay reagents, e.g. peptides mimic haptens, peptides specific for immunocomplexes and novel substitutes to small molecule antibody.

- Avoiding chemical based synthesis of structural variants of haptens and production of antibody *in vivo*.

Acknowledgement

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Hee Joo Kim

Thank You !