浙江大學
ZHEJIANG UNIVERSITY

Towards the on-site detection of neonicotinoids using a lateral flow device

Part A: Development of a neonicotinoid lateral flow device and its application on tea

Institute of Pesticide and Environmental Toxicology (IPET), Zhejiang University, Hangzhou

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yirongguo@zju.edu.cn

2017.10.17, Xi'an, China

1 General Introduction of IPET

2 Immunoassay Researches in IPET

3 Rapid strip tests for neonicotinoids

- **Founded in 1897, a key national university**
- **Ranked as one of the first-class universities in China**
- **The motto: “Seeking Truth and Pursuing Innovation”**

http://www.zju.edu.cn/english

Zhejiang University

College of Agriculture and Biotechnology

Dept. Plant protection

- Inst. Applied Entomology
- Inst. Phytopathology
- Inst. Pesticide & Environmental Toxicology (IPET)

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Labs of IPET

Zi Jing Gang Campus

- Residue analysis
- Environmental Toxicology
- Pollution control & Bioremediation

Hua Jia Chi Campus

- Synthesis
- Formulation
- Bioassay
- Neuro-toxicology

Joint - Lab ZJU+ZAFU

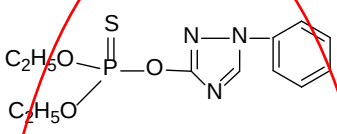
- Eco-toxicology
- Ecological Risk Assessment

Immunoassay Researches in IPET (since 1998)

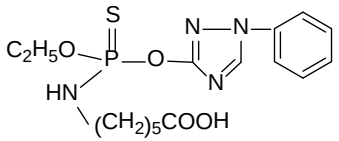
- **Development of techniques for rapid detection of low-molecular-mass contaminants, such as pesticides, antibiotics, biotoxins.**
  - Design and synthesis of small molecule haptens
  - Preparation of hapten-carrier protein conjugates as antigens
  - Production of polyclonal & monoclonal antibodies
  - ELISA; Gold immunochromatographic assay; Chemiluminescence/Fluorescence immunoassay
  - Recombinant antibody engineering: sequence analysis & structure modeling

Hapten synthesis for antibody development

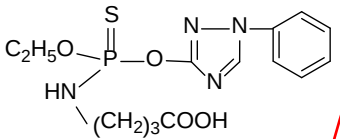
Organophosphorus, pyrethroid, neonicotinoid and carbamate insecticides:



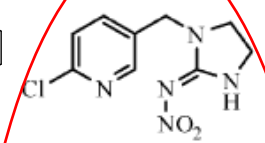
Triazophos



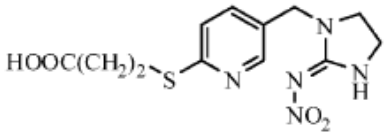
THHe



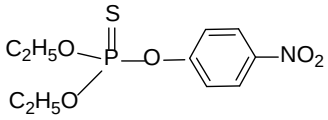
THBu



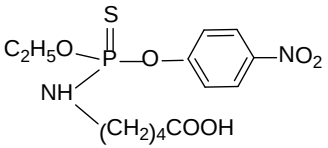
Imidacloprid



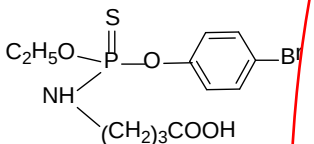
Imidacloprid hapten



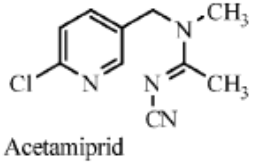
Parathion



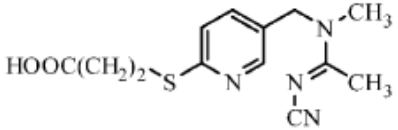
PA0204



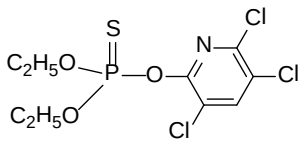
PA0304



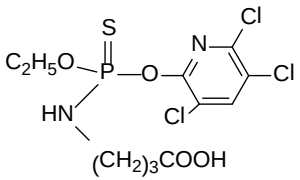
Acetamiprid



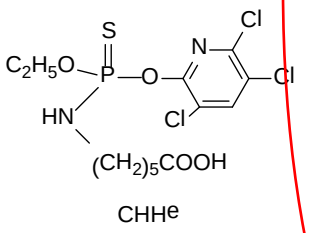
Acetamiprid hapten



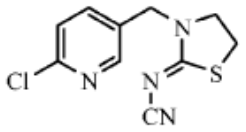
Chlorpyrifos



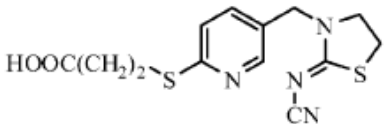
CHBu



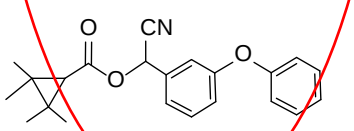
CHHe



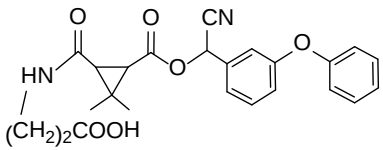
Thiacloprid



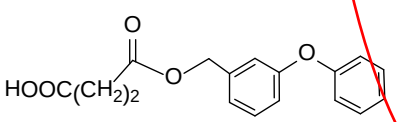
Thiacloprid hapten



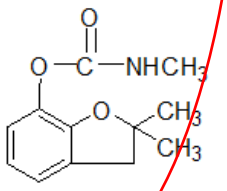
Fenpropathrin



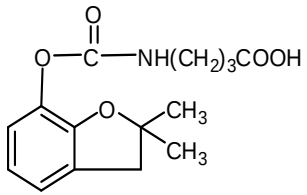
NC-S-2



NC-S-7

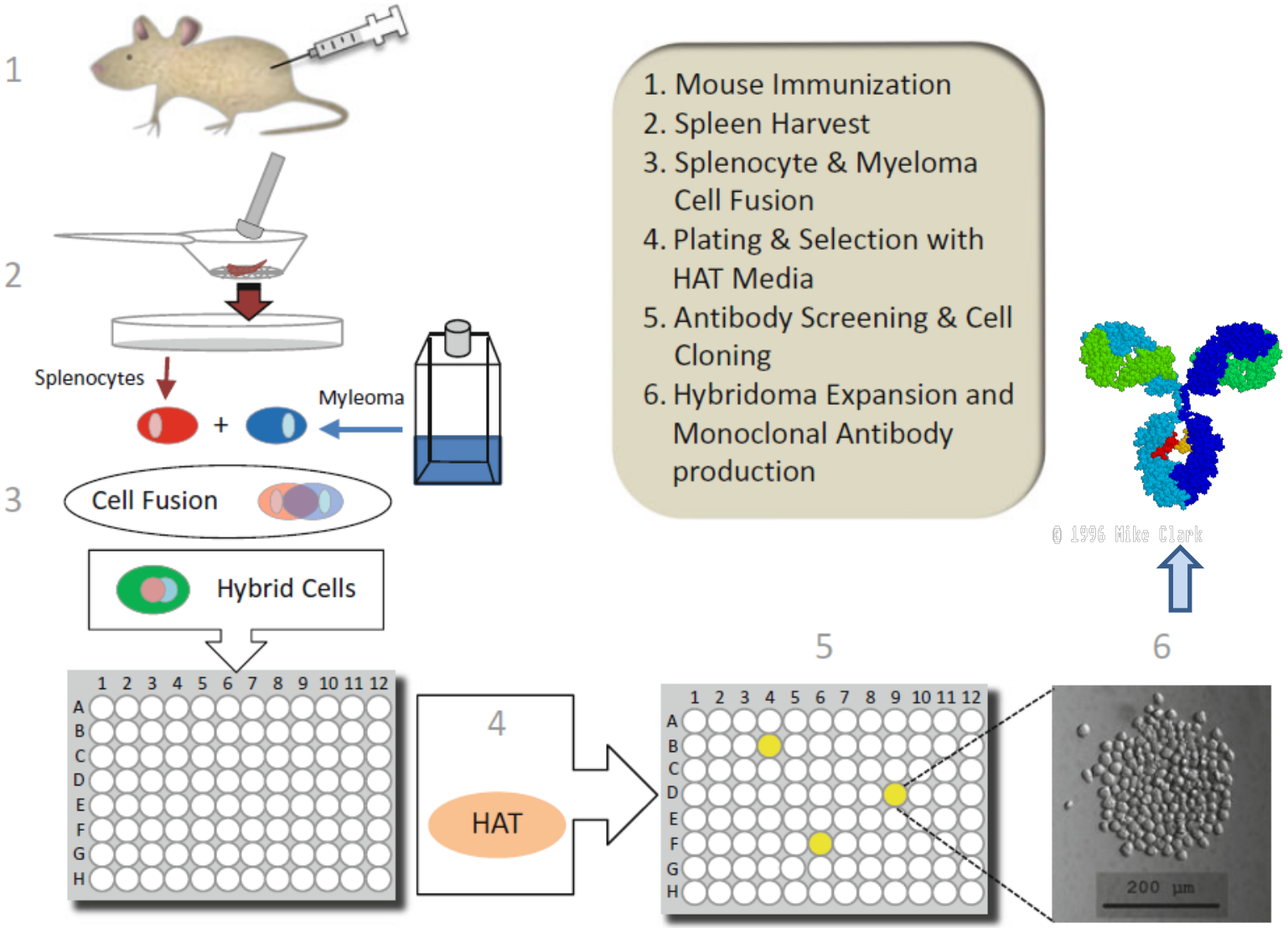


Carbofuran



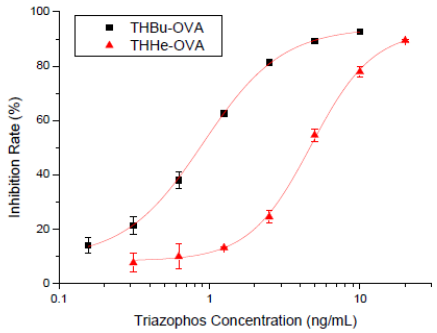
BFNP

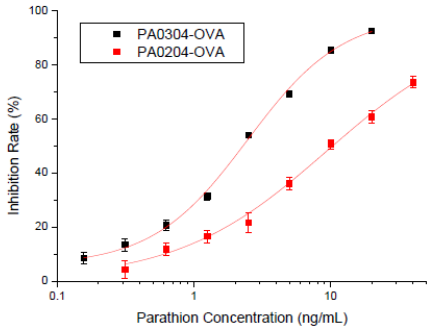
Production of mAbs against pesticides

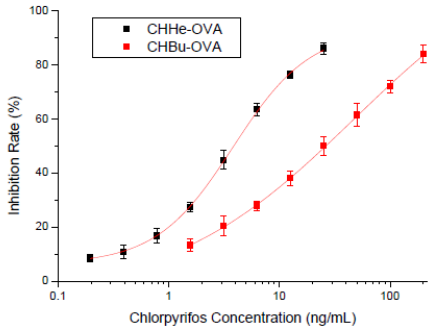


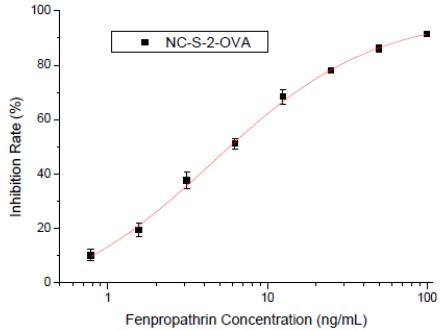
Cited from: Robert M. Hnasko and Larry H. Stanker, Hybridoma Technology, Springer Protocols, New York, 2015

Antibody characterization









In a lot of cases, heterologous competitive ELISAs gave higher sensitivity.

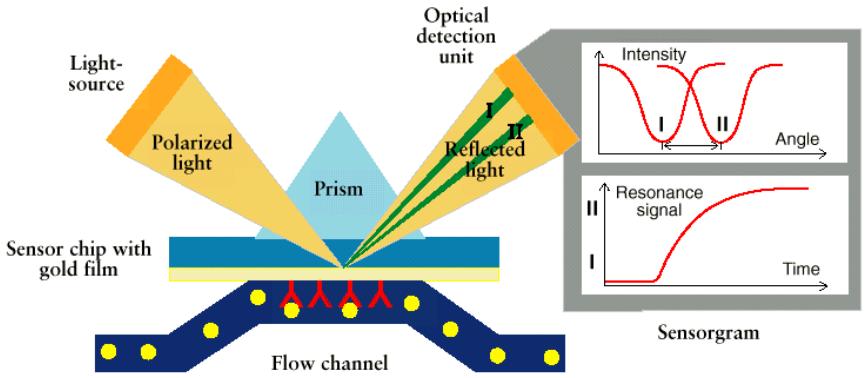
The mAbs were generally specific to the target analytes, except for minor cross-reactions to very similar chemicals.

Pesticide	Clone name	Isotype	IC_{50} (ng/mL) in this work	IC_{50} (ng/mL) in literatures
Triazophos	THHe-QA2-8C10	IgG1, *lambda*	0.91	2.54
Parathion	PA0204-QA1-7C2	IgG1, *kappa*	2.46	2.94; 7.06
Chlorpyrifos	CHBu-QA2-13C7	IgG1, *kappa*	3.72	24; 3.5
Fenpropathrin	NC-S-2-QA2-1G9	IgG2a, *kappa*	6.22	14.03

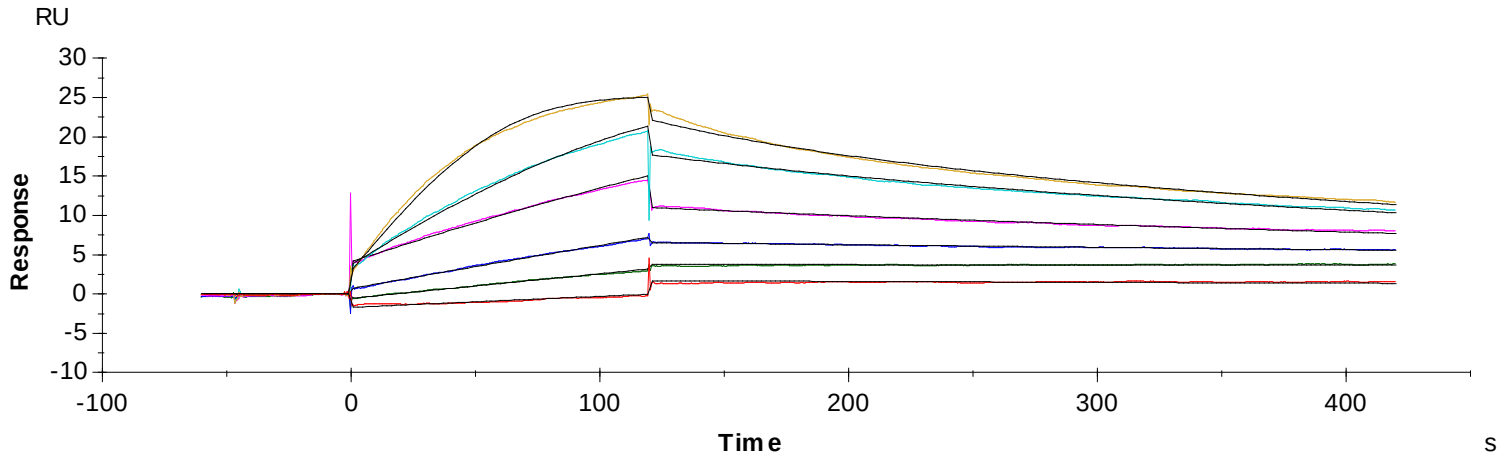
Journal of Zhejiang University SCIENCE B, **2016**, 17, (4), 282-293

Antibody affinity measurement

Biacore
(Bio-molecule interaction analysis core-technology)
SPR biosensor: T200



Taking anti-triazophos mAb as an example:



Sensorgram of the capture of THBu-OVA (0, 4.25-137.5 nM) by the mAb immobilized on the sensor chip

ka (1/Ms)	kd (1/s)	*K*D (M)
4.783E+5	0.004430	9.263E-9

Science of The Total Environment, 2018, 783-791

Immunoassay products for pesticide detection

➢ **Previous works mainly focused on single-test for rapid detection of insecticides such as: carbofuran, ethyl-parathion, triazophos, chlorpyrifos, *et al.***

ELISA kits

Lateral-flow test strips

➢ **Drawbacks:** one assay only for one analyte,
hard to reach the purposes of rapid screening for multi-residue in one sample.

Recent researches on multi-analyte immunoassays for several pesticides

Strategy 1:

Combinations of analyte-specific Abs (multi-Ab approach)

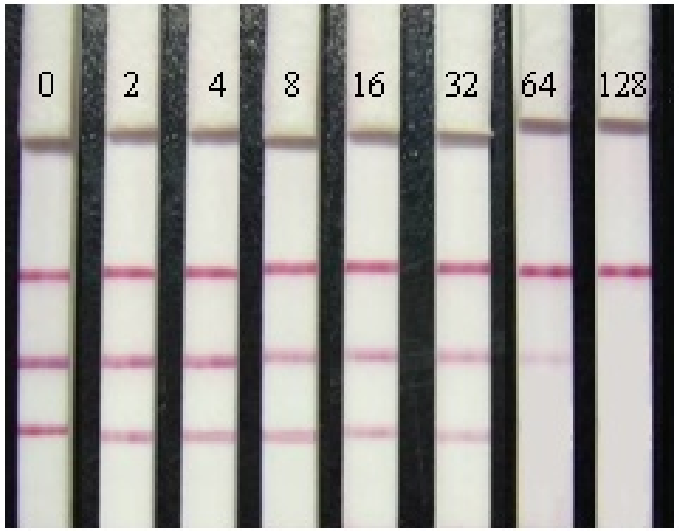
Strategy 2:

Applications of group-specific/ broad-selective Abs

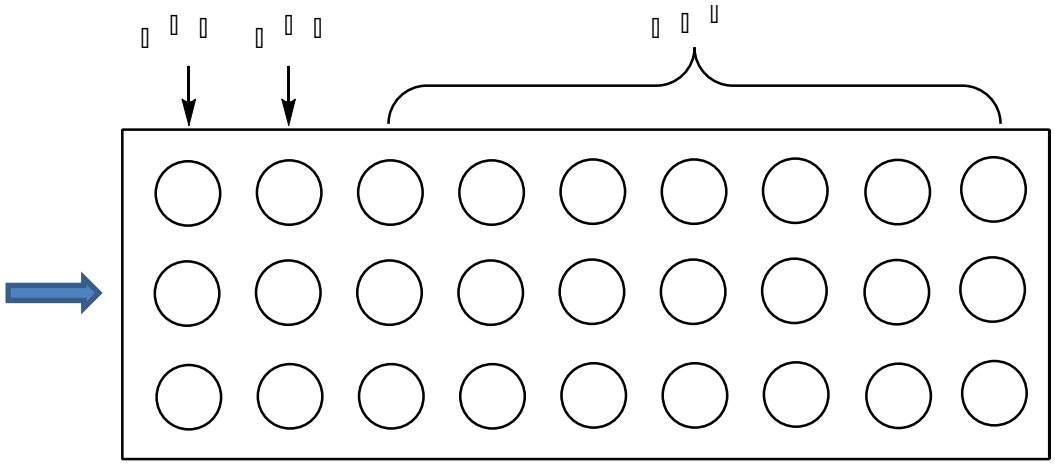
Multiple Immunoassays for Pesticides

1) Combinations of several specific Abs

Gold-labeled anti-carbofuran monoclonal antibody (McAb1) and gold-labeled anti-triazophos monoclonal antibody (McAb2)



Dual-pesticide immunostrips

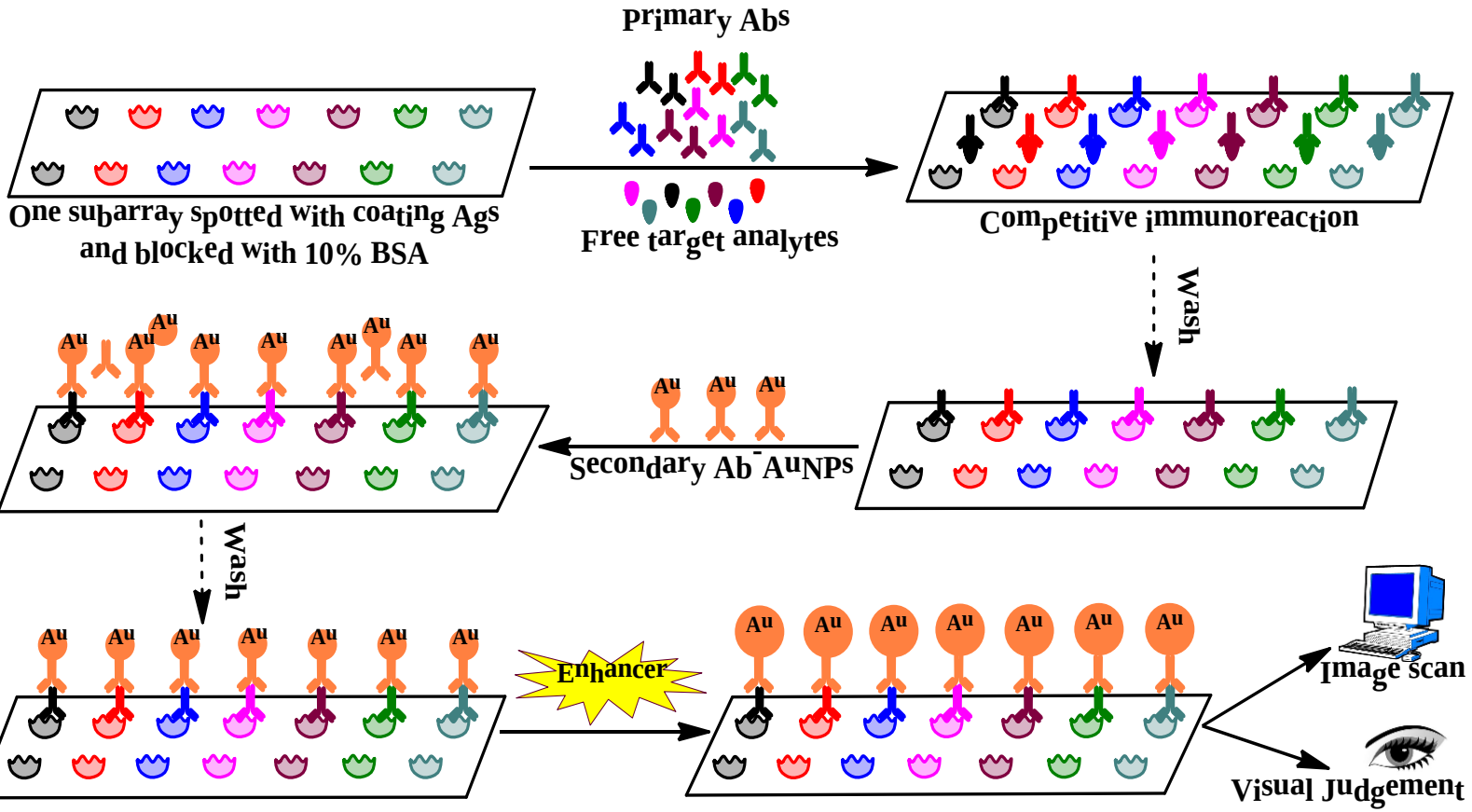


Immunochip for multi-pesticide test

Analytical Biochemistry ***2009,*** *389, (1), 32-39*
(Cited by more than 70 times until August 2017)

Multiple Immunoassays for Pesticides

1) Combinations of several specific Abs



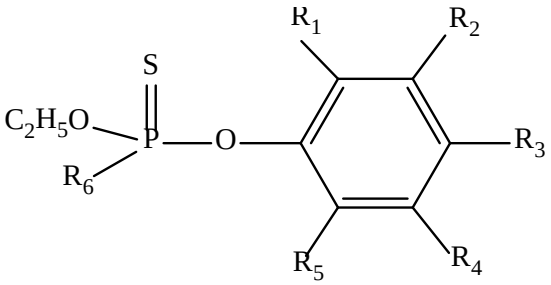
Multi-residue detection of 7-9 pesticides using a sensitive immunochip assay based on nanogold enhancement

Analytica Chimica Acta **2016, 938, 146-155.**

Multiple Immunoassays for Pesticides

2) Application of group-selective/broad-specific Abs

Parathion-ethyl hapten



Compound	R1	R2	R3	R4	R5	R6
PA0204	H	H	NO_2	H	H	$NH(CH_2)_4COOH$
PA0203	H	H	NO_2	H	H	$NH(CH_2)_3COOH$
PA0304	H	H	Br	H	H	$NH(CH_2)_3COOH$
PA0314	CH_3	H	H	H	H	$NH(CH_2)_3COOH$
PA0318	F	H	NO_2	H	H	$NH(CH_2)_3COOH$
p-nitrobenzoic acid	H	H	NO_2	H	H	----

Heterologous competitive ELISAs for selection of group-specific mAbs

Pesticide	Clone 2G6（PA0314-OVA）		Clone 7B2（PA0304-OVA）	
	IC_{50}（μg/L）	Cross-reactivity（%）	IC_{50}（μg/L）	Cross-reactivity（%）
Parathion-ethyl	12.4	100	21.6	100
Parathion-methyl	18.2	68.1	9.4	229.8
Fenitrothion	16.1	77.0	6.9	313.0

Multiple Immunoassays for Pesticides

2) Application of group-selective/broad-specific Abs

Sensitivity and LOD for OP pesticides using mAb 2H6 and PA0314-OVA

Compound	IC_{50} value(ng/mL) [a]	CV (%)[b]	IC_{10} value(ng/mL) [a]	CV (%)[b]
Parathion	20.32 ±1.65	9.27	5.67 ± 0.87	17.46
Methyl-parathion	21.44 ± 1.06	5.62	6.73 ± 0.94	15.88
Fenitrothion	42.15 ± 3.10	8.39	8.42 ± 1.14	15.38
Isocarbophos	58.85 ± 2.51	4.87	8.65 ± 1.50	19.78

Recoveries of mixed OP pesticides from spiked soil samples

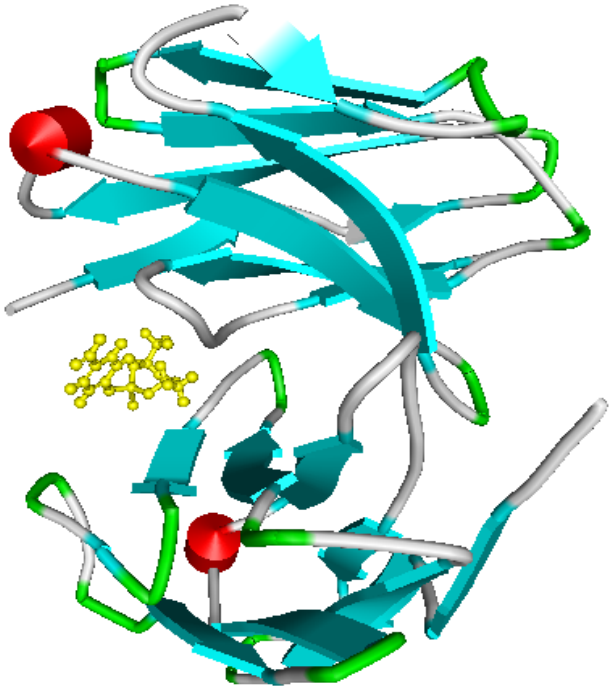
Analyte	Spiked (ng/mL)	Measured (ng/mL) [a]	Recovery (%)	CV (%)
Mixture of Parathion +	10 + 10	18.71 ± 1.49	93.55	7.06
Methyl-parathion	10 + 20	20.82 ± 1.05	69.40	4.46
	20 + 10	22.34 ± 0.68	74.48	2.68
	20 + 20	34.49 ± 1.98	86.22	5.07
Mixture of the four OP pesticides	10+10+10+10	55.20 ± 9.35	137.99	14.98

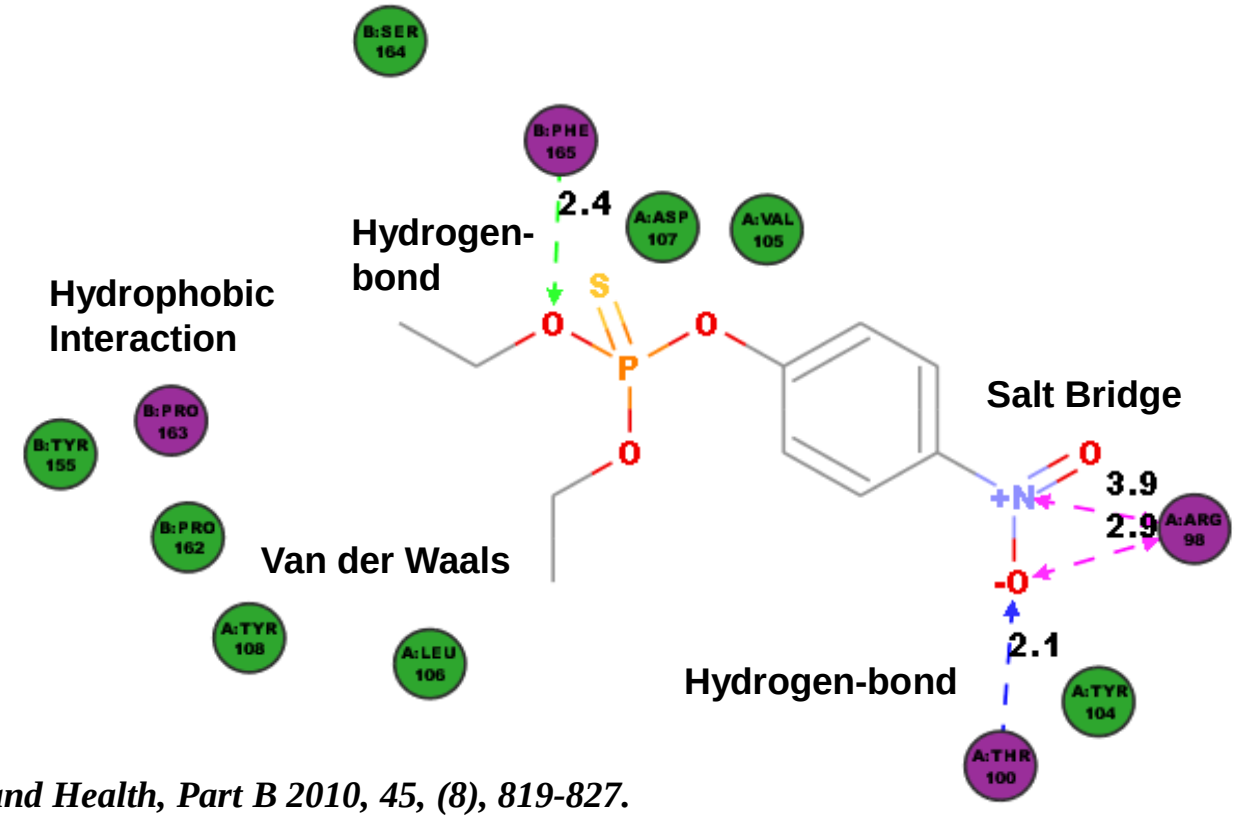
Journal of Agricultural and Food Chemistry **2010, 58, (9), 5658-5663.**

Interaction between Ab and pesticide

Discovery Studio
(Accelrys Co., USA)

Homology modeling
Dynamics Simulation
Molecular Docking





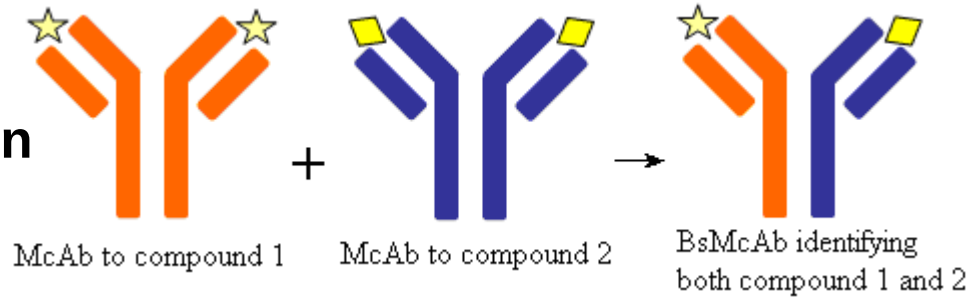
Journal of Environmental Science and Health, Part B 2010, 45, (8), 819-827.

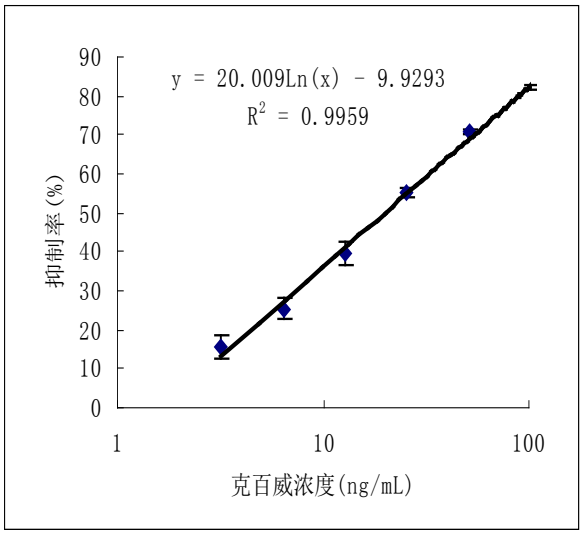
Food and Agricultural Immunology 2014, 1-11.

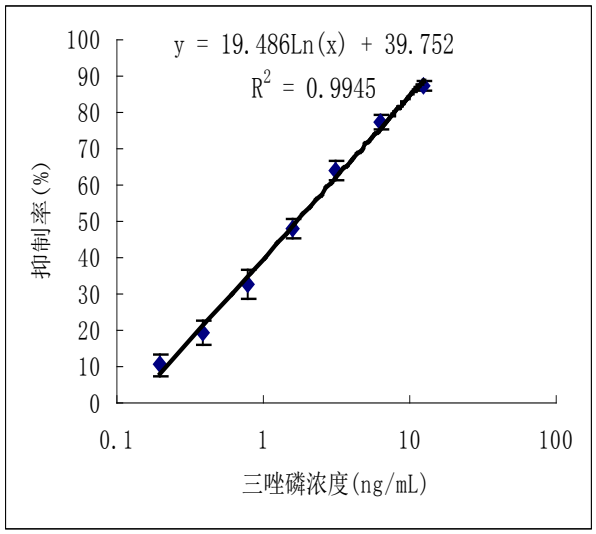
Multiple Immunoassays for Pesticides

3) Bispecific monoclonal antibody

Tetradomas preparation







Carbofuran IC50=20 ng/mL

Triazophos IC50=1.7 ng/mL

Standard curves by direct competitive ELISA

Journal of Food Science 2009, 74, (1), T1-T6.

Multiple Immunoassays for Pesticides

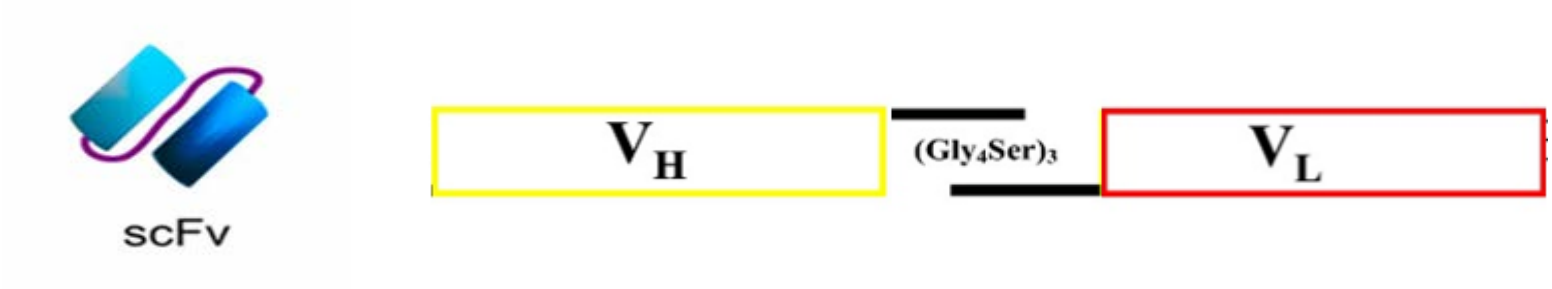
4) Combined ScFvs for multi-pesticide recognition

THHE-QA2-8C10-C10-A3-E6-B8-E3 (triazophos) IgG1 lambda

PA0204-QA2-4C6-F12-D3-E12-B3-D3-E4（parathion）IgG1 kappa

CHBU-QA2-13C7-2-G2-G9-B3-G12（ chlorpyrifos ）IgG1 kappa

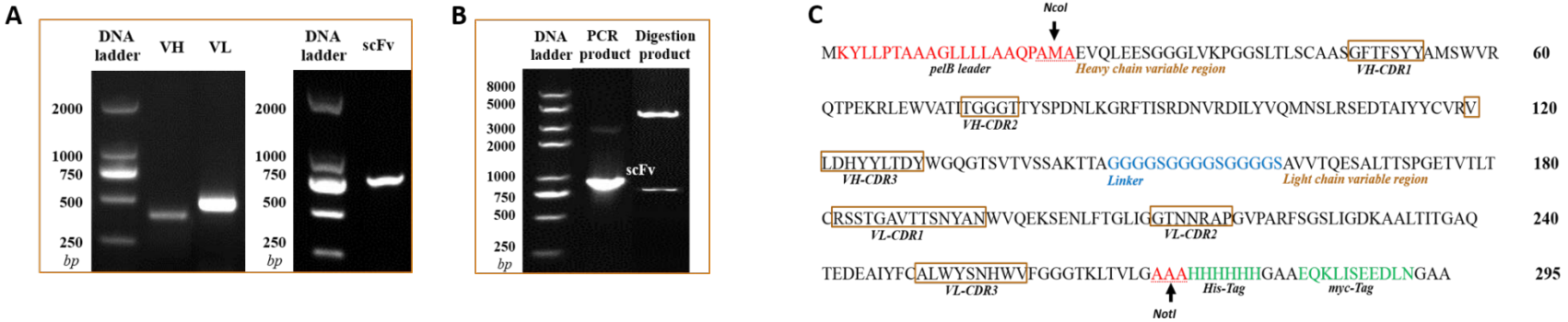
NC-S-2-QA2-1C3-F12-1-A9-D2-D10-2A4-1G9（fenpropathrin）IgG2a kappa



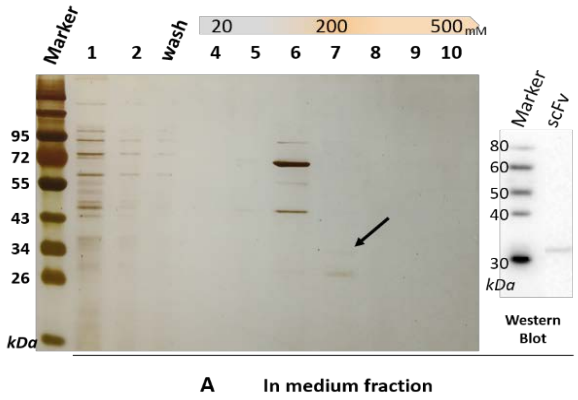
Anti- triazophos / parathion / chlorpyrifos ScFvs

Articles under review

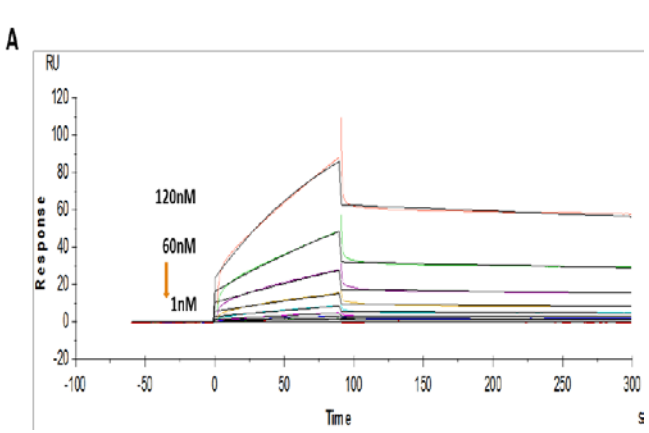
Expression, characterization of a high-affinity single-chain variable fragments antibody specific to triazophos



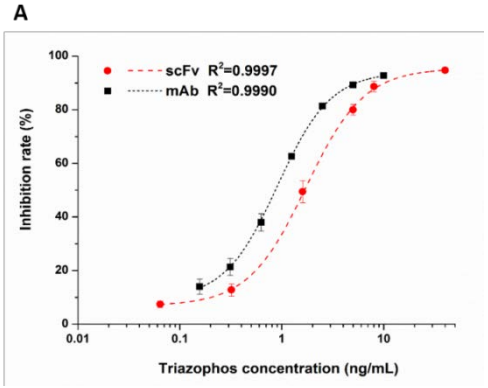
Construction of the scFv against triazophos for protein expression in *E. coli*.



SDS-PAGE and Immunoblotting of soluble scFv



Affinity determination by SPR



ELISA standard curves for triazophos

International Journal of Molecular Sciences 2016, 17, (6), 823.

Recent works on rapid strip tests for neonicotinoids

Step 1:

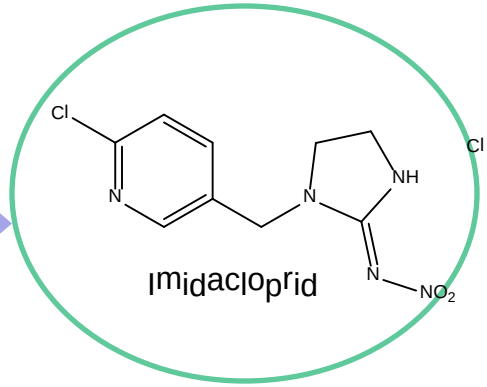
Production of Abs for neonicotinoids

Step 2:

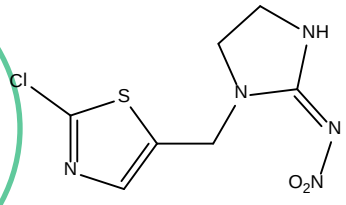
Development of immunostrips for neonicotinoids

Commonly-used neonicotinoid insecticides

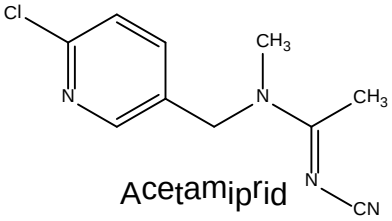
Pioneer



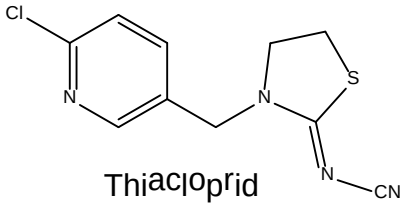
Imidacloprid



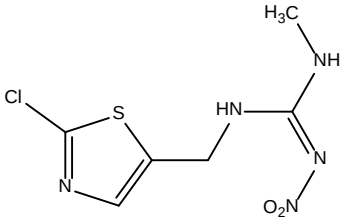
Imidaclothiz



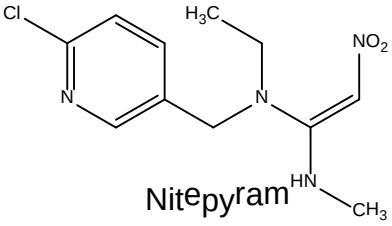
Acetamiprid



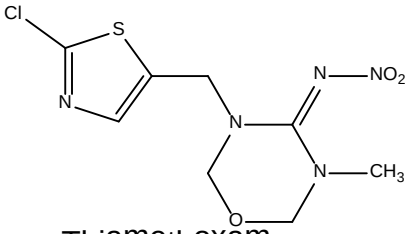
Thiacloprid



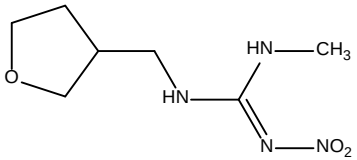
Clothianidin



Nitepyram



Thiamethoxam



Dinotefuran

Type 1: chlorothiazole ring

Type 2: chloropyridine ring

Review of Enzyme-Linked Immunosorbent Assays (ELISAs) for Analyses of Neonicotinoid Insecticides in Agro-environments

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Table 2. Cross-Reactivities of Developed ELISAs and Assessed Commercial ELISA Kits Against Structurally Related Neonicotinoid Analogues

	imidacloprid									acetamiprid	
	PoAb				MoAb			NR[a]	MoAb	MoAb	
	ic-ELISA	ic-ELISA	ic-ELISA	ic-CL-ELISA	dc-ELISA	ic-ELISA	ic-ELISA	dc-ELISA kit	dc-ELISA kit	dc-ELISA	dc-ELISA kit
imidacloprid	100 (35)[b]	100 (17.3)	100 (2.7)	100 (15)	100 (6.4)	100 (1.6)	100 (6.2)	100 (1.05)	100 (5)	0.3	0.62
acetamiprid	–[c]	7.1	0.3	***19***[d]	0.3	0.6	0.6	***24***	0.21	100 (1.3)	100 (0.8)
thiacloprid		1.5						***81***	0.83		***40***
thiamethoxam						NC[e]	<0.1	0.05	<0.05		<0.02
clothianidin			<0.01			3.6	3.6	0.07	***11.9***		0.1
dinotefuran											
nitenpyram											
imidaclothiz											
ref	13	19	22	29	20	21	30	17	31	20	32

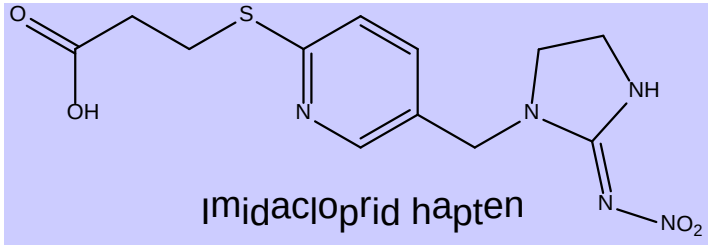
Different imidacloprid-Abs exhibited various cross-reactivities to other neonicotinoids.

	thiacloprid	thiamethoxam				clothianidin			dinotefuran	imidaclothiz
	PoAb	PoAb	MoAb			MoAb	PoAb		MoAb	MoAb
	ic-ELISA	dc-ELISA	FIA	ic-ELISA	dc-EL[illegible]t	dc-ELISA	ic-ELISA	ic-CL-ELISA	dc-ELISA kit	ic-ELISA
imidacloprid	0.23	0.8	NC	<0.01	0.09[illegible]	<0.1	0.8	0.4	<0.1	***91.7***
acetamiprid	0.72	<0.4	0.01	0.01	0.084	<0.1	<0.05	<0.02	<0.1	<0.05
thiacloprid	100 (10)								<0.1	<0.05
thiamethoxam	<0.01	100 (9.0)							<0.1	<0.05
clothianidin	0.12	1.8							***184***	
dinotefuran	0.02	<0.25							100 (7)	
nitenpyram	<0.01				<0.01	<0.1	<0.05	<0.02	<0.1	<0.05
imidaclothiz	<0.01						0.6	0.4		100 (87.5)
ref	23	24	25	30	33	26	27	27	34	28

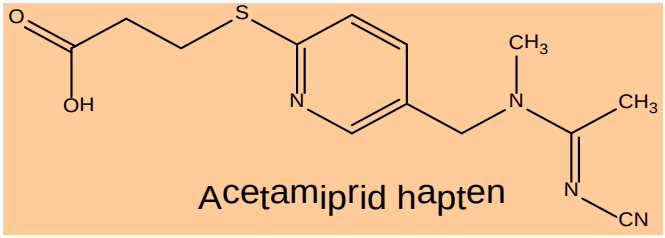
A broad-specific or generic Ab for neonicotinoids ?

[a]Not reported. [b]Units of IC_{50} value were unified in nanograms per milliliter. [c]Not reported. [d]Bold and italic figures denote insecticides that showed significant cross-reactivity. [e]No competition.

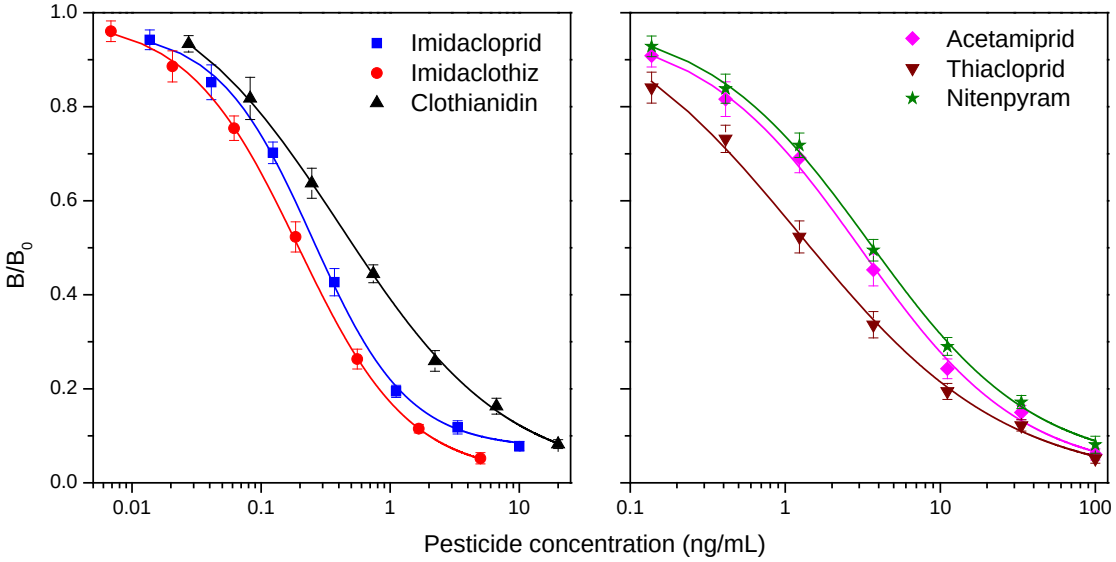
Recent production of mAbs for neonicotinoids



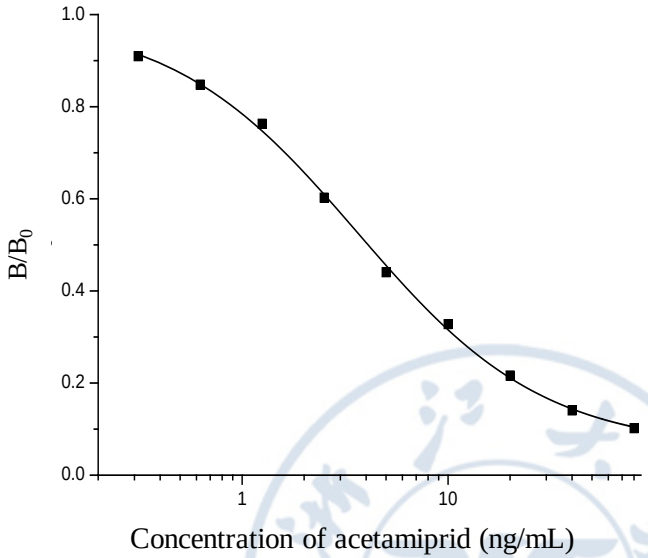
Imidacloprid hapten



Acetamiprid hapten

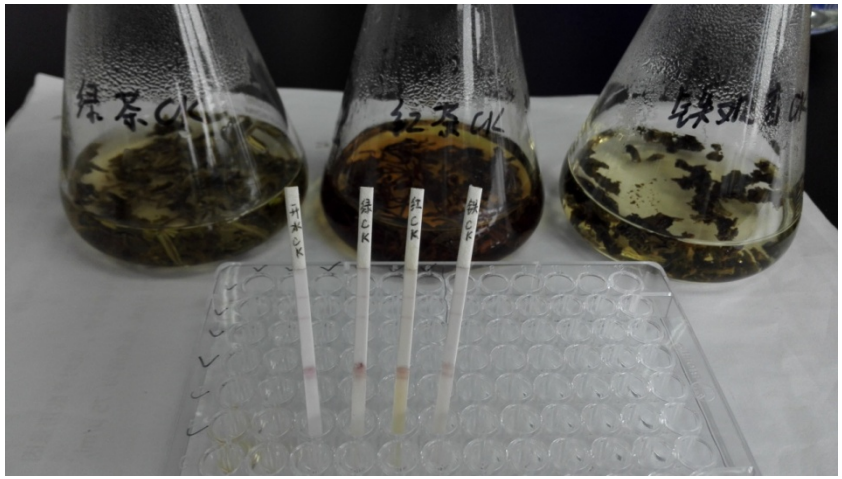


Standard curves for 6 neonicotinoids by icELISAs based on a broad-specific mAb (IC50: 0.48-7.16 ng/mL)



Standard curve of acetamiprid by icELISA (IC50: 3.62 ng/mL)

Gold immunostrips for rapid test of neonicotinoid insecticide residues in tea infusion samples

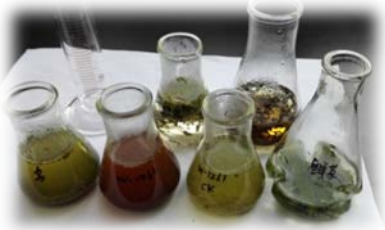


Green tea, black tea, oolong tea…

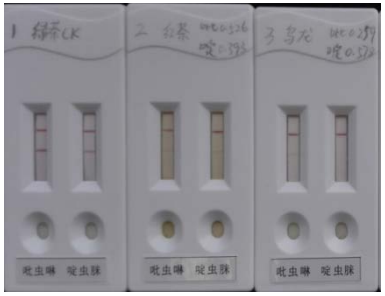
Pesticide	Visual LOD (ng/mL)
Imidacloprid	5
Imidaclothiz	5
Clothianidin	5

Chinese Patent:
ZL201510609069.9

Strip tests for imidacloprid and acetamiprid residue in tea

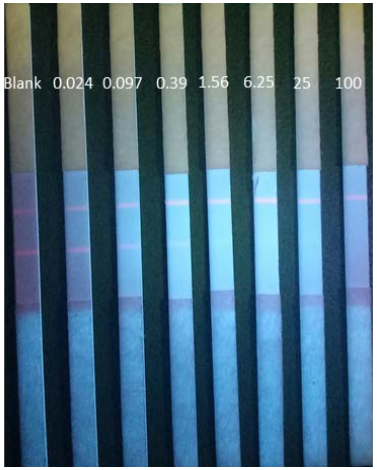


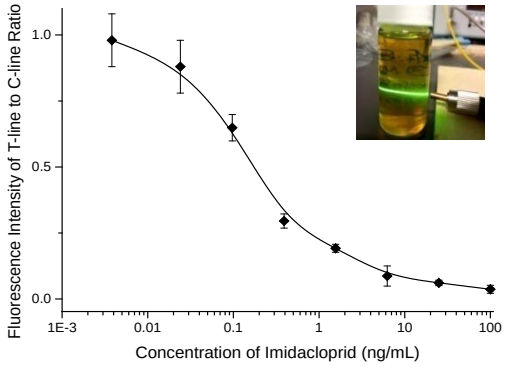
5 min



Immunostrips with other nanoparticles, such as quantum dots, up-conversion phosphors

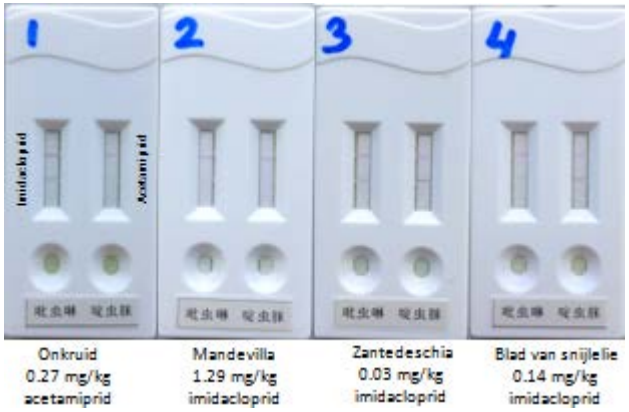
Articles under revision





Strip tests for neonicotinoids in flowers to protect honey bees

Dr. Jeroen Peters from RIKILT–Wageningen University & Research



Strip test compared with instrumental analyis by RIKILT

Thanks a lot for your attention !

Welcome !