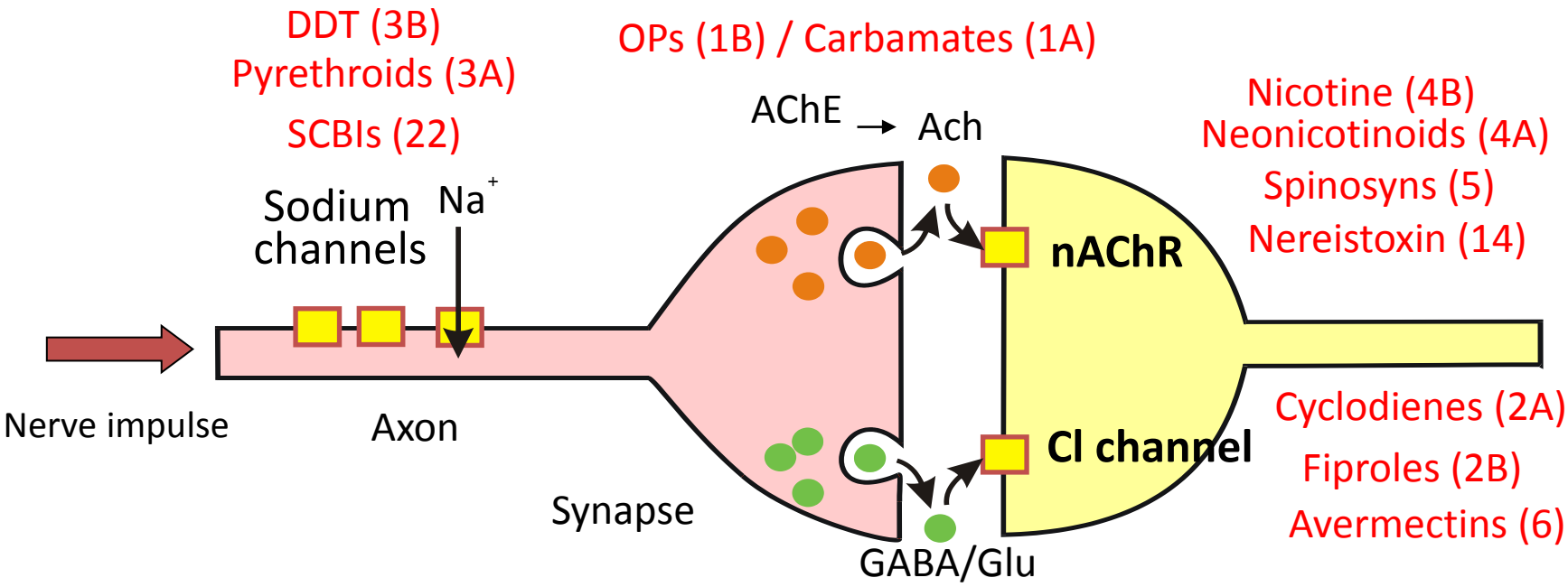
Mechanisms of insecticide resistance: Historical and more recent developments in target site resistance to insecticides

Martin Williamson & Chris Bass

Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

Insecticide targets and MoA classes (nerve & muscle)

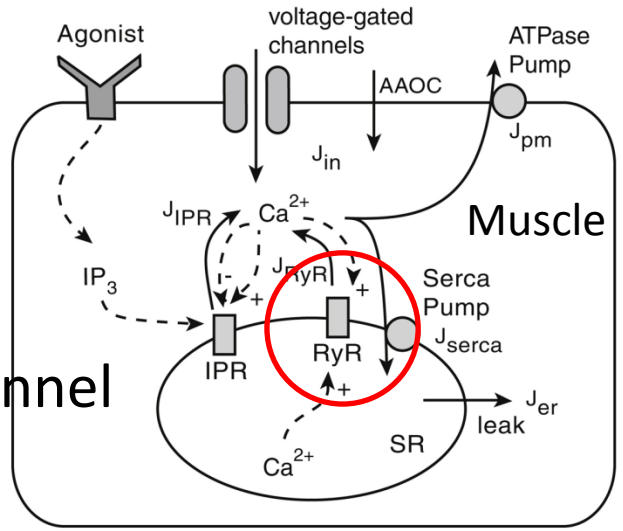


Other targets:

- Growth / development
- Respiration
- Midgut

Diamides (28)

RyR Ca^+ channel



Overview of target site mutations (nerve & muscle)

Target	cpds	TSR?	mutations	Species
GABA / Cl channels	cyclodienes fiproles	yes yes	rdl (A302S/G) (A302N, R340Q)	many planthoppers
Acetylcholinesterase	OPs / CMs	yes	many	many
Sodium Channel	pyrethroids	yes	many	many
Glu / Cl channels	avermectins	yes	G314D / G326E	Spider mites
nACh Receptor	neonicotinoids spinosyns	yes yes	Y151S (α1 + α3) R81T (β1) ko + G275E (α6)	*N. Lugens* *M. persicae, A. gossypii* Several + WFT
RyR / Ca^{+} channel	diamides	Yes	G4946E	*P. xylostella*

Why study target site resistance?

- Identify mutations responsible for causing resistance
- Develop DNA-based assays for resistance monitoring
- Study insecticide / target interactions
    - Investigate species selectivity
    - Further design opportunities?
    - Target-based screening for new compounds

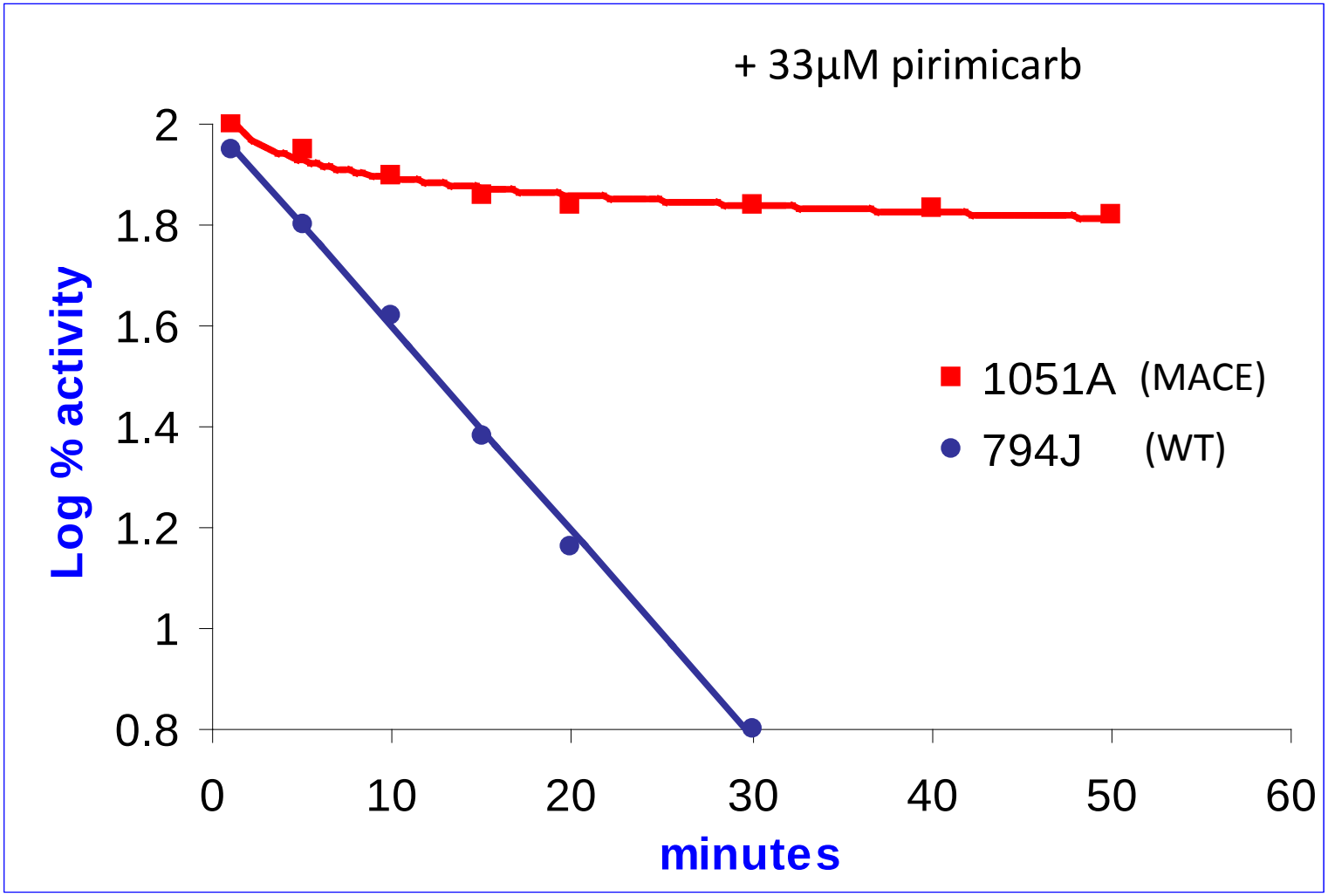
Sensitivity of *M. persicae* clones to pirimicarb

Clone	E4 level	AChE	LC50 ppm	RF
US1L	S	S/S	23	1
T1V	R2	S/S	200	9
1051A	R2	S/R	2800	120
1200Q	R2	R/R	>10000	>500

Mechanism = MACE

(modified acetylcholinesterase)

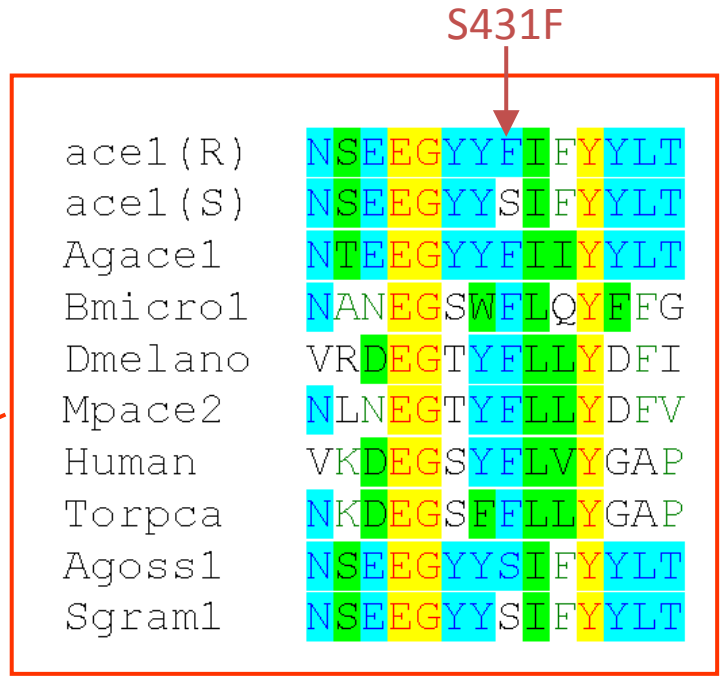
AChE activities of wild-type and MACE aphids with pirimicarb



MACE is caused by a single point mutation in the ace1 gene

- confers resistance to dimethylcarbamates

```
          1                                                  50
ace1(R)   DDPLVIHTNKGQIRGITQAATTGKLVDARLGIPYAKKPIGDLRFRHPRPI
ace1(S)   DDPLVIHTNKGQIRGITQAATTGKLVDARLGIPYAKKPIGDLRFRHPRPI
         51                                                 100
ace1(R)   DRWDNTNPETVFNCTTPPNTCVQIFDTLFGDFPGATMWNPNSPVSEDCLY
ace1(S)   DRWDNTNPETVFNCTTPPNTCVQIFDTLFGDFPGATMWNPNSPVSEDCLY
         101                                                150
ace1(R)   INVVVPKPRPQNAAVMVWIFGGGFYSGSATLDIYDPKVLVSEENVILVSM
ace1(S)   INVVVPKPRPQNAAVMVWIFGGGFYSGSATLDIYDPKVLVSEENVILVSM
         151                                                200
ace1(R)   QYRVASLGFLYFDTEDVPGNAGLFDQLMALQWVHENIKLFGGNPNNVTLF
ace1(S)   QYRVASLGFLYFDTEDVPGNAGLFDQLMALQWVHENIKLFGGNPNNVTLF
         201                                                250
ace1(R)   GESAGAVSVSLHLLSPLSRNLFNQAIMESGSSTAPWAILSREESYSRGLR
ace1(S)   GESAGAVSVSLHLLSPLSRNLFNQAIMESGSSTAPWAILSREESYSRGLR
         251                                                300
ace1(R)   LARAMGCPDDRNEIHKTVECLRKANSSTMVEKEWDHVAICFFPFVPVVDG
ace1(S)   LARAMGCPDDRNEIHKTVECLRKANSSTMVEKEWDHVAICFFPFVPVVDG
         301                                                350
ace1(R)   AFLDDYPQKSLSTNNFKKTNILMGSNSEEGYYFIFYYLTELFKKEENVVV
ace1(S)   AFLDDYPQKSLSTNNFKKTNILMGSNSEEGYYSIFYYLTELFKKEENVVV
         351                                                400
ace1(R)   SRENFVKAIGQLNPNADAAVKSAIEFEYTDWFSPNDPEKNRNALDKMVGD
ace1(S)   SRENFVKAIGQLNPNADAAVKSAIEFEYTDWFSPNDPEKNRNALDKMVGD
         401                                                450
ace1(R)   YQFTCNVNEFAHKYALTGNNVYMYYFKHRSLNNPWPKWTGVMHGDEISYV
ace1(S)   YQFTCNVNEFAHKYALTGNNVYMYYFKHRSLNNPWPKWTGVMHGDEISYV
         451                                                500
ace1(R)   FGDPLNPNKRYEIEEIELSKKMMRYWTNFAKTGNPSKTFEGSWVTPKWPV
ace1(S)   FGDPLNPNKRYEIEEIELSKKMMRYWTNFAKTGNPSKTFEGSWVTPKWPV
         501                                                550
ace1(R)   HTAYGKEFLTLDTNNTSIGVGPRLEQCAFWKNYVPDLMAISKSMKSDKNC
ace1(S)   HTAYGKEFLTLDTNNTSIGVGPRLEQCAFWKNYVPDLMAISKSMKSDKNC
         551                  578
ace1(R)   TTISGGTKTYMIELSLWTIVMTTAVLML
ace1(S)   TTISGGTKTYMIELSLWTIVMTTAVLML
```

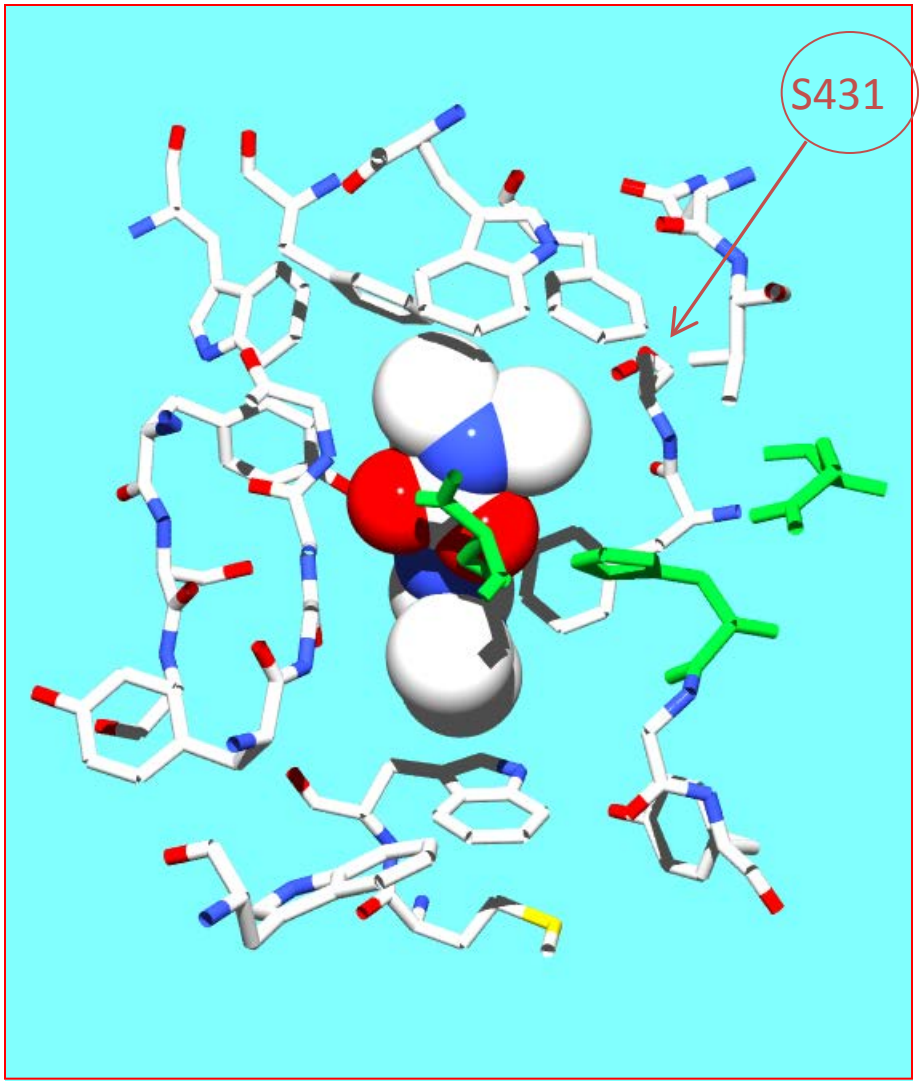


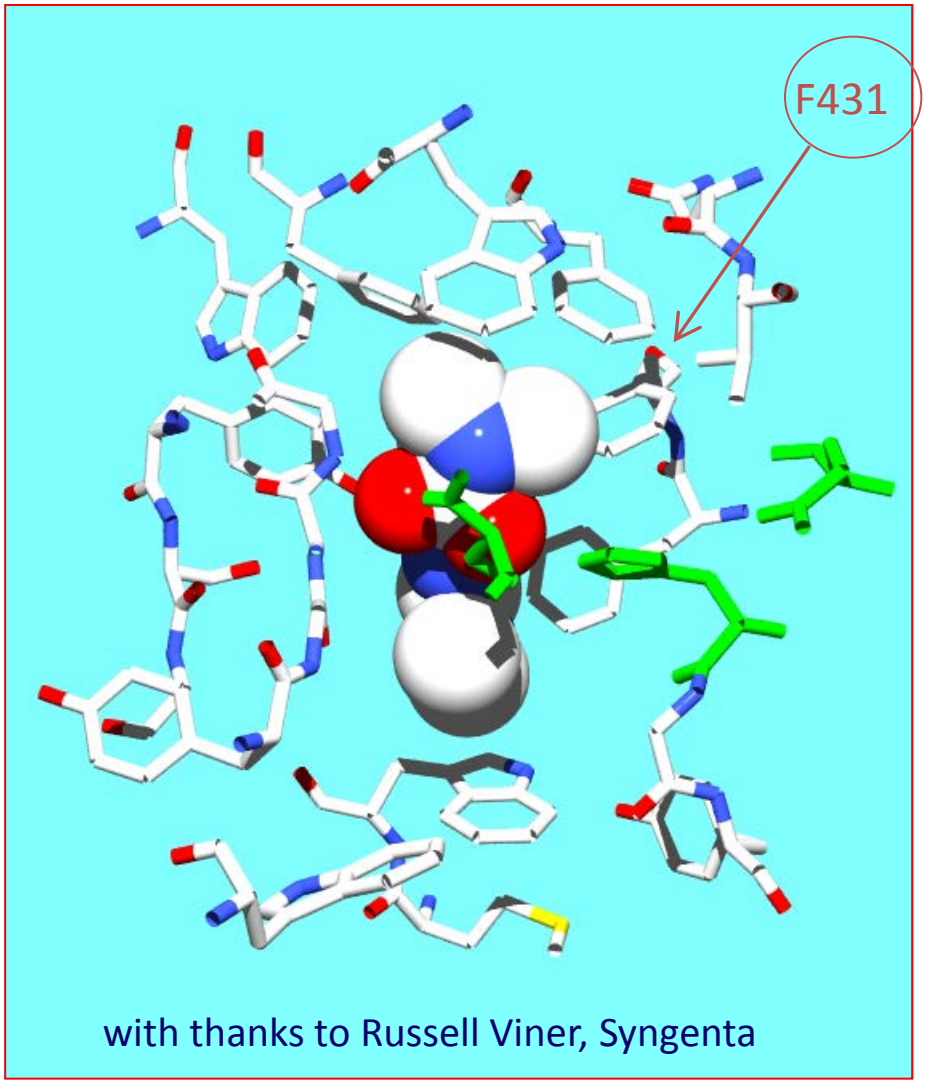
NB only aphids have a serine at residue 431, other insects and mammals have a phenylalanine.

Does this explain aphid selectivity for dimethylcarbamates?

Nabeshima et at 2003, Andrews et al 2004

Modelling the effect of the S431F mutation on pirimicarb binding

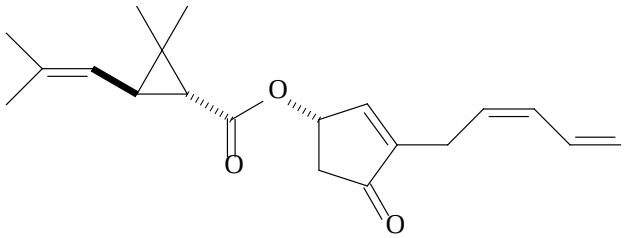


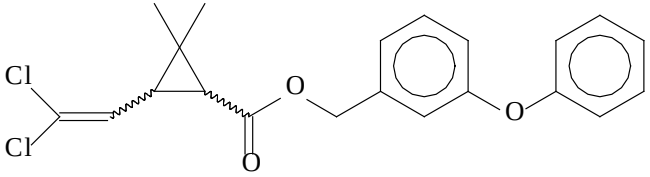


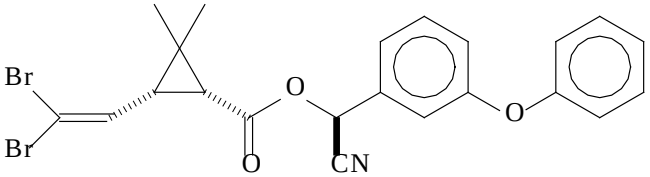
with thanks to Russell Viner, Syngenta

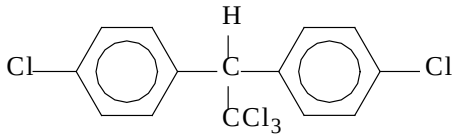
shows that it is possible to ‘design’ pest-specific compounds

Natural pyrethrins & synthetic pyrethroids



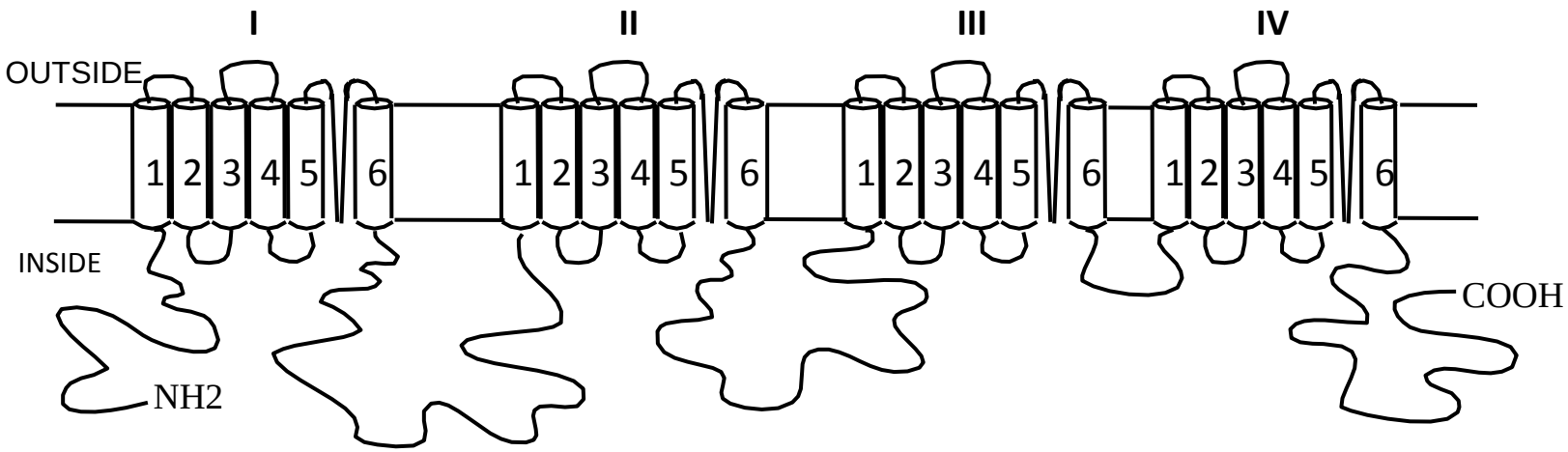


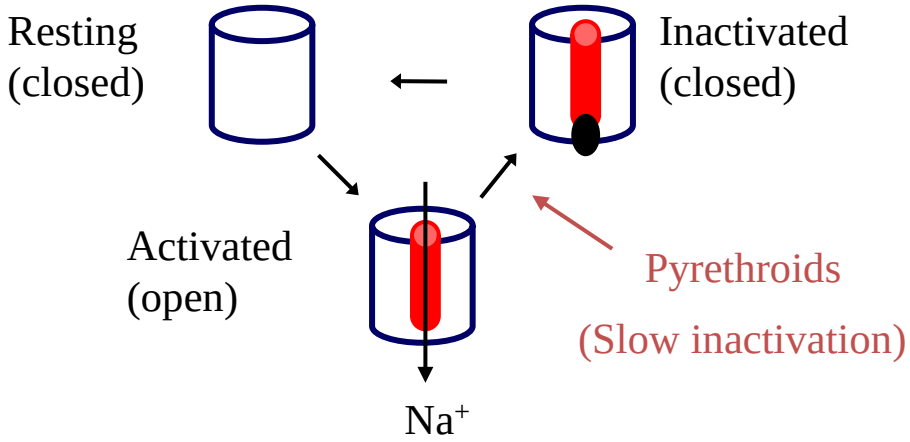




- Rothamsted pyrethroids still account for ~50% of the pyrethroid market
- over $30 billion dollar sales over patent life

Voltage-gated Sodium Channel



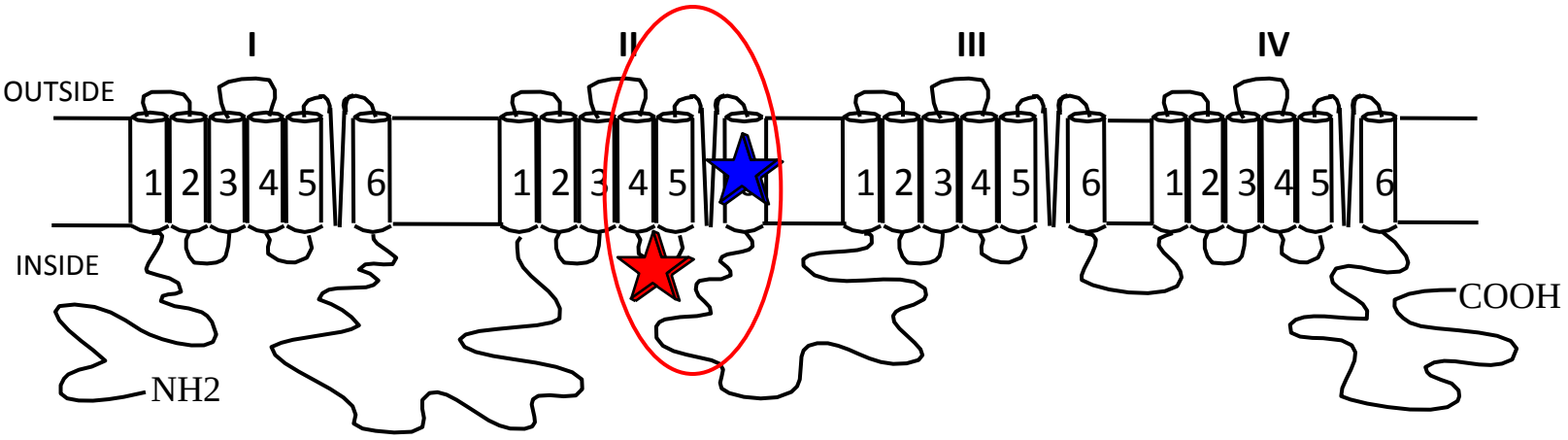


Resistance to Pyrethroids
- knockdown resistance (kdr)

- Reduced sensitivity to DDT was recognised in 1950s in housefly and termed “knockdown resistance” (*kdr*).

- Allelic forms of *kdr* include super-kdr (*s-kdr*) which confers up to 500-fold resistance to deltamethrin. *kdr* and *s-kdr* are recessive traits mapping to chromosome 3 in houseflies.

Sodium channel mutations in *Musca domestica*



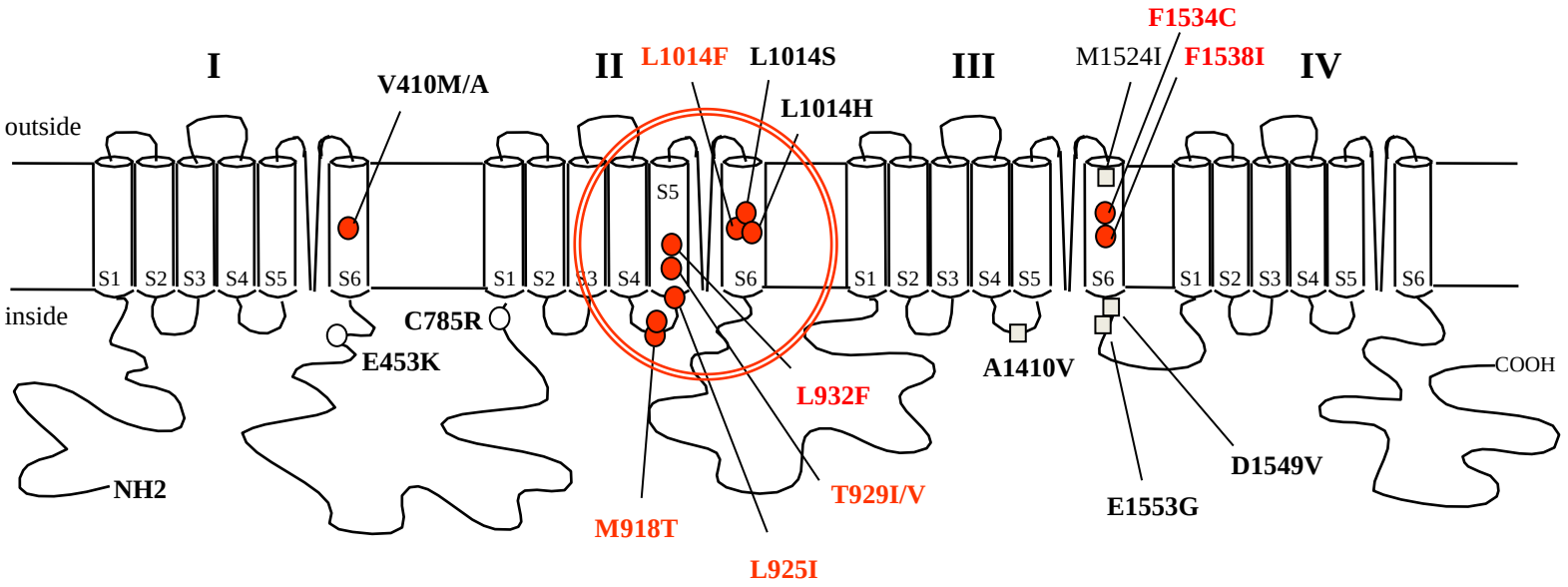
Leu 1014 to Phe (kdr mutation)

Met 918 to Thr (super-kdr mutation)

Williamson et al. (1996) *Molecular & General Genetics 252*, 51-60.

ROTHAMSTED RESEARCH

Current range of Na channel mutations implicated in resistance

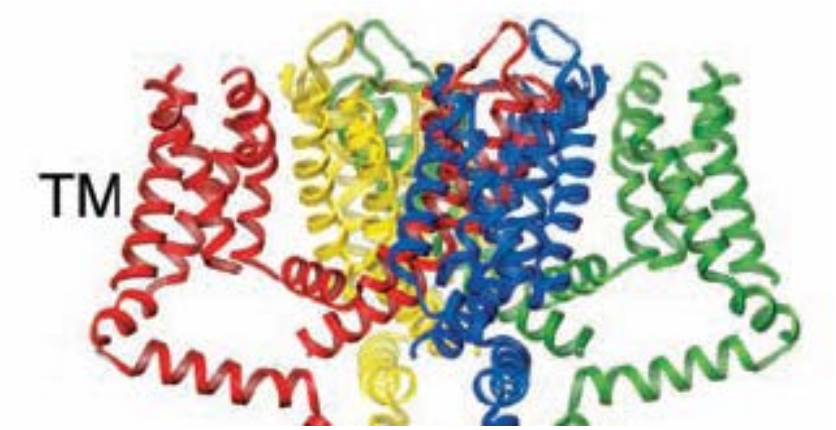


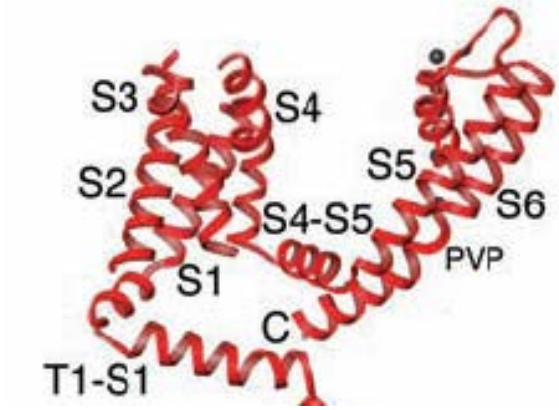
Aphis gossypii
Bemisia tabaci
Blattella germanica
Cydia pomonella
Drosophila melanogaster
Frankliniella occidentalis
Helicoverpa armigera
Heliothis virescens
Liriomyza huidobrensis
Liriomyza sativae
Leptinotarsa decemlineata

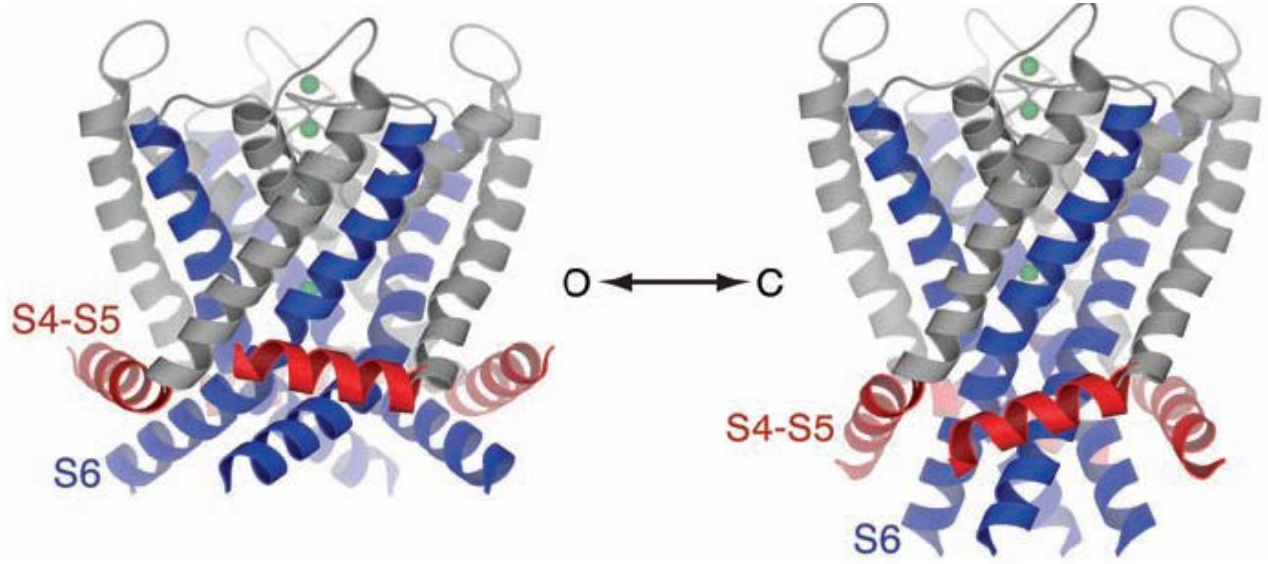
Meligethes aeneus
Myzus persicae
Plutella xylostella
Sitophilus zeamais
Spodoptera littoralis
Tetranychus evansi
Tetranychus urticae
Thrips tabaci
Triatoma infestans
Tuta absoluta
Trialeurodes vaporariorum

Aedes aegypti
Anopheles gambiae
Blattella germanica
Boophilus microplus
Cimex lectularius
Ctenocephalides felis
Culex pipiens
Hematobia irritans
Musca domestica
Pediculus capitis
Sarcoptes scabiei
Stomoxys calcitrans

Crystal Structure of Kv1.2 Shaker Voltage-gated Potassium Channel

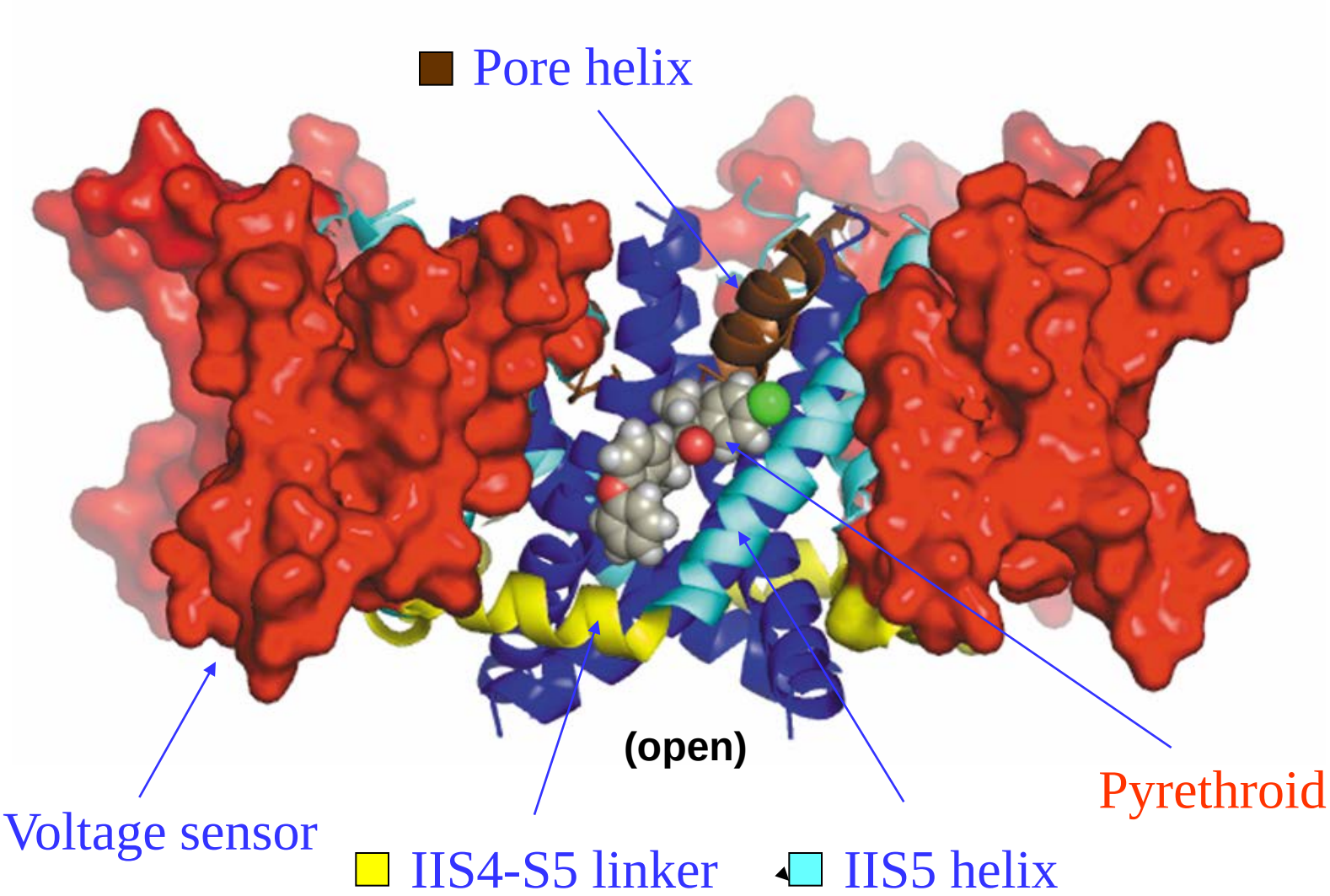






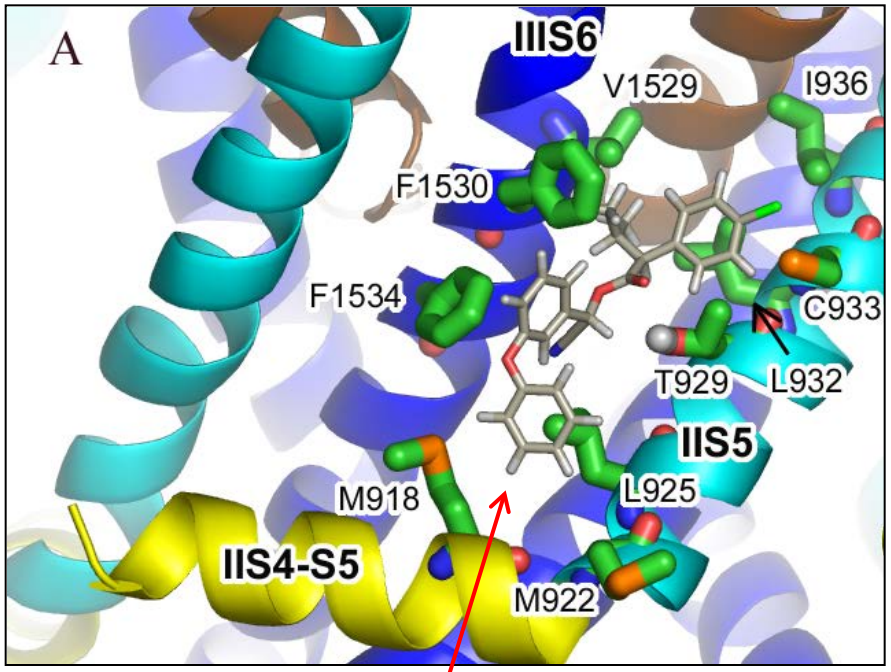
Long et al (2005) Science 309, 897-903

Homology model for housefly sodium channel

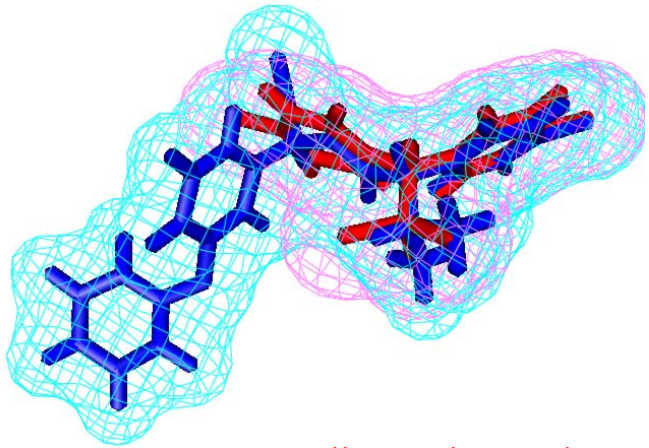


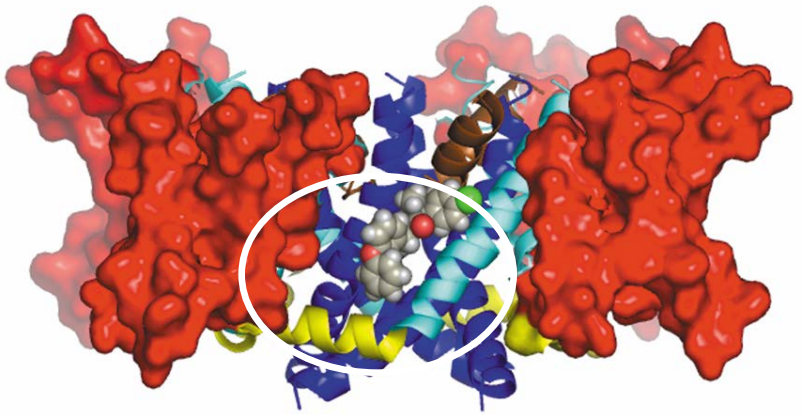
O'Reilly et al. Biochemical Journal (2006) 396, 255-263.

Identifying a binding site for pyrethroids & DDT

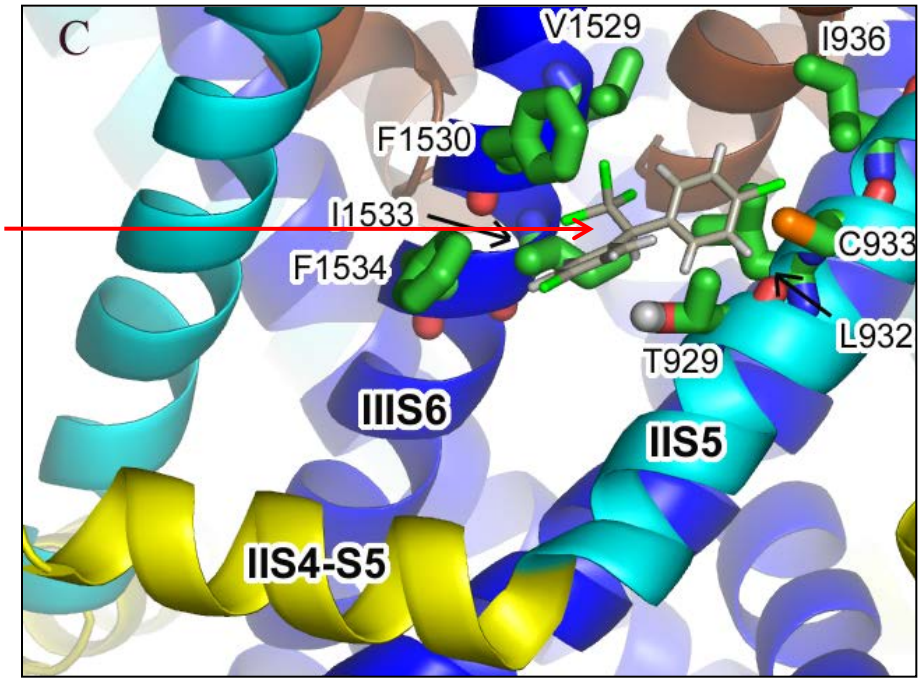


Fenvalerate





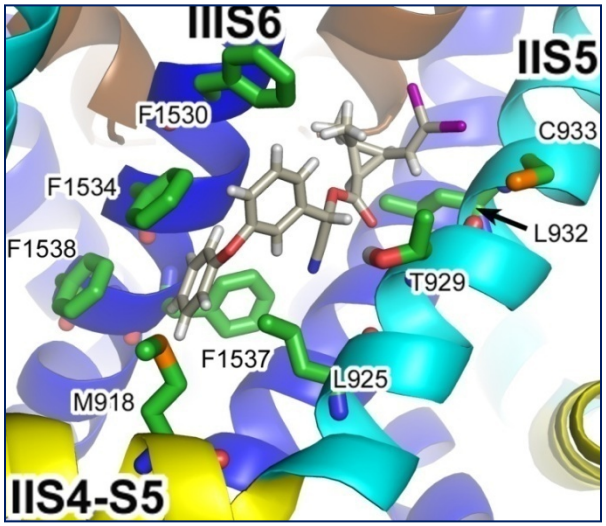
DDT

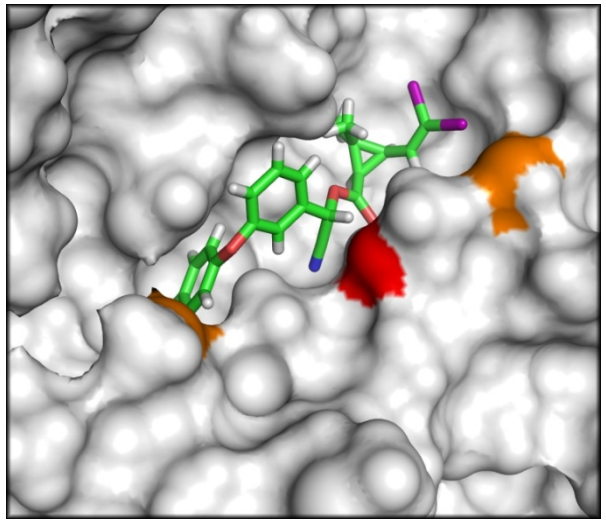


O'Reilly et al. Biochemical Journal (2006) 396, 255-263.

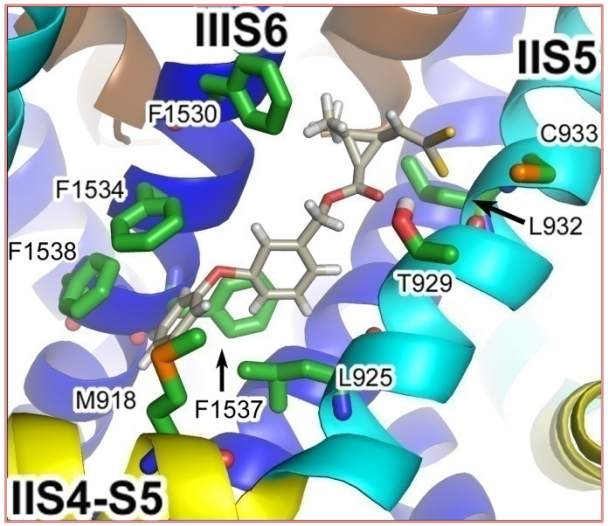
Docking studies with pyrethroids & DDT

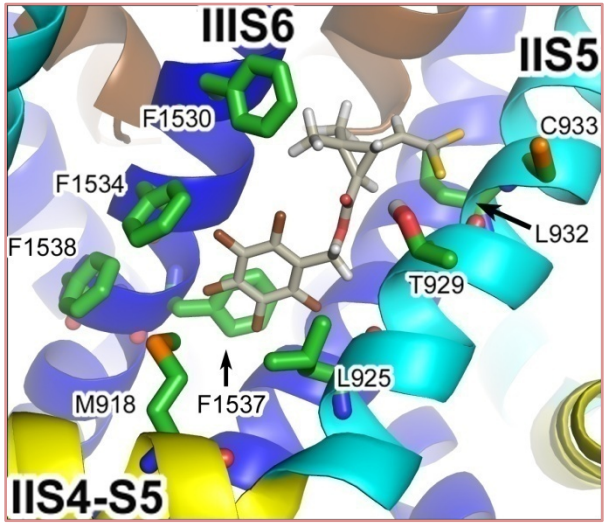
Deltamethrin



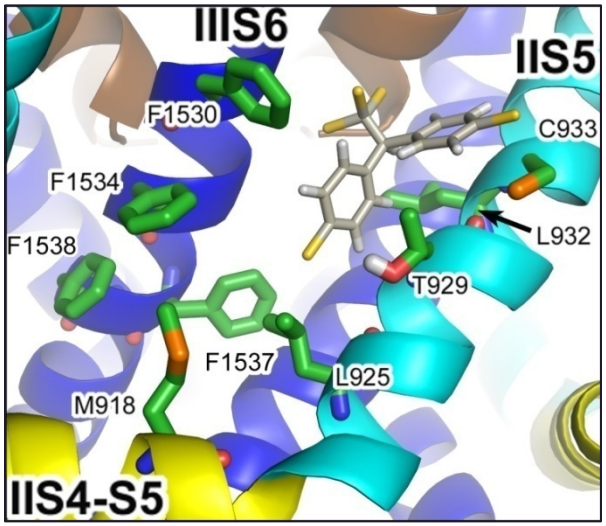


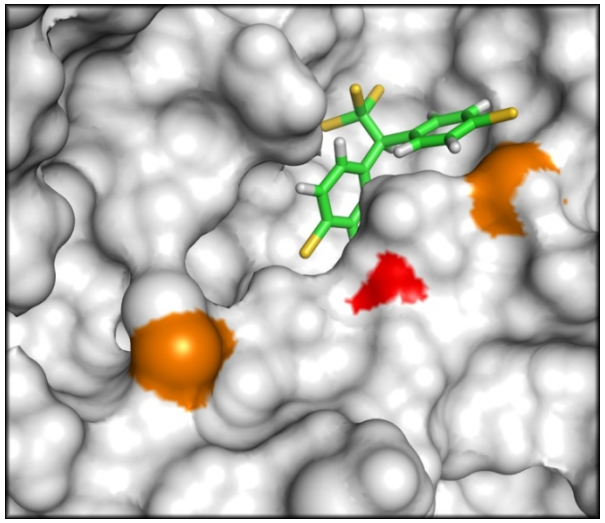
Permethrin





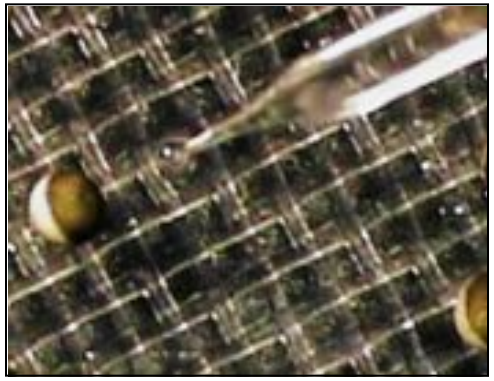
DDT

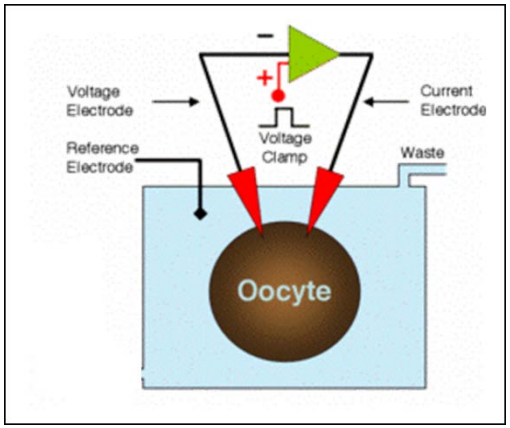


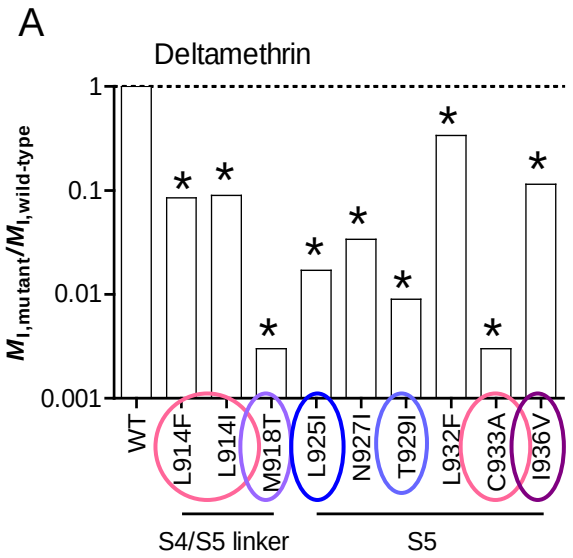


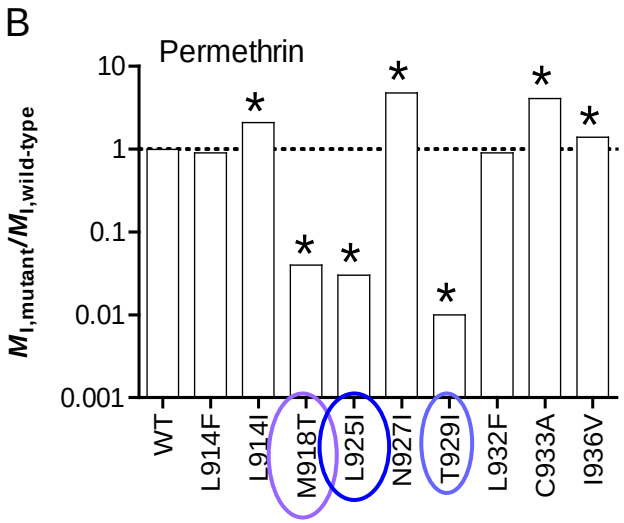
Fenfluthrin

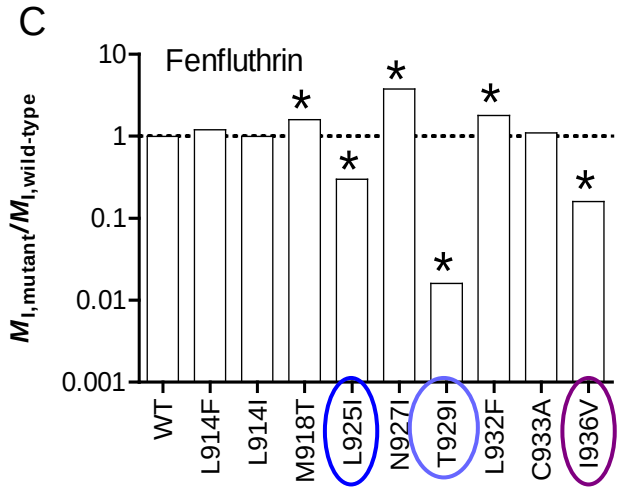
Testing the model experimentally

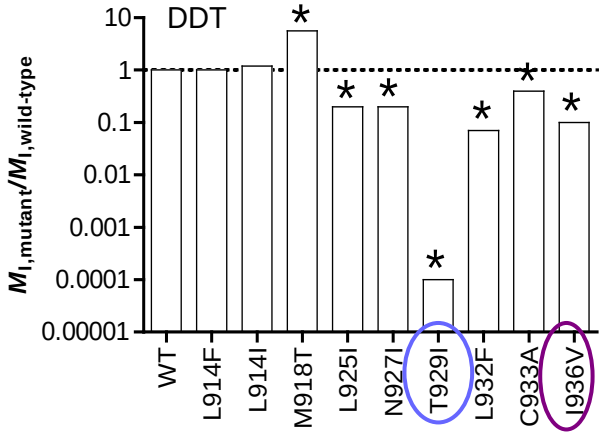












Usherwood et al. (2009) *FEBS Letters*

Summary

We have developed a homology model of the housefly sodium channel and used it to predict a binding site for pyrethroids and DDT.

The model address a number of key features of the known action of pyrethroids:

1. The binding site is located in a hydrophobic cavity delimited by the IIS4-S5 linker and the IIS5/IIIS6 helices that is accessible to lipid-soluble insecticides.
2. The binding site is formed during activation (opening) of the sodium channel and is consistent with observations that pyrethroids bind preferentially to open channels.
3. The binding of pyrethroids is predicted to stabilise the open state of the channel and is consistent with pyrethroid-induced tail currents observed following membrane repolarisation.
4. DDT occupies a restricted area of the binding cavity and the reduced number of contacts explains the lower potency of this compound compared to pyrethroids.
5. The binding pocket includes several known mutation sites in the IIS4-S5 linker, IIS5 helix and IIIS6 helix that cause reduced sensitivity to pyrethroids/DDT in resistant insect strains.
6. The model identifies other key residues in these helices that are likely to contribute to the insect/mite selectivity of these compounds.

Target-site resistance to neonicotinoids

IRAC MoA Classification v 7.2, February 2012[1]		
Main Group and Primary Site of Action	**Chemical Sub-group or exemplifying Active Ingredient**	**Active Ingredients**
4 **Nicotinic acetylcholine receptor (nAChR) agonists** Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects}	**4A** Neonicotinoids	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam,
	4B Nicotine	Nicotine
	4C Sulfoxaflor	Sulfoxaflor

Nicotinic acetylcholine receptor

- target for neonicotinoid insecticides

Vertebrates: 17 subunit genes

muscle-type: $\alpha_2\beta\gamma\delta$

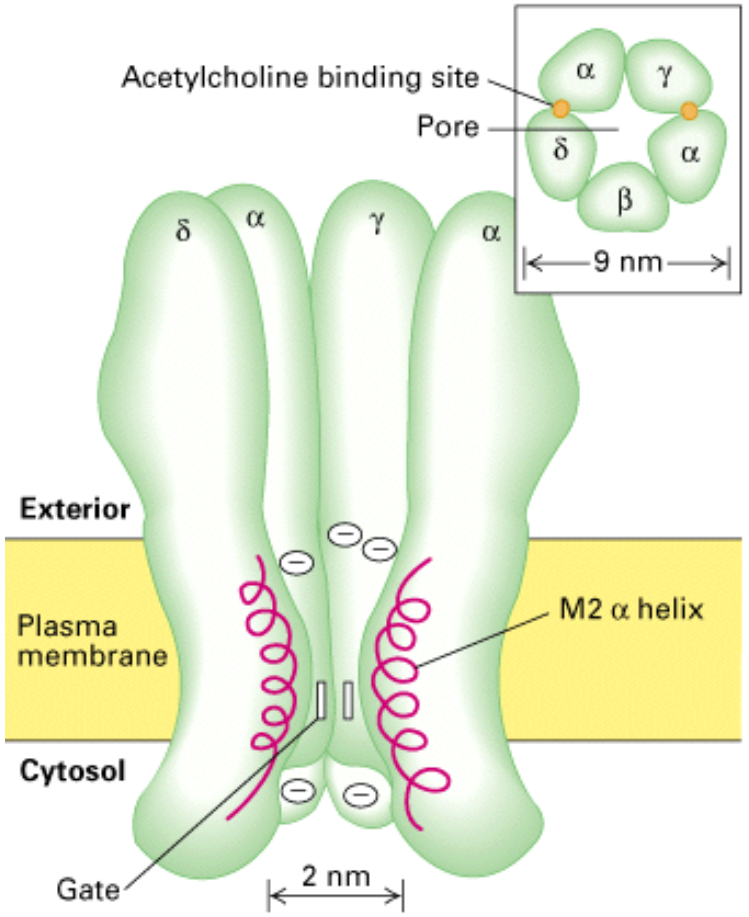
neuronal: various types

(hetero/homo pentamers)

Invertebrates: 10+ subunit genes

neuronal: various types?

Mature muscle receptor is a hetero-pentamer of non-identical subunits



Target-site resistance in brown planthopper

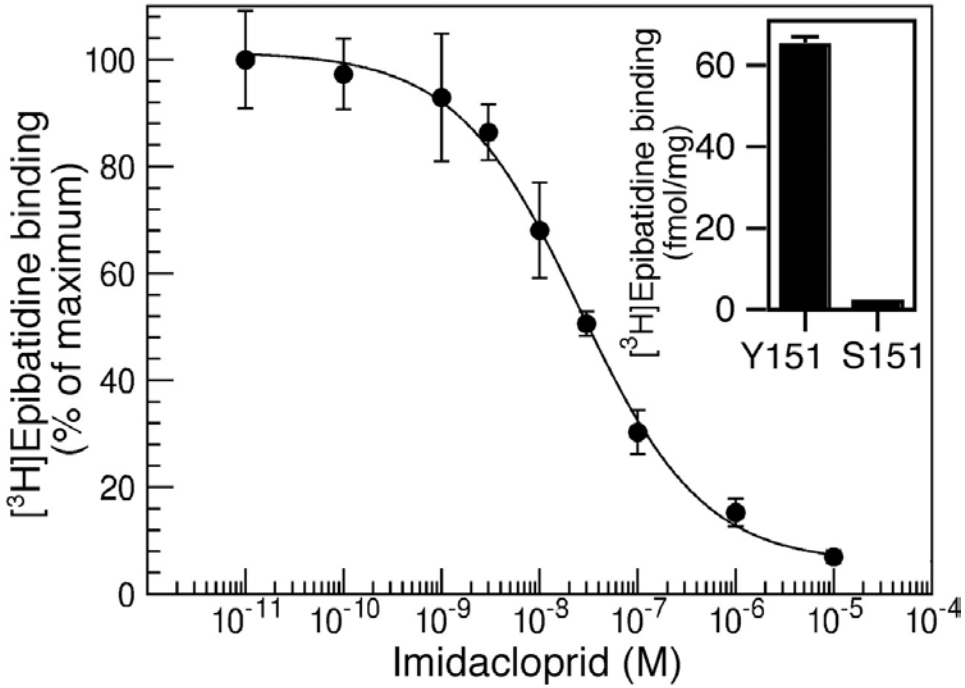
PNAS

A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper)

Zewen Liu*, Martin S. Williamson†, Stuart J. Lansdell‡, Ian Denholm†, Zhaojun Han*§, and Neil S. Millar‡

*Key Laboratory of Monitoring and Management of Plant Diseases and Insects, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China; †Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom; and ‡Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, United Kingdom

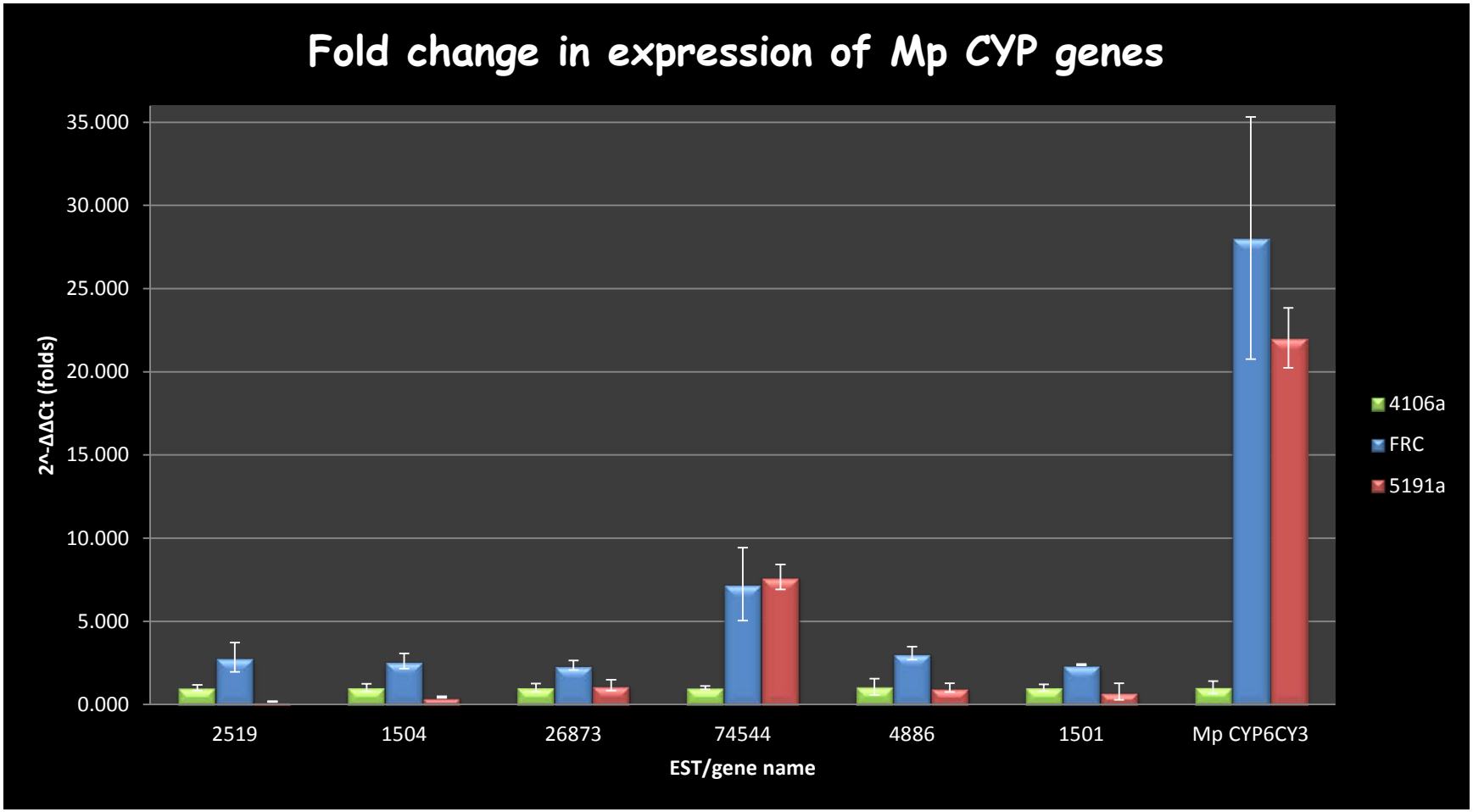
Communicated by John E. Casida, University of California, Berkeley, CA, April 18, 2005 (received for review September 24, 2004)



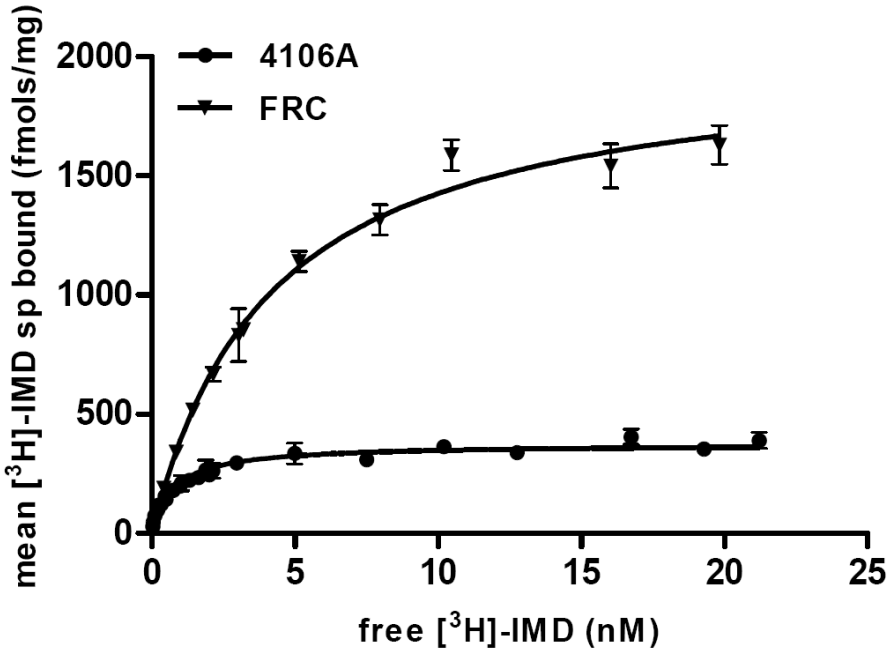
Imidacloprid resistance in field collected *Myzus persicae*

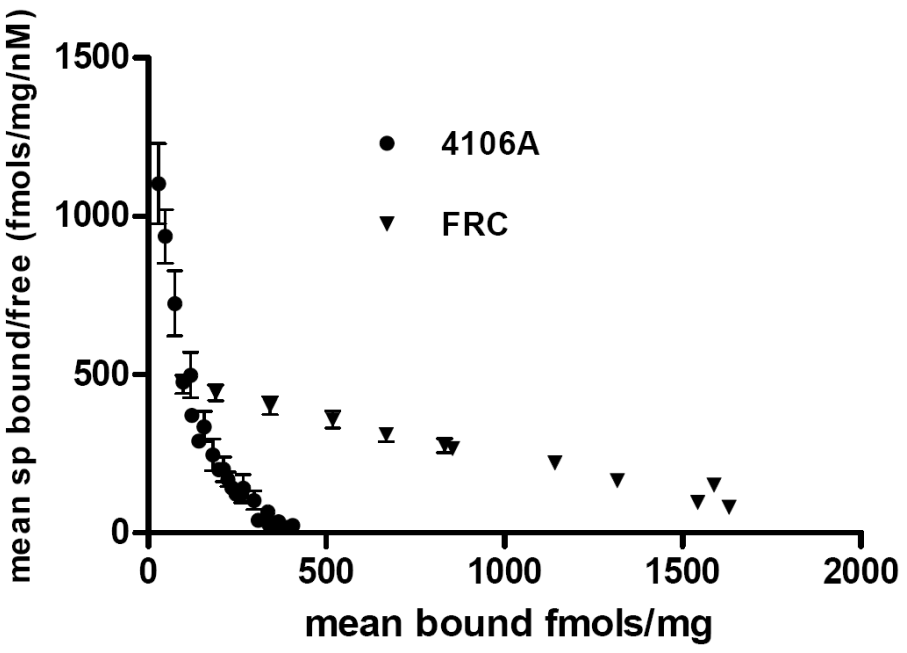
Microarray Analysis

Description	Fold change	Parent Sequence ID	Probe name
carboxylesterase; esterase E4	290.98	contig3118	M_persicae3118a
LOC100168312 similar to carboxylesterase; esterase FE4 [Acyrthosiphon pisum]	127.91	454Myzus_30192	CUST_8482_PI410703081
carboxylesterase; esterase E4	62.07	contig720	M_persicae720a
carboxylesterase; esterase FE4	55.25	contig9215	M_persicae9215a
carboxylesterase; esterase E4	43.60	contig720	M_persicae720b
carboxylesterase; esterase E4	42.39	contig4586	M_persicae4586a
cytochrome p450	13.60	contig749	M_persicae749a
cytochrome p450	13.26	contig749	M_persicae749b
cytochrome p450	11.01	contig497	M_persicae497b
cytochrome p450	10.40	contig497	M_persicae497a
LOC100168115 similar to cytochrome P450 CYP6AX1 protein [Acyrthosiphon pisum]	10.18	454Myzus_77428	CUST_29688_PI410703081
cytochrome p450	9.15	contig5173	M_persicae5173a
ref|XM_001952692.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 (LOC100163313)	4.36	454Myzus_27444	CUST_7241_PI410703081
ref|XM_001947885.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein (LOC100160895)	2.83	454Myzus_26873	CUST_6689_PI410703081
ref|XP_001944530.1| PREDICTED: similar to cytochrome P450 4A7 [Acyrthosiphon pisum]	2.78	contig2519	M_persicae2519a
ref|XM_001944991.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 4A7 (LOC100162710)	2.70	454Myzus_27229	CUST_7033_PI410703081
ref|XM_001944991.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 4A7	2.67	contig1504	M_persicae1504a
ref|XM_001944495.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 4A7 (LOC100158738)	2.65	454Myzus_82930	CUST_33643_PI410703081
ref|XM_001944991.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 4A7	2.55	contig1504	M_persicae1504b
ref|XM_001947885.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein (LOC100160895)	2.55	contig643	M_persicae643a
ref|XM_001947885.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein (LOC100160895)	2.52	DW362118.1	CUST_63_PI304494170
ref|XM_001947885.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein (LOC100160895)	2.40	contig643	M_persicae643b
ref|XM_001948546.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein	2.35	454Myzus_74544	CUST_27625_PI410703081
ref|XM_001948386.1| PREDICTED: Acyrthosiphon pisum similar to cytochrome P450 CYP6AX1 protein	2.28	contig4886	M_persicae4886a
ref|NM_001163211.1| Acyrthosiphon pisum cytochrome P450 protein	2.10	contig1501	M_persicae1501a


Fold change in expression of Mp CYP genes
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
2^-ΔΔCt (folds)
2519
1504
26873
74544
4886
1501
Mp CYP6CY3
EST/gene name
4106a
FRC
5191a

Comparative saturation isotherms of 3H-Imidacloprid binding



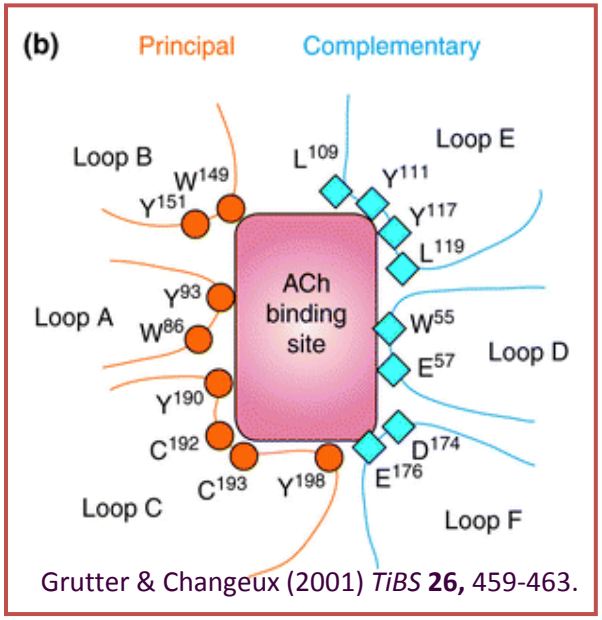


Clone	High affinity		Low affinity		
	kd (nM)	Bmax (fmols/mg)	kd (nM)	Bmax (fmols/mg)	Hill value (n_H)
FRC	-	-	4.14	2016	1
4106A	0.083	100	1.7	298	0.78

FRC contains a single point mutation (R81T) in the Mpβ1 subunit

	α-type subunits							β	α/β	α/β
	*	*	*							
Drosophila melanogaster	Dα1 (ALS)	Dα2 (SAD)	Dα3	Dα4	Dα5	Dα6	Dα7	Dβ1 (ARD)	Dβ2 (SBD)	Dβ3
Myzus persicae	Mpα2	Mpα1	Mpα3	Mpα4		Mpα7-2	Mpα7-1	Mpβ1		

	Amino Acid Number of *Myzus persicae β1* Subunit							
Species	77	78	79	80	81	82	83	84
Homo sapiens β2	N	V	W	L	**T**	Q	E	W
Gallus gallus β2	N	V	W	L	**T**	Q	E	W
Rattus norvegicus β2	N	V	W	L	**T**	Q	E	W
Drosophila melanogaster β1	C	V	W	L	**R**	L	V	W
Anopheles gambiae β1	N	V	W	L	**R**	L	V	W
Bemisia tabaci β1	N	V	W	L	**R**	L	V	W
Locusta migratoria β1	N	V	W	L	**R**	L	V	W
Heliothis virescens β1	N	V	W	L	**R**	L	V	W
Ctenocephalides felis β1	N	V	W	L	**R**	L	V	W
Myzus persicae 4106A β1	N	V	W	L	**R**	L	V	W
Myzus persicae 5191A β1	N	V	W	L	**R**	L	V	W
Myzus persicae FRC β1	N	V	W	L	**T**	L	V	W



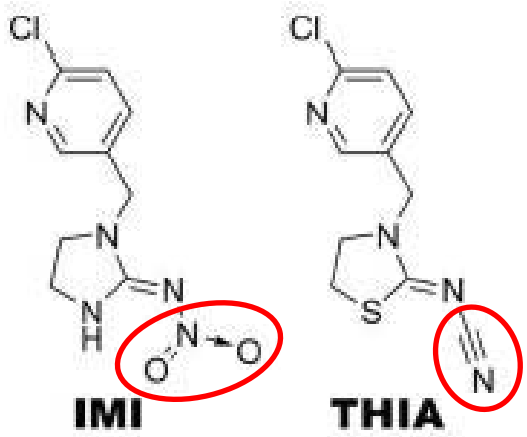
Grutter & Changeux (2001) *TiBS* **26,** 459-463.

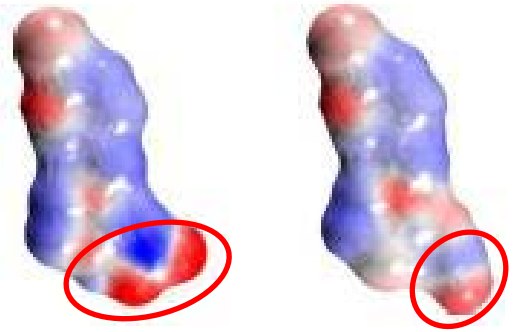
Loop D

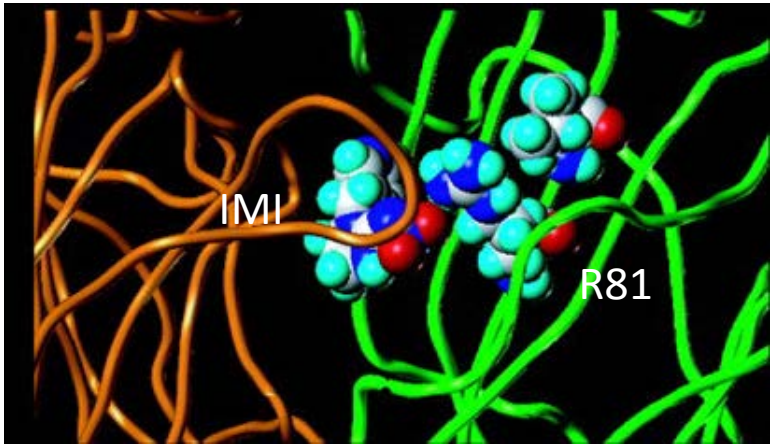
Bass C, Puinean AM, Andrews M, Cutler P, Daniels M, Elias J, Paul VL, Crossthwaite AJ, Denholm I, Field LM, Foster SP, Lind R, Williamson MS, Slater R (2011) Mutation of a nicotinic acetylcholine receptor β subunit is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *BMC Neurosicence* 12:51.

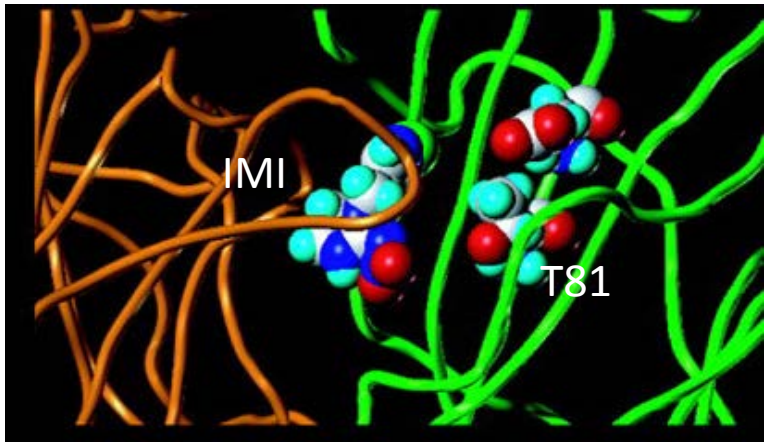
Neonicotinoid insecticides – Insect selectivity

electronegative pharmacophore (nitro/cyano group) of neonicotinoids









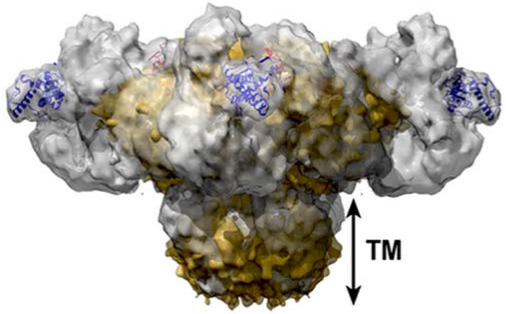
Shimomura et al. (2006) Role in the selectivity of neonicotinoids of insect-specific basic residues in loop D of the nicotinic acetylcholine receptor agonist binding site. Mol Pharmacol 70:1255-63.

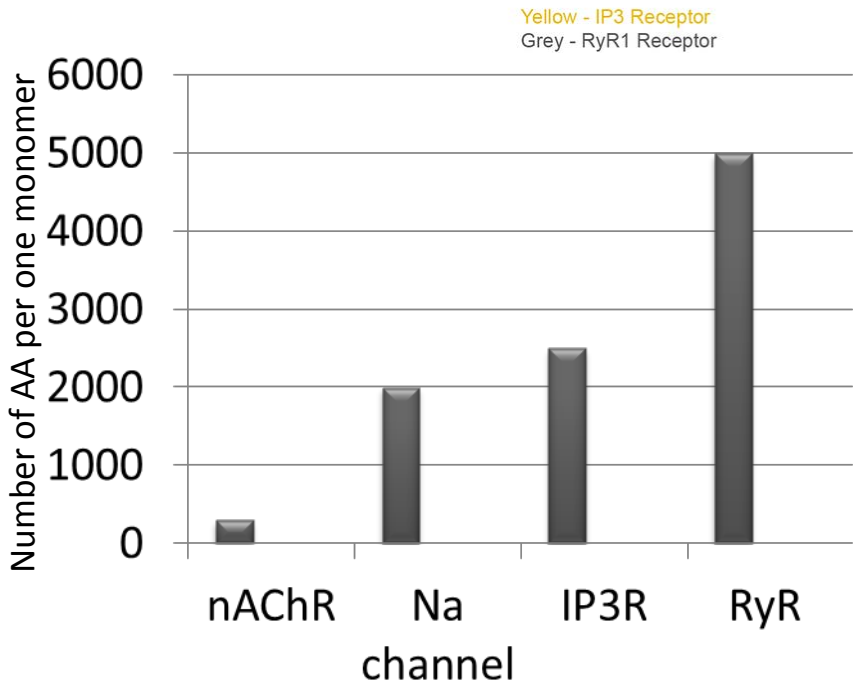
Target-site resistance to diamide insecticides

IRAC MoA Classification v 7.2, February 2012[1]		
Main Group and Primary Site of Action	**Chemical Sub-group or exemplifying Active Ingredient**	**Active Ingredients**
28 **Ryanodine receptor modulators** Nerve and muscle action {Good evidence that action at this protein complex is responsible for insecticidal effects}	Diamides	Chlorantraniliprole, Cyantraniliprole, Flubendiamide

Ryanodine receptors (RyRs)

- Ryanodine receptor is a ER/SR calcium release channel
- There are 3 genes coding for different isoforms in mammals; other vertebrates have 2 isoforms.
- Invertebrates have only one gene encoding RyR
- RyR cDNA is >15 Kb
- Functional channel is formed of 4 monomers each consisting of approximately 5000 amino acids
- Approx. 47% homology between mammalian and insect channels





Sharma, P., et al., *Structural determination of the phosphorylation domain of the ryanodine receptor.* The FEBS journal, 2012. **279**(20): p. 3952-64.

Diamide Resistant *Plutella*

- Two populations of highly resistant *Plutella* from south east Asia screened for mutations associated with resistance
  - Thailand strain (ThaiR) – Syngenta Crop Science
  - Philippines strain (Sudlon) – Bayer Crop Protection
- Partial sequencing done covering entire transmembrane region and diamide sensitivity region described in Silkworm RyR by Kato *et al* 2009

Troczka B, Zimmer CT, Elias J, Schorn C, Bass C, Davies TGE, Field LM, Williamson MS, Slater R, Nauen R (2012) Resistance to diamide insecticides in diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. *Insect Biochemistry and Molecular Biology* 42, 873-80.

Bioassay results

Bioassay experiments done by members of Bayer Crop Science and Syngenta Crop Protection

Log-dose probit-mortality data for two diamide insecticides tested against 2nd/3rd instar larvae of four strains of *P. xylostella* in a leaf-dip bioassay (72h).

Compound	**Strain**	**LC_{50}-value [mg/L^{-1}]**	**95% FL[a]**	**Slope (±SD)**	**RR[b]**
Chlorantraniliprole	HS	0.048	0.026-0.15	1.3 ± 0.068	-
	Sudlon	>200	-	-	>4100
	Thai S	0.30	0.25 - 0.38	5.1 ± 0.83	-
	Thai R	>60	-	-	>200
Flubendiamide	HS	0.15	0.086-0.30	3.8 ± 0.30	-
	Sudlon	>200	-	-	>1300
	Thai S	0.08	0.06 - 0.11	3.8 ± 0.59	-
	Thai R	>60	-	-	>750

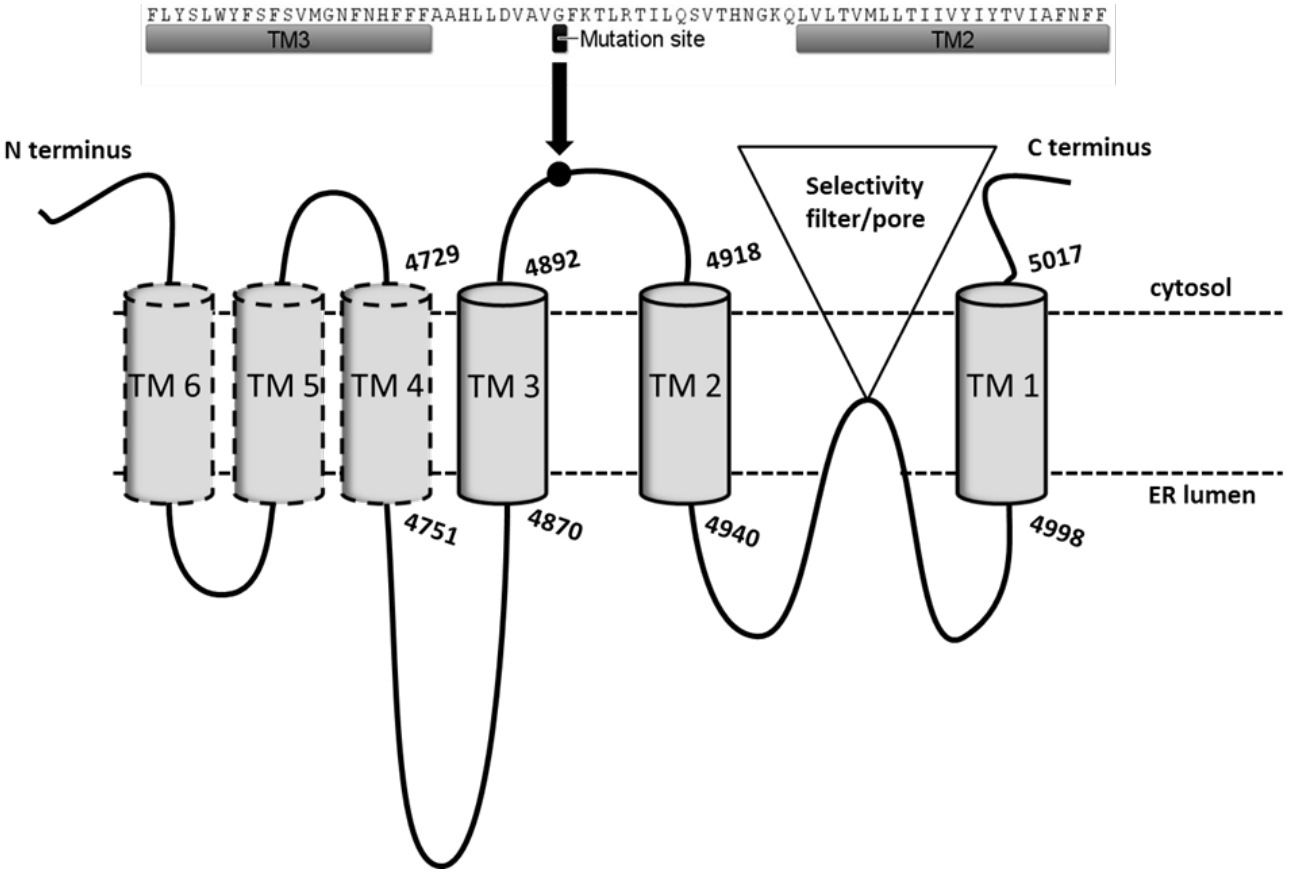
[a]95% Fiducial limits, [b]RR=resistance ratio (LC_{50} of strain Sudlon or ThaiR divided by LC_{50} of strain HS or ThaiS, respectively)

Sequencing results

Sequencing of transmembrane region and a small portion of N-terminus revealed a mutation resulting in amino acid change G to E at a position 4946. The mutated coding triplet varies between the Sudlon and Thai R strain:

HS/Thai S	**GGG**
Sudlon	**GAA**
ThaiR	**GAG**

Mapping the mutation onto channel structure



Summary – the study of target-site resistance

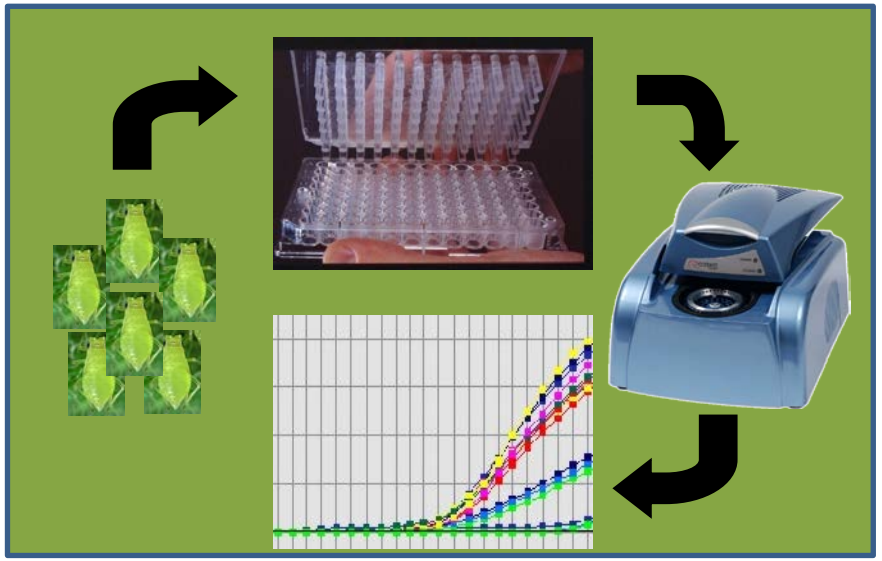
- Develop mutation-based assays for resistance monitoring

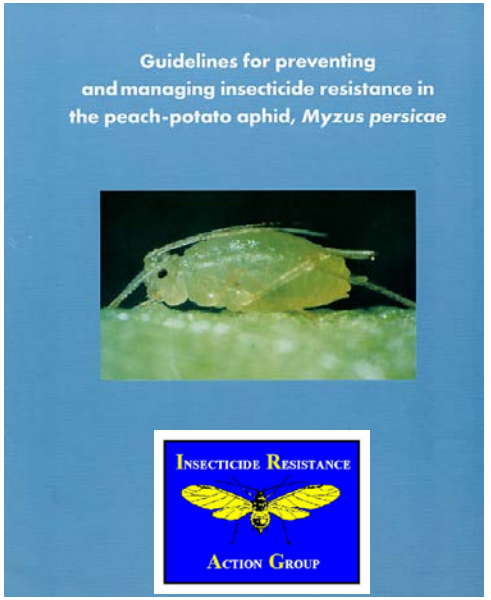
- Study insecticide / target interactions

- Investigate species selectivity

- Further design opportunities?

- Target-based screening for new compounds





Acknowledgments

Emyr Davies, Bartek Troczka, Alin Puinean, Steve Foster, Ian Denholm, Lin Field

Christoph Zimmer, Corinna Schorn, Ralf Nauen

Melanie Andrews, Penny Cutler, Miriam Daniels, Jan Elias Verity Paul, Andrew Crossthwaite, Rob Lind, Russell Slater

Andy O'Reilly, Bonnie Wallace

Ian Mellor, Ian Duce, Peter Usherwood