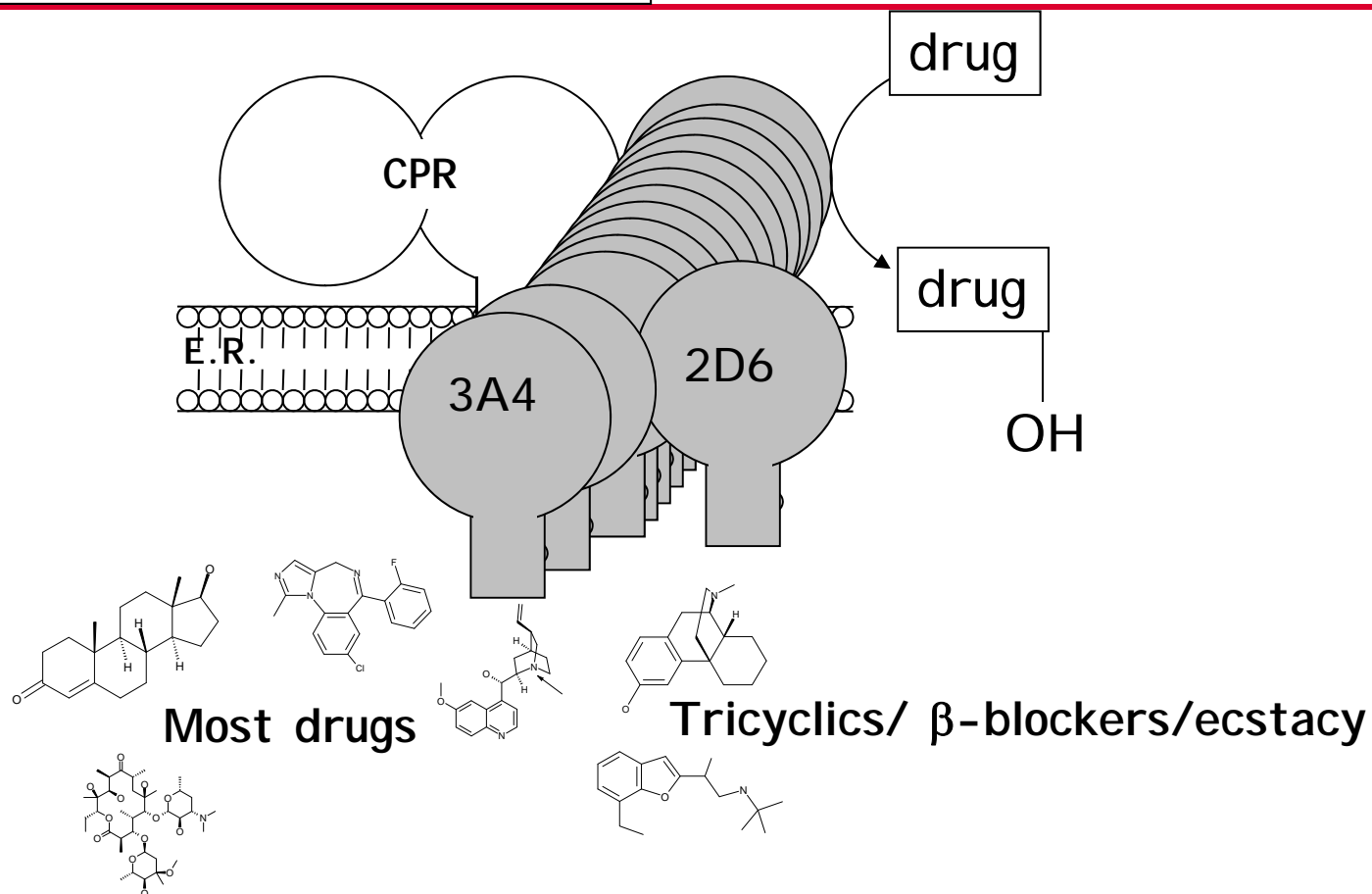**LSTM**
LIVERPOOL SCHOOL
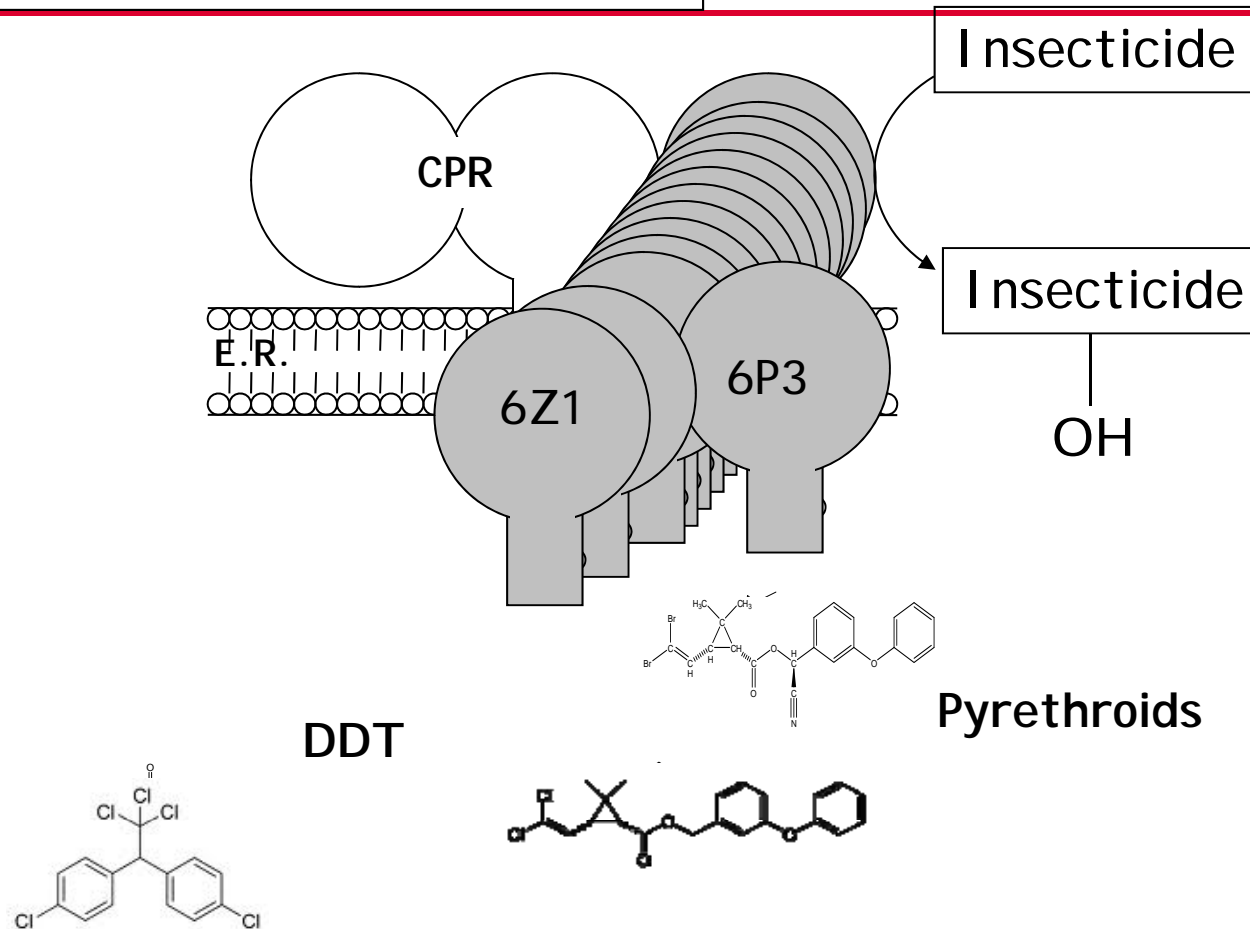OF TROPICAL MEDICINE

Forewarned is forearmed:

development of pyrethroid activity based protein profiling probes (PyABPs) to predict insecticide resistance liabilities

Mark J.I. Paine , Hanafy M. Ismail, Janet Hemingway , David Hong, Paul M. O`Neill,

Drug Discovery: The first Commandment
Thou shalt determine if your drug is metabolised by P450

CPR

drug

drug

OH

E.R.

3A4   2D6

Most drugs

Tricyclics/ β–blockers/ecstacy

Insecticide

CPR

Insecticide

E.R.

6Z1   6P3

OH

DDT

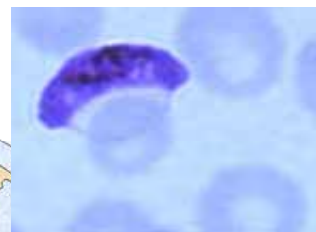Pyrethroids

Malaria remains a global problem
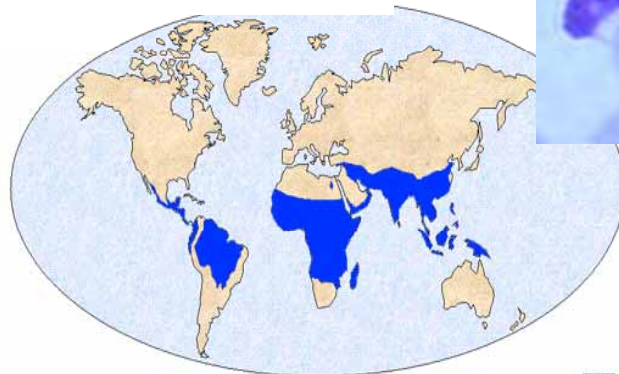
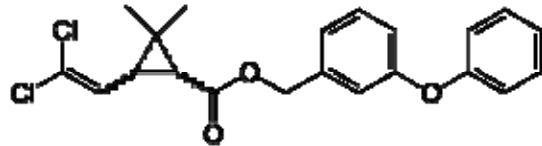Reservoir → **Pathogen** → Vector → 'host'

Distribution of malaria

- 400 – 900 m cases
- 3 m people die
- Mainly children
- Tropical Regions
- Perpetuates poverty

Bednets are frontline tools for malaria control BUT only pyrethroids can be used

RESISTANCE MAP

Key:
- ○ An. funestus
- ◎ An. gambiae s.l.
- △ An. arabiensis
- □ An. gambiae s.s.
- ■ L1014F
- ■ L1014S
- ■ L1014F + L1014S
- · Esterase and/or P450

- > 50 m nets distributed in Africa
- Provides personal and community protection
- long lasting, low toxicity, fast acting
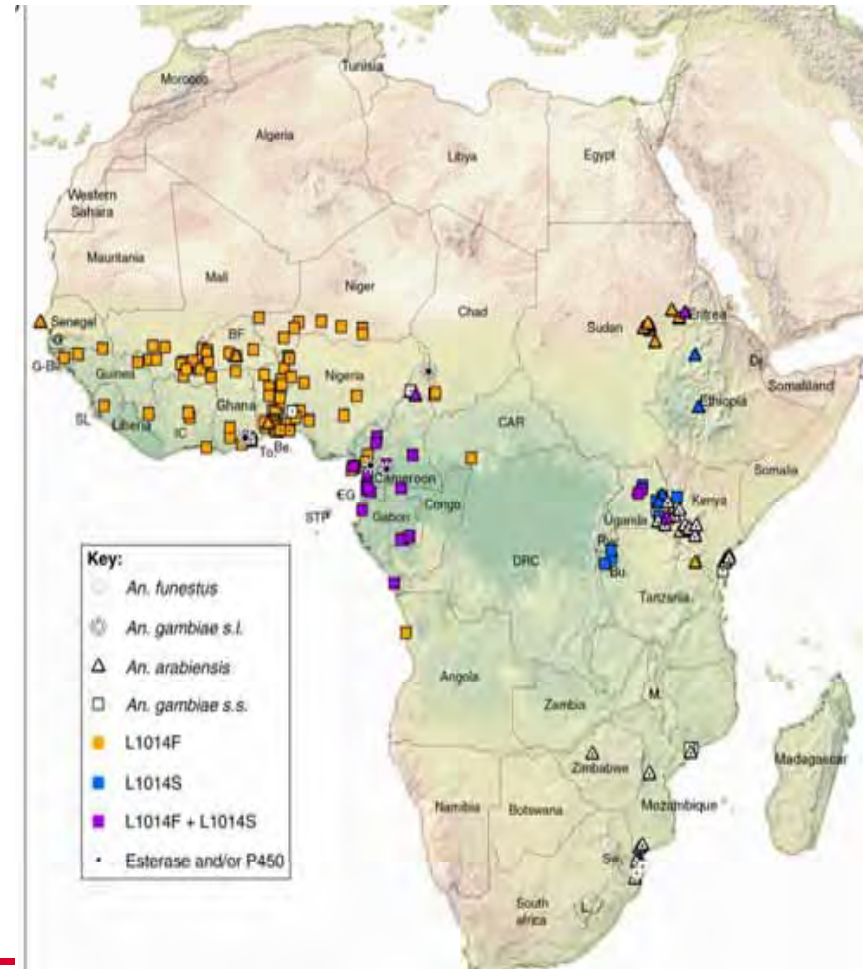
Source: Ranson et al. 2010

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DDT Photo's kindly provided by Dr Vijay Kumar, RMRI, Patna

DDT Spray operation
Preparing DDT mix — Stirrup pump — Measuring 6ft limit — Spraying

Insecticide	Class	Target	IRS	ITN
Alpha-cypermethrin	Pyrethroid	Sodium channel	✓	✓
Bifenthrin	Pyrethroid	Sodium channel	✓	
Cyfluthrin	Pyrethroid	Sodium channel	✓	✓
Deltamethrin	Pyrethroid	Sodium channel	✓	✓
Etofenprox	Pyrethroid	Sodium channel	✓	✓
Lambda-cyhalothrin	Pyrethroid	Sodium channel	✓	✓
Permethrin	Pyrethroid	Sodium channel		✓
DDT	Organochlorine	Sodium channel	✓	
Malathion	Organophosphate	ACE	✓	
Fenitrothion	Organophosphate	ACE	✓	
Perimiphos-methyl	Organophosphate	ACE	✓	
Bendiocarb	Carbamate	ACE	✓	
Propoxur	Carbamate	ACE	✓	

MALARIA CONTROL RESTS ON TWO TARGETS

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Resistance monitoring is essential

WHO Resistance testing in 2014

By the time resistance is spotted it is usually too late – resistance genes are widespread
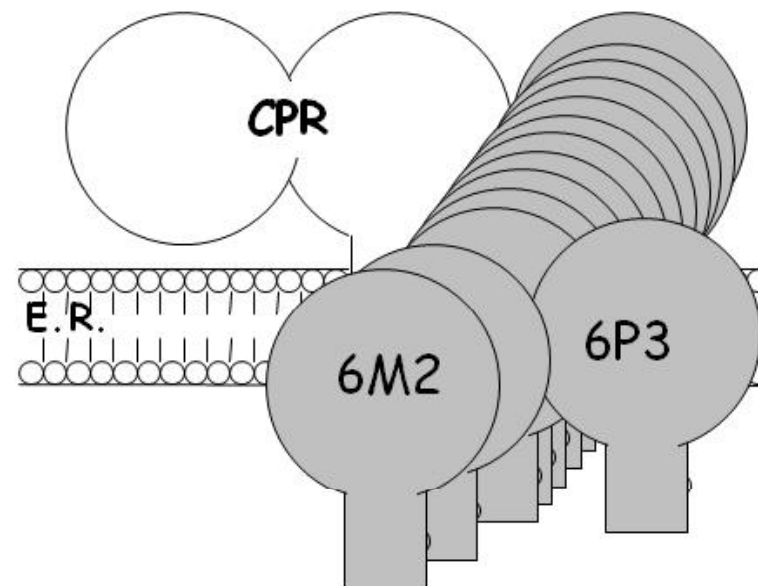
P450's play a <u>major</u> role in metabolic resistance to pyrethroids: WHICH ONES?

Phenotype

↓

Candidate

↓

Validate

↓

Technology

↓

Field test

P450s key for:
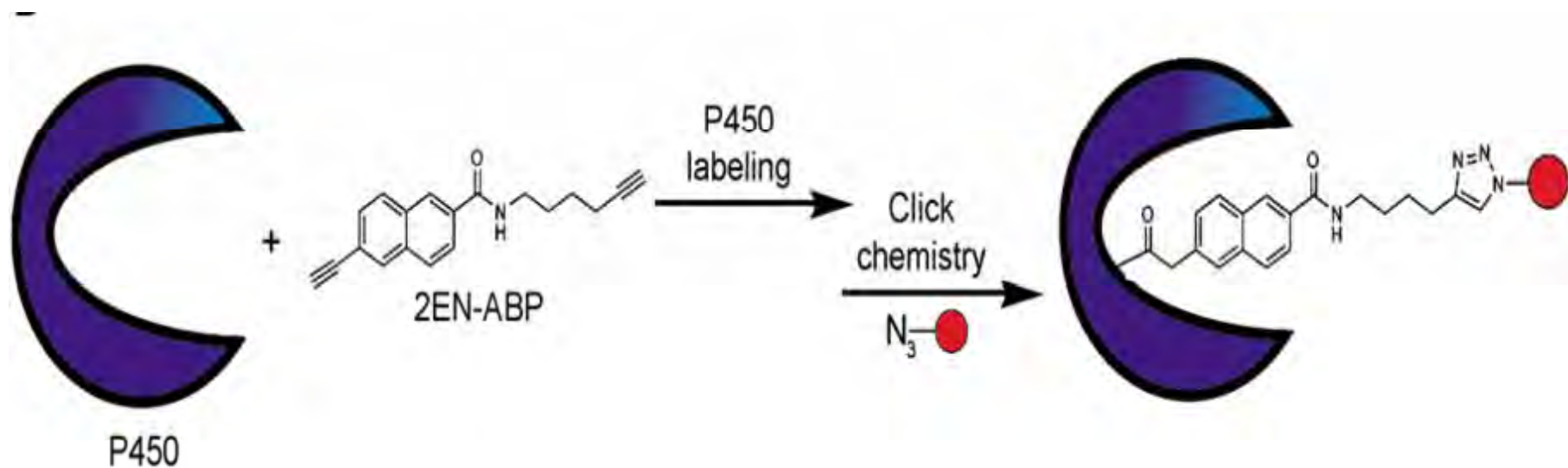1. Diagnostics
2. Insecticide
   development

Challenges
- 100 – 200 P450s
- SNP frequency ~50 nt

CPR

E.R.

6M2

6P3

AIM: fast track the identification of P450s associated with insecticide metabolism
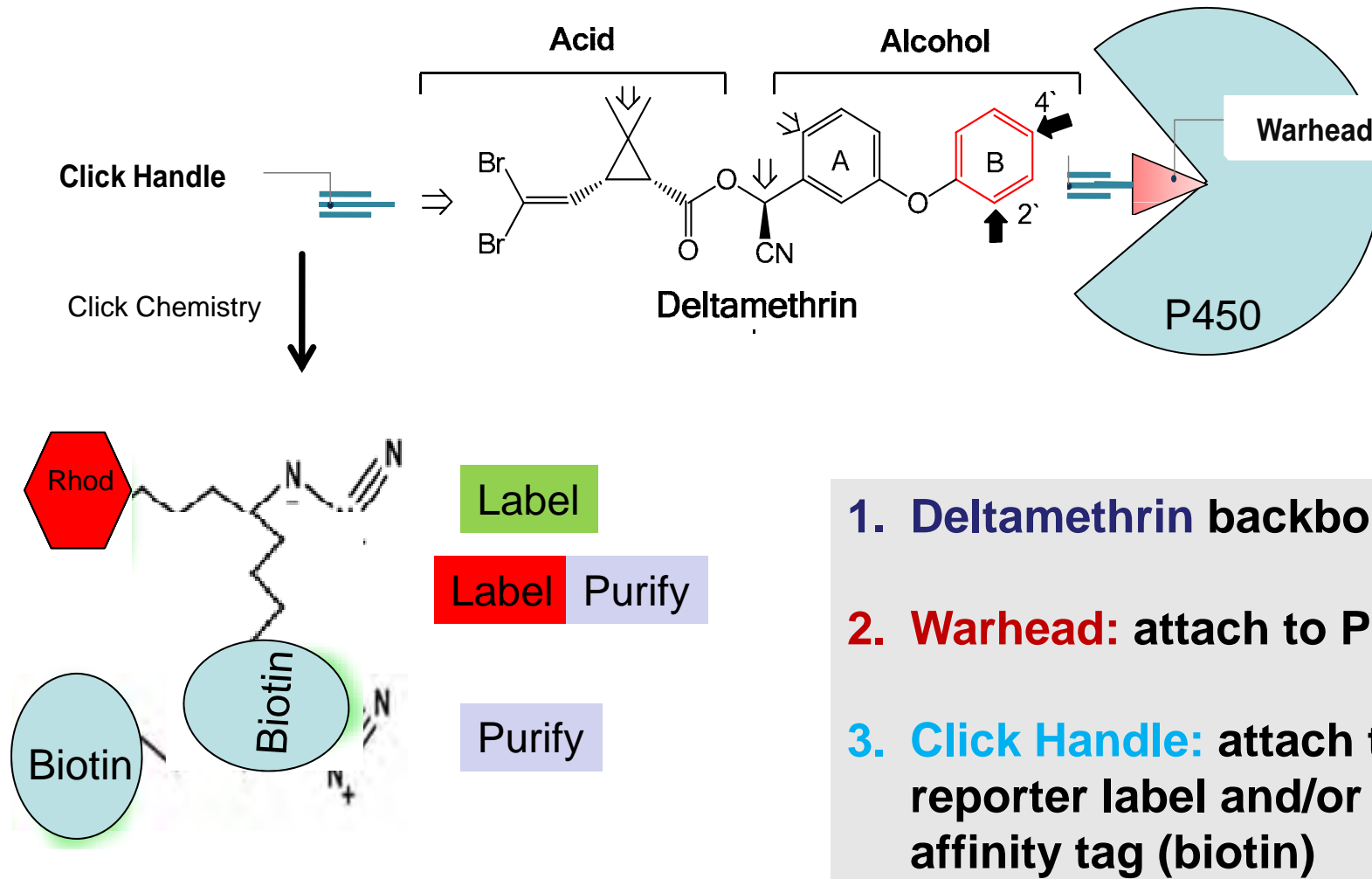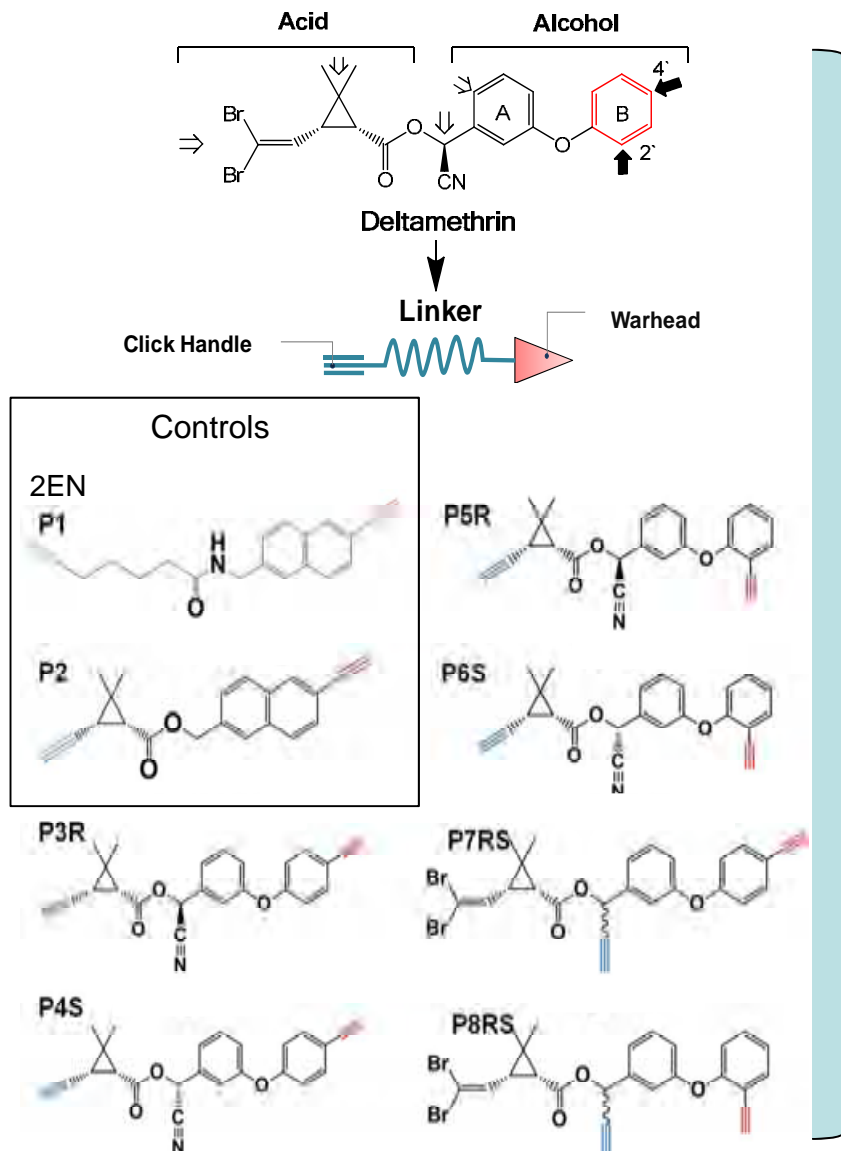
✓ Activity Based Protein Profiling Probes can target P450s

Wright A. T. and Benjamin F. Cravatt (2007) Chemistry & Biology 14, 1043–1051
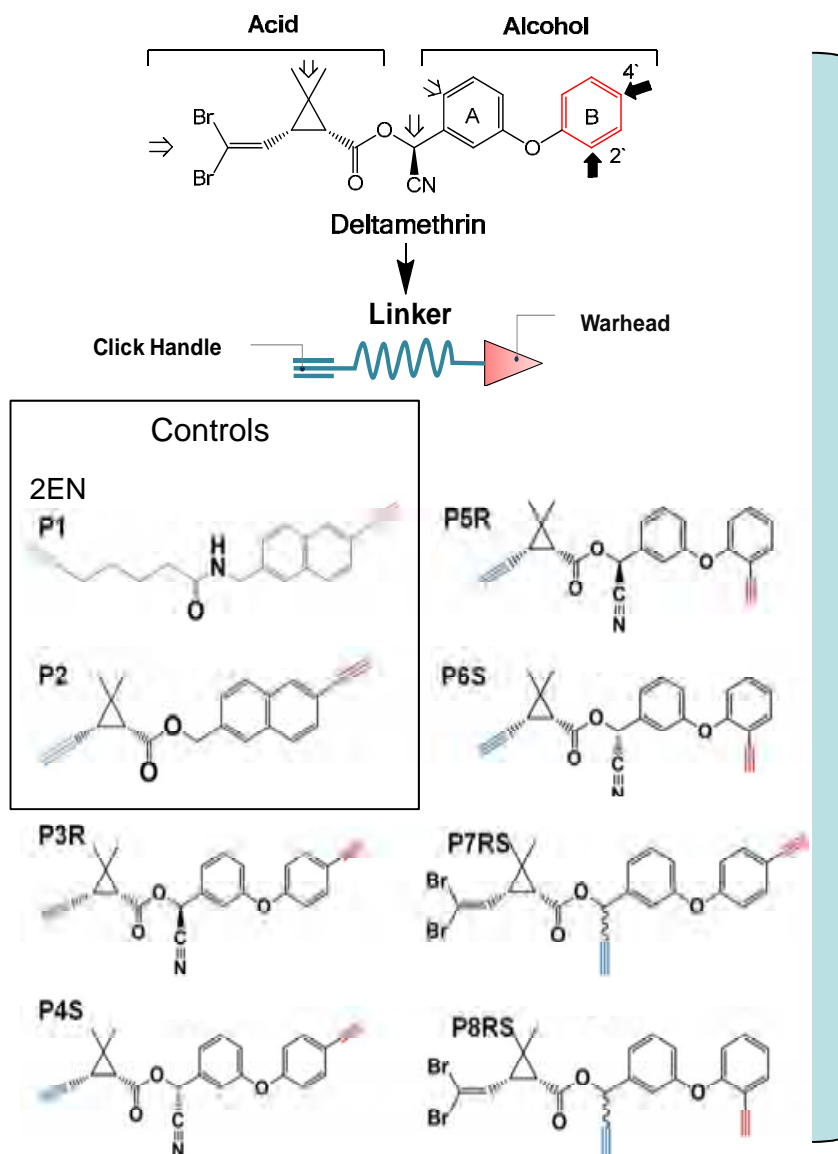
Pyrethroid Activity Based Probes
PyABP: Design features

Label

Label Purify

Purify

1. **Deltamethrin** backbone

2. **Warhead:** attach to P450

3. **Click Handle:** attach to reporter label and/or affinity tag (biotin)

PyABP Suite

- **Warhead** and **click handle** positioned at major and minor sites of attack
- **Based on knowledge of deltamethrin breakdown by CYPs 6M2 and 6P3**

An. gambiae P450s +/- NADPH

Screened against mosquito P450s associated with Py resistance – metabolisers AND non metabolisers
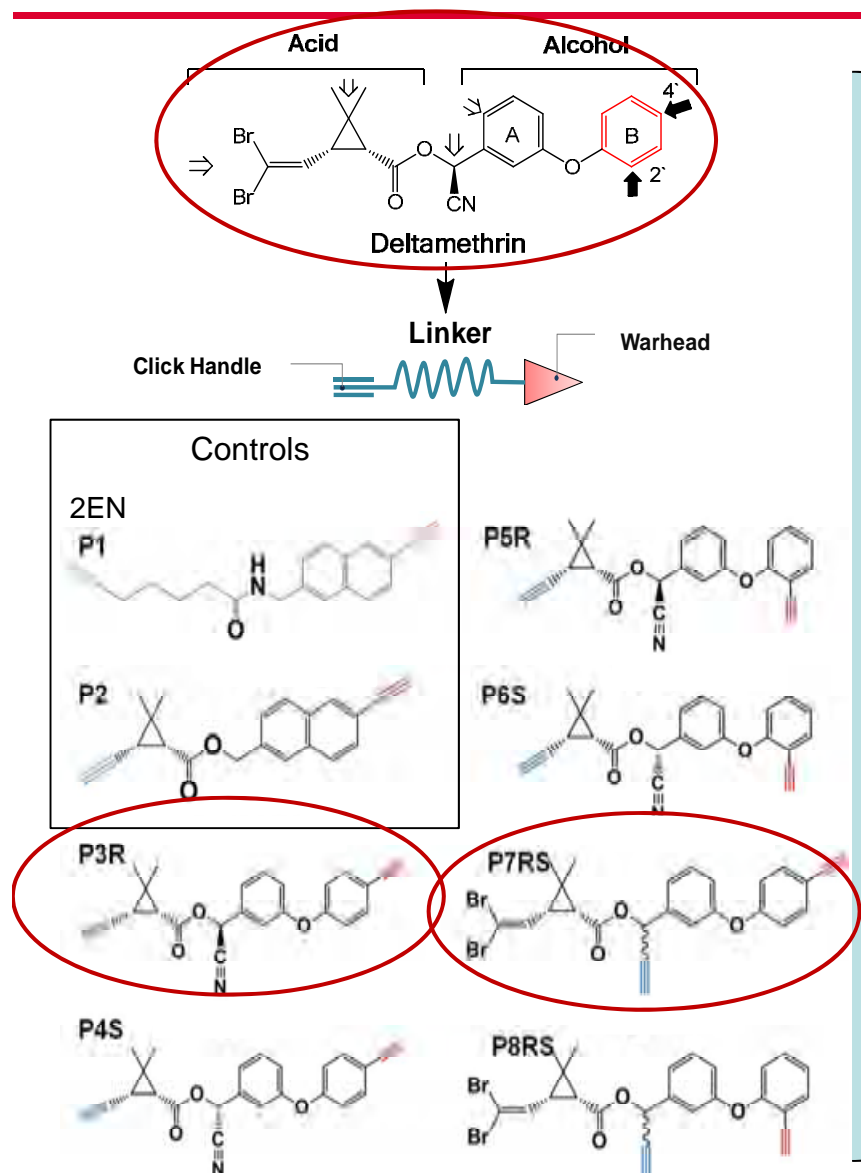
Screened *An. gambiae* P450s +/- NADPH
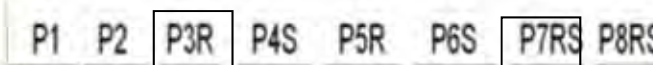
Con   Delta-ABP

P450	Metaboliser
6P3	Yes
6M2	Yes
6Z2	No

Selective for deltamethrin metabolisers

Warhead best on major 4' site of attack

SAR INFORMATIVE

Are Py-ABPs really activity based?

Acid   Alcohol

Deltamethrin

Click Handle — Linker — Warhead

Tag Free Click Chemistry ABP Probes Design

Control: 2EN-ABP

P1, P2, P3R, P4S, P5R, P6S, P7RS, P8RS
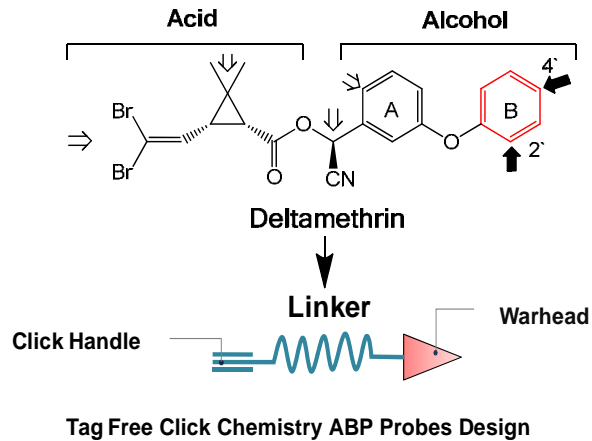
Validated with Dundee CPR and b5 KO mice (Henderson, Finn, Wolf)

b5 affects PyABP metabolism

CPR/b5		-CPR/b5		CPR/-b5		
+	−	+	−	+	−	NADPH

P7

P8
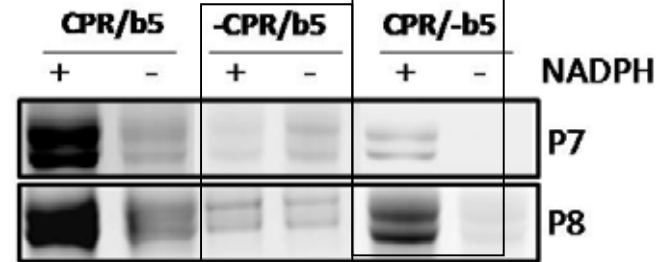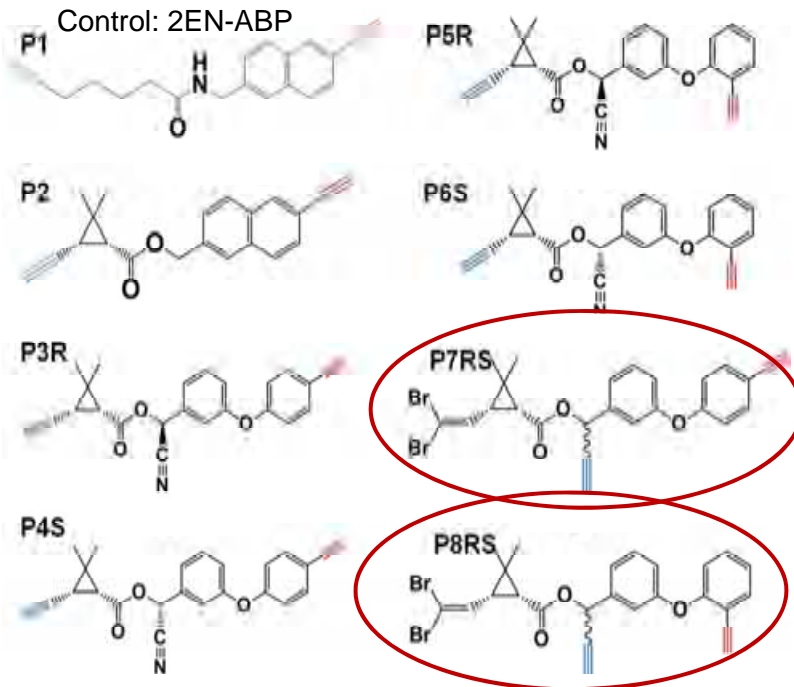
CPR needed for metabolism

Do PyABPs work?

Acid

Alcohol

Deltamethrin

Linker

Click Handle   Warhead

Tag Free Click Chemistry ABP Probes Design

Control: 2EN-ABP

P1

P2

P3R

P4S

P5R

P6S

P7RS

P8RS

Pre affinity   Post affinity purification

+   -   +   -   +   - NADPH

P450>>

Laser imaged   Coomassie stained
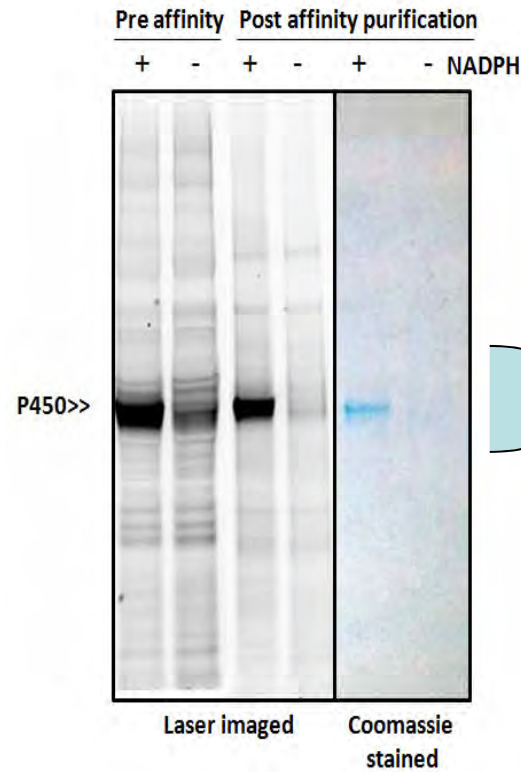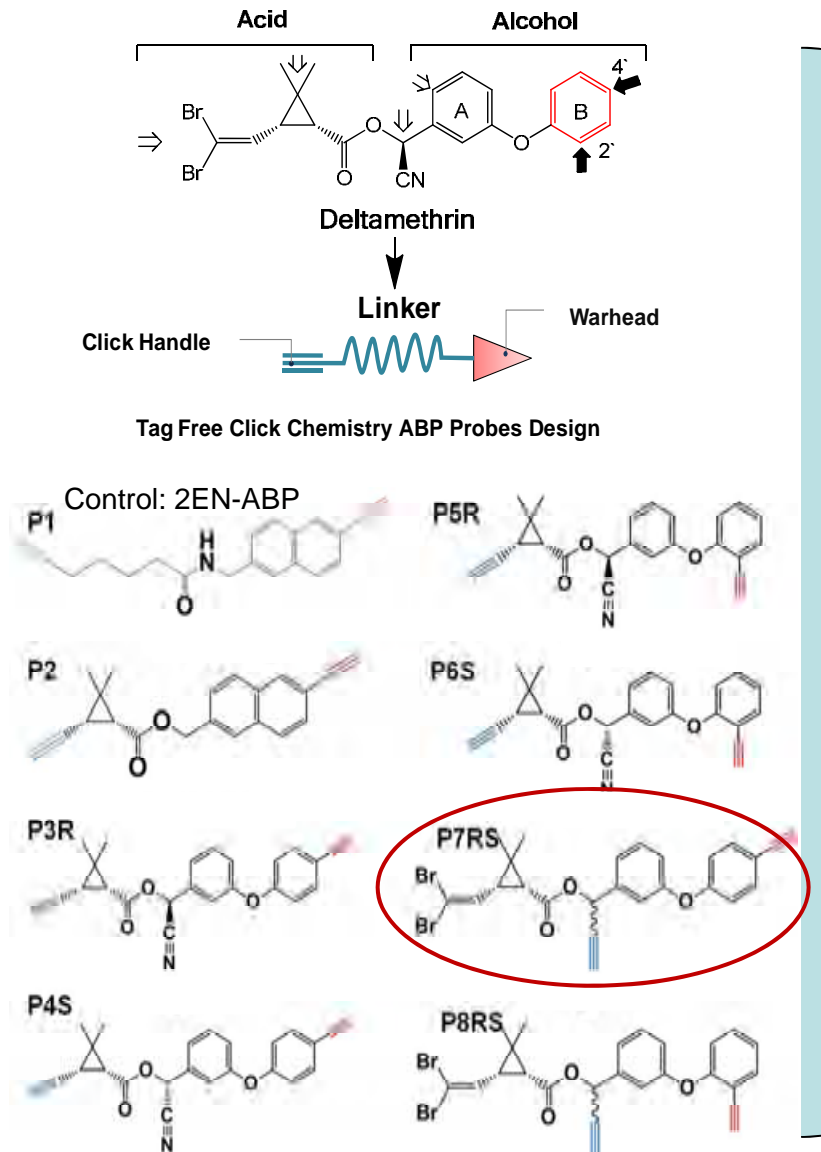
Cut Band

⇩

Tryptic Digest

⇩

LC/MS/MS

⇩

Peptide ID

Enzymes interacting with P7RS

Gel extraction

Protein	Mass KDa	emPAI
CYP2C11	57.1	9.48
CYP2C6	56	3.85
CYP2C23	56.3	1.02
Ugt2b37 similar to UDP-g2B5 precursor	49.4	0.85
UDP-g 2B5	60.6	0.74
CYP2C13	49.6	0.74
CYP2C7	56.2	0.72
CYP2D10	57	0.62
CYP2D1	57.1	0.62
CYP2D26	56.6	0.62
UDP-g2B3	60.5	0.58
FMO3	59.9	0.5
Epoxide hydrolase	52.5	0.42
UDP-g2B4	61	0.42
CYP2D3	56.9	0.38
Aldh3a2 Fatty aldehyde dehydrogenase	54.0	0.33
CYP2C13 (male-specific)	55.8	0.31
CYP2B3	56.3	0.31
CYP4V3	60.5	0.29
CYP2C7	53.2	0.26
CYP2A2	56.3	0.24
CYP4F4	60.0	0.23
CYP2C70	56.1	0.18
CYP2A1	55.0	0.18

On-bead digestion

Protein	Mass KDa	Average emPAI (+NADPH)*
CYP2C11	57.1	25.41
CYP2D10	57	3.85
CYP2D1	57.1	2.69
CYP2D3	56.6	2.11
CYP2D26	56.6	1.6
UDP-g 2B5	60.6	1.05
UDP-g 2B2	60.9	1.03
UDP-g 2B3	60.5	0.85
CYP2D18	56.6	0.5
CYP2C6	56	0.49
CYP2D4	56.7	0.46
Aldh3a2 Fatty aldehyde dehydrogenase	54	0.46
Epoxide hydrolase	52.5	0.37
UDP-g 2B4	61	0.24
CYP2B3	56.3	0.16
CYP2C23	56.4	0.15
CYP3A2	57.7	0.14
FMO3	59.9	0.12
CYP2C70	56.1	0.12
Protein disulfide-isomerase	56.9	0.1
CYP2C7	56.2	0.09
UDP-g 2B1	60.4	0.06
CYP4V3	60.5	0.02

LSTM

P7RS pulls down a network of insecticide metabolising enzymes

Enzyme	Py Activity
CYP2C11	K_M 32 µM, V_{max} 206 min^{-1}
CYP2C6	and K_M 22 µM, V_{max} 150 min^{-1}
CYP2C13	Yes
CYP2D1	Yes
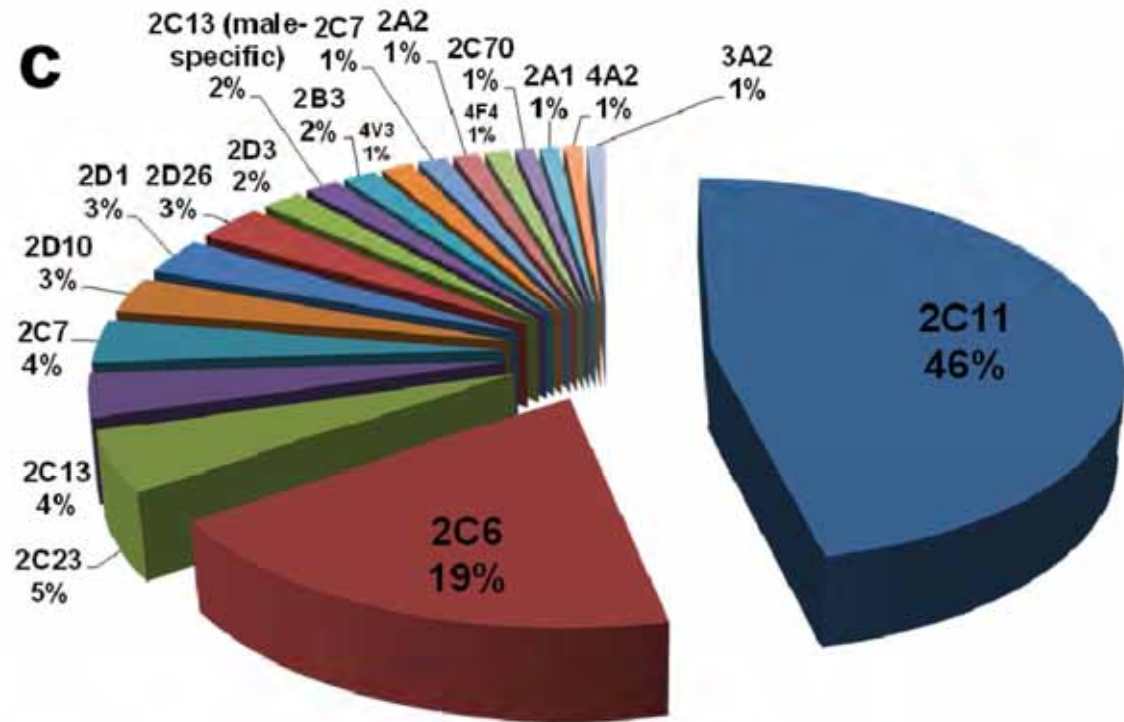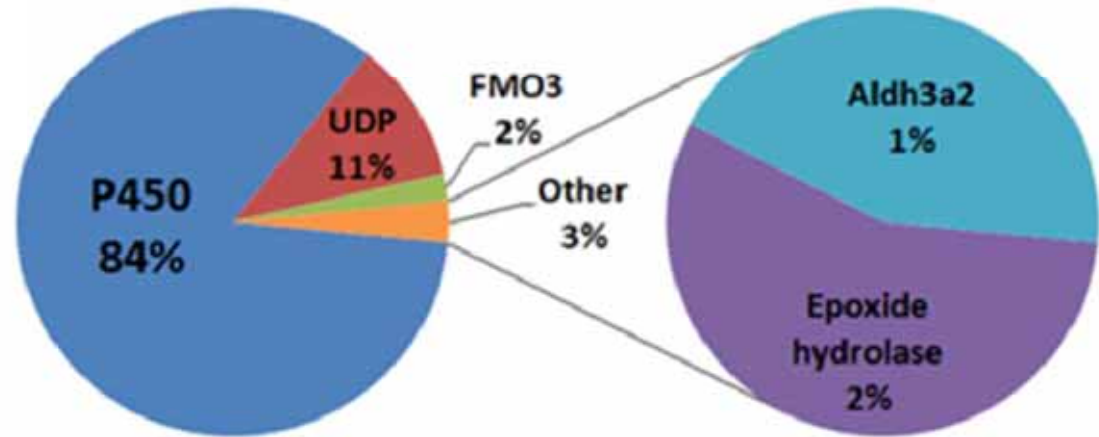
Interaction networks of CYP2C11 and UDP-g 2B5 according to STRING software (http://string-db.org/).
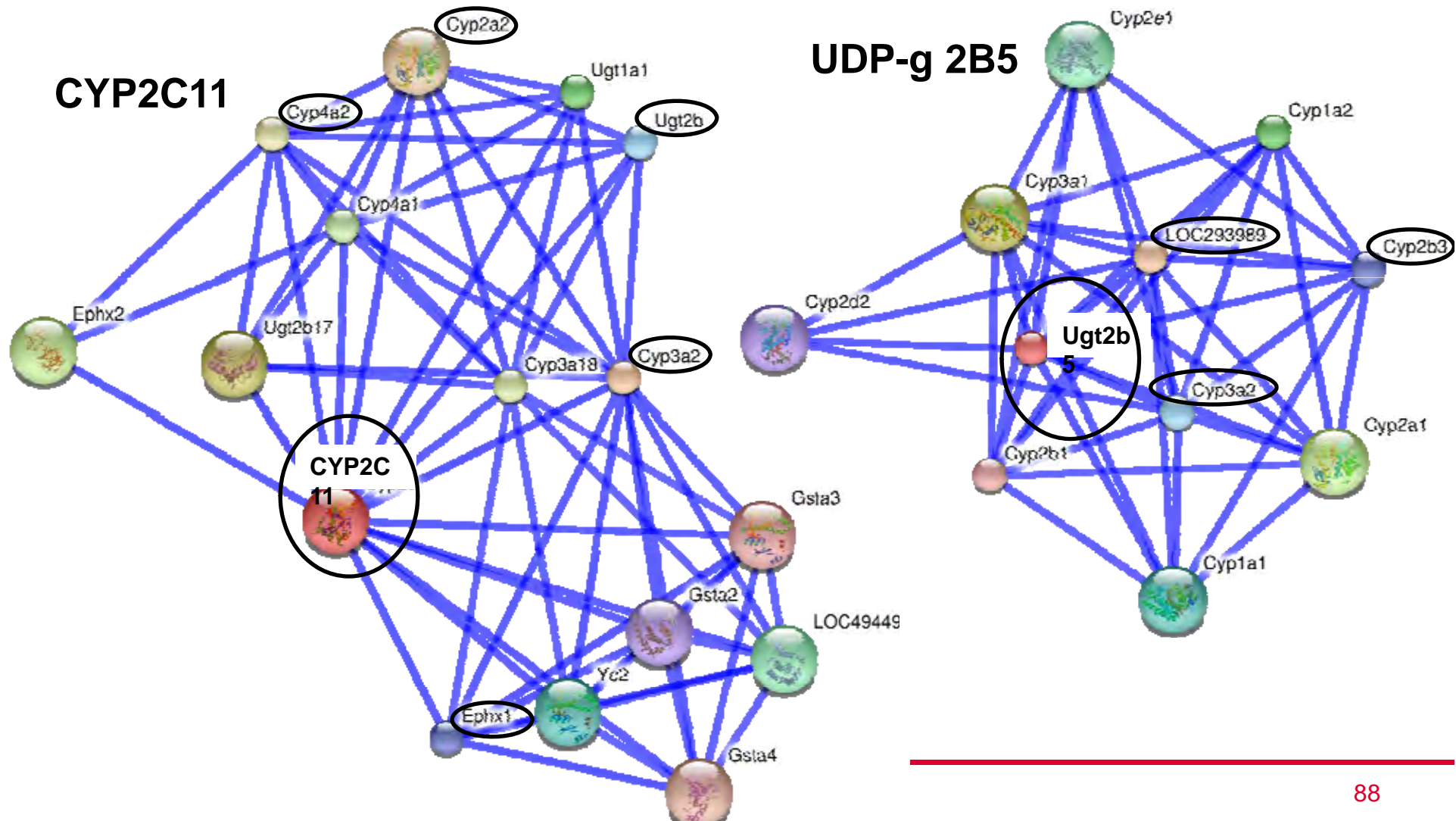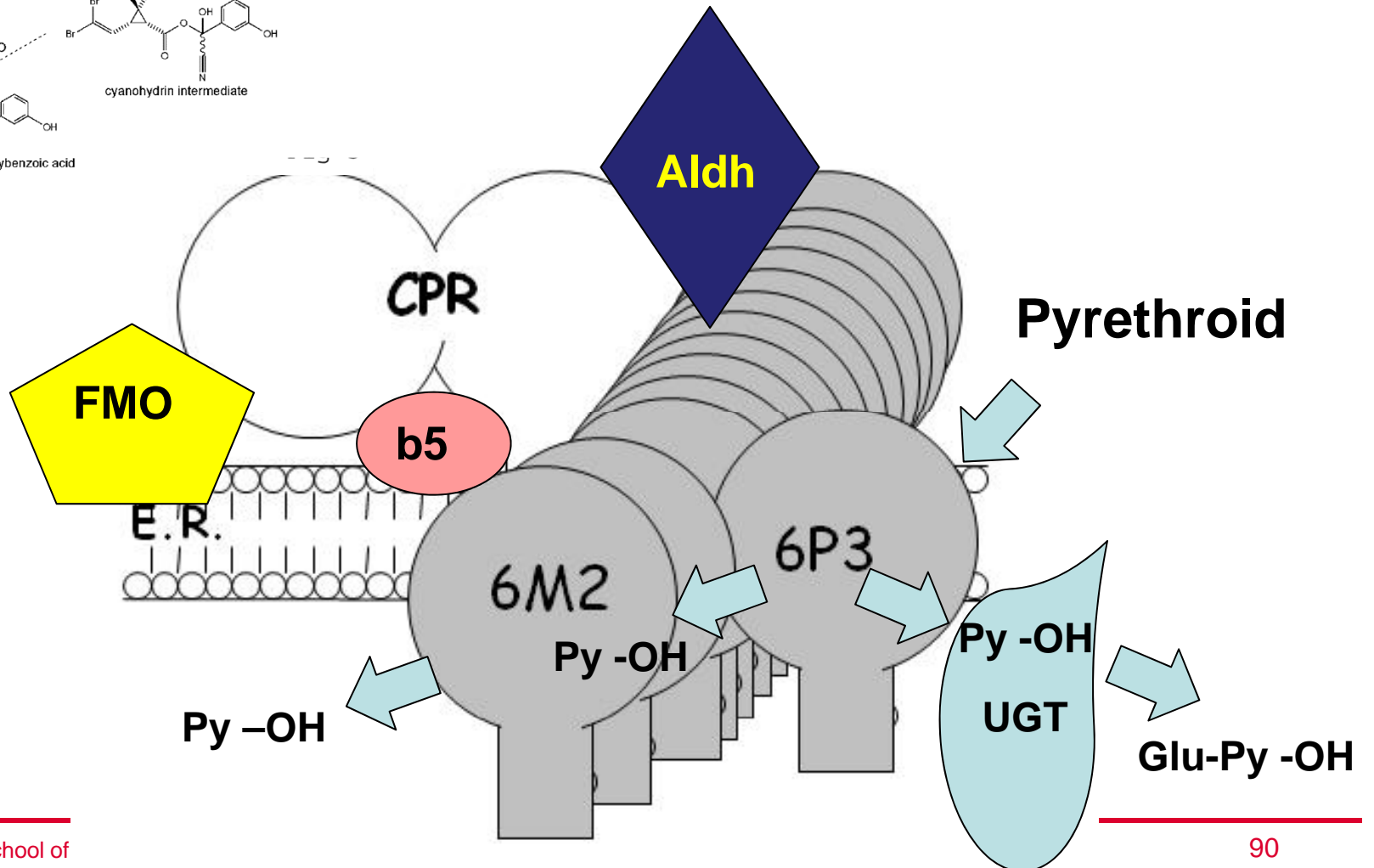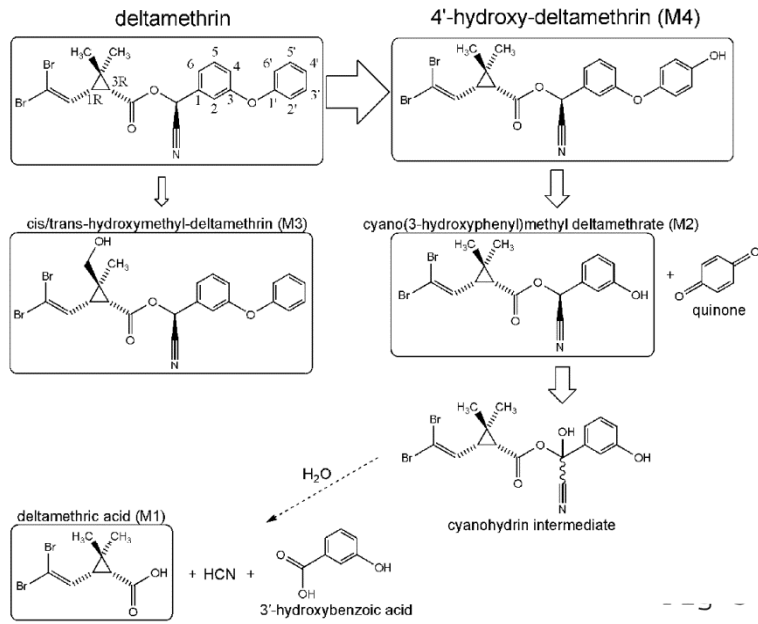
CONCLUSIONS

**Synthesized a new panel of ABPs, PyABPs, directed towards pyrethroid metabolizing P450s**

- Interactions with mosquito P450s confirmed pyrethroid specificity and provided useful SAR of Py-P450 interaction

- mouse liver KOs indicated modulation of activity by b5

- P7RS was capable of detecting deltamethrin metabolizing P450s in a complex rat proteome, and other enzymes associated with xenobiotic metabolism

- Suggests the probe has captured a **'pyrethrome',** an associated network of enzymes involved in pyrethroid metabolism.

- **PyABPs** useful for assessing P450-pyrethroid interactions in a wide range of species and biological systems,
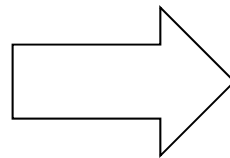
Pyrethrome

LSTM

Next step is to use P7RS against *An gambiae*

Tororo | Kisumu
+ − | + − NADPH

Pre-affinity purification

Post-affinity purification
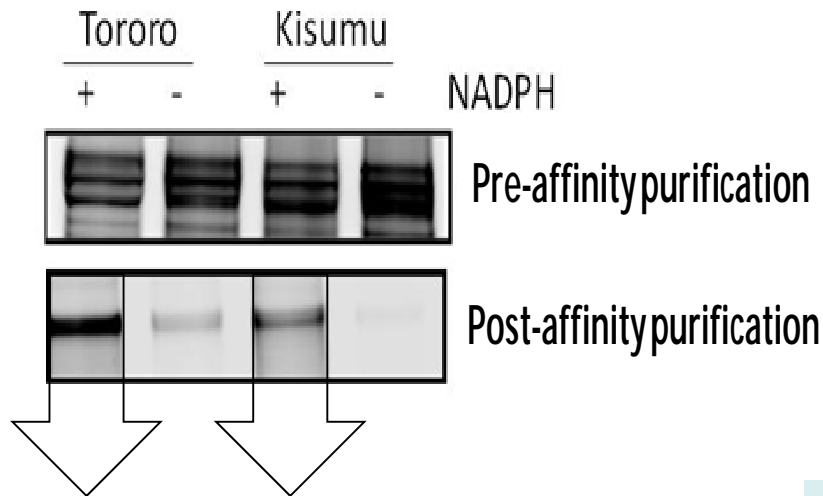
An gambiae pyrethrome?

- mechanism
- diagnostics
- SAR
- new insecticide

?

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Hanafy Ismail
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U of Liverpool
Paul O'Neill
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