Detection of organophosphate residues using a biosensor

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How to detect OP and carbamate ?

1- Gas chromatography and Mass spectroscopy

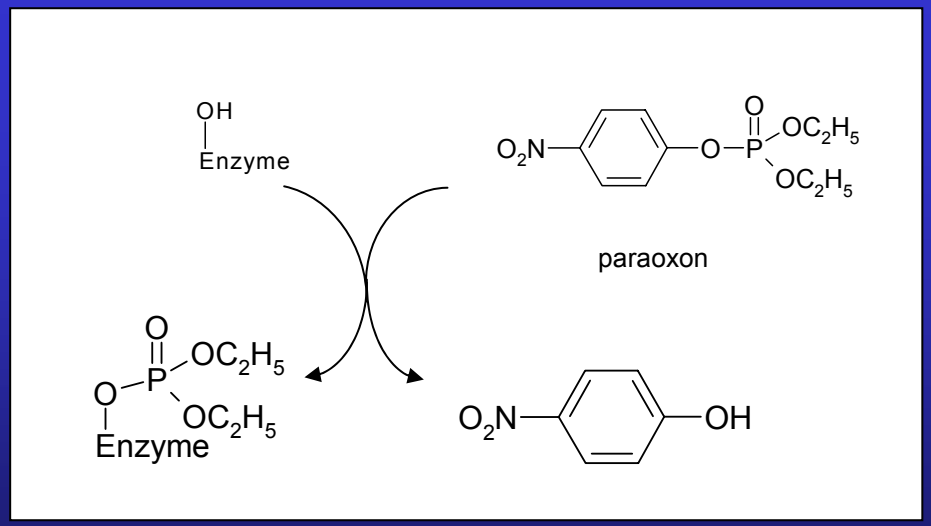
- expensive
- time consuming
- not suitable for field or home use
- quantitative and qualitative estimation

2- Biosensors

- rapid assay
- low cost
- approximative estimation

Inhibition of acetylcholinesterase depends on the amount of organophosphate

➔ Amount of remaining free enzyme is correlated to the amount of insecticide.



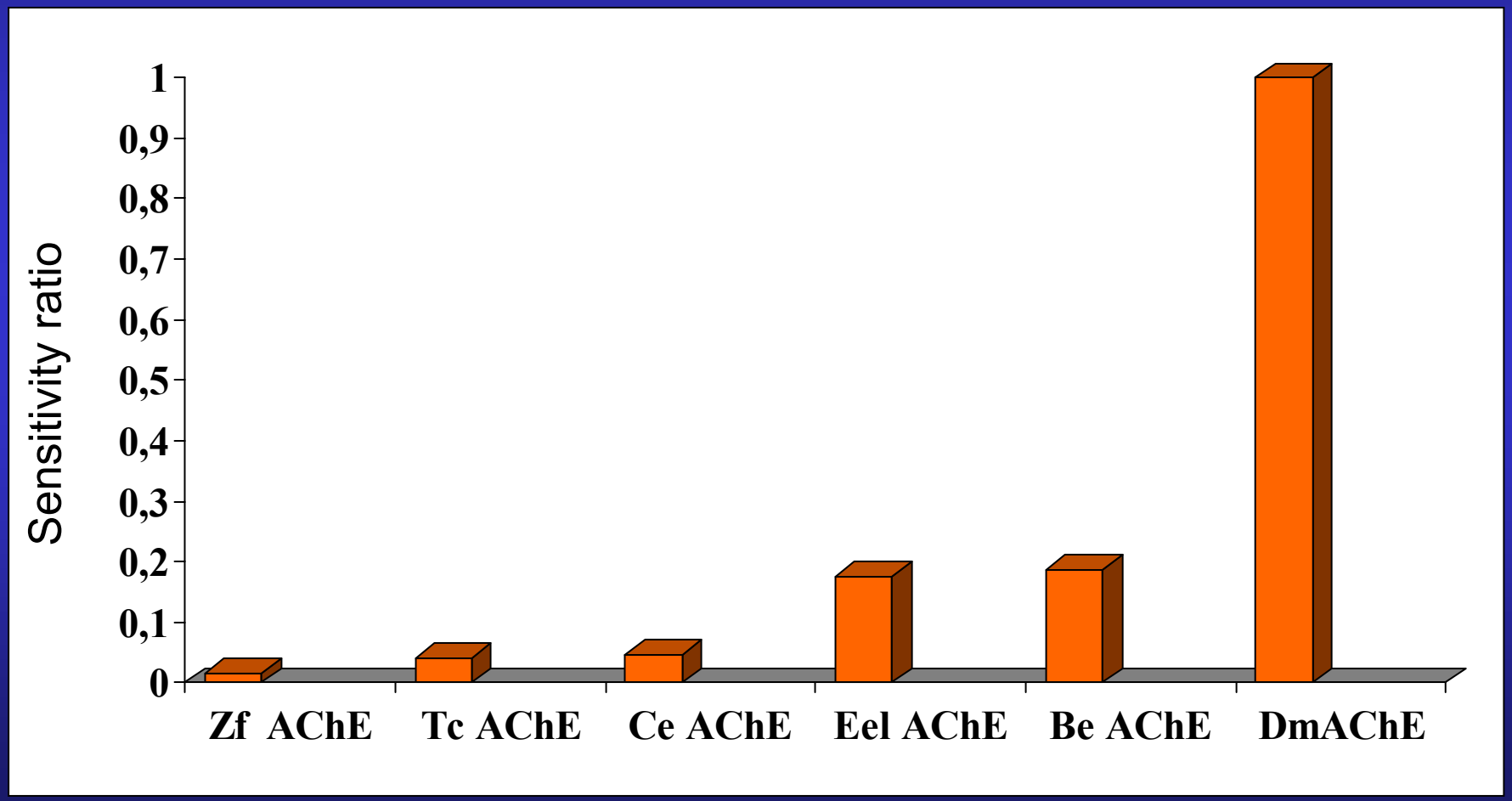
Obtention of sensitive acetylcholinesterase

Methodology

- Choice of the most sensitive AChE

- Site-directed mutagenesis

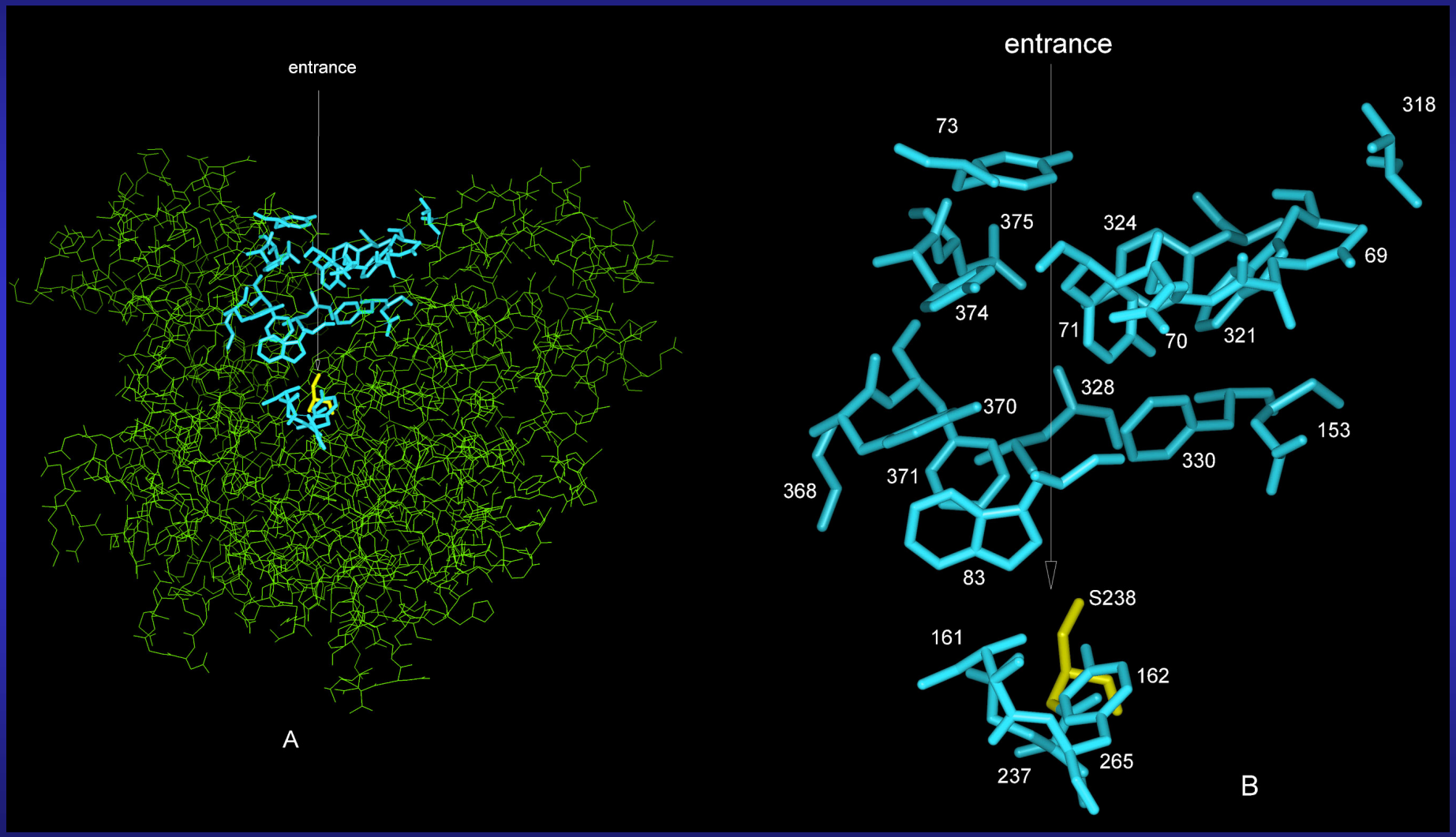
Drosophila AChE is the most sensitive to insecticide



Site-directed mutagenesis of the cDNA encoding DmACHE

- Mutagenesis at position confering insecticide resistance
- Modelling directed mutagenesis (docking)
- Effect of backbone length
- N-glycosylation site at the entrance of the active site
- Alanine-scanning mutagenesis
- Residue-scanning at selected positions
- Combination of favorable mutations in the same enzyme

Site directed mutagenesis



Drosophila AChE mutants conferring the best sensitivity

Insecticides	Dm AChE	mutants Dm AChE			Electric eel AChE	
	ki	Amino acids	$ki_{(mut)}$	$ki_{(mut)}/ki$	$ki_{(e.e)}$	$ki_{(mut)}/ki_{(e.e)}$
Aldicarbe	0.013	E69Y, Y71D	0.094	7	0.013	7
Azinphos metyloxon	66	E69Y	692	10.5	0.36	1922
Carbaryl	0.19	Y370A	2.687	14	0.065	41
Carbofuran	5.36	I161V	11.5	2.14	4	3
Chlorpyriphos oxon	840	Y324A	1621	2	2.85	569
Diazinon oxon	50	E69Y	777	15.5	0.14	5550
Dichlorvos	1.8	E69Y, Y71D	548	300	0.0019	288000
Malaoxon	2.5	E69Y, Y71D, F330L	72	29	0.114	632
Methamidophos	0.00067	D375Δ, F376L	0.012	18	0.0018	7
Methiocarbe	0.425	E69W	3.67	8.5	0.067	55
Omethoate	0.042	E69W	2.32	55	0.001	2320
Paraoxon	1.462	E69Y, F330L	18.9	13	0.287	66
Pirimicarb	0.206	E69W	0.850	4	0.0022	386

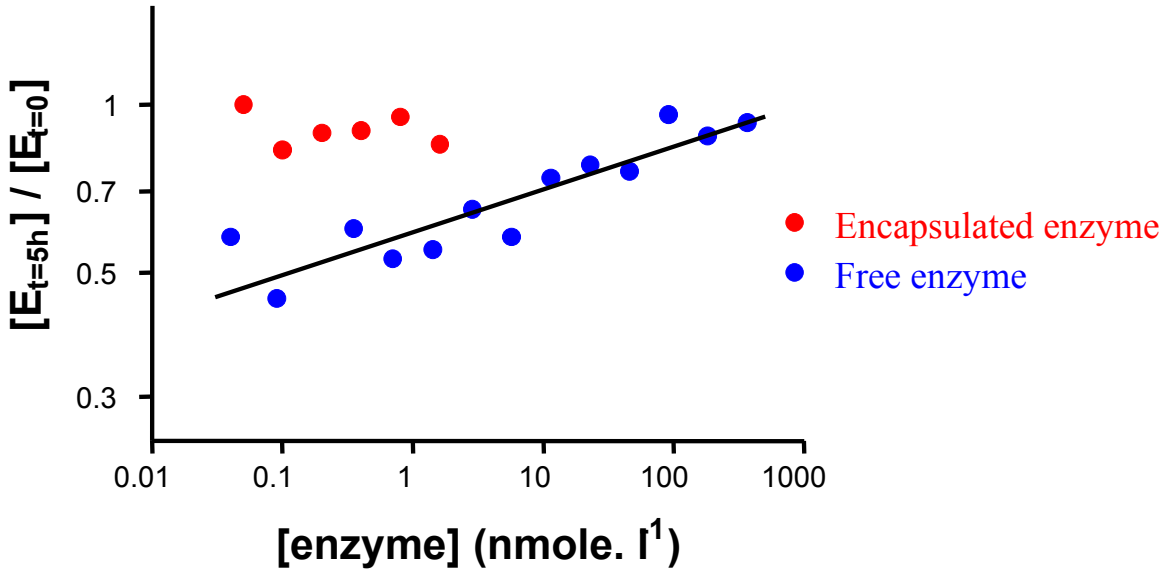
Stabilisation by *in vitro* mutagenesis

- ***- Adding proline residues in the peripheral loops***
- ***- Changing the repartition of hydrophilic and hydrophobic residues.***
- ***- Increasing the number of glycosylation sites***

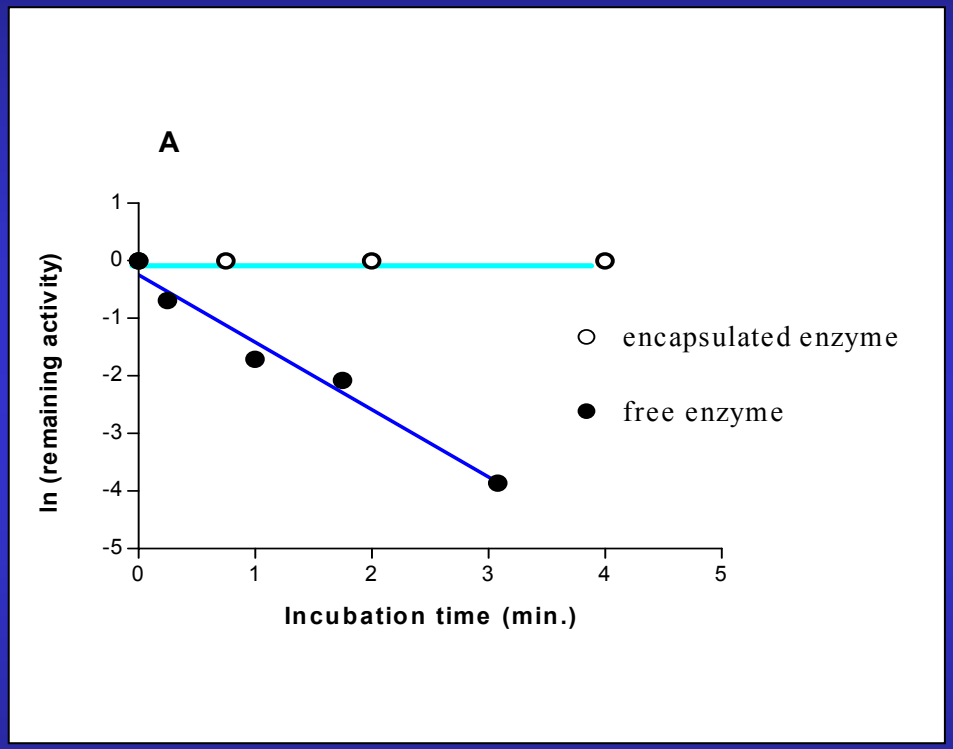
Increasing AChE stability with a new disulfide bond

t50 en min.	20% acetonitrile	50°C	4M urea	Pronase
327C/375C	3.6	363	168	22
Drosophila	1.7	6	14	14

protection by encapsulation in liposomes



Protection of acetylcholinesterase to proteolysis by encapsulation in liposome



Remaining activity following incubation of AChE with 0.5 mg/ml pronase at 25°C

AChE encapsulation into liposomes with OmpF porins

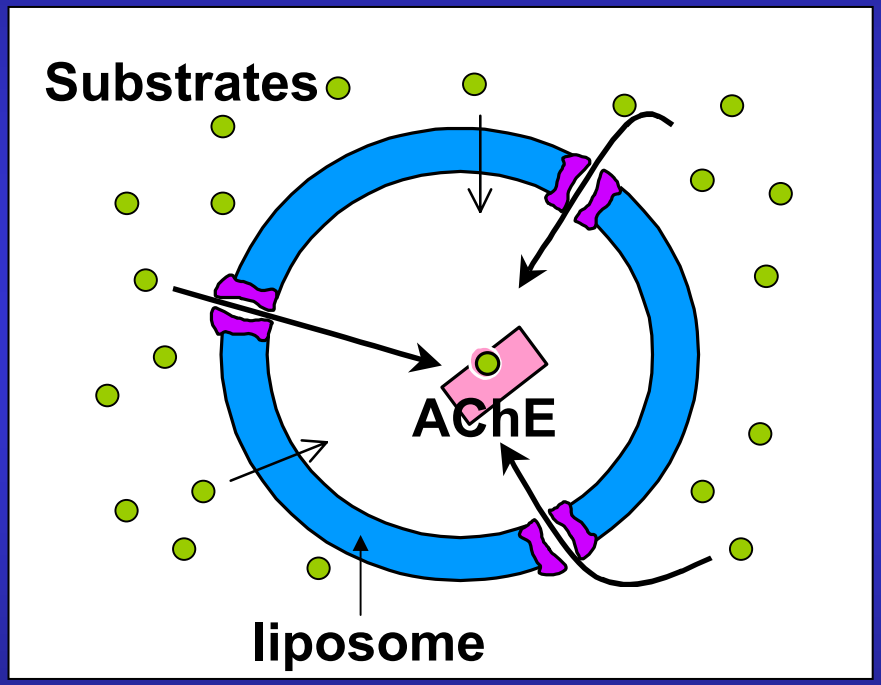
OmpF porin

- proteic channel

- from membrane of Gram - bacteria

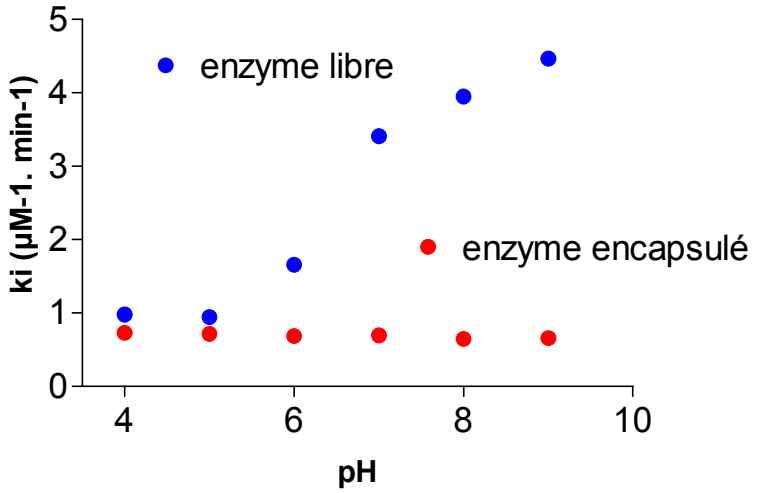
- stable trimere

-molecules < 400 Da



L ’encapsulation protège contre les effets de matrice

			ki paraoxon (µM-1.min-1)	
		enzyme libre		enzyme encapsulée
		0,87		0,45
10 mM propidium		2,79		0,47
10 mM Cd+		0,48		0,48
10 mM acetylcholine		0,01		0,48



Sorting out molecules reacting with an enzyme by encapsulation in liposome

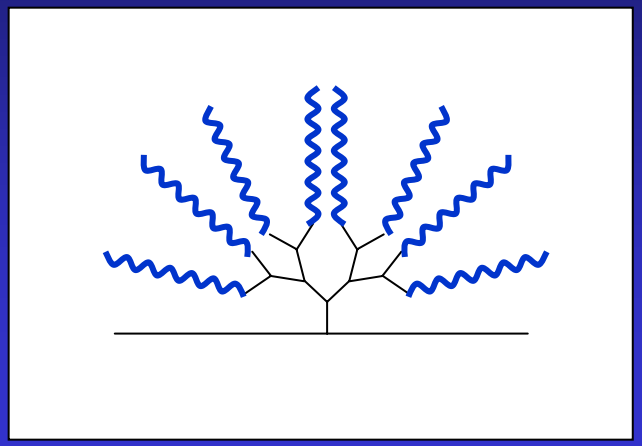
Enzyme encapsulation allows:

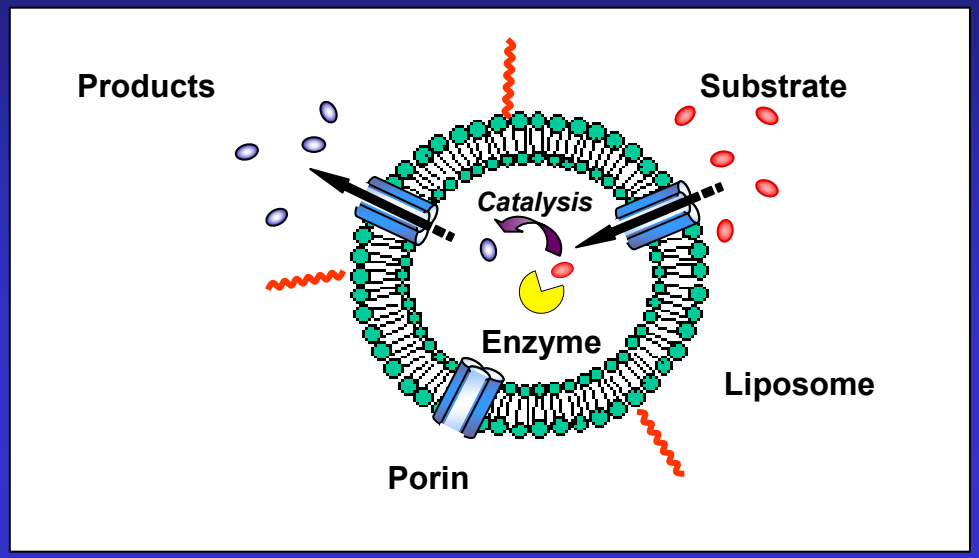
- to sort out substrates.
  Charge substrate such as acetylcholine does not cross the lipid bilayer but non charge substrate (nitrophenylacetate, organophosphates) pass through the membrane
  ($k > 1000\ sec^{-1}$)

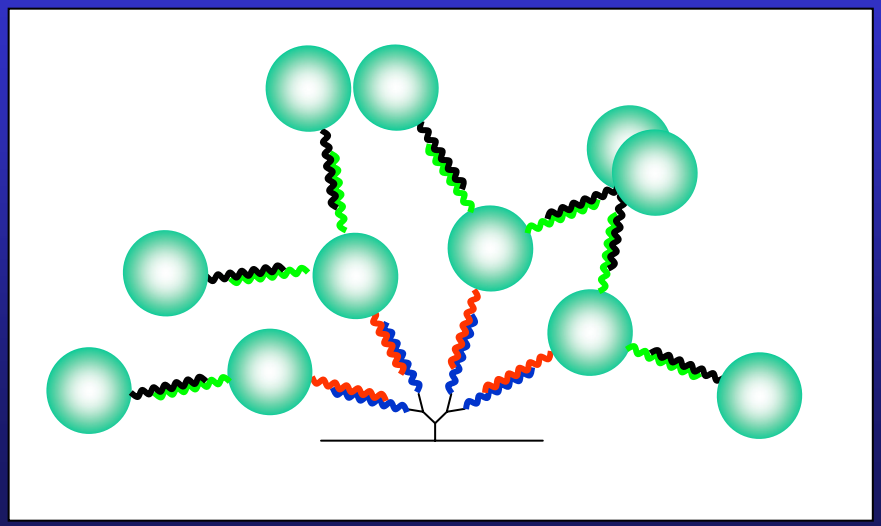
- To protect the enzyme from charge molecules (matrix effect) .

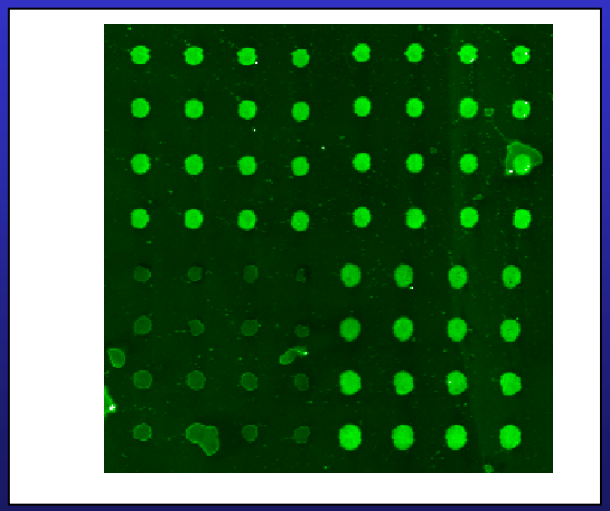
- To keep the enzyme in optimal microenvironment (pH, ionic strength).

Encapsulation of proteins allows to bind the protein on surfaces









Biosensor with acetylcholinesterase

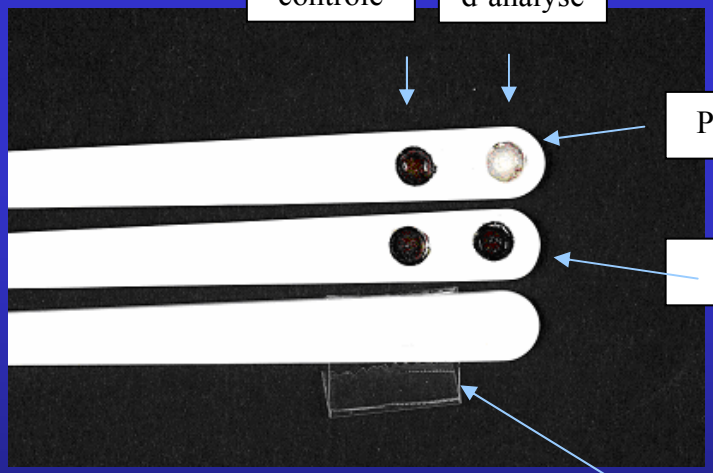
Different steps

- Activation of organophosphothioates (10 min.)
- Inactivation of the activator (5 min.)
- Inhibition of the enzyme (1 to 16 hours)
- Activity of the enzyme (15 min.)

Détection d'insecticide dans l'eau et les aliments
Mis au point de kits

Kit de détection des résidus dans l'eau

Kit de détection dans les aliments de nourissons



AMPEROMETRIC BIOSENSOR (manufactured by screen- printing)

1st layer : **silver film** (conductor film)

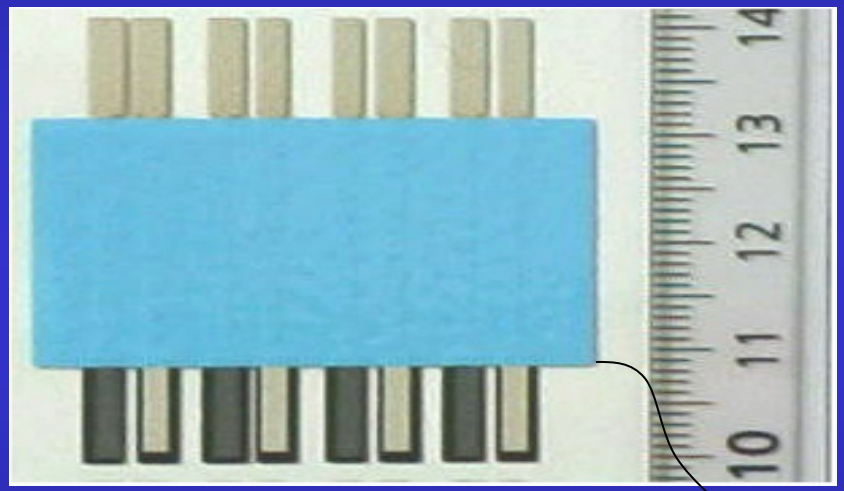
2nd layer : **carbon film**

3rd layer: **reference layer** (Ag/AgCl)

4th layer **graphite layer with TCNQ as a mediator**

5th layer : **insulating paint**

6th layer (working layer) : **AChE paste over the fourth layer**



B87 B73 B3 B4

Enzymes

Biosensor Sensitivities

Sensitivity in ppb (ng/ml)

Enzyme B394
test solution 10 ml

	Liquid			sticks	
incubation time	1 hour	16 hours		1 hour	16 hours
Chlorpyrifos ethyl	0,1	**0,1**		100	**10**
Dichlorvos	1	**1**		100	**10**
Malaoxon	1	**0,1**		100	**10**
Malathion	1	**0,1**		100	**10**
Paraoxon	1	**0,1**		100	**10**
Parathion	1	**0,1**		100	**10**
Carbofuran	10	**10**		100000	**100000**
Pirimicarbe	1000	**1000**		>100000	**>100000**
profenofos	10	**10**		100000	**10000**
Carbaryl	100	**1**		>100000	**100000**
Metamidofos	>1000	**>1000**		>10000	**>10000**