New strategies in the fight against Ciguatera

Hélène Martin-Yken, Camille Gironde, Hélène Taiana Darius, Christophe Furger, Mireille Chinain, Etienne Dague

New strategies in the fight against Ciguatera

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LISBP Toulouse.

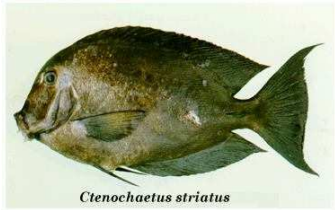
CIGUATERA

Poissons dangereux à la consommation

- ★ Pêches et ventes interdites en tous lieux et en tous temps.
- ★ Pêches et ventes interdites au nord du 16,5° parallèle (cf. carte).
- ★ Pêches et ventes interdites, quel que soit le lieu de pêche, si le poids dépasse 1 kg.

Poissons interdits à la pêche et à la vente
(Arrêté préfectoral n°2002-1249)

Vector fish of ciguatera toxins


Ctenochaetus striatus


Neso unicornis


Cheilinu


Neso brevirostris


Caranx sexfasciatus


Lutjanu

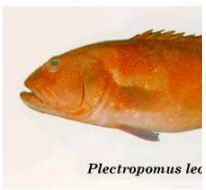

Lutjanus rivulatus


Glabrilutjanus nematophorus


Scar


Gymnothorax undulatus


Plectorhynchus punctatissimus


Plectropomus le

POISSONS VÉNÉNEUX

ARCHIPEL DE LA GUADELOUPE

La pêche et la vente de ces poissons sont interdites en tout temps et tous lieux.

TÉTRODONS ET DIODONS
Puffer, Spotfin Burrfish, Spotfin Porcupinefish.
Chilomycterus et *Diodon*

BARRACUDA BÉCUNE
Great Barracuda
Sphyraena barracuda

CARANGUE JAUNE
Yellow Jack
Caranx bartholomaei

GRANDE SÉRIOLE
Greater Amberjack
Seriola dumerili

SÉRIOLE LIMON - BABIANE
Almaco Jack
Seriola rivoliana

La même interdiction s'applique aux poissons pêchés au nord du parallèle 16° 50' de latitude Nord, appartenant aux espèces suivantes :

CARANGUE NOIRE
Black Jack
Caranx lugubris

CARANGUE FRANCHE CARANGUE BLEUE
Bar Jack
Caranx ruber

CARANGUE GROS-YEUX MAYOL
Horse Eye Jack
Caranx latus

MURÈNE CONGRE VERT
Green Moray
Gymnothorax funebris

VIEILLE À CARREAUX
Yellow fish Grouper
Mycteroperca venenosa

VIEILLE MORUE
Tiger Grouper
Mycteroperca tigris

VIEILLE VARECH
Mutton Hamlet
Alphestes afer

VIEILLE BLANCHE
Red Grouper
Epinephelus morio

La même interdiction s'applique aux poissons ci-dessous, quel que soit le lieu de pêche, si leur poids dépasse 1 kg.

PAGRE DENTS DE CHIEN
Dog Snapper
Lutjanus jocu
Concerné par les deux interdictions

VIVANEAU OREILLES NOIRES
Blacklin Snapper
Lutjanus buccanella

PAGRE JAUNE
Shoolmaster Snapper
Lutjanus apodus

Extrait de l'Arrêté Préfectoral n°2002/1249 du 19/08/2002 - Annexes n°2 et 3.

Dessins : © Th. Péan le Brun 2006 avec le concours de l'association Green Sub et de l'APSBT.

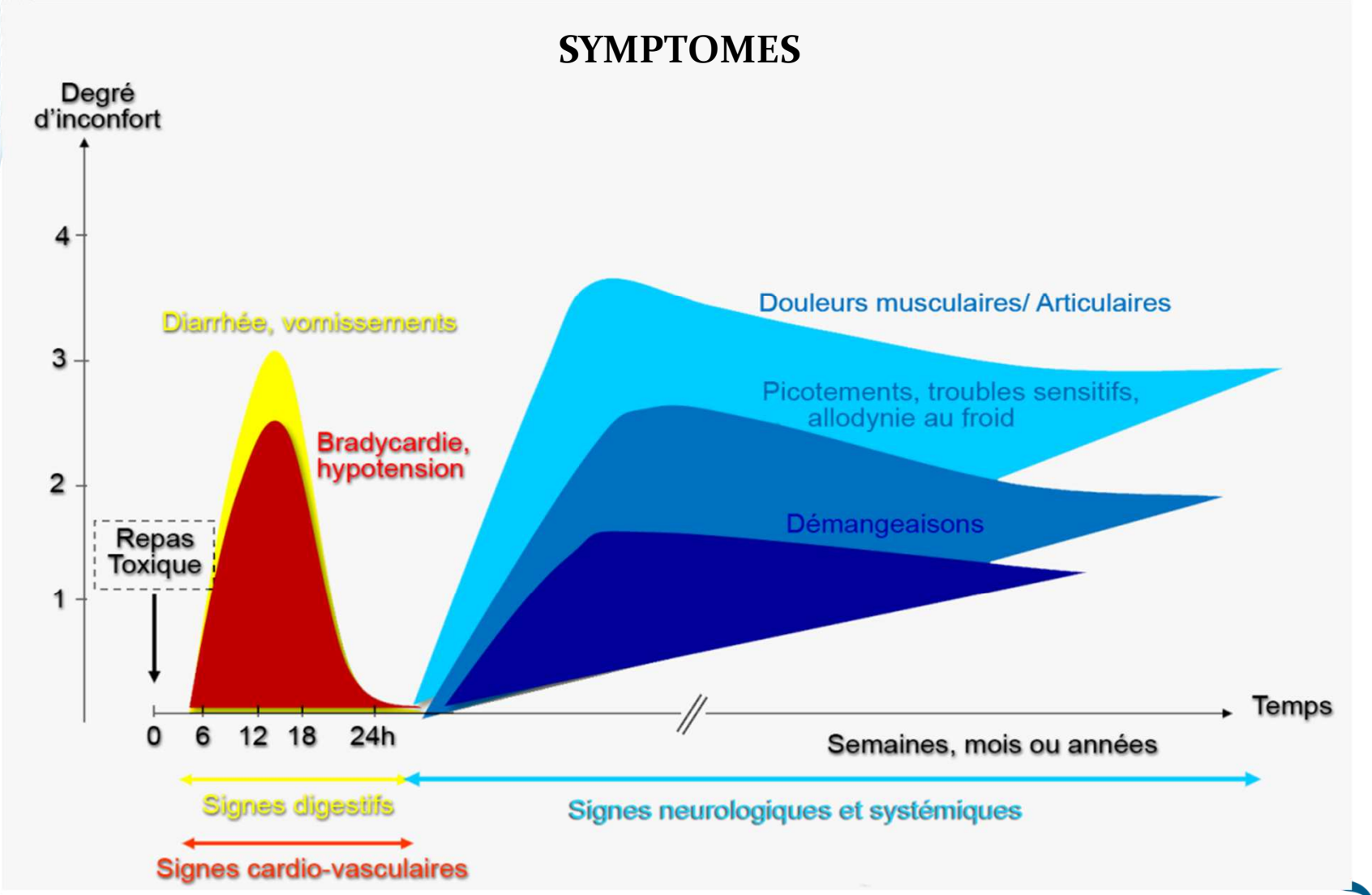
Ciguatera Food Poisening

- Considered as the **most frequent food poisoning of marine origine worldwide** *(~200 000 cases /year).*

- **Symptoms** : digestive, **neurological** (hypotension, bradycardia, paresthesia, cold allodynia, myalgia, arthralgia) and **cardiac,** potentially lethal.

- **Long term** prevalence and **body sensitization** effect = increase of symptoms severity upon next exposure, cross effects (ex : alcohol).

Ciguatera Food Poisoning



History :

- First mention : Chen Tsang Chi, chinese doctor of the T'ang dynasty (~650).

- From the XVth century, Colomb, Magellan, Cortes, Vasco de Gama describe the disease in the Caribbeans ; Fernandes de Quiros, Cook, Bligh,… in Pacific.

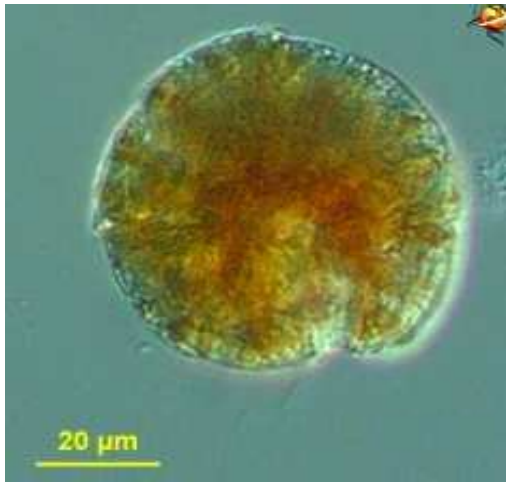
- First precise description of a ciguatoxin intoxication : James Cook, Sept. 7th 1774 Nouvelles-Hébride.

- The term ciguatera : attributed to cuban Felipe Poey, 1866 for a neuro-digestive intoxication due to a marine gasteropode (*Livona pica*) whose cuban name is « cigua ».

THE CAUSAL AGENT : CIGUATOXINES (CTXS)

- ***Among the most powerful marine biotoxines***
  (1 microgramme can kill a man)

- Produced by the dinoflagellate micro-algae *Gambierdiscus toxicus,* upon corral reefs damage



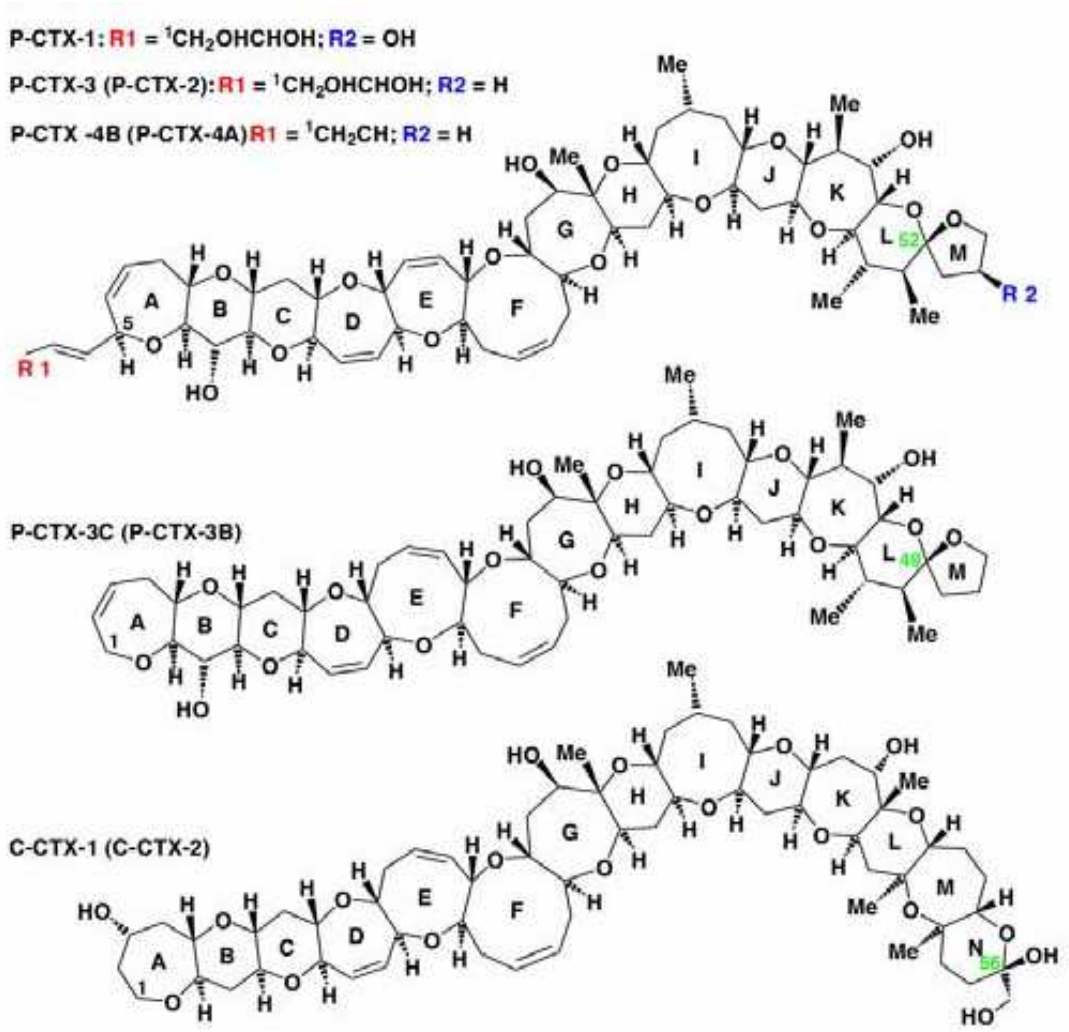
Gambierdiscus toxicus, (B Anderson, MBL.)

- Polycyclic polyethers, liposoluble, MW~1100 Da

- 3 famillies : Pacific = P-CTXs, Caribbean = C-CTXs, Indian Ocean = I-CTXs.

No simple detection test available !!!

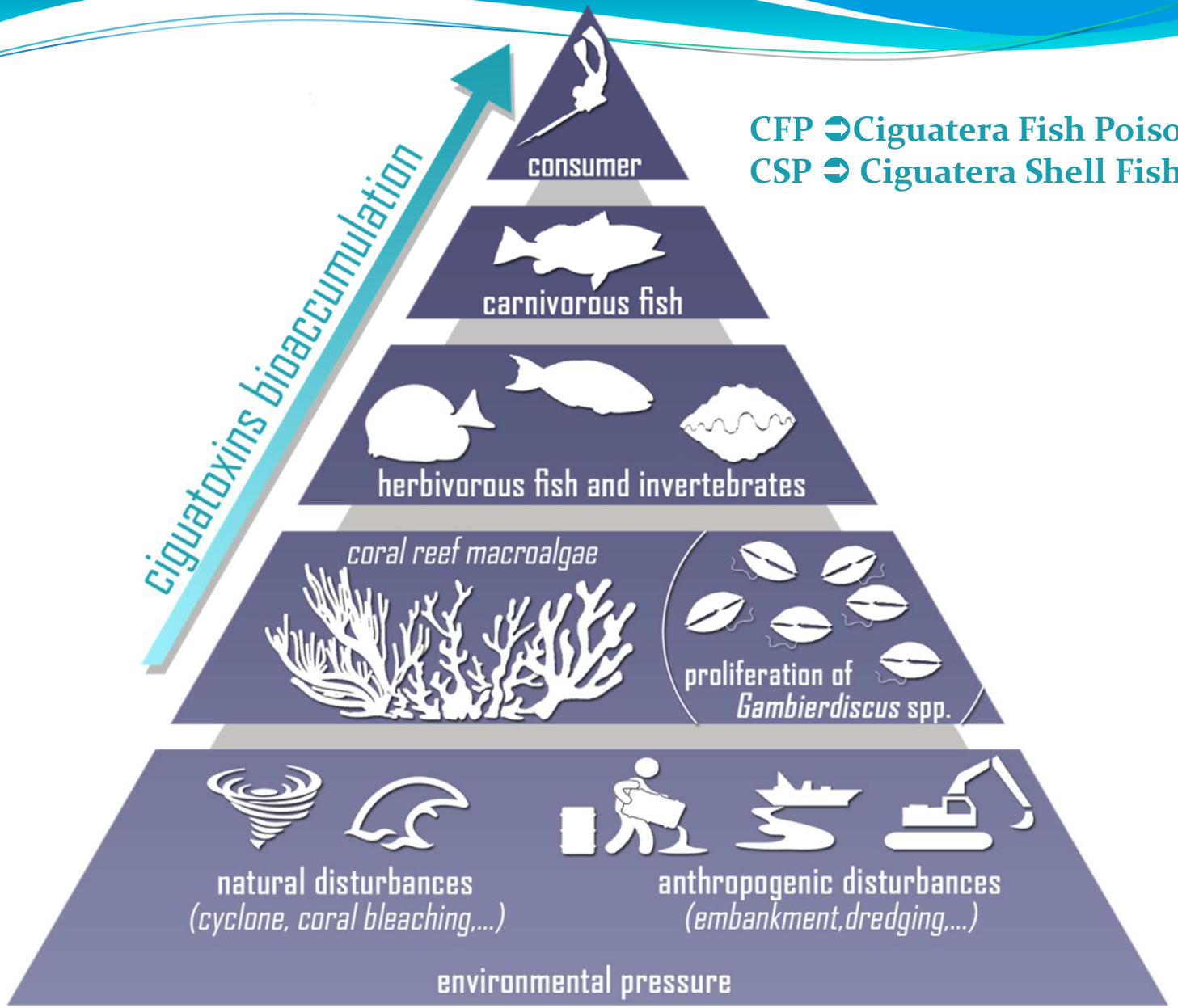
Ciguatoxines Molecular Structures



Pacific Ciguatoxines

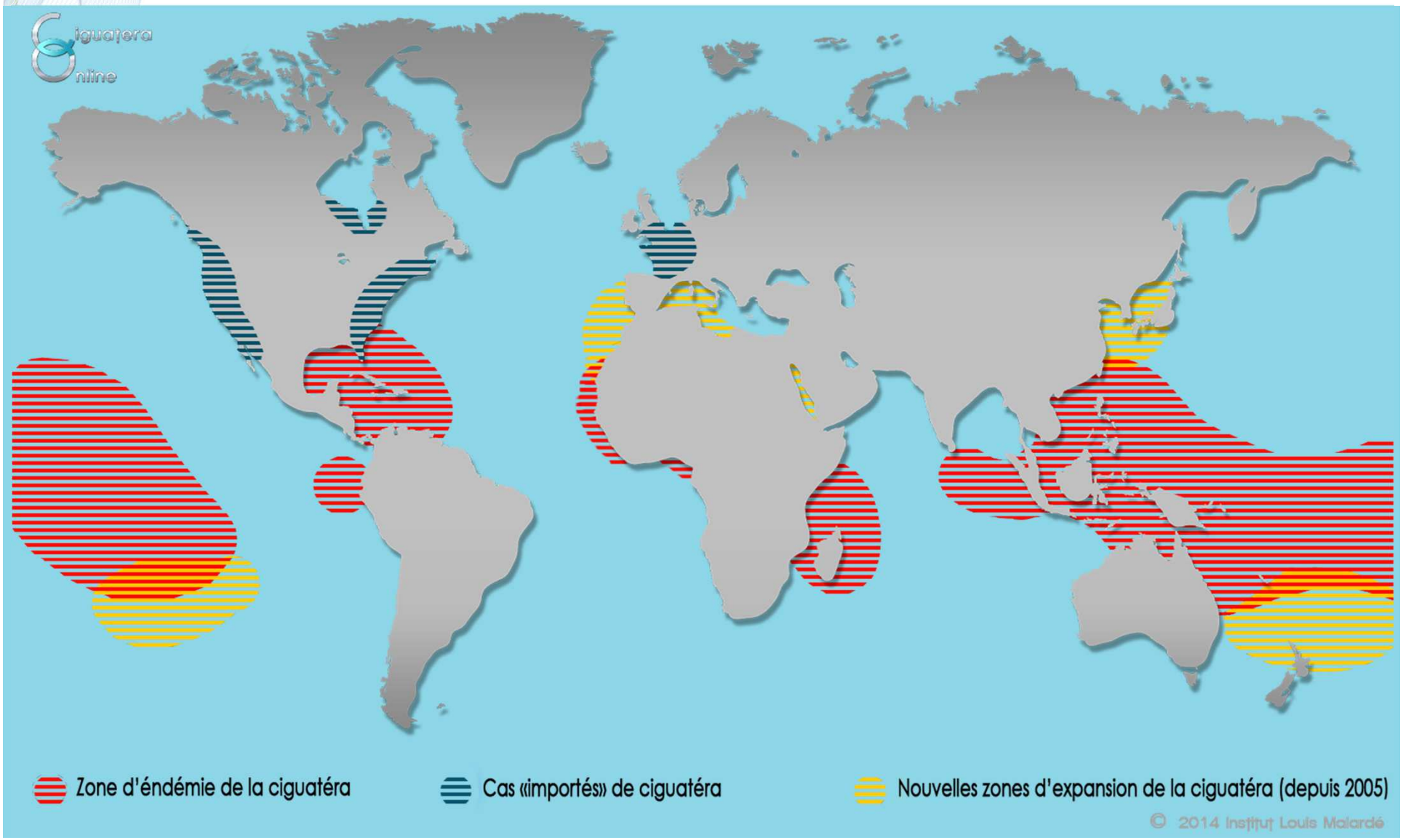
Caribbean Ciguatoxines

(Nicholson and Lewis, Marin Drugs, 2006).

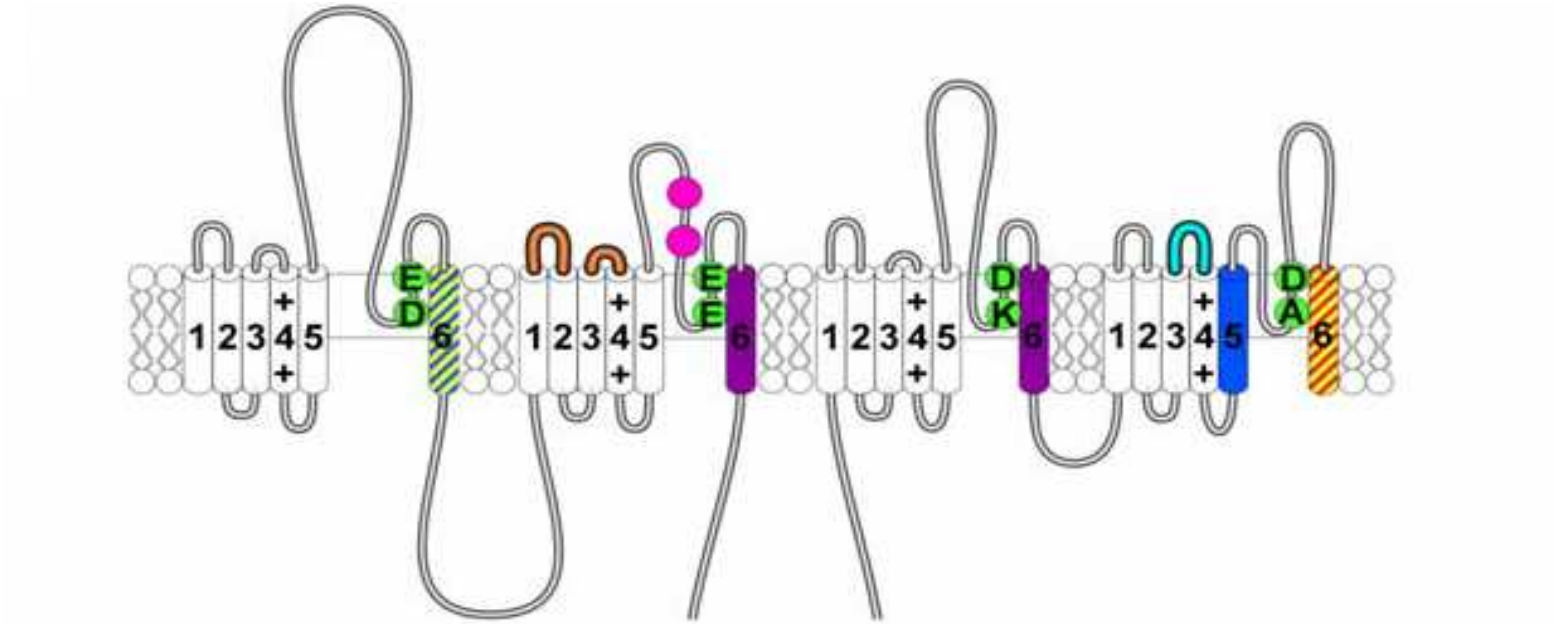


CFP ➲Ciguatera Fish Poisoning
CSP ➲ Ciguatera Shell Fish Poisoning

World Repartition of Ciguatera



CTXs Targets : Voltage Gated Sodium Channels



Site 1 Tetrodotoxin
Saxitoxin

Site 1 μ-Conotoxins

Site 2 Batrachotoxin
Veratridine

Site 3 Scorpion α-toxins,
Sea anemone toxins,
Spider δ-toxins

Site 4 Scorpion β-toxins
Magi 5

Site 5 Ciguatoxin
Brevetoxin

Site 7 Pyrethroids

Drugs Local anaesthetics
Anticonvulsants

(Nicholson and Lewis, Marin Drugs, 2006).

CTXs Targets :
Voltage Gated Sodium Channels

- VGSC play key roles **in electric signal propagation** in excitable tissues (nerves, heart, muscles).

- Mechanism : local activation of VGSCs provokes a transiant depolarization, responsible of the ascendant phase of the action potential.

- **CTXs stay fixed and prevent repolarization of VGSCs.**

CTXs Detection : problematic

- Current assays : heavy, difficult, long, expensive, and/or unethical (*Mouse bioassay, RBA, CBA, HPLC…)*

→ ***URGENT NEED FOR A SIMPLE DETECTION TOOL !!!***

- Can we design a biosensor for Ciguatoxins ?

- Yeast based bio sensors : interesting for several reasons

Developping yeast-based Biosensors

Scientific Questions :

- Cell wall permeability/reactivity towards toxins?

  Investigation tools : cell wall mutants, tagged toxins.

- Membrane target (receptor)? Binding?

  Investigation tools : Cloning,

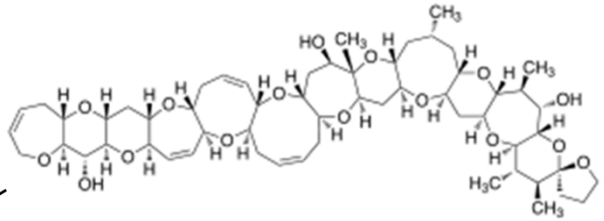
  SMFS with Atomic Force Microscopy.

- Cellular signalisation pathways?

  Investigation tools : reporters, Fluorescent proteins,

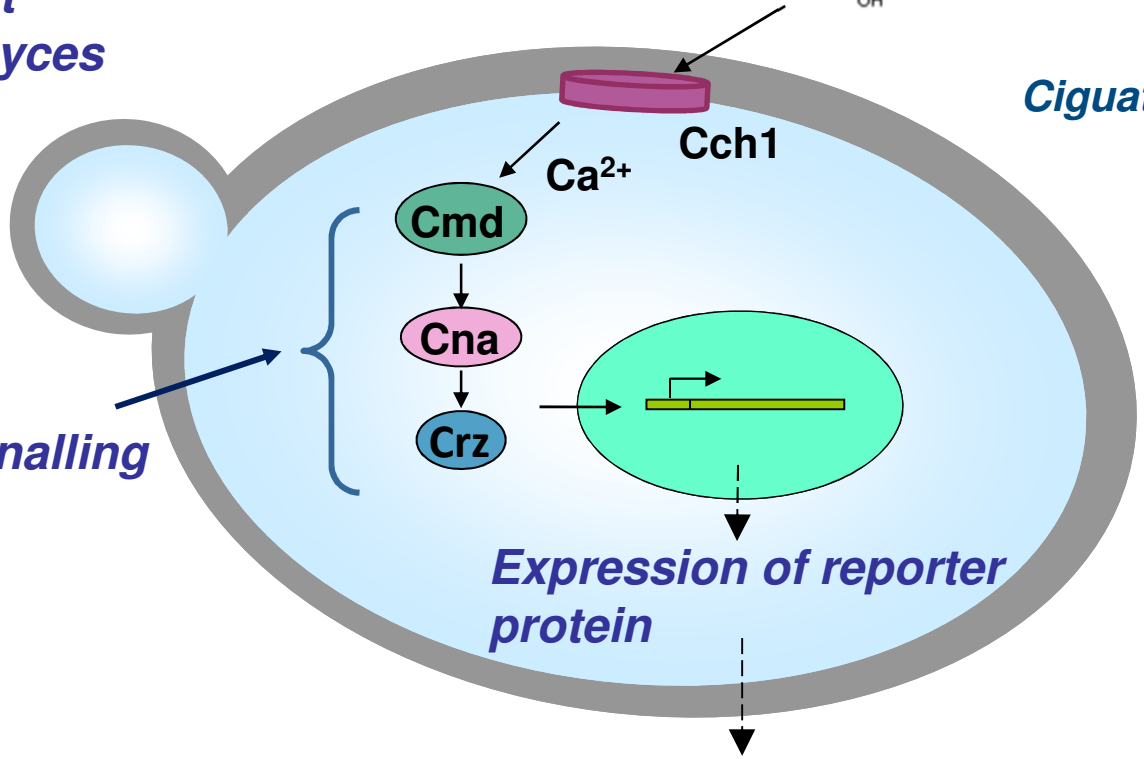
  western blotting, RT-PCR.

Biosensor strains design



Ciguatoxins

Model Yeast Saccharomyces cerevisiae

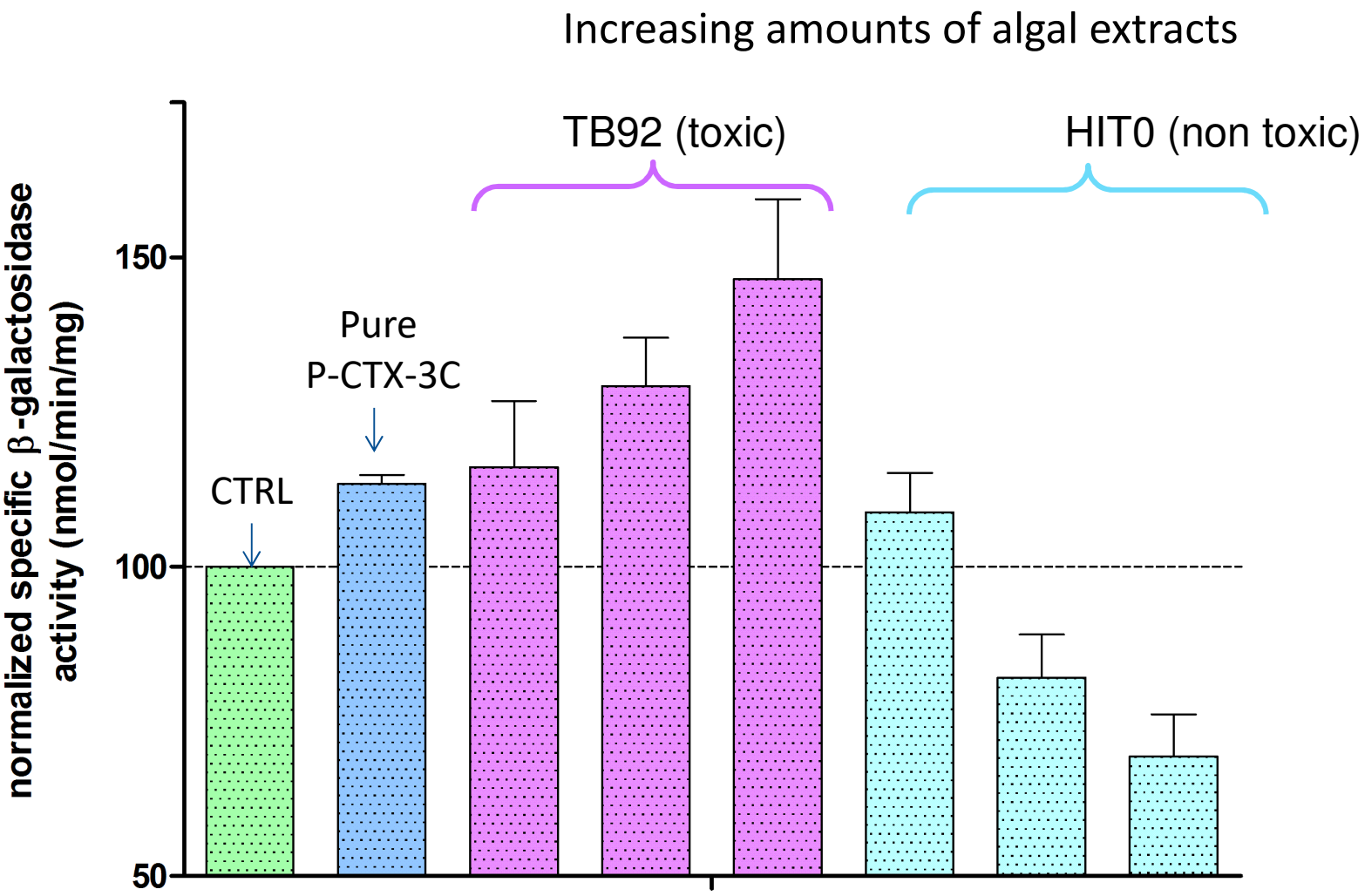


Cellular signalling pathway

Expression of reporter protein

Signal (fluorescence or colorimetry)

Response to Algal extracts



(Martin-Yken et al., Environmental Research, April 2018)

Perspectives :

Understanding yeast cellular response to CTXs

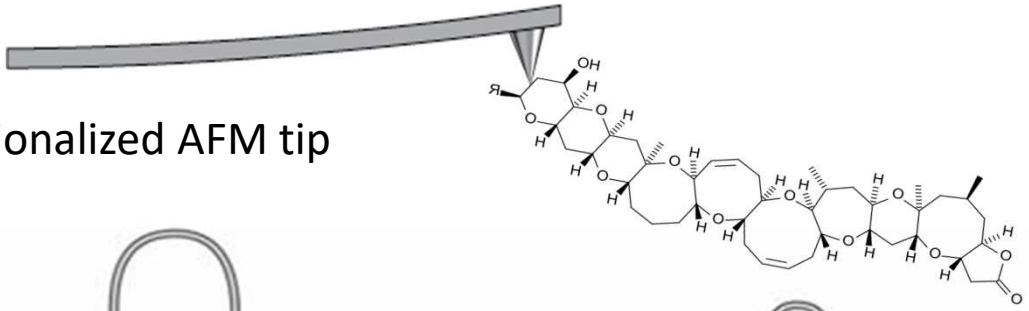
- Cell wall transfert
- Competition CTXs - Rosmarinic acid and tetrodotoxin.
- Inhibitory Effect of HIT0 extracts ????
- Activators / repressors / blokers of VGSC and Cch1
- Hyper-active point mutants of Cch1 and Crz1, Chimera Cch1-VGSC

Response Optimisation

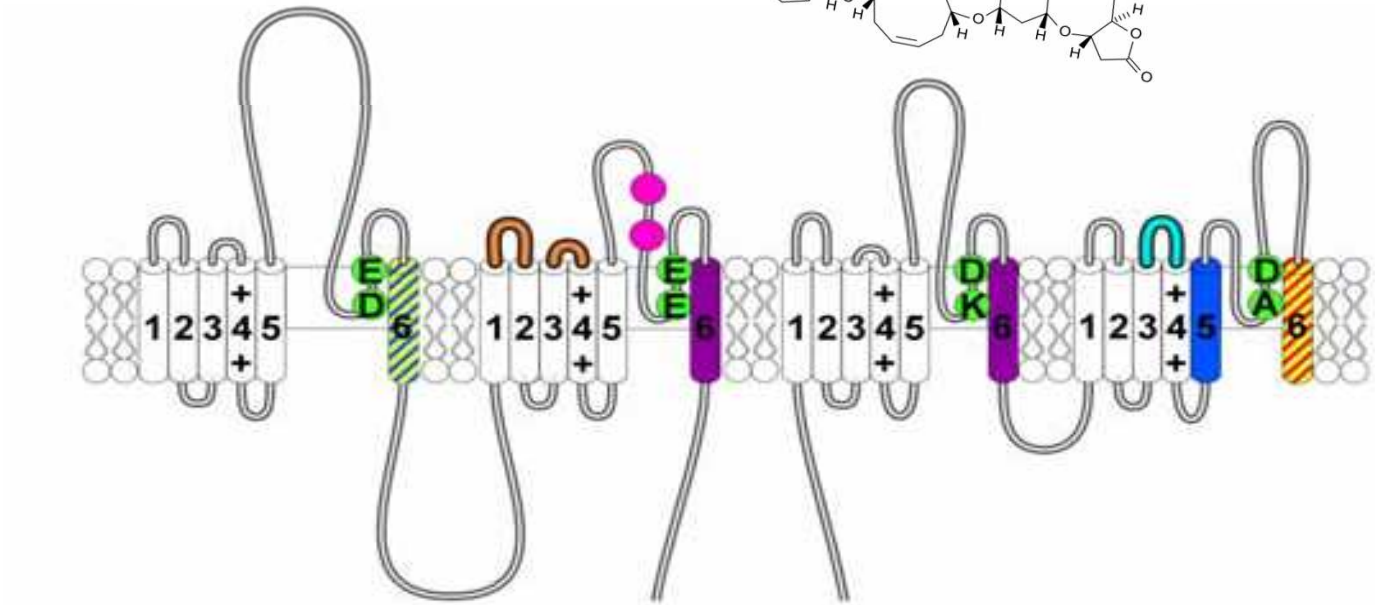
Developp a generic approach, apply to other toxins and drugs binding VGSCs.

Future applications : build a bio-sensor : huge interest for Pharmacology and Environmental domains

NeuroSens Project : LISBP-LAAS
Using Atomic Force Microscopy to study Neurotoxines Binding on VGSCs



Functionalized AFM tip



LISBP
Ingénierie des Systèmes Biologiques et des Procédés

NeuroSens :

A totally Innovating Approach, very powerfull and precise.

➡ Informations on nature and intensity of molecular interactions between **key receptors of our neuronal and cardiac cells** and ligands of pharma. and env. interest.

➡ *Ex vivo* testing of the inhibition of toxin fixation by **potential therapeutic molecules**

➡ Apply to other toxines (brevetoxines, ciguatoxines, rosmarinic acid, tetrodotoxin…)

Collaborations :

- Institut Louis Malardé, Papeete (French Polynesia) :

  Dr Mireille Chinain, Dr Hélène Taiana Darius.

- IRD Polynésie française : Dr Dominique Laurent.

- LAAS CNRS Toulouse :

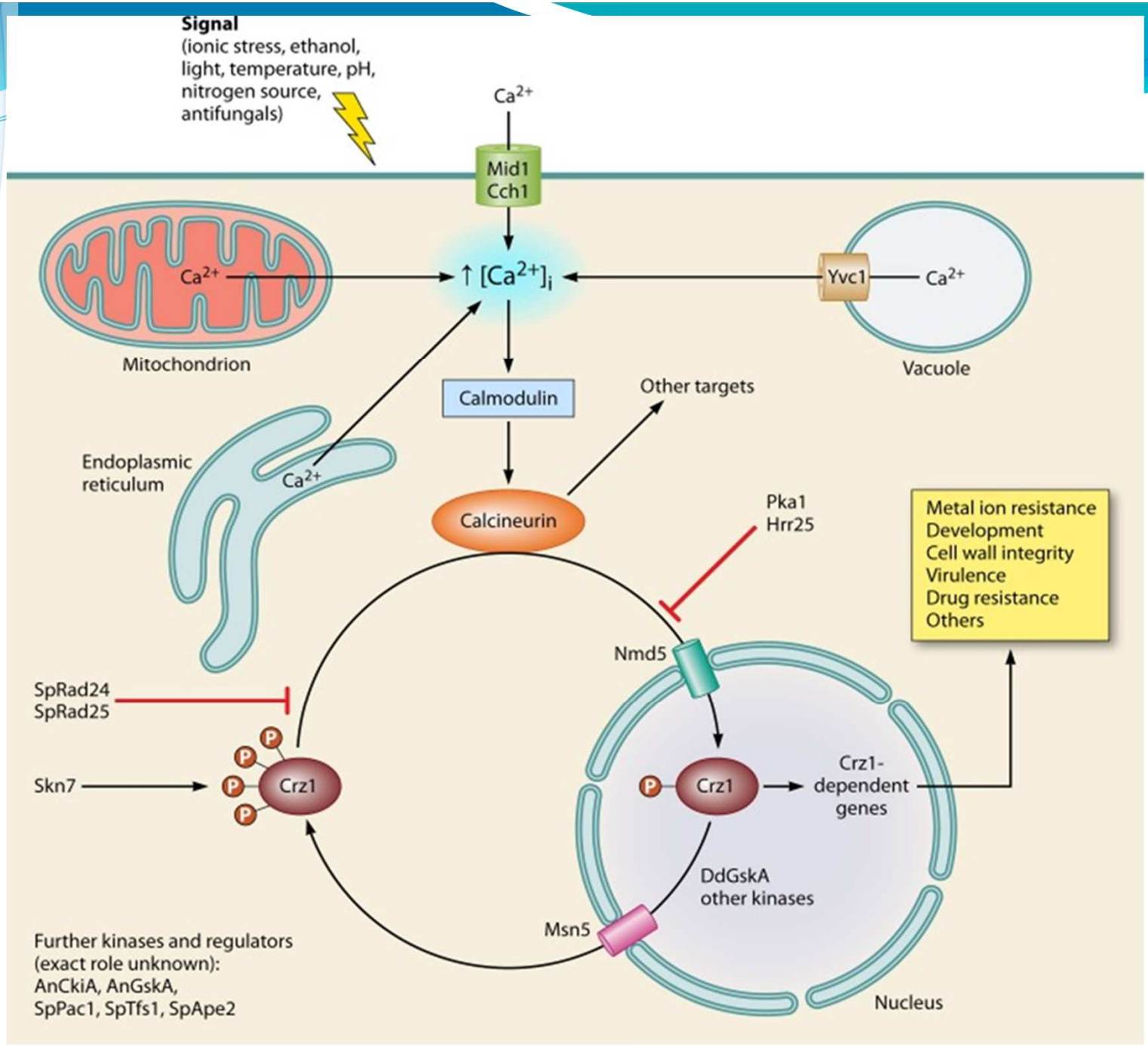
  Dr Etienne Dague, Dr Emmanuelle Trévisiol,
  Dr Christophe Furger, Camille Gironde.

Thanks for your attention !

Interested in Microbial Adhesion ?

*Come to **BioPhysAdh meeting, 10-11 septembre, Toulouse.***

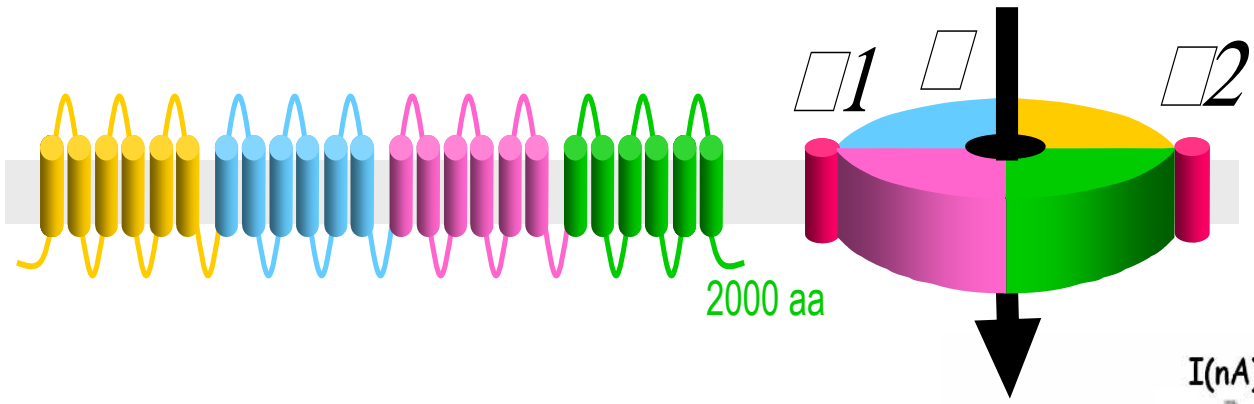
website : https://biophysadh.sciencesconf.org


Signal
(ionic stress, ethanol, light, temperature, pH, nitrogen source, antifungals)
Ca2+
Mid1
Cch1
Ca2+
↑ [Ca2+]i
Yvc1
Ca2+
Mitochondrion
Vacuole
Calmodulin
Other targets
Endoplasmic reticulum
Ca2+
Calcineurin
Pka1
Hrr25
Metal ion resistance
Development
Cell wall integrity
Virulence
Drug resistance
Others
Nmd5
SpRad24
SpRad25
Skn7
Crz1
Crz1
Crz1-dependent genes
DdGskA
other kinases
Msn5
Further kinases and regulators (exact role unknown):
AnCkiA, AnGskA,
SpPac1, SpTfs1, SpApe2
Nucleus

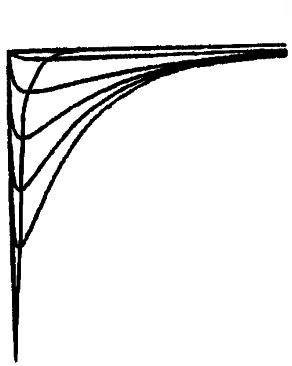
(Thewes, 2014)

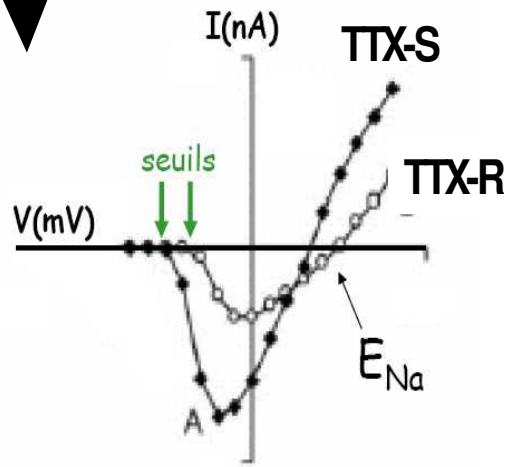
CTXs Targets : Voltage Gated Sodium Channels

rôles dépolarisation de la membrane du neurone et propagation du PA

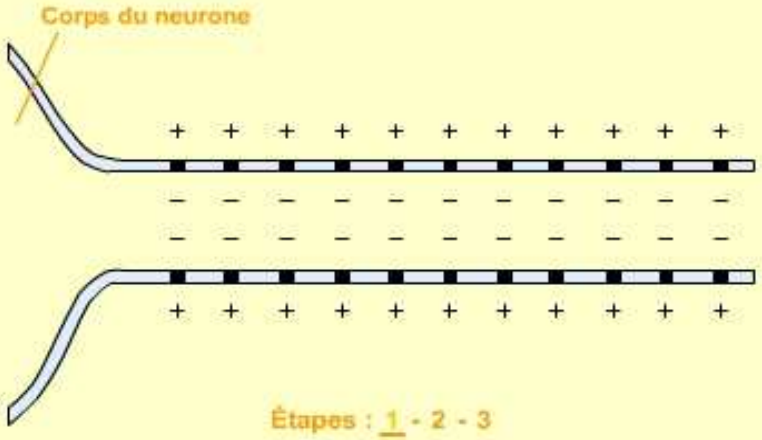


bloqueurs tétrodotoxine
anesthésiques locaux
anti-convulsivants
anti-arythmiques

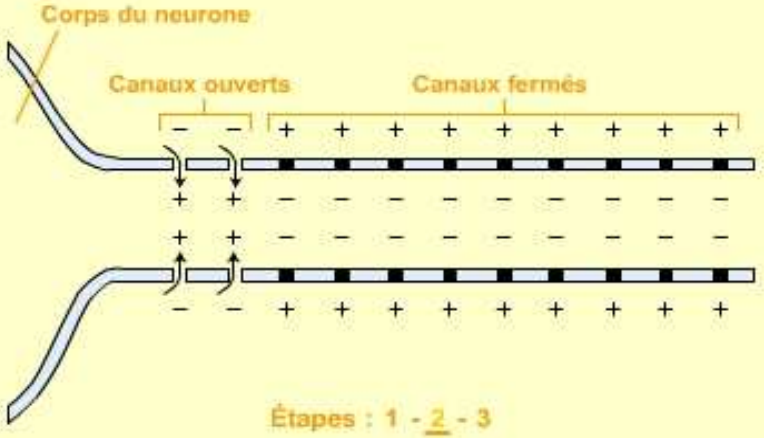




1. À l'état de repos, les canaux de la membrane du neurone créent une répartition inégale des charges : davantage de charges négatives à l'intérieur et plus de charges positives à l'extérieur.



2. L'influx nerveux, en ouvrant et fermant certains canaux, va inverser le potentiel électrique de part et d'autre de la membrane : durant un bref instant, l'intérieur devient plus positif que l'extérieur.



3. Le potentiel de repos est rapidement rétabli par le travail d'autres canaux. Mais déjà, dans la région voisine, le phénomène se répète, propageant ainsi l'influx nerveux le long de l'axone du neurone.