Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

03 – Mechanisms @proteins, part 1

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www.recetox.cz

Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Major mechanisms (modes of action) to be discussed in detail

- **Proteins** and inhibition of enzymatic activities
- Mitotic poisons & microtubule toxicity
- Ligand competition – receptor mediated toxicity

- **Membrane** nonspecific toxicity (narcosis)
- Toxicity to membrane gradients

- **DNA** toxicity (genotoxicity)

- **Complex** mechanisms
  - Oxidative stress – redox toxicity
  - Defence processes as toxicity mechanisms and biomarkers - detoxification and stress protein induction
  - Toxicity to signal transduction

Proteins and enzyme inhibitions → toxicity mechanisms

Proteins as targets to toxicants

Structure

– primary (sequence of aminoacids, AA),

- secondary, tertiary, quarternary (folding – important for functions)

Proteins - large/long – key target for number of toxicants!

= polypeptides - tens to thousands of AA

Peptides (small, “πεπτός, "digested“, 2x AA to e.g. 20x AA)

may have various functions (e.g. protective - glutathione)

Key functions of proteins

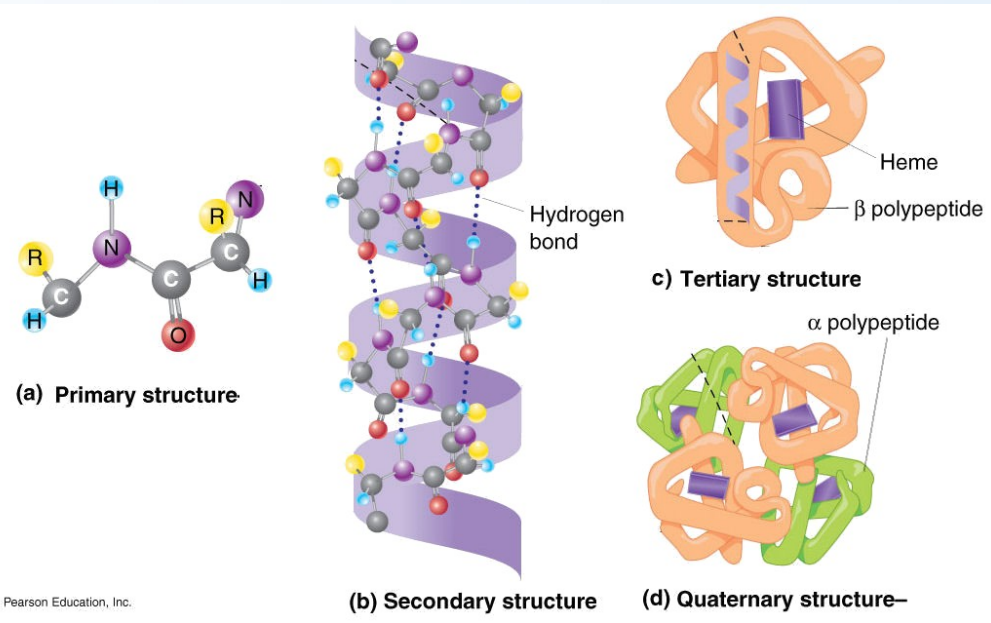
STRUCTURE and PROTECTION

CATALYSIS (enzymes)

TRANSFER (information and mas

- receptors, channels, transporte

... student should know examples..



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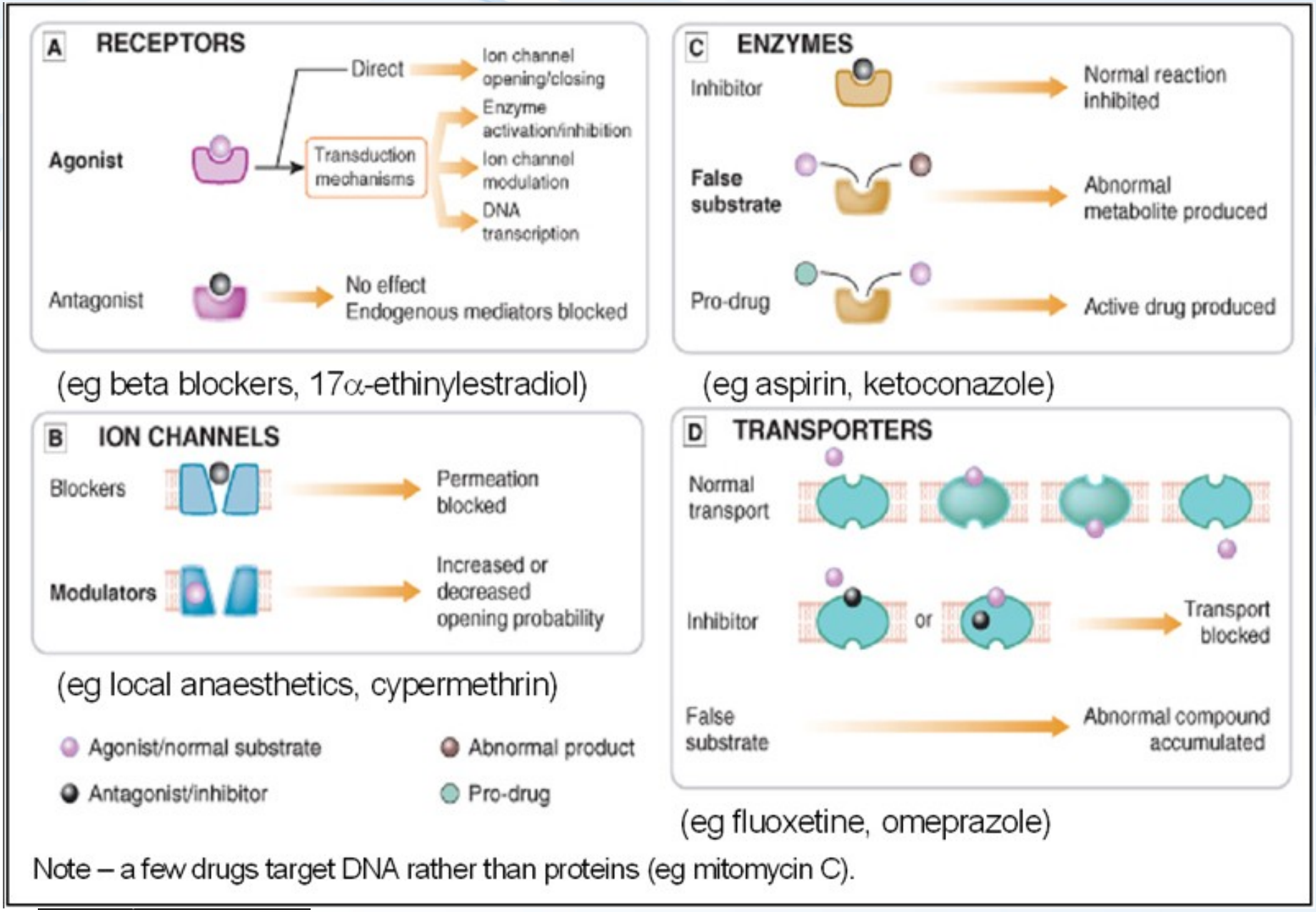
Overview - interactions of small molecules with proteins



CATALYTICAL PROTEINS = Enzymes

- Catalysis - what is it?
  ... student should know

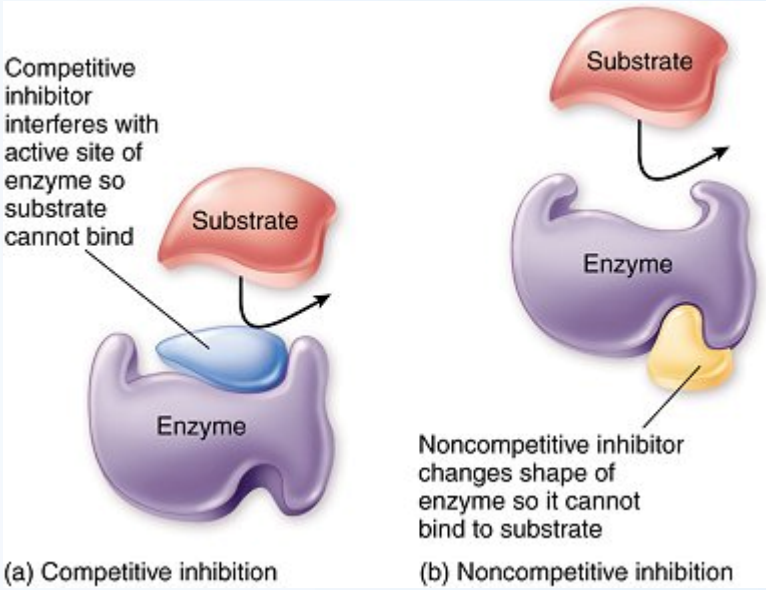
- Thousands of enzymes (vs. millions of compounds)
  - present in body fluids, membranes, cytoplasm, organelles..

  ... student should know key examples

- Enzymology – science of enzymes
  - includes also **interactions** of enzymes with small molecules (xenobiotics)

Enzymes *vs* toxicants

- **Interactions** that make a chemical compound an enzyme (or protein) inhibitor
  - Competitive vs. non-competitive
    - active site vs. side domains
  - Specific vs nonspecific
    - affinity of the inhibition .... is determined by the effective concentration (lower the effective concentrations → higher the affinity)
  - Nonspecific inhibitions
    - **Most of the chemical toxicants** (!)
    - Compound interacts with functional groups on the surface of the protein (reactive toxicity) or affects the environment (high osmomolarity, changing pH)

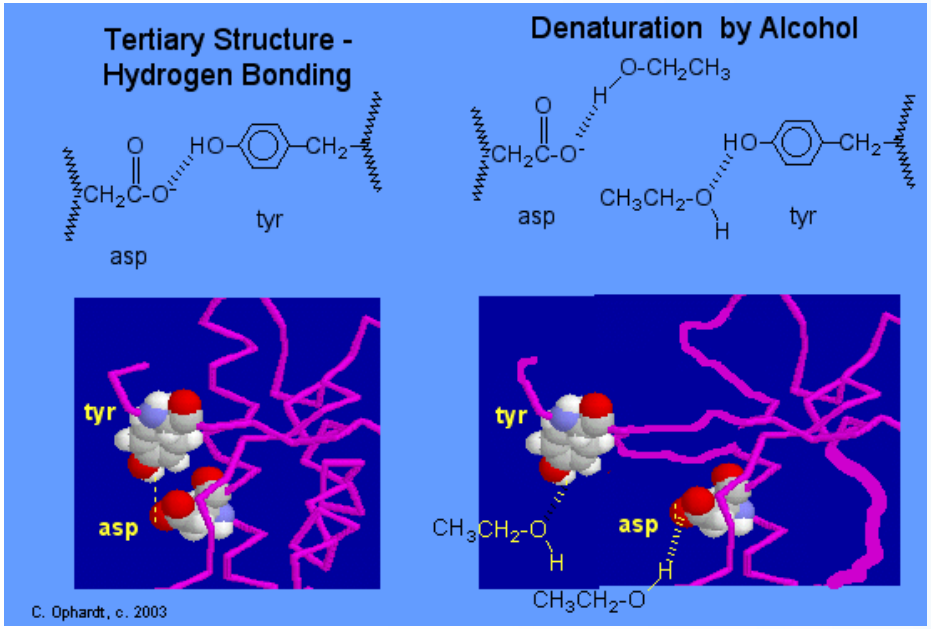


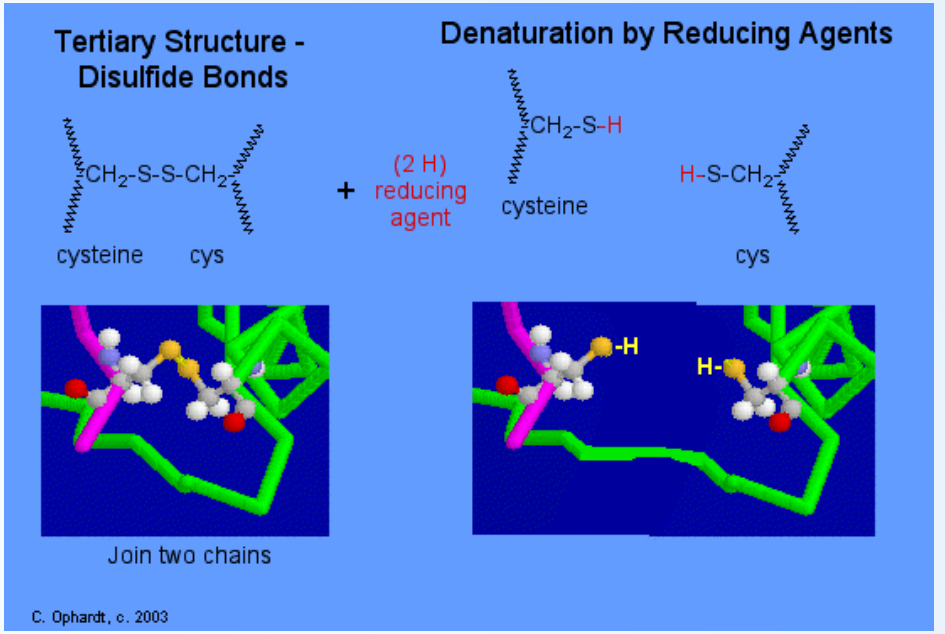
Non-specific interactions & denaturation

Most common interactions (and some examples)

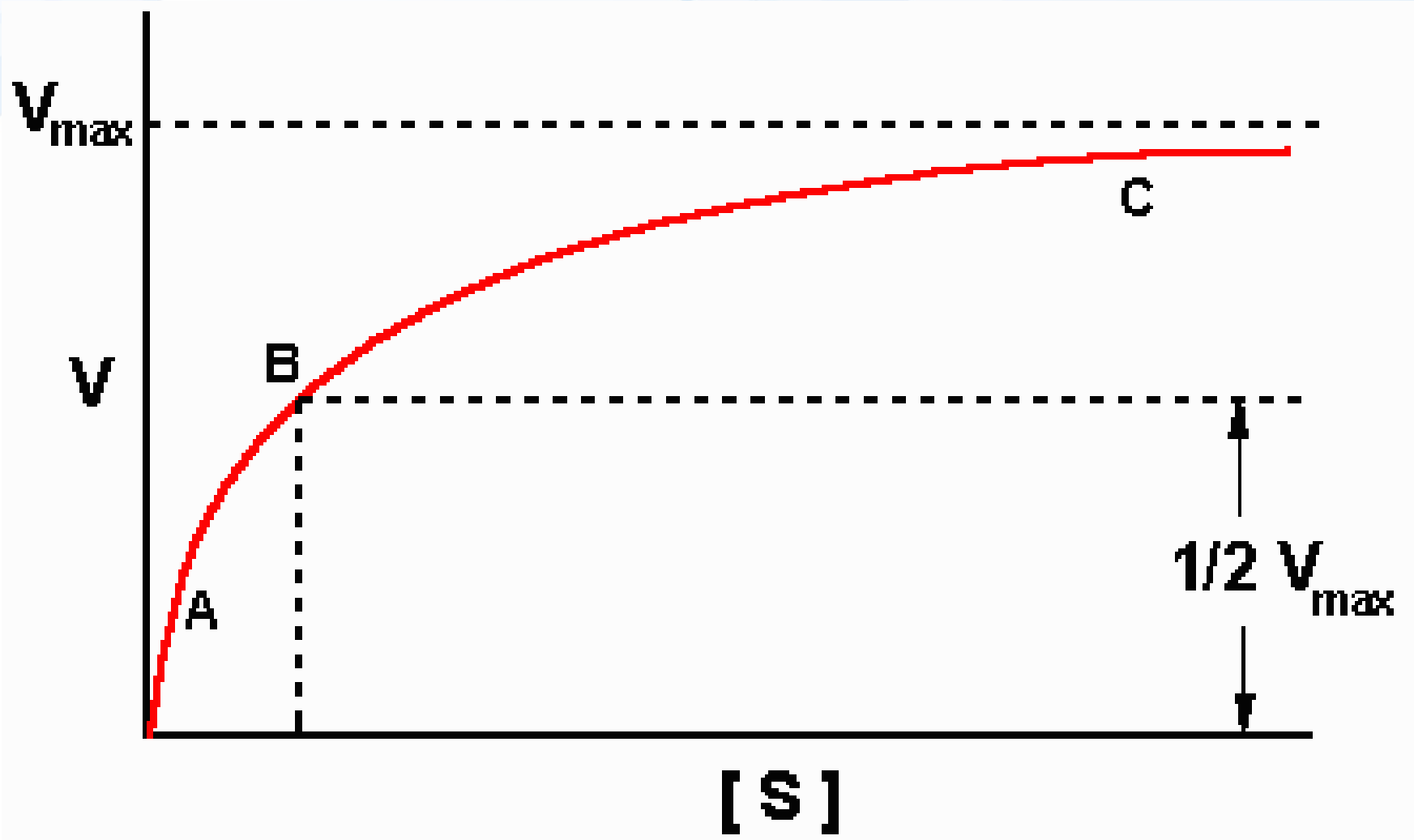
Hydrogen bond disruption	alcohols, amines
Ion bonds	acids (COOH), alkalic compounds (amines) toxic metals Hg^{+2}, Pb^{+2}, Cd^{+2} , Ag^{+1} Tl^{+1}, carbonyls
S-S bonds	toxic metals

See also http://www.elmhurst.edu/~chm/vchembook/568denaturation.html

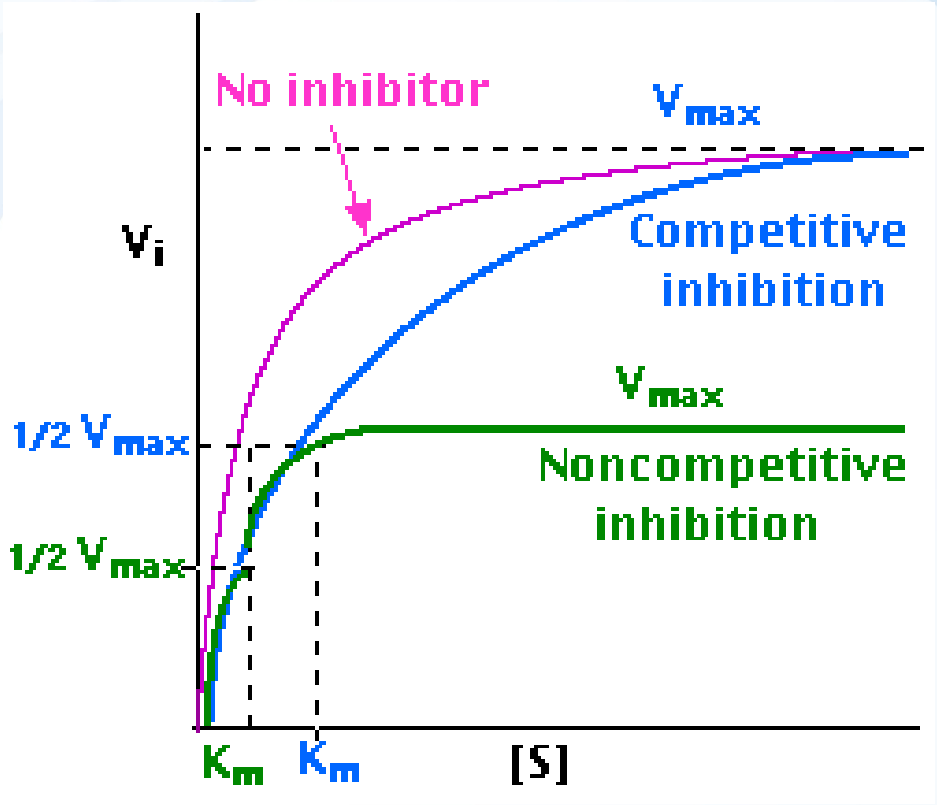




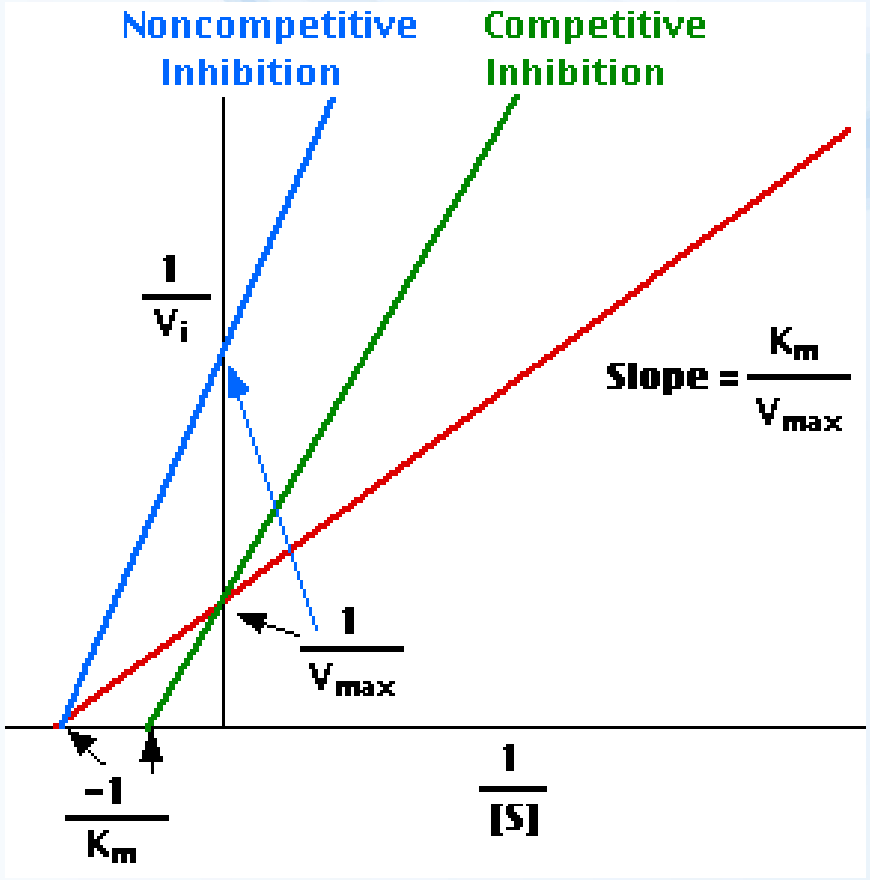
Kinetics of the enzyme reaction (Michaelis Menten)



Michaelis Menten INHIBITIONS



The kinetics informs
about the nature of the interaction !



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Enzyme inhibitions by toxicants – overview of key examples

Acetylcholinesterase (organophosphate pesticides)

Microsomal Ca^{2+}-ATPase (DDE)

Inhibition of hemes – respiratory chains (cyanides)

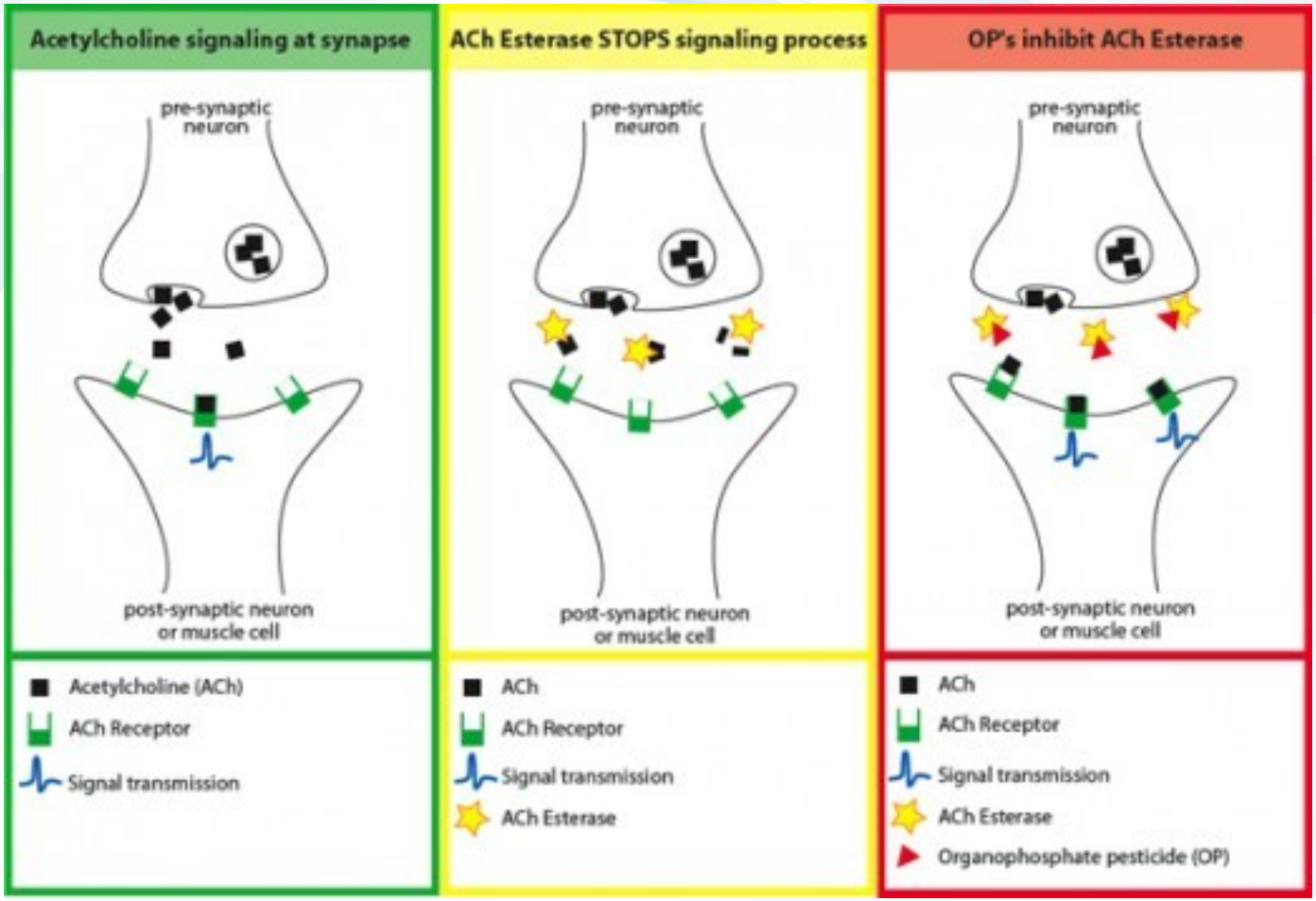
d-Aminolevulinic Acid Dehydratase (ALAD) inhibition
(lead - Pb)

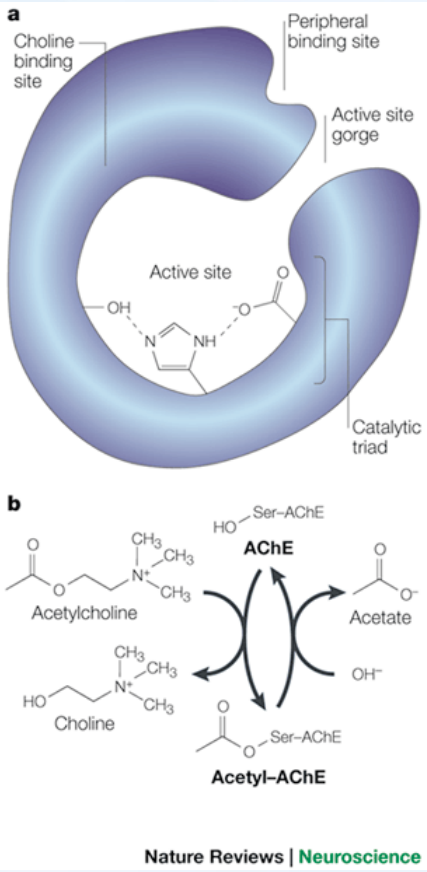
Inhibition of proteinphosphatases *(microcystins)*

Glyphosate (roundup) action

Enzyme inhibitions are beyond many other mechanisms
→ see e.g. CELL REGULATIONS etc.

Acetylcholinesterase inhibition by organophosphates





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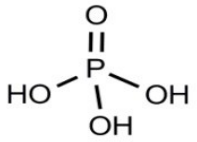
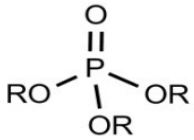
Acetylcholinesterase inhibition by organophosphates (and carbamates)

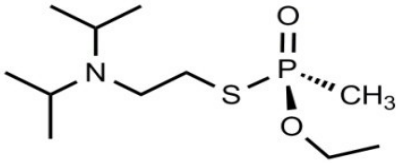
Nerve gases



Phosphoric acid

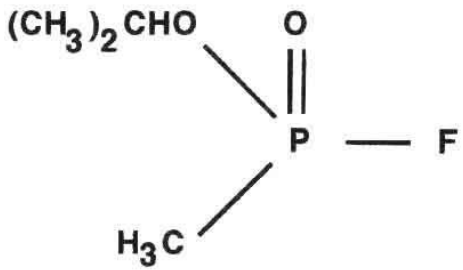


'Organophosphate'

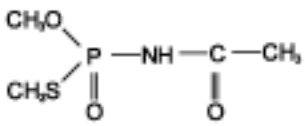


VX

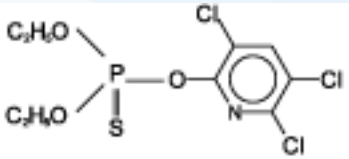
SARIN / GB NERVE AGENT
Isopropoxymethylphosphoryl Fluoride



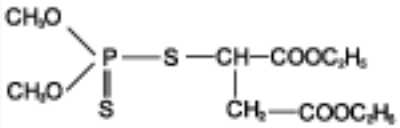
Insecticides - OPs



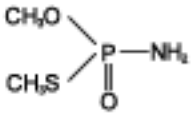
Acephate



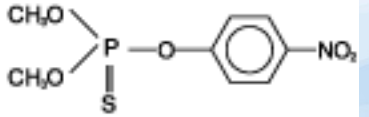
Chlorpyrifos



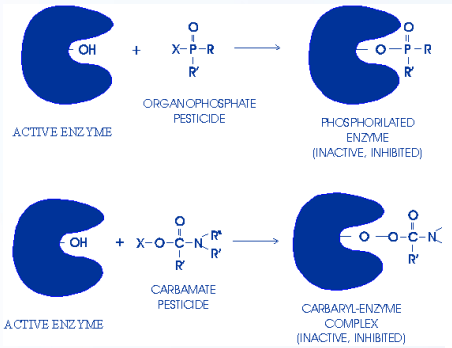
Malathion



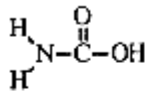
Methamidophos



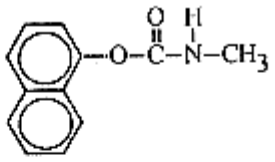
Parathion-methyl



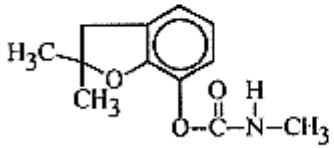
Insecticides - Carbamates



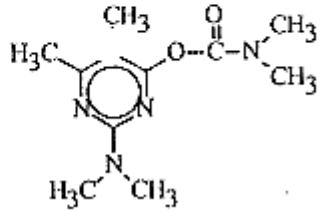
Carbamic acid



Carbaryl



Carbofuran

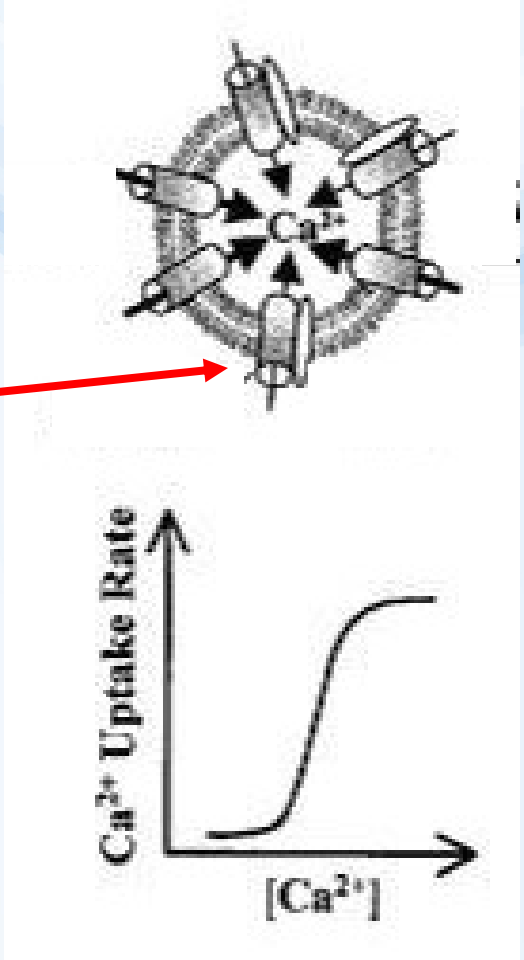


Pirimicarb

Inhibition of Ca^{2+}-ATPase by DDE

Ca2+ in cells

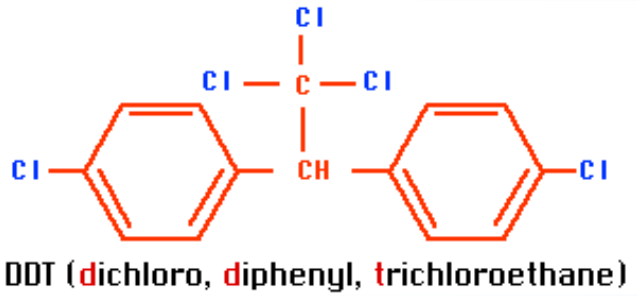
* general signalling molecule (see later)
* stored in (endo-/sarcoplasmatic reticulum)
* assures contractility of muscles
* concentrations regulated by Ca^{2+}-ATPase



DDE

→ calcium metabolism in bird eggs

→ egg shell thinning



DDT (dichloro, diphenyl, trichloroethane)

DDE

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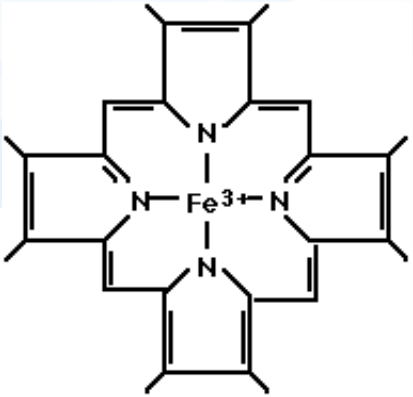
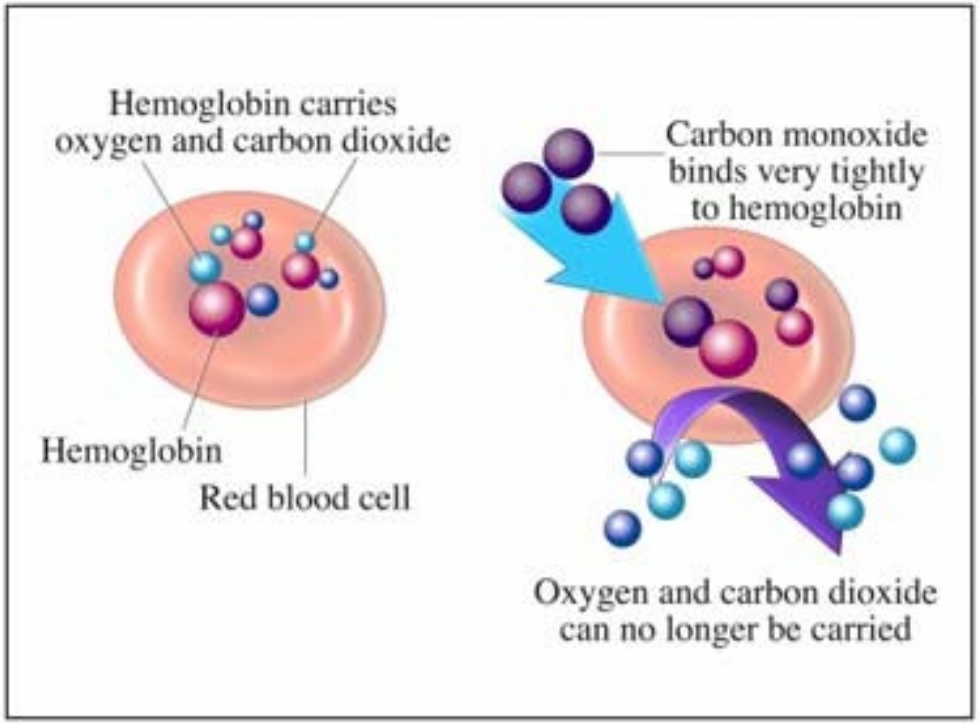
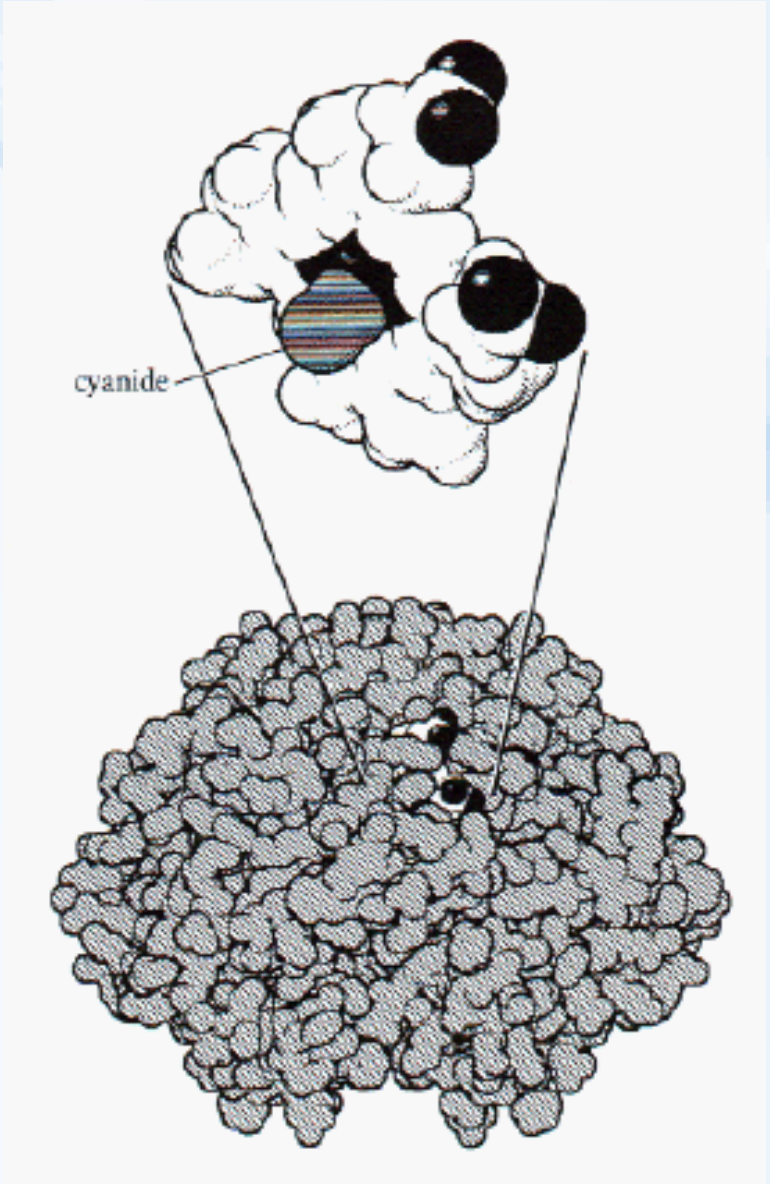
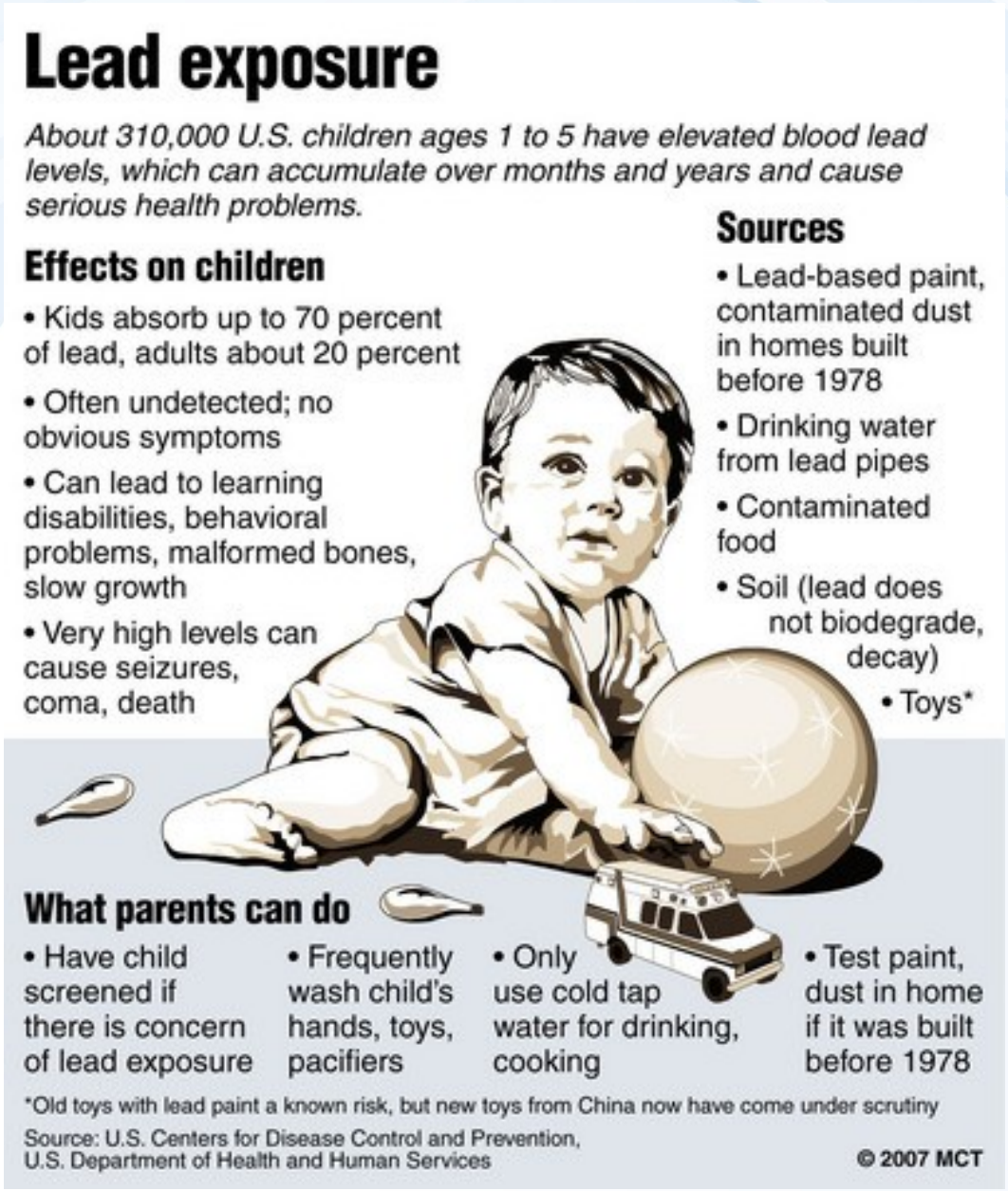
Inhibition of hemes – e.g. Haemoglobin, Mitchochondria, CYP450 etc. (cyanide HCN, carbon monooxide – CO)







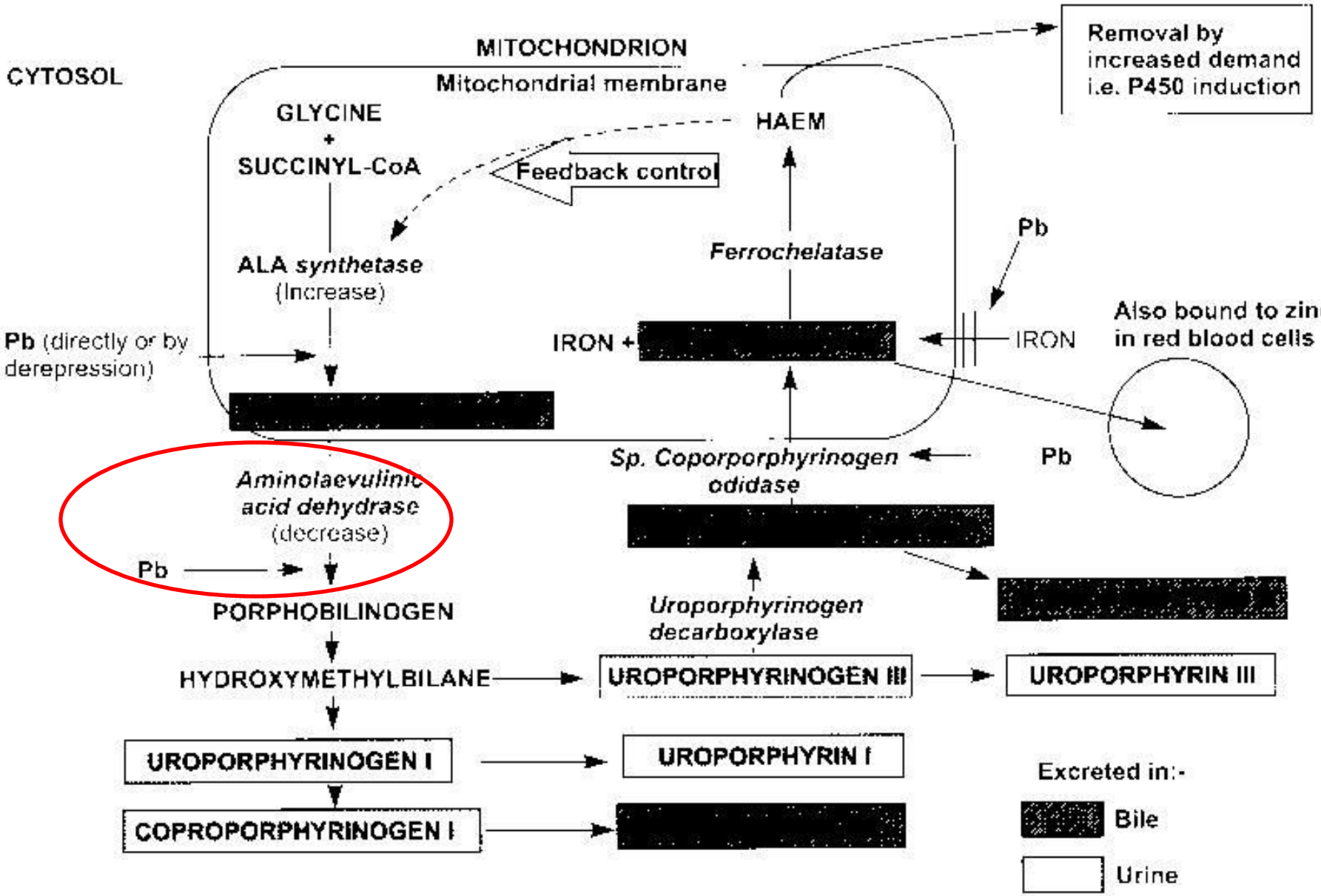
ALAD inhibition by lead (Pb)



Problem mostly in the USA

Ban of Pb-containg petrols

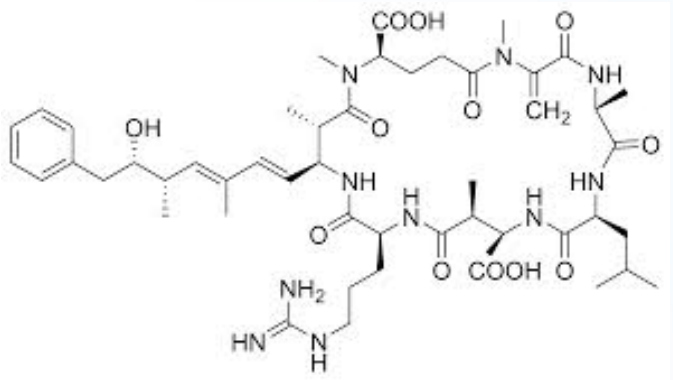
ALAD inhibition by lead (Pb) – inhibition of HAEM (!) synthesis



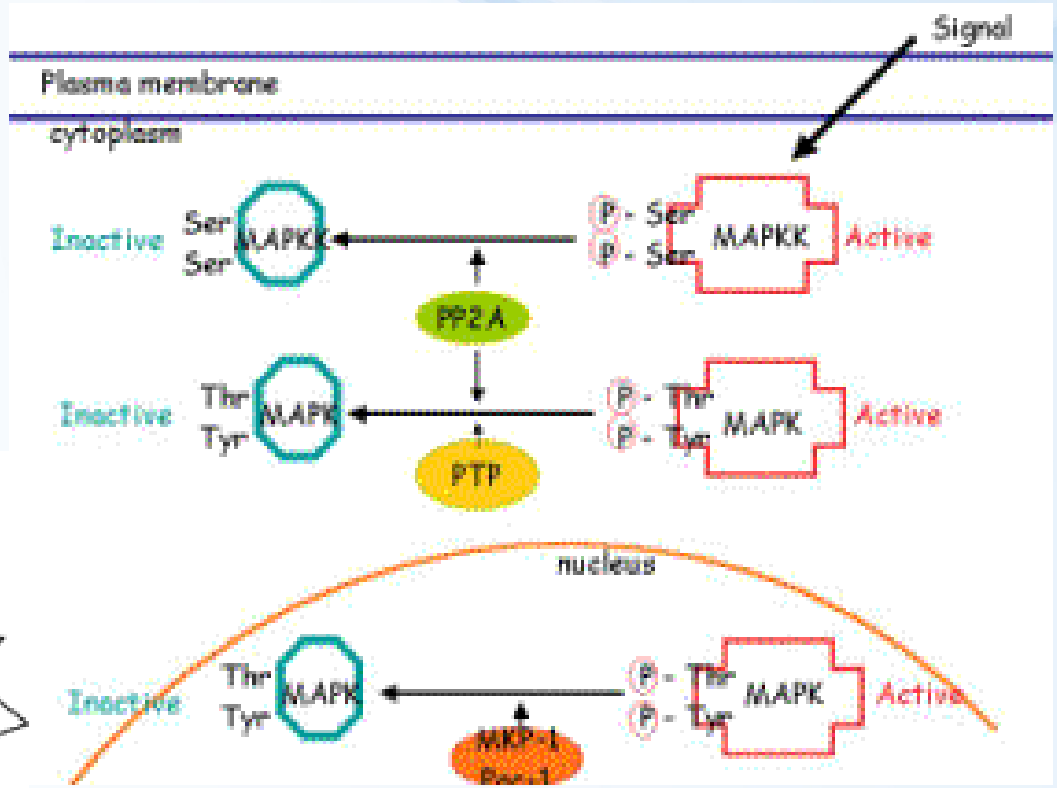
Inhibitions of PROTEINPHOSPHATASES by microcystins

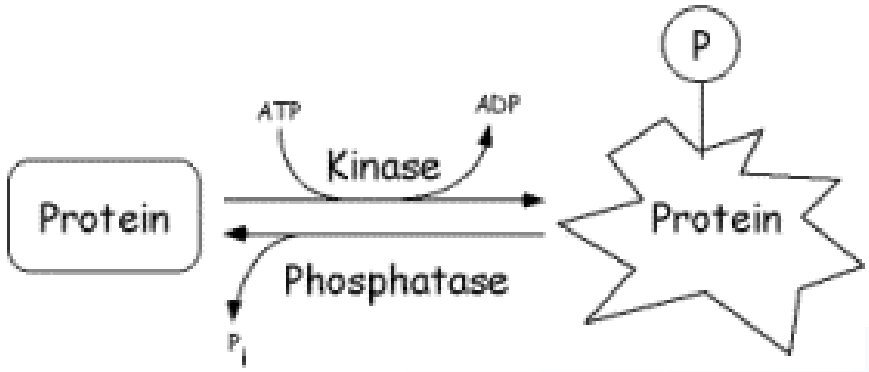
Microcystins (7x AA – heptapeptides)

Cyanobacterial toxins produced in eutrophied waters (water blooms) up to tons/reservoir

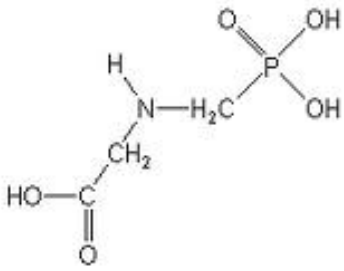


PPases – signalling enzymes (see further)





Glyphosate action



N-(phosphonomethyl)glycine

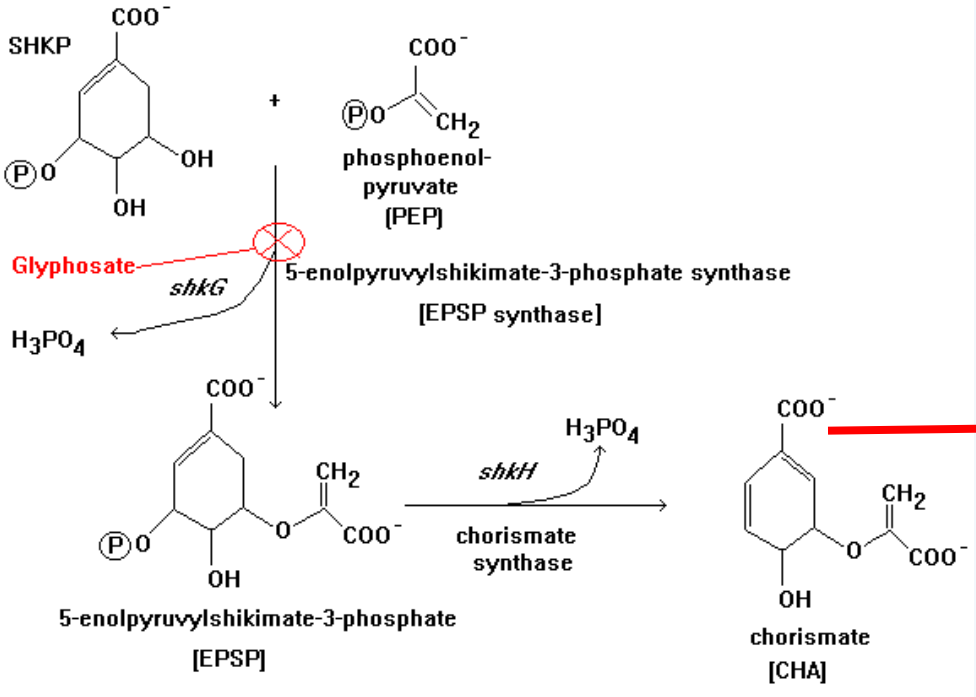
Broad-spectrum herbicide („**RoundUp**“)

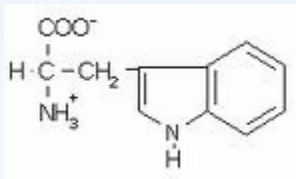
Selective inhibition of ESPs 5-*enol*pyruvylshikimate-3-phosphate synthase;

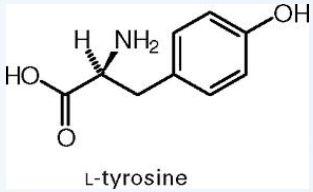
(synthesis of aromatic AAs – Tyr, Trp, Phe)

Uptake via leafs - only to growing plants

„Non-toxic“ to other organisms (no ESPs in animals, AA-like chemical - rapid degradation)







Structural proteins (CYTOSKELETON) as target for toxicants

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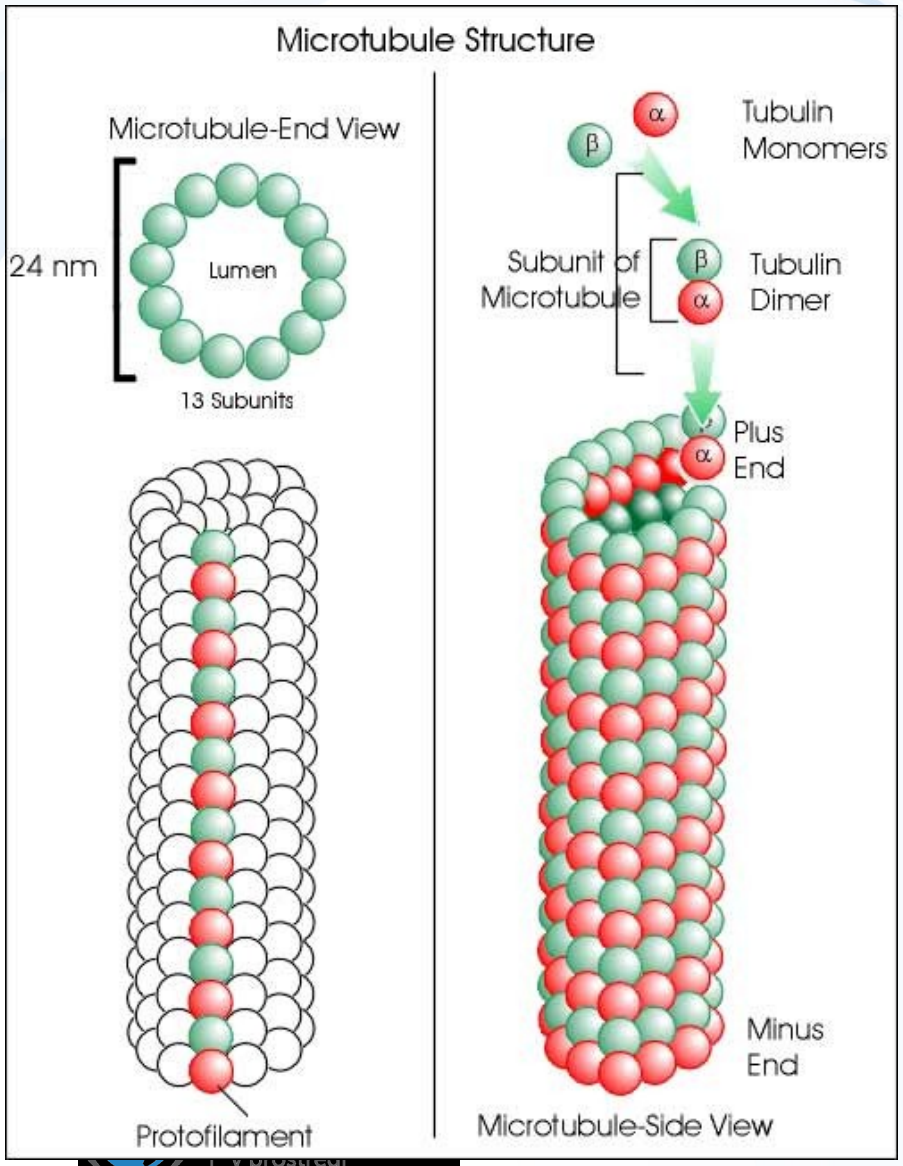
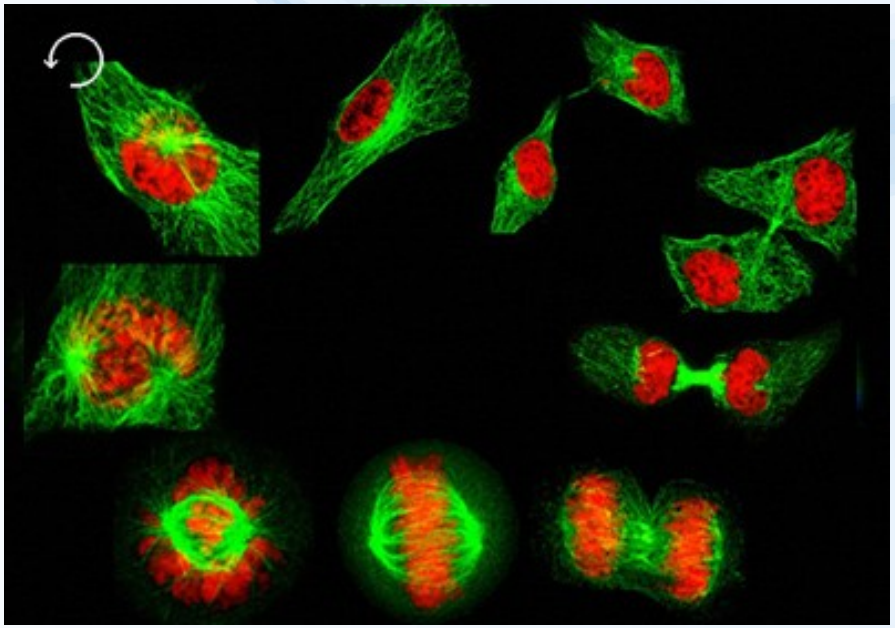
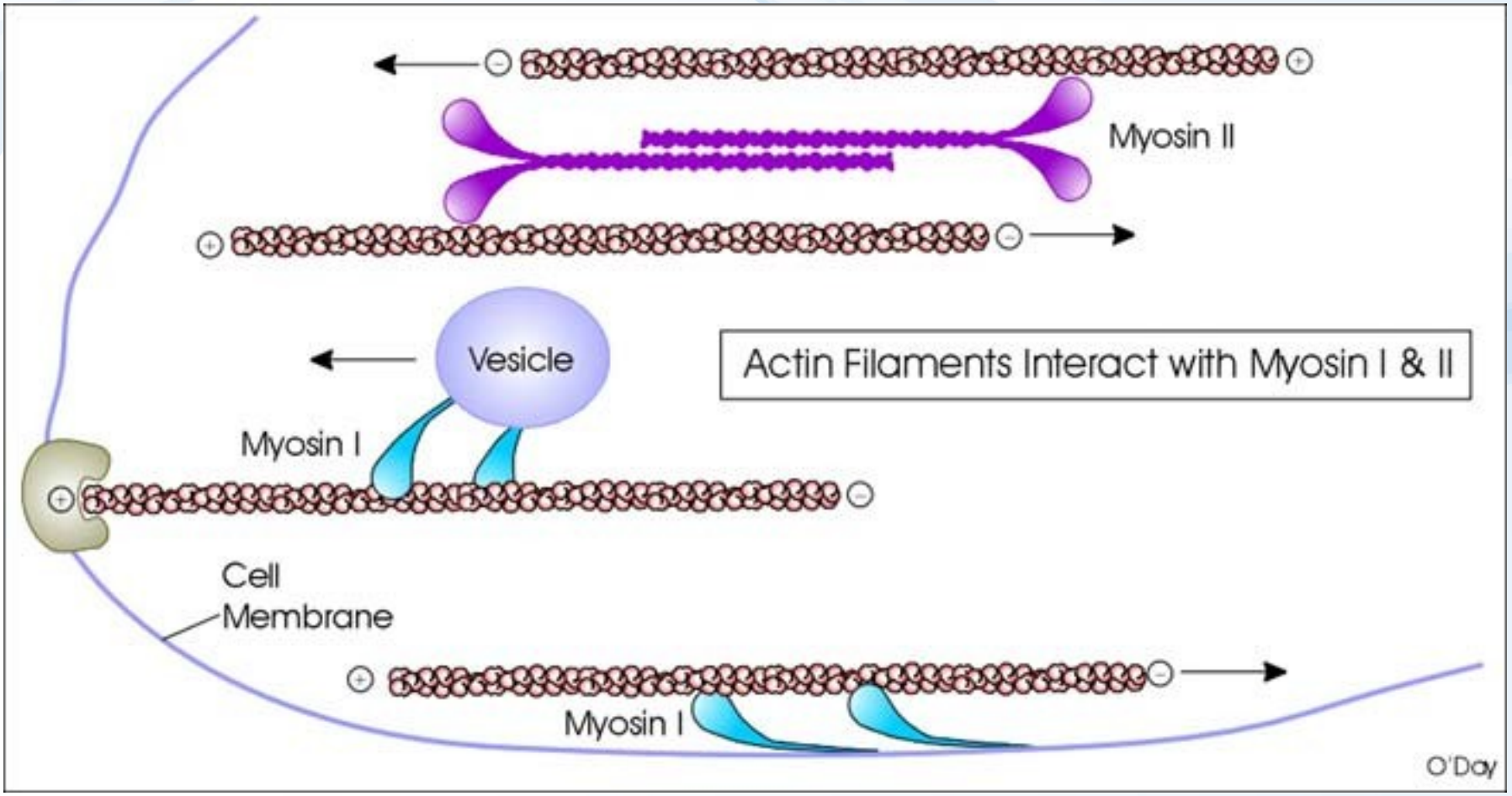
Structures of microtubules – dynamic de/polymerization



Visualization of microtubules during cell division – separation of chromosomes

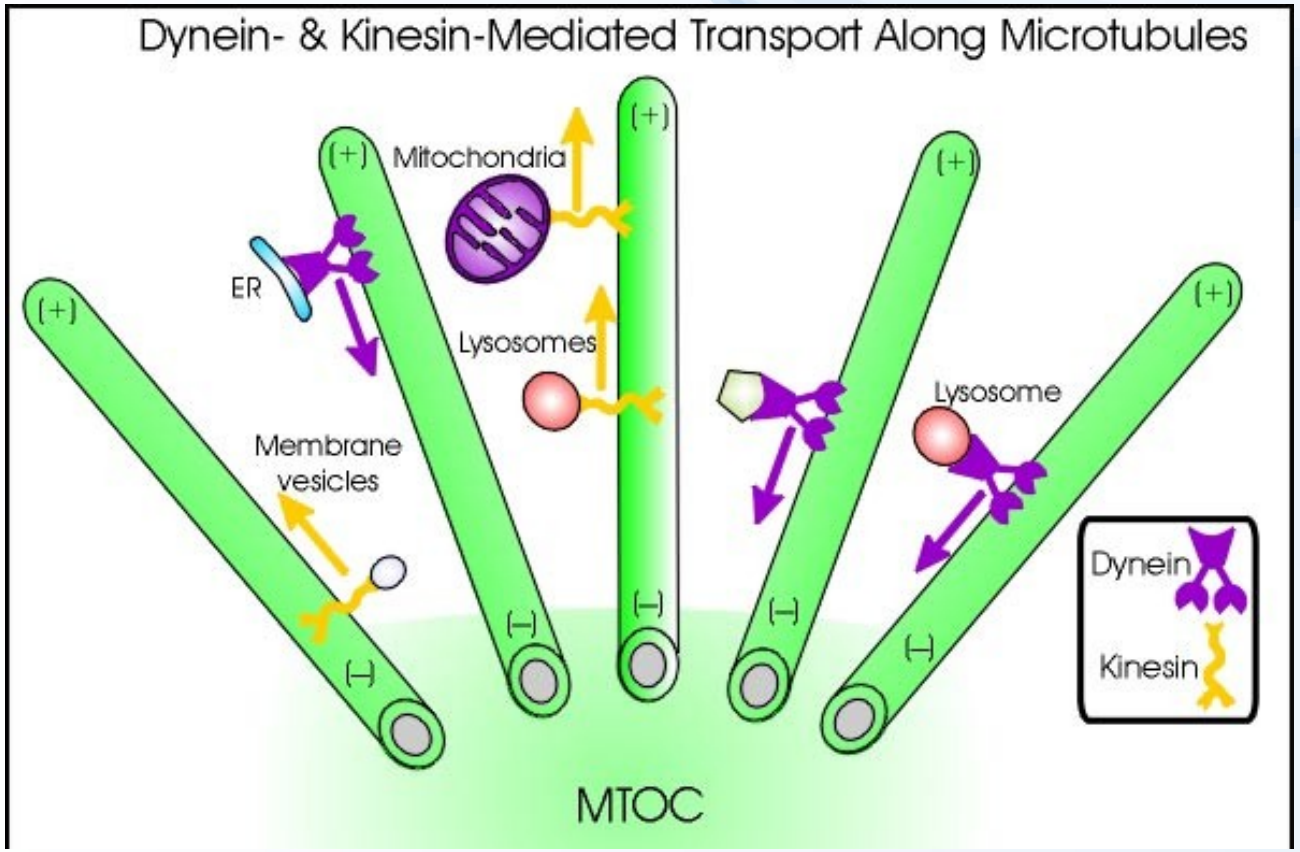


Structure of actin-myosin system

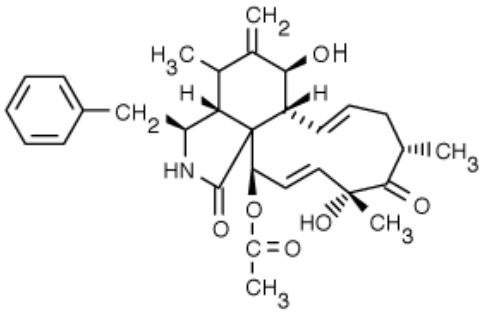


Cytoskeleton – functions

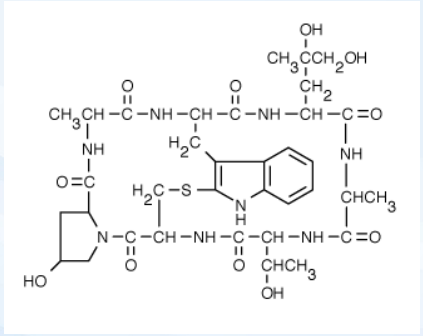
- intracellular transport
- cell replication and division (mitosis:chromosomes)
- muscle movement
- membrane (vesicles) fusion



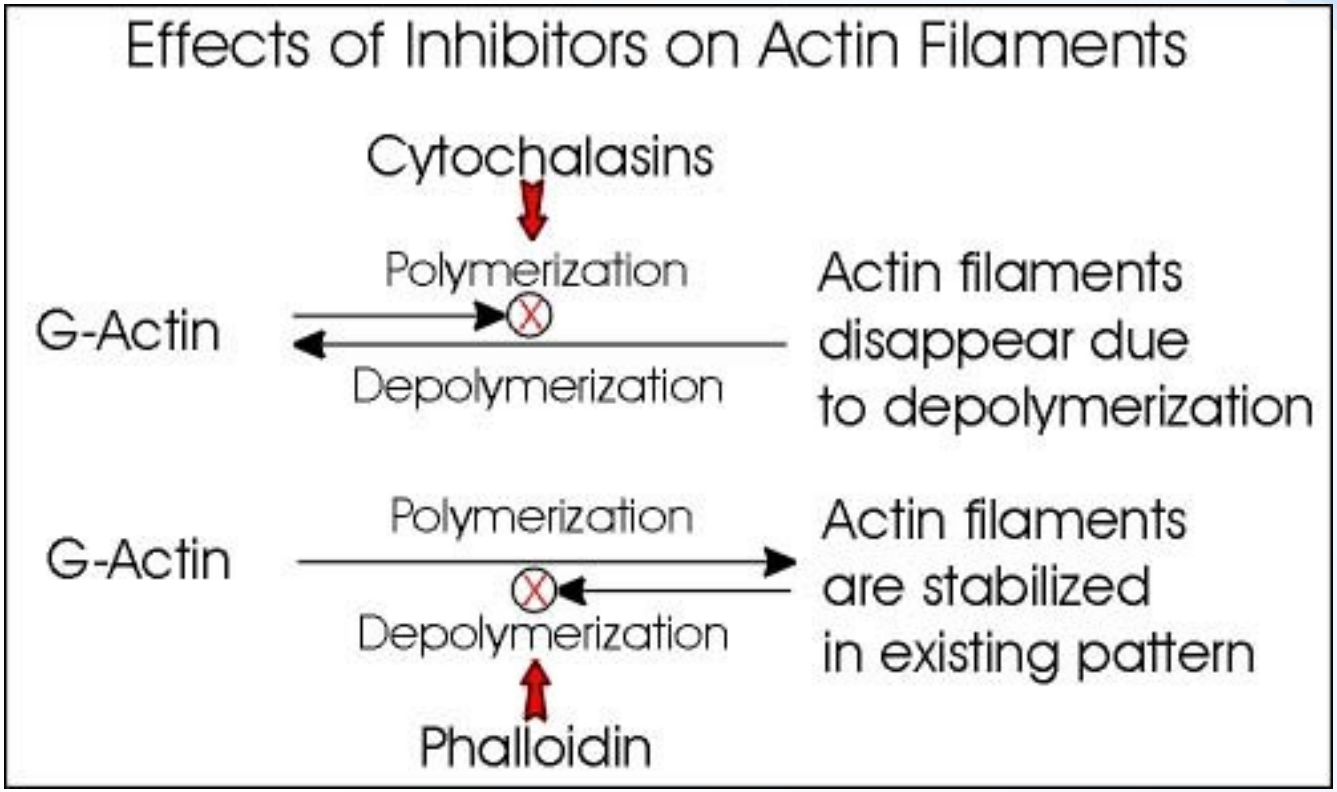
ACTIN – toxin effects on (DE)POLYMERIZATION



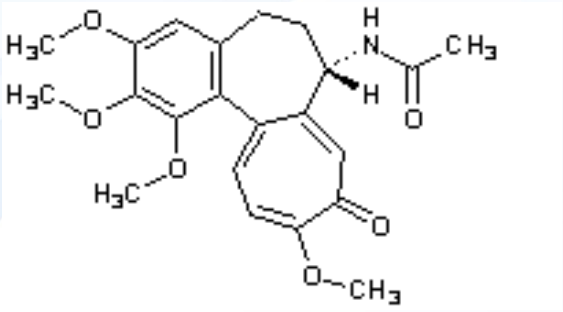
cytochalasin D
(fungal toxin)



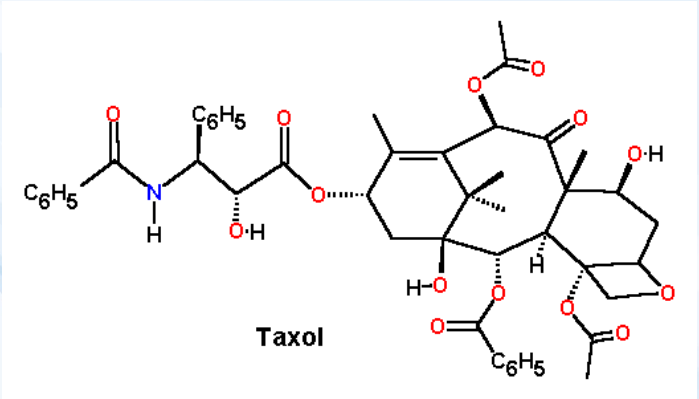
Phalloidin
(death cap - Amanita phalloides)



TUBULIN – toxin effects on (DE)POLYMERIZATION



Colchicine



taxol

Effects of Inhibitors on Microtubules

Colchicine

Tubulin dimers ⇄ Polymerization / Depolymerization → Microtubules disappear due to depolymerization

Tubulin dimers → Polymerization / Depolymerization ← Taxol → Microtubules are stabilized in existing pattern