



Centrum pro výzkum
toxických látek
v prostředí

BIOMARKERS AND TOXICITY MECHANISMS 03 – Mechanisms @proteins, part 1

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



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, quaternary (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

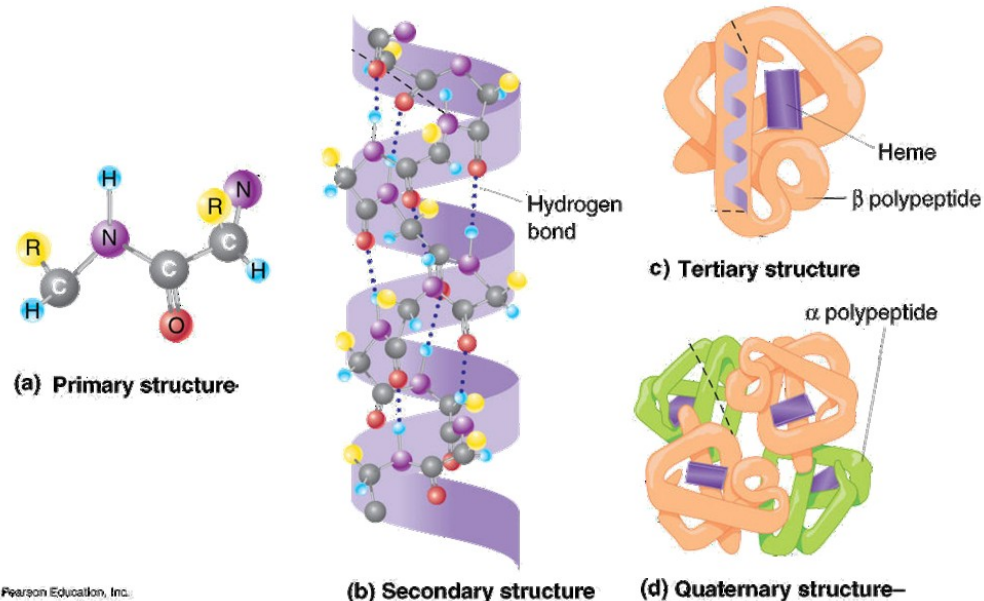
STRUCTURE and PROTECTION

CATALYSIS (enzymes)

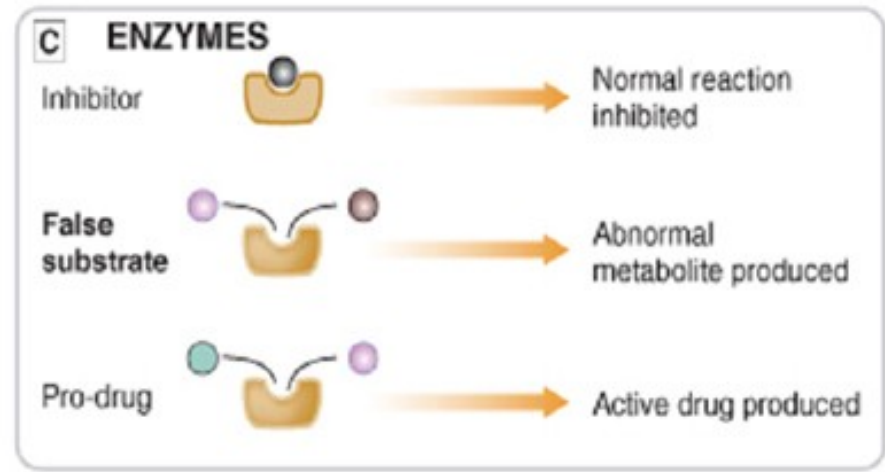
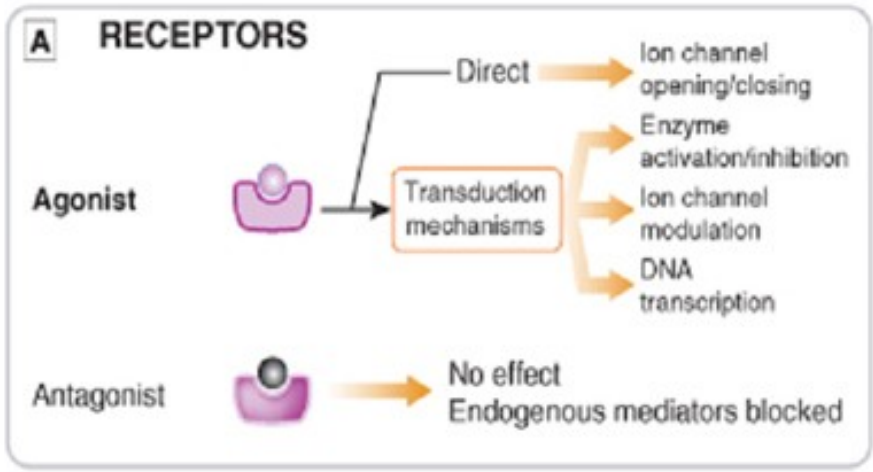
TRANSFER (information and mass)

- receptors, channels, transporters

... student should know examples..

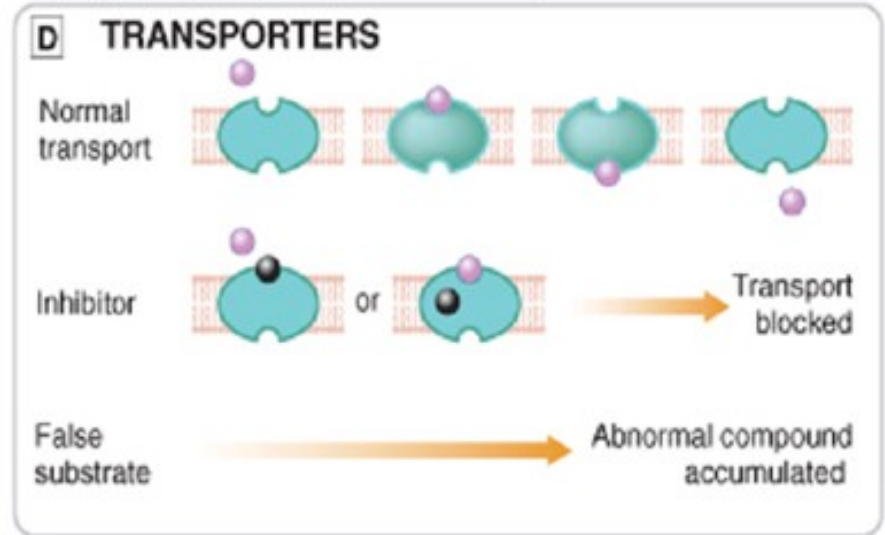
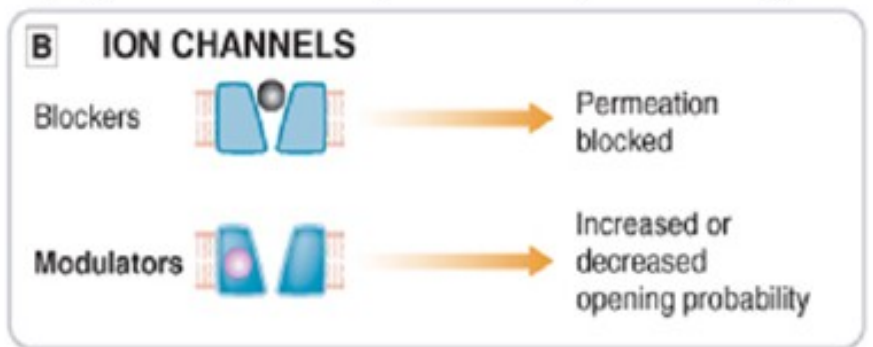


Overview - interactions of small molecules with proteins



(eg beta blockers, 17α -ethinylestradiol)

(eg aspirin, ketoconazole)



(eg local anaesthetics, cypermethrin)

(eg fluoxetine, omeprazole)

- Agonist/normal substrate
- Antagonist/inhibitor
- Abnormal product
- Pro-drug

Note – a few drugs target DNA rather than proteins (eg mitomycin C).

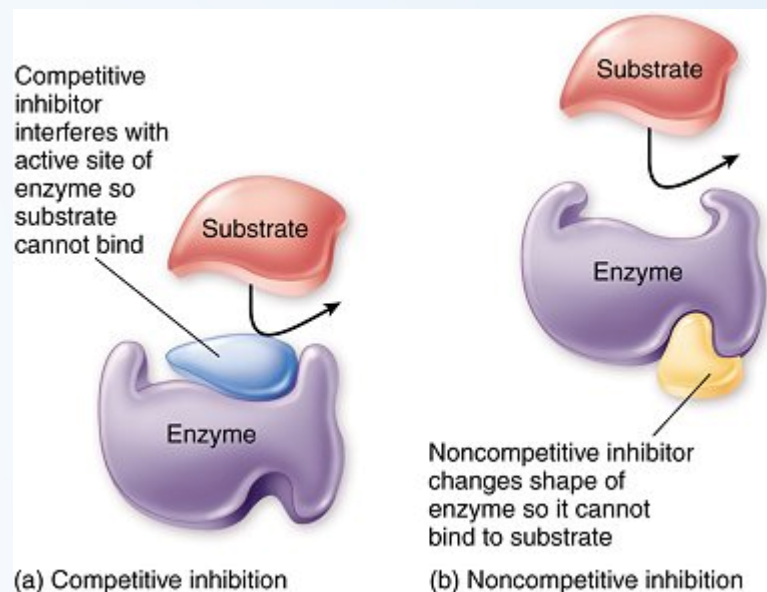
CATALYTICAL PROTEINS = Enzymes

- **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 osmolarity, changing pH)



Non-specific interactions & denaturation

Most common interactions (and some examples)

Hydrogen bond disruption

Ion bonds

S-S bonds

alcohols, amines

acids (COOH), alkalic compounds (amines)

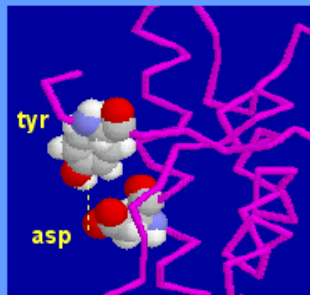
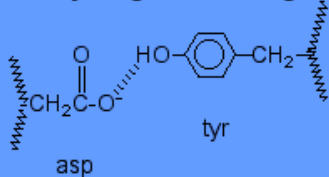
toxic metals Hg^{+2} , Pb^{+2} , Cd^{+2} , Ag^{+1} Tl^{+1} ,

carbonyls

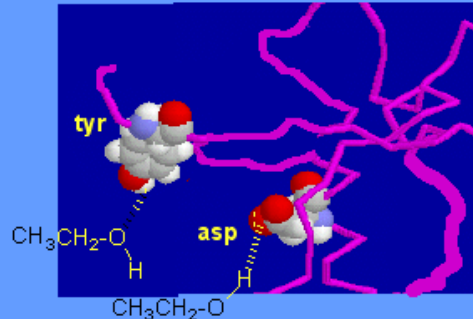
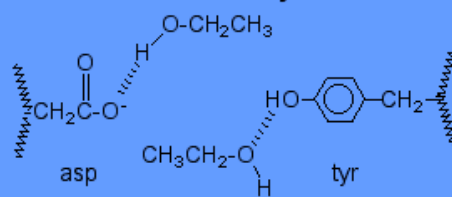
toxic metals

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

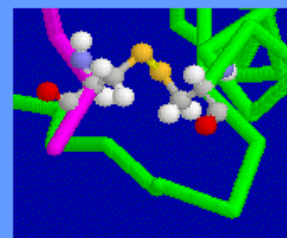
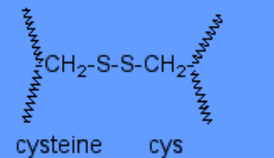
Tertiary Structure - Hydrogen Bonding



Denaturation by Alcohol

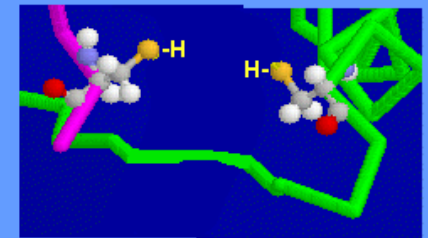


Tertiary Structure - Disulfide Bonds

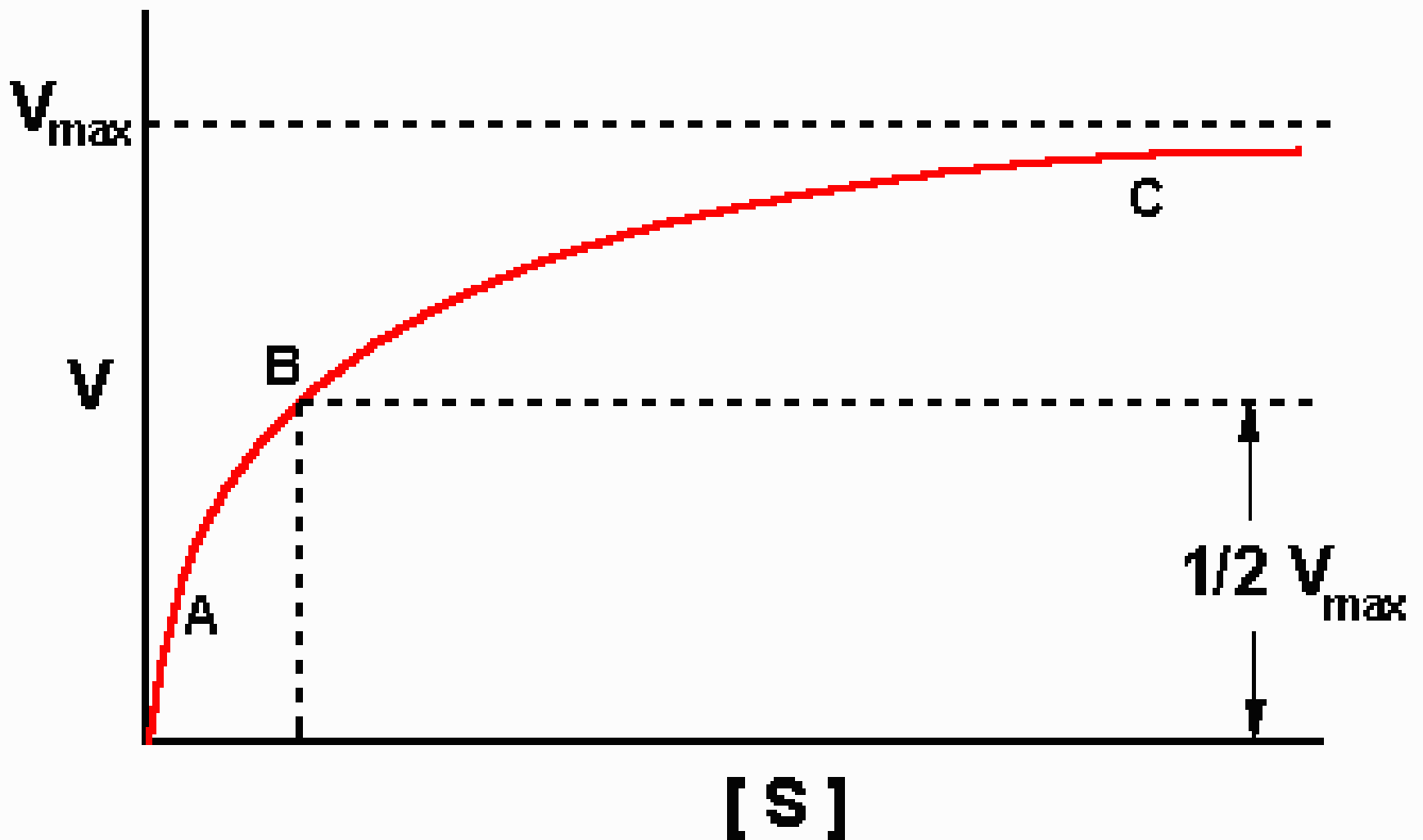


Denaturation by Reducing Agents

+ (2 H)
reducing
agent

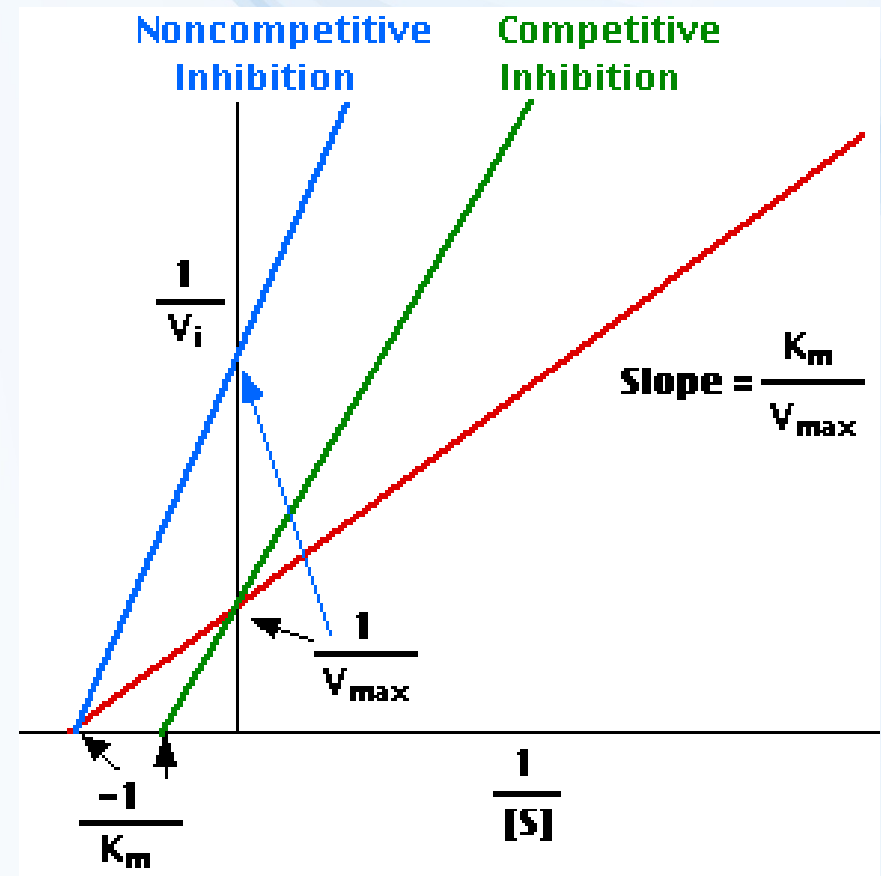
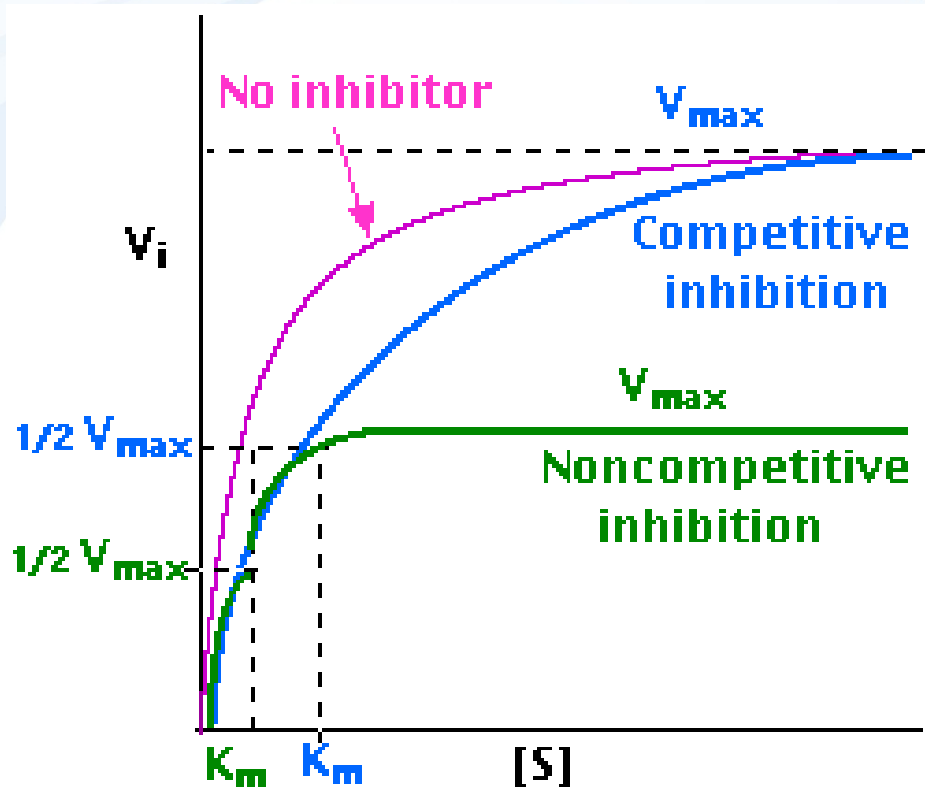


Kinetics of the enzyme reaction (Michaelis Menten)



Michaelis Menten INHIBITIONS

The kinetics informs about the nature of the interaction !



Enzyme inhibitions by toxicants – overview of key examples

Acetylcholinesterase (organophosphate pesticides)

Microsomal Ca²⁺-ATPase (DDE)

Inhibition of hemes – respiratory chains (cyanides)

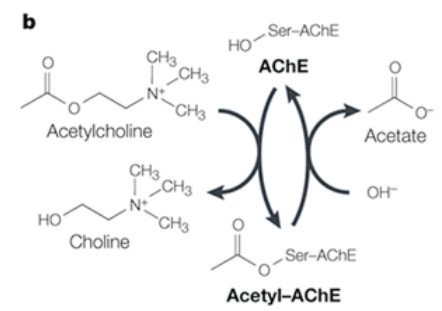
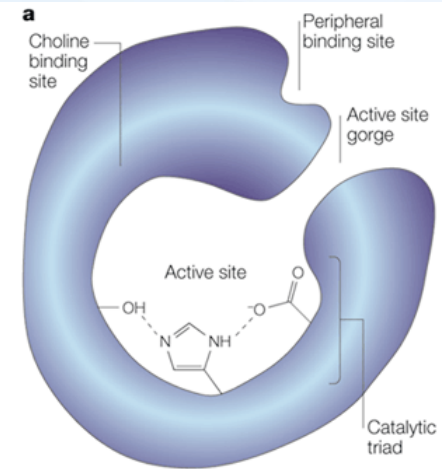
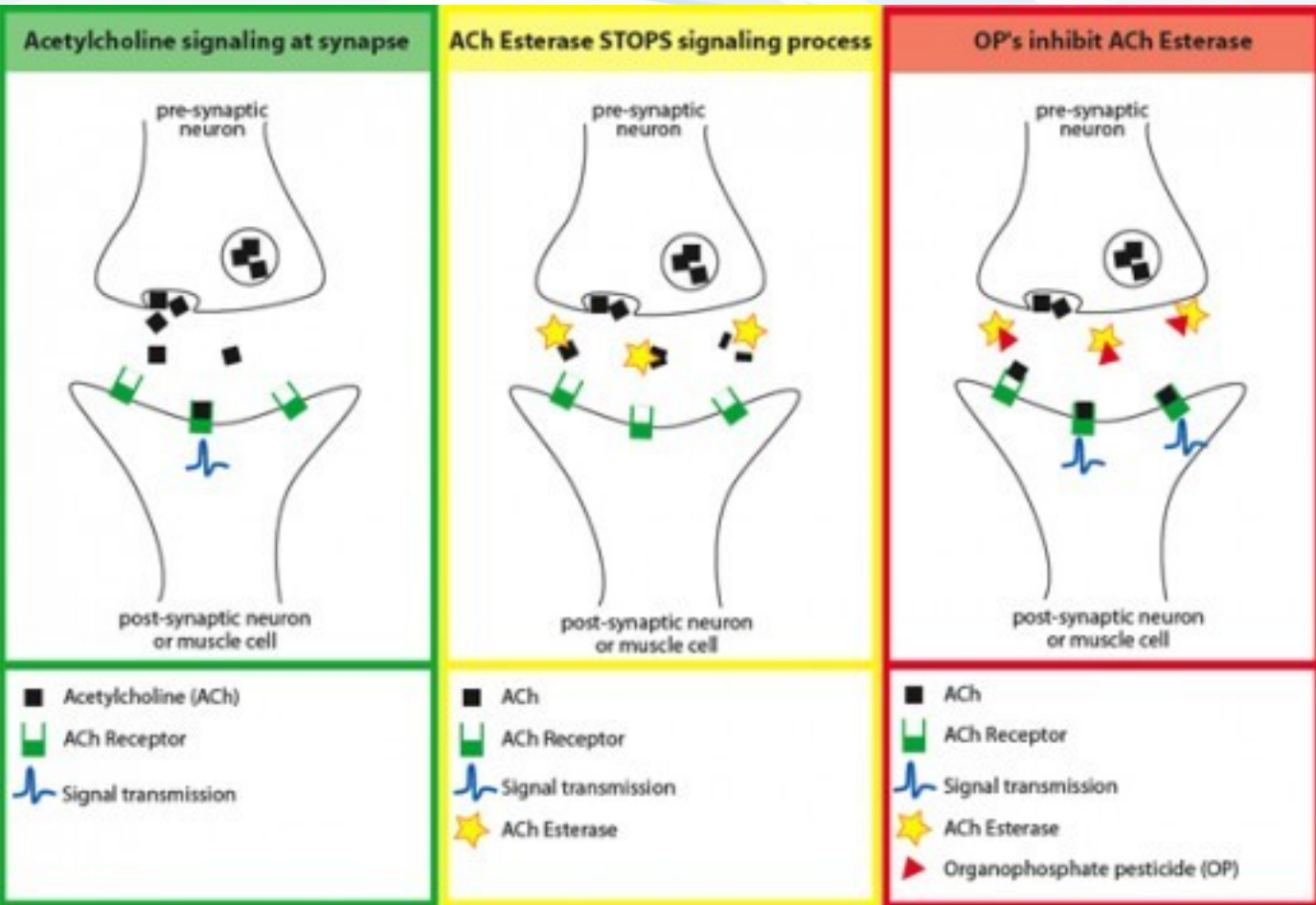
d-Aminolevulinic Acid Dehydratase (ALAD) inhibition
(lead - Pb)

Inhibition of proteinphosphatases (*microcystins*)

Glyphosate (roundup) action

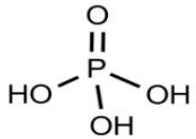
Enzyme inhibitions are beyond many other mechanisms
→ see e.g. *CELL REGULATIONS* etc.

Acetylcholinesterase inhibition by organophosphates

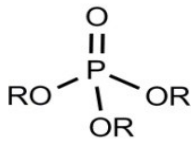


Acetylcholinesterase inhibition by organophosphates (and carbamates)

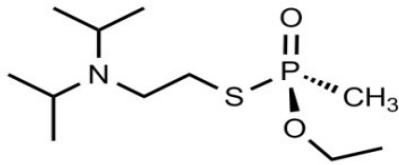
Nerve gases



Phosphoric acid



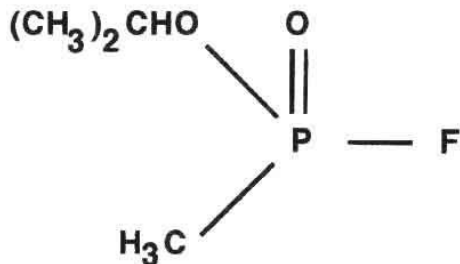
'Organophosphate'



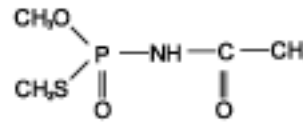
VX

SARIN / GB NERVE AGENT

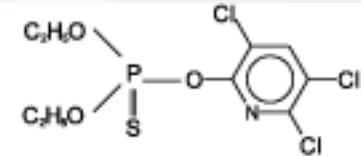
Isopropoxymethylphosphoryl Fluoride



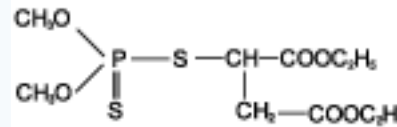
Insecticides - OPs



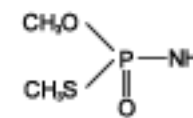
Accphate



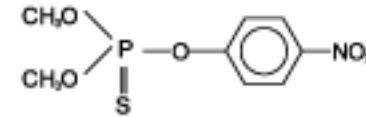
Chlorpyrifos



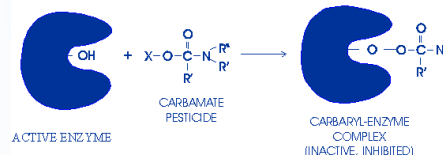
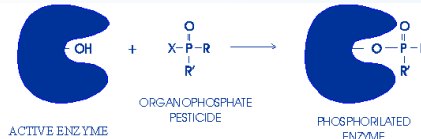
Malathion



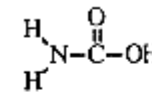
Methamidophos



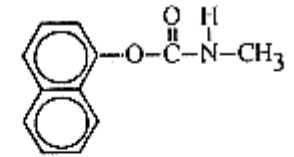
Parathion-methyl



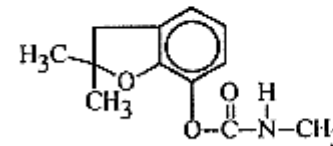
Insecticides - Carbamates



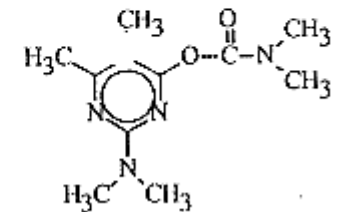
Carbamic acid



Carbaryl



Carbofuran



Pirimicarb

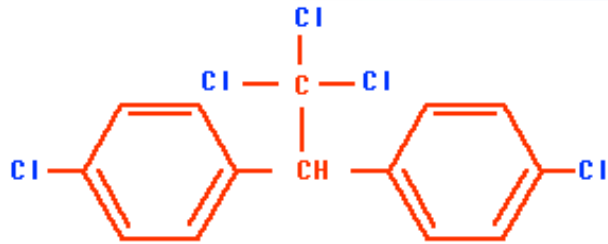
Inhibition of Ca^{2+} -ATPase by DDE

Ca^{2+} in cells

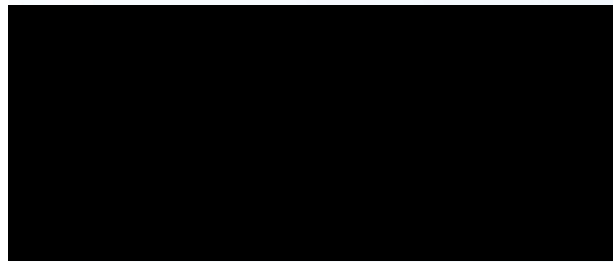
- * general signalling molecule (see later)
- * stored in (endo-/sarcoplasmic reticulum)
- * assures contractility of muscles
- * concentrations regulated by Ca^{2+} -ATPase

DDE

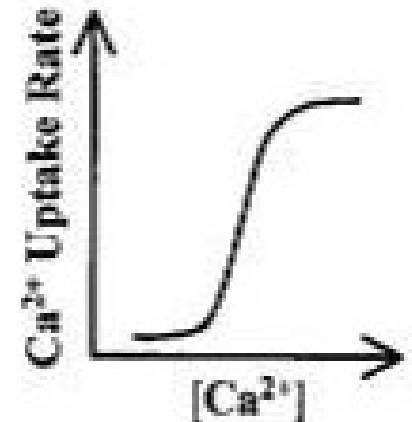
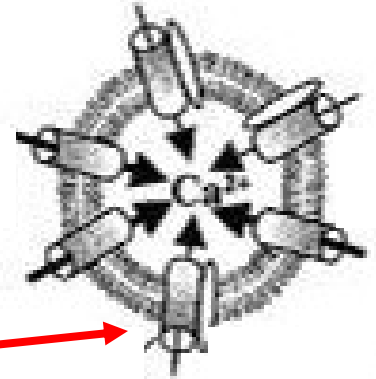
- calcium metabolism in bird eggs
- egg shell thinning



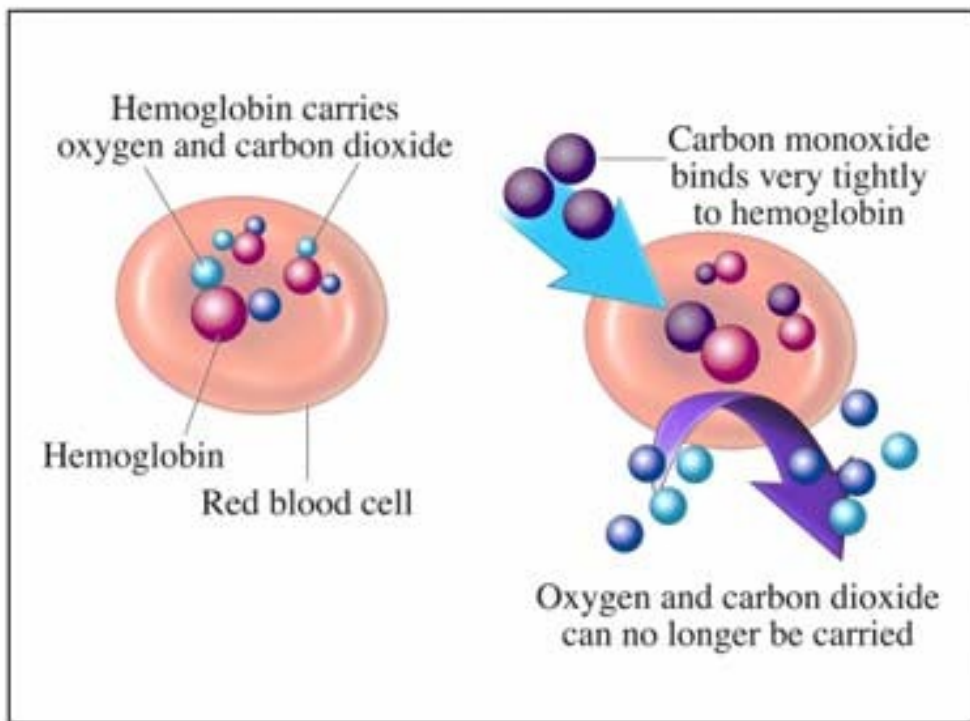
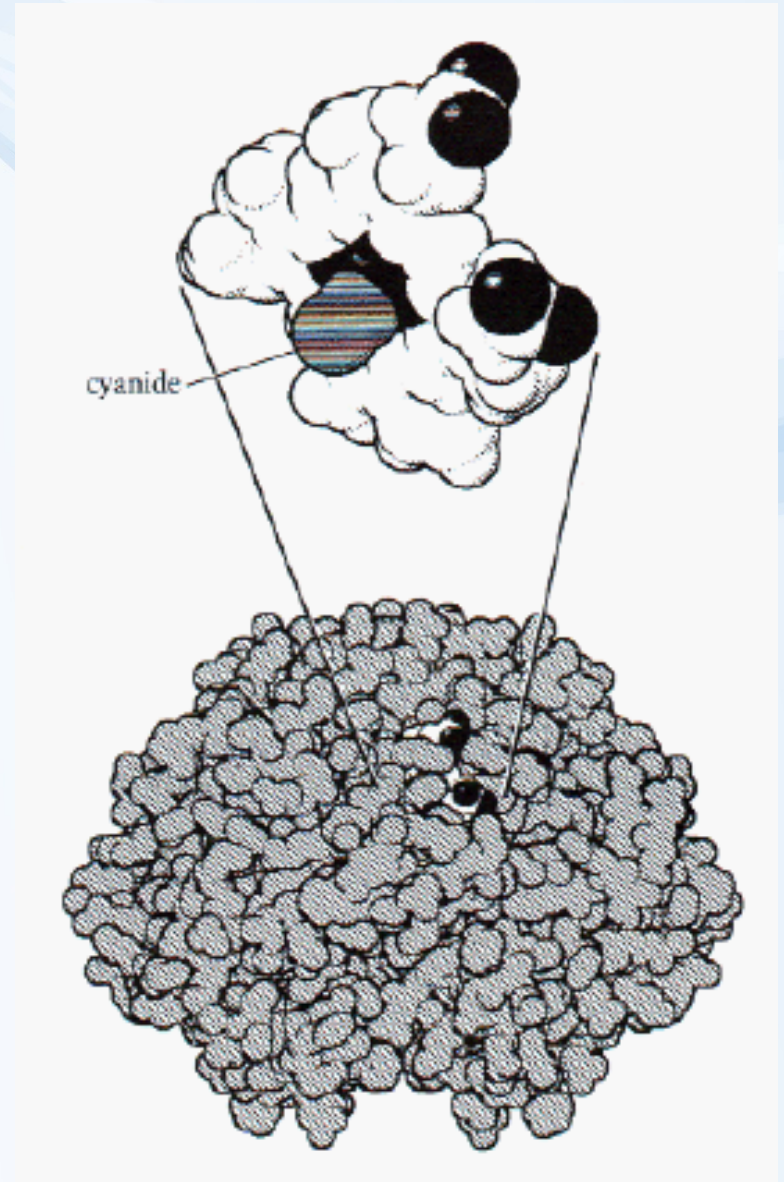
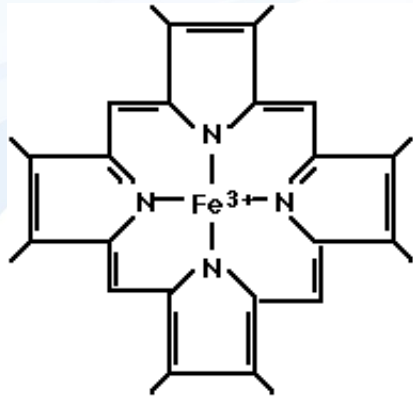
DDT (dichloro, diphenyl, trichloroethane)



DDE



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



ALAD inhibition by lead (Pb)

Lead exposure

About 310,000 U.S. children ages 1 to 5 have elevated blood lead levels, which can accumulate over months and years and cause serious health problems.

Effects on children

- Kids absorb up to 70 percent of lead, adults about 20 percent
- Often undetected; no obvious symptoms
- Can lead to learning disabilities, behavioral problems, malformed bones, slow growth
- Very high levels can cause seizures, coma, death

Sources

- Lead-based paint, contaminated dust in homes built before 1978
- Drinking water from lead pipes
- Contaminated food
- Soil (lead does not biodegrade, decay)
- Toys*



What parents can do

- Have child screened if there is concern of lead exposure
- Frequently wash child's hands, toys, pacifiers
- Only use cold tap water for drinking, cooking
- Test paint, dust in home if it was built before 1978

*Old toys with lead paint a known risk, but new toys from China now have come under scrutiny

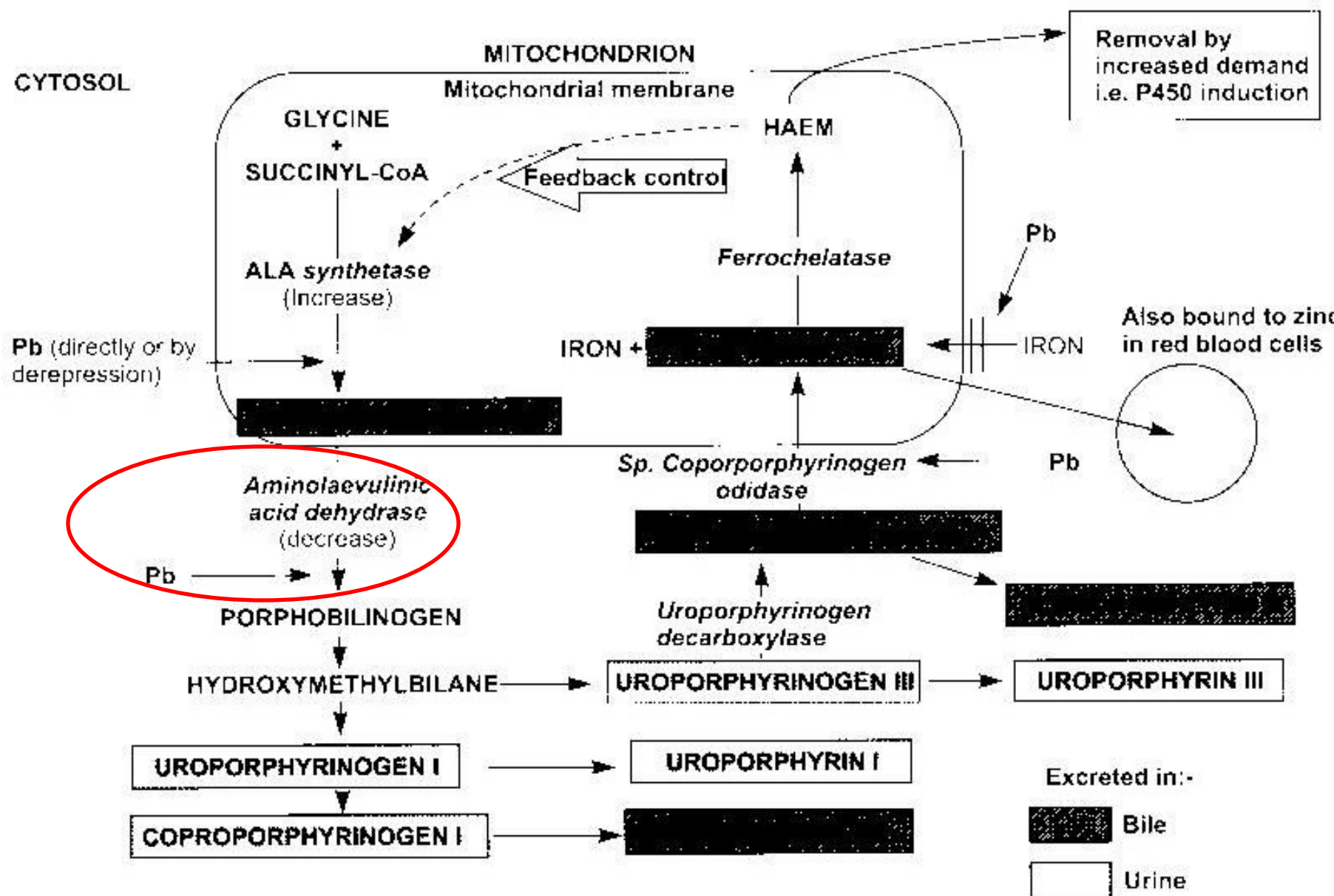
Source: U.S. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services

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Problem mostly in the USA

Ban of Pb-containing petrols

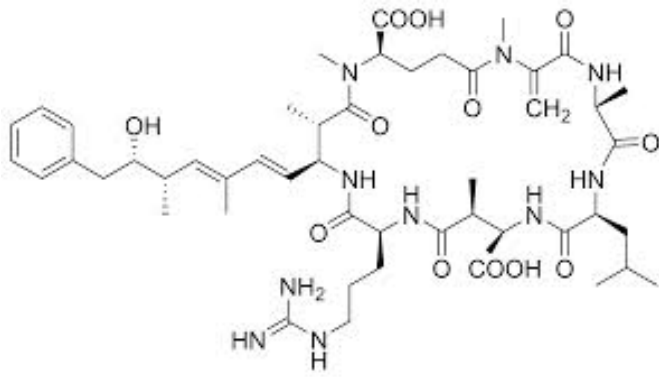
ALAD inhibition by lead (Pb) – inhibition of HAEM (!) synthesis



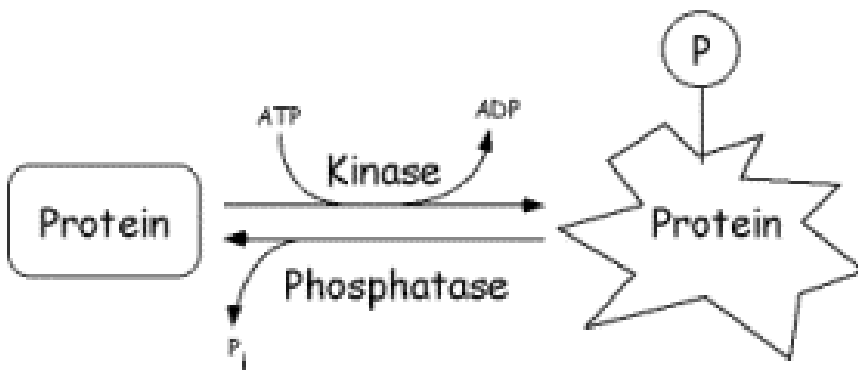
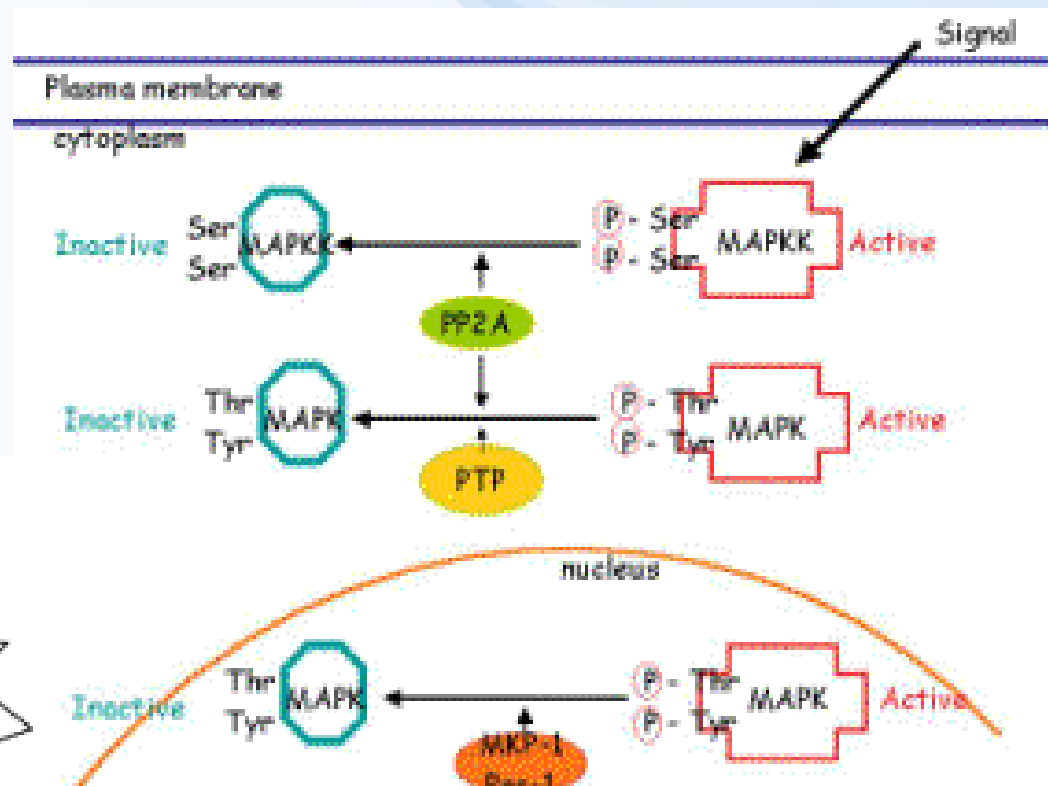
Inhibitions of PROTEINPHOSPHATASES by microcystins

Microcystins (7x AA – heptapeptides)

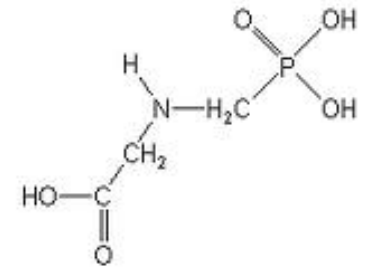
Cyanobacterial toxins produced in eutrophied waters (water blooms) up to tons/reservoir



PPases – signalling enzymes (see further)



Glyphosate action



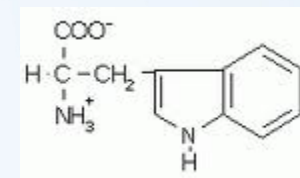
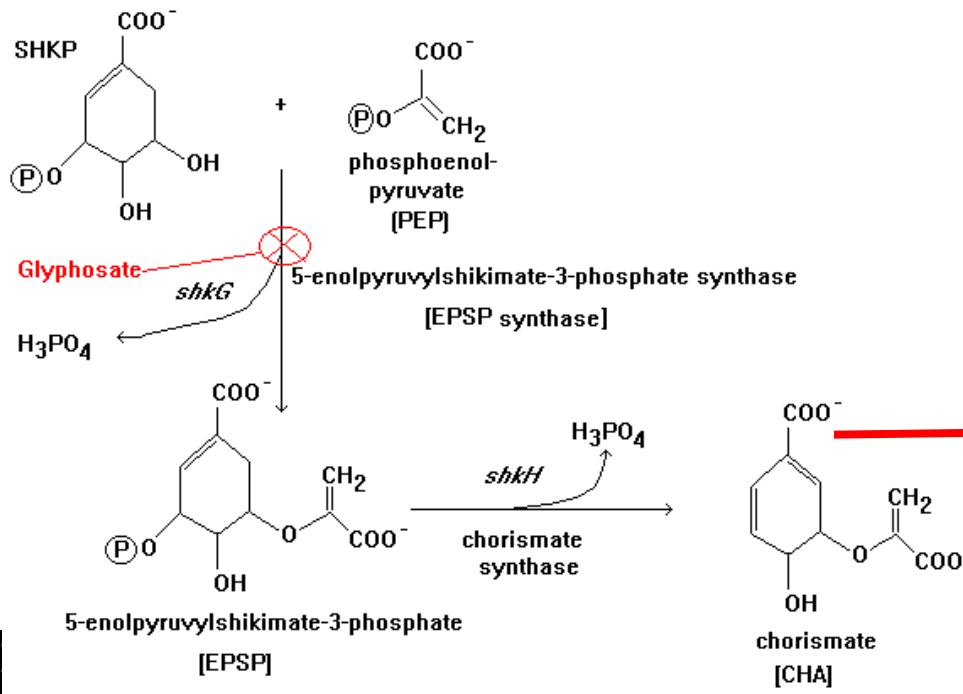
N-(phosphonomethyl)glycine

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

Selective inhibition of ESPs 5-*enol*pyruvylshikimate-3-phosphate synthase;
(synthesis of aromatic AAs – Tyr, Trp, Phe)

Uptake via leaves - only to growing plants

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



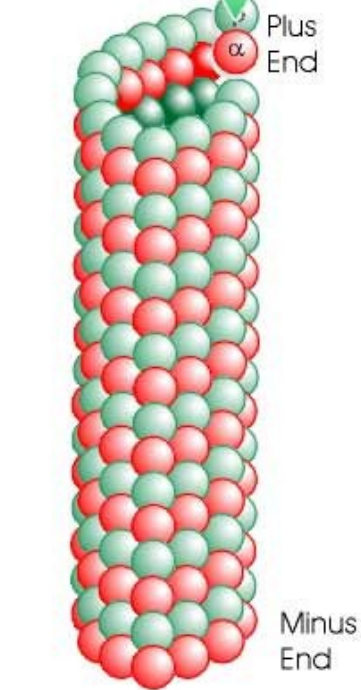
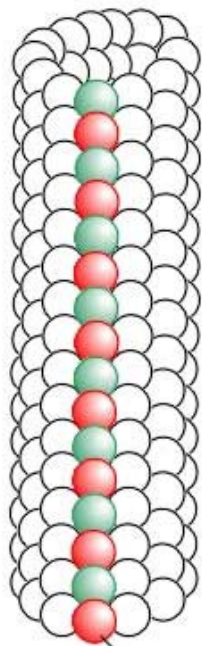
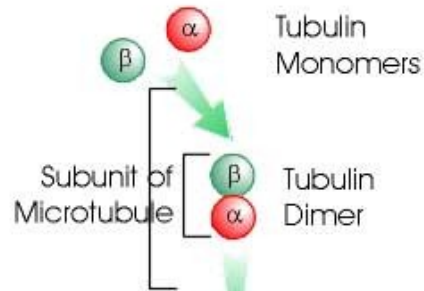
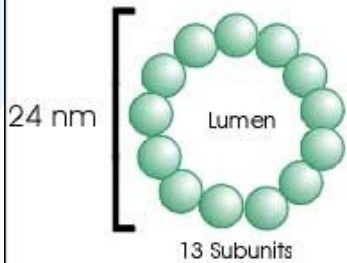
Structural proteins (CYTOSKELETON) as target for toxicants



Structures of microtubules – dynamic de/polymerization

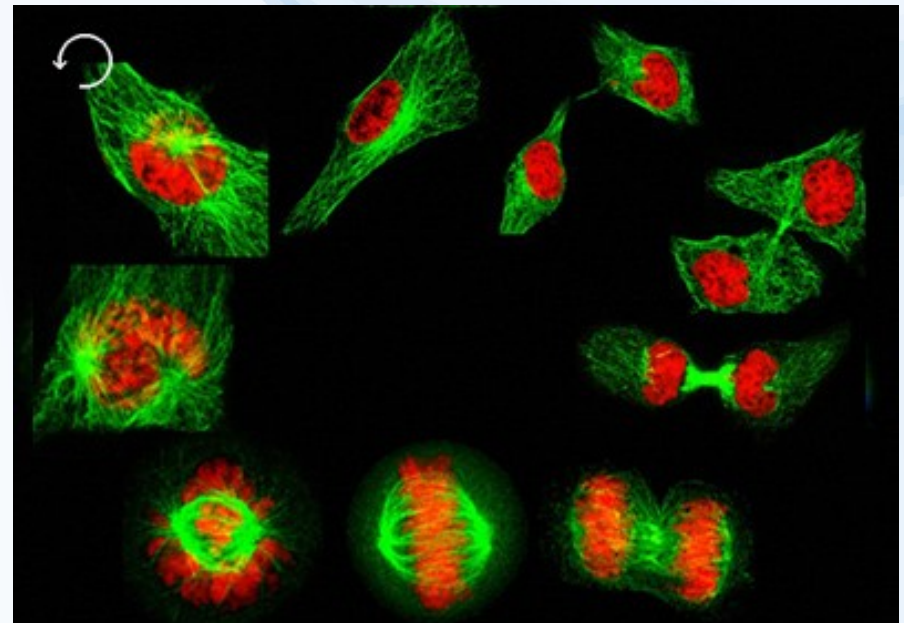
Microtubule Structure

Microtubule-End View

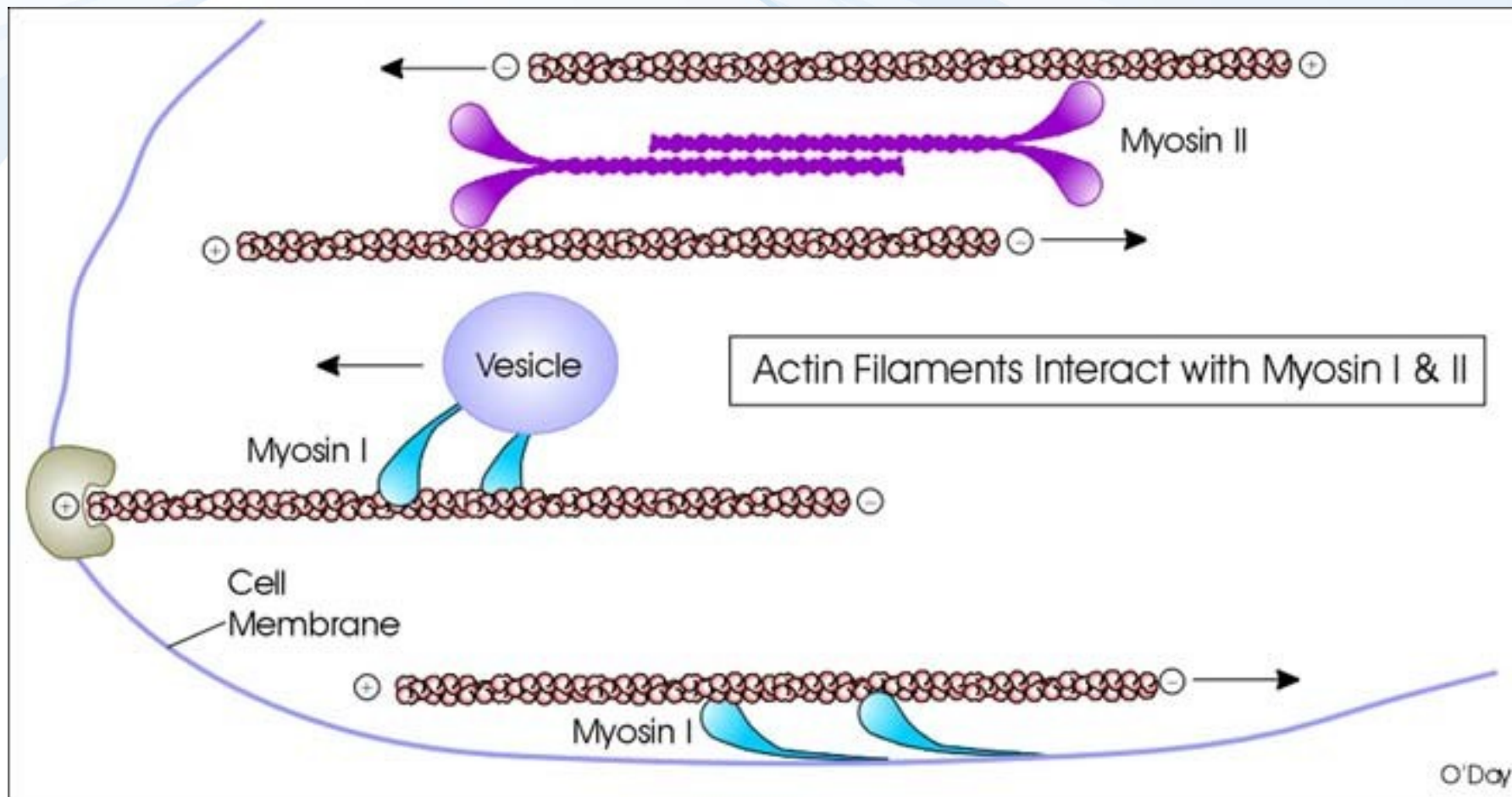


Microtubule-Side View

Visualization of microtubules during cell division – separation of chromosomes

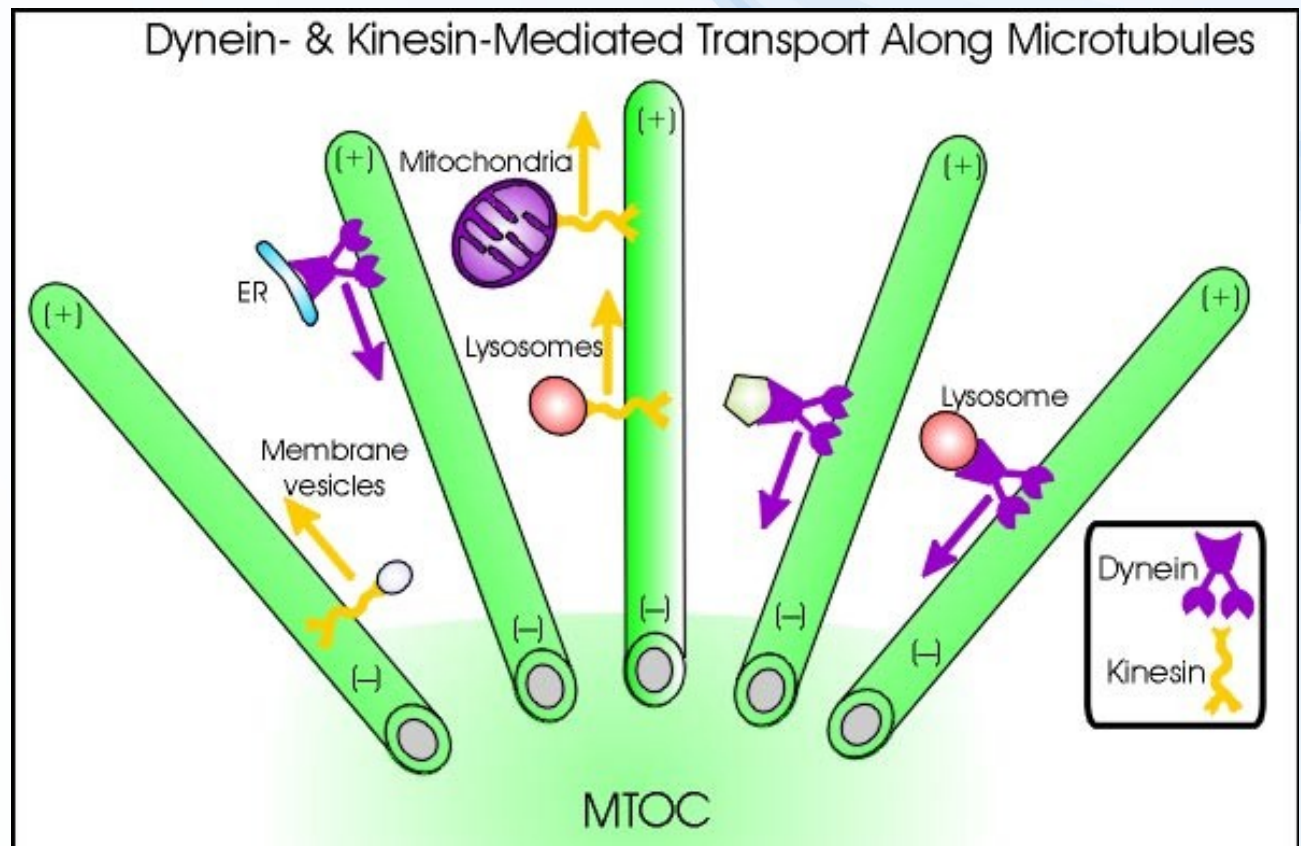


Structure of actin-myosin system

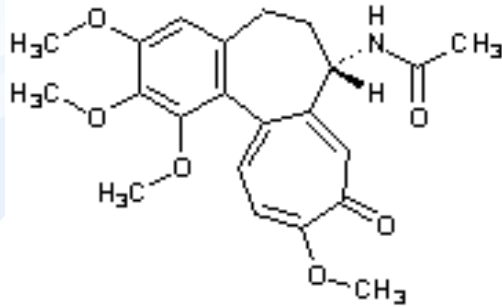


Cytoskeleton – functions

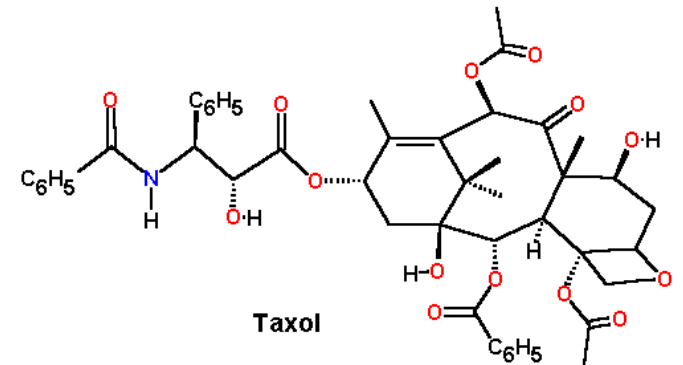
- intracellular transport
- cell replication and division (mitosis:chromosomes)
- muscle movement
- membrane (vesicles) fusion



TUBULIN – toxin effects on (DE)POLYMERIZATION



Colchicine



Taxol

taxol



Effects of Inhibitors on Microtubules

