HYBRID POLYFUNCTIONAL MOLECULES

From concept to multifunctional drugs

Vladimir Bezuglov

Laboratory of Oxylipins
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry
Russian Academy of Sciences, Moscow, Russia

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Headlines

- Current paradigm: ‘magic bullet’
- New paradigm: informational drug
- Polypotency and multifunctionality
- Design of hybrid polyfunctional molecules
- Simple construction: binary drugs
- Complex hybrid molecules
- Non-covalent complexes: a road to individuality
- Perspectives

‘Magic bullet’ concept

‘Magic bullet’ - chemical substances that can be used to destroy pathogenic organisms by virtue of their particular affinity for such pathogens.

Nowadays,‘magic bullets’ are more frequently envisaged as being side-effect-free drugs specifically targeted to particular individuals with particular diseases.(NRDD, 2004)

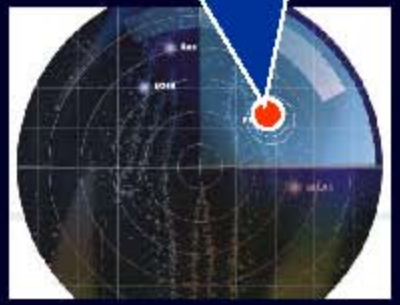
Paul Ehrlich

“to use synthetic chemistry to modify the starting material chemically in various ways and analyse the resulting products for their quality to heal” – *Paul Ehrlich*

‘Magic bullet’ concept

One target – one drug

A human suffers from a lot of diseases. Each disease has only one molecular target (one gene).



Paul Ehrlich

Genomics, proteomics …
Computer aided drug design
Combinatorial synthesis
Throughput screening

A human as a molecular machine

A wide range of ‘magic bullets’

Drugs prescribed to one patient during a year

Анаприлин
Сердол
Метопролол

Коринфар, кордафлекс
Амлодипин, нормодипин

Энап
Берлинприл

Индапамид, арифон
Вирошпирон

Кларитин
Антистен
Аллопуринол

Детралекс
Диклофенак
КардиАСК, тромбо-АСС

Оликард-ретард,
моночинкве-ретард,
пектрол

Цистон
Клотримазол
Пентоксифилин
Пирацетам
Вимпоцетин
Бромгексин
Афлубин
БиоМакс

A wide range of ‘magic bullets’

Shortcomings of MONOfunctional ‘magic bullets’

- In reality, monofunctional compound does not exist. (‘Magic bullet’ designer often ignore this fact).
- Therapeutic effect is a result of ACTIVE drug-organism INTERaction.
- Any drug disturbs informational state of organism influencing various receptor systems.
- Organism affect drugs via inner environment properties and determines healing effect via the state of receptor and transport systems.
- Designed as monofunctional the drug, as a rule, can’t overcome the organism resistance that forces dosage increasing.

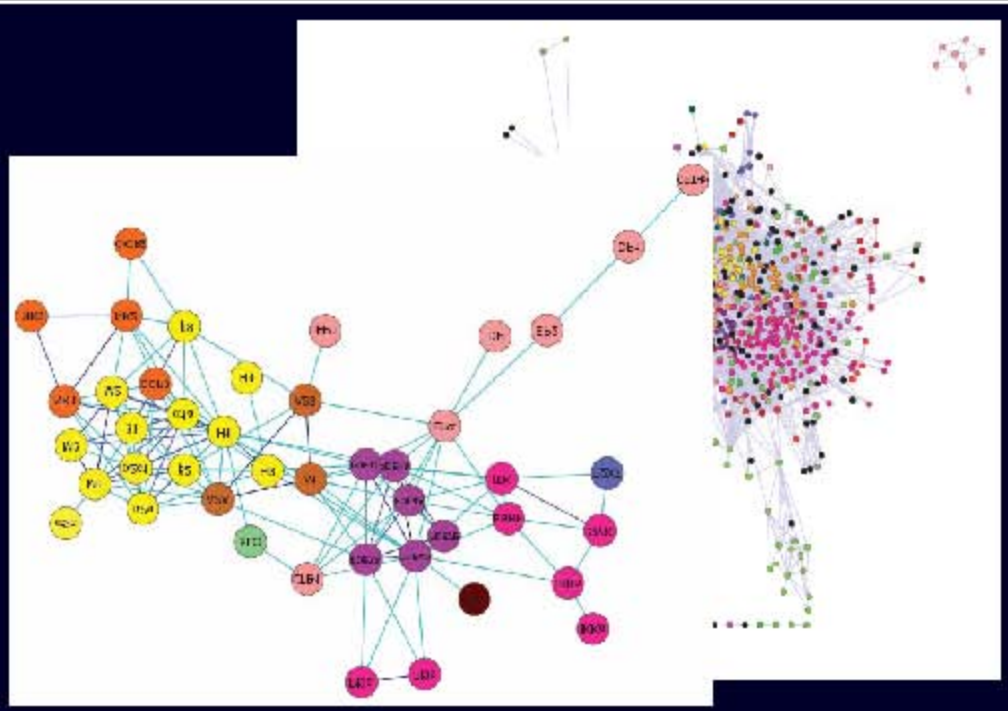
More than a half of drugs are withdrawn from the market due to sudden toxic effects

Drug	Company	Disease/indication	Toxicity	Action
Baycol	Bayer	High cholesterol levels	Rhabdomyolysis	Withdrawn 2001
Duract	Wyeth-Ayerst	Pain	Liver toxicity	Withdrawn 1998
Fen-phen	Wyeth-Ayerst	Obesity	Cardiac arrhythimia	Withdrawn 1998
Lotronex	GlaxoSmithKline	Irritable bowel syndrome	Ischemic colitis	Withdrawn 2000
Propulsid	Janssen	Abnormal gastrointestinal motility	Cardiac arrhythmia	Withdrawn 2000
Raplon	Organon	Anesthesia	Bronchospasm	Withdrawn 2001
Rezulin	Parke-Davis/ Warner-Lambert	Type II diabetes	Liver toxicity	Withdrawn 2000
Seldane	Hoechst	Allergy	Cardiac arrhythmia	Withdrawn 1998
Serzone	Bristol-Myers Squibb	Depression	Liver toxicity	Selective withdrawal 2003
Trovan	Pfizer	Anti-microbial	Liver toxicity	New restrictions
Zyflo	Abbott	Asthma	Liver toxicity	New restrictions

New paradigm: multifunctional drugs

One drug –
many targets

"A human has only one chronic disease, that manifests in multiplicity of symptoms and is a result of intervention of imperfect mind into delicate regulation processes within the organism"
Sergei Konovalov



System biology
Network pharmacology
Informational biology

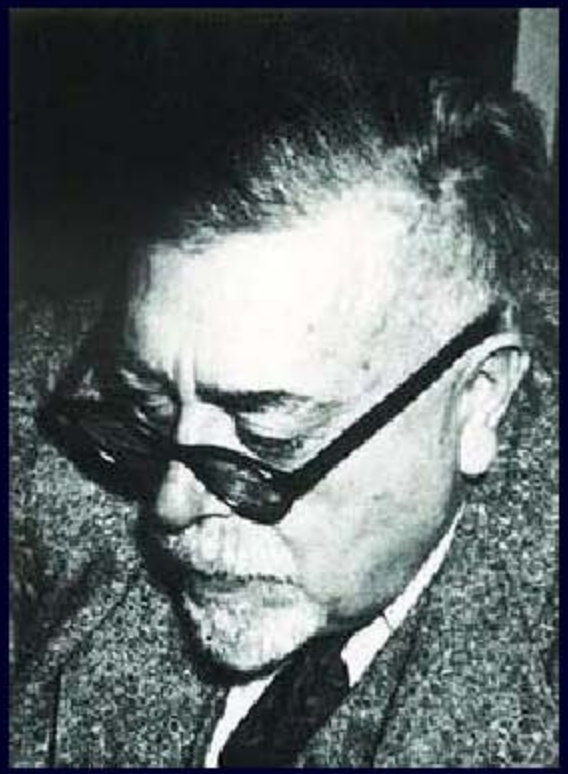
Informational-energetic Theory

Founded by Sergey Konovalov (SPb, Russia)

- A human is mainly an informational entity.
- His physical body is govered and supported by complex multylayer informational framework.
- Disturbances in this framework lead to instability of regulatory system, breaks in informational threads and links.
- At the physical level all information flows are realized through fluxes of signal molecules. Their excess or shortage is a key component of illness manifestation.

Information

'Information is information, neither matter nor energy. No materialism that fails to take account of this can survive the present day.'
Wiener, N. (1961).
Cybernetics, or control and communication in animal and machine, 2nd Ed. Cambridge, MA: MIT press, P. 132.



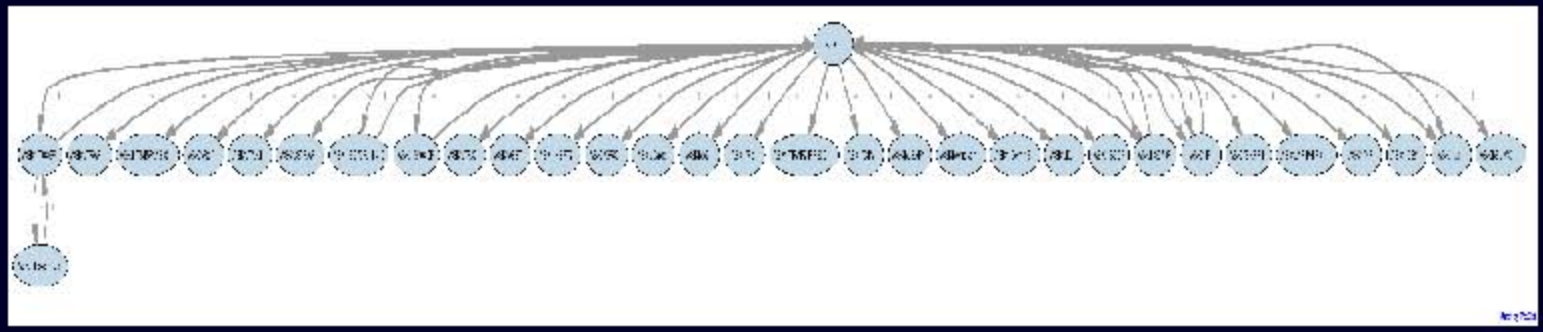
Polypotency and multifunctionality

- All chemical compounds acting within the living bodies are polypotent (by definition).
- Multifunctionality is a result of interaction of polypotent chemical substances with various molecular targets – receptors.
- Receptor – any molecule capable to transmit its structural changes, evoked by ligand, downstream the informational network.
- Signal molecules – polypotent chemical substances forming information flow.
- To enlarge the potency spectra of signal molecules we are to combine them creating multifunctional drugs.

Pharmacological strategies

- Independent dosage of the drugs – COMPLEX DRUG THERAPY
- Fixed-ratio combination of two (or more) drugs in one dosage form - COMBINED DRUGS
- Combination two or more mechanisms of action in one molecule – MULTIFUNCTIONAL DRUGS
- Conjugation of two monofunctional drugs in one molecule – HYBRID DRUGS
- Combination of several multifunctional drugs in one molecule – HYBRID MULTIFUNCTIONAL DRUGS

An example of polyfunctionality of endogenous compounds

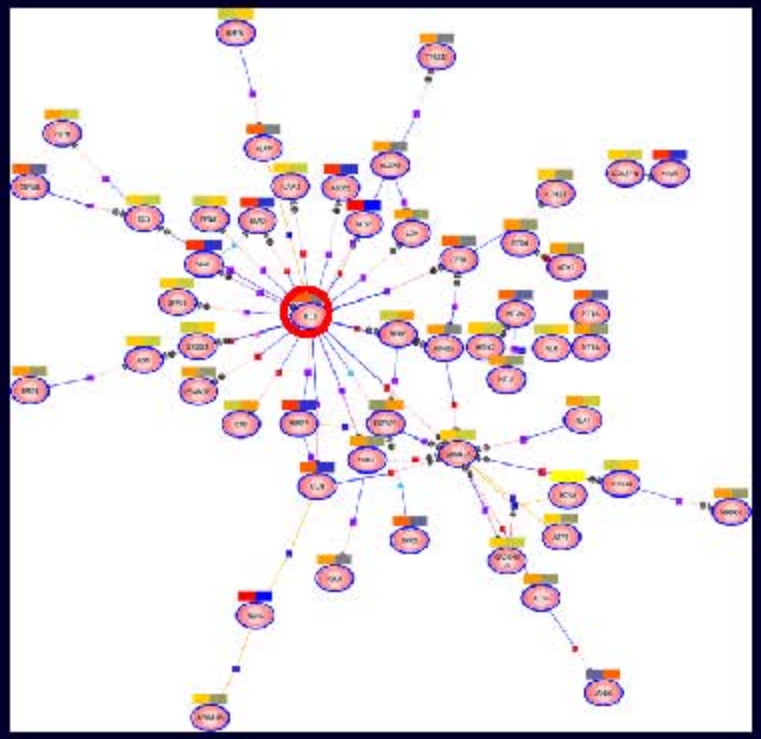


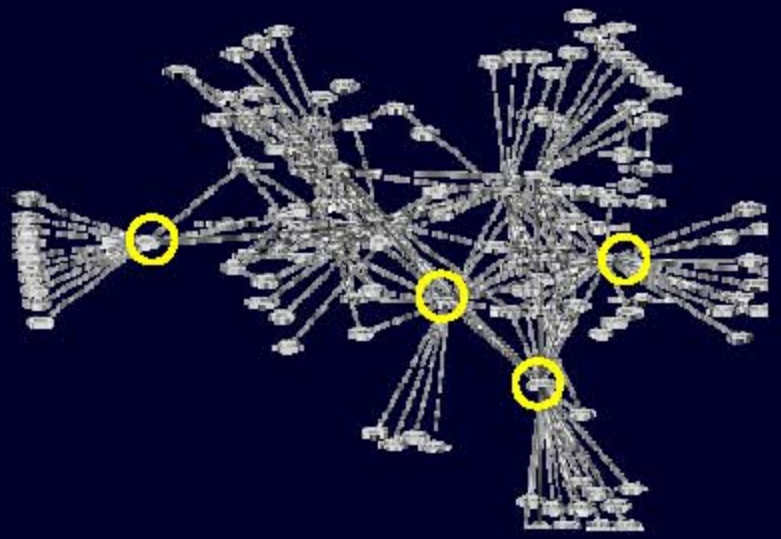
Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase (MAPK) kinase kinase which activates the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways. ASK1 is preferentially activated in response to various types of stress, such as oxidative stress and endoplasmic reticulum stress, and has pivotal roles in a wide variety of cellular responses, including apoptosis and cell differentiation.

This molecule exists in 32 states, has 40 transitions between these states and has 7 enzyme functions

A. Matsuzawa, K. Takeda, H. Ichijo, *UCSD-Nature Molecule Pages*, 2010

Network hubs a good objects for therapeutic manipulations





Some proteins acts as information collectors, processors and transmitters (network hubs)

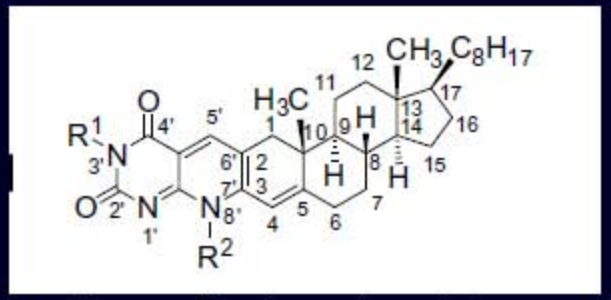
Hybrid (binary) drugs

Drug 1 — Linker — Drug 2

Hybrid drug
(two together)

Drug 1 | Drug 2

Chimeric drug
(two within a one)

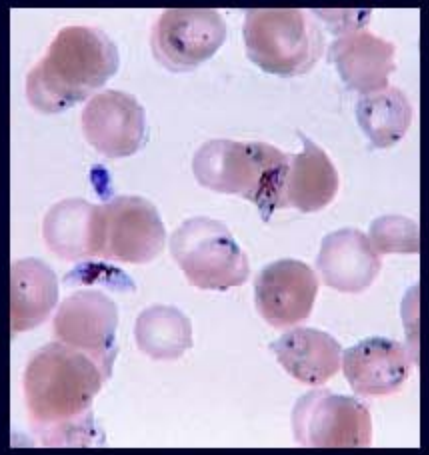


deazaflavin–steroid
antiproliferative
A.R. Shrestha, et al, 2008

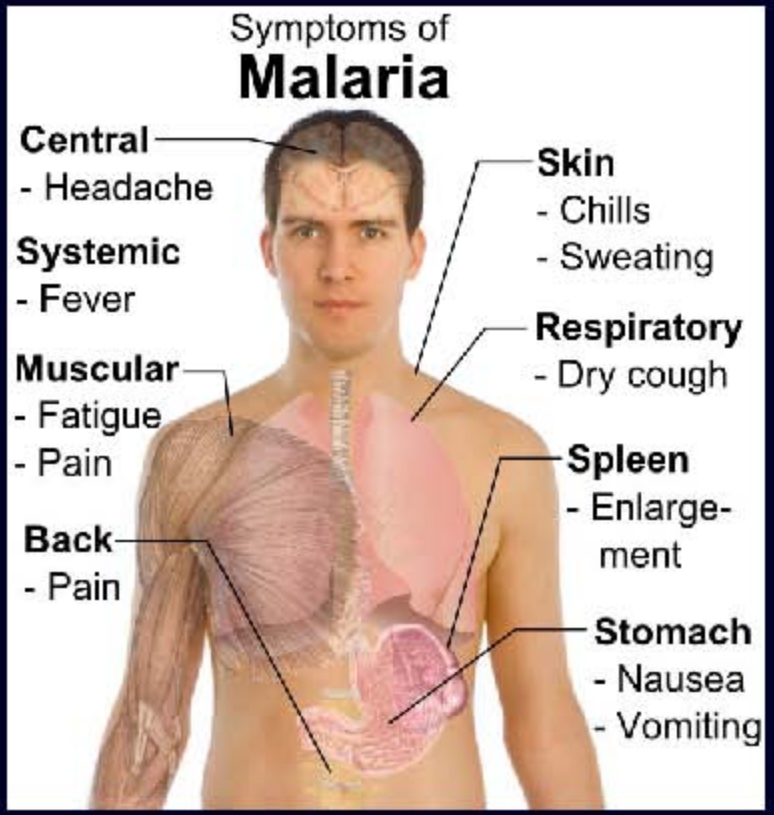
Hybrid antimalarian drug

Malaria kills between 1 and 2 million people per year

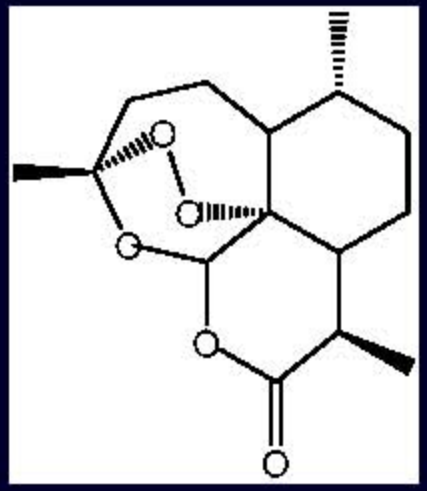
Anopheles stephensi



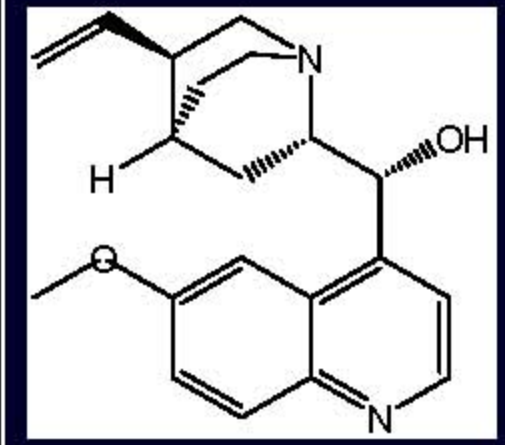
Plasmodium falciparum



Hybrid antimalarian drug



Artemisinin



Qinine

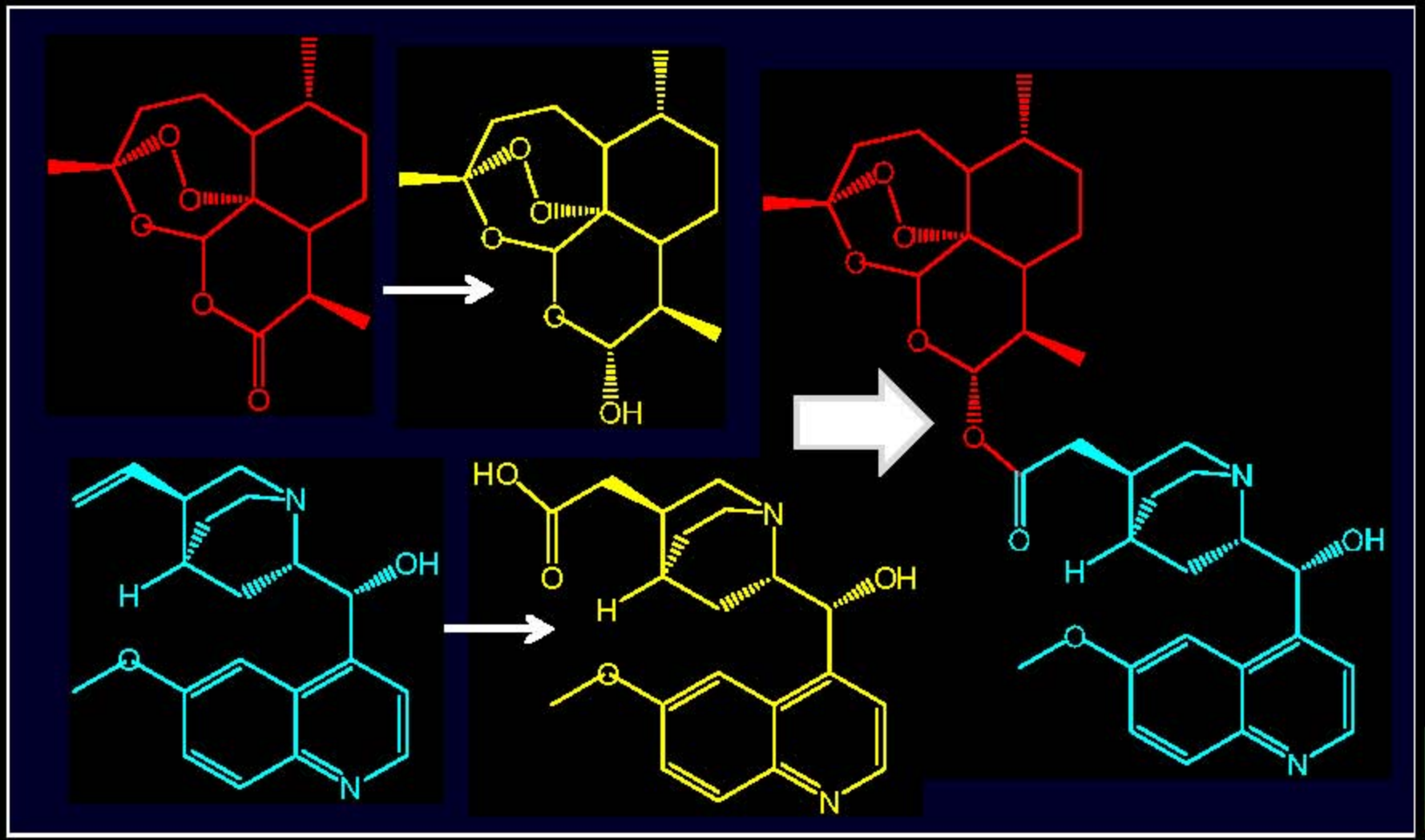
Artemisia annua

Cinchona pubescens

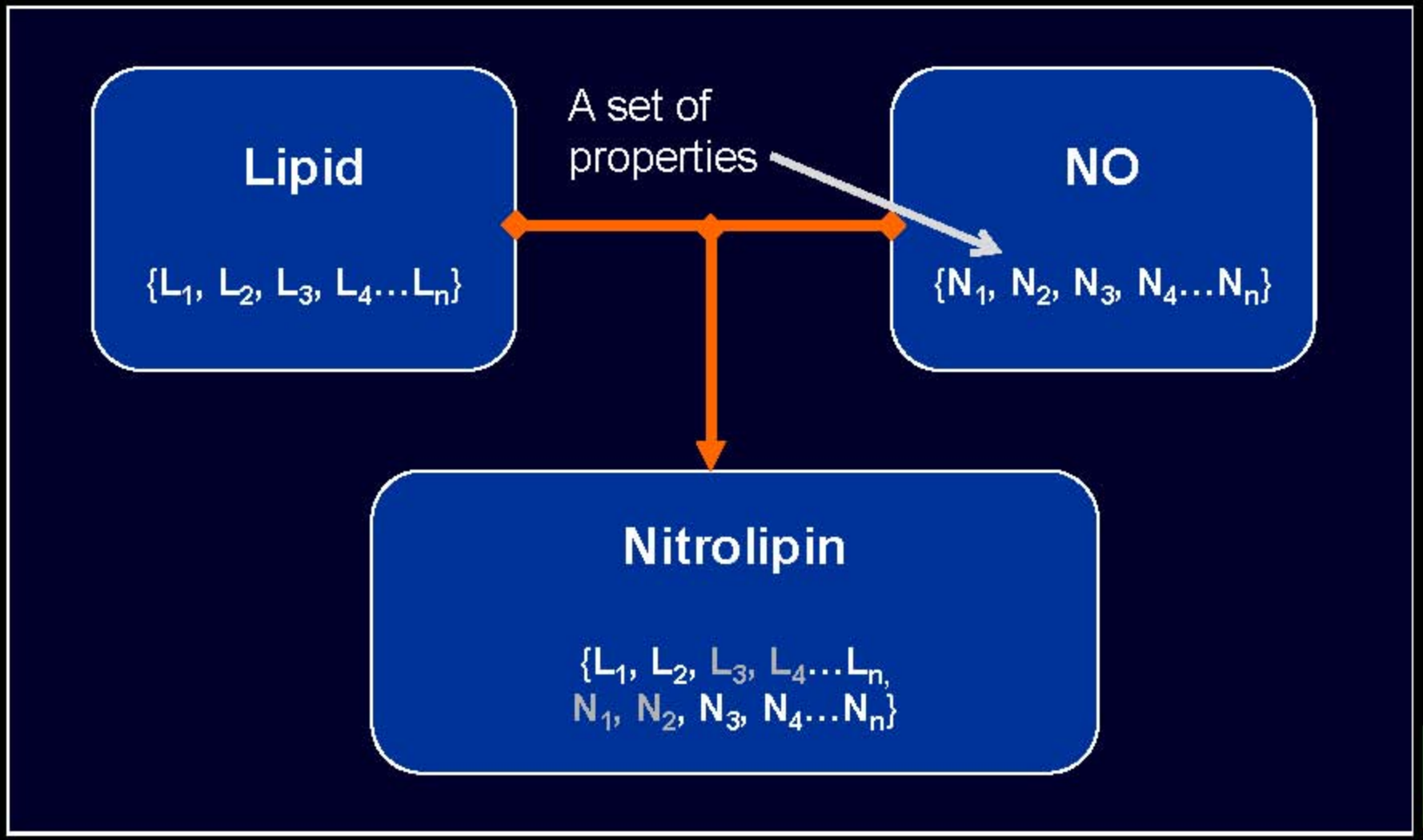
Combinations of artemisinins, which kill parasites rapidly but are also rapidly excreted, with longer half-life antimalarial agents are favoured in order to achieve full eradication of parasites and prevent the recrudescence commonly found with artemisinin monotherapy.

J.J. Walsh, D. Coughlan, N. Heneghan, C. Gaynora and A. Bell, Trinity College Dublin, Ireland (BMCL, 2007)

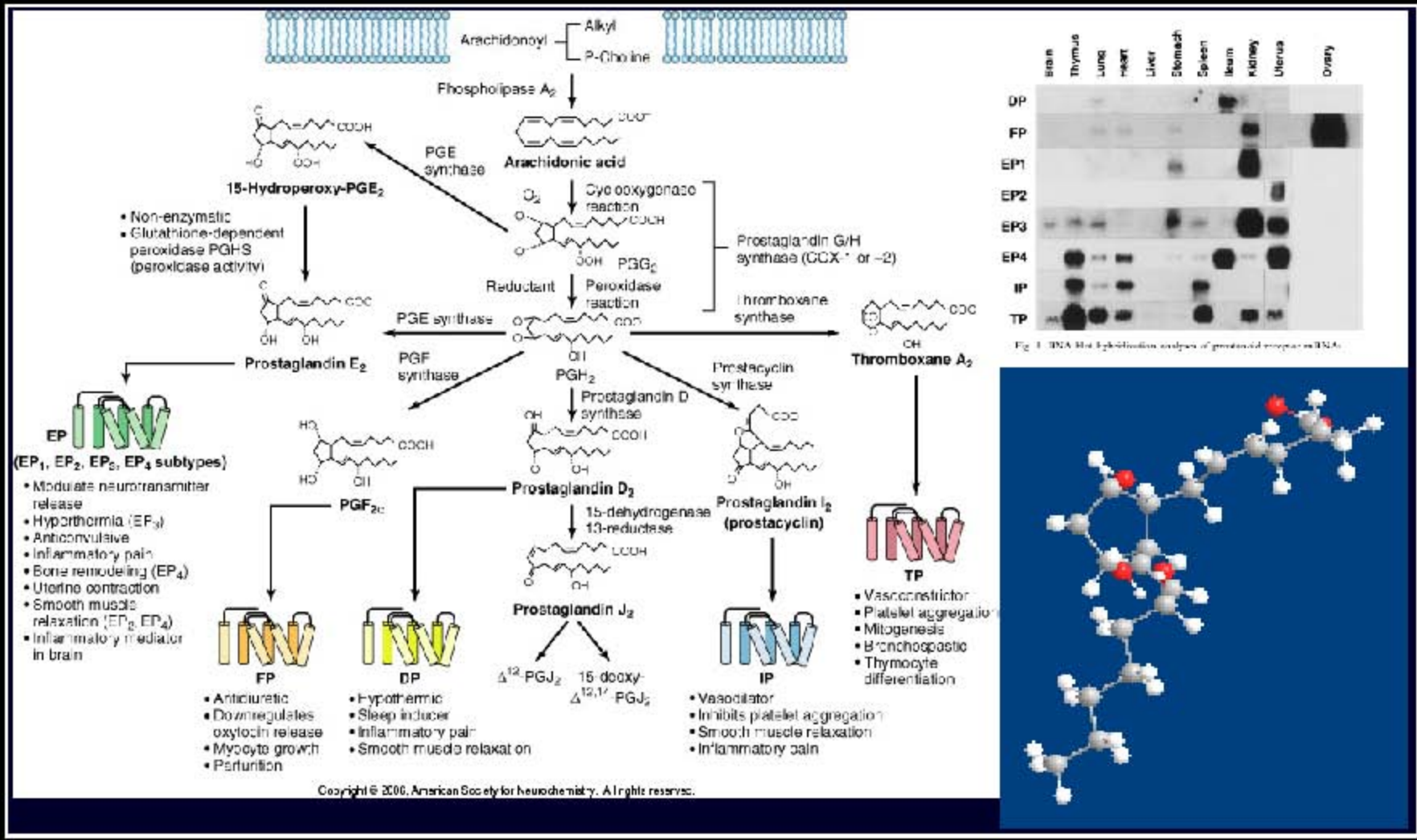
Hybrid (binary) antimalarian drug



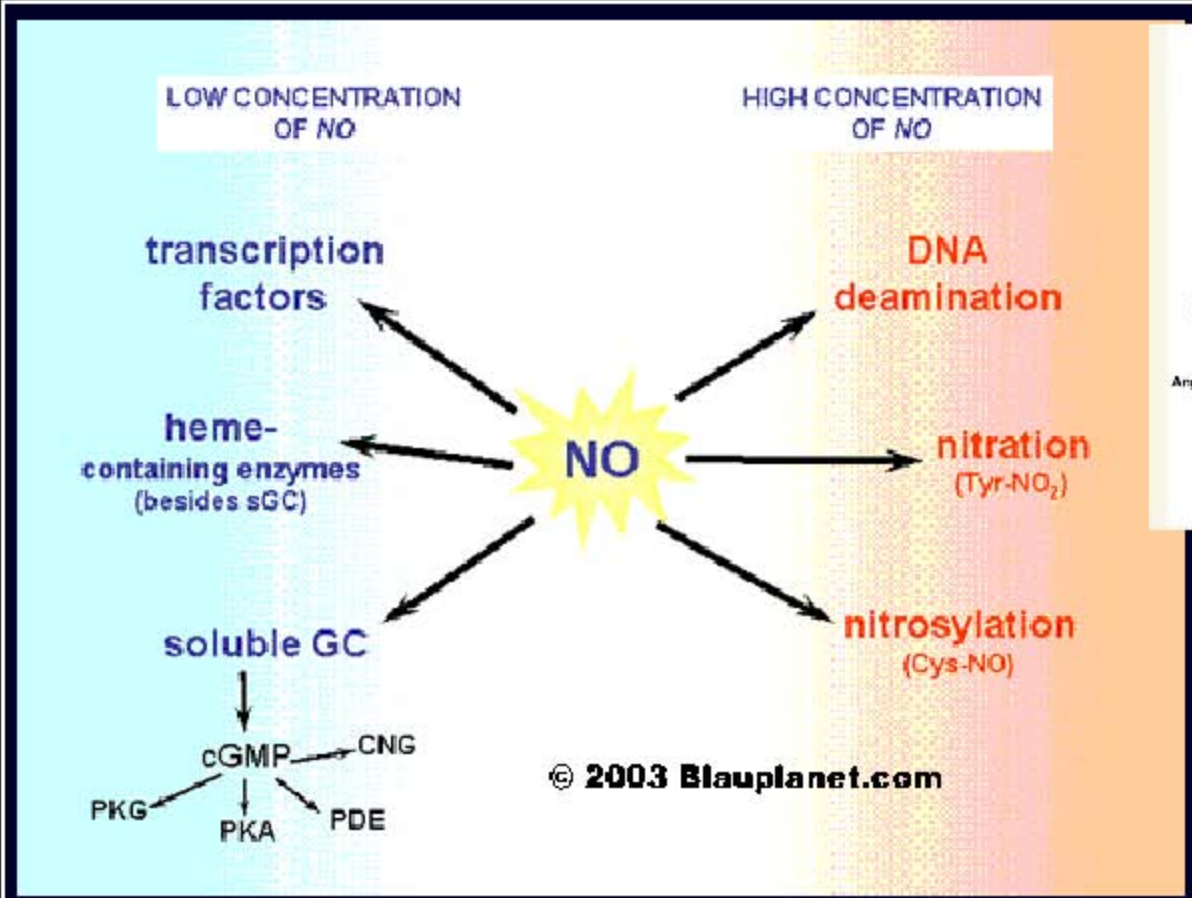
Hybrid NO-donating polyfunctional compounds

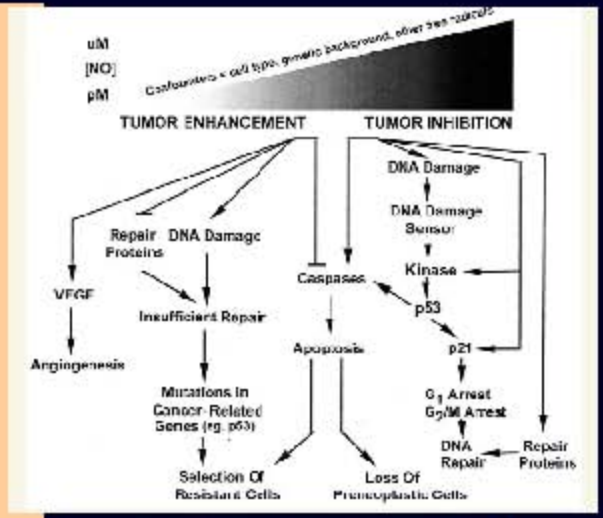


Prostaglandins – polyfunctional compounds

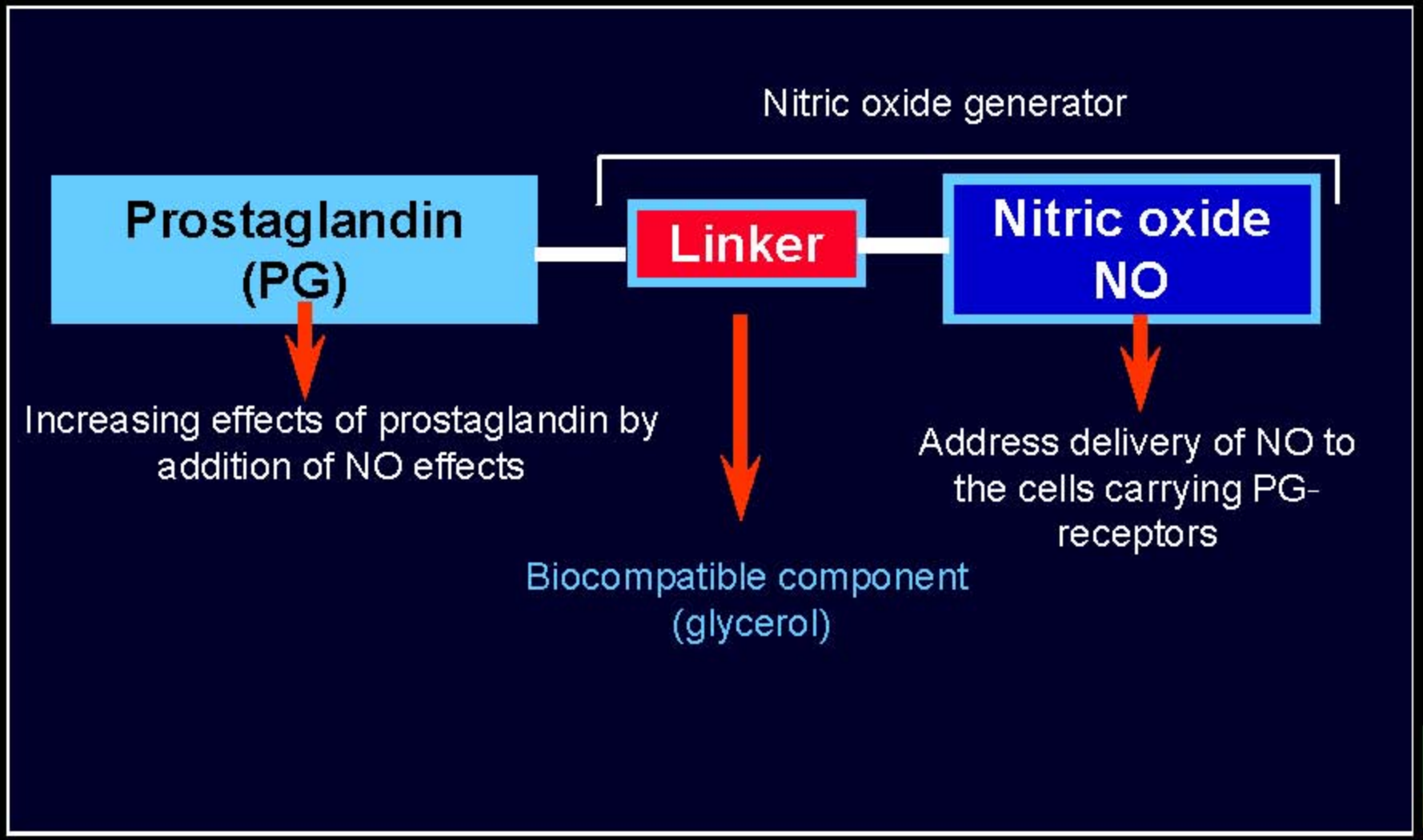


Polyfunctional nitric oxide (NO)





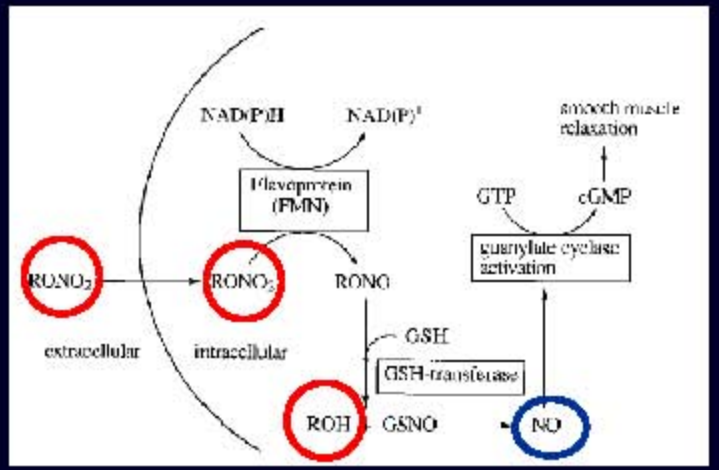
Design of NO-PGs



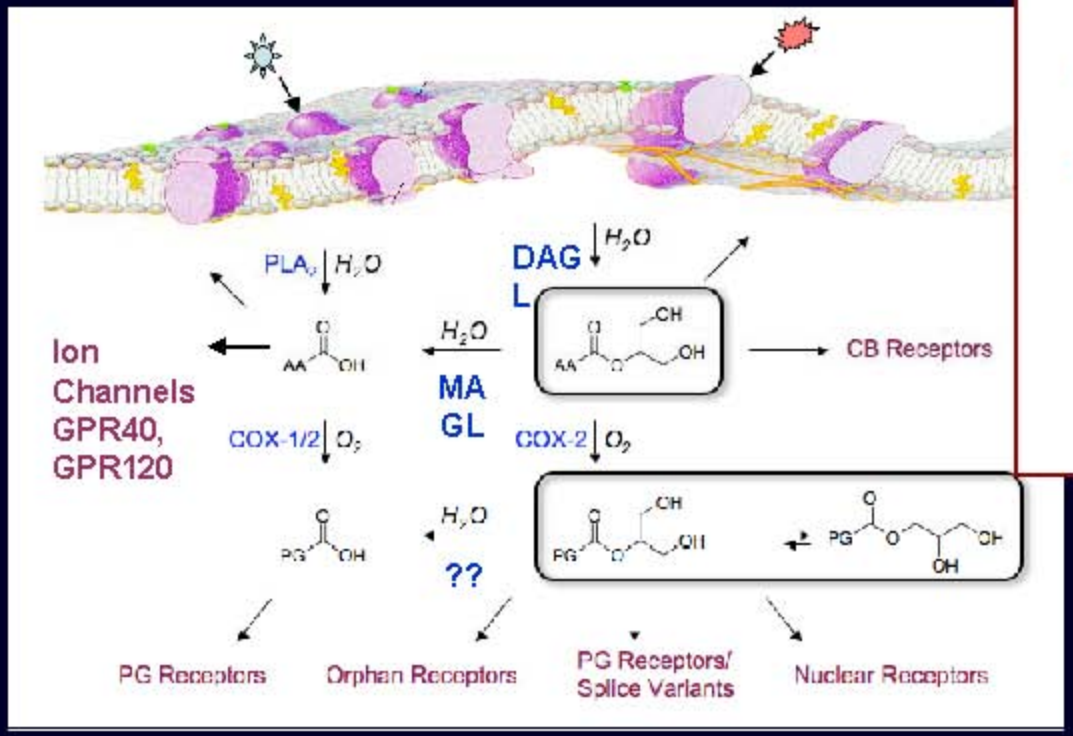
Why organic nitrates? -O-NO_2

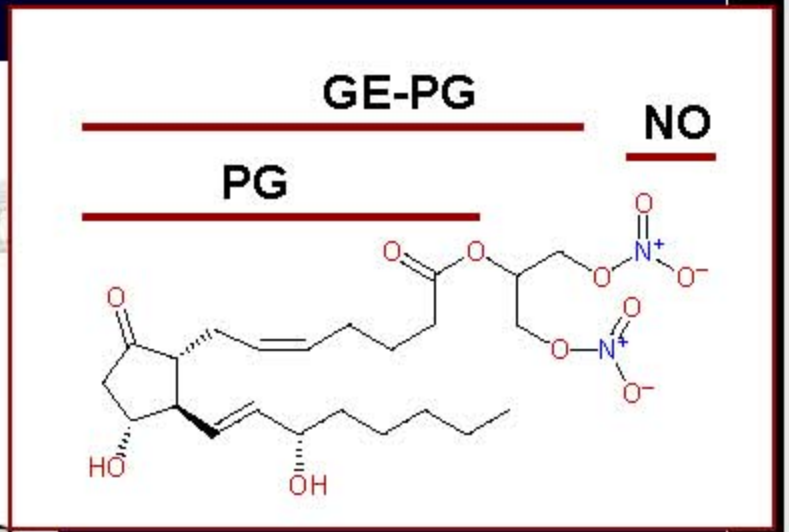
- Absence of spontaneous NO generation
- NO releasing is thiol –depending process (protein participation)
- Bioactivation releases hydroxyl group
- Conversion into nitroester changes ligand properties and pharmacokinetic of natural compounds
- Perhaps are endogenous compounds

→ Possibility of address delivery



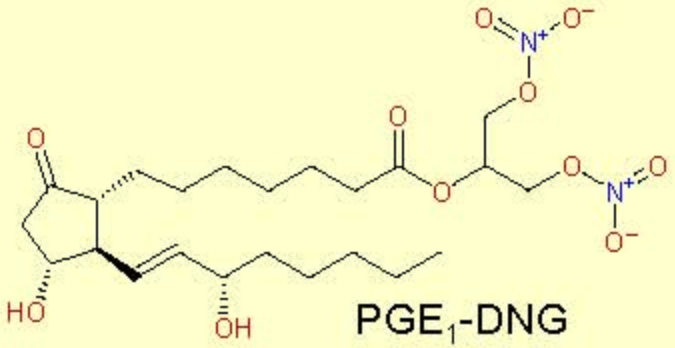
Endocannabinoids → prostaglandins



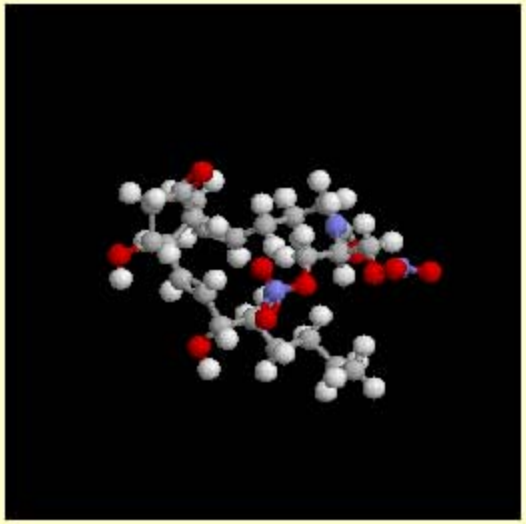


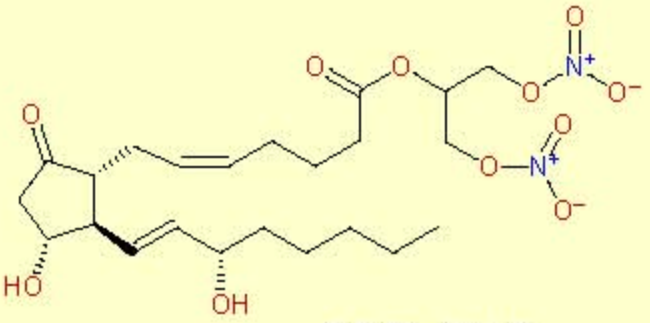
Adapted from Carol A. Rouzer and Lawrence J. Marnet,JBC 2008

Dinitroglycerol esters of prostaglandins

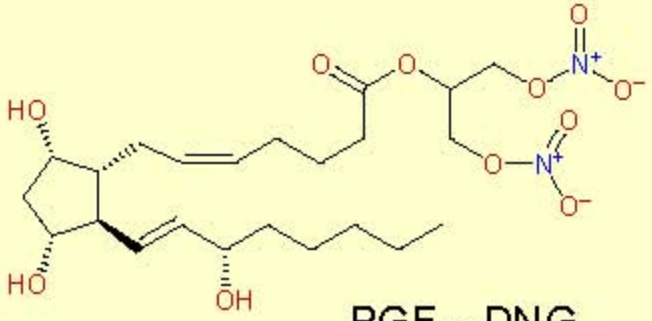


PGE_1-DNG
prostanit





PGE_2-DNG
nitroproston



$PGF_{2\alpha}$-DNG
nitroprost-F

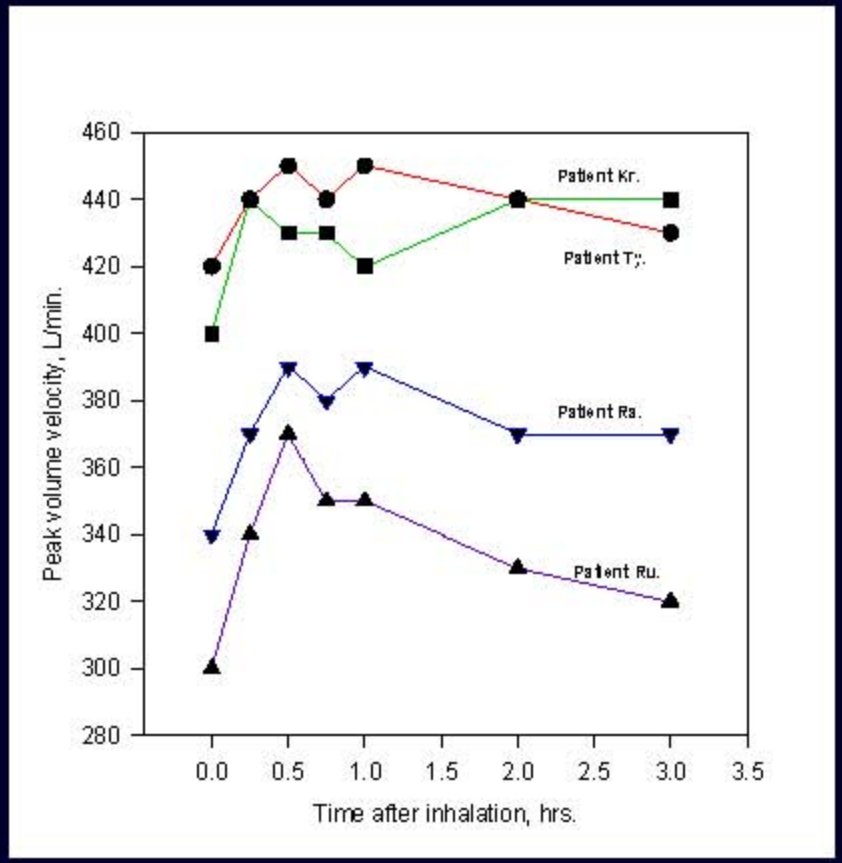
Prostanit as smooth muscle modulators

PGE$_1$-DNG

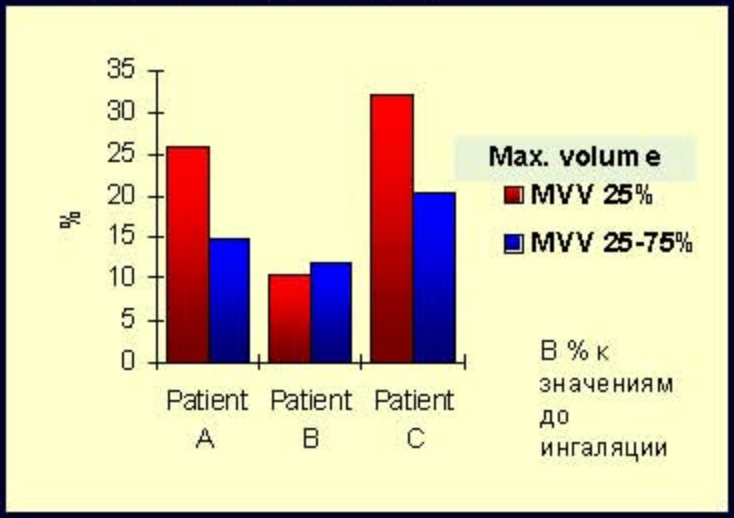
Object	*Activity (EC_{50}, μM)*	
	PROSTANIT®	**PGE$_1$**
Rat aorta	2.0±0.9 relaxation	0.16±0.11 contraction
Rat uterus contraction	0.33±0.08	2.7±0.8
Rat stomach contraction	0.3±0.12	0.04±0.01

Nitroproston as bronchodilatator

PGE_2-DNG

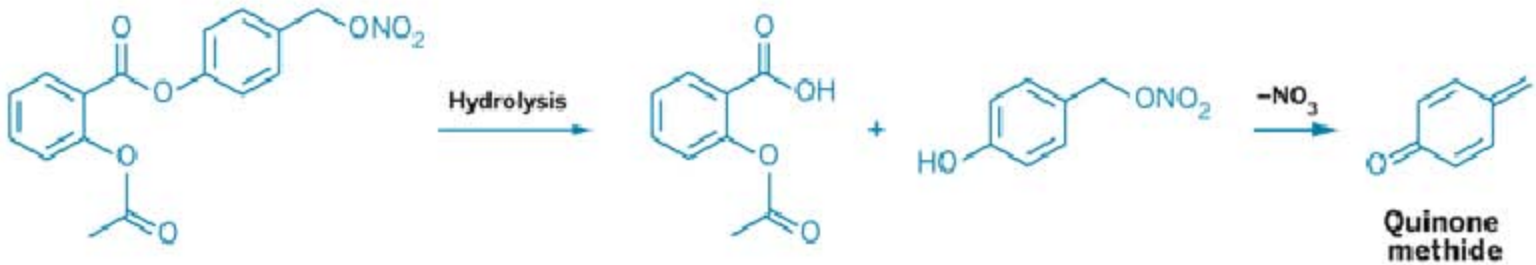


Single dose of nitroproston as a solution via inhalation (7.5 µg per inhalation) evoked long lasting increase of maximal volume velocity of expiration.
Patients with obstructive bronchitis and asthma.



Some comments about linker

SURPRISE This aspirin-NO donor hybrid doesn't behave as expected. After hydrolysis, the fragment containing the spacer and the $-NO_3$ group undergoes a 1,6-elimination to form quinone methide.



N.Hulsman,et al. *JMC, 2007*

Peptolipins as hybrid multifunctional substances

Lipid

$\{L_1, L_2, L_3, L_4 \ldots L_n\}$

A set of properties

Peptide

$\{P_1, P_2, P_3, P_4 \ldots P_n\}$

Peptolipin

$\{L_1, L_2, L_3, L_4 \ldots L_n, P_1, P_2, P_3, P_4 \ldots P_n\}$

The medical problem

Insult

- Disturbances of cerebral blood flow

  Increasing of local blood flow avoiding system effect
- High risk of thrombosis due to massive releasing of pro-aggregants

  Application of mild antiaggregants
- Consequence of ischemic damage of the brain

  Application of neuroprotectors

Selection criteria

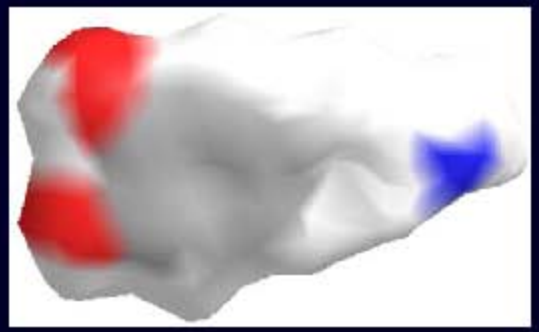
- increasing of local cerebral flow
- minimal influence upon arterial blood pressure
- absence of individual sensitivity
- marked anti-aggregation (anti-platelet) effect
- neuroprotection

Composition of multifunctional drug

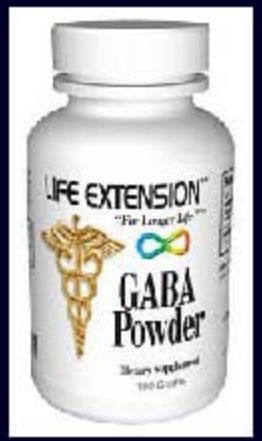
- 1) neurotropic peptide with positive clinical history (neuroprotection)
- 2) neuroactive lipid (cerebral blood flow, anti-aggregation, neuroprotection)
- 3) linker – low molecular weight drug substance increasing cerebral blood flow

1) Met-Glu-His-Phe-Pro-Gly-Pro

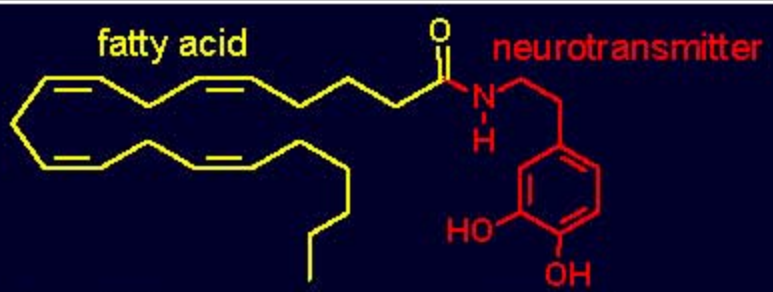
2) N-acyldopamines



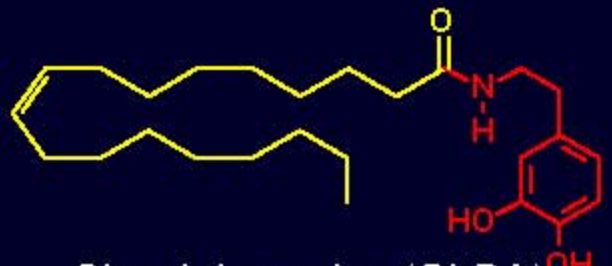
3) GABA



Polypotency of acyldopamines



Arachidonoyl dopamine (AA-DA)



Oleoyl dopamine (Ol-DA)

Agonists

Cannabinoid receptors - CB1, CB2

Vanilloid receptor – TRPV1

GPR 119 (Ol-DA)

Antagonists

Cold and menthol receptor 1 - TRPM8 (AA-DA)

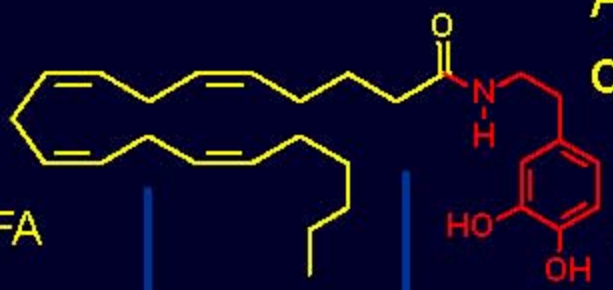
Other activities

Inhibition the HIV-1-LTR promoter (LTR-Luc)

Inhibition T-type Ca^{2+}-channels

Structure-Activity Relationship of NADA at Cannabinoid Receptor 1

Arachidonoyl dopamine



FA

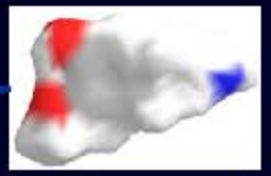
Activity strongly depends on FA moiety

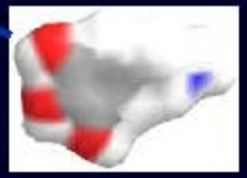
	Ki, nM
AA-DA	100
22:5-DA	425
EPA-DA	475
Pin-DA	1050
18:4-DA	2000
Lin-DA	2150

	Ki, nM
AA-DA	100
alphadiMe-AA-DA	10000

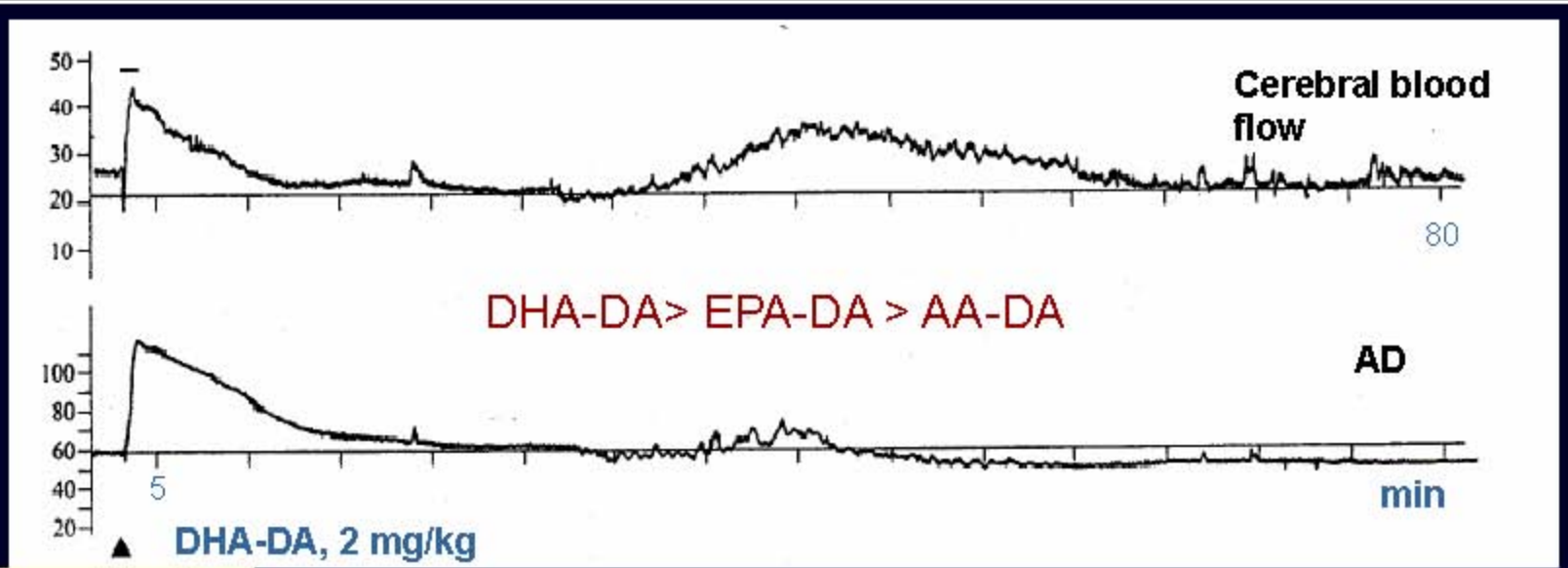
Headgroup geometry

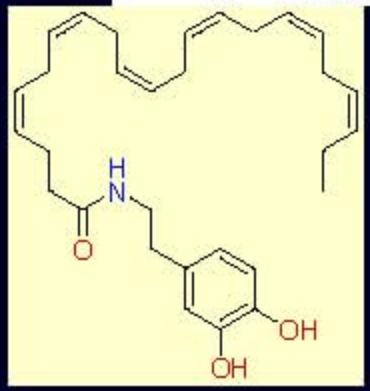
	Ki, nM
AA-DA	100
AA-4mDA	190
AA-3mDA	310
AA-6DA	10000





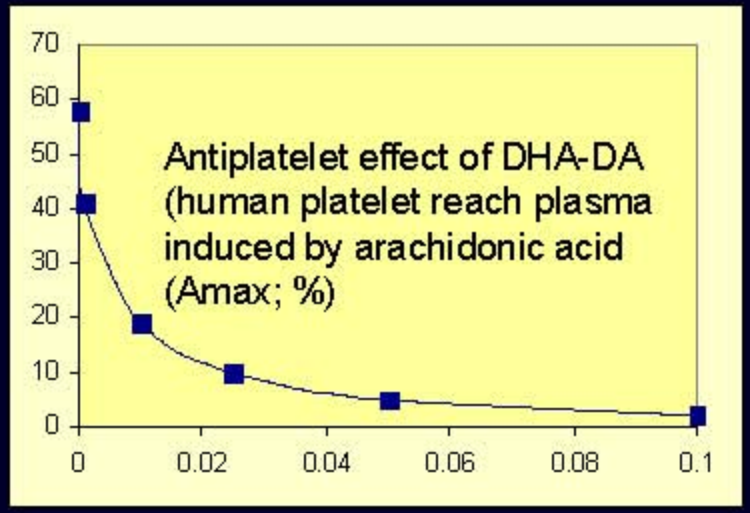
Lipid component of peptolipin

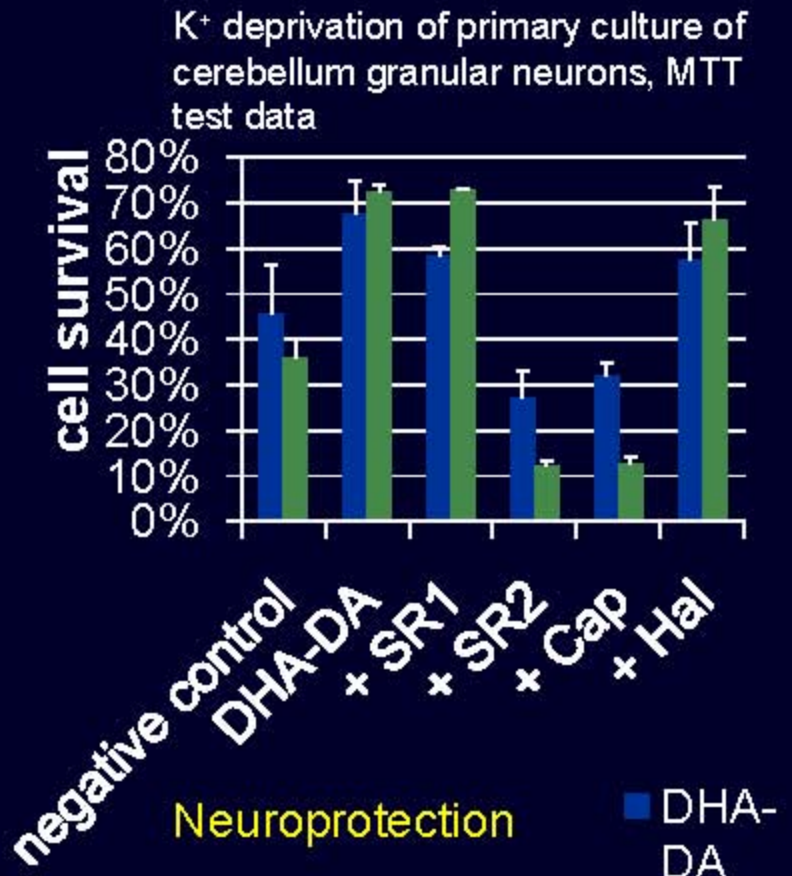




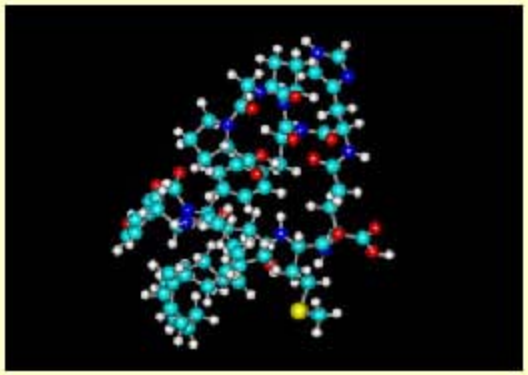
Docosahexaenoyl dopamine (DHA-DA)

Lipid component of peptolipin

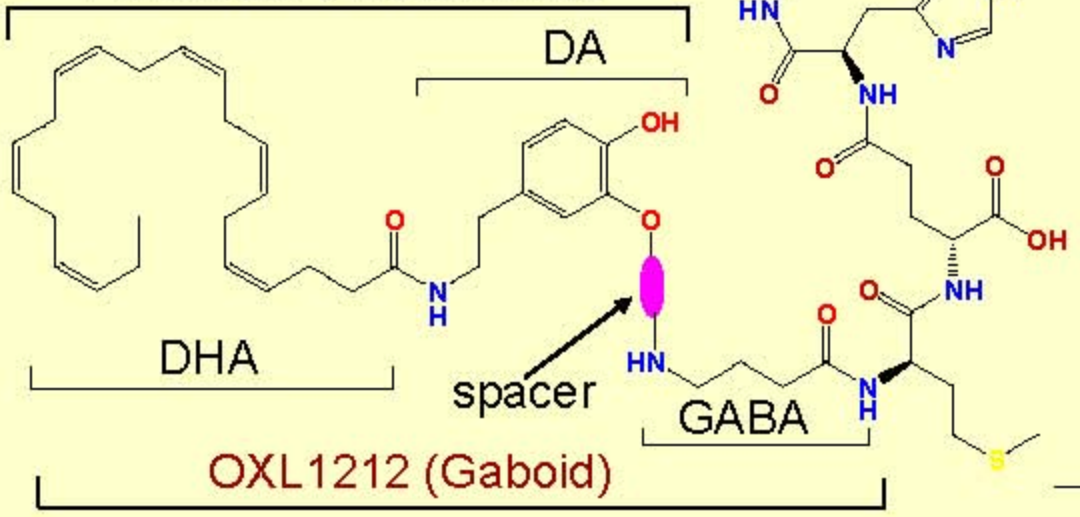




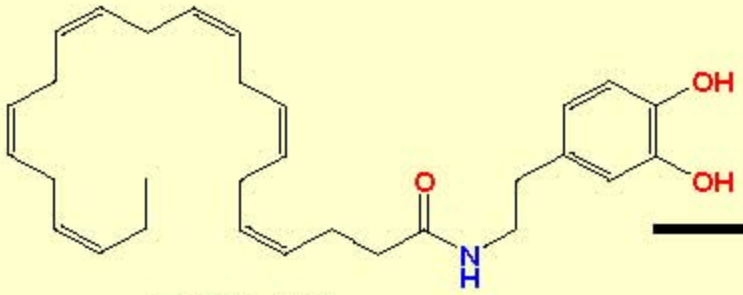
Final structure of multifunctional peptolipin



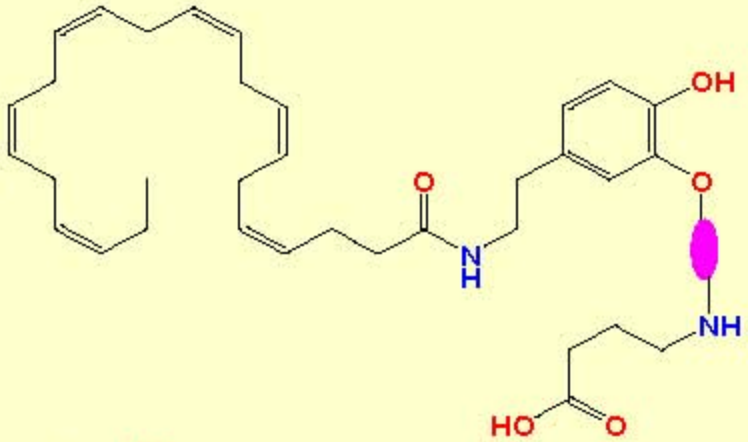
Neurolipin (Dopaminoid)



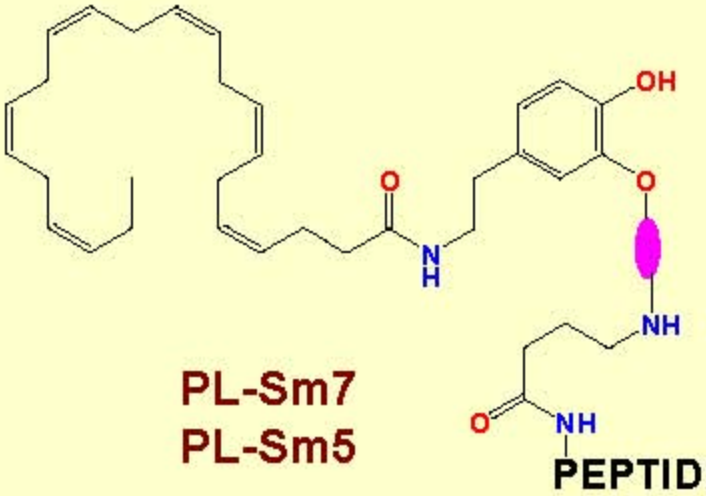
Synthetic approach to peptolipins



DHA-DA



OXL1212

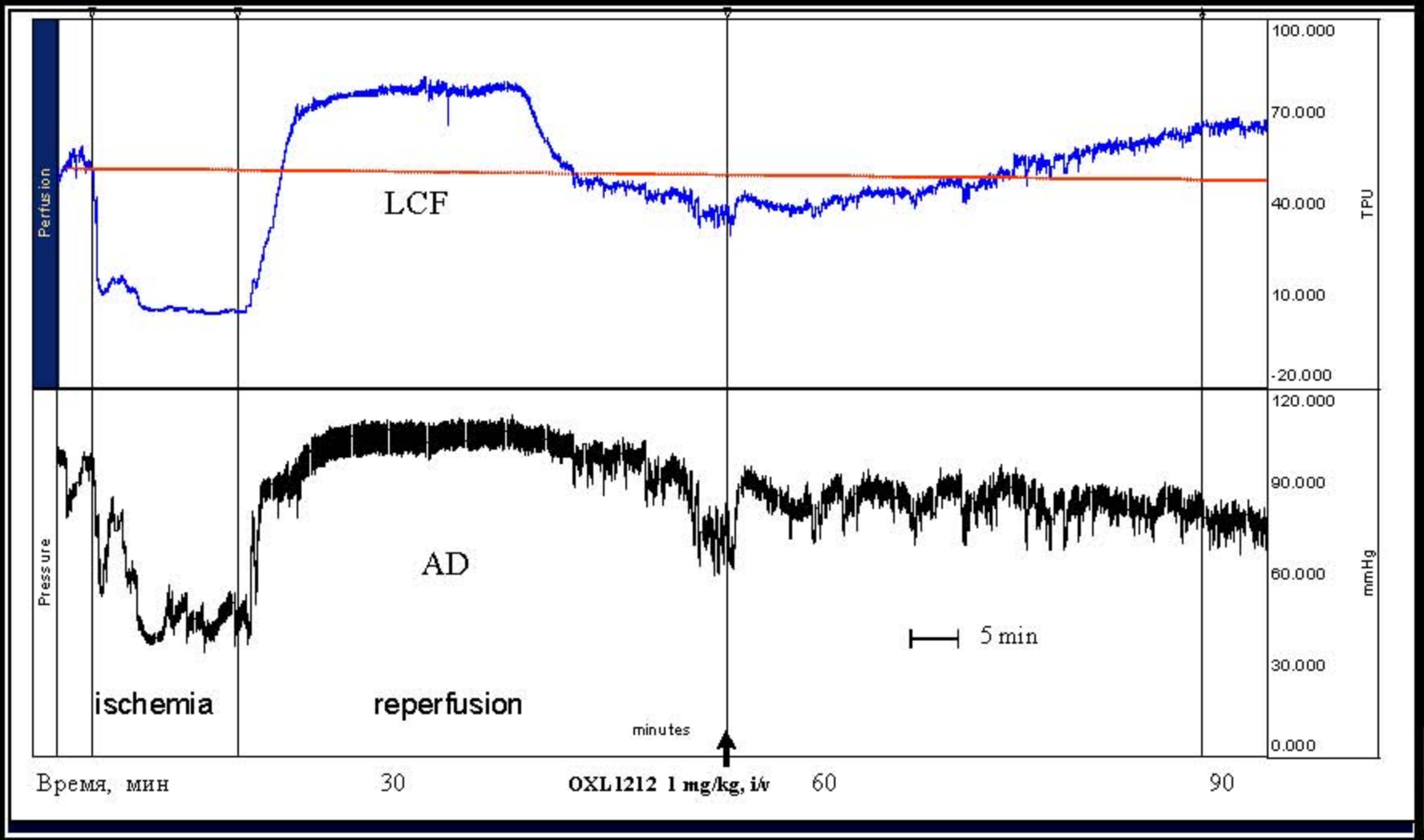


PL-Sm7
PL-Sm5

PEPTIDE

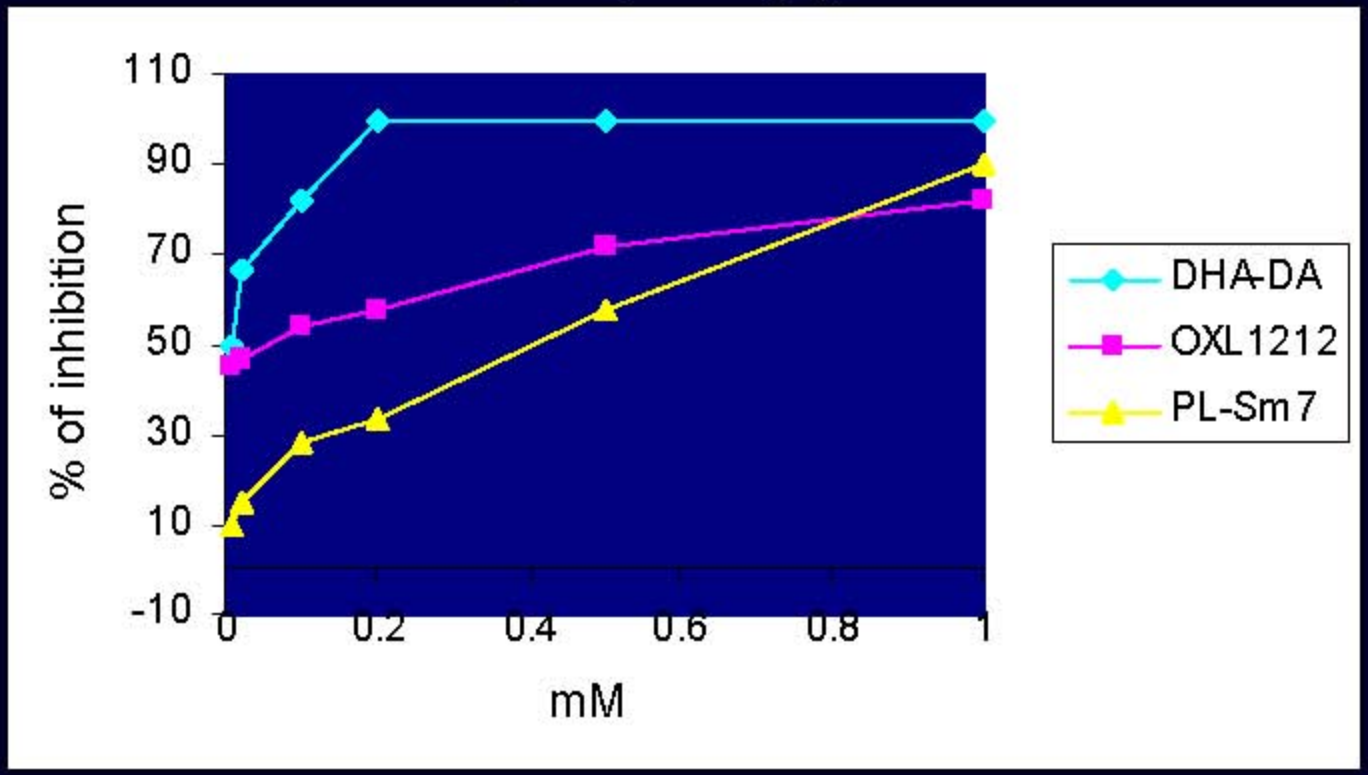
Химический синтез

Llipid part of peptolipin (OXL1212) increases local cerebral flow (LCF) in rats with transient global ischemia



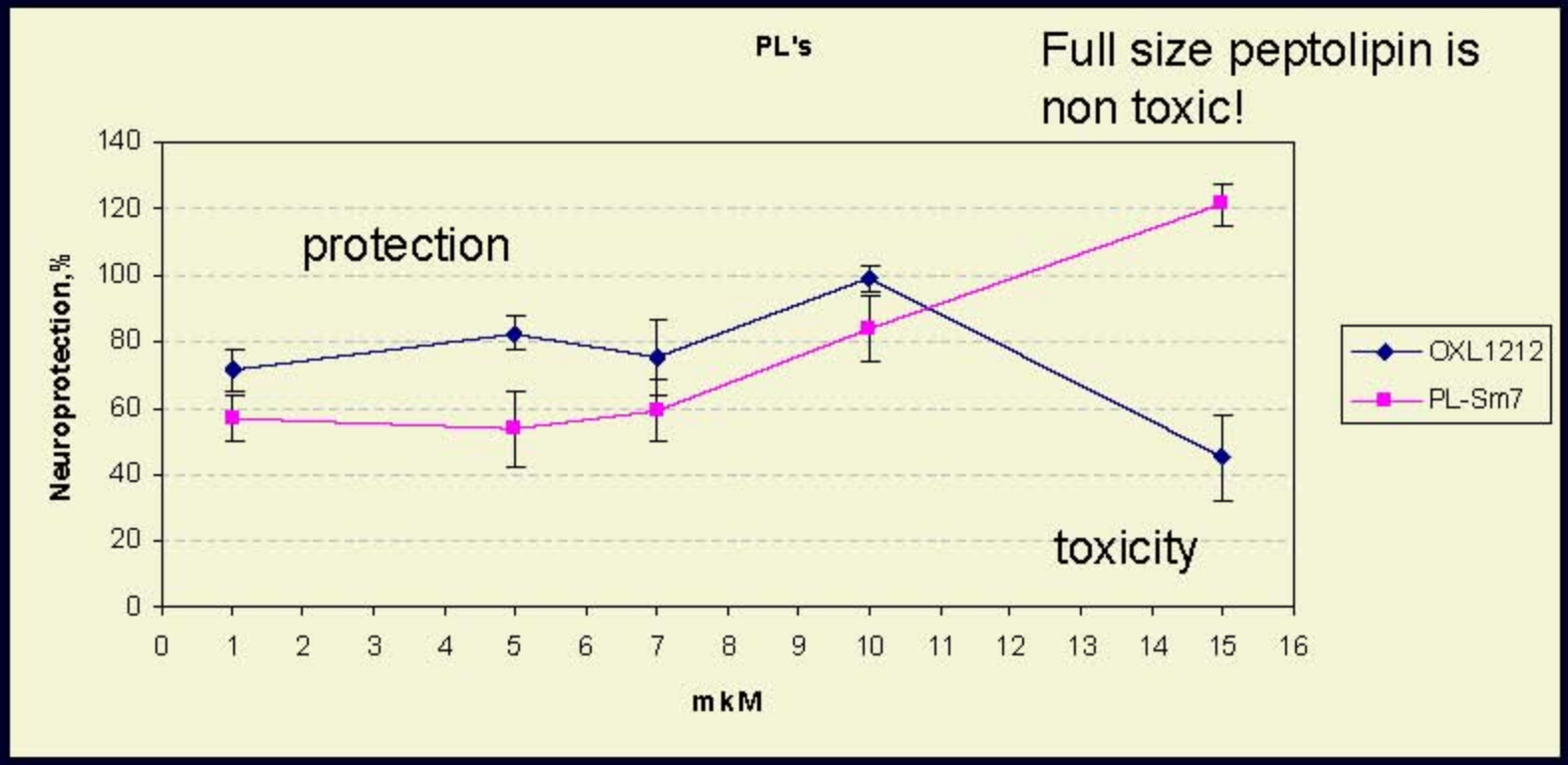
Antiplatelet activity of peptolipin and its components

Platelet reach human plasma, inducing by arachidonic acid



Full size peptolipin possesses antiplatelet activity

Neuroprotection by peptolipin and its component in K^+-serum deprivation model



Rat granular cerebellum neurons harvested 7 days. Incubation with(without) compound in a milieu with decreased K+ (9mM) и FBS 2%. Survived cells counted by MTT-test.

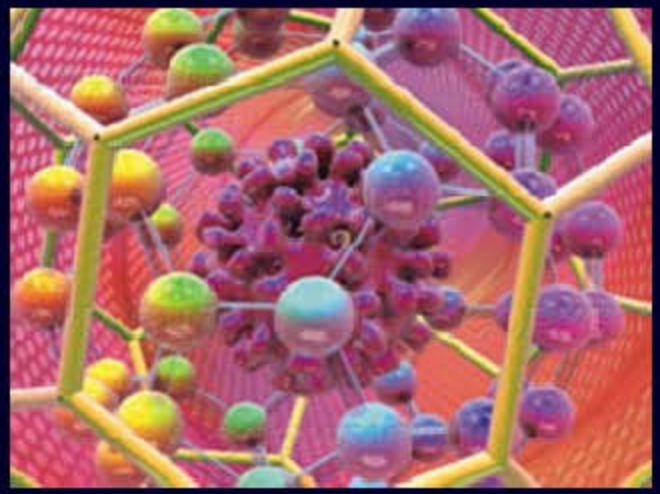
Non-covalent complexes: a road to individuality

Can we proof that drug molecule acts as each taken separately?

How to achieve the monomolecular form of drug compound?

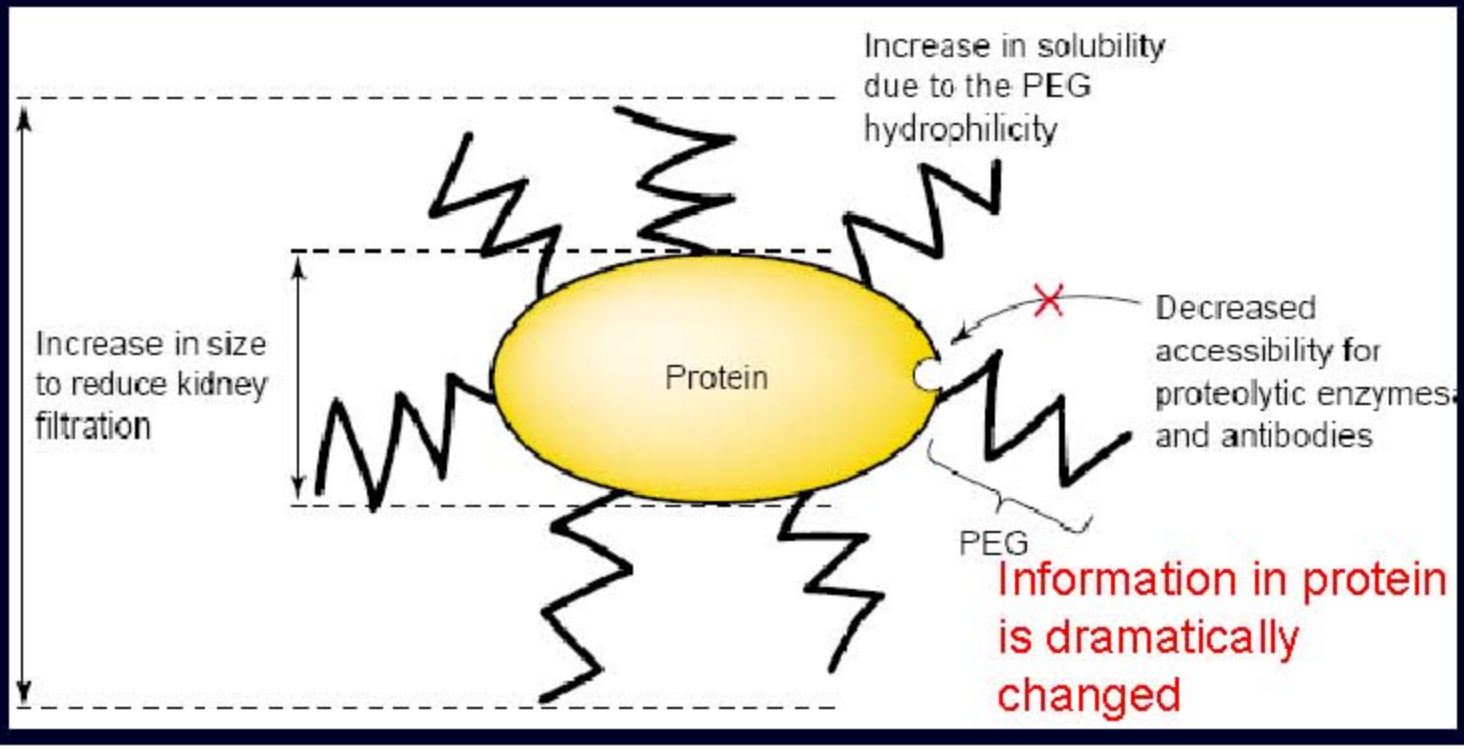
Shortcomings of recombinant proteins

- Instability (in vitro and vivo)
- Nonresistance towards proteases
- Low clearance time
- Immunogenity
- Side effects (especially at long-term application)



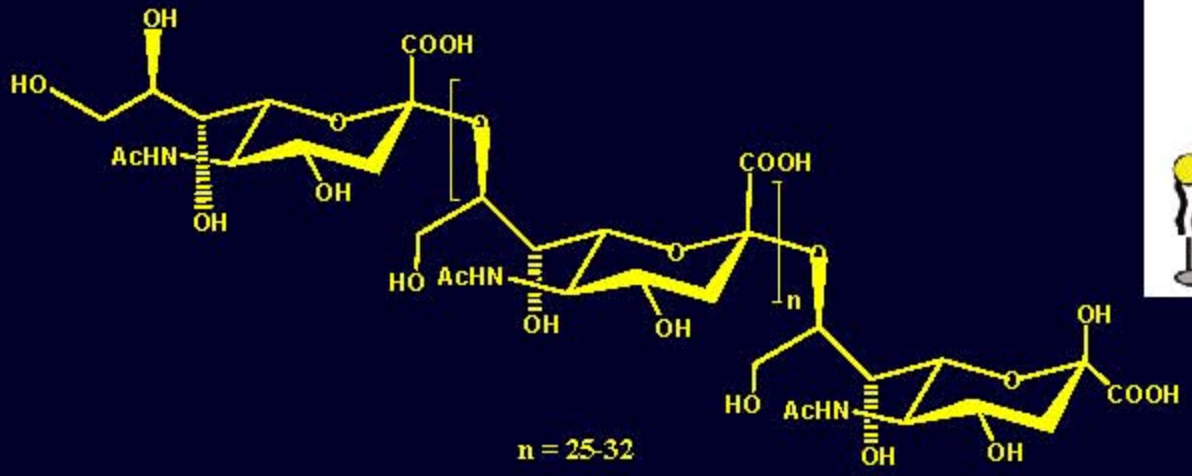
PEGylation: individuality that was changed

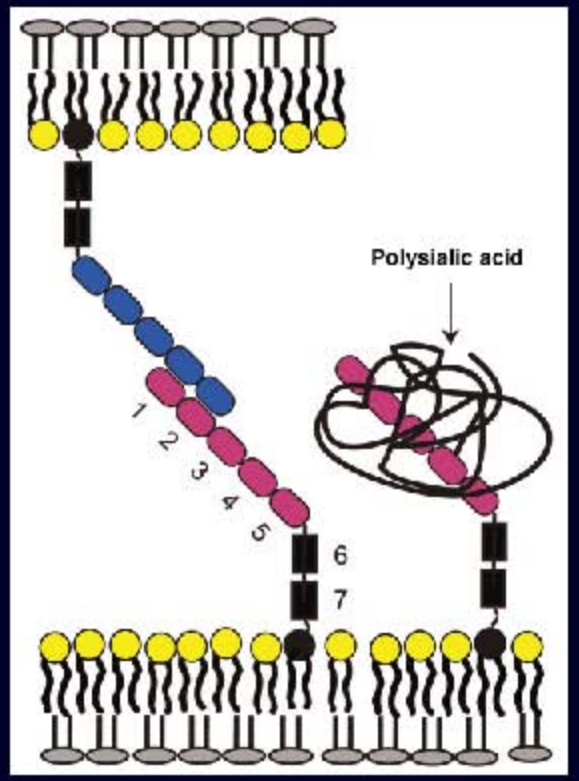
PEGylation is the process of covalent attachment of polyethylene glycol (PEG) polymer chains to a drug or therapeutic protein.



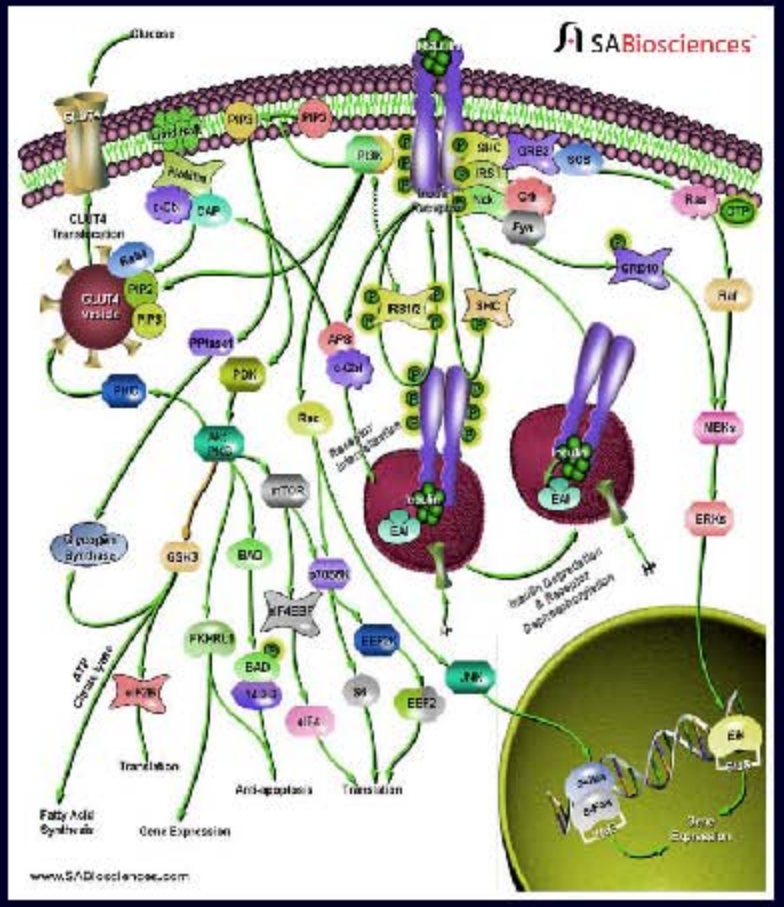
Polysialic acid (PSA) – soluble biopolymer involved in neural cell adhesion

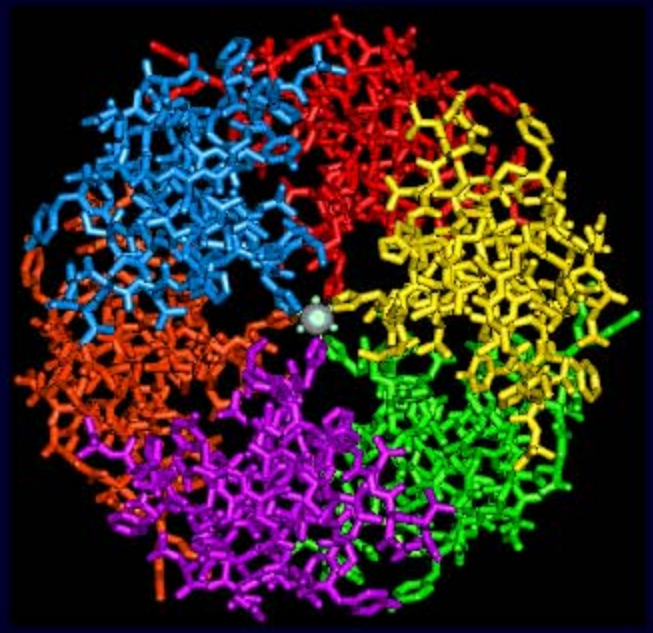
N-acetyl neuraminic acid

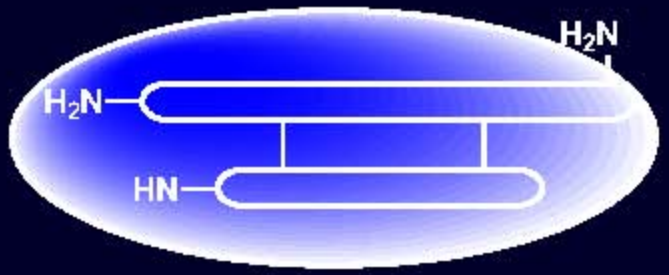




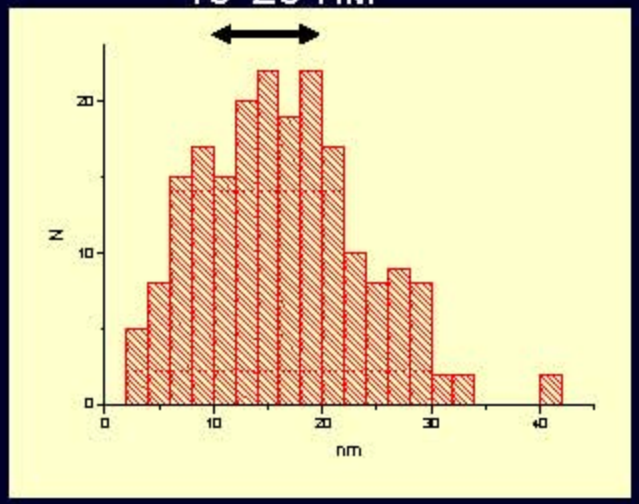
Non-covalent complexes of insulin with polysialic acid



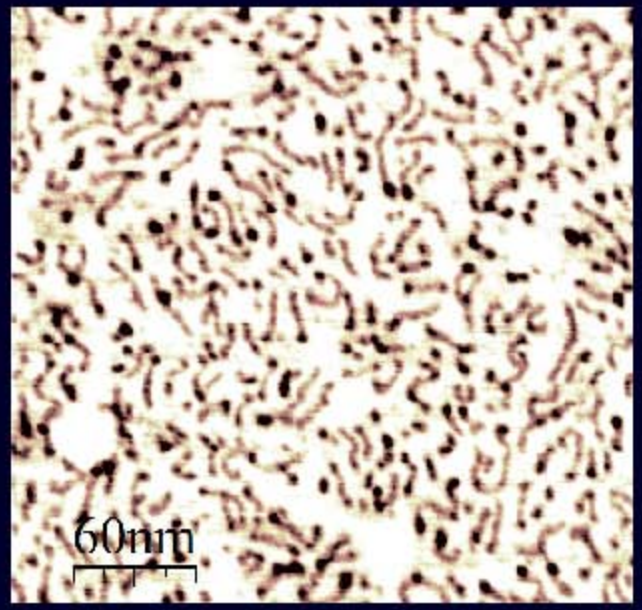


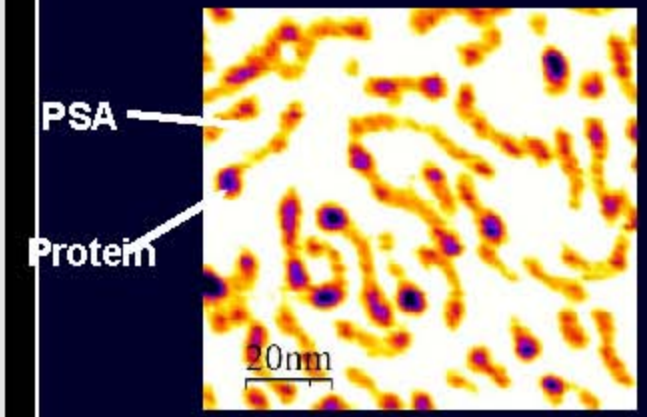


Monomolecular complexes of insulin with PSA



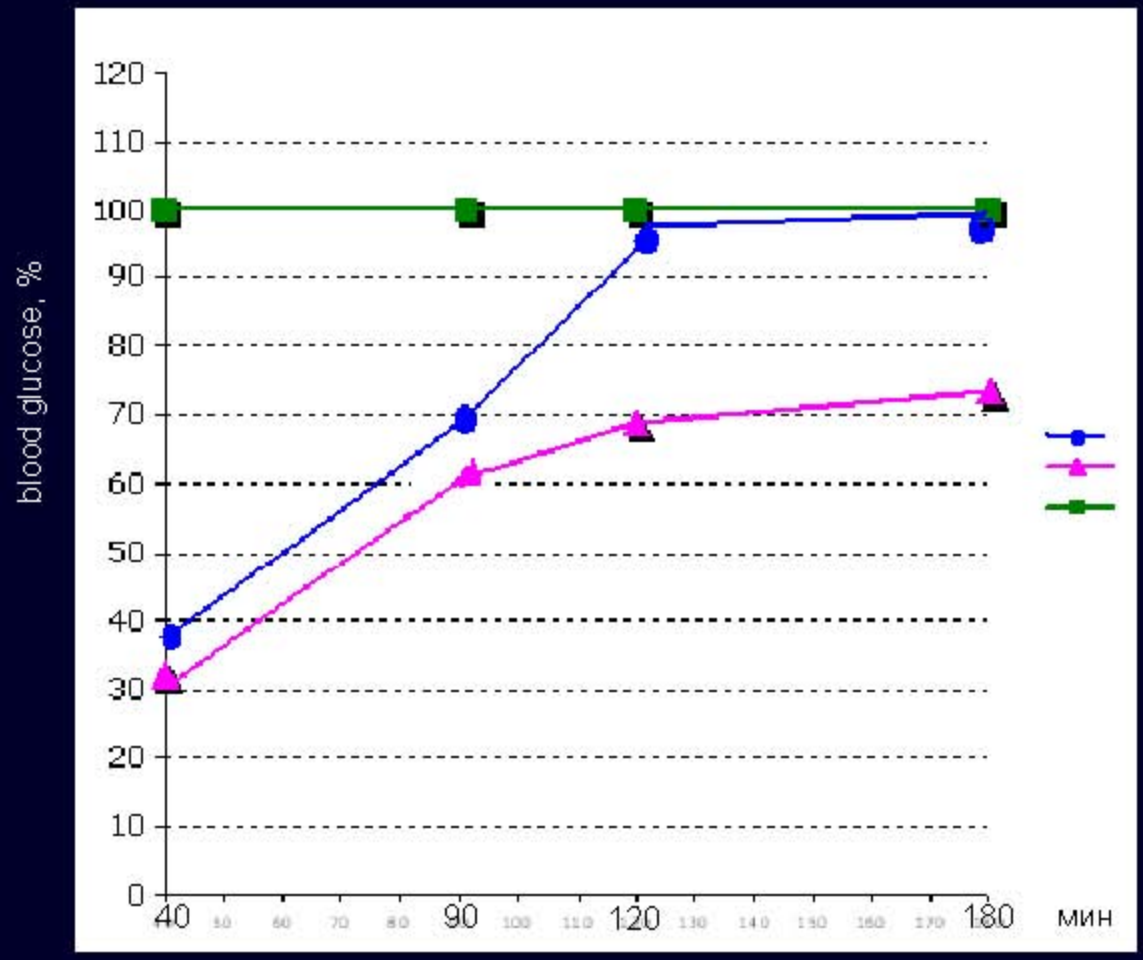
More than 60% of PSA are carrying one molecule of protein





High resolution AFM of insulin-PSA nanocomplex

Prolongation without dosage increasing



Two approaches

Molecular surgery – to kill the enemy.
Paradigm – magic bullets.

Molecular therapy – to restore the power of organism.

Paradigm – informational drugs.

Illness ↔ Health

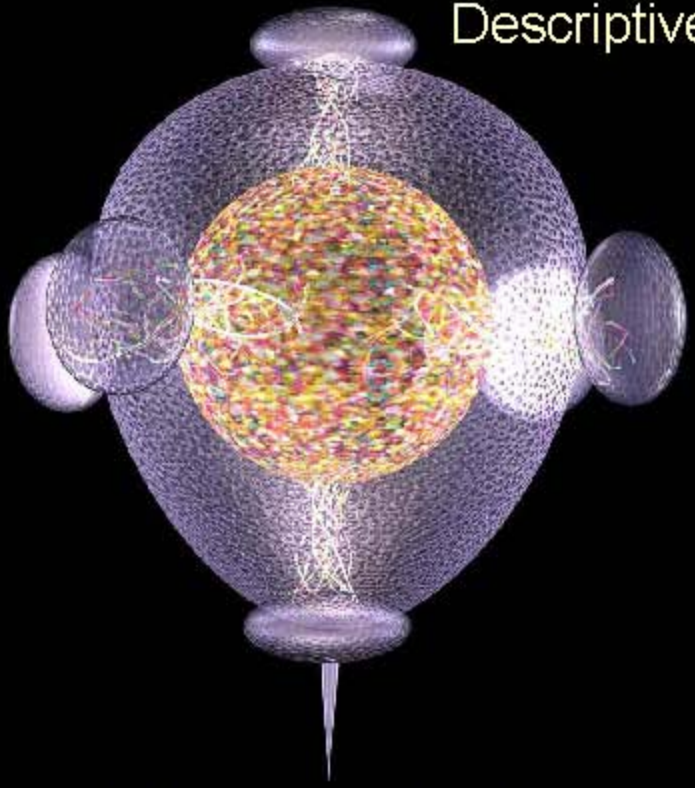
Illness is a way how an organism survived in inadequate environment conditions

We are to help our organism instead of struggling against it.

Informational drug

- Capable to repair abnormalities of informational framework.
- Combines the structures of two (or more) endogenous signal molecules. One of them is lipid or peptide.
- Absence of foreign molecular fragments in the structure.

A NEW ERA IN BIOLOGY



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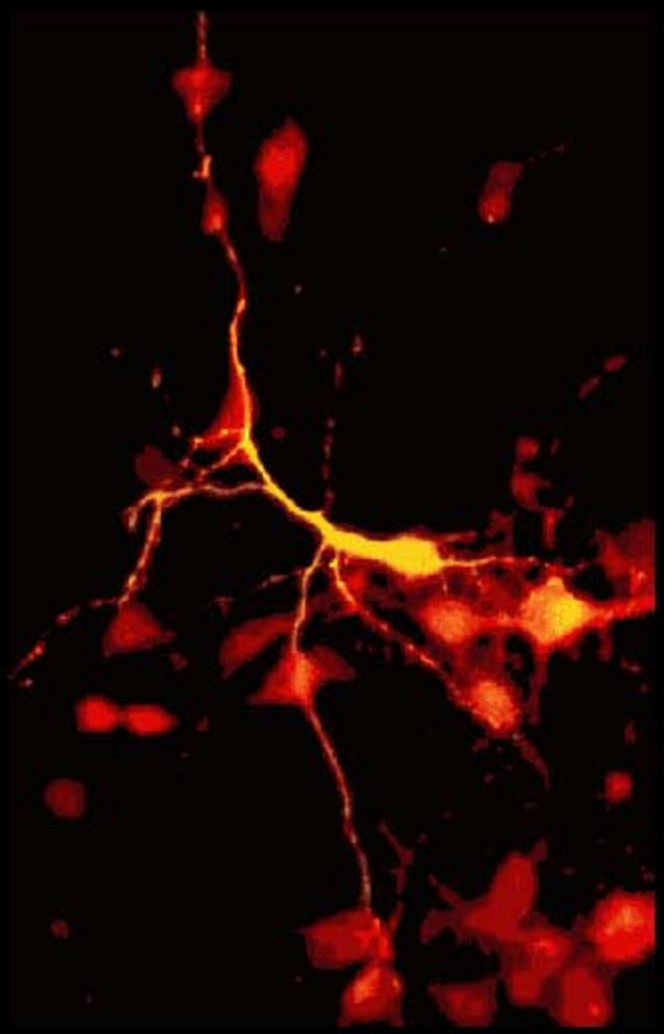

"The illness is too negligible to give up."
Sergei Konovalov