Biochem 03
November 9, 2009

- **Signal Transduction: short acting signals.**
  - **Nitric Oxide** is a fast acting gaseous signaling molecule responsible for a variety of functions.
  - **Nitric Oxide Synthase, NOS** the enzyme which synthesizes NO occurs in three isoforms and has an complex structure.
  - **Reaction catalyzed**: reaction of the amino acid arginine with molecular oxygen to form citrulline and NO.

What does Nitric Oxide do?

- control of blood pressure (fast acting)
  - Smooth muscle relaxant
  - Dilates blood vessels
  - One step in multi-step pathway
- implicated in learning, memory
- control of the inflammatory response

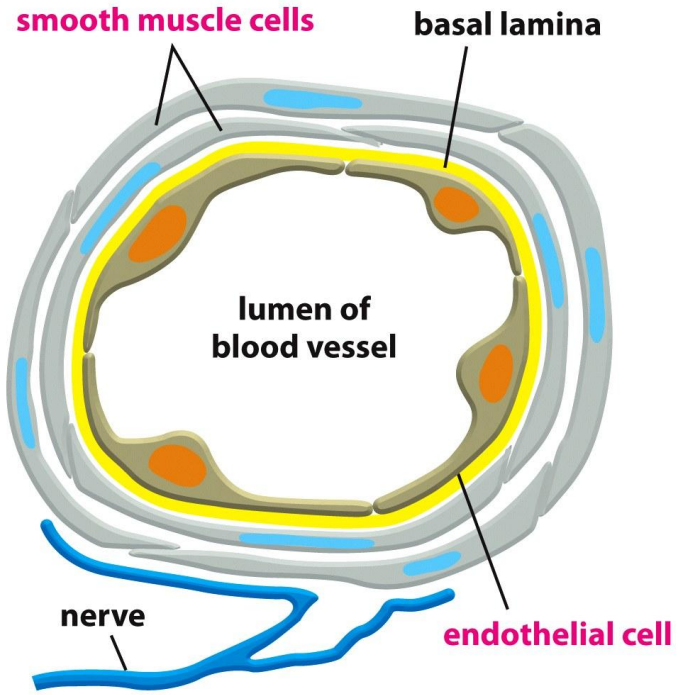


Figure 16-11a Essential Cell Biology 3/e (© Garland Science 2010)

What does Nitric Oxide do?

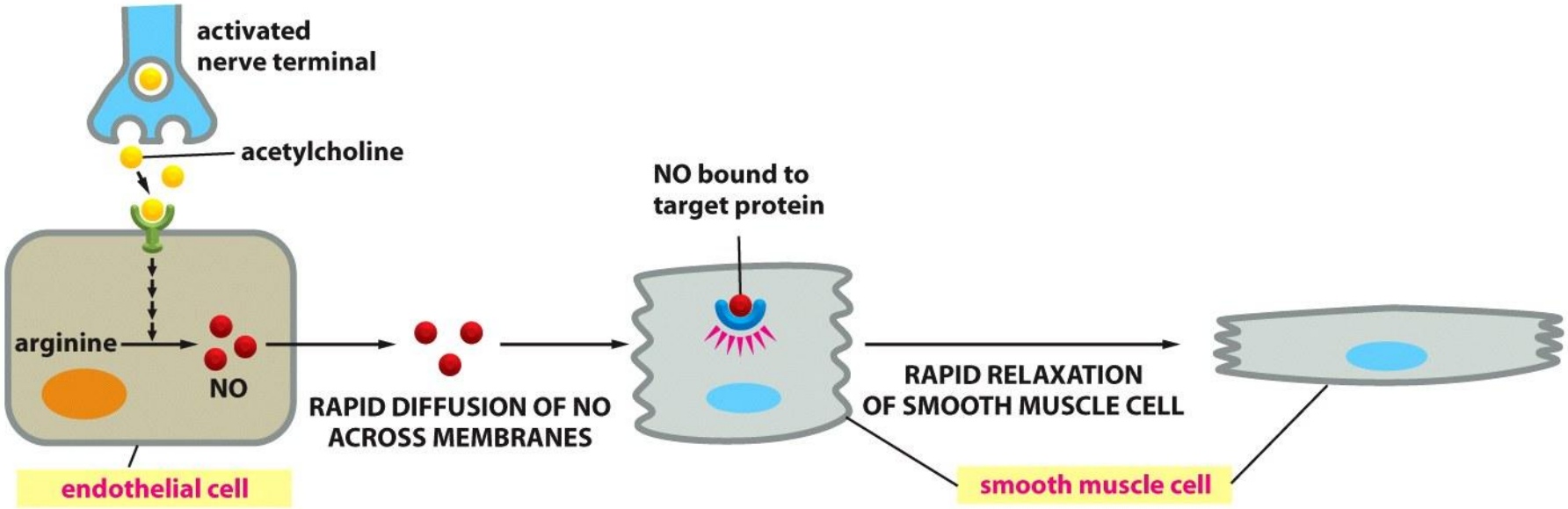
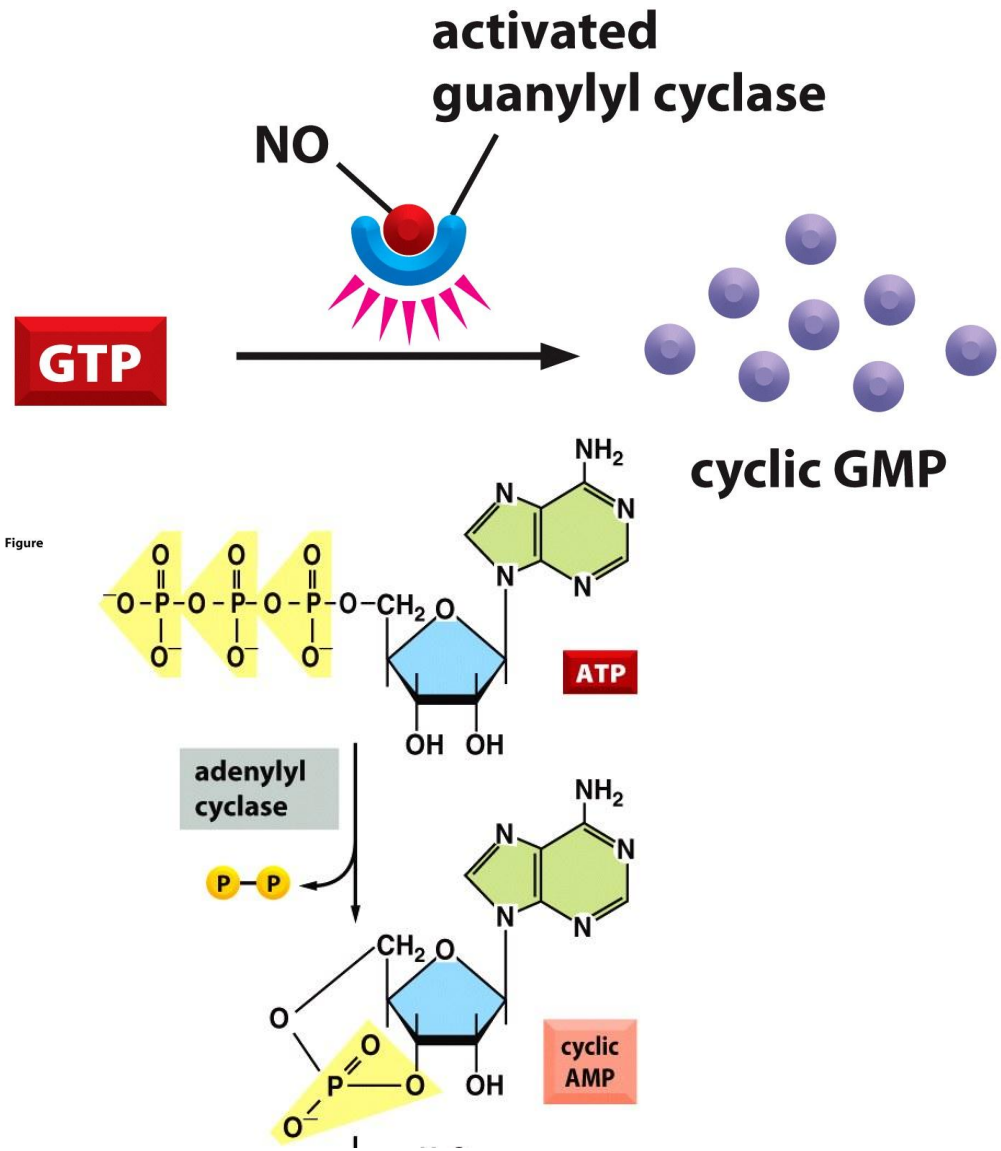


Figure 16-11b Essential Cell Biology 3/e (© Garland Science 2010)

- As a gas, which diffuses rapidly, crosses membranes, readily soluble, NO acts FAST!
- Within minutes, signal disappears because NO is so reactive (radical)

What does Nitric Oxide do?

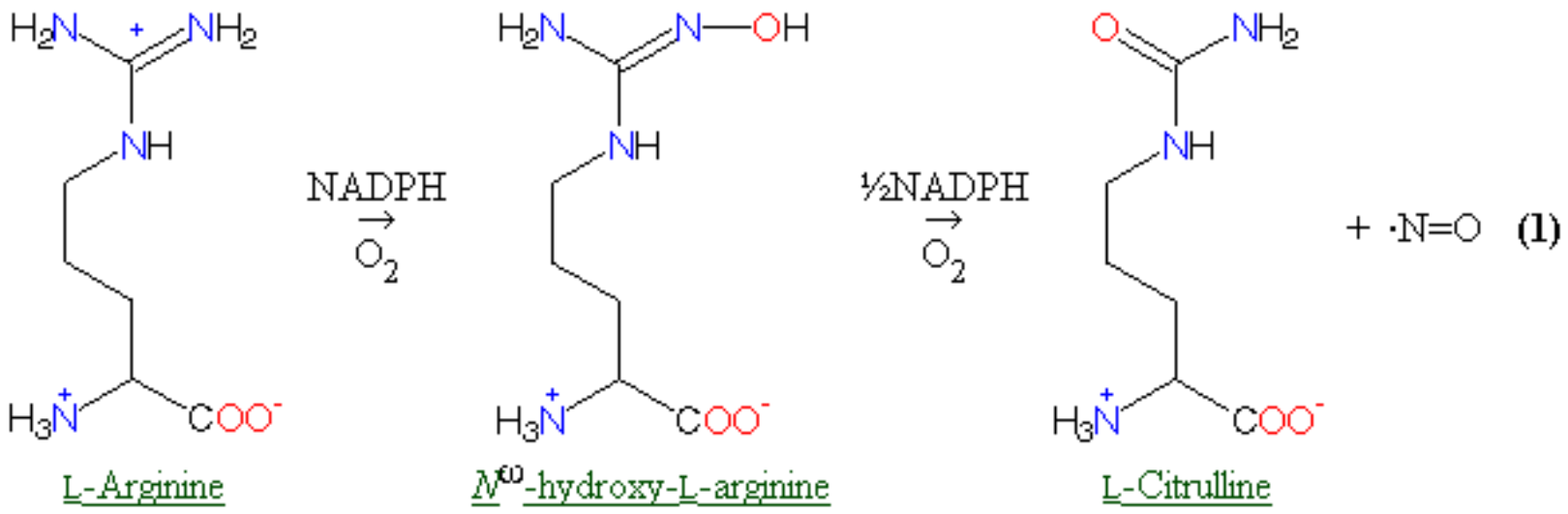
- NO activates many enzymes
  - One of these is guanylyl cyclase, which produces cyclic GMP



How is Nitric Oxide Made?

- by NO Synthase, NOS
  - **occurs in three distinct isoforms**:
    - neuronal **NOS, nNOS** in the brain and nerve cells implicated in olfaction, learning
    - epithelial **NOS, eNOS** in the linings of the blood plasma membranes, regulates blood pressure
    - inducible **NOS, iNOS** in the inflammatory response, floods the body with NO, powerfully destructive
  - **calcium regulation**
    - Both nNOS and eNOS are constitutive (always in the cell) and calcium modulated, through calmodulin
    - iNOS, is induced upon trauma, does not need Ca(II) for activation. Massive amounts of NO are produced and can lead to catastrophic cell death (septic/toxic shock)

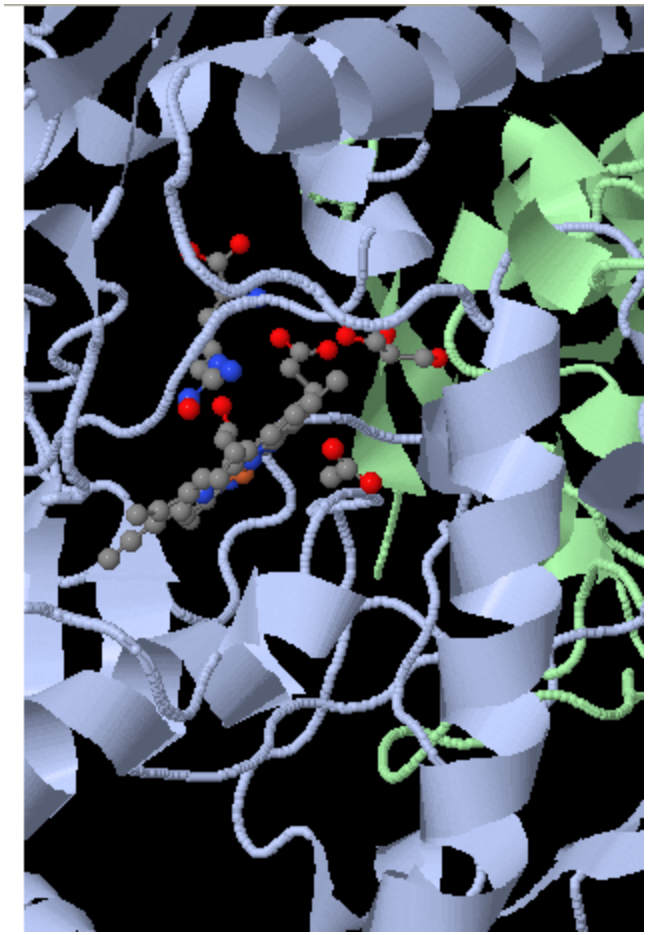
How is Nitric Oxide Made?



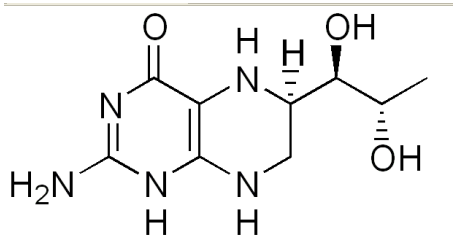
- Enzyme binds oxygen and arginine, causes a complicated two step reaction
- One set of products are citrulline and NO
- Second products are 2 molecules of water (not shown)
- LOTS of cofactors are involved

Structure of NOS * two distinct domains

N-terminal oxidase or catalytic domain pdbid:5nse

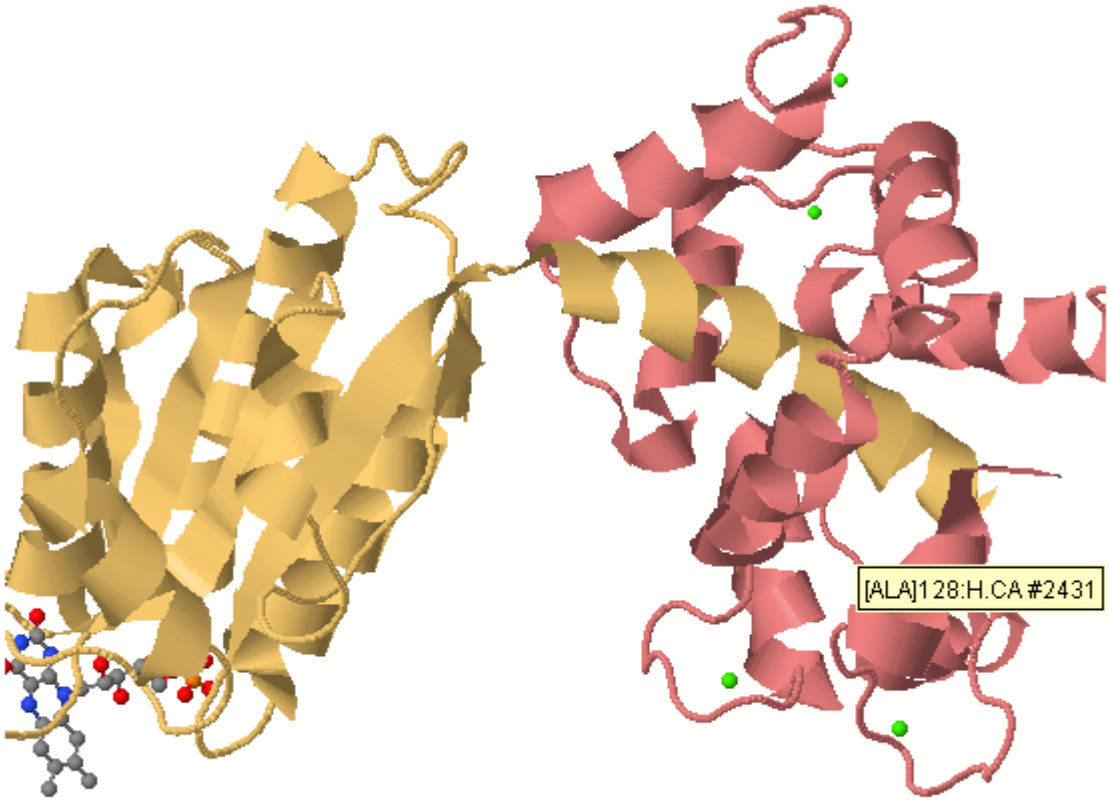


- structure like a glove
- binds substrates arginine and O_2 and contains:
  - a heme group ~ accessible to solvent close to O_2 and Arg
  - another cofactor molecule*
  - sulfate or phosphate which adjusts 2nd structure
  - Zn(II) ion which resides at the dimer interface
  - *



Structure of NOS * two distinct domains

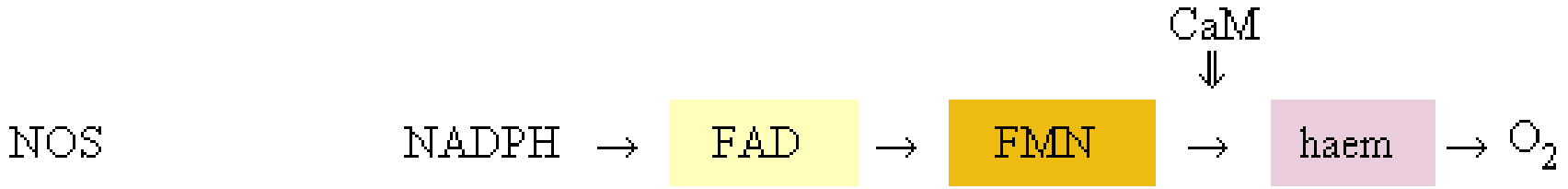
C-terminal reductase domain **3hr4 shown w. CaM(pink)**



- Bound cofactor molecules are all bound close to one another to bring e- in
  - NADPH
  - FMN
  - FAD
- CaM binding displaces inhibitory loop

Structure of NOS * two distinct domains and a linker

- All NOS isozymes are catalytically self-sufficient. The electron flow in the ·NO synthase reaction is shown below
- CaM binding to nNOS has been shown to regulate catalytic activity by triggering electron flux from FMN to heme, thereby coupling the oxygenase and reductase domains.
- The continual activity of iNOS is explained by its exceptionally high avidity for CaM.



What does NO do in memory?

Formation of olfactory memories mediated by nitric oxide

K. M. Kendrick, R. Guevara-Guzman, J. Zorrilla, M. R. Hinton, K. D. Broad, M. Mimmack & S. Ohkura

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Sheep learn to recognize the odours of their lambs within two hours of giving birth, and this learning involves synaptic changes within the olfactory bulb[1,2]. Specifically, mitral cells become increasingly responsive to the learned odour, which stimulates release of both glutamate and GABA (γ-aminobutyric acid) neurotransmitters from the reciprocal synapses between the excitatory mitral cells and inhibitory granule cells[1]. Nitric oxide (NO) has been implicated in synaptic plasticity in other regions of the brain as a result of its modulation of cyclic GMP levels[3–7]. Here we investigate the possible role of NO in olfactory learning. We find that the neuronal enzyme nitric oxide synthase (nNOS) is expressed in both mitral and granule cells, whereas the guanylyl cyclase subunits that are required for NO stimulation of cGMP formation[8] are expressed only in mitral cells. Immediately after birth, glutamate levels rise, inducing formation of NO and cGMP, which potentiate glutamate release at the mitral-to-granule cell synapses. Inhibition of nNOS or guanylyl cyclase activity prevents both the potentiation of glutamate release and formation of the olfactory memory. The effects of nNOS inhibition can be reversed by infusion of NO into the olfactory bulb. Once memory has formed, however, inhibition of nNOS or guanylyl cyclase activity

Why is NOS a drug target?

- Drug companies filing for patents,
  - Pfizer making a fortune with Viagra.
  - See links in CourseInfo

Male mice without the gene that enables the brain to make a key neurotransmitter attack each other relentlessly, even fatally.

Inhibit iNOS and NOT eNOS and you can prevent septic shock!

Structural characterization of nitric oxide synthase isoforms reveals striking active-site conservation

Thierry O. Fischmann[1], Alan Hruza[1], Xiao Da Niu[2], James D. Fossetta[2], Charles A. Lunn[2], Edward Dolphin[2], Andrew J. Prongay[1], Paul Reichert[1], Daniel J. Lundell[2], Satwant K. Narula[2] and Patricia C. Weber[1]

Crystal structures of human endothelial nitric oxide synthase (eNOS) and human inducible NOS (iNOS) catalytic domains were solved in complex with the arginine substrate and an inhibitor *S*-ethylisothiourea (SEITU), respectively. The small molecules bind in a narrow cleft within the larger active-site cavity containing heme and tetrahydrobiopterin. Both are hydrogen-bonded to a conserved glutamate (eNOS E361, iNOS E377). The active-site residues of iNOS and eNOS are nearly identical. Nevertheless, structural comparisons provide a basis for design of isozyme-selective inhibitors. The high-resolution, refined structures of eNOS (2.4 Å resolution) and iNOS (2.25 Å resolution) reveal an unexpected structural zinc situated at the intermolecular interface and coordinated by four cysteines, two from each monomer.

Recent patent applications in nitric oxide research I

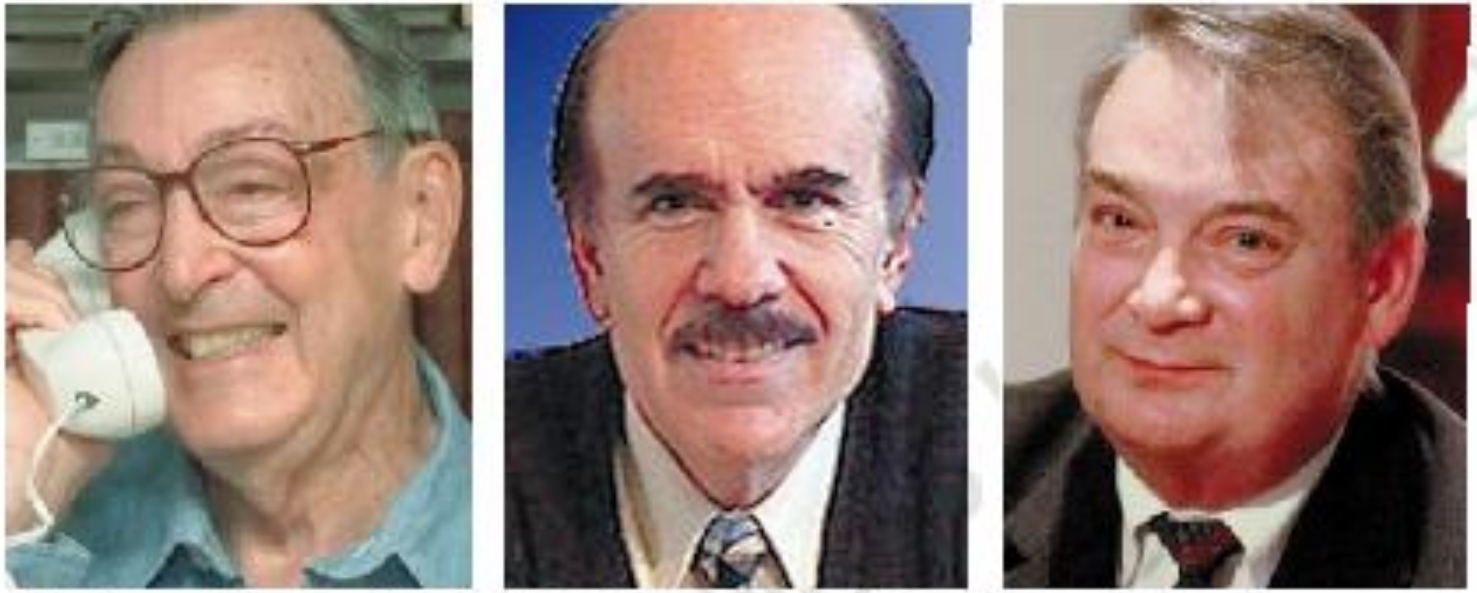
- WO 9839291 New 3- or 4-thiol cyclopentane derivatives: topoisomerase II and IGE inhibitors and apoptosis inducers used for treating, e.g., bacterial infections, allergies, and nitric oxide-mediated diseases, including hypertension, cerebral ischemia, and hyperdipichemia
- WO 9838320 New isolated bromelain component protein; used for treating cancers or diseases responding to increased nitric oxide production or as a vaccine adjuvant or antimicrobial agent
- WO 9837079 New nitric oxide synthase inhibiting N-heterocyclic compounds; used to treat, e.g., inflammatory and autoimmune diseases, multiple sclerosis, stroke, Parkinson's disease, and Alzheimer's disease
- WO 9835667 Treatment or prophylaxis of type-2 diabetes by administration of amino-guanidine or other nitric oxide synthase inhibitors.
- WO 9834955 Hemoglobin(s) modified with S-nitroso groups, and related compounds; used in treatment of ischemic injury, hypertension, angina, reperfusion injury, or inflammation.

Recent patent applications in nitric oxide research II

- WO 9834626 Compounds for reducing ischemia/reperfusion injury, using a combination of an inhibitor of iNOS induction, a nitric oxide donor, and an endopeptidase inhibitor.
- WO 9833519 Nucleic acid encoding arginine deiminase of *Mycoplasma arthritidis*; useful for treating tumors, cancers, and nitric oxide-related conditions.
- WO 9830537 New (imino-ethyl-amino)ethyl-homocysteine inhibits nitric oxide synthase; used to treat arthritis, asthma, ileus and migraine.
- WO 9830220 Prevention of opioid tolerance, particularly to morphine, in the clinical management of moderate to severe pain, by administering an inducible nitric oxide inhibitor.
- WO 9827108 New amide compounds for treatment of nitric oxide-mediated diseases such as cardiovascular ischemia, diabetes, cerebral infarction, and asthma.
- FR 2757864 Antibodies specific for nitrosylated protein(s); used to treat, prevent, or diagnose disorders involving nitric oxide, e.g., infection, septic shock, cancer, autoimmune disease, etc.

NO resources for you

- Irony in Alfred Nobel's world
- http://www.nobel.se/medicine/laureates/1998/press.html
- great structural resource/biblio http://metallo.scripps.edu/PROMISE/NOS.html



The winners: Robert Furchgott (left), Louis Ignarro and Ferid Murad.

Nobel for NO 1998

- " Nitric oxide (NO) is a gas that transmits signals in the organism. Signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell represents an entirely new principle for signalling in biological systems. The discoverers of NO as a signal molecule are awarded this year's Nobel Prize. "