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iNOS: An Effector in Immune Response

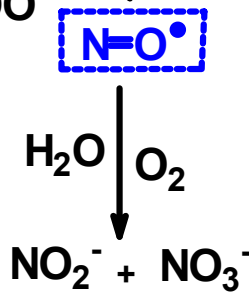
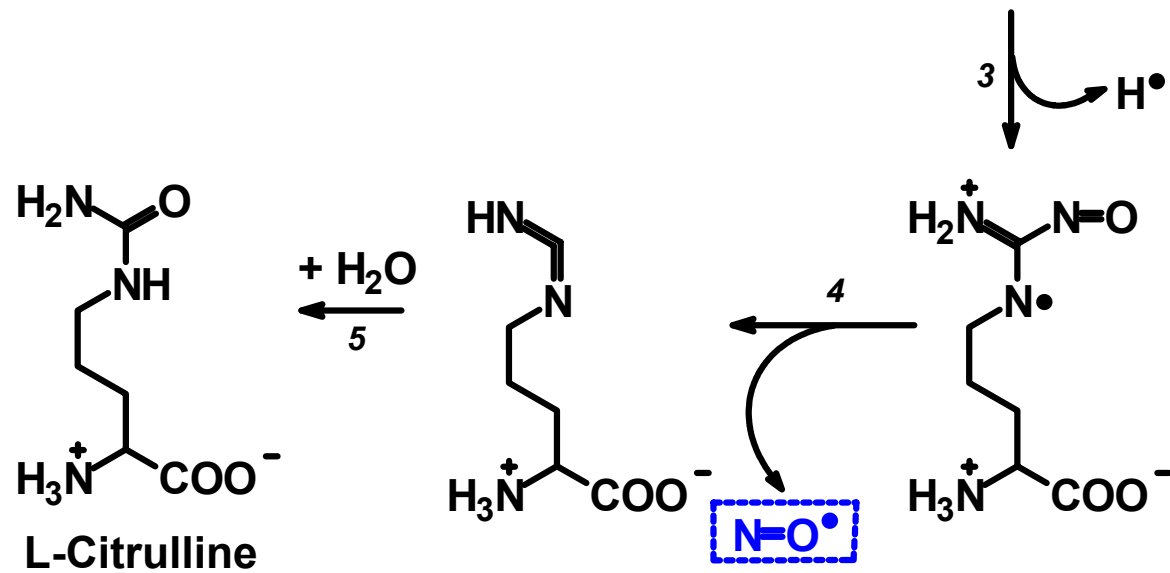
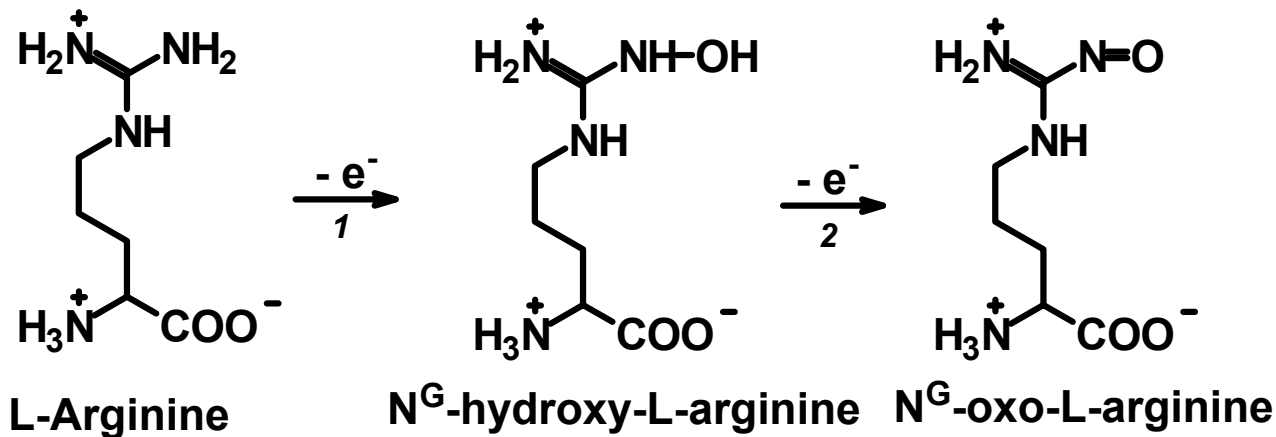
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What is NOS?

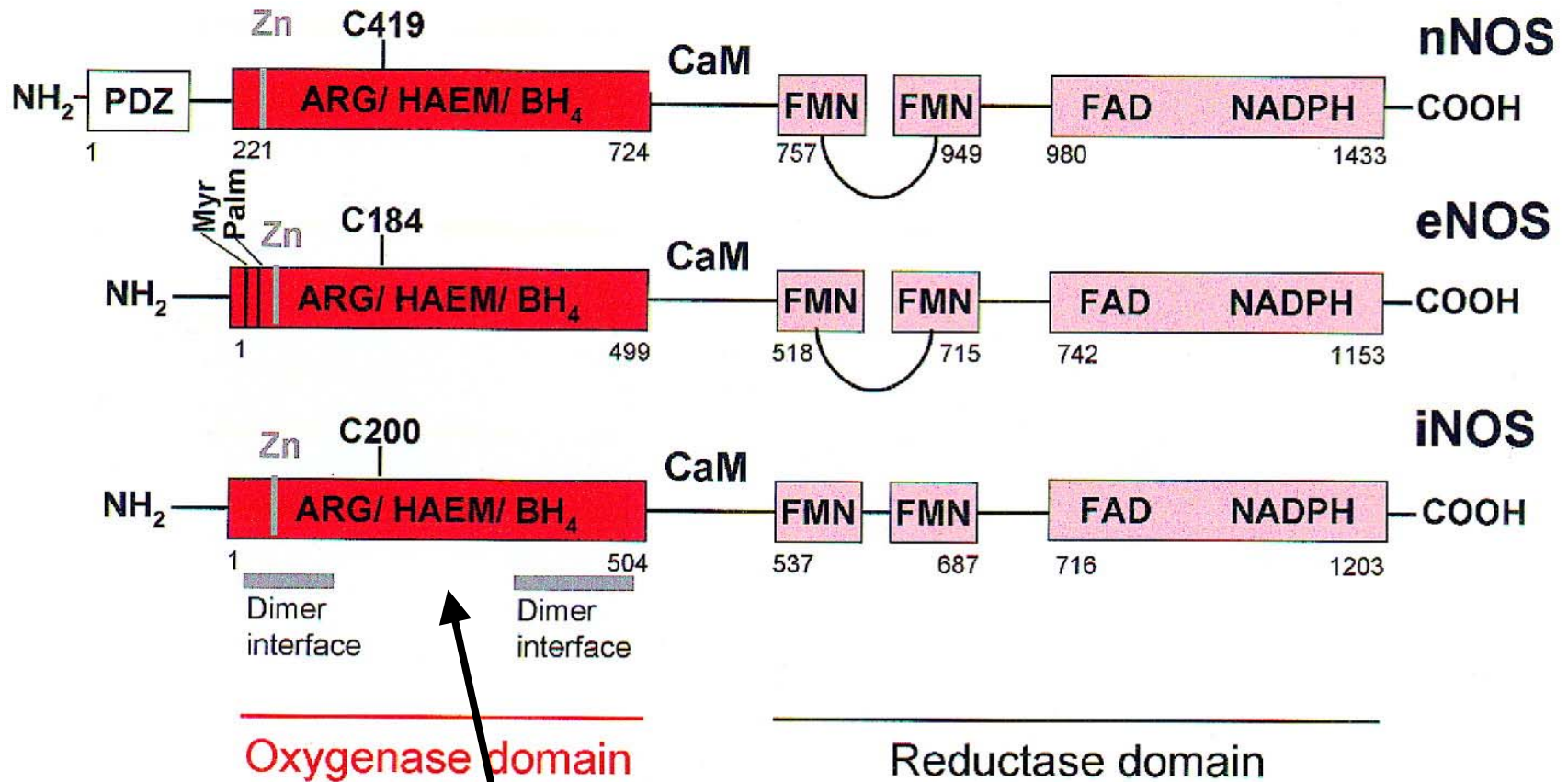
- Nitric oxide synthase (NOS) is an enzyme that facilitates the 5-electron oxidation of L-arginine to L-citrulline
- Three known isoforms:
 - Endothelial NOS (eNOS)
 - Inducible NOS (iNOS)
 - Neuronal NOS (nNOS)
- eNOS and nNOS are constitutively active
- iNOS is inducible



NOS
production of
NO[•]

Domain Structures of NOS isoforms

(Alderton *et al.* (2001))



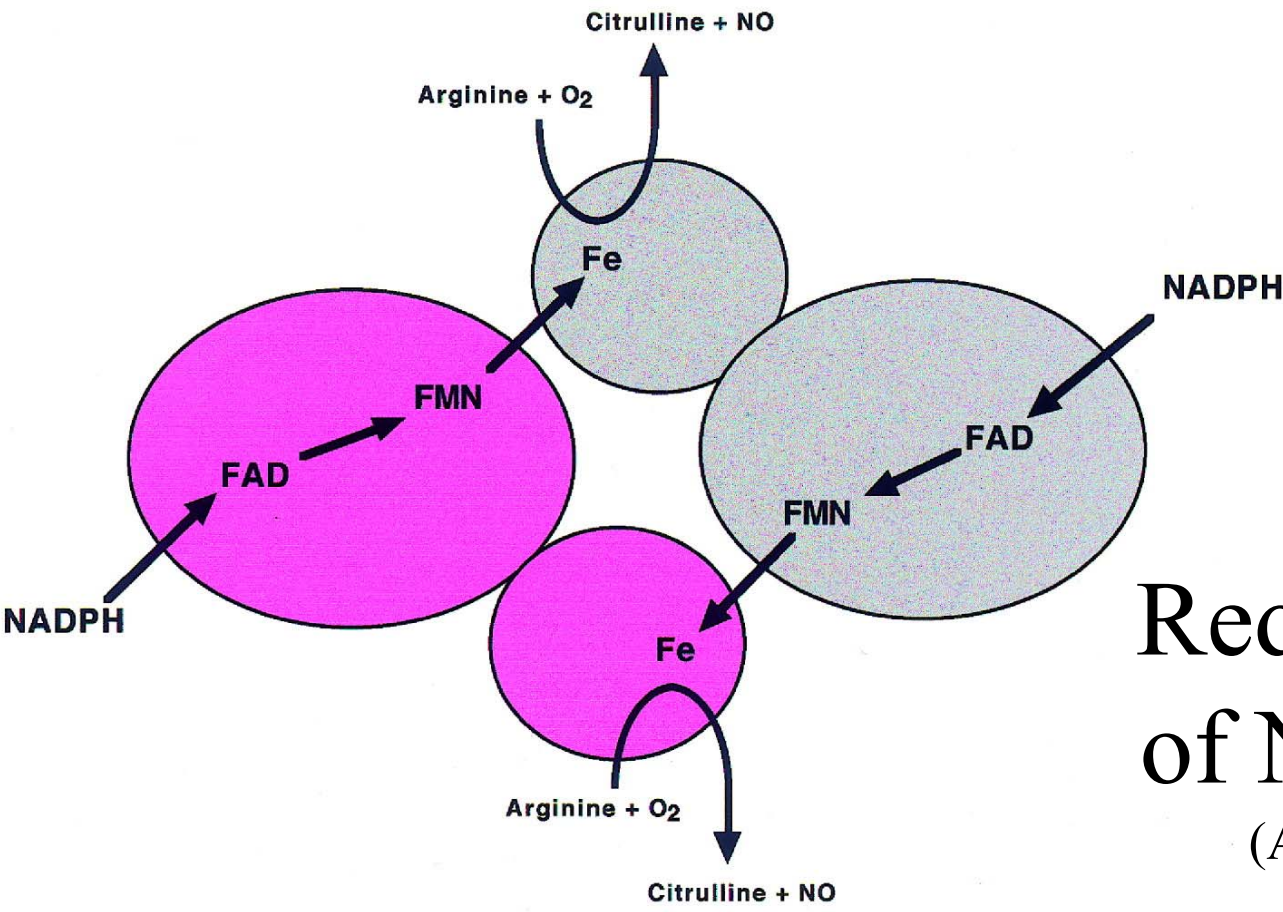
Forms a novel α - β fold described as a “catcher’s mitt” with the heme group in the palm

Heme Importance in iNOS -- Background

- Located in the oxygenase domain
- Iron is penta-coordinate, axial co-ordination to the proximal Cys²⁰⁰
- Buried in the protein's interior
- Makes extensive van der Waals interactions with hydrophobic and aliphatic side chains

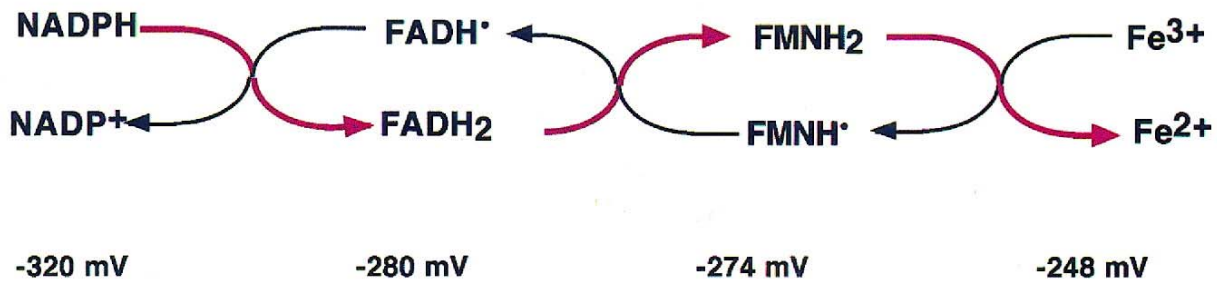
Heme Importance in iNOS – Background

- Lowest reduction potential of the “NOS pathway” – making it the final electron acceptor
- The heme group then gets oxidized as it passes electrons to L-arginine in order to make L-citrulline and NO•



Redox Potentials of NOS pathway

(Alderton *et al.* (2001))



iNOS

Hummel, S.G.

iNOS and Immune Response – 1

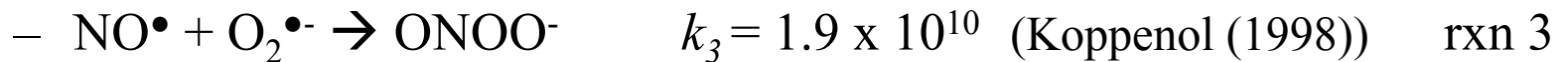
- iNOS produces very large, toxic amounts of NO^\bullet in a sustained manner
 - NO^\bullet levels surpass normal physiological concentrations (Xie *et al.* (1994))
- eNOS and nNOS produce NO^\bullet within seconds and their activities are short acting (Guzik *et al.* (2003))

iNOS and Immune Response – 2

- NO• kills microorganisms and nitrosylates macromolecules (Guzik *et al.* (2003))
- Toxic properties play a role in the pathogenesis of septic shock (Parratt (1997))
- Within a few seconds of formation, NO• is oxidized to nitrites and nitrates; reaction catalyzed by oxy-hemoglobin or oxy-myoglobin
 - $\text{NO}\bullet + \text{O}_2 \rightarrow \text{NO}_3^-$ rxn 1
 - $2\text{NO}\bullet + \text{O}_2 + 4\text{H}_2\text{O} \rightarrow 4\text{NO}_2^- + 4\text{H}^+$ rxn 2

iNOS and Immune Response – 3

- NO• from myeloid cells is usually generated in conjunction with superoxide (O₂•⁻) to form peroxynitrite (ONOO⁻) (Channon *et al.* (2002)) (Guzik *et al.* (2002))



- ONOO⁻ can mediate cytotoxic effects such as DNA damage, LDL oxidation, isoprostane formation, tyrosine nitration, inhibition of aconitase, and mitochondrial respiration ((Ischiropoulos and al-Mehdi. (1995))

NO• is a potent immunoregulator

- NO• role is still somewhat ambiguous
- Believed to inhibit expression of cellular proliferation and growth genes (Kroncke *et al.* (2001))
 - For example, NO• inhibits Ig-E mediated secretory functions of mast cells including histamine release, which is a hallmark of allergic inflammation (Eastmond *et al.* (1997))

Summary of iNOS Biology – 1

- iNOS is one of three NOS isoforms and produces high concentrations of NO•
- The haem unit of NOS plays a central role in the oxidation of L-arginine to L-citrulline *via* its redox potential
- NO• production from iNOS is crucial in the immune response
 - high concentrations of NO• allow for increased NO• reactivity and toxicity

Summary of iNOS Biology – 2

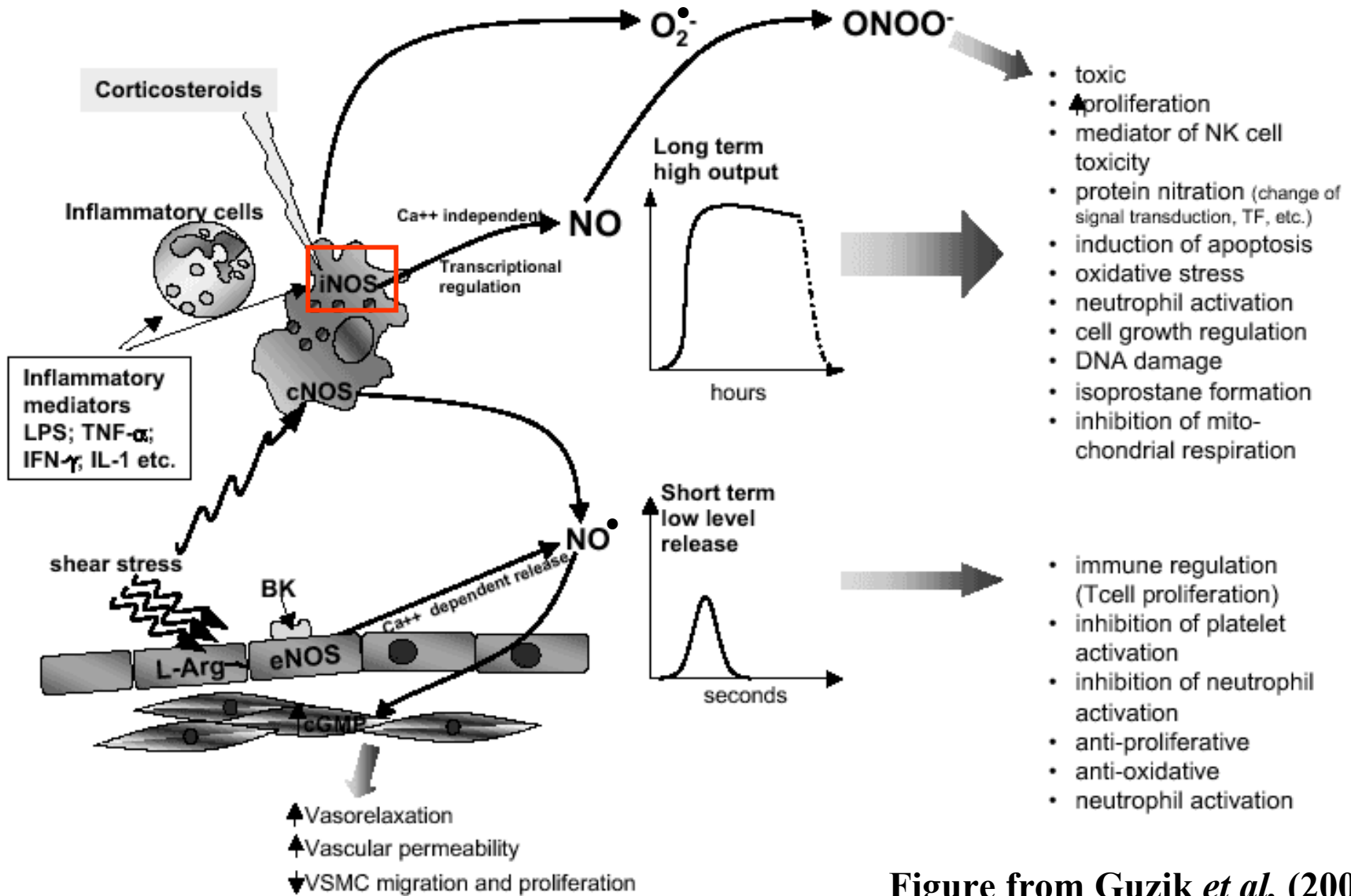


Figure from Guzik *et al.* (2003)

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