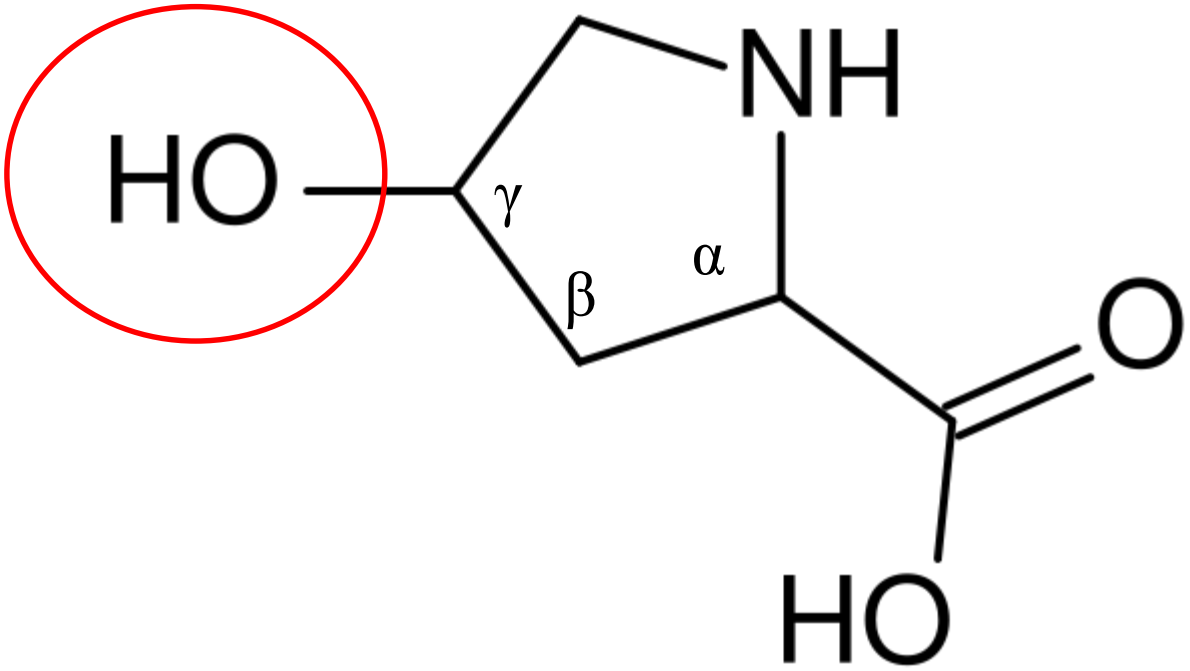
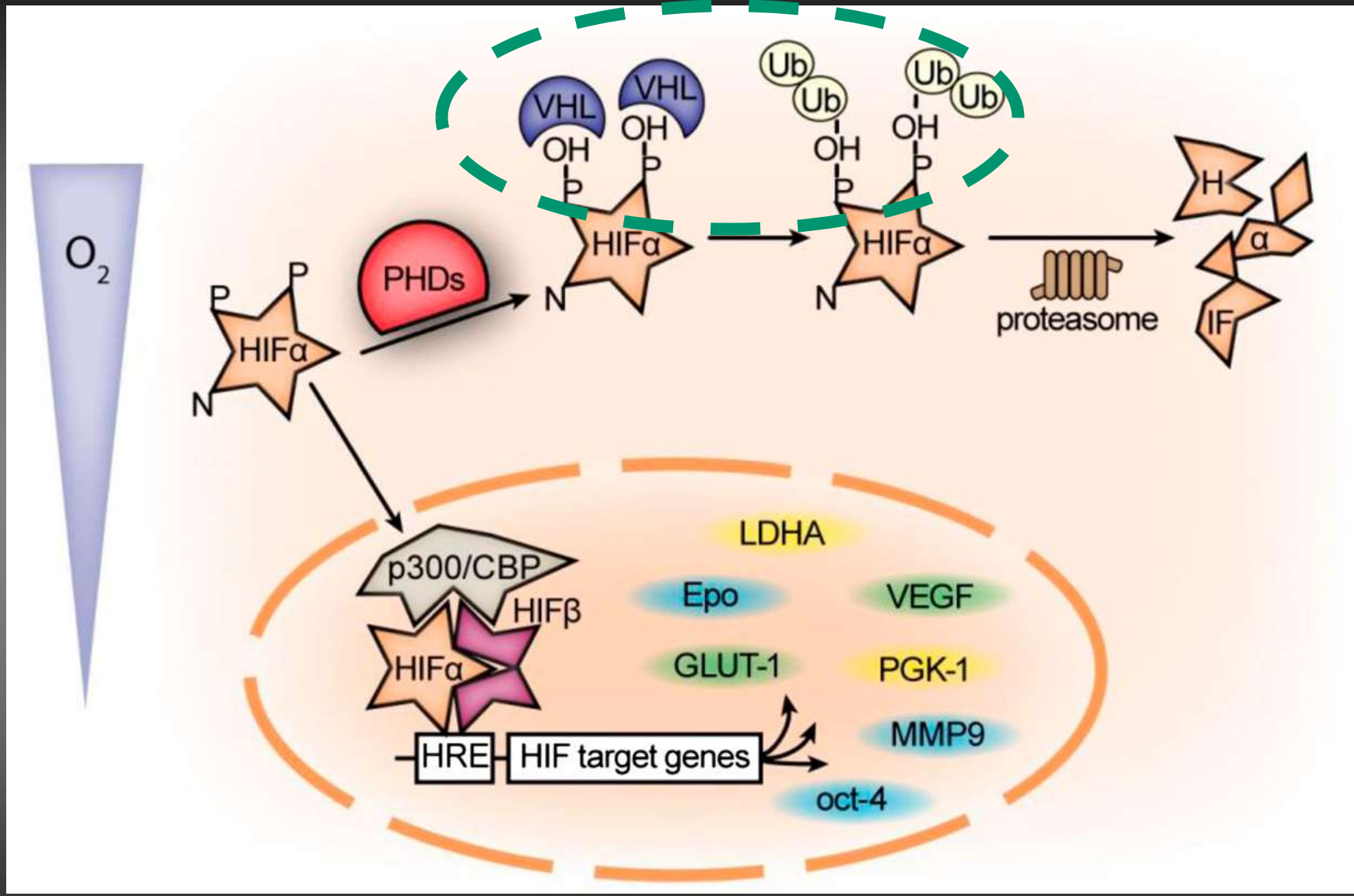
O2 sensing

Regolazione di HIF ?

Idrossiprolina



4-hydroxypyrrolidine-2-carboxylic acid


O2
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
H
α
IF
proteasome
PHDs
P
P
HIFα
N
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

- The hypoxia-sensing mechanism involves oxygen limited hydroxylation of prolyl residues in the N- and C-terminal oxygen-dependent degradation domains (NODD and CODD) of HIFα isoforms,

catalysed by prolyl hydroxylases (PHD 1-3)

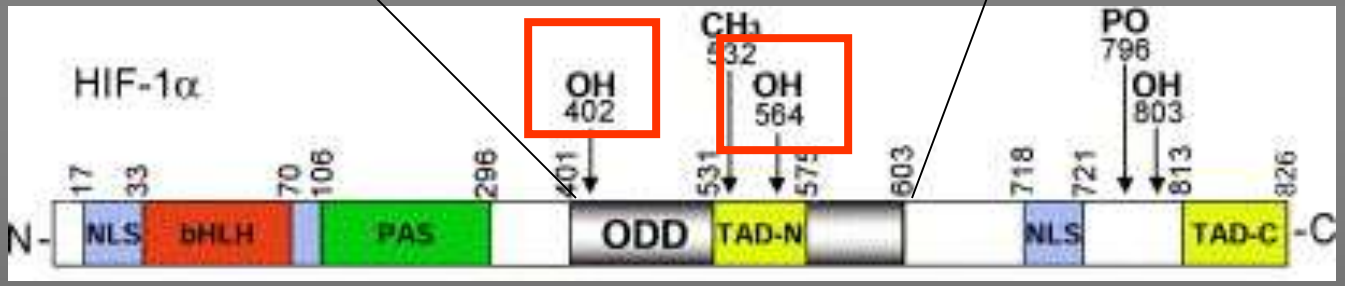
Struttura di HIF1

α

Prolina-Idrossilasi

Pro402—OH
α
Pro564—OH

Sequenza di idrossilazione

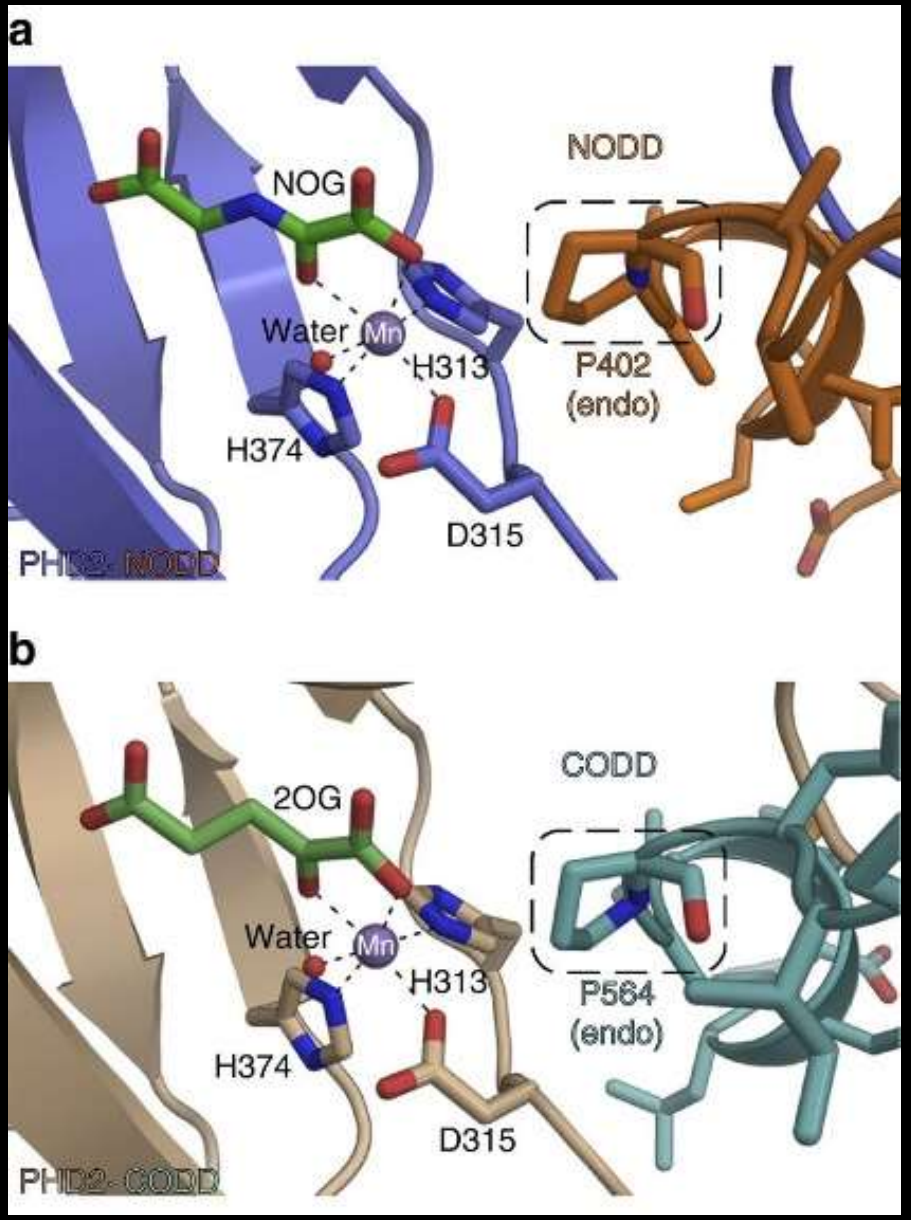


Conserved binding modes of the Pro402NODD/Pro564CODD to PHD2

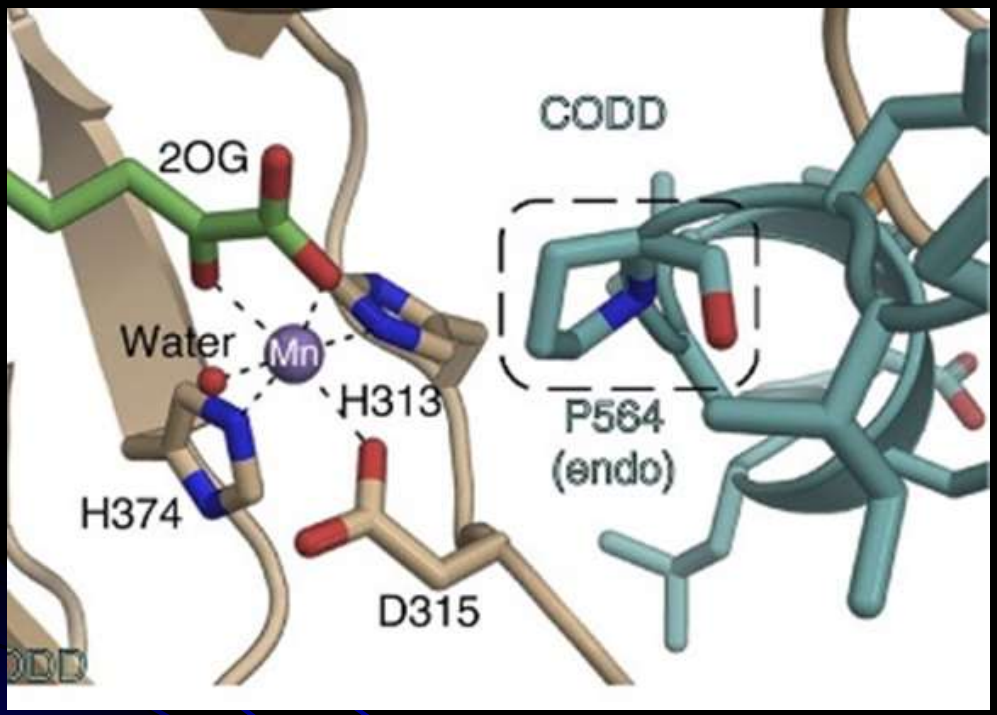
NODD
P402

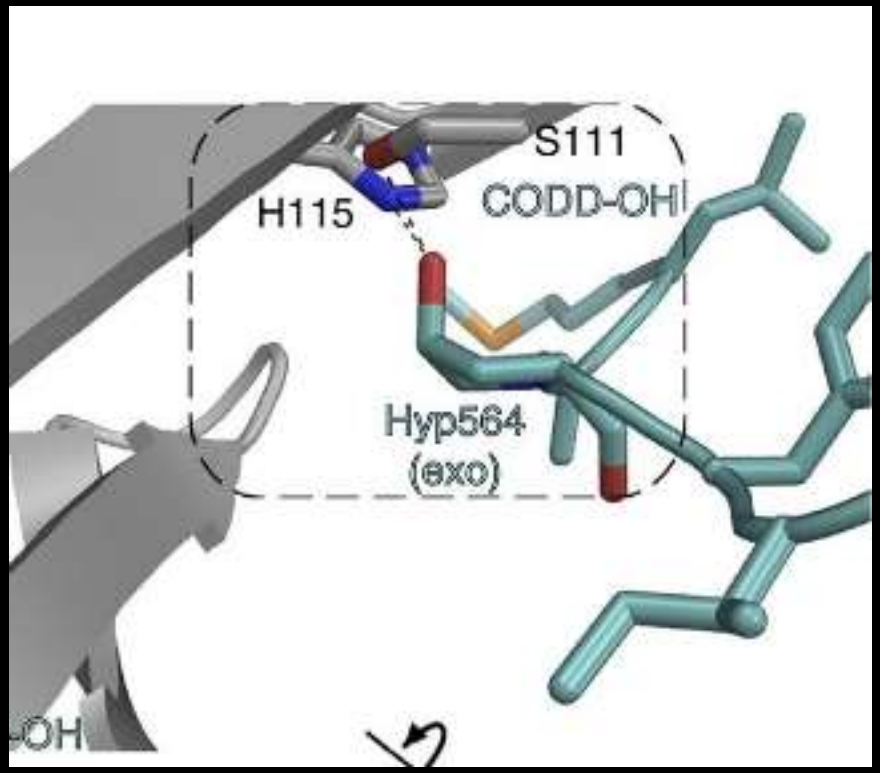
Lo stesso sito catalitico di PHD2 lega similmente NODD e CODD

CODD
P564



Binding of proline (P) hydroxyproline/Hyp (CODD) to PHD VHL





Ruolo chiave istidine

H313 H115

Le prolil idrossilasi (PHD) sono finemente regolate

Concentrations of O2 in tissues
- range 10–30 μM-

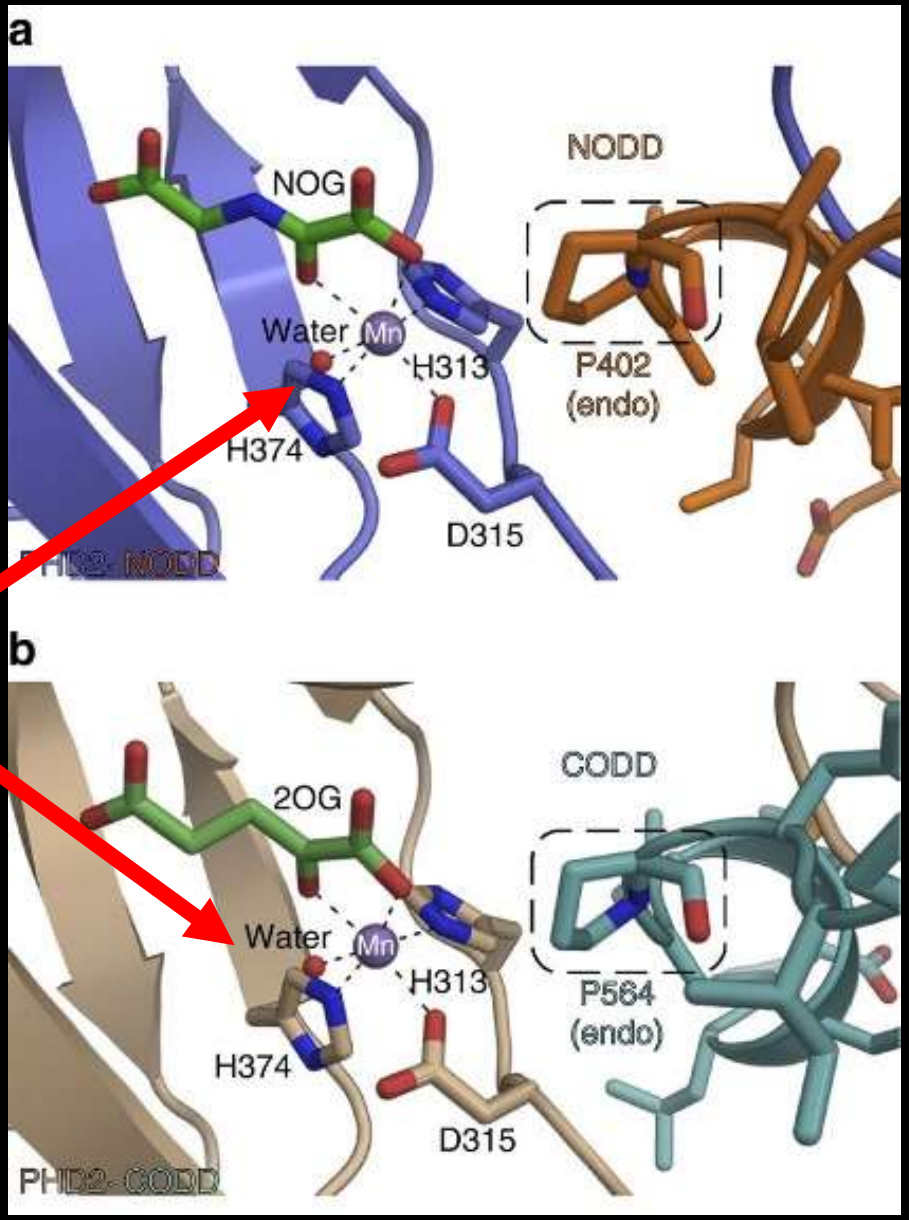
- below the Km for O2 of the PHD

Concentrations of oxygen is limiting for enzyme activity over the entire physiological range

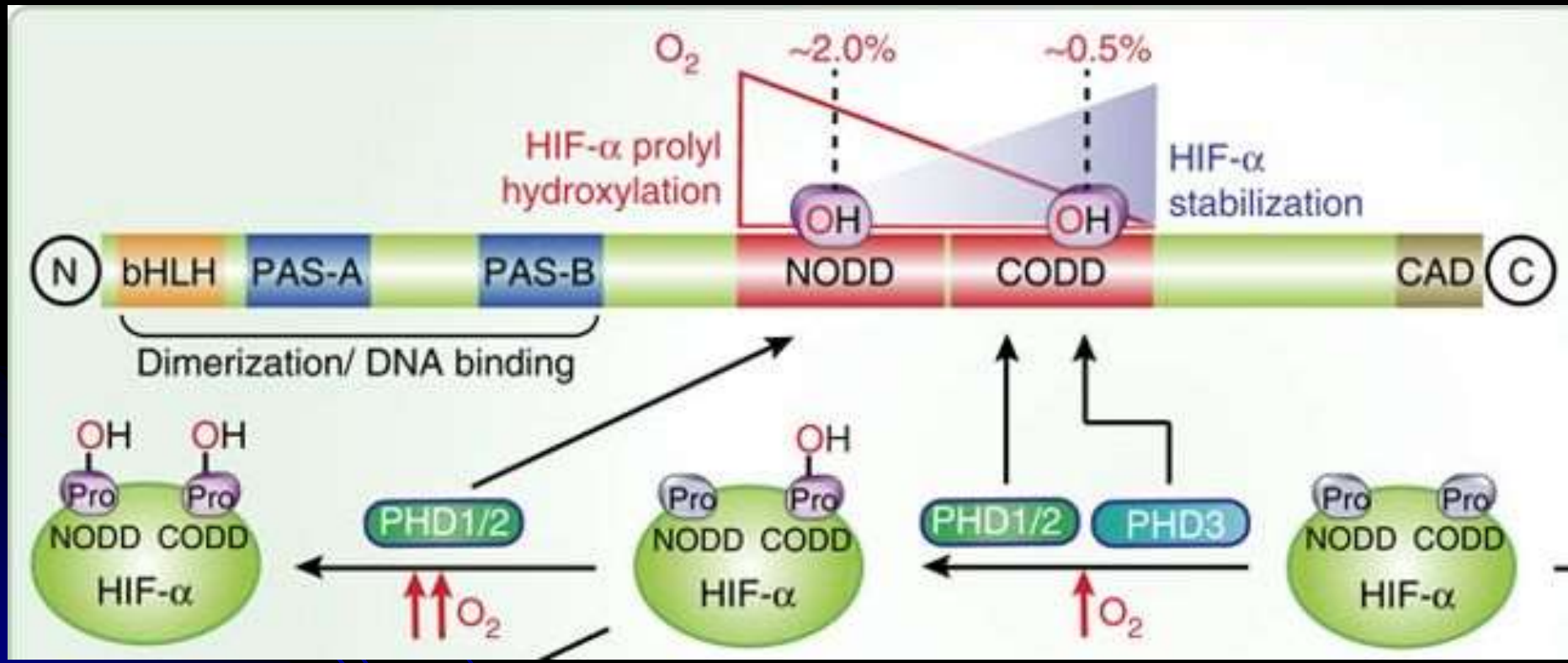
La Km è quella concentrazione di substrato a cui la V. 0 è pari a metà della Vmax

Binding of O2 is proposed to be limiting in PHD-ODD catalysis

The metal (Mn) bound water replaced by O2 in catalysis



NODD hydroxylation is more sensitive (2% 02) than CODD (0.5% 02) to hypoxia



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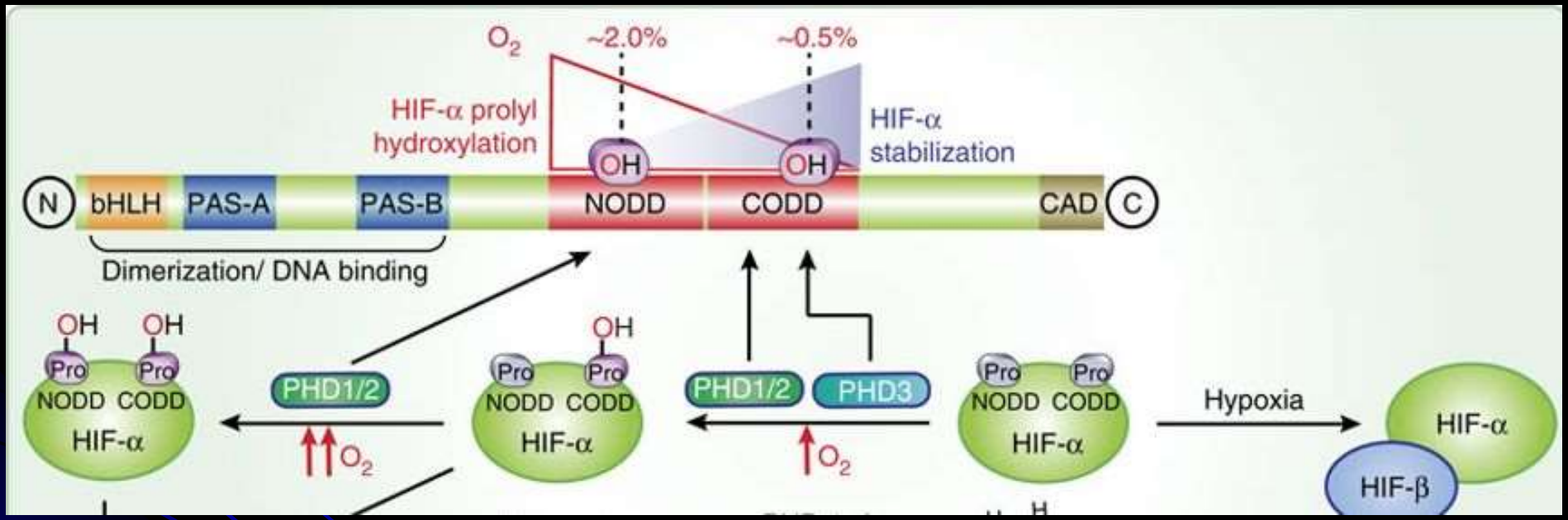
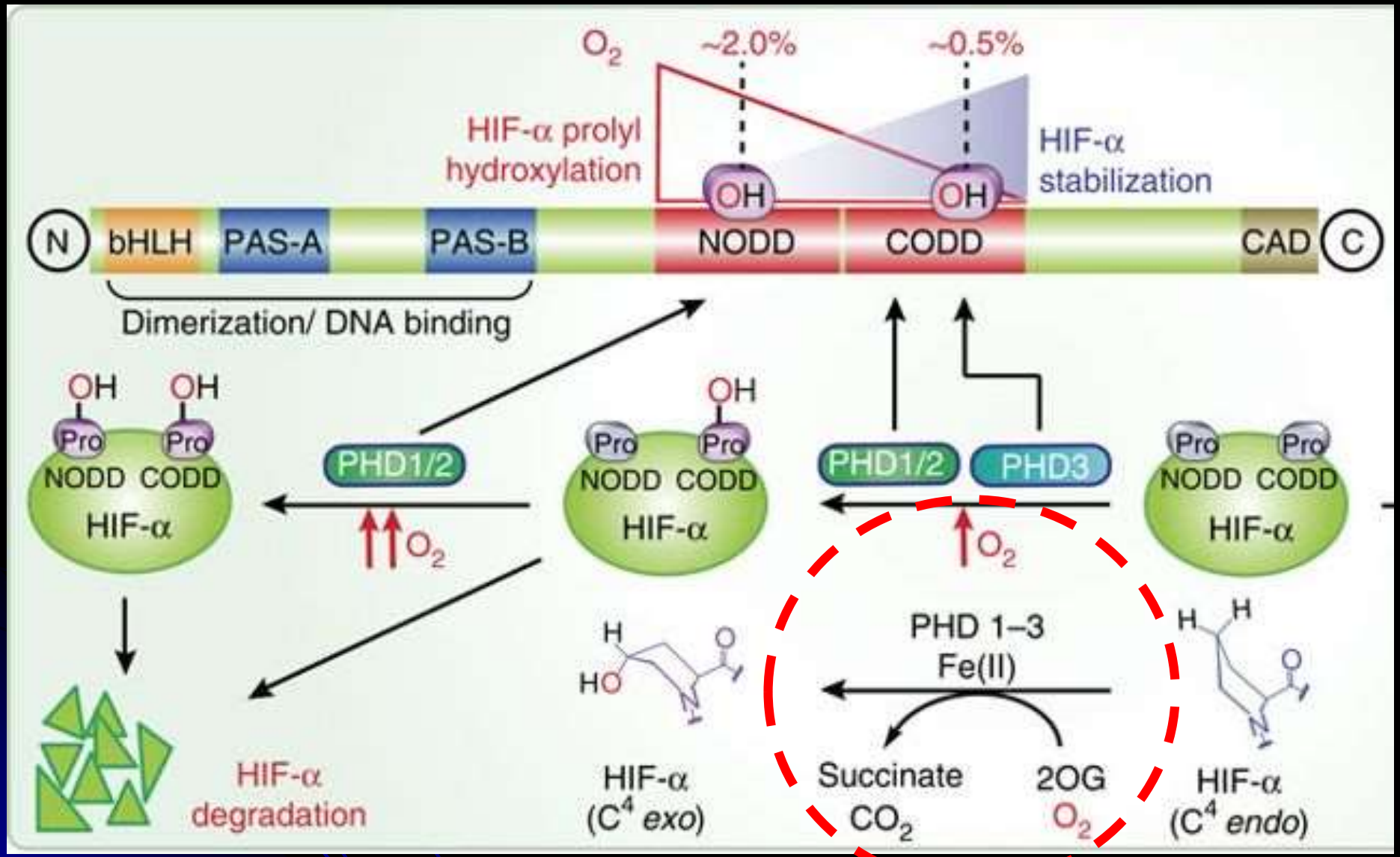
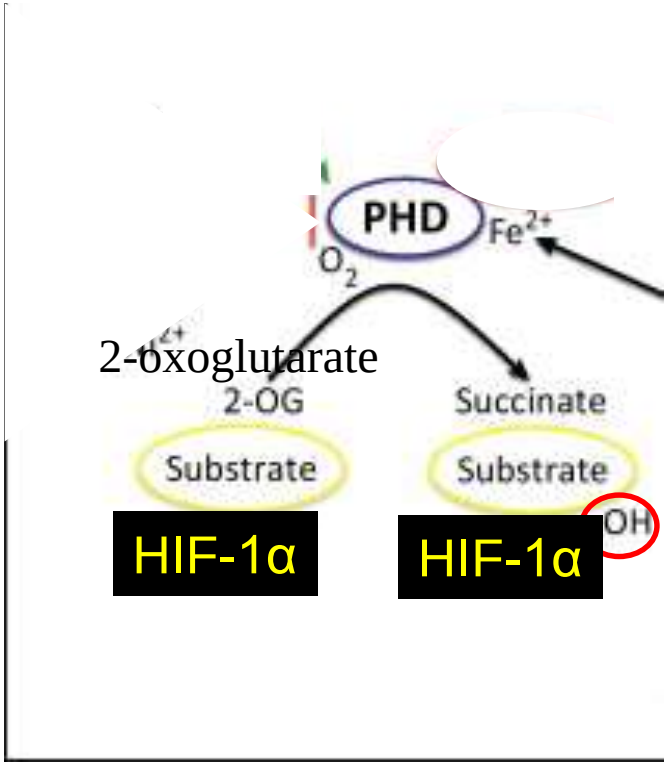


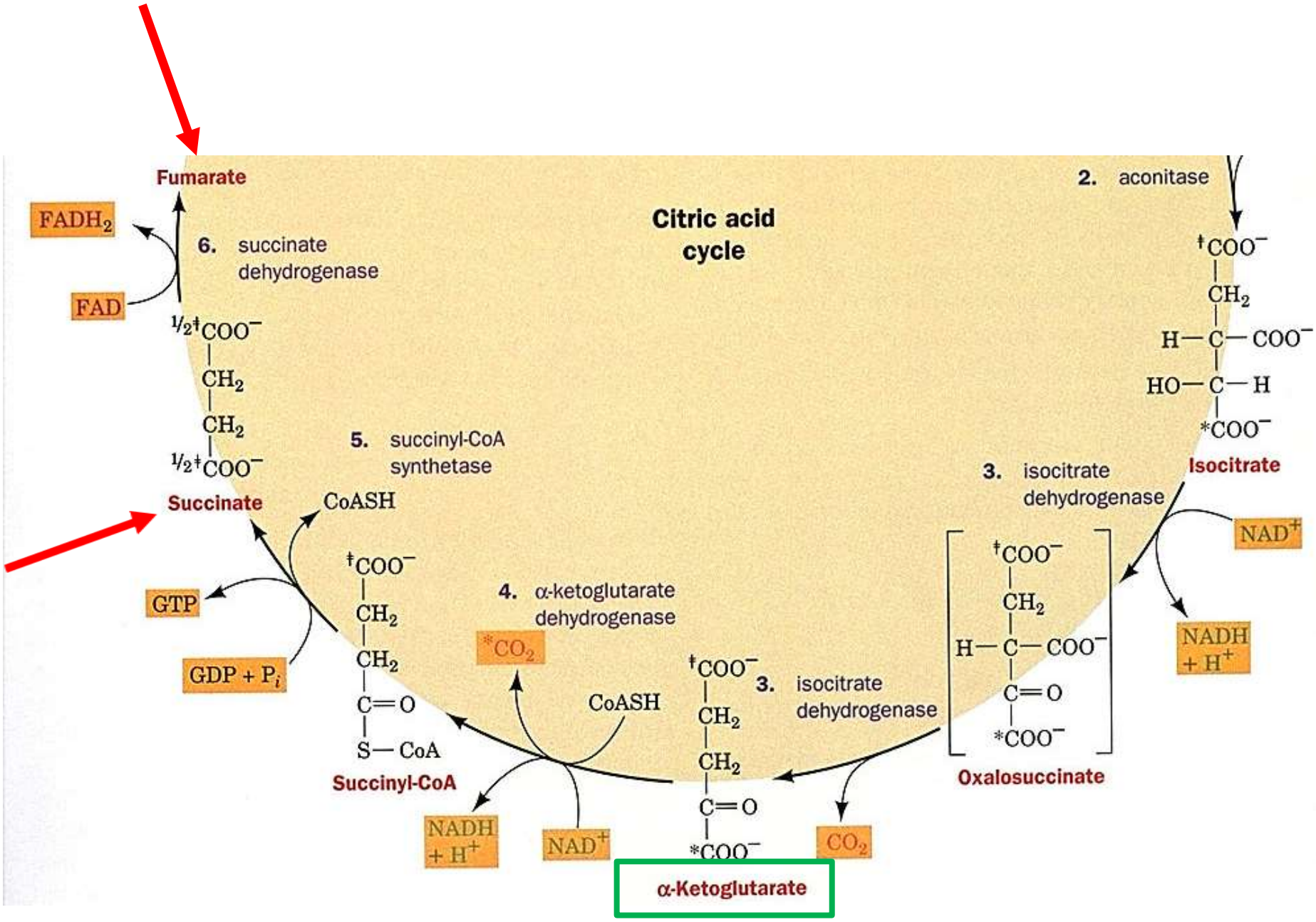

O2
~2.0%
~0.5%
HIF-α prolyl hydroxylation
HIF-α stabilization
OH
OH
N
bHLH
PAS-A
PAS-B
NODD
CODD
CAD
C
Dimerization/ DNA binding
OH
OH
Pro
Pro
NODD CODD
HIF-α
PHD1/2
O2
OH
Pro
Pro
NODD CODD
HIF-α
PHD1/2
PHD3
O2
Pro
Pro
NODD CODD
HIF-α
HIF-α degradation
H
HO
HIF-α
(C4 exo)
PHD 1–3
Fe(II)
Succinate
CO2
2OG
O2
H
H
HIF-α
(C4 endo)

Prolyl hydroxylase domain enzyme (PHD) activity

PHDs are α-ketoglutarate/2-oxoglutarate (2-OG)-dependent hydroxylase and the cofactors oxygen and iron to hydroxylate substrates



g **Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes** Brian W. Won


Citric acid cycle
Fumarate
FADH2
FAD
6. succinate dehydrogenase
Succinate
2. aconitase
Isocitrate
3. isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
5. succinyl-CoA synthetase
CoASH
GTP
GDP + Pi
Succinyl-CoA
4. α-ketoglutarate dehydrogenase
*CO2
CoASH
3. isocitrate dehydrogenase
NADH + H+
NAD+
CO2
α-Ketoglutarate

Prolyl hydroxylase domain enzyme (PHD) activity

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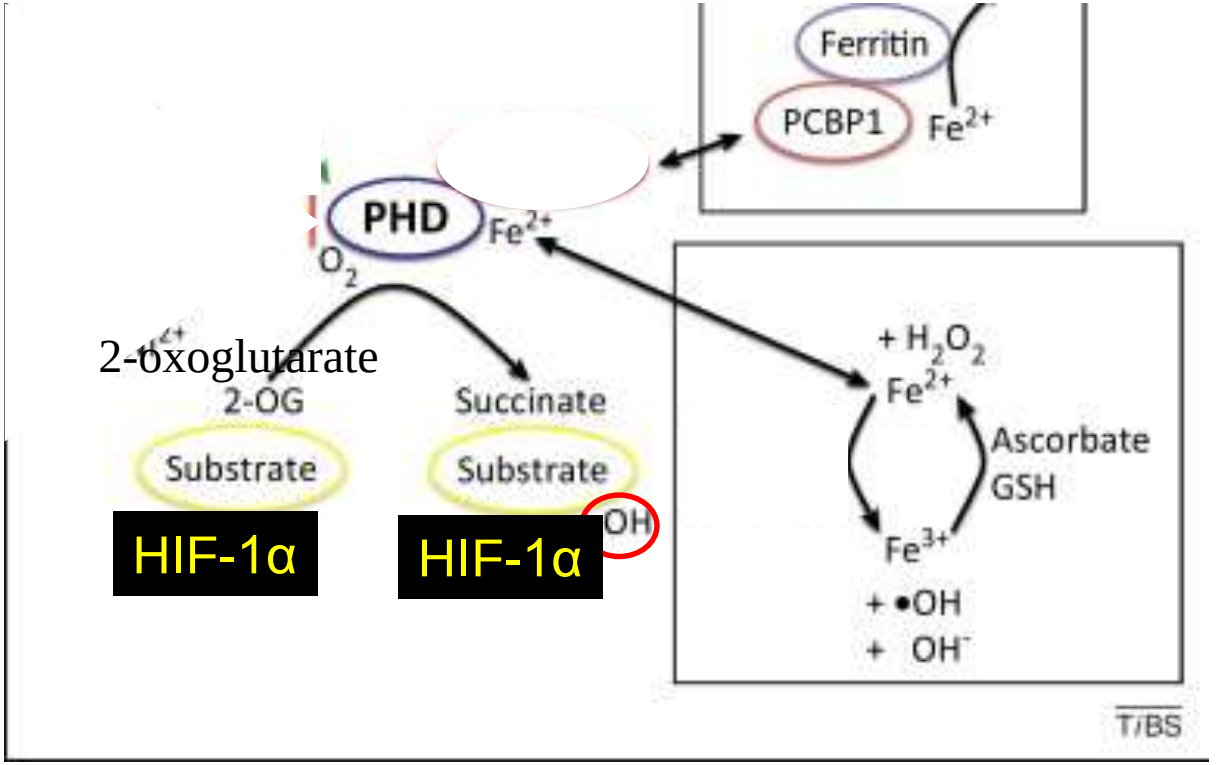


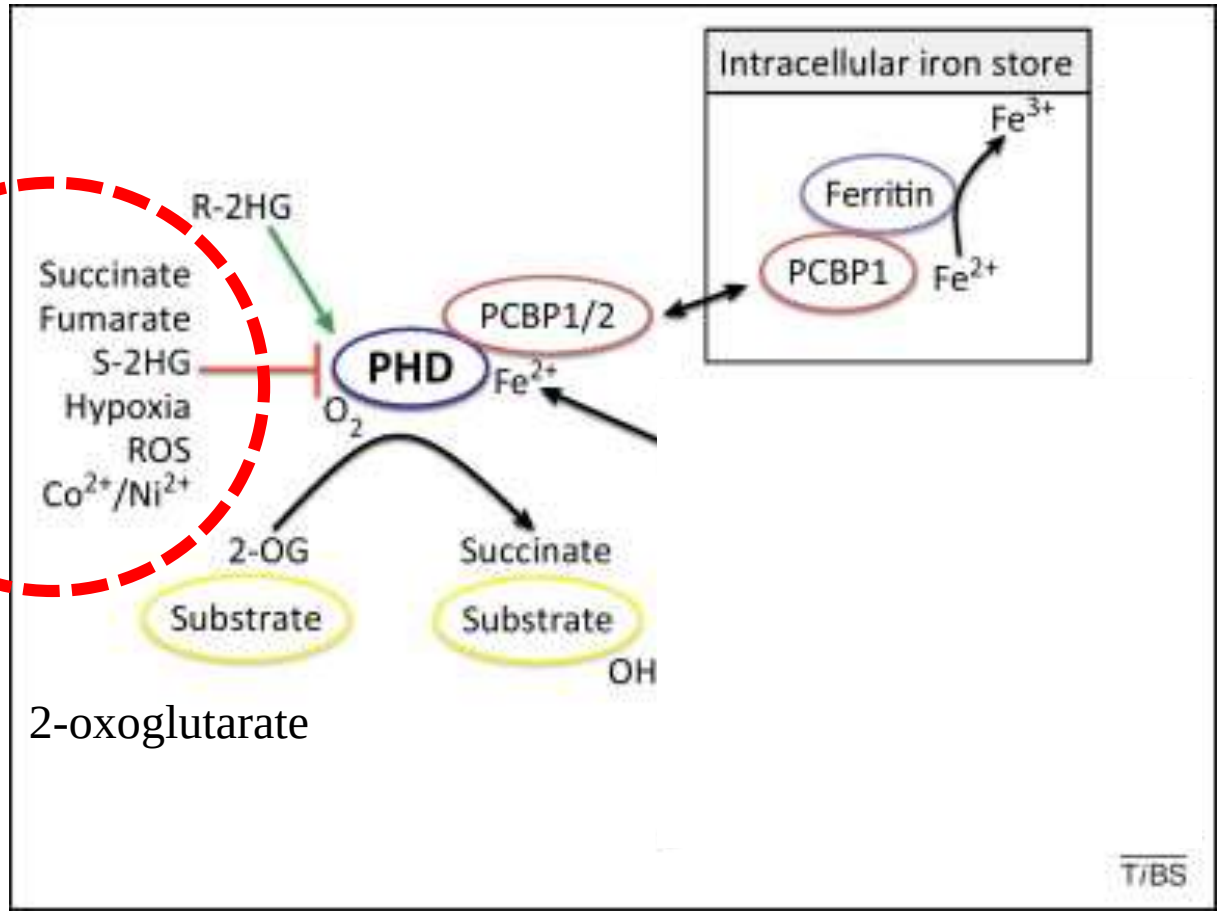
Fe2+, which binds the proline substrate and the oxygen molecule, undergoes oxidation

Ascorbate /glutathione maintains iron in the active site of PHDs in the reduced (ferrous) state

PCBP1 delivers Fe2+ to PHDs and iron to ferritin for intracellular iron storage

g **Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes** Brian W. Won

Regulation of prolyl hydroxylase domain enzyme (PHD) activity **INHIBITORS**

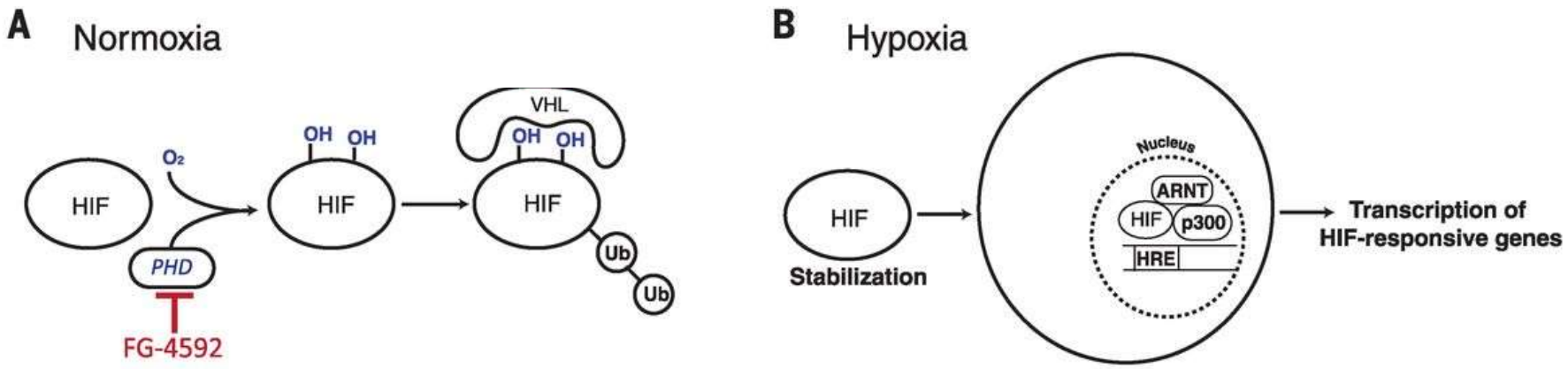


reactive oxygen species (ROS) can disrupt oxygen interaction with PHDs.

g **Emerging novel functions of the oxygen-sensing prolyl hydroxylase domain enzymes** Brian W. Won

INIBITORI FARMACOLOGICI DI PHD

activation of the HIF response by PHD inhibition



FG-4592 acts as competitive antagonists of 2-oxoglutarate, a cofactor that accepts one oxygen from molecular dioxygen to become succinate as the second oxygen forms trans-4-hydroxyproline

Published by AAAS

Isha H. Jain et al. Science 2016;352:54-61

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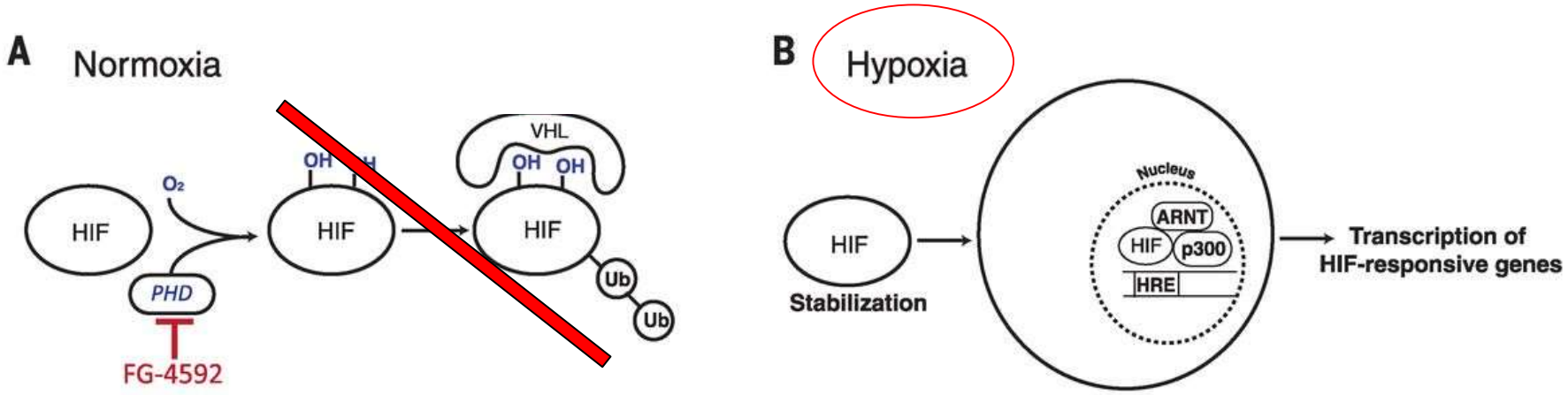

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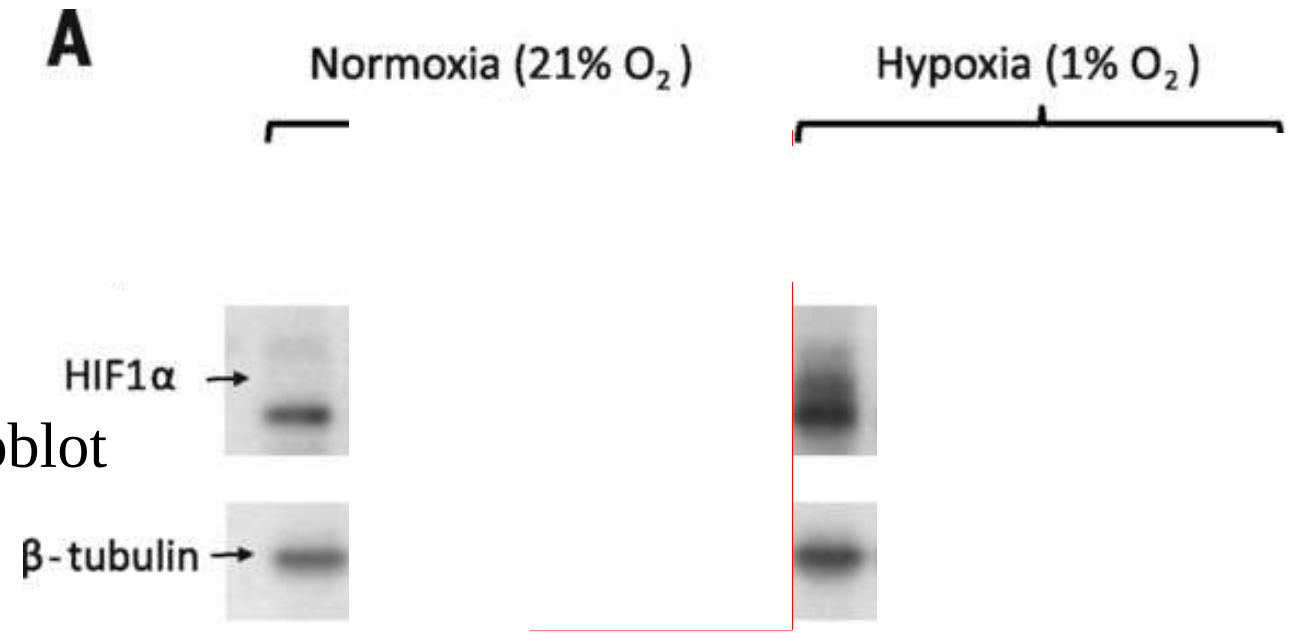
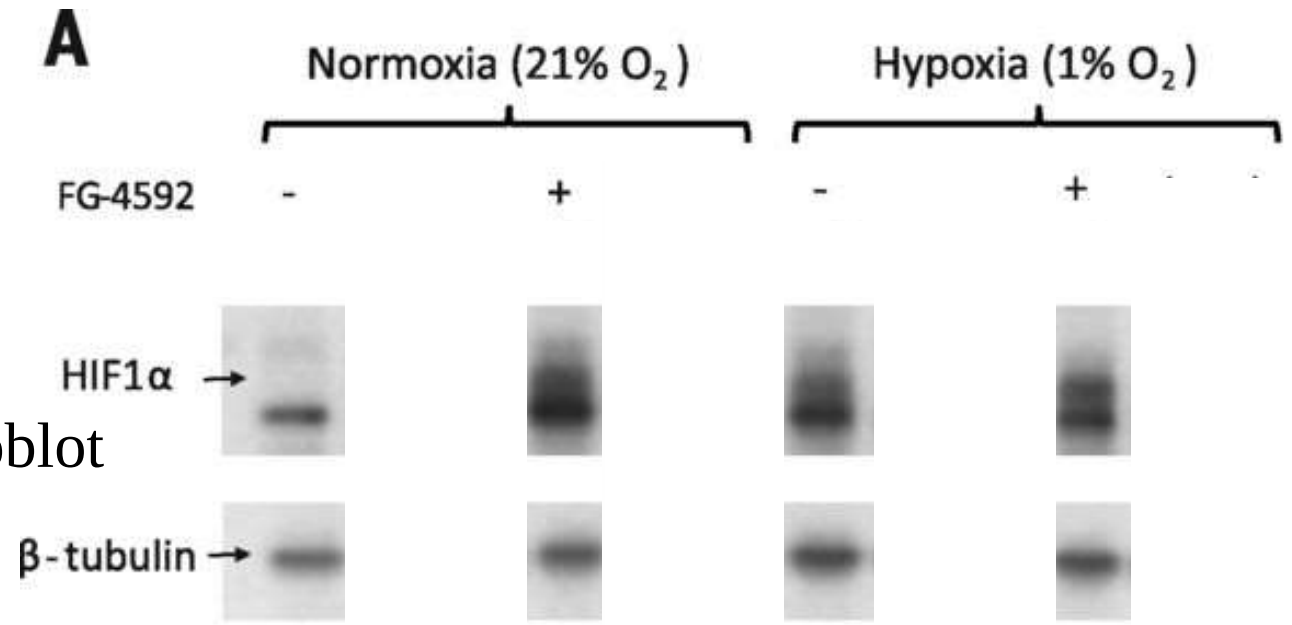


Immunoblot

Isha H. Jain et al. Science 2016;352:54-61

Fig. 3 FG-4592 causes normoxic stabilization of HIF1α and rewires energy metabolism.

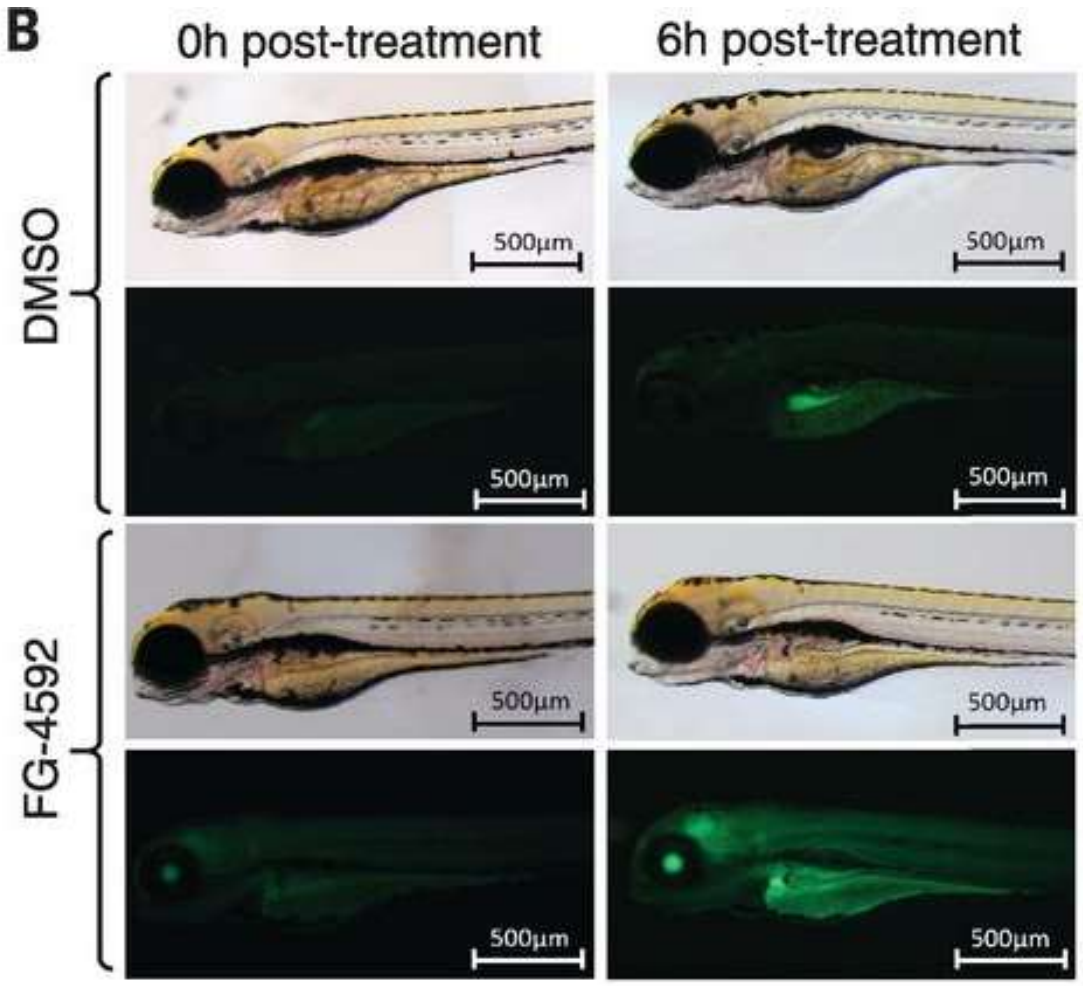


Immunoblot

± FG-4592 under normoxia (21% O2) or hypoxia (1% O2)
FG-4592 administration stabilizes HIF1α even during normoxia.

Isha H. Jain et al. Science 2016;352:54-61

FG-4592 treatment activates the HIF response in zebrafish embryos



HIF-responsive promoter -EGFP embryos

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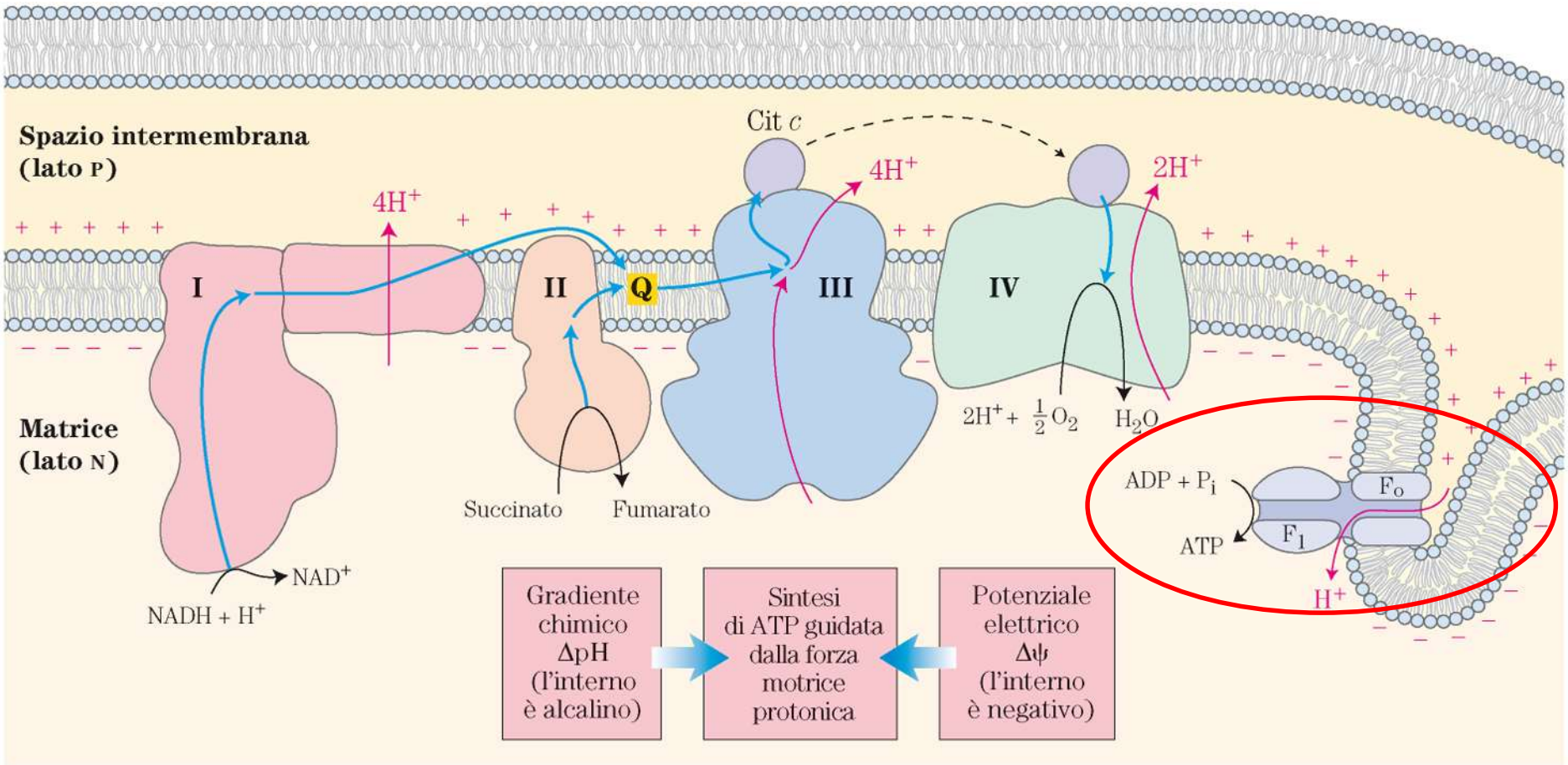
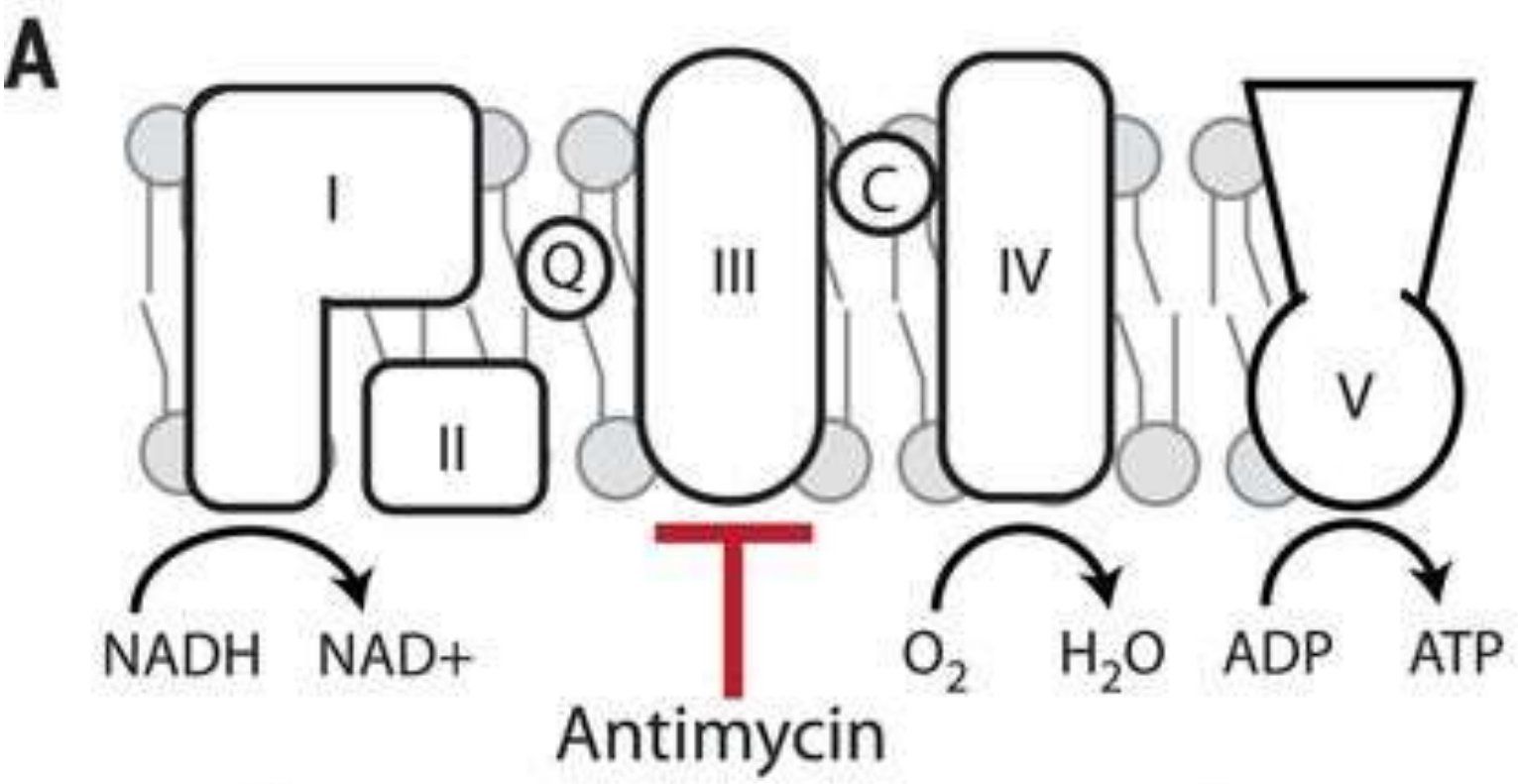
FG-4592 treatment activates the HIF response in zebrafish embryos and alleviates death caused by Respiratoty Chain inhibition.

Isha H. Jain et al. Science 2016;352:54-61

La fosforilazione ossidativa nel mitocondrio



Respiratoty Chain inhibition



Isha H. Jain et al. Science 2016;352:54-61

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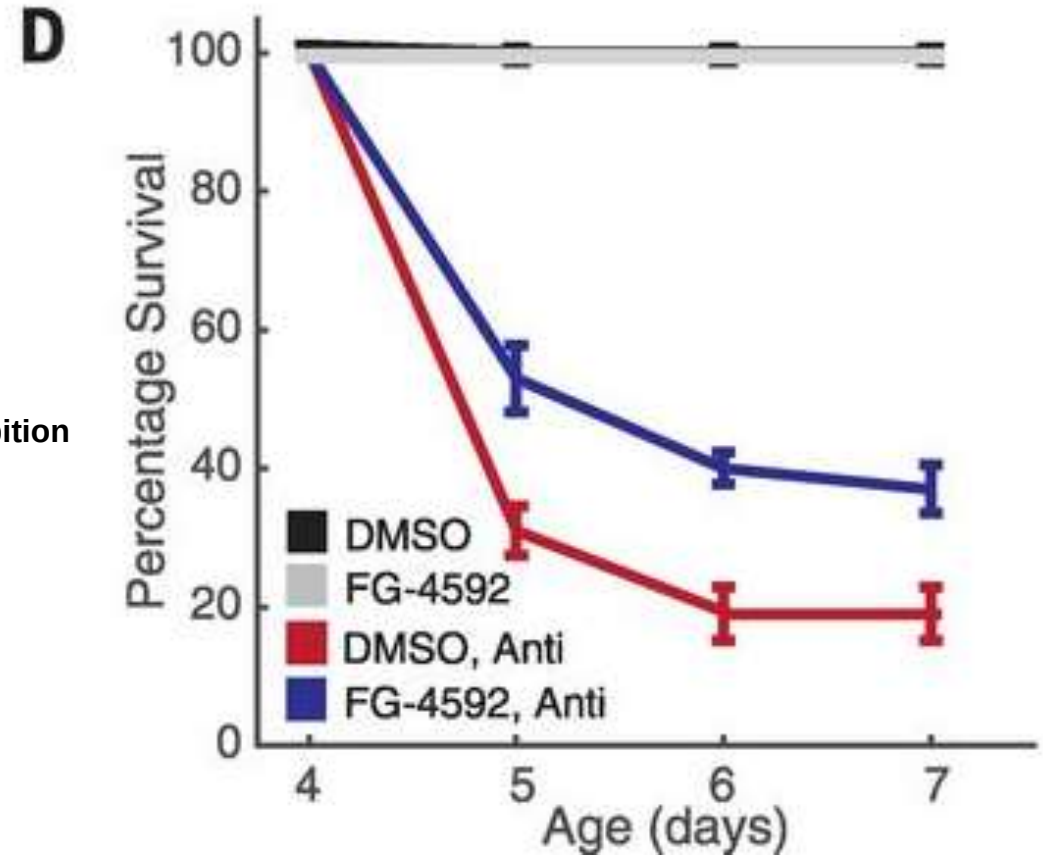
Antimycin=Anti
Respiratoty Chain inhibition



RC inhibition by 2.5 nM antimycin in 4 days post fertilization (dpf) embryos results in significant death within the first 24 hours of treatment

Coexposure of antimycin with FG-4592 (2.5 μM) doubles embryo survival, whereas FG-4592 alone has no impact.

Exposure to FG-4592 rescues antimycin-induced zebrafish embryonic death.

Retinopathy of prematurity (ROP) causes 100,000 new cases of childhood blindness each year.
ROP is initiated by oxygen supplementation necessary to prevent neonatal death.

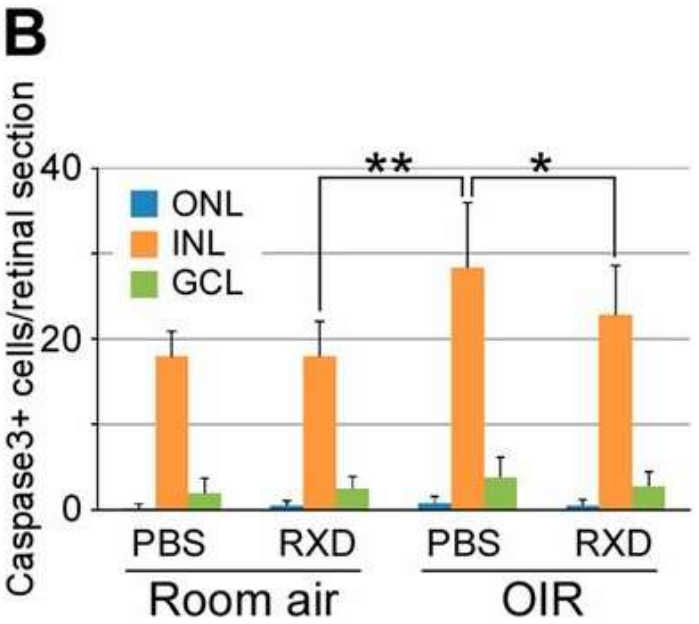
hypoxia-inducible factor (HIF) stabilization via HIF prolyl hydroxylase inhibition

retinal HIF stabilization - Retinopathy

hypoxia-inducible factor (HIF) stabilization via HIF prolyl hydroxylase **(PHD) inhibition** using the isoquinolone **Roxadustat**

Effect of Roxadustat on neural retina apoptosis.

outer nuclear layer (ONL),
inner nuclear layer (INL)
ganglion cell layer (GCL)



Quantification of active caspase 3-positive cells demonstrates statistically significant reduction in apoptosis in the inner nuclear layer of animals treated with Roxadustat (RXD) FG-4592

George Hoppe et al. PNAS 2016;113:E2516-E2525

hypoxia-inducible factor (HIF) stabilization via HIF prolyl hydroxylase **(PHD) inhibition** using the isoquinolone Roxadustat

This provides a rationale for protecting the severely premature infant from oxygen toxicity.