Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

Materiale didattico di supporto

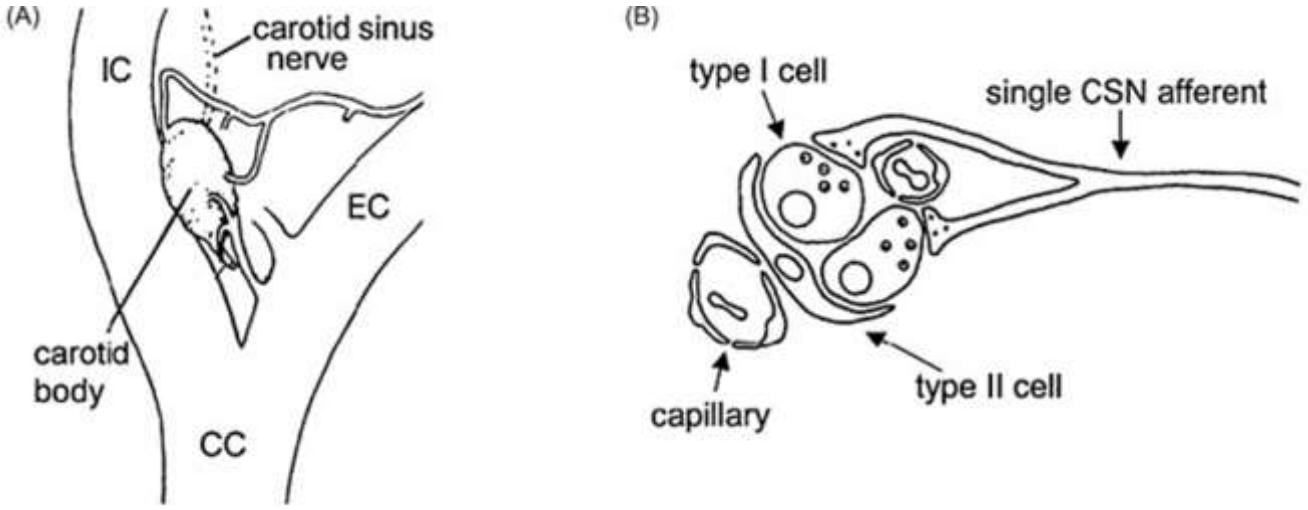
Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

Macromolecole della risposta alla pressione parziale di ossigeno

In mammals, O2 sensing occurs at many levels, leading to both **acute and chronic** adaptation

Acute .. seconds…..

The carotid body, which is located at the bifurcation of the internal and external carotid arteries, contains highly specialized chemosensory cells.
These cells depolarize in response to reduction in arterial blood PO2 (hypoxemia)



Acute .. seconds…..

1. **depolarization in** response to reduction in arterial blood PO2 (hypoxemia)
2. **stimulation of the brain stem centers** that control the respiratory and cardiovascular systems,
3. **rapid changes in ventilation, heart rate, and blood pressure** that serve to
4. **increase O2 uptake in the lungs** and O2 delivery to the tissues.

.. minutes … hours

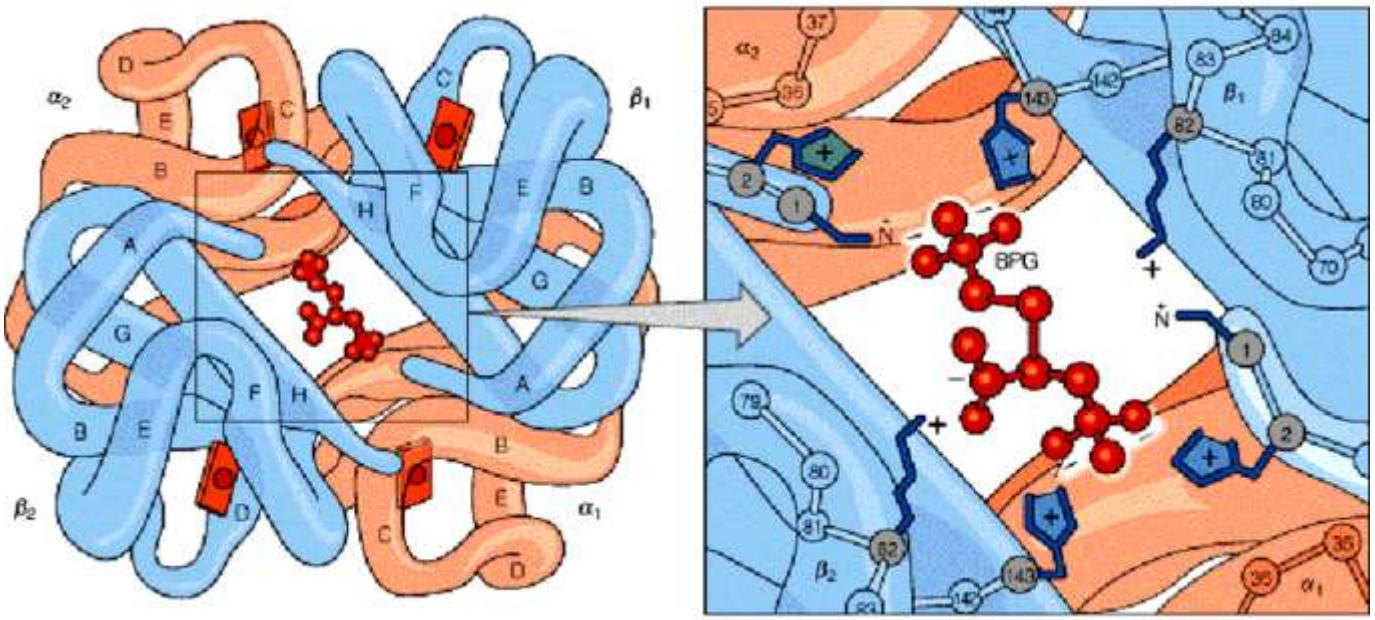
.. hours

2,3 Bisphosphoglycerate (BPG)

- 2,3,BPG is involved in acclimatization to hypoxia as in high altitude

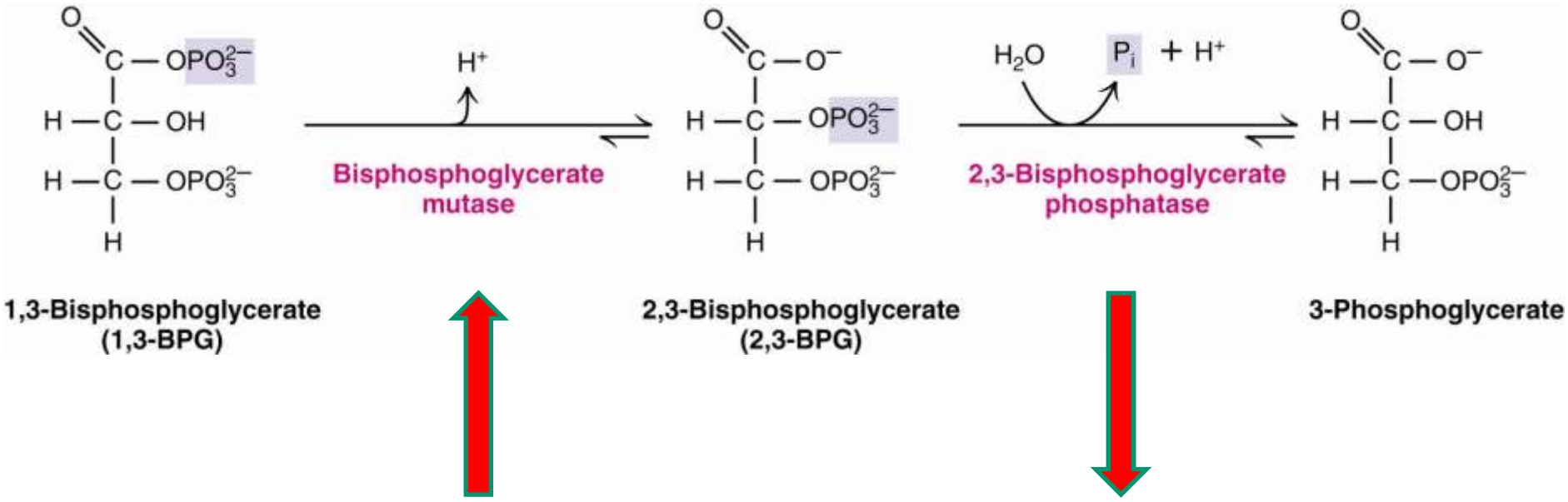
2,3 Bisphosphoglycerate (BPG)

- BPG binds in the cavity between β-Hb subunits and Stabilizes T-conformation

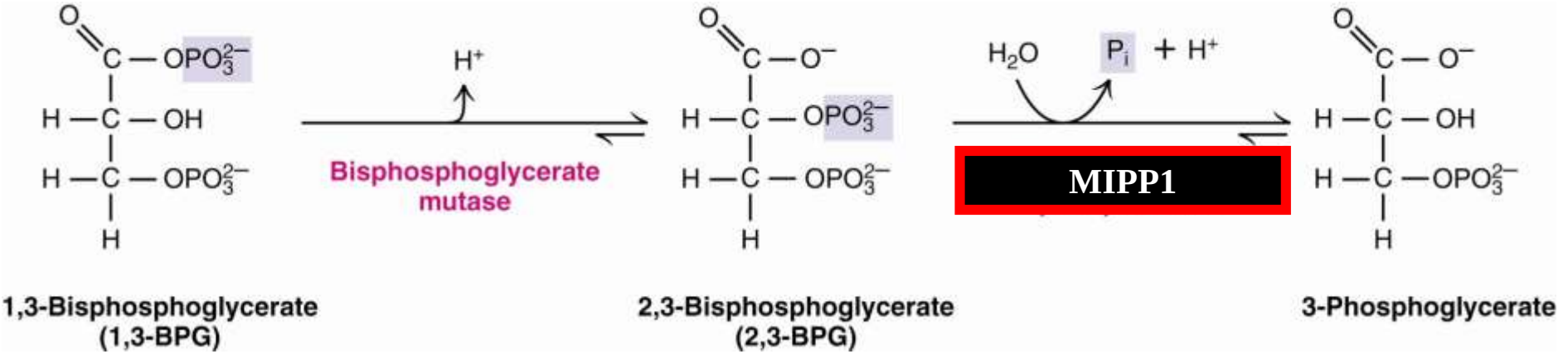


2,3-BPG is a glycolytic intermediate in RBCs

Erythrocyte synthesis and hydrolysis



Erythrocyte decomposition of 2,3-BPG

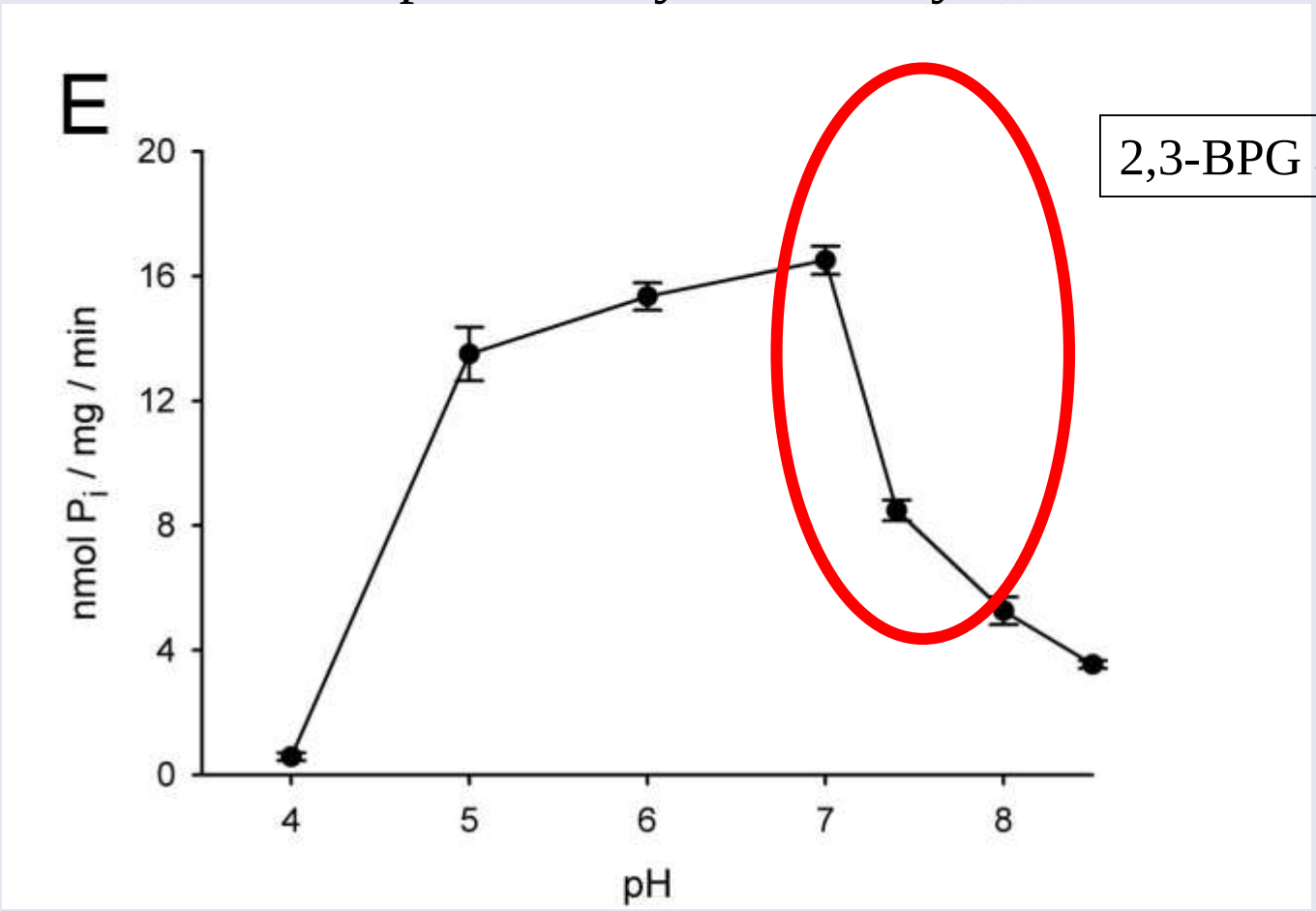


multiple inositol polyphosphate phosphatase (MIPP1)

Un’altra fosfatasi

Jaiesoon Cho et al. PNAS 2008;105:16:5998-6003

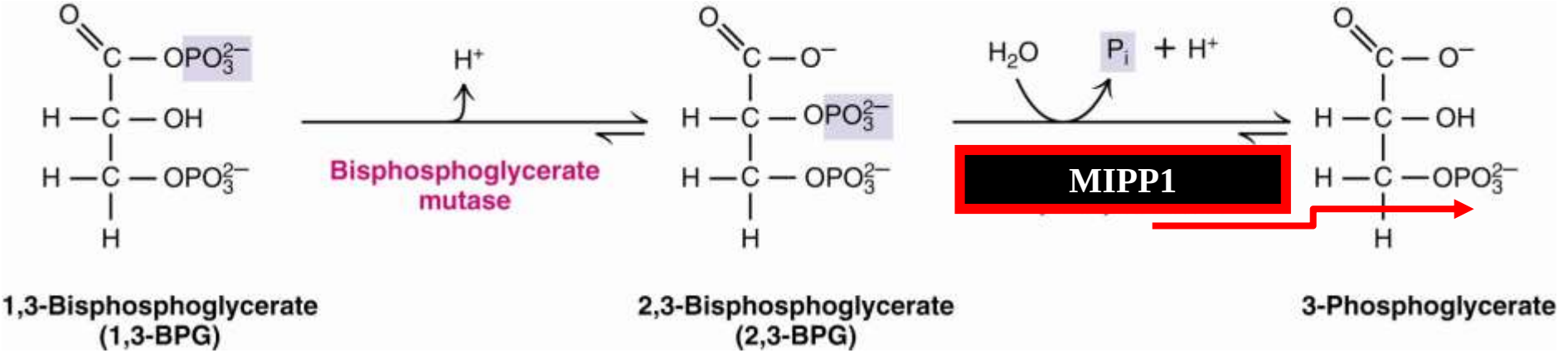
The effect of pH on enzyme activity of MIPP1



2,3-BPG as substrate

activity decreases 50% when pH rises from 7.0 to 7.4

Erythrocyte decomposition of 2,3-BPG



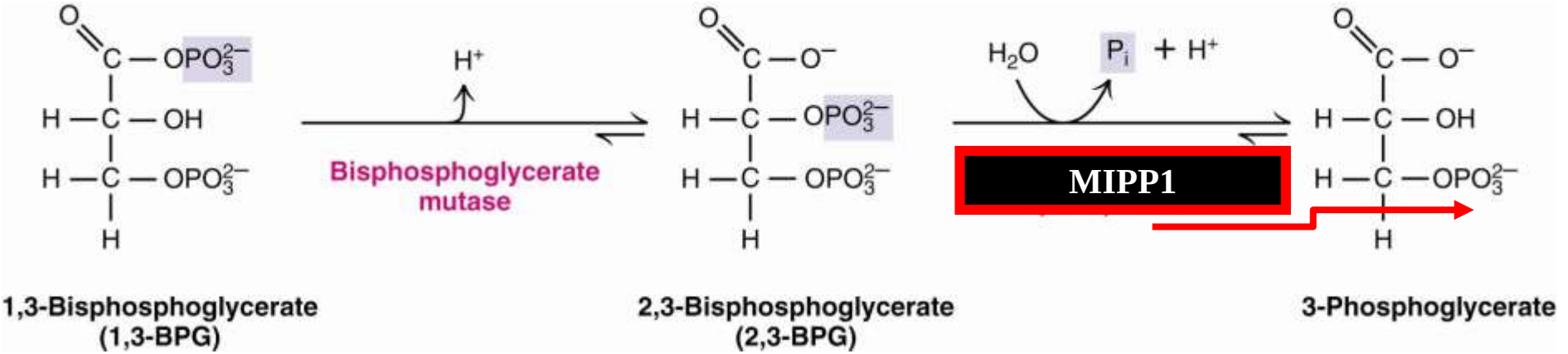
Un'altra fosfatasi

multiple inositol polyphosphate phosphatase (MIPP1)

activity decreases 50% when pH rises from 7.0 to 7.4

2,3-BPG ↑ MIPP1 activity ↓ pH ↑

Erythrocyte regulation of 2,3-BPG



Un'altra fosfatasi

multiple inositol polyphosphate phosphatase (MIPP1)

activity decreases 50% when pH rises from 7.0 to 7.4

↑ O2 release → ↑ 2,3-BPG → ↓ MIPP1 activity → ↑ pH

homeostatic mechanism for elevating 2,3-BPG levels, thereby enhancing oxygen release to tissues

L’effetto Bohr: pH bassa ⟶ bassa affinità ⟶ rilascio di O_2

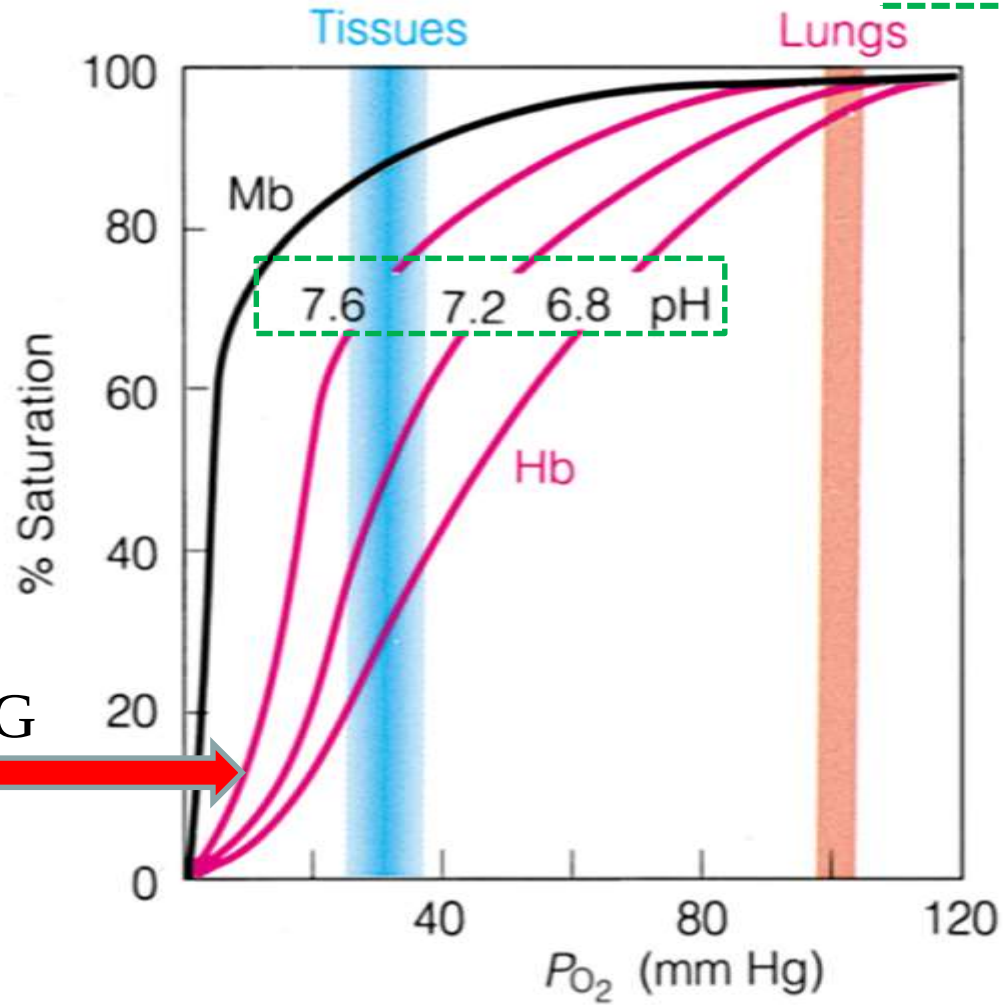
-His-H^+

Terminal-NH_3^+

pH

MIPP1 activity

2,3-BPG



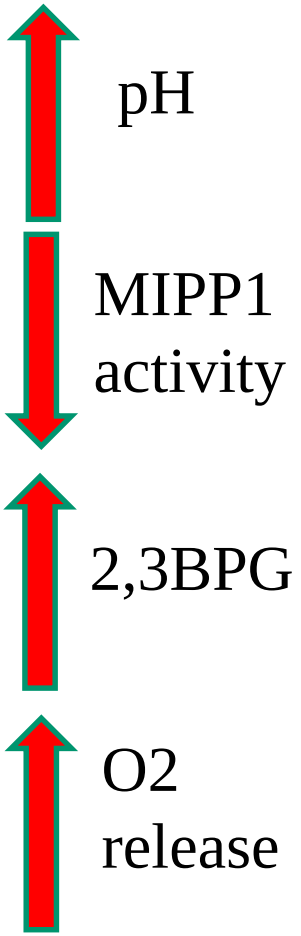
homeostatic mechanism for elevating 2,3-BPG levels enhancing oxygen release to tissues

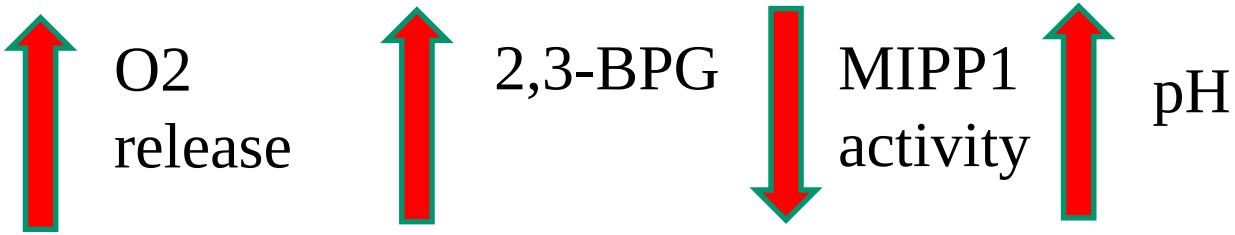
As hemoglobin releases oxygen, its affinity for H^+ increases, causing intracellular alkalinization. Hyperventilation-induced alkalosis at high altitude

Increased intracellular pH drives a feedback loop

increasing levels of 2,3-BPG

facilitating more oxygen release





homeostatic mechanism for elevating 2,3-BPG levels enhancing oxygen release to tissues

O2 sensing

.. Days….

RNA transcription

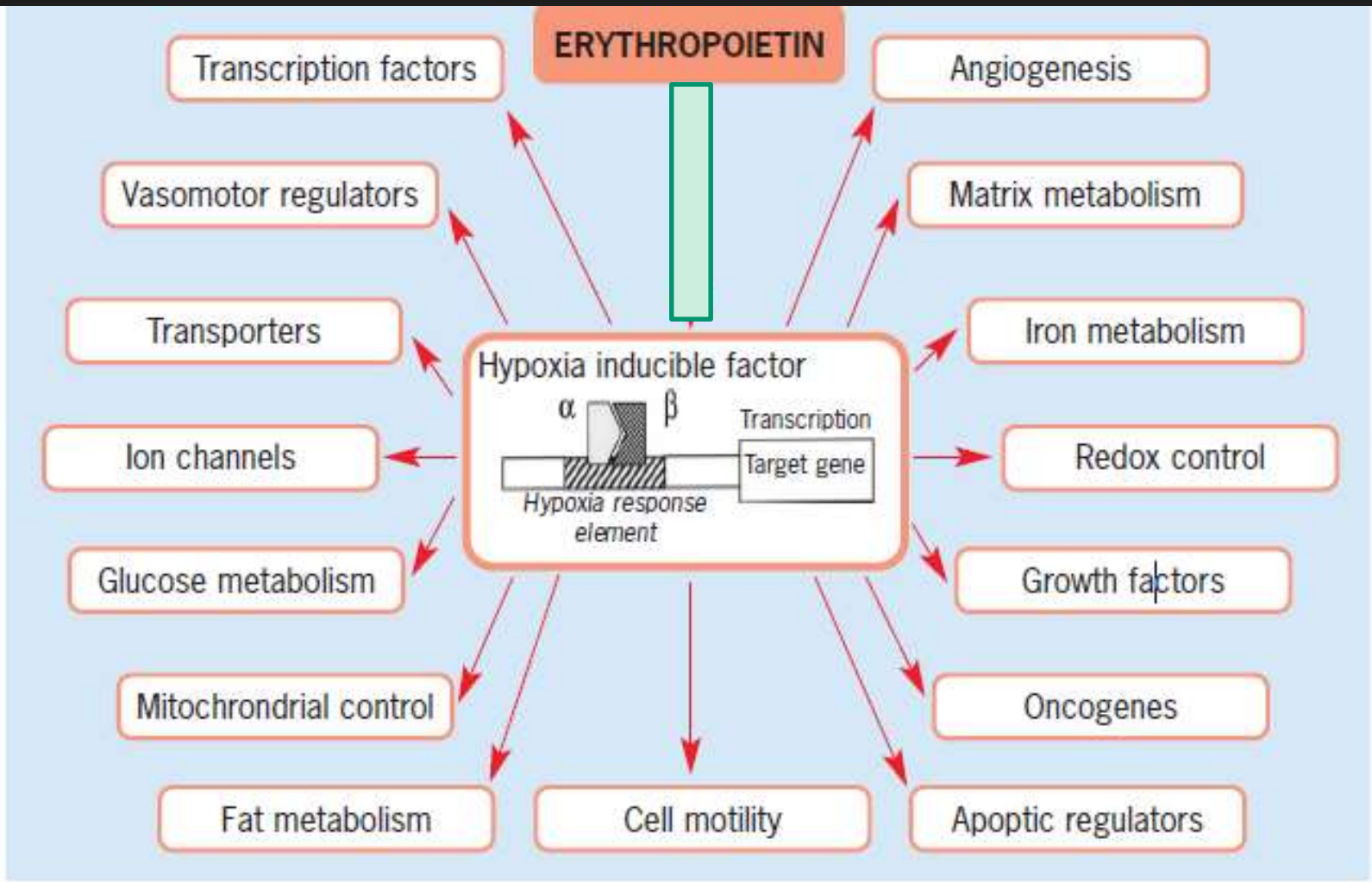
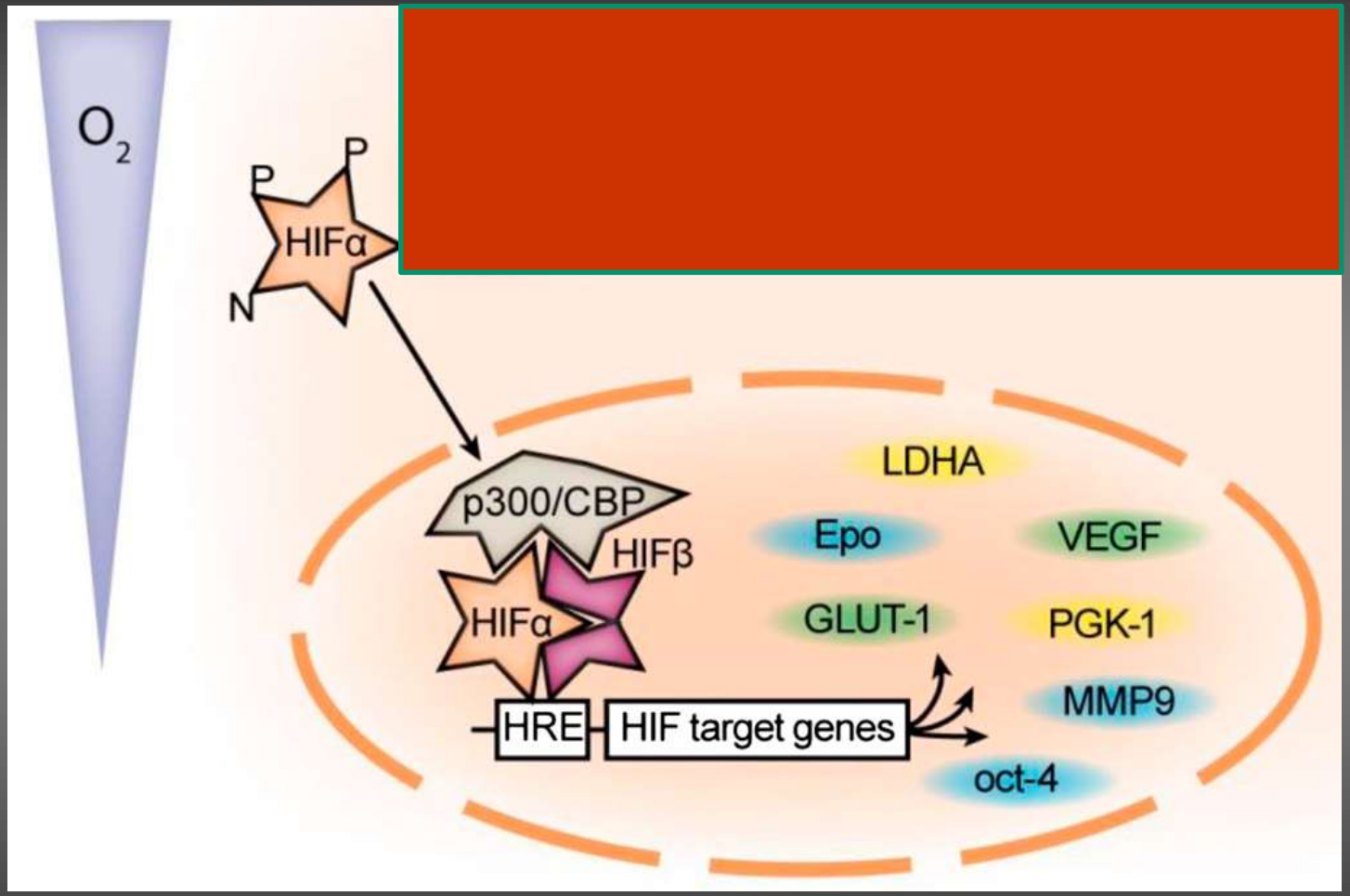
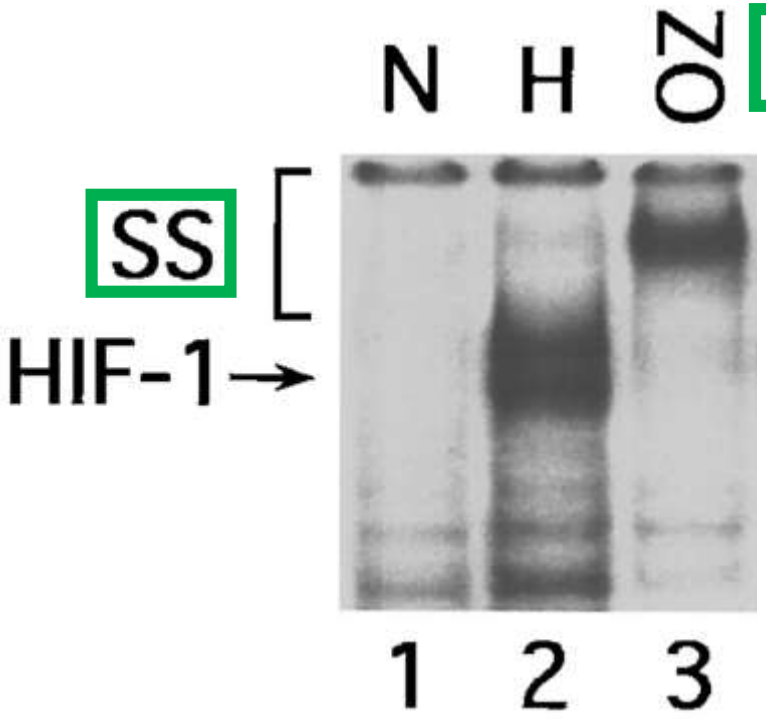


Fig 1. The hypoxia-inducible factor (HIF) transcriptional cascade directly regulates genes with key functions in a broad range of processes. The complex binds in a sequence-specific manner to control elements in DNA, termed hypoxia-response elements, at target gene loci.


O2
P
P
HIFα
N
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

HIF-1 DNA binding.

normoxic (*N*) and hypoxic (*H*) HeLa cells



+Ab anti Hif super shift SS

HRE 5'-GGTAGGCCACGTGACCGGGTA-3'

Semenza Wang et al 1992 1996;271:32253-32259

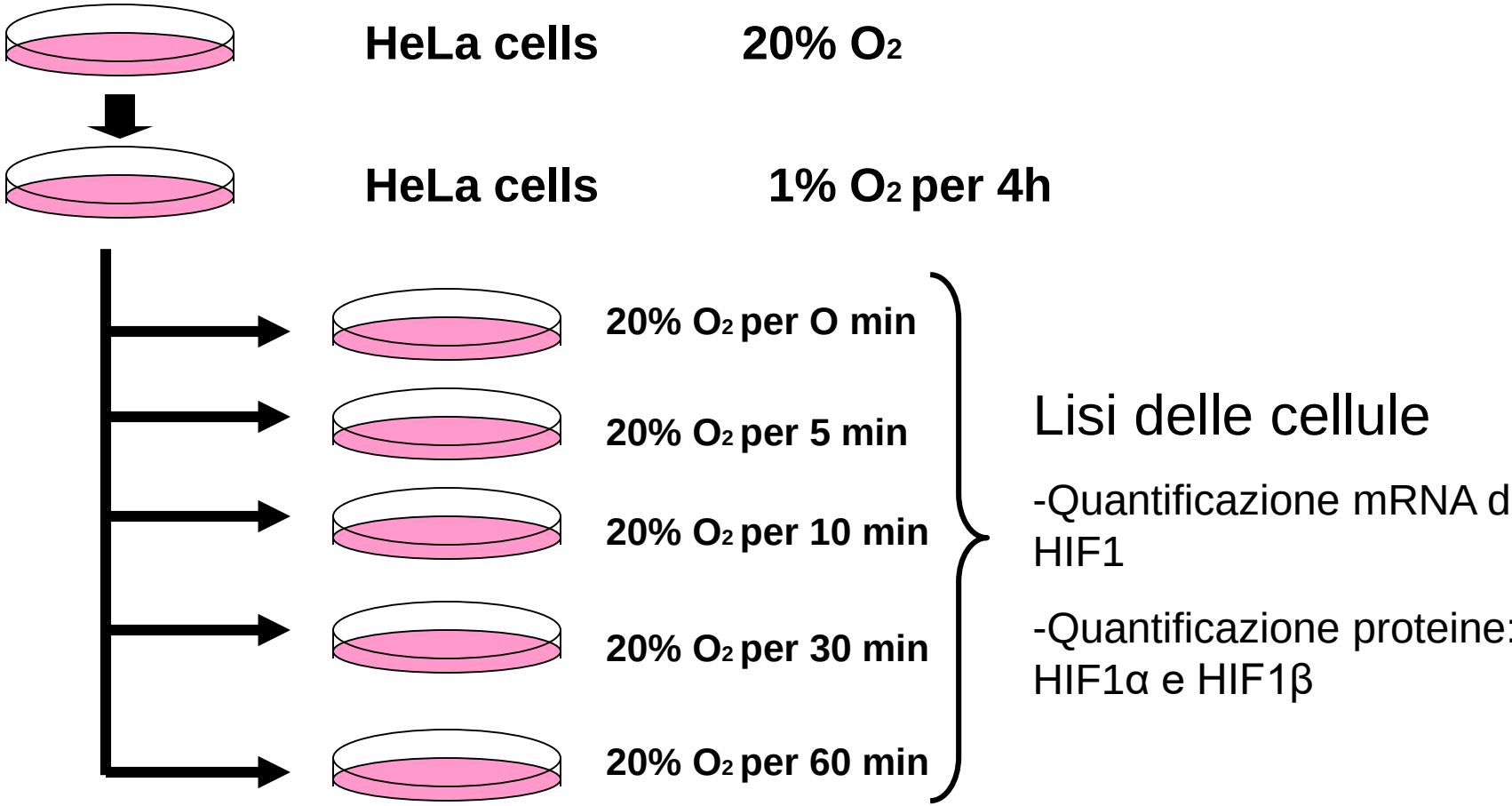
JBC JOURNAL OF BIOLOGICAL CHEMISTRY

O2 sensing

Regolazione di HIF ?

Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Eric Huang et al. - JBC 1996



Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Huang et al. - JBC 1996

H=hypoxia; N=normoxia

Sonda indigerita

HIF1α è espresso a livello di mRNA.

Activation of Hypoxia-inducible Transcription Factor Depends Primarily upon Redox-sensitive Stabilization of Its α Subunit

Huang et al. - JBC 1996

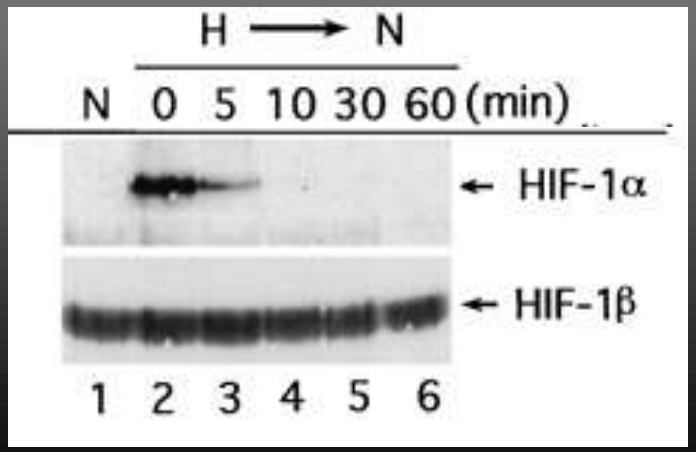
H=hypoxia; N=normoxia

Sonda indigerita

HIF1α è espresso a livello di mRNA.

Quantificazione proteine → **Western blot**

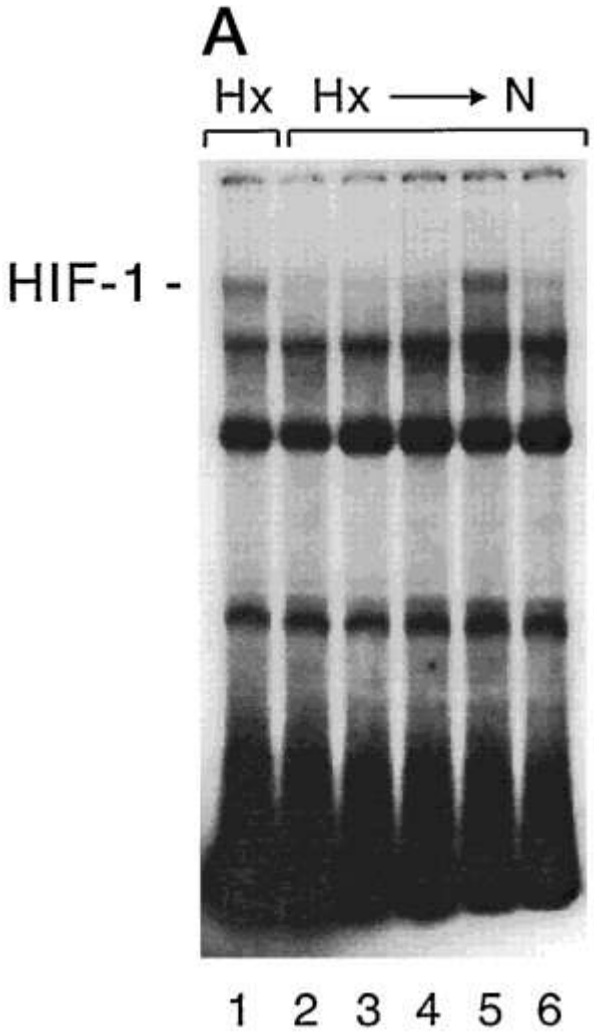
Ibridazione con anticorpi anti HIF1α e HIF1β



HIF1α è presente solo in condizioni di ipossia

HIF1β è sempre presente

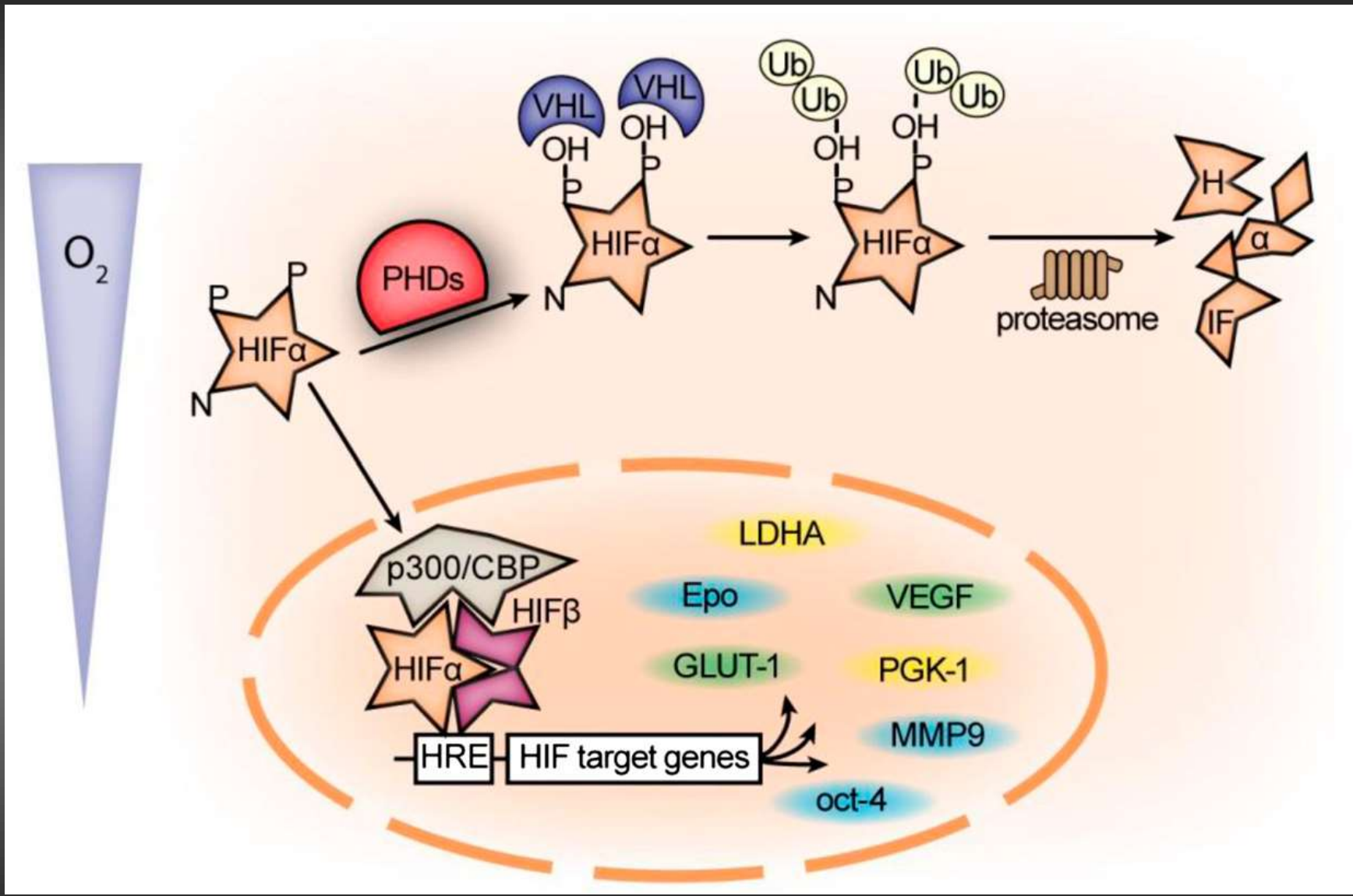
Protease inhibitors decrease the rate of degradation of the HIF-1 complex.



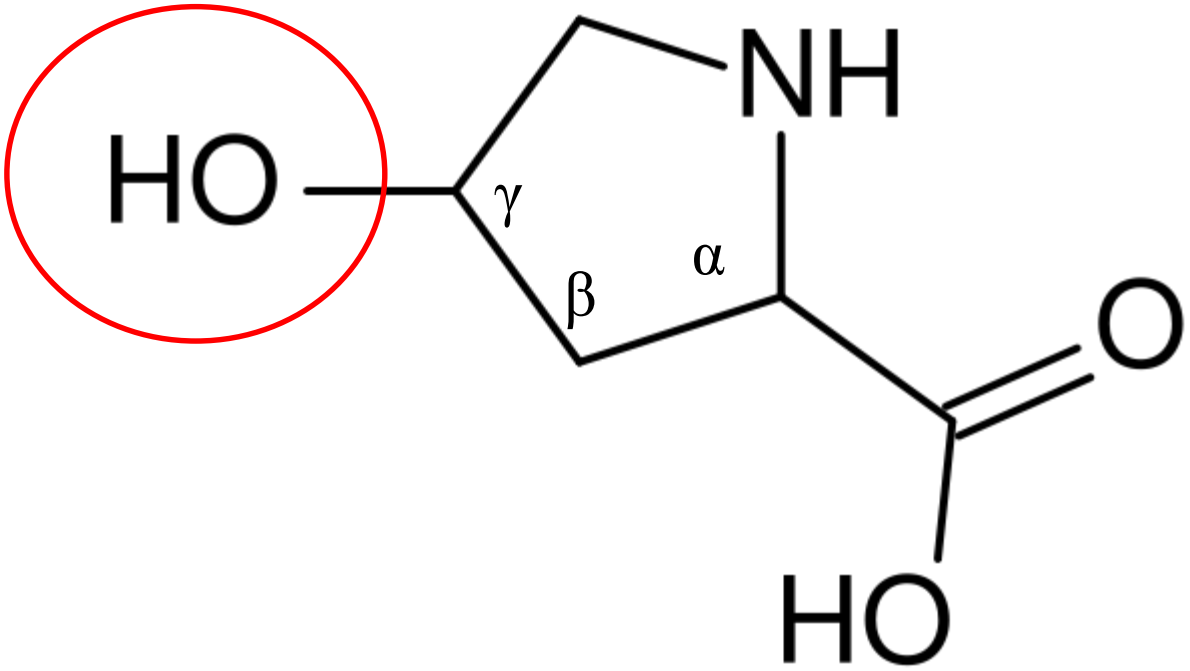
hypoxia Hx for 6 h **1** followed by 30 min at normoxic conditions N **2**
Protease inhibitors **3-6** were added after 15 min at normoxic conditions

5 Proteasome inhibitor

Susana Salceda, and Jaime Caro J. Biol. Chem. 1997;272:22642-22647

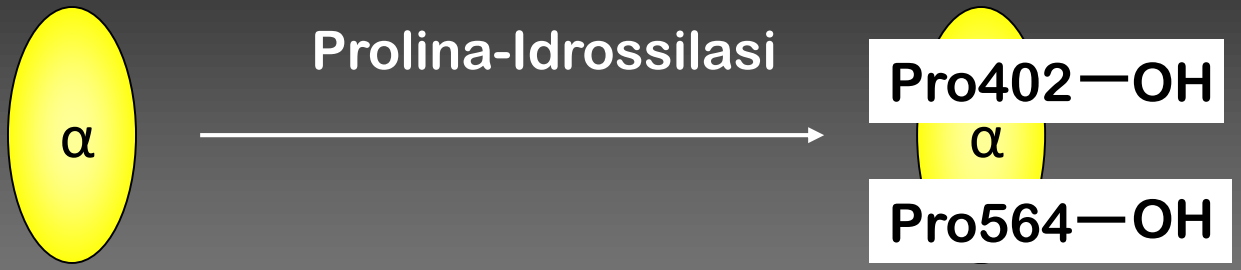

O2
P
P
HIFα
N
PHDs
VHL
VHL
OH
OH
P
P
HIFα
N
Ub
Ub
Ub
Ub
OH
OH
P
P
HIFα
N
proteasome
H
α
IF
p300/CBP
HIFβ
HIFα
HRE
HIF target genes
LDHA
Epo
VEGF
GLUT-1
PGK-1
MMP9
oct-4

Idrossiprolina

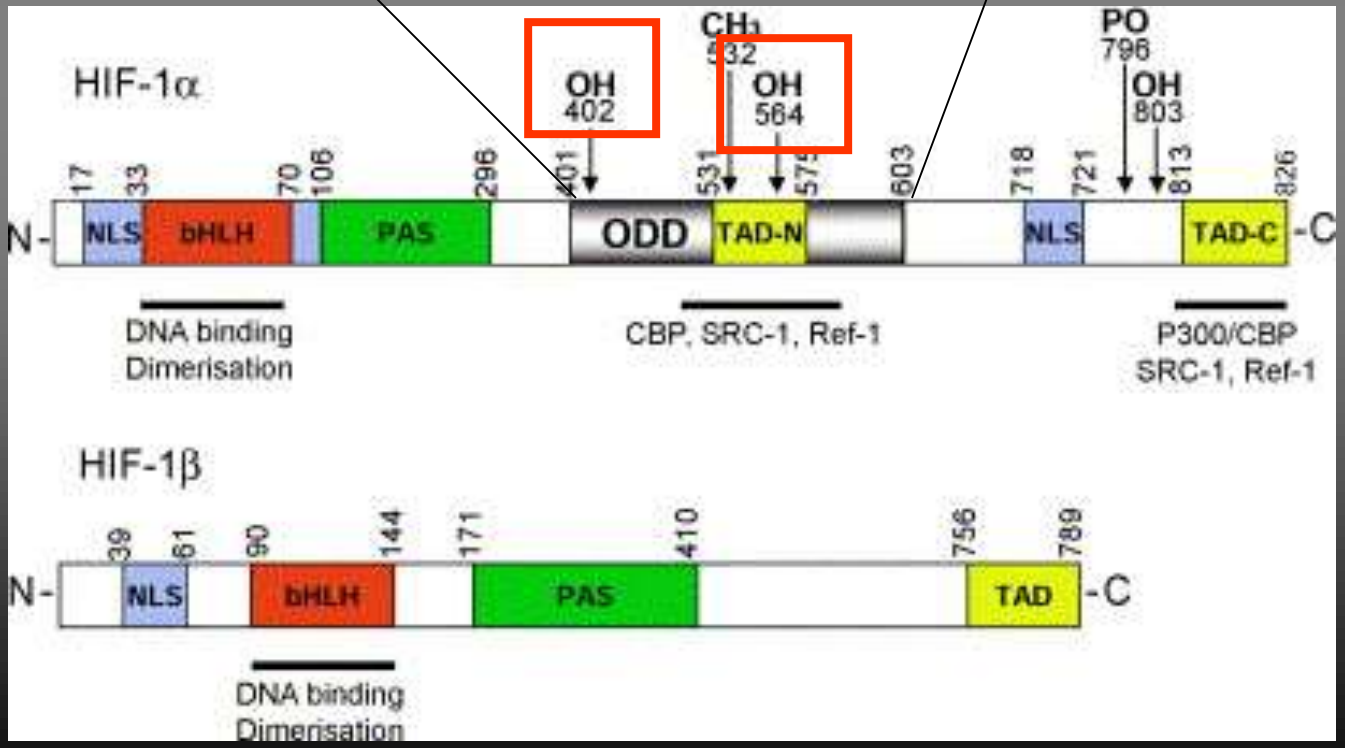


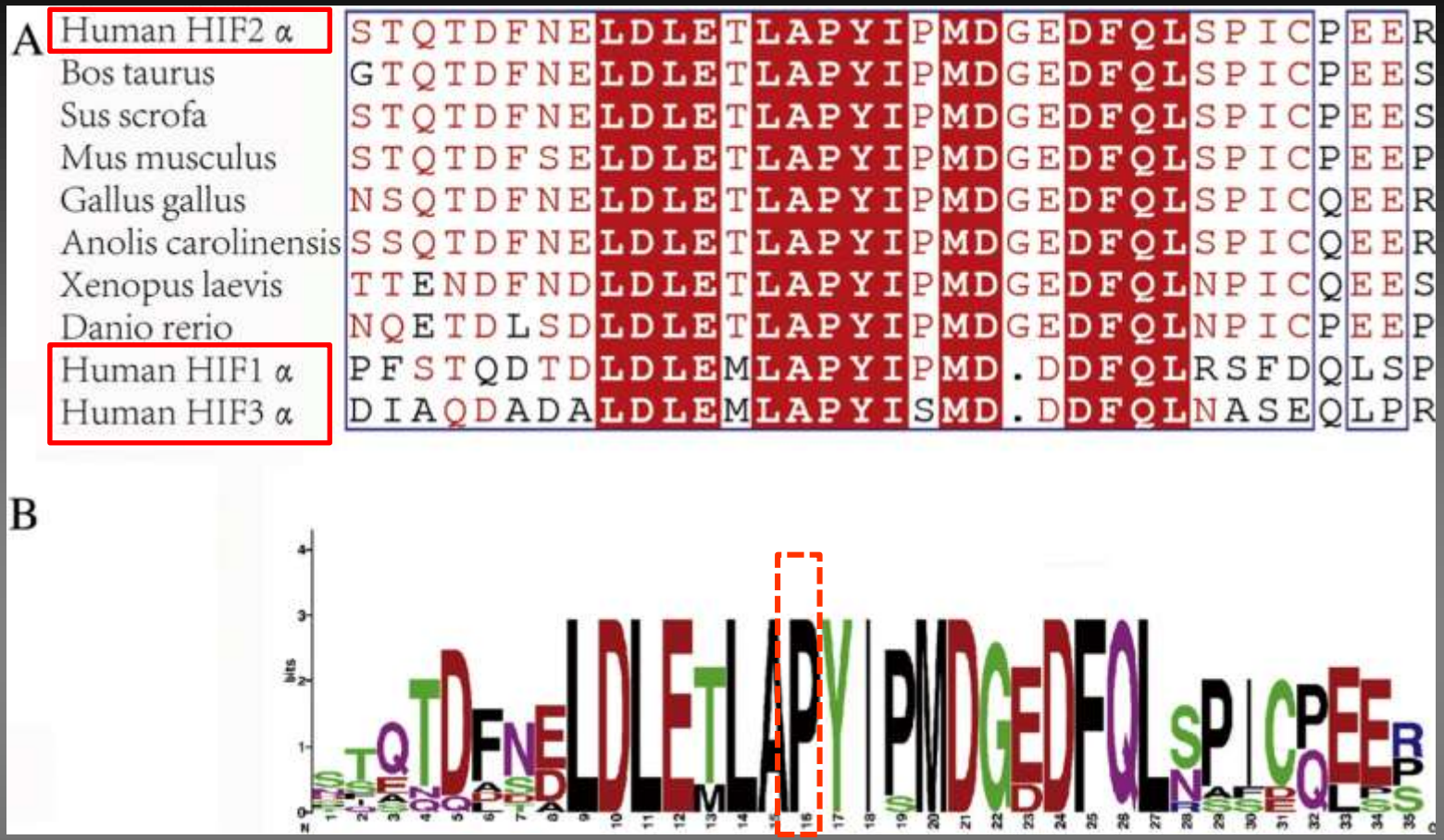
4-hydroxypyrrolidine-2-carboxylic acid

Struttura di HIF1



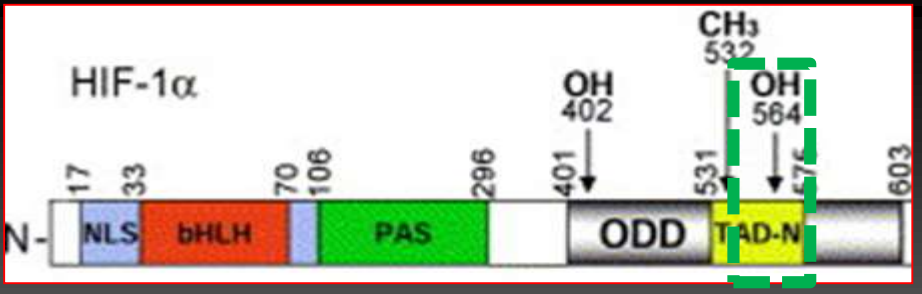
Sequenza di idrossilazione



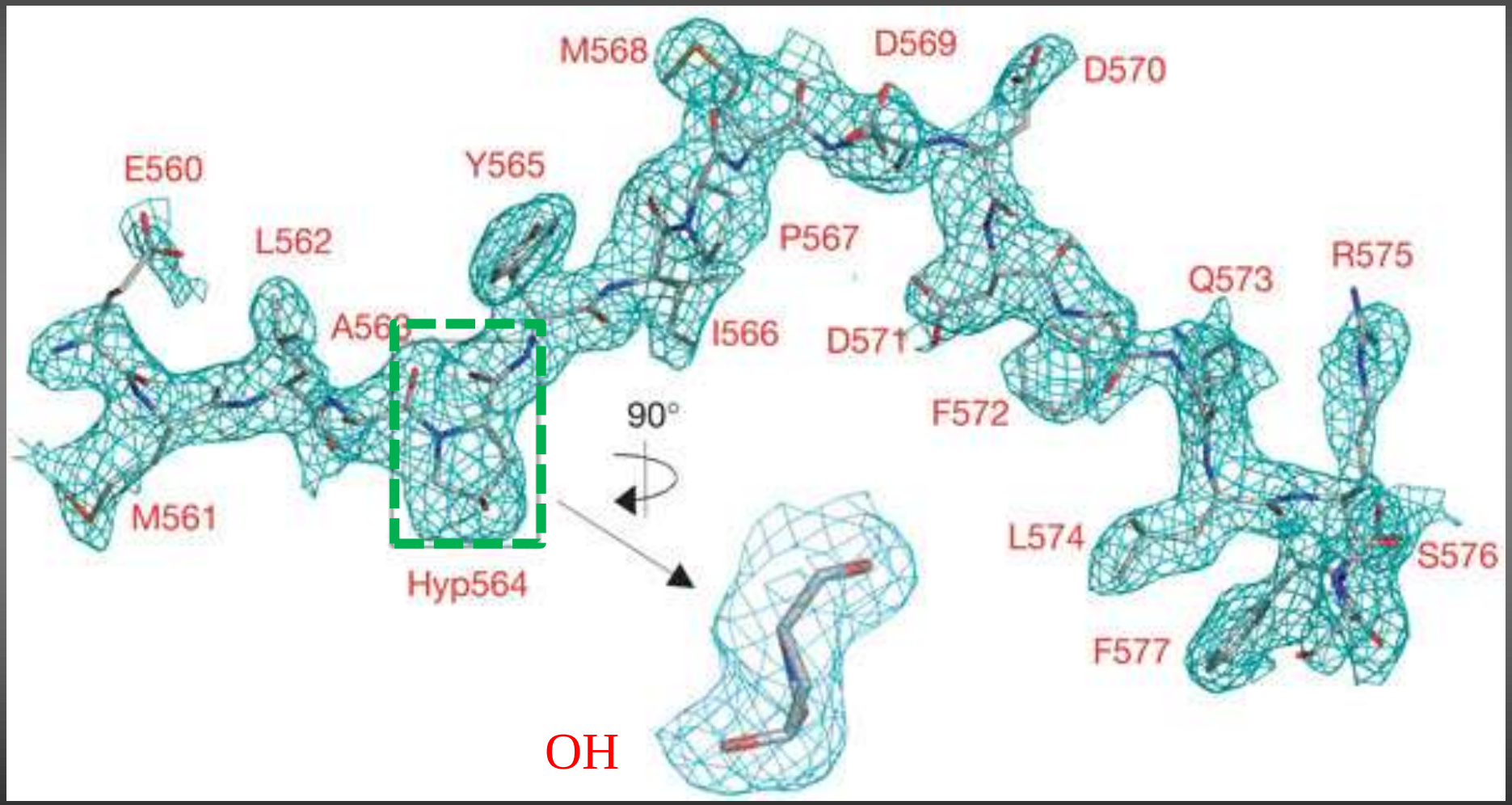


Amino acid sequence alignments of the HIF2α **CODD** (residue numbers: 516–550) domains of various species

Riconoscimento specifico dell'idrossiprolina da parte del complesso di VHL

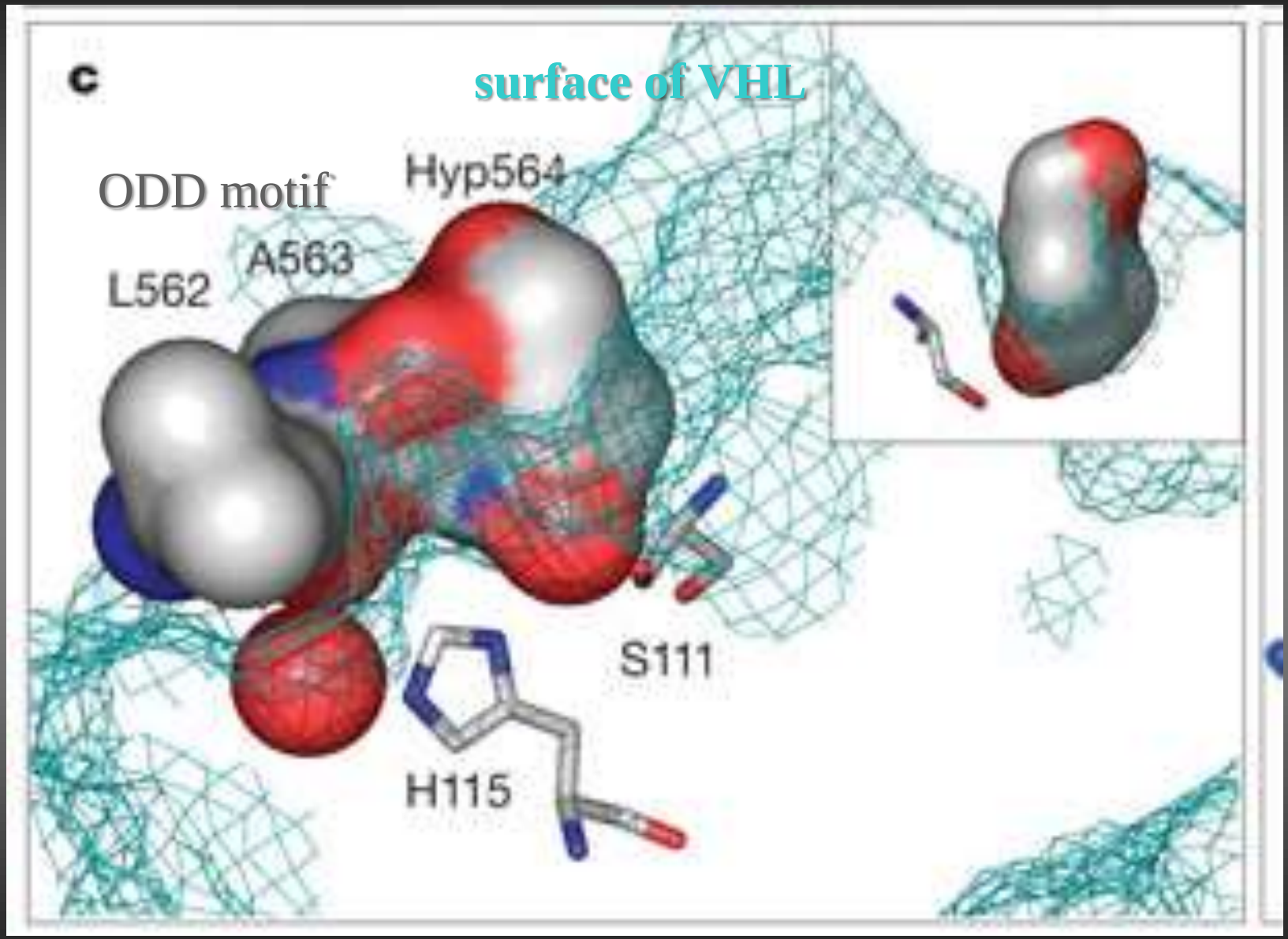


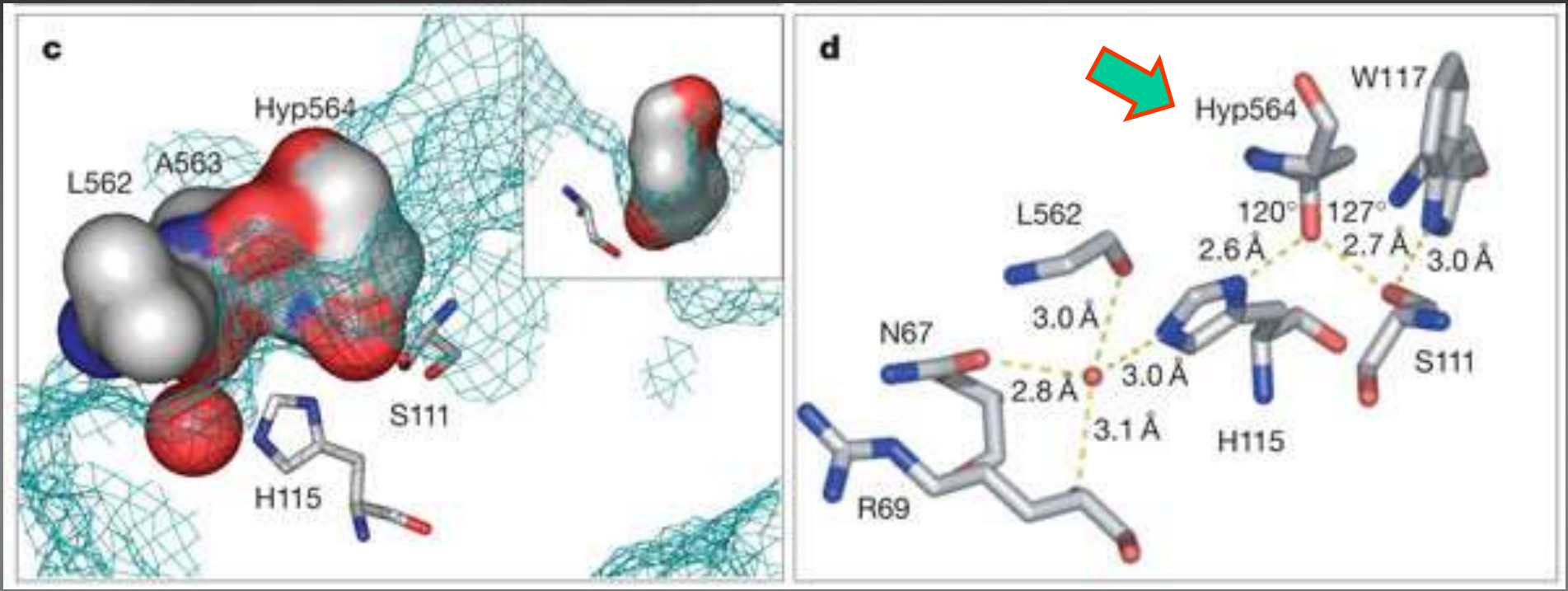
The boomerang-shaped CODD peptide (Hif)



CODD: Carboxyl oxygen dependent domain

Hyp-binding pocket (VHL)

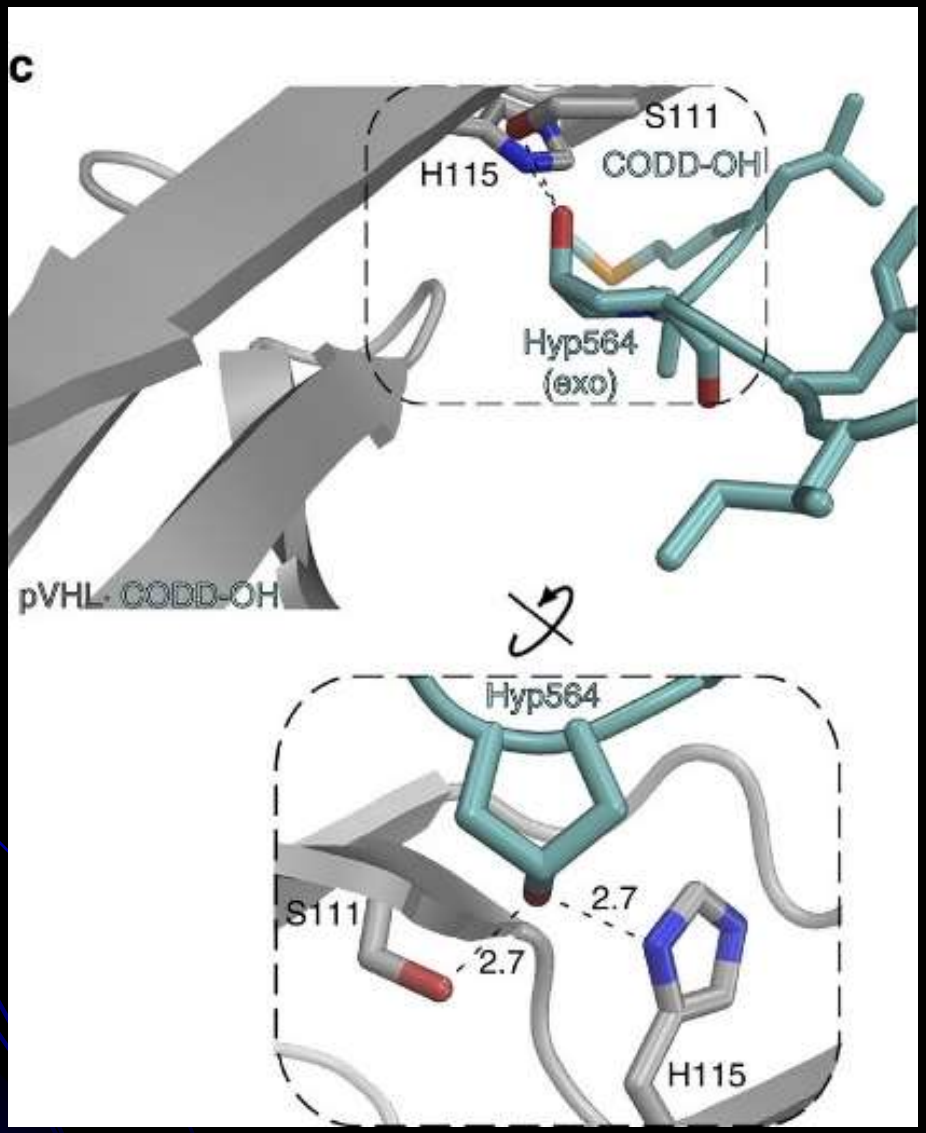




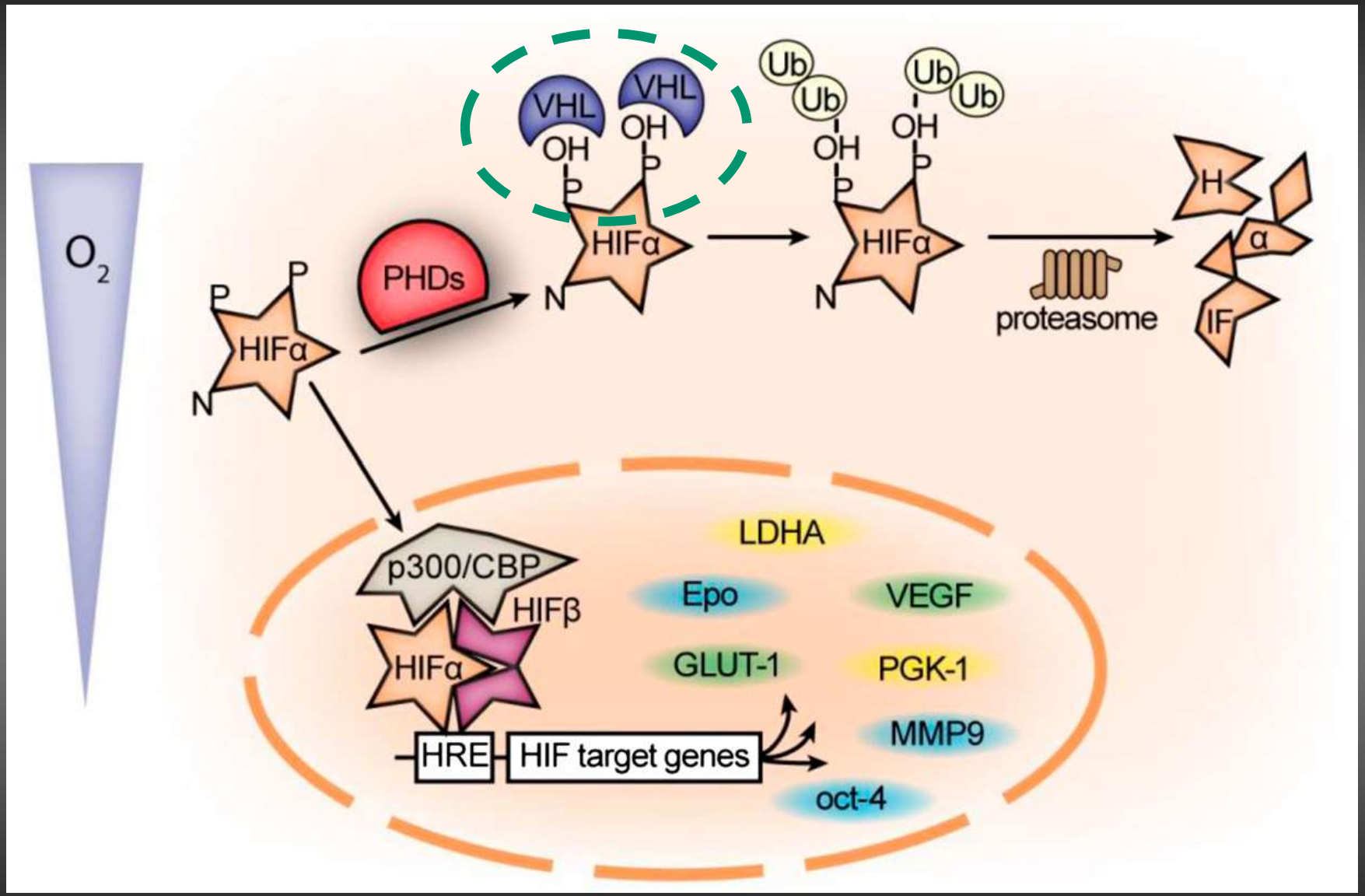
The hydrogen-bonding network (VHL) involved in binding of the Hyp564 hydroxyl group (Hif)

red sphere = key water molecule

Binding of proline hydroxyproline/Hyp (CODD-OH) to the VHL



the difference in kd for hydroxylated versus non-hydroxylated CODD is ~1,000-fold
(33 nM versus 34 μM)


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

STOP