

WHAT IS HYPEROXIA, NORMOXIA AND HYPOXIA TO CELLS:

Why researchers should care about
environmental oxygen and how it
influences results

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WEBINAR CONTENTS

1. Basics to CO₂ incubators
2. Oxygen terminology
3. Oxygen and HIF1 transcription factor
4. Oxygen emerging role
5. The future consideration for cell research

GENERAL BASICS ABOUT AIR AND O₂

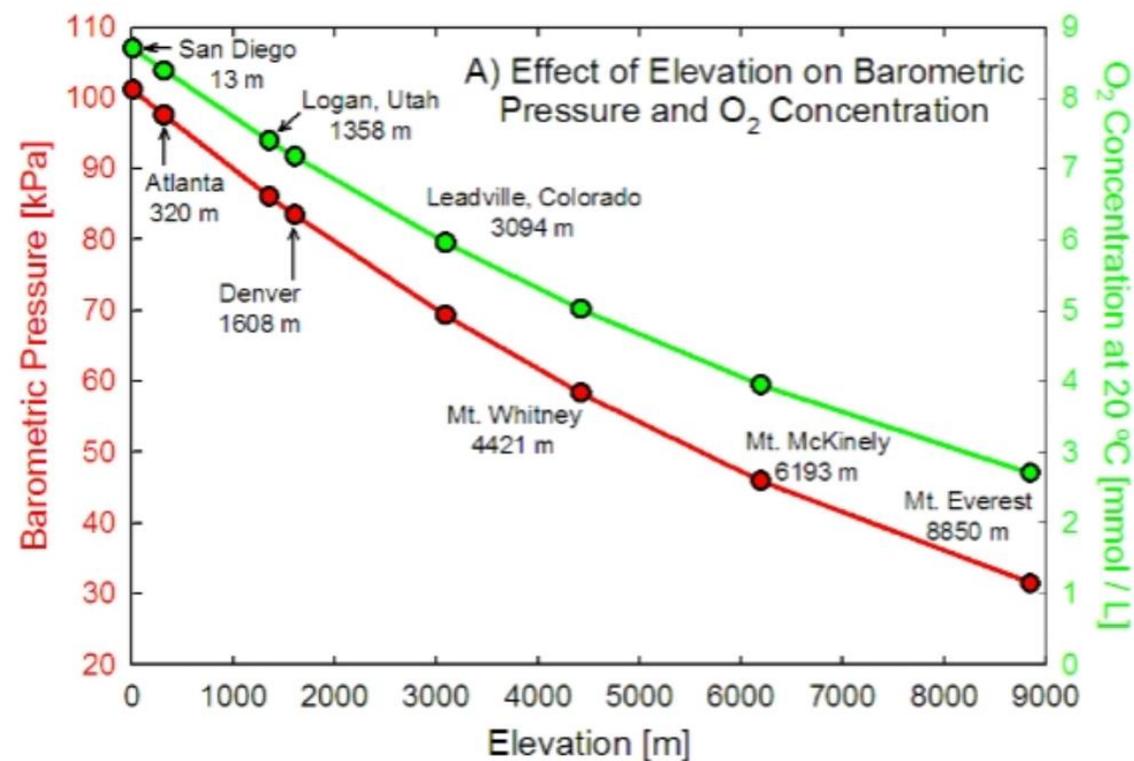
- Air e.g. 'dry air' by volume percentage contains
 - 78.09% nitrogen (N₂)
 - 20.95% oxygen (O₂)
 - 00.93% argon (Ar)
 - 00.04% mainly carbon dioxide (CO₂) and trace amount of other gases (Ne, He, CH₄, Kr, H₂)
- At sea level air 1% humidity
- Cell incubators
 - Temperature mostly at 37°C for mammalian cells
 - CO₂ levels displayed as percentage
 - Passively 100% humidity

CELL CULTURE IN CO₂ INCUBATORS

- CO₂ incubators have lower internal oxygen level
- Why?
 - 5% CO₂
 - 100% Humidity
 - Remaining volume of gas filled with 'air'
- Basic physics

DRY AIR - PRESSURE - OXYGEN LEVELS

- Altitude (pressure) affects the gas amount (mol) not the concentration (%)
- Sea level air pressure is 101.3 kPa (kN/m²)
 - Medical/biology often uses mmHg (or Torr)
 - Equivalent 760mmHg
- Sea level 20.9% O₂ = 159mmHg pO₂
 - 500m altitude, pressure 713.2 mmHg
 - pO₂ 149.1mmHg



AIR vs. CO₂ INCUBATOR AIR

CO₂, Humidity and O₂

Inside an incubator relative air humidity is ideally kept at 100%

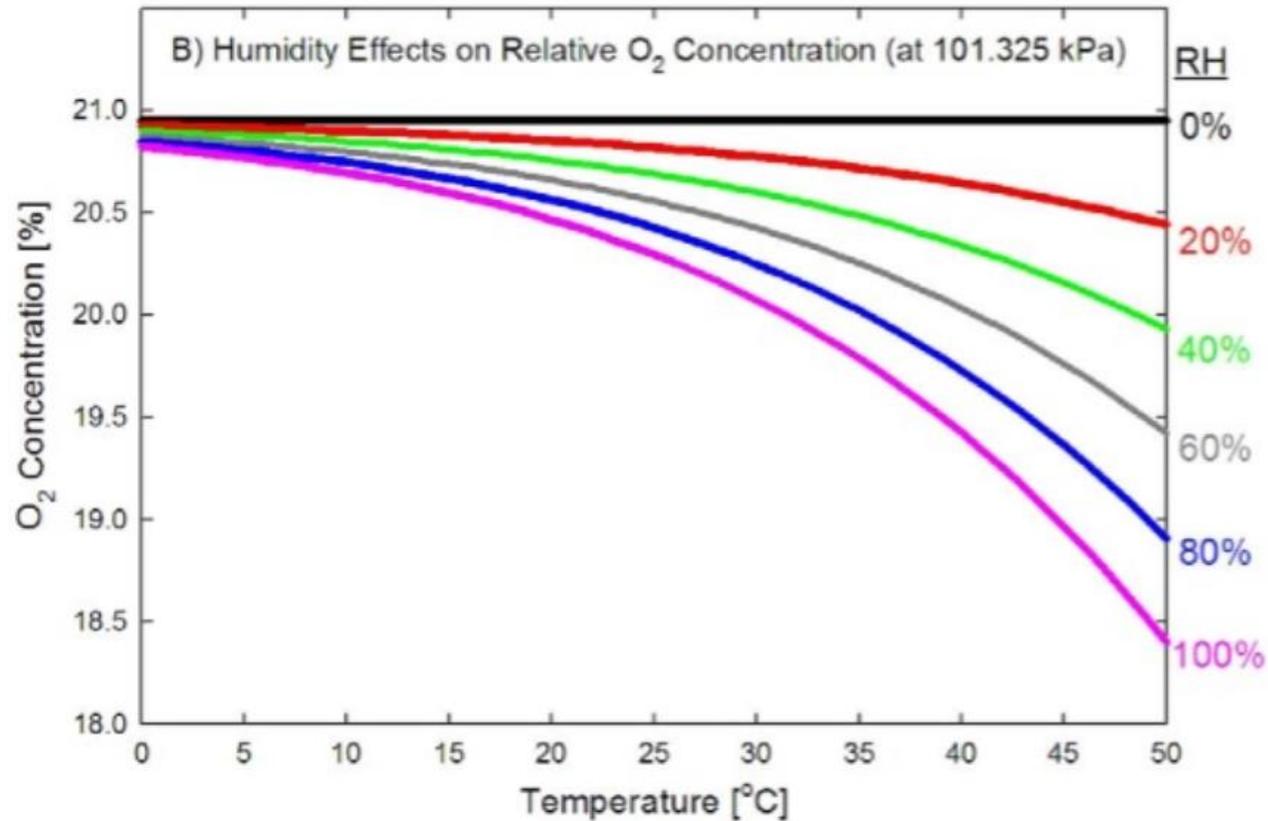
- Prevention of cell culture media evaporation
- At 37°C p_{H₂O} is 47 mmHg >> 6.2% V/V

Inside an incubator relative CO₂ is standardly kept at 5.0% V/V

- Prevention of cell culture media acidification by carbonate buffer
- At 37°C and sea level air pressure pCO₂ is 38 mmHg



AIR O₂ LEVELS RELATIVE TO TEMPERATURE AND HUMIDITY INFLUENCE



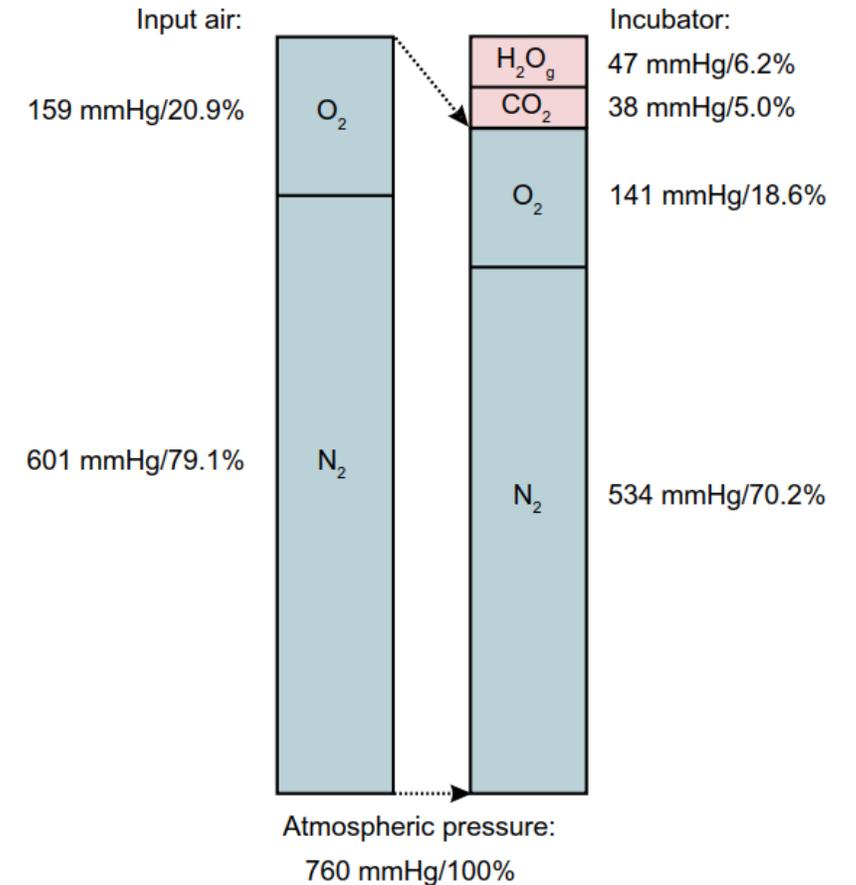
I AM THE LAW, THE PHYSICS LAWS

- Dalton's law of partial pressures
- Henry's law of partial pressures
- Bunsen's constant
- Fick's law of Diffusion



DALTON'S LAW OF PARTIAL PRESSURES

- Of a mixture of non-reacting gases, the total pressure exerted is equal to the sum of the partial pressures of the individual gases
- Inside an incubator at 37°C and 760mmHg pressure
 - p_{H_2O} is 47 mmHg \gg 6.2% V/V
 - p_{CO_2} is 38 mmHg \ll 5.0% V/V
 - Leaving 675mmHg \gg 88.8% V/V for the other gasses
 - 20.9% O_2 outside 'air' \gg p_{O_2} 141 mmHg of the pressure \gg 18.6% inside incubator's gas composition



HENRY'S LAW OF PARTIAL PRESSURES

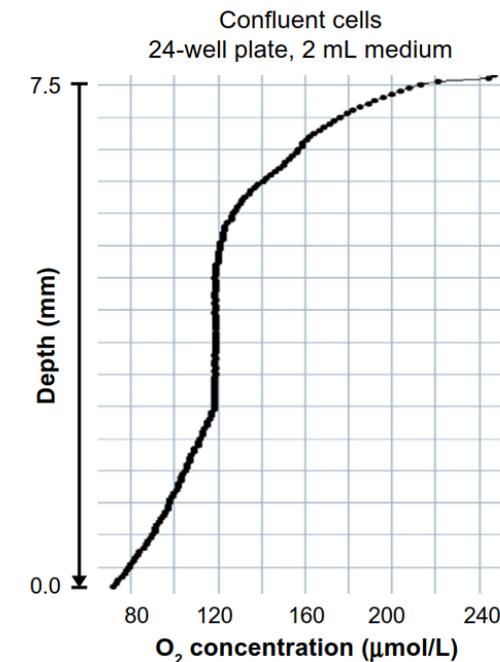
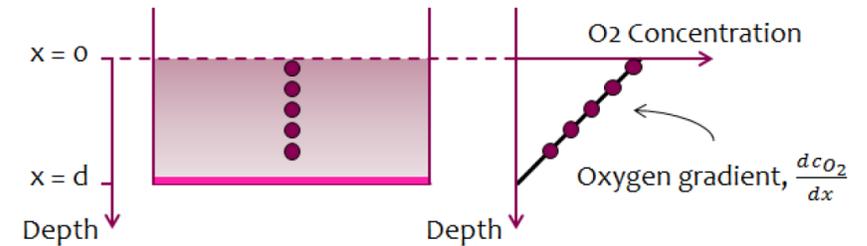
GAS TO LIQUID PHASE O₂ LEVELS

- The amount of dissolved gas in the liquid is proportional to its partial pressure in the gas phase
- Solubility / **Bunsen's constant** (temperature and liquid dependent)
- Assuming cell culture media similar to blood plasma
 - 1.26 μM O₂ / 1 mmHg at 37°C
- Incubator pO₂ 141 mmHg
 - 177.66 μM O₂ **CAN** dissolve in the media

FICK'S LAW OF DIFFUSION

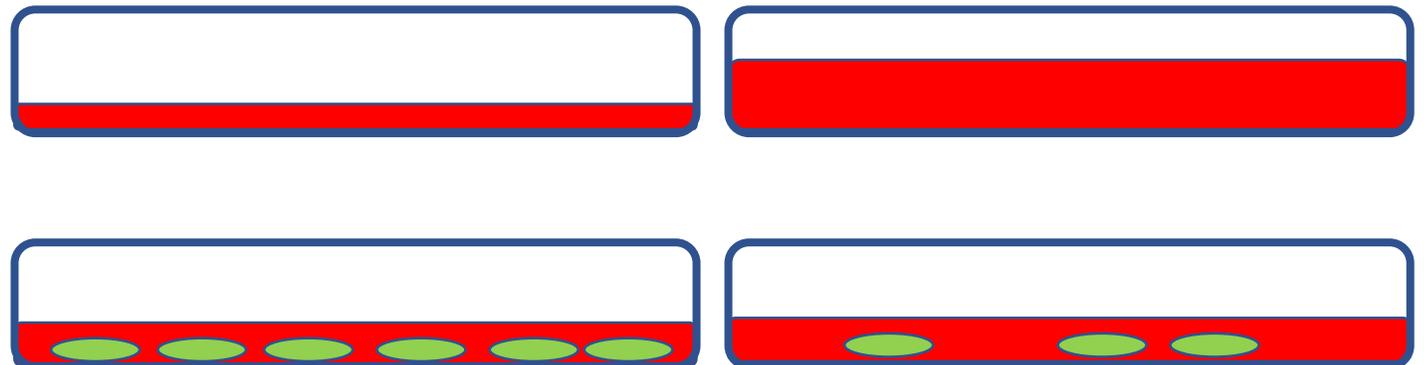
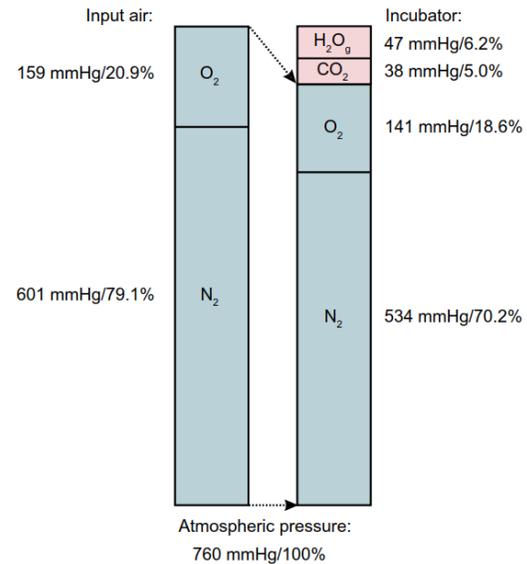
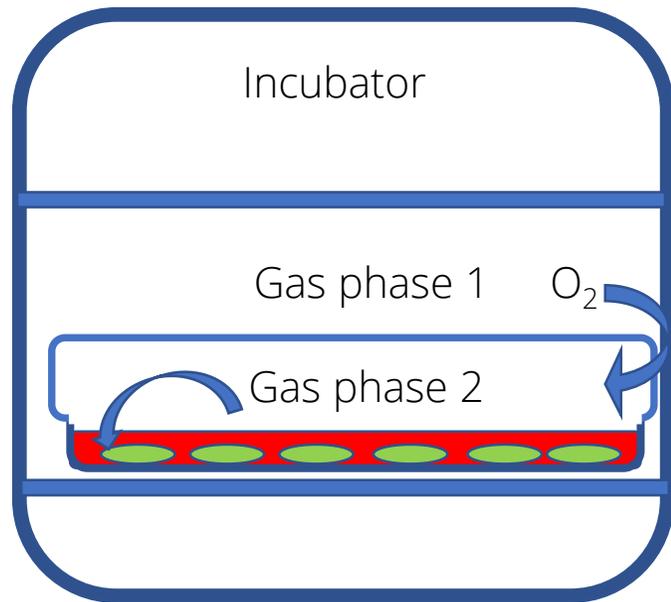
LIQUID PHASE O₂ LEVELS

- Henry's law is only valid in homogeneous liquids (stirring) or surface area of the liquid!
- Diffusion occurs from high to low proportional to the concentration gradient (partial pressure)
- O₂ will diffuse based on Fick's law
 - Rule of thumb Oxygen diffusion is ~100-200µm in tissue
 - Cell culture media 500µm or more
- Lower Cell's pericellular oxygen concentration
- Higher CO₂ solubility
 - 1140 µM CO₂ can dissolve in the media



PRACTICALITY INSIDE CELL INCUBATOR

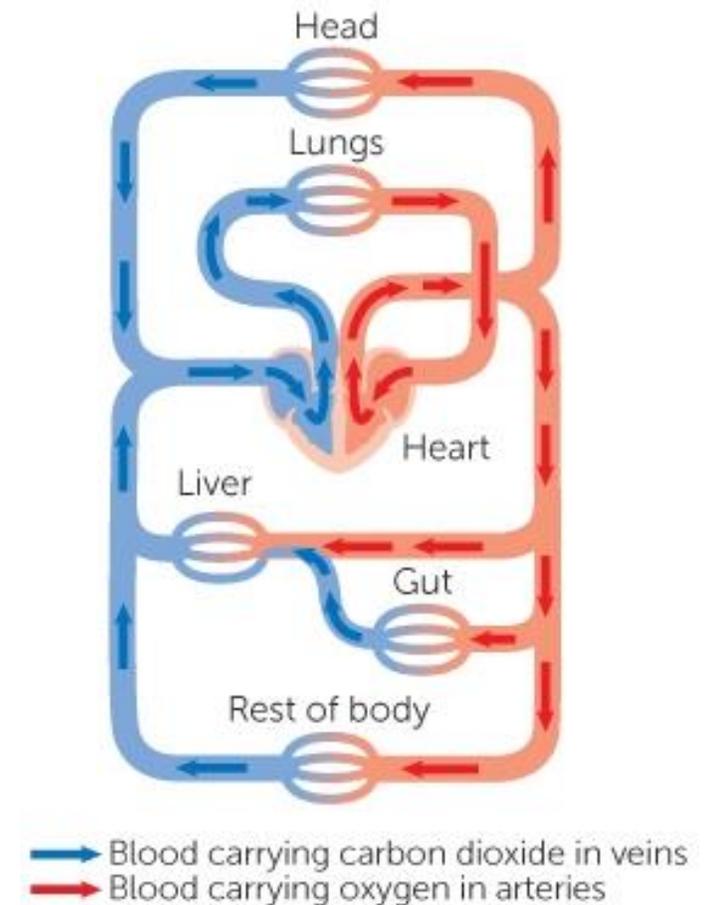
- Passive diffusion
- 1 liquid phase
- 2 gas phases



WHY BOTHER WITH OXYGEN LEVELS?

Research fields/subjects related to O₂ levels

- Sleep apnea
- Cancer cell research
- Pulmonary research (COPD, asthma)
- Reactive Oxygen Species effects (ROS)
- Muscle physiology (endurance, general metabolism)
- Brain stroke, seizure
- Heart infarct (Ischemia / reperfusion)
- Embryonal development



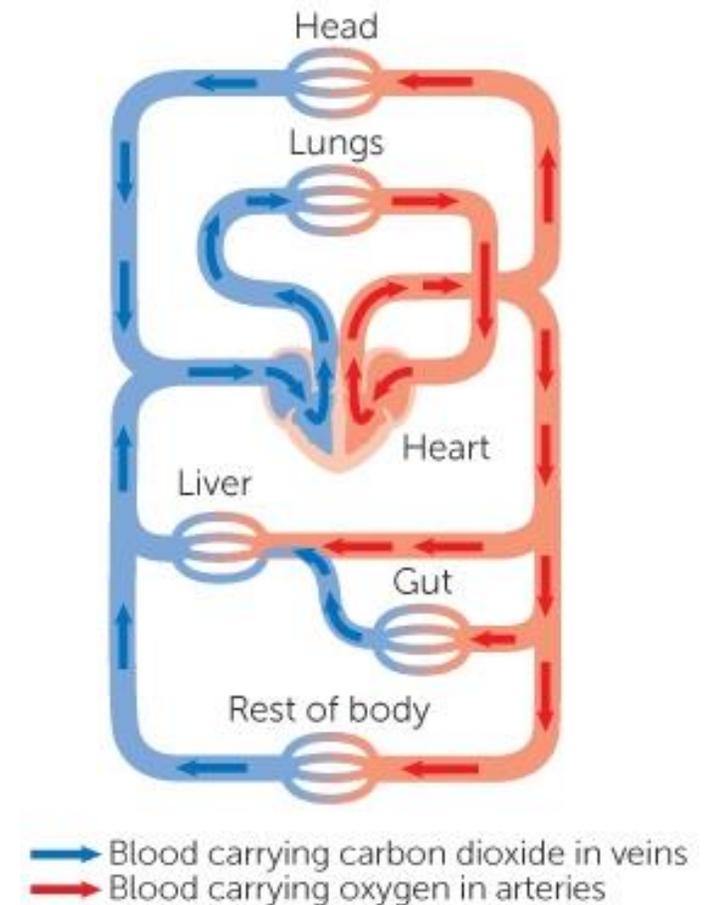
WHY BOTHER WITH OXYGEN LEVELS?

In vivo O₂ levels

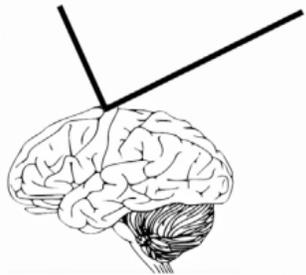
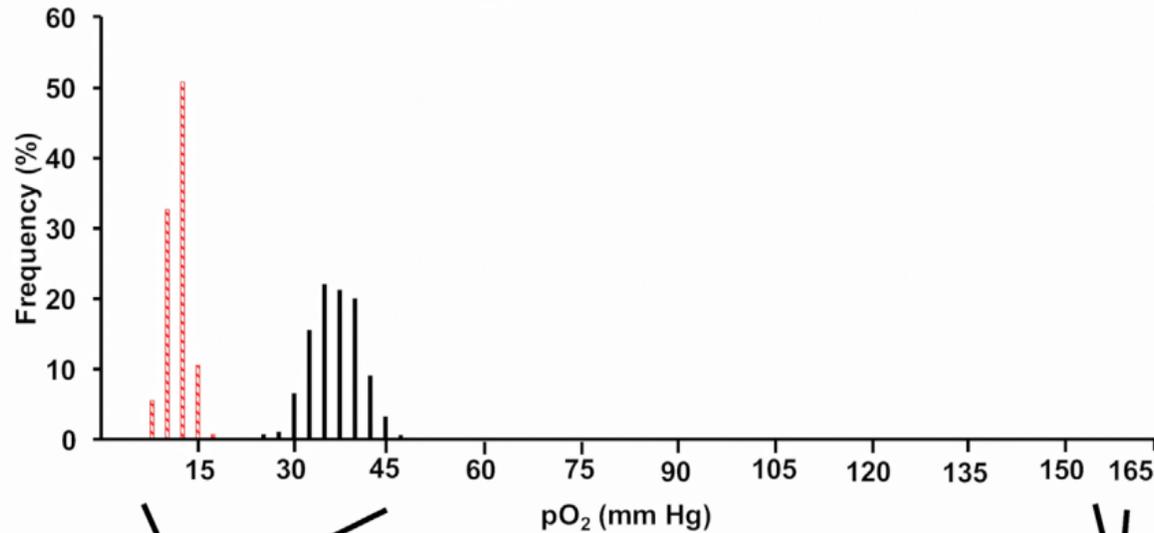
- Tissue level
 - Vascularization
 - Blood flow speed
 - Cell (type) composition
 - Microbiota
 - Damage e.g. injury or disease
- Cell level
 - Cell type
 - Activation
 - Proliferation
 - Differentiation
 - Migration

CELL / TISSUE EXPOSURE/ ACCESS TO O₂

- Breathing pO₂ 159mmHg
- Lung alveolar tissue ~100mmHg pO₂
- Arterial blood carrying ~75- 100mmHg pO₂
- Venous is between 30-40mmHg pO₂
- What are the implications of maintaining cells *in vitro* at higher pO₂ 141mmHg?



PRACTICAL EXAMPLE *in vivo* vs. *in vitro*



***In vivo* brain pO₂**
25-50 mm Hg (normoxia)
8-18 mm Hg (hypoxia)



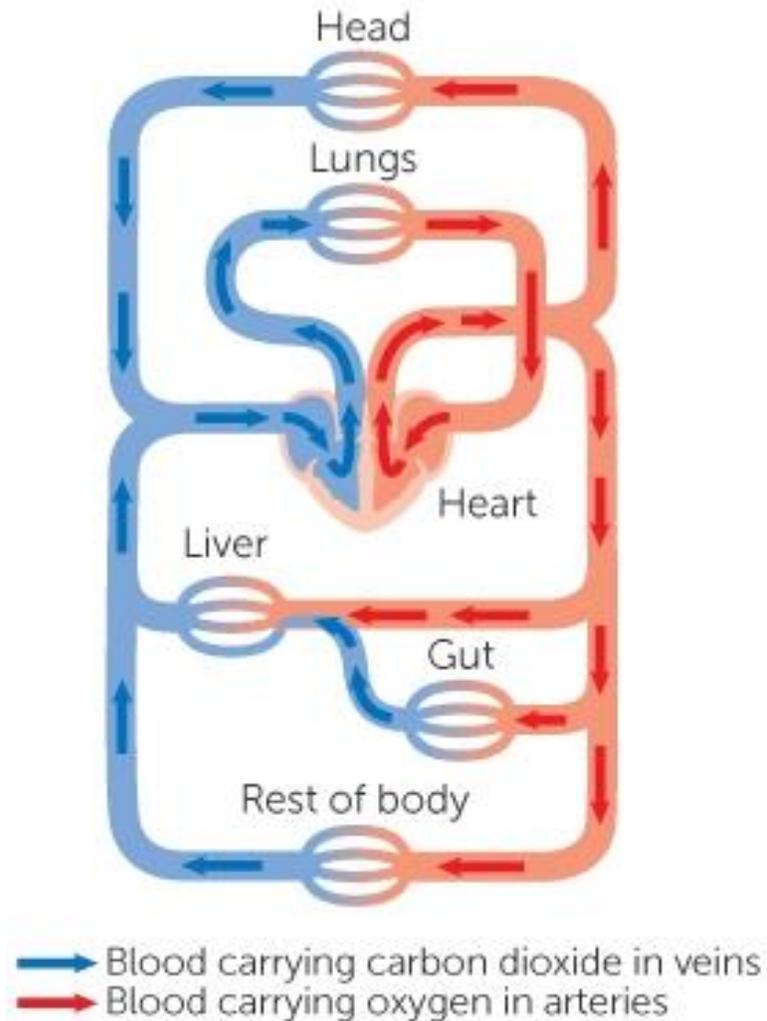
***In vitro* pO₂**
~160 mm Hg
(atmospheric air)

- oxygen tension plays an important role in cellular function
- *in vitro* environment should more closely resemble *in vivo* conditions
- tissue oxygenation in particular brain regions can vary

OXYGEN TERMINOLOGY

- **Hypoxia** is medically defined as: “a deficiency of oxygen reaching the tissues of the body.”
 - In its extreme form, where oxygen is entirely absent, the condition is called anoxia
- **Hyperoxia** is when cells, tissues and organs are exposed to an excess supply of O₂
- **Normoxia** not clearly defined though ‘assumed’ as to be the oxygen concentration at sea level
- Relevance in relation to the tissues/organs in the body >> **Physoxia**

ORGANS AND PHYSOXIA



	pO ₂	
	mmHg	%
Air	160	21.1
Inspired air (in the tracheus)	150	19.7
Air in the alveoli	110	14.5
Arterial blood	100	13.2
Venous blood	40	5.3
Cell	9.9–19	1.3–2.5
Mitochondria	<9.9	<1.3
Brain	33.8 ± 2.6	4.4 ± 0.3
Lung	42.8	5.6
Skin (sub-papillary plexus)	35.2 ± 8	4.6 ± 1.1
Skin (dermal papillae)	24 ± 6.4	3.2 ± 0.8
Skin (superficial region)	8 ± 3.2	1.1 ± 0.4
Intestinal tissue	57.6 ± 2.3	7.6 ± 0.3
Liver	40.6 ± 5.4	5.4 ± 0.7
Kidney	72 ± 20	9.5 ± 2.6
Muscle	29.2 ± 1.8	3.8 ± 0.2
Bone marrow	48.9 ± 4.5	6.4 ± 0.6

ORGANS AND PHYSOXIA

Cell	HYPOXIA	PHYSOXIA	HYPEROXIA
Kidney	<25mmHg	72mmHg	110mmHg
Intestinal tissue	<25mmHg	58mmHg	86mmHg
Skin (superficial region)	1mmHg	8mmHg	> 30mmHg

- Implications of culturing cells at non-physoxia pO₂ 141mmHg?

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KNOW AND DEFINE YOUR 'NORMOXIA' AND 'HYPOXIA'

- We are scientists we want to be the first to find new things not 'be unique'
 - Others need to be able to **reproduce** our great results to validate our findings and for that we need to standardize
- Transition from % O₂ to kPa or mmHg pO₂
- Culturing AND handling in O₂ regulated environment

HypoxyLAB™

- Compact and efficient
- Delivers “true physoxia” using the partial pressure of oxygen, imitating *in vivo* oxygen state
- Chamber rapidly equilibrates while consuming less gas
- **MODULATE Oxygen profiles!**
- Optional – digital microscope add-on

<https://www.scintica.com/products/oxford-optronix/hypoxia-workstation/>

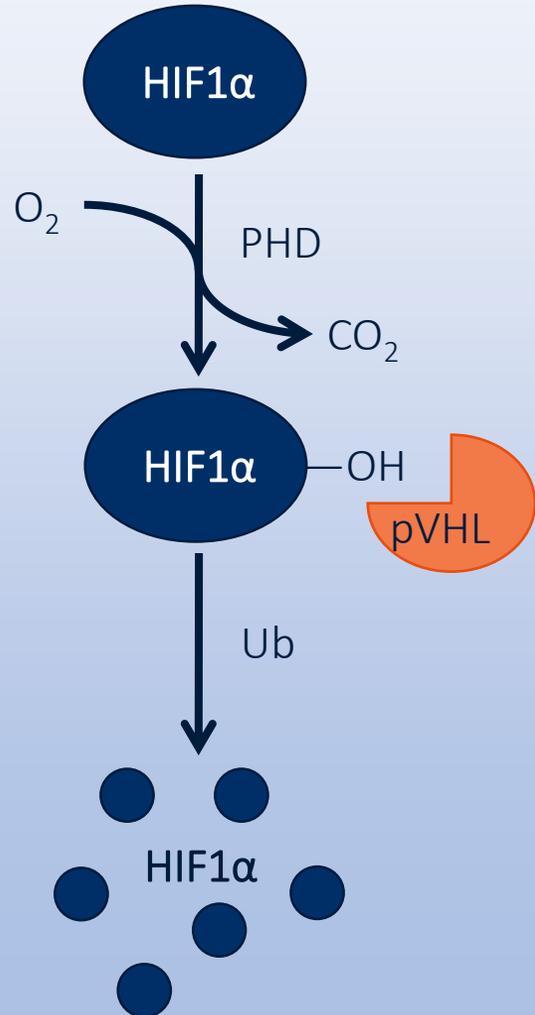


PHYSOXIA VS. HYPOXIA: HOW DOES A CELL KNOW

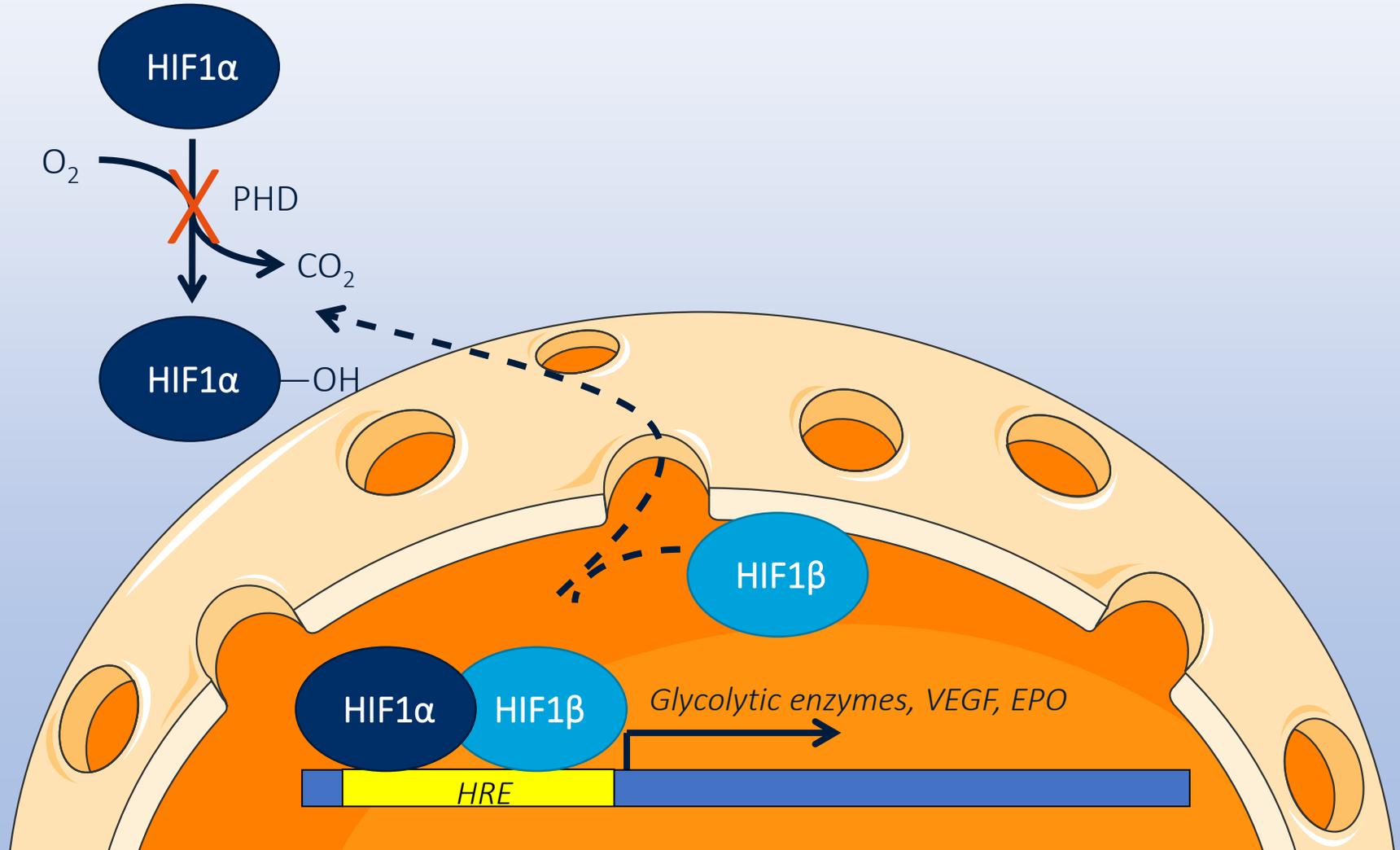
- 'Normoxia' \neq 'Physoxia' and likely considered hyperoxic
 - (Incubator air 141mmHg/18.9% vs. cells range ~40-100mmHg/ ~5-12% O₂)
- How do cells 'see' as hypoxia / physoxia / hyperoxia
- Hypoxia Inducible Factor (HIF)-1 is sort of the oxygen sensor of the cell

Hypoxia Inducible Factor (HIF)-1 α

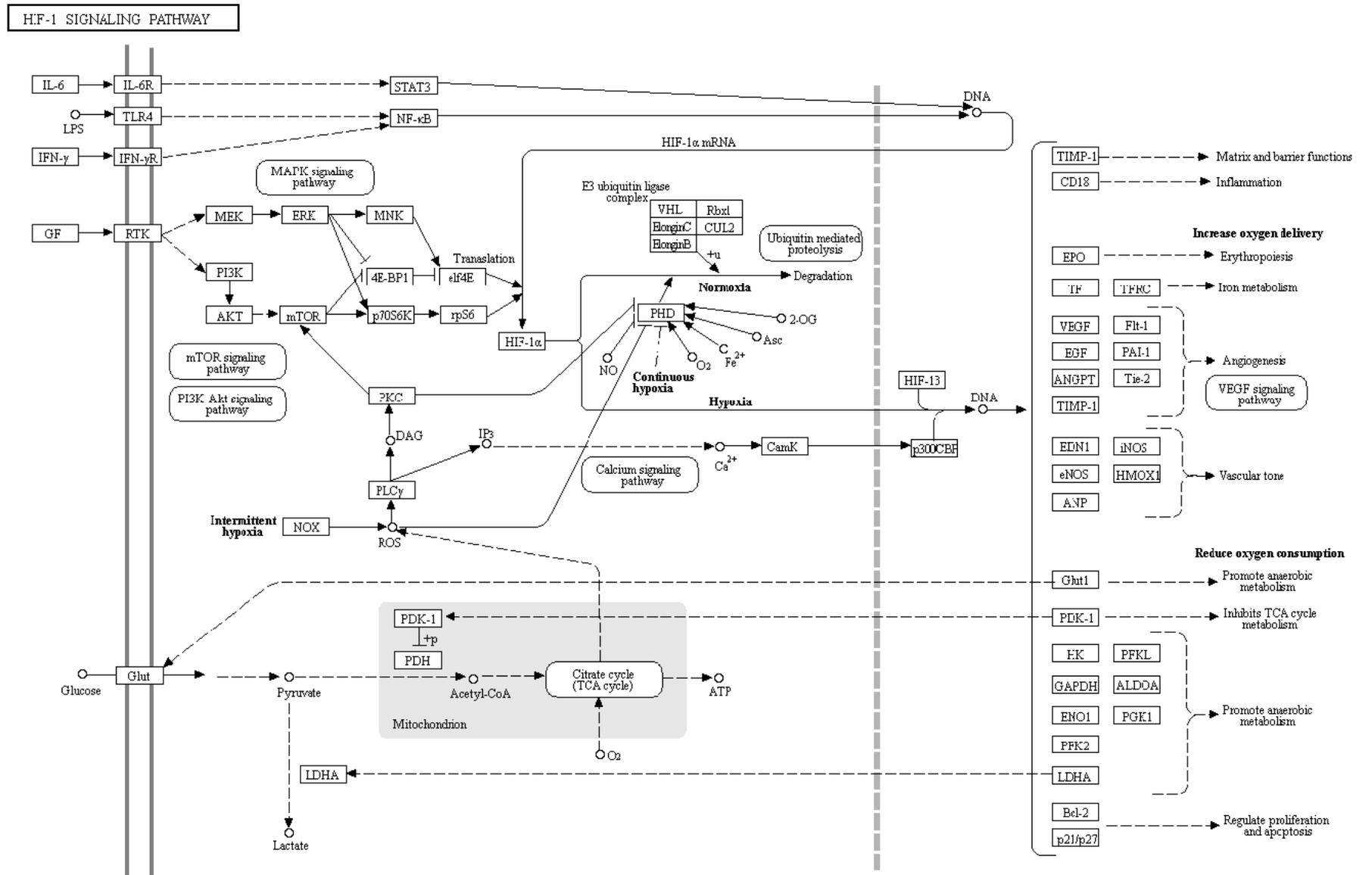
Normoxia



Hypoxia



HIF-1 SIGNALING PATHWAY

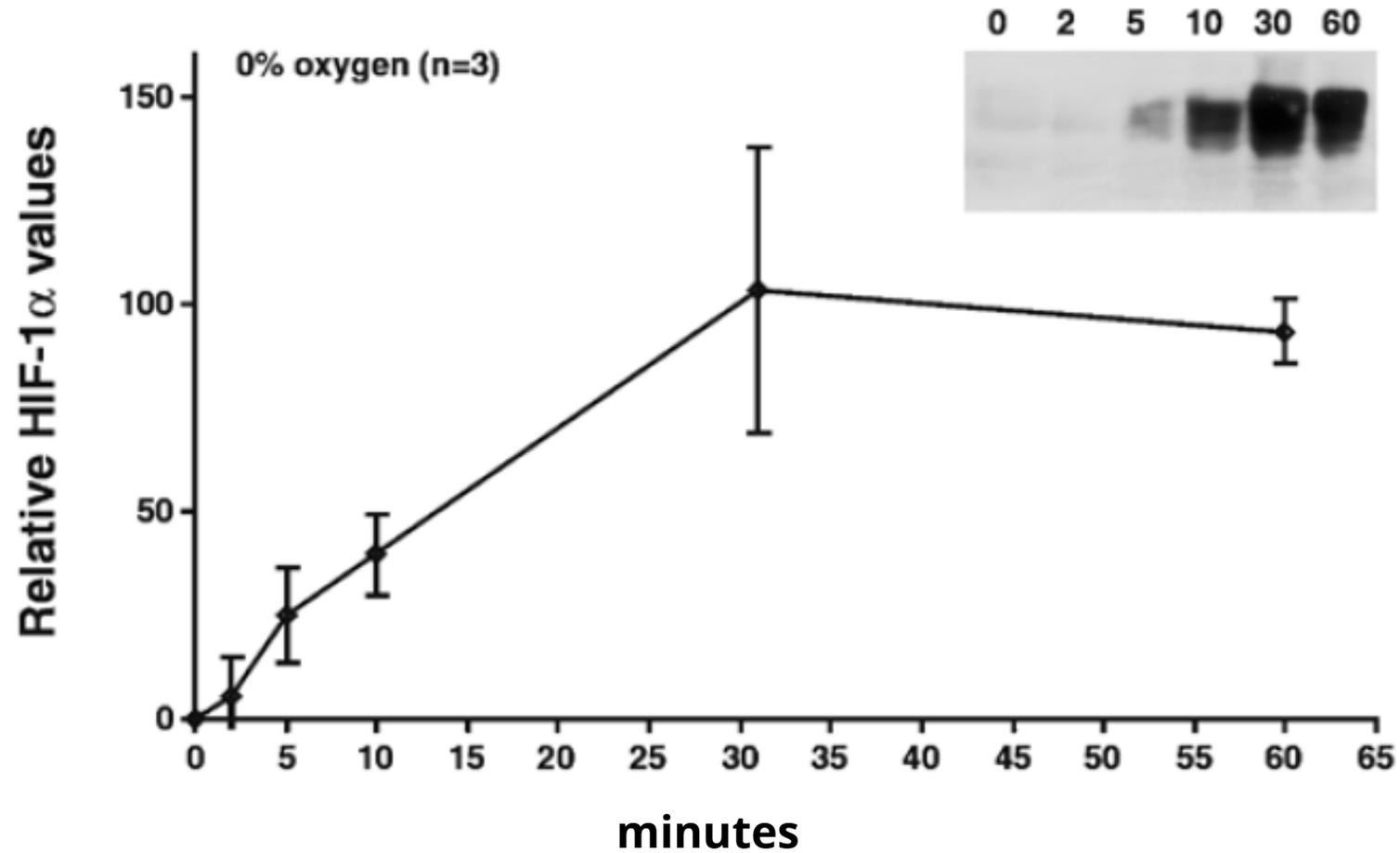


66 6/29/18
Kanehisa Laboratories

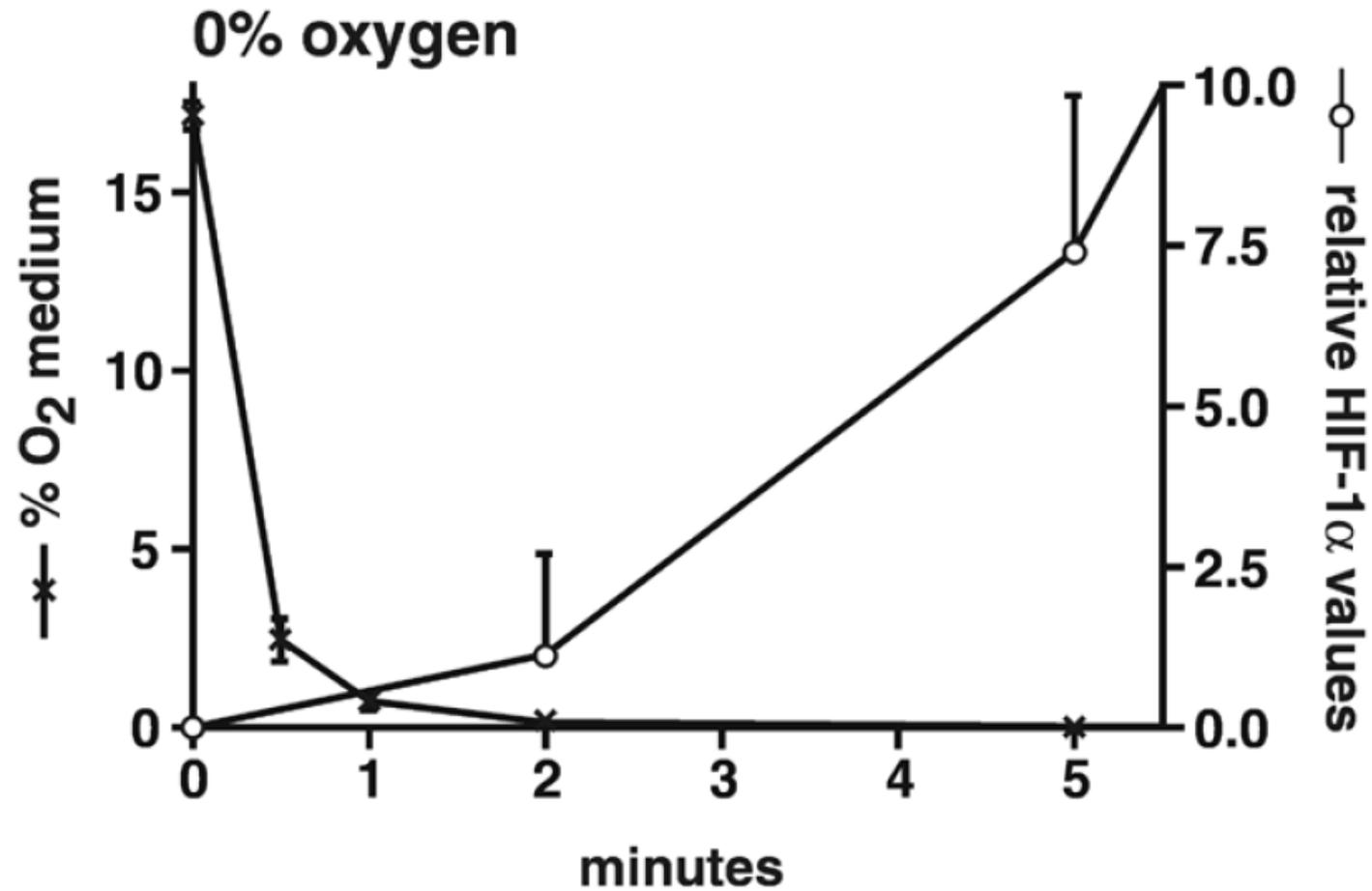
HIF-1 Co-FACTOR DRIVEN GENE EXPRESSION OF:

- Matrix and barrier function genes
- Inflammation
- Increase oxygen delivery (EPO, Heme)
- Angiogenesis
- Vascular tone
- Reduction of oxygen consumption
- Promote anaerobic metabolism
- Regulate cell proliferation and apoptosis

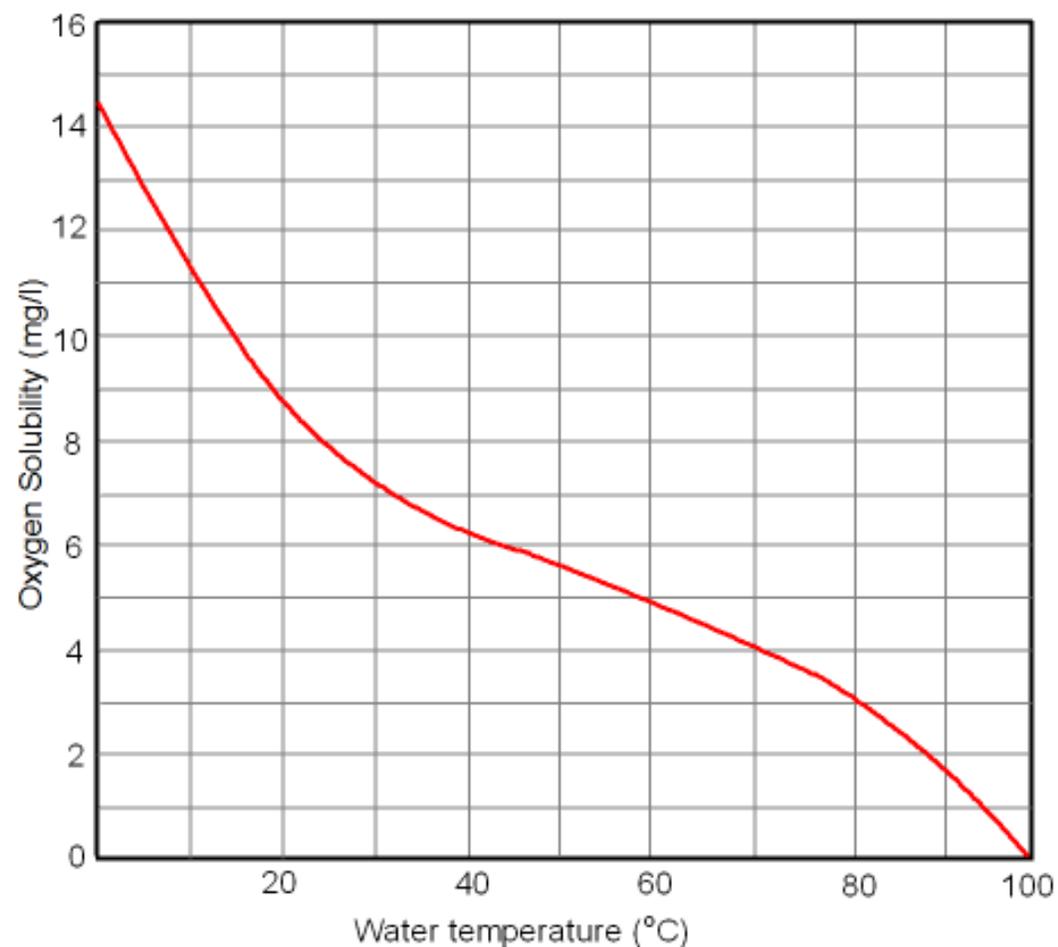
HIF-1 α RESPONSE O₂ NORMOXIA TO ANOXIA



HIF-1 α RESPONSE TO DEOXYGENATING MEDIA



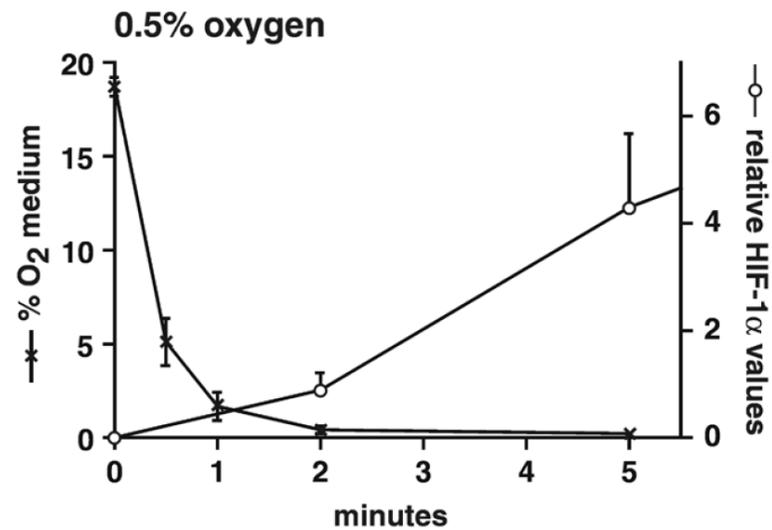
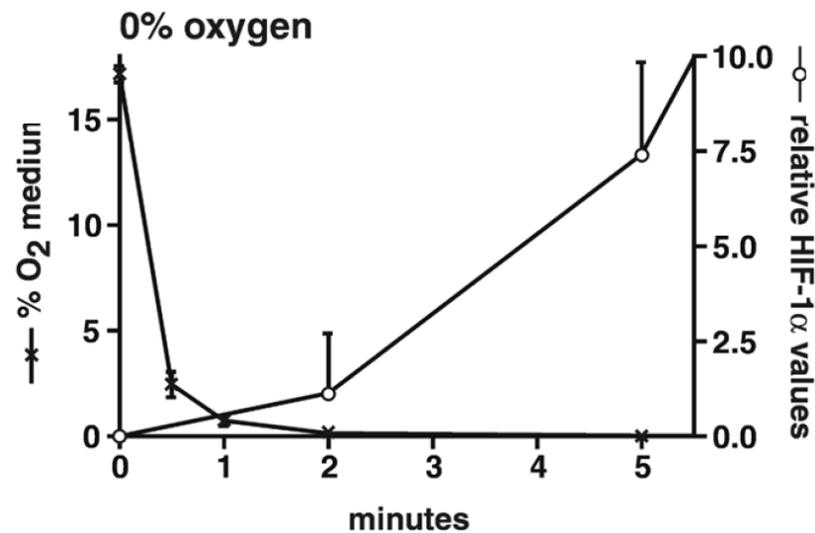
DE-GASSING/DE-OXYGENATING MEDIA



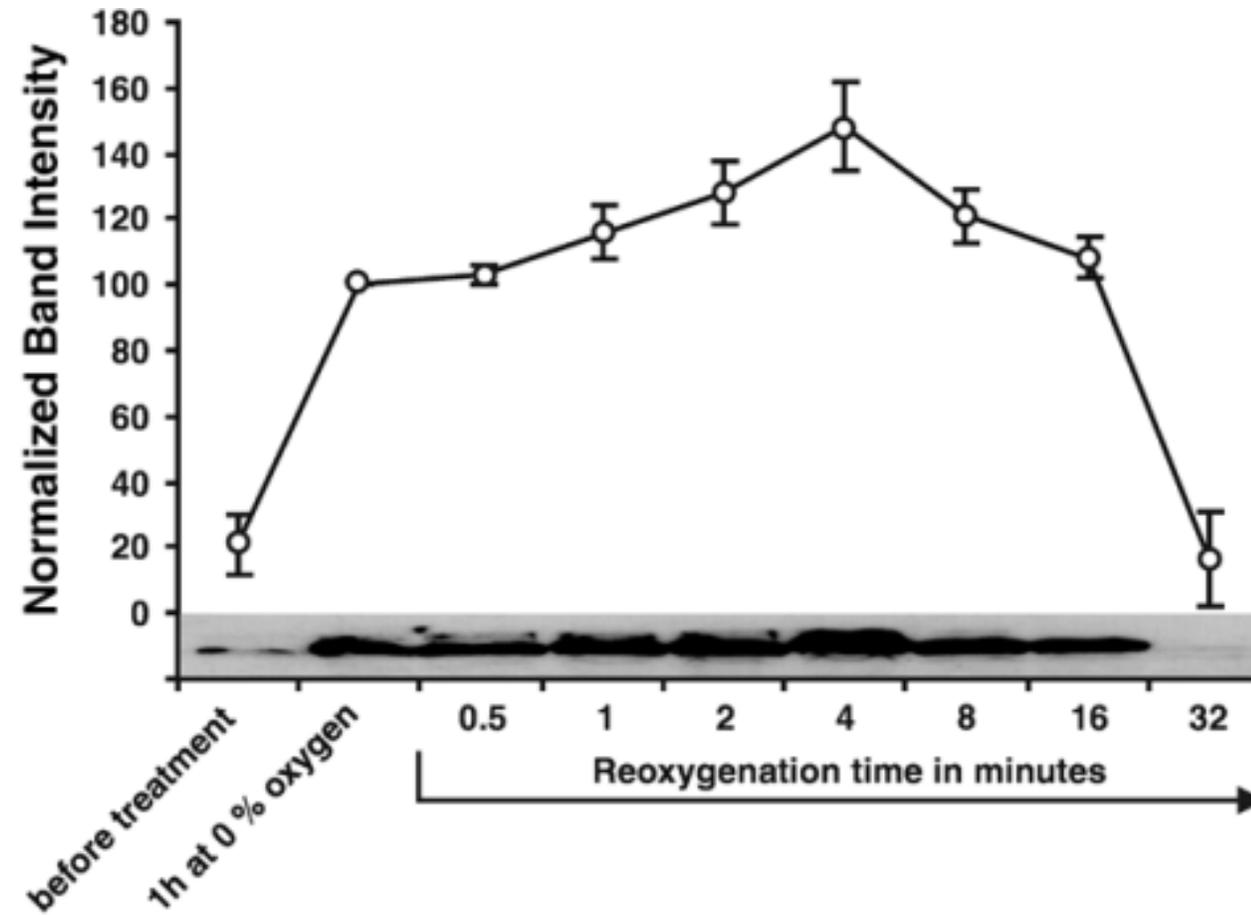
- O₂ dissolves less in higher temperatures
- Work smooth and fast **do not** bubble your media
- Device for deoxygenating and storing prepped media (HypoxyCOOL)

HypoxyCOOL

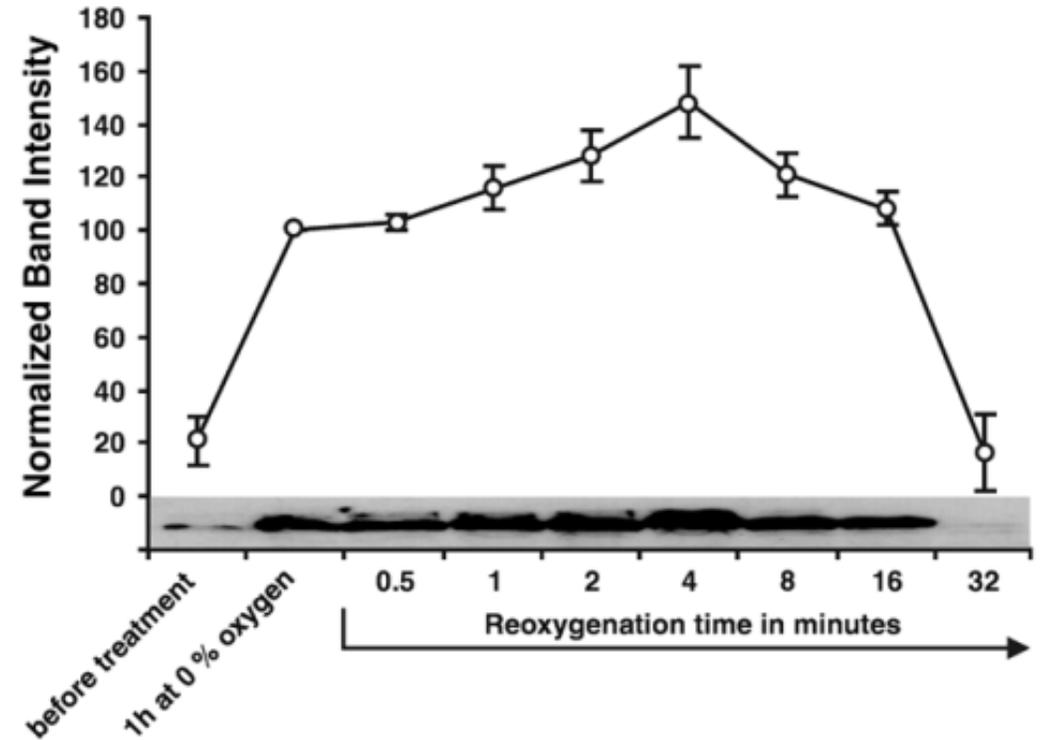
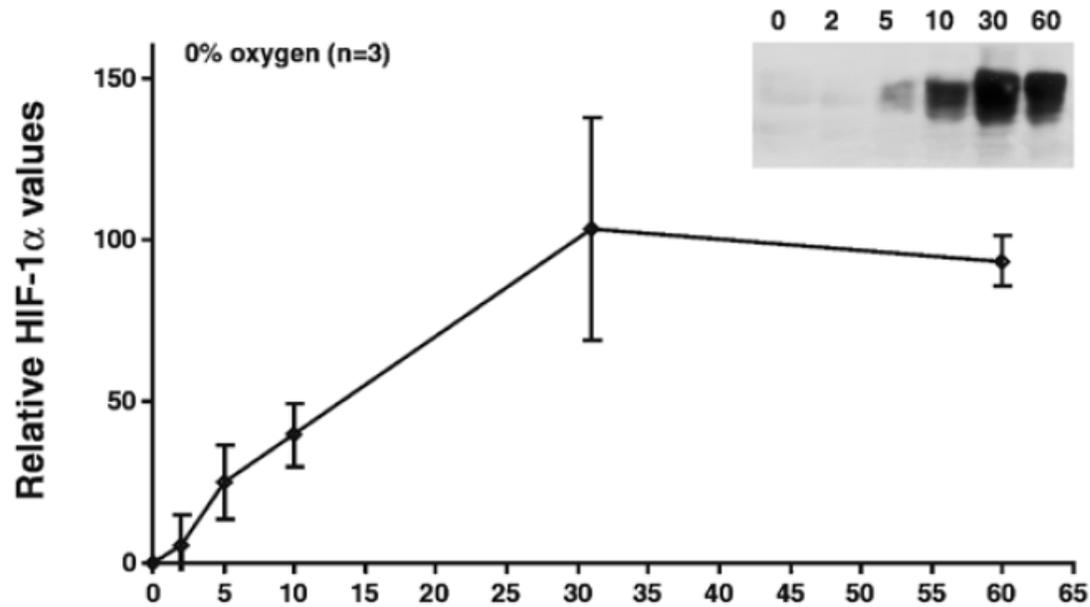
- Accelerated oxygen conditioning for tissue culture media



HIF-1 α RESPONSE TO REOXYGENATION



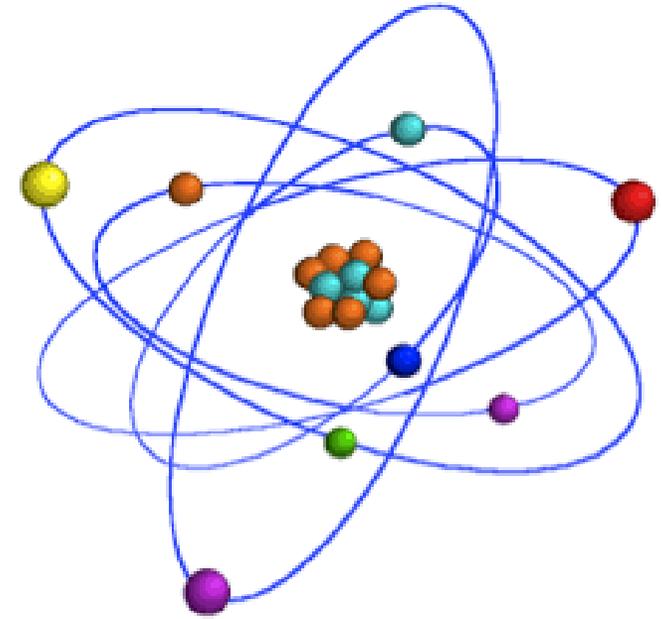
HIF-1 α RESPONSE TO DE- AND RE-OXYGENATION



- Setting Anoxia takes +30 minutes for cell to experience relative stable phase
- Re-oxygenation takes between 15-30 minutes for HIF-1 α to be fully degraded

O₂ free and bound in the blood

- O₂ Concentration of Blood
 - Dissolved O₂
 - Bound to heme (Hgb)
- $= 0.003 * pO_2 \text{ mmHg} + 1.4 * [\text{Hgb}] * [\%O_2\text{Sat}]$
 - Hgb (14 g/dL, 140 g/L)
 - Dissolved O₂ 0.3 mL/dL
 - Bound to heme (Hgb) 19.6 mL/dL
 - normal value: about 20 mL/dL



FREE RADICAL SCAVENGERS



DETERMINE O₂ WITH OXYLITE

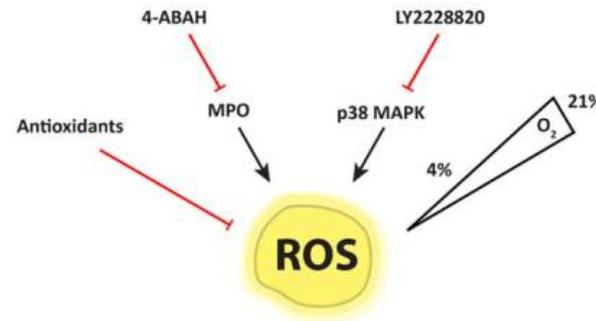
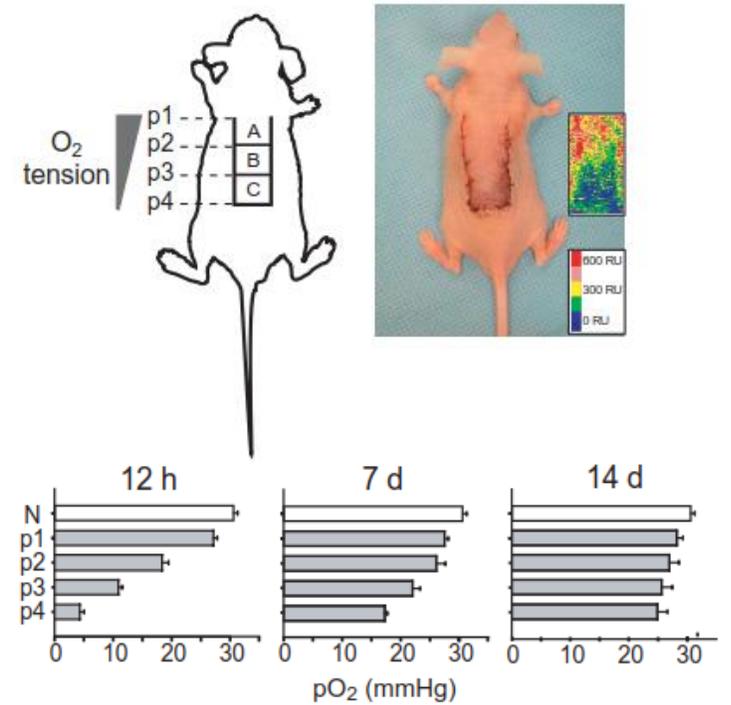
- OXYLITE
 - NON-oxygen consuming determination
 - Oxygen (pO₂)
 - Temperature monitor
 - Glass fiber probes
- Works both *In vitro* & *In vivo*
- Integratable with the HypoxyLab

<https://www.scintica.com/products/oxford-optronix/oxygen-monitors/>

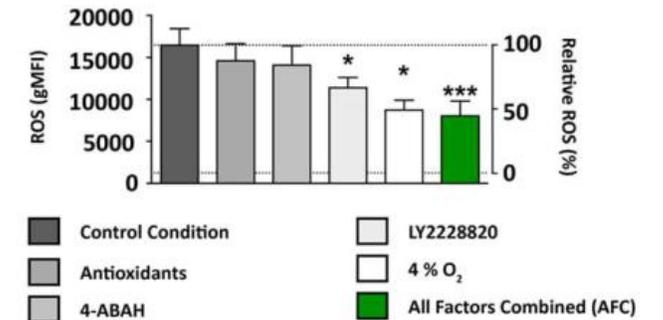


OXYGEN (HIF-1 α) RELATED SCIENTIFIC PUBLICATIONS

- Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1
 - Ceradini *et al.* 2014 Nature Medicine
- Reactive Oxygen Species Impair the Function of CD901 Hematopoietic Progenitors Generated from Human Pluripotent Stem Cells
 - Rönn *et al.* 2016 Stem Cells

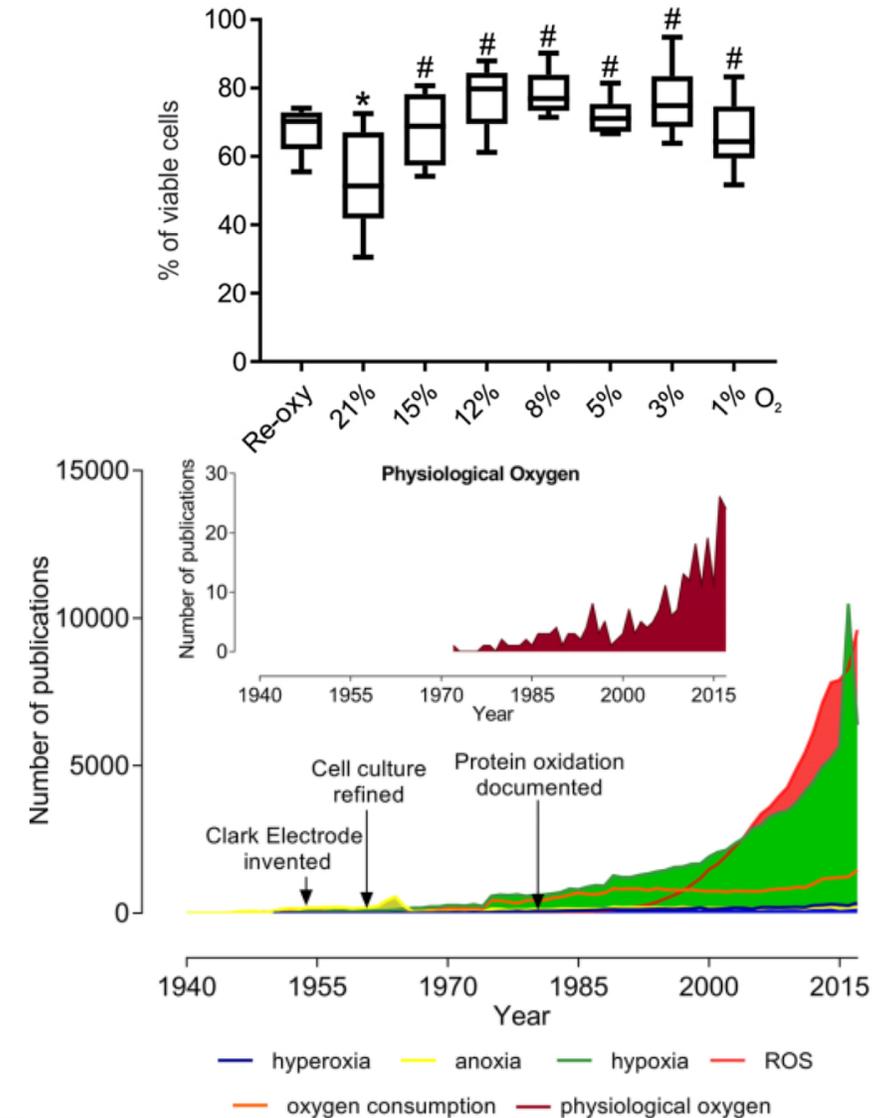


ROS Reduction, hPSC-Derived, CD43/45⁺CD34⁺



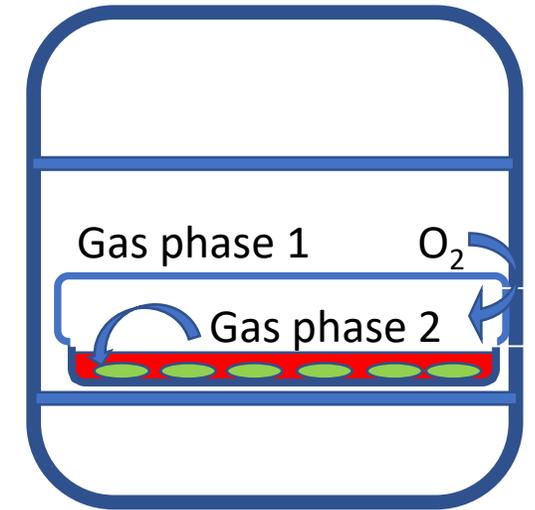
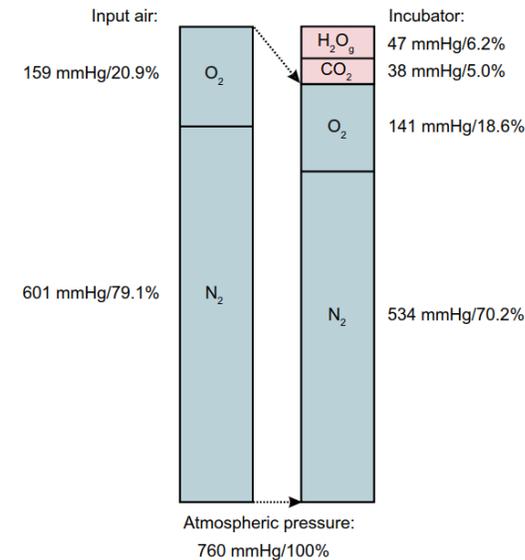
OXYGEN RELATED SCIENTIFIC PUBLICATIONS

- Physioxic human cell culture improves viability, metabolism, and mitochondrial morphology while reducing DNA damage
 - Timpano *et al.* 2019 FASEB
- Defining physiological normoxia for improved translation of cell physiology to animal models and humans
 - Keeley *et al.* 2019 Physiol Rev



SUMMARY

- % >> SI units kPa / mmHg for O₂ level
- Incubator at 37°C, 760mmHg pressure
 - p_{H₂O} is 47 mmHg >> 6.2% V/V
 - p_{CO₂} is 38 mmHg << 5.0% V/V
 - 'Air' 675mmHg >> 88.8% V/V
- Incubator pO₂ 141mmHg (Normoxia) >> 18.6%



The Physics Laws & constant

1. Dalton's Law of Partial Pressures
2. Henry's Law of Partial Pressures
3. Bunsen's Constant
4. Fick's Law of Diffusion

PHYSOXIA

- Breathing ~159 mmHg pO₂
- Lung alveolar tissue ~100 mmHg pO₂
- Arterial blood carrying ~75 - 100 mmHg pO₂
- Venous is between ~30 - 40 mmHg pO₂



TAKE HOME MESSAGES / FOOD FOR THOUGHT

- As scientists be the first to find new things not 'be unique'
 - Reproducible findings that can be validated
 - What are my cell culture O₂ levels and can I regulate O₂ levels to improve my reproducible results
- Reevaluate my previously obtained results
 - They are still usable, though you need to be aware of factors that could have skewed your perception/interpretation on the obtained results due to oxygen differences.

Q&A SESSION:



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To ask a question, click the Q&A Button, type your question and click send. Any questions that are not addressed during the live webinar will be answered following the event.

Thank you for participating!

