Otto Heinrich Warburg (1883–1970), was a German physiologist, medical doctor and Nobel laureate. He earned double doctorates in chemistry and medicine, and won the Nobel Prize in 1931, for his research into cellular respiration, showing that cancer thrives in anaerobic (without oxygen) or acidic conditions. His father was a highly respected physicist, and in Warburg's childhood such luminaries as Albert Einstein, Max Planck, Emil Fischer, and Walther Nernst were frequent dinner guests. OW was one of the twentieth century's leading biochemists. He was nominated an unprecedented three times for the Nobel prize for three separate achievements.

"Cancer, above all other diseases, has countless secondary causes. But, even for cancer, there is only one prime cause. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar."

Metal-O$_2$ Inorganic Biological Chemistry


B. G. Malmstroem (1997) *A life with the metals of life*. Selected Topics in the History of Biochemistry Comprehensive Biochemistry, 40, 277-331

M. Saraste (1999) *Oxidative Phosphorylation at the fin de siècle*. SCIENCE, 283, 1488-1493


Dioxygen Activation

\[ 3\Sigma^+ \]

\[ \sigma_p \]

\[ \pi^* \]

\[ \sigma_p \]

\[ \pi \]

\[ \sigma_s \]

\[ 3\Sigma^+ \]

\[ \text{E}_0 \text{ vs. NHE at pH 7.25} \]

\[ -0.33 \text{ V} \]

\[ +0.94 \text{ V} \]

\[ +0.38 \text{ V} \]

\[ +2.31 \text{ V} \]

\[ \text{O}_2^- \]

1.49 pm

112 pm

1554 cm\(^{-1}\)

133 pm

1145 cm\(^{-1}\)

\[ \text{H}_2\text{O}_2 \]

1.49 pm

842 cm\(^{-1}\)

\[ \cdot\text{OH}+\text{H}_2\text{O} \]

\[ 2\text{H}_2\text{O}_3 \]
Activation of $O_2$ – Reaction Types

- Reversible binding of $O_2$ – Myoglobin, Hemoglobin (Fe), Hemocyanin (Cu-Cu)
- $O_2^-$ dismutation – Superoxide Dismutase (Mn, Fe, Ni, Cu, Zn)
  \[ O_2^- + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \]
- $H_2O_2$ decomposition – Catalase (Mn, heme-Fe)
  \[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]
- Oxygenases (focus on Monooxgenase Cytochrome P450)
  \[ R-H + O_2 + NADPH + H^+ \rightarrow R-OH + H_2O + NADP^+ \]
- Oxidases (2-electron reduction to $H_2O_2$; Fe, Cu)
  \[ O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \] (focus on Cu enzyme Galactose Oxidase)
- Oxidases (4-electron reduction to $H_2O$; heme-Fe, Cu)
  \[ O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \] (focus on Cu enzyme Ascorbic Acid Oxidase and Fe,Cu enzyme Cytochrome $c$ Oxidase)
**O$_2$ activation by Metallo-Oxygenases**

Mechanisms involve the formation of an initial O$_2$ adduct (superoxo), conversion to a metal–peroxide (peroxo), and subsequent O–O bond cleavage to yield a high-valent oxidant (oxo). Oxygen atoms involved are shown in red. M, metal; P, porphyrin.

Reversible $O_2$ Binding
Myoglobin and Hemoglobin

The iron must be in the Fe(II) (ferrous oxidation) state.
Binding of $O_2$ rearranges the electronic distribution and alters the d orbital energy.

This causes a difference in the absorption spectra.

Bluish for deoxy Hb, Redish for Oxy Hb

Measuring the absorption at 578 nm allows an easy method to determine the percent of $O_2$ bound to hemoglobin.
Hemocyanin (reversible $O_2$ binding)

Oxygenated Cu site, see Que, Tolman, NATURE (2008) 455, 333; oxygenated form has a blue colour, $\mu$-$\eta^2:\eta^2$-peroxo binding mode

Cu,Zn Superoxide Dismutase (SOD)

PDB code 1SPD http://en.wikipedia.org/wiki/Superoxide_dismutase

\[ M^{(n+1)+}-SOD + O_2^- \rightarrow M^{n+}-SOD + O_2 \]
\[ M^{n+}-SOD + O_2^- + 2H^+ \rightarrow M^{(n+1)+}-SOD + H_2O_2 \]
HUMAN ERYTHROCYTE CATALASE


Porphyrin Radical Cation
O=Fe(IV)-E(·+) is involved in the reaction mechanism
http://en.wikipedia.org/wiki/Catalase

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Fe(III)}-\text{E} &\rightarrow \text{H}_2\text{O} + \text{O}=\text{Fe(IV)}-\text{E(·+)} \\
\text{H}_2\text{O}_2 + \text{O}=\text{Fe(IV)}-\text{E(·+)} &\rightarrow \text{H}_2\text{O} + \text{Fe(III)}-\text{E} + \text{O}_2
\end{align*}
\]
Tyrosine acts as axial ligand

Maté et al. (2001) (Messerschmidt, Huber, Poulos, Wieghardt (eds)) Handbook of Metalloproteins, John Wiley & Sons, LTD

Fe(IV)=O state
Cytochrome P450

Trans-Effect - Tuning reactivity

A ligand X \textit{trans} to a second ligand Y can influence the stability of the M-Y bond. With X being a strong Lewis base, the M-Y bond will be weakened.

\[
\text{RH} + \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{R-OH} + \text{H}_2\text{O}
\]
Typical Reactions of Cytochrome P450 (O-Transfer)

J. Rittle, M. T. Green (2010), SCIENCE 330, 933-936
Cytochrome P450 – important enzyme for detoxification of organic compounds

- Single \( b\)-type heme group
- Cys thiolate as proximal axial ligand
- Triangular prism
- No domain structure, but a more \( \alpha\)-helical half and a half containing \( \beta\)-strands.
- High degree of structural conservation within the family.
Camphor substrate complex of Cytochrome P450

Multiple substrate complexes of P450s were readily obtained, but no intermediates of the reaction cycle.

In the P450cam substrate complex, CO binds to heme, presumably analogous to $O_2$.  

Cytochrome P450cam; PDB-ID 3CPP  
Cytochrome P450 Reaction Cycle

Fe(II)OH₂ → Fe(III)O₂⁻ → Fe(III)OH → Fe(III)-OOH → Fe(V)=O

H⁺ + e⁻ → Fe(III)OH

Fe(II)OH₂ + O₂ → Fe(III)OH + H₂O

compound I

key intermediate
Galactose Oxidase, a Cu-Ligand Radical Enzyme

\[
RCH_2OH + O_2 \xrightarrow{\text{GalOx}} RCHO + H_2O_2
\]

2 different EPR signals (metal-centered and ligand centered) observed during reaction cycle.
Galactose Oxidase - Mechanism

Que, Tolman (2008) NATURE 455, 333
Galactose Oxidase - Mechanism

Que, Tolman (2008) NATURE 455, 333
History: The discovery of a new Copper Center


1939: Laccase, a Blue Copper-Protein Oxidase from the Latex of Rhus succedanea
1959: An Electron Spin Resonance Study of the State of Copper in Fungal Laccase (a blue Multi-Copper oxidase)

Type 1 Blue Copper Electron Transfer Center
Plastocyanin: Blue Type 1 Cu Site

Function: Electron Transfer Protein/Photosynthesis

Covalent Cu-Cys π-bond is mainly responsible for its unique properties
El Solomon, Inorg. Chem. 2006, 45, 8012-8025

PDB Code: 1PLC
HC Freeman, 1978

Cu(II) Spin-Distribution

41% Cu
38-45% S
Ascorbic acid oxidase (AOX)
Multi-Copper oxidase (8Cu/homodimer) PDB code 1AOZ

(5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one
Type 1 Cu ET center in AOX

A. Messerschmidt, Handbook of Metalloproteins (2001)

Entrance point for electrons
β-barrel structural module
Trinuclear Cu center in AOX
Dioxygen reduction site
Reduced Trinuclear Site and Peroxide Adduct of AOX
Longe Range Electron Transfer through Protein

Early experiments by H Gray and J Winkler/Caltech

RA Marcus Nobel Prize Chemistry 1992

Ru-Complex covalently attached to Fe protein cytochrome c
Electron transfer: Marcus Theory

\[ k_{\text{ET}} = \frac{|H_{AB}|^2}{\hbar^2} \sqrt{\frac{\pi \hbar}{\lambda k_B T}} e^{\frac{(\lambda+\Delta)^2}{4\lambda k_B T}} \]

\[ \Delta G^0 = \text{Driving force} \quad (\propto \text{difference of potentials donor vs acceptor}) \]

\[ H_{AB} = \text{Electronic Coupling} \quad (\text{“overlap of orbitals”}) \]

\[ \lambda = \text{Reorganization energy} \quad (\propto \text{structural change upon ET}) \]
Low (zero) Reorganization Energy

Low-Spin Heme center

199.4 pm $\text{Fe}^{III}$ $\text{N}_{\text{His}}$ $\text{N}_{\text{His}}$ $\text{N}_{\text{His}}$

200.9 pm $\text{Fe}^{II}$ $\text{N}_{\text{His}}$ $\text{N}_{\text{His}}$

Reorganization Energy
in Cytochromes $\leq 4$-$5$ kcal/mol

Opening of Substrate Binding Sites in Enzyme

3,4 Protocatechuate Dioxygenase

Cytochrome c oxidase (COX), a redox-driven proton pump

Proton-Coupled Electron Transfer in COX

\[
4 \text{cyt} c^{2+} + 8 H^+ + O_2 \rightarrow 4 \text{cyt} c^{3+} + 4 H_0^+ + 2 H_2O
\]
Cytochrome c oxidase


O$_2$ + 4H$^+$ + 4H$_i^+$ + 4e$^-$ → H$_2$O + H$_2$O + 4H$_o^+$ (+ 818 mV)

N$_2$O + 2H$^+$ 2e$^-$ → N$_2$ + H$_2$O (+1355 mV)
2NO + 2H$^+$ + 2e$^-$ → N$_2$O + H$_2$O (+1175 mV)

metals (CuA, Fe-heme, Mg, Zn)

e$^-$ transfer (redox; tyrosyl radical ?), H$^+$transfer (pump)

metal centers: CuA → ET; Fe-CuB → O$_2$ reduction
Cytochrome/Heme Types

Heme $a$

Heme $b$

Heme $c$
Cytochrome c oxidase

M Saraste Science (1999) 283,1488-1493

Published by AAAS
Mitochondrial Cytochrome c oxidase (COX)
(representation of the monomer from bovine heart/13 subunits)
Tsukihara et al., SCIENCE 1995, 269, 1069; Yoshikawa et al., SCIENCE 1998, 280, 1723

Bacterial COX from Pseudomonas denitrificans
(three subunits; Iwata et al., NATURE, 1995, 376, 660)
Metal Centers in bacterial COX

Cu Fe Mg
NO Reductase (NOR)

Site of O₂ Reduction
(Fe(III)-Cu(II) State – covalent link Tyr-His)
Active Sites of NOR (A) and COX (B)
Schematic Representation of the Catalytic Cycle
(no release of toxic ROS; tyrosine radical Y)

Fe^{III} \text{HO}^- \text{-Cu}^{+II} \rightarrow \text{Fe}^{+II} \text{Cu}^{+I} \rightarrow \text{Fe}^{+II} \text{-O}_2 \text{Cu}^{+I} \rightarrow \text{Y} \cdot \text{Fe}^{+IV} = \text{O}^{2-} \text{-HO}^- \text{-Cu}^{+II}
Proton Transfer Pathways
(D Asp 124 & E Glu 78) in *P. denitrificans* COX
Aspartate 51 (D51/51) – Site of Proton Pumping?
(observation of a redox induced conformational change)
Sir Humphry Davy (1800)
Researches, chemical and philosophical—chiefly concerning nitrous oxide or dephlogisticated nitrous air, and its respiration.

What Is Nitrous Oxide?
A former nitrous addict and expert on addiction explains why laughing gas is no joke....
By Maia Szalavitz | @maiasz | January 26, 2012.
Actress D... M....’s trip to the emergency room was reportedly spurred by symptoms of a seizure triggered by inhaling the drug nitrous oxide, more commonly known as laughing gas or whippits.

N₂O has many uses....
Nitrous Oxide – Dinitrogen Monoxide
Laughing Gas – Sweet Air

\[
\begin{align*}
\Theta & \leftrightarrow \Theta \\
\mid & \equiv N – \bar{O} \\
\end{align*}
\]

A Structurally Characterized Nitrous Oxide Complex of Vanadium
\[ \text{N}_2\text{O} \rightarrow \text{NO}_x \rightarrow \text{Ozone effects} \]

Stratosphere

\[ \text{N}_2\text{O} \rightarrow \text{Climate effects} \]

Troposphere

**MENACING GAS**

Agriculture is the primary source of \text{N}_2\text{O} emissions worldwide

<table>
<thead>
<tr>
<th>THOUSANDS OF METRIC TONS</th>
<th>N\textsubscript{2}O EMISSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural soils</td>
<td>8,005</td>
</tr>
<tr>
<td>Other agricultural activities</td>
<td>885</td>
</tr>
<tr>
<td>Manure management</td>
<td>728</td>
</tr>
<tr>
<td>Fossil fuel combustion</td>
<td>703</td>
</tr>
<tr>
<td>Production of adipic &amp; nitric acids</td>
<td>531</td>
</tr>
<tr>
<td>Biomass combustion</td>
<td>108</td>
</tr>
<tr>
<td>Other nonagricultural activities</td>
<td>60</td>
</tr>
</tbody>
</table>

**NOTE:** Projected values for 2010, **SOURCE:** EPA

http://epa.gov/climatechange/emissions/usinventoryreport.html
Bacterial Nitrous Oxide Reductase is a purple Copper Enzyme

\[ \text{N}_2\text{O} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{N}_2 + \text{H}_2\text{O} \]

\[ E'_o = +1.35 \text{ V} \]

kinetically inert molecule

~ 59 kcal/mol activation barrier
Bacterial Nitrous Oxide Reductase is a head-to-tail homodimer, with 6 Cu/monomer

There are two novel Copper-Sulfur sites (A) the dinuclear CuA, (B) the tetranuclear CuZ
Protein Crystallography and in crystallo Chemistry

• Crystals grown in N₂/H₂ atmosphere, flash-frozen in liquid nitrogen

• N₂OR crystals are stable, high diffraction quality

Data collection at Paul Scherrer Institute, Swiss Light Source, Villigen (CH)
In Bio EPR nothing compares to the returns from a standard X-band instrument (9-10 GHz)

W Zumft
Microbiologist

CuA is Mixed-Valence Cu$_2$S$_2$ Rhomb, S=1/2
formal oxidation state of Cu 1.5$^+$, 1 unpaired electron/2Cu

F Neese

Cu-Cu Bond? (Metallic Cu)
CuA is also present in respiratory Cytochrome c oxidase

Cu Fe Mg

Electron entrance

Pathways for electrons & protons

O₂ reduction site
O₂ → H₂O
Evolution and Bioengineering through Loop directed Mutagenesis
From a Blue Mononuclear Cu to a Purple Dinuclear CuA
CuZ is a Copper Sulfide ($S^{2-}$) complex which is oxygen-sensitive. First Xtal structures showed oxygen-ligands coordinated instead of sulfide. CuZ was proposed to activate $N_2O$ upon binding at position X.


$$\begin{align*}
\text{Cu}_4^{II}S^{2-} & \quad 6^+ \\
\text{Cu}_3^{II}\text{Cu}_1^{I}S^{2-} & \quad 5^+ \\
\text{Cu}_2^{II}\text{Cu}_2^{I}S^{2-} & \quad 4^+ \\
\text{Cu}_1^{II}\text{Cu}_3^{I}S^{2-} & \quad 3^+ \quad S=1/2 \\
\text{Cu}_4^{I}S^{2-} & \quad 2^+ \quad S=0
\end{align*}$$
When prepared/crystallized under the strict exclusion of O$_2$, CuZ is a Cu$_4$S$_2$ Cluster
Upon binding of N$_2$O (Xtal under N$_2$O pressure) the ligands of CuA rearrange and His 583 flips to ligate Cu1 of CuA.
Catalysis: CuA and CuZ operate *in concert*.

Xtal under $\text{N}_2\text{O}$ pressure.
Take Home Message

“Dear Lord, I fall upon my knees and pray that all my syntheses may cease to be inferior to those conducted by bacteria”


Bacteria are outstanding Chemists
To advance in SCIENCE and ARTS, YOU need a good EDUCATION, YOU must have IDEAS, even some crazy ones, however, 95-98% will be HARD WORK...