MiP*school* 2008
Schröcken, July 2008
http://www.mitophysiology.org/index.php?id=mip-textbook

Mitochondrial Respiratory Physiology.

Mitochondrial respiratory control: Electron transport system, oxidative phosphorylation and leak – *ETS*, *OXPHOS* and *LEAK*.

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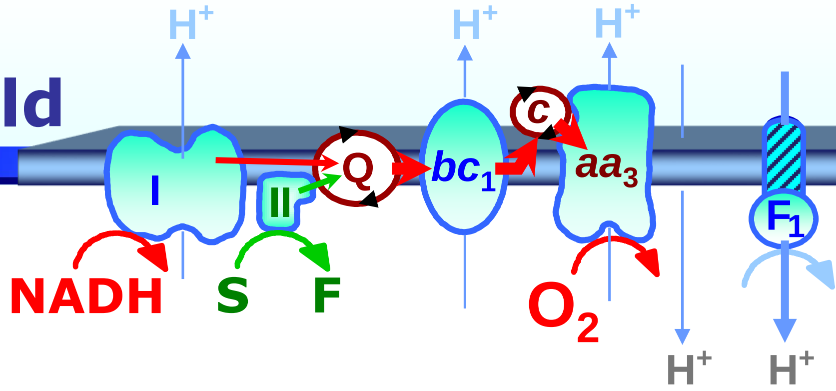
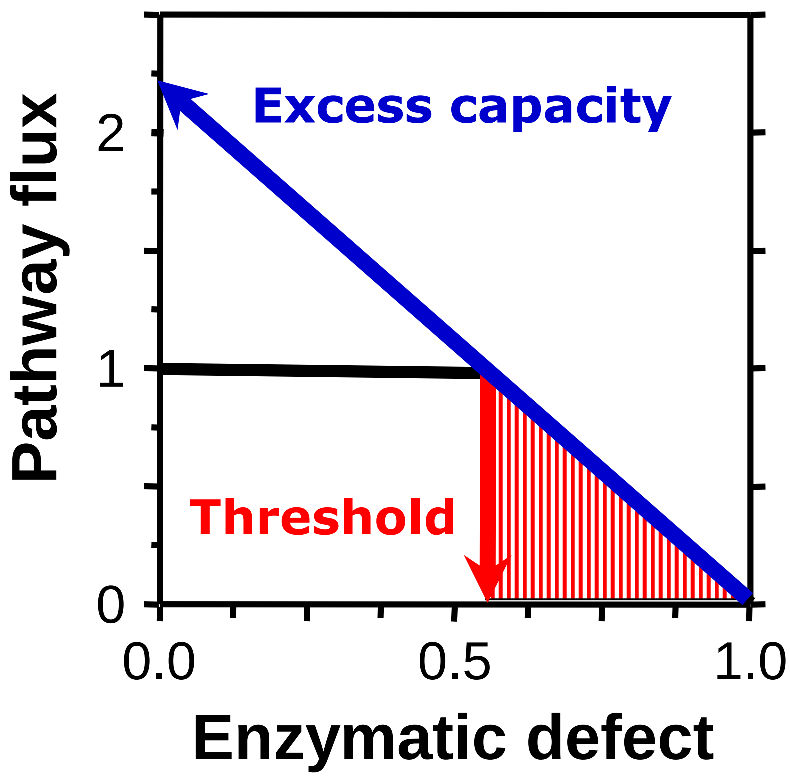
Excess Capacity and Biochemical Threshold

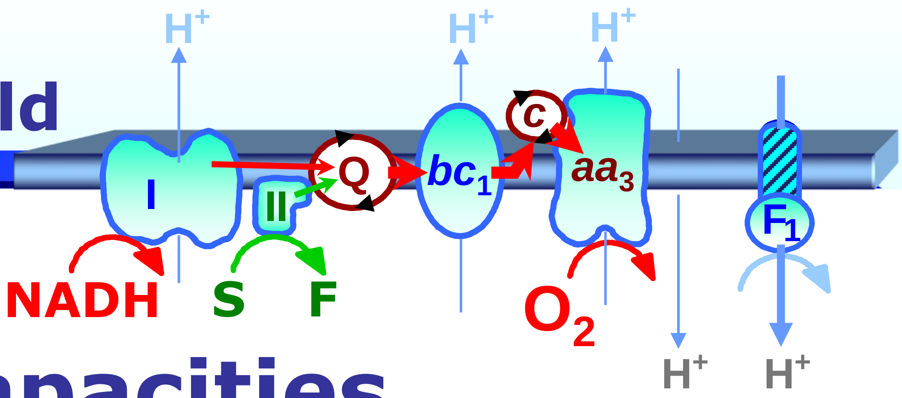


Excess capacity: Insurance against a specific enzymatic injury.

Biochemical threshold: Cellular function is buffered against a specific enzymatic defect.



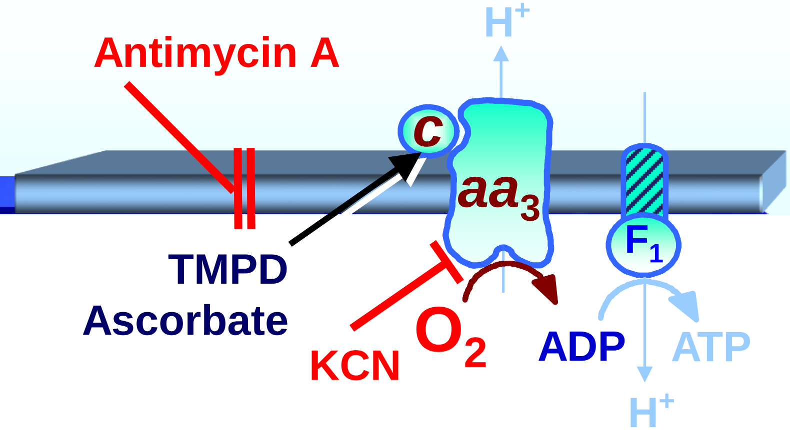
Excess Capacity and Biochemical Threshold



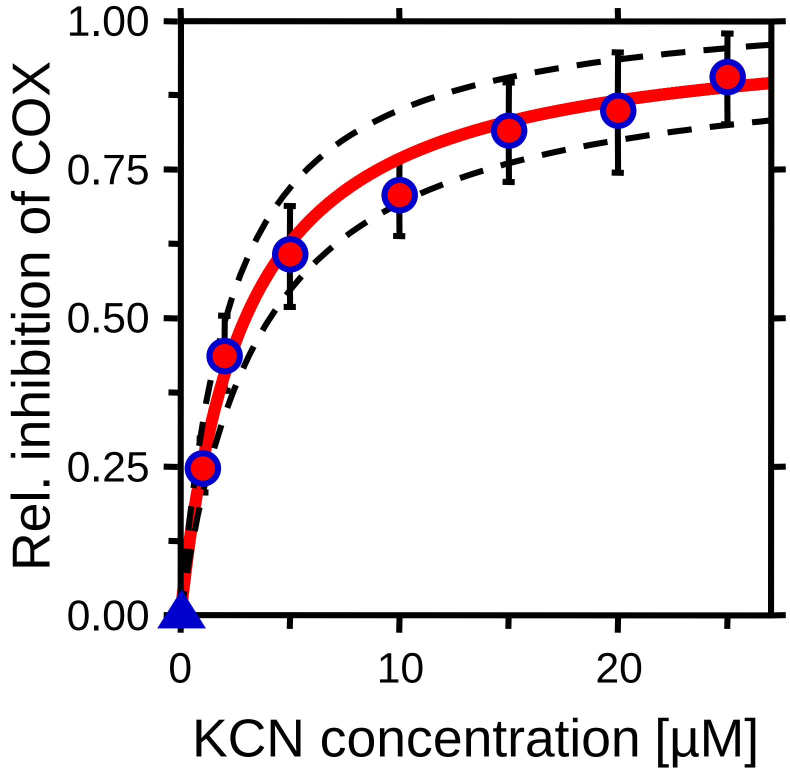
Different excess capacities imply tissue-specific (in)sensitivity to enzymatic defects in:

- **genetic mitochondrial disorders**
- **aging**
- **ischemia-reperfusion injury**
- **degenerative diseases**

Cytochrome *c* Oxidase



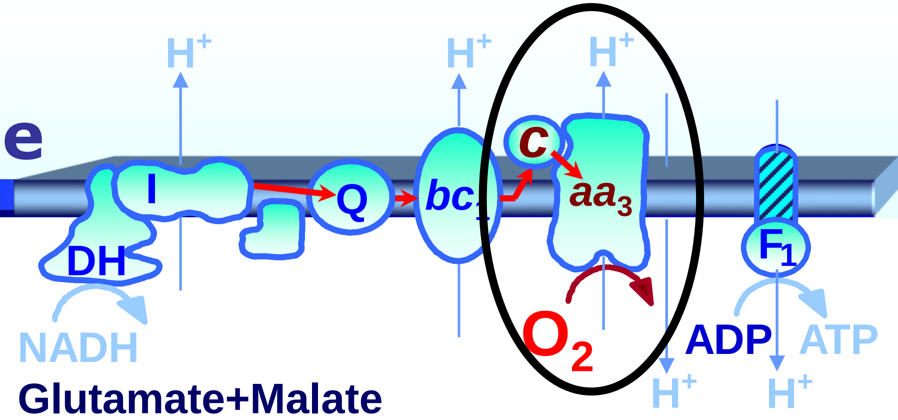
KCN Titration



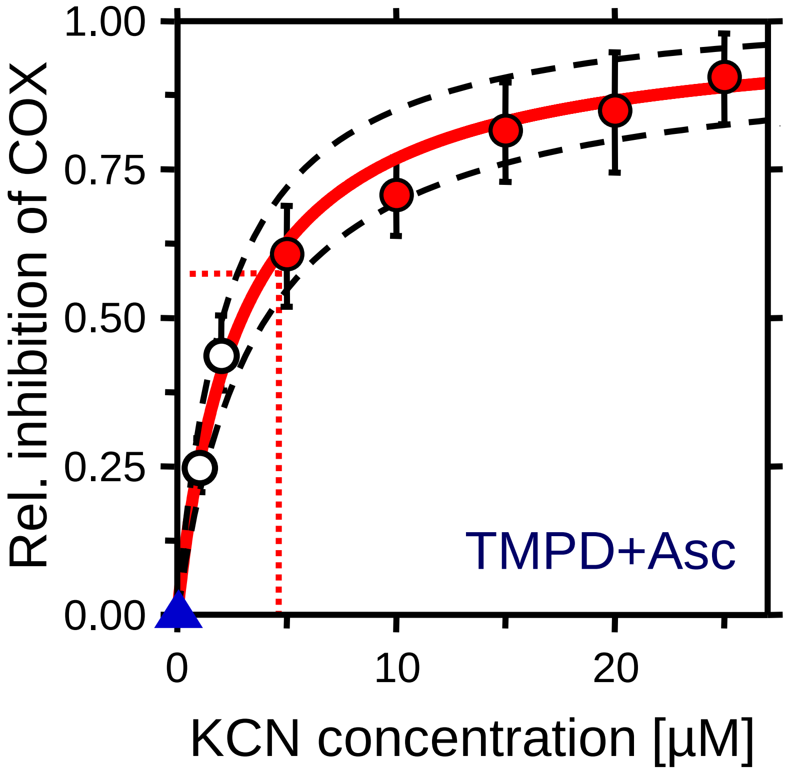
Isolated step in intact isolated mitochondria:

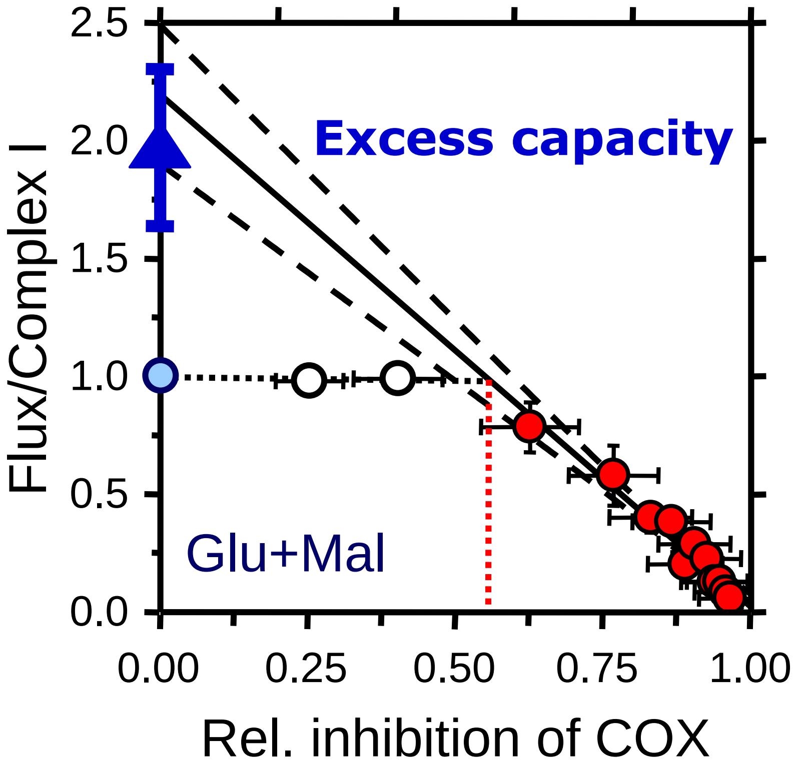
- ▲ TMPD+Ascorbate
- ● Cyanide titration

Electron Transport Chain and Cytochrome *c* Oxidase



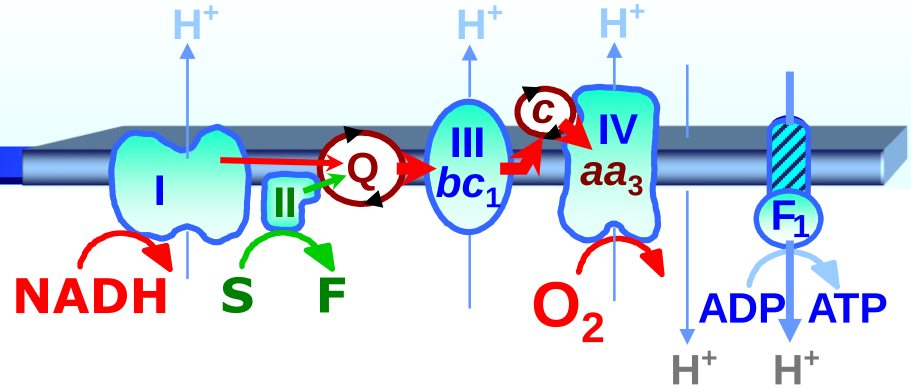
Excess capacity





0.5 mM TMPD + 2 mM ascorbate: 2-fold relative COX capacity

Electron Transport System



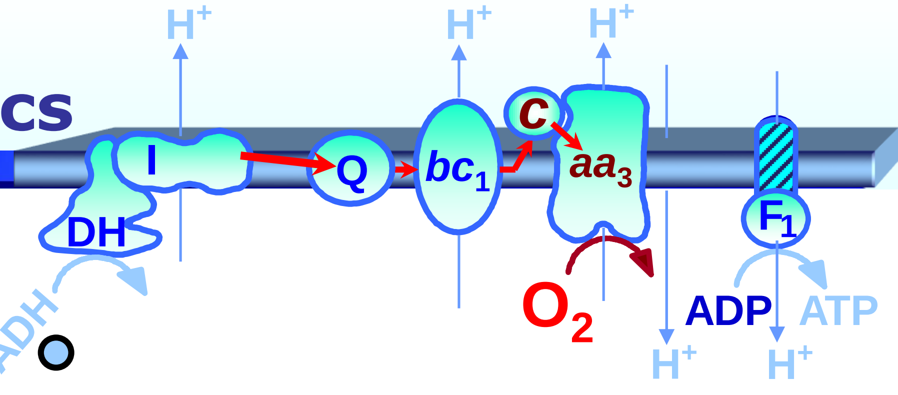
Which metabolic state represents electron transport capacity?

A. **Definition of *ETS* capacity.**

B. **Measurement in mitochondria and permeabilized cells.**

C. **Measurement in intact cells.**

Conventional Protocol
Derived from Bioenergetics



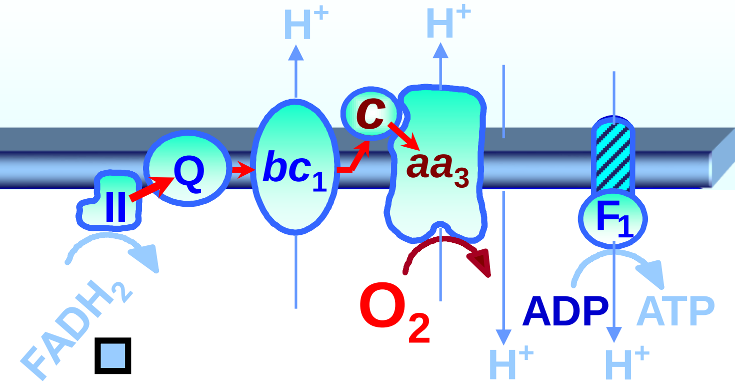
Electron Transport Chain

Bioenergetic paradigm (1):

Respiratory capacity in State 3, feeding electrons specifically into complex I

Conventional Protocol Derived from Bioenergetics

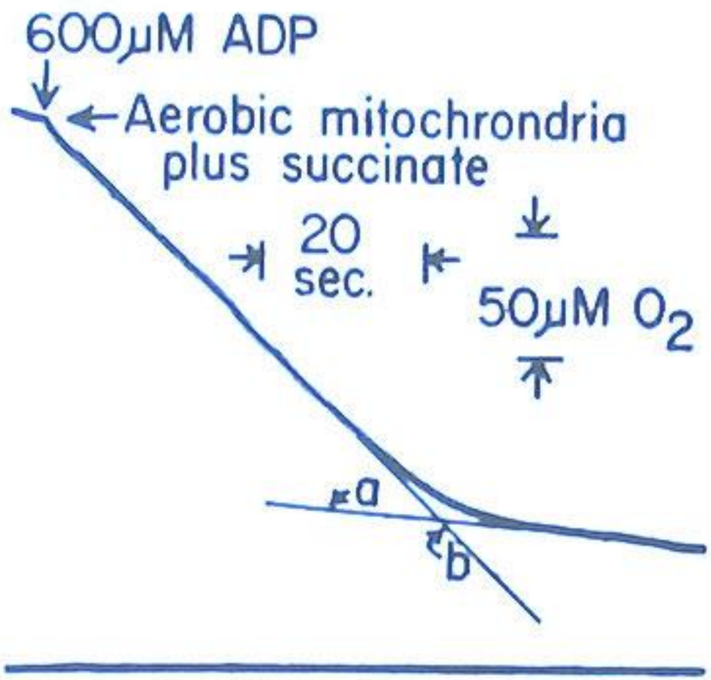
Electron Transport Chain



Bioenergetic paradigm (1):

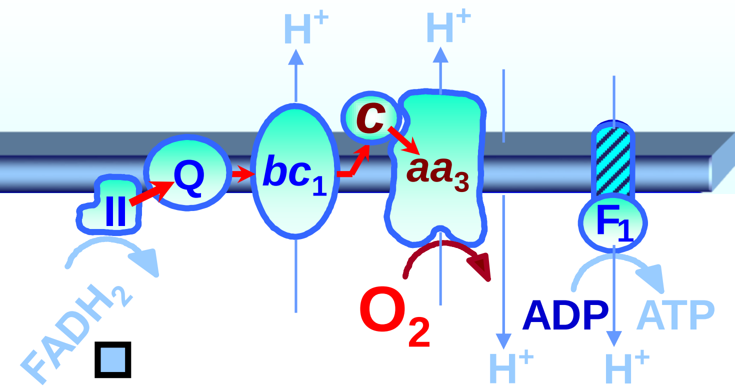
Respiratory capacity in State 3, feeding electrons specifically into

- **complex I, *or***
- **complex II**



Conventional Protocol Derived from Bioenergetics

Electron Transport Chain



Bioenergetic paradigm (1):

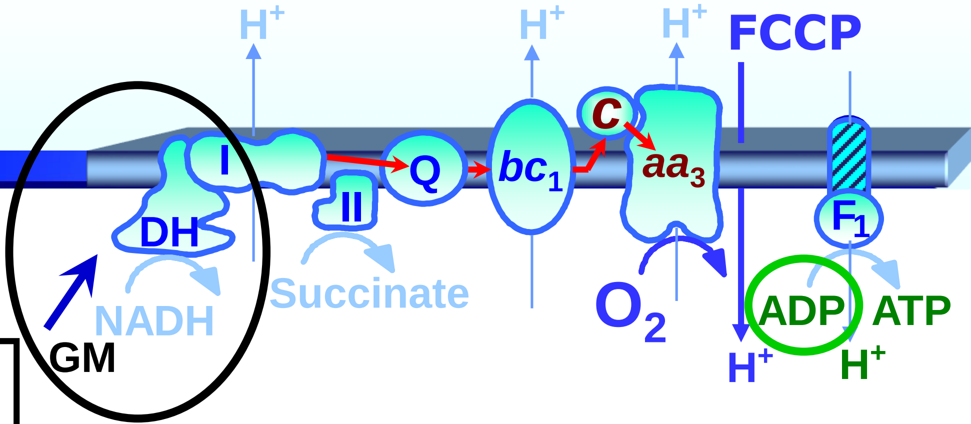
Respiratory capacity in State 3, feeding electrons specifically into

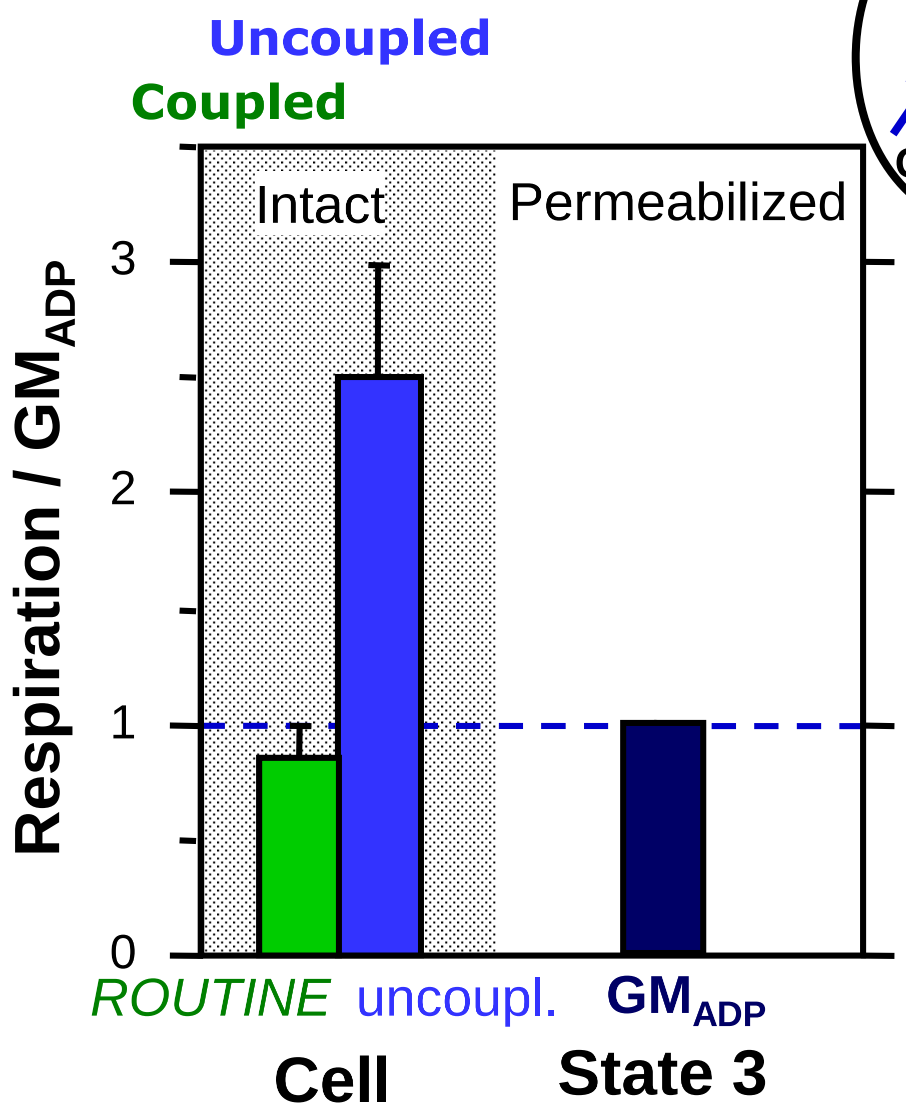
complex I, *or*

complex II (rotenone+succinate)

Then we are surprised to find ...

Intact versus Permeabilized Cells

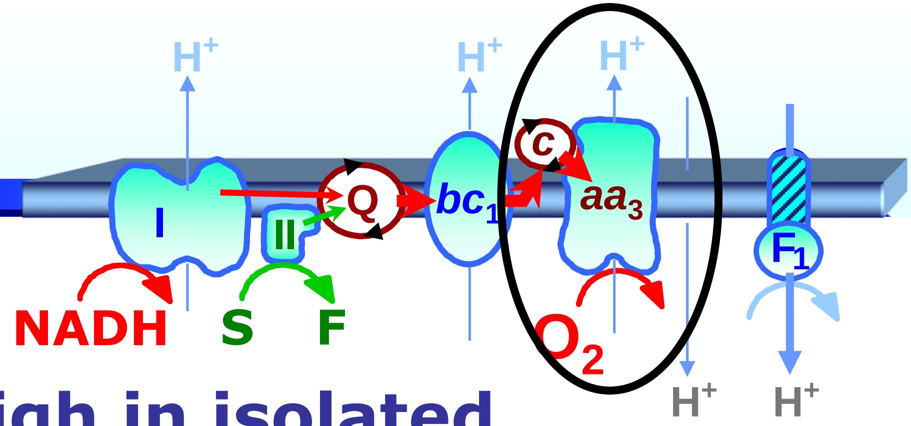




In permeabilized cells, State 3 respiration (Glutamate+Malate) is short of representing respiratory capacity of *intact* uncoupled cells.

Fibroblasts NIH3T3

Contoversy
on Isolated Mitochondria



COX excess capacity is high in isolated mitochondria, with corresponding phenotypic threshold.

- Letellier et al (1994) *Biochem. J.* 302: 171.
- Gnaiger et al (1998) *BBA* 1365: 249
- Rossignol et al (2003) *Biochem. J.* 370: 751.
- Antunes et al (2004) *PNAS* 101: 16774.

But **low COX excess in intact cells „raises the critical issue of how accurately the data obtained with isolated mitochondria reflect the *in vivo* situation“.**

- Villani, Attardi (1997) *PNAS* 94: 1166.

Bioenergetic paradigm (2) of substrate/uncoupler combinations which yield maximum flux in:

- **Intact cells:**
  Villani and Attardi (1997) *PNAS*
- **Permeabilized muscle fibers:**
  Kunz et al (2000) *JBC*
- **Isolated mitochondria:**
  Rasmussen et al (2001) *AJP*

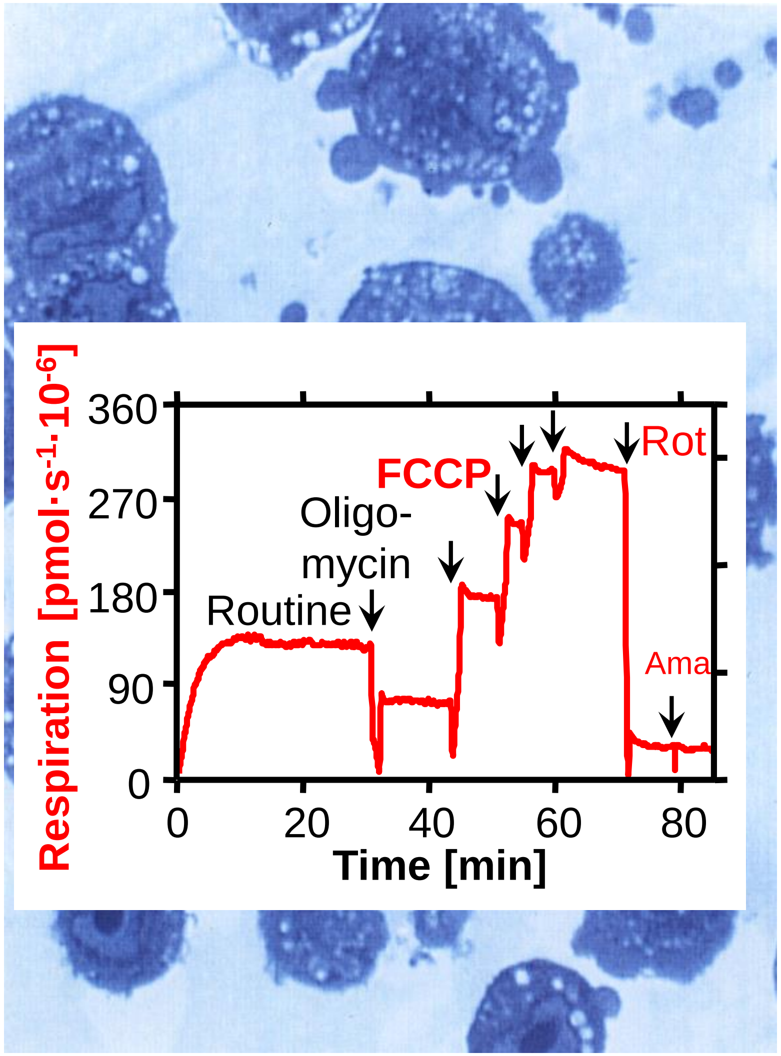
Oxidative Phosphorylation in Top Gear

Gold standard to assess maximum aerobic capacity in cultured cells:

→Uncoupled flux

- **Villani, Attardi (1997) *PNAS* 94: 1166**

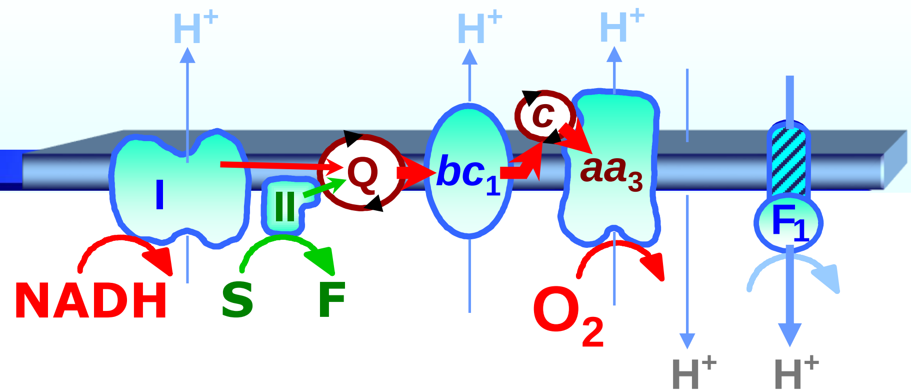
But intact cells do not have uncoupled mitochondria !



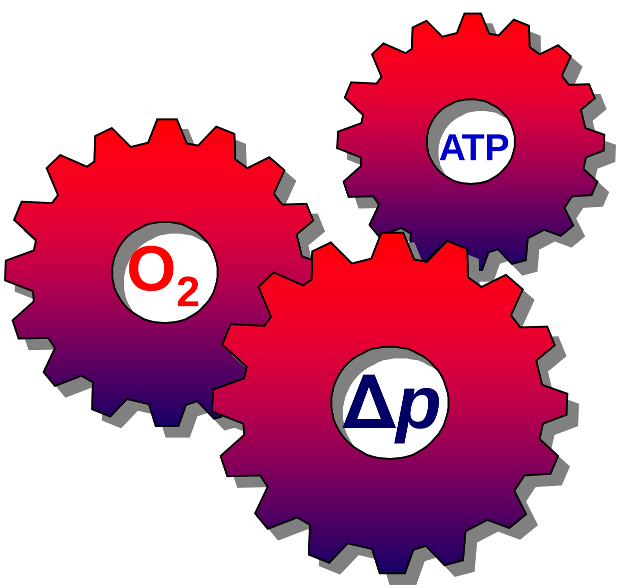
Gold standard to assess maximum aerobic capacity in humans:

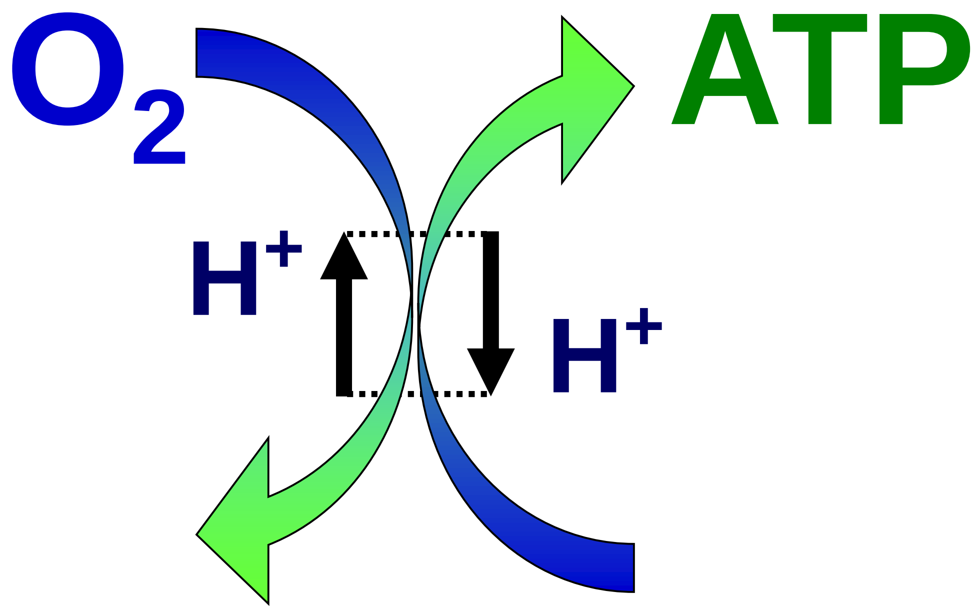
$\rightarrow VO_{2\ max}$

Electron Transport Coupled to ATP Synthesis

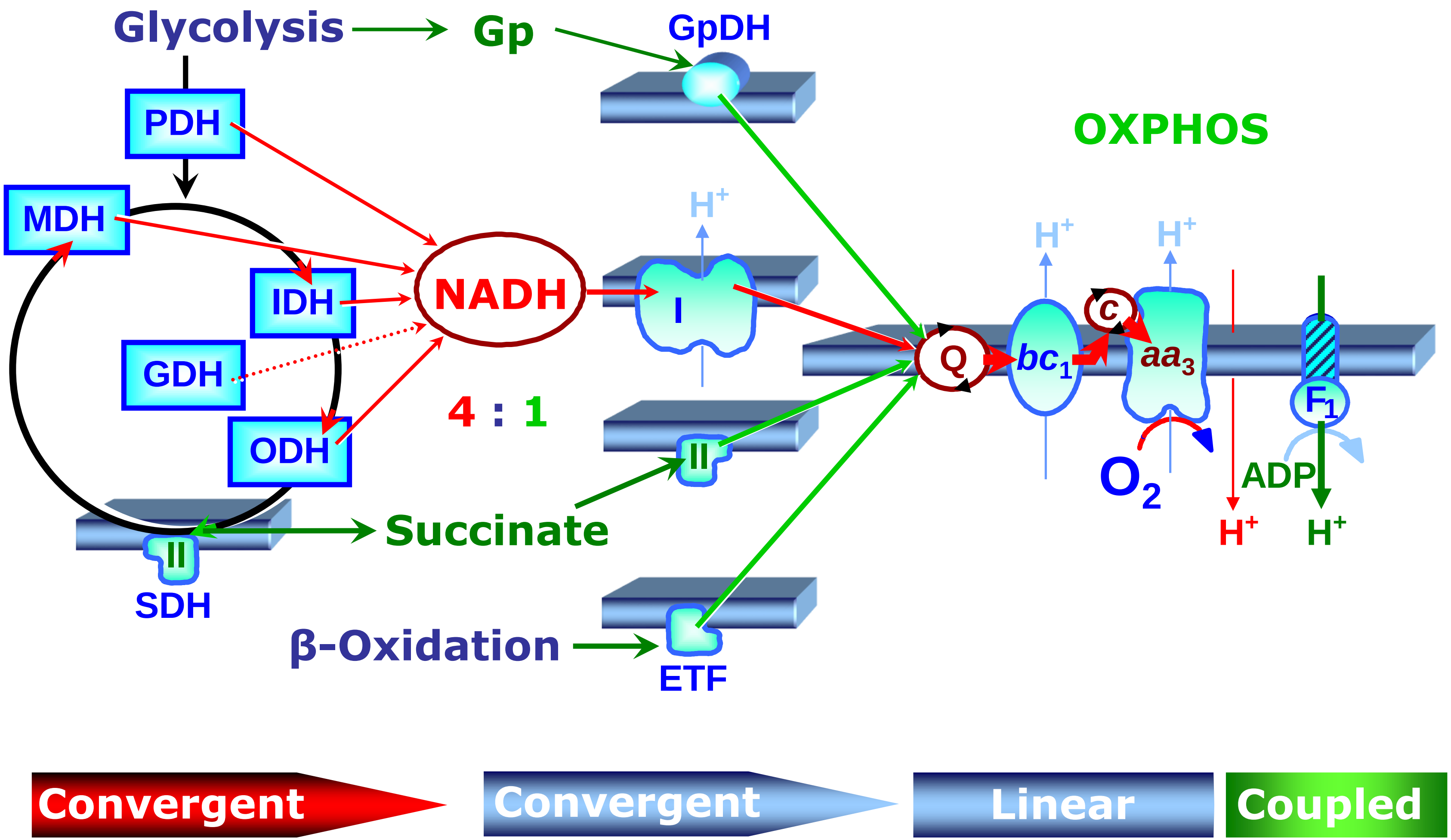


Oxidative Phosphorylation: Coupling

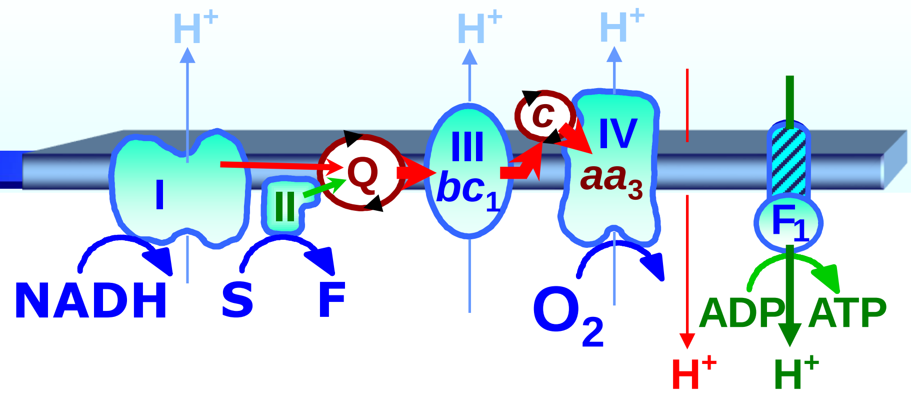




Mitochondrial Pathways
Convergent Redox and ET System



Question 1



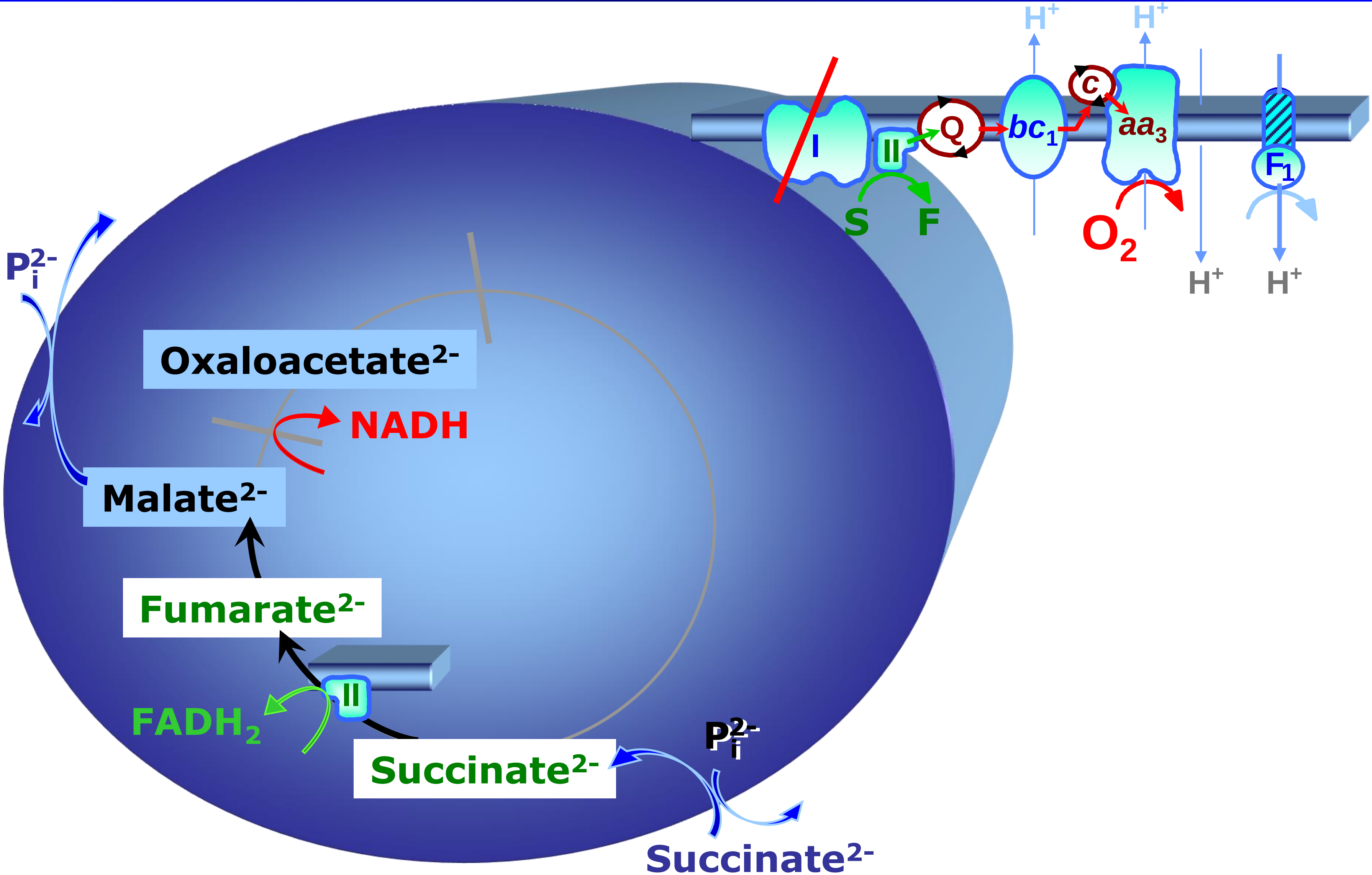
ETS

How do we measure mitochondrial electron transport capacity?

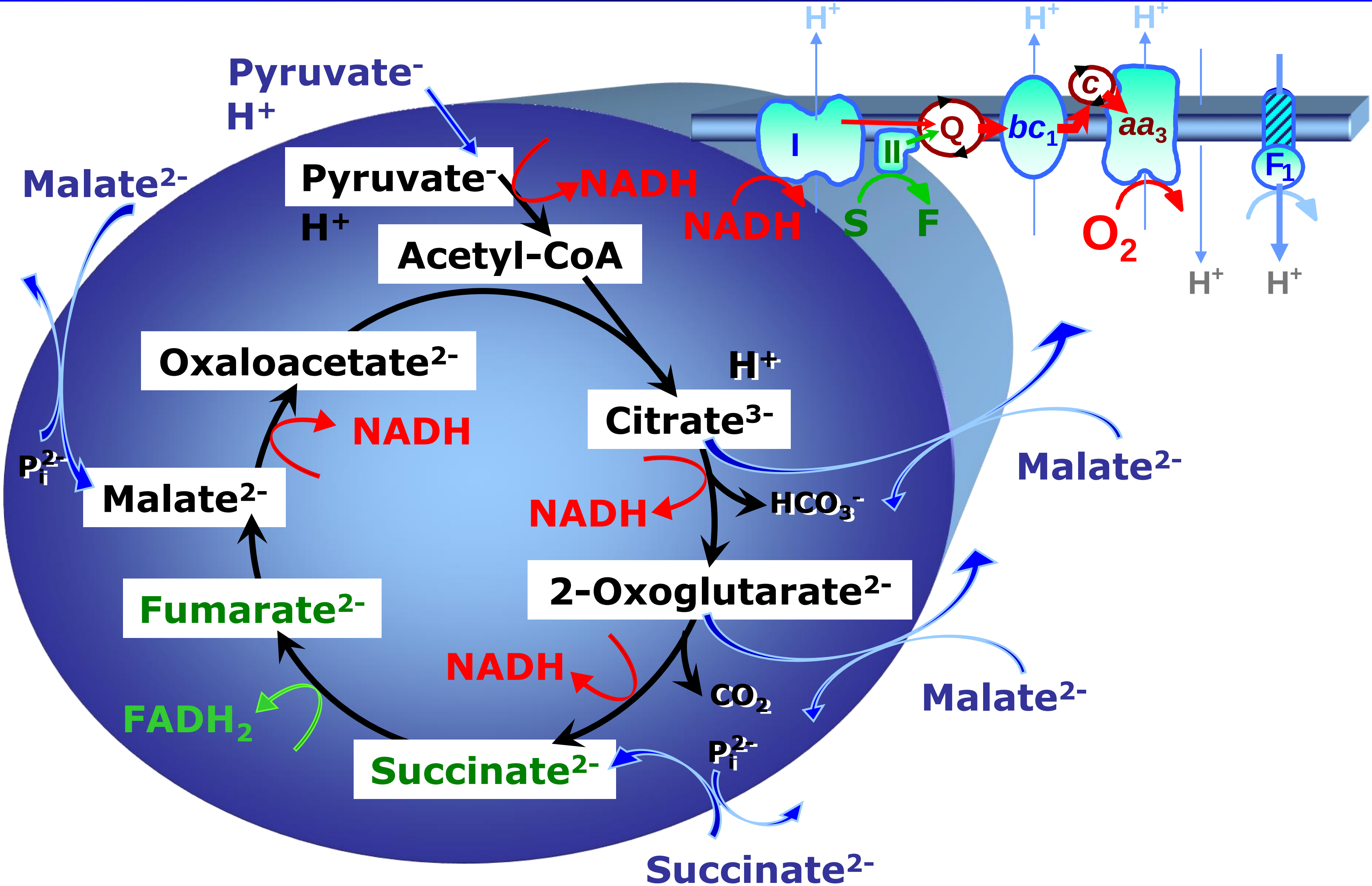
A. Mitochondria

B. Intact cells

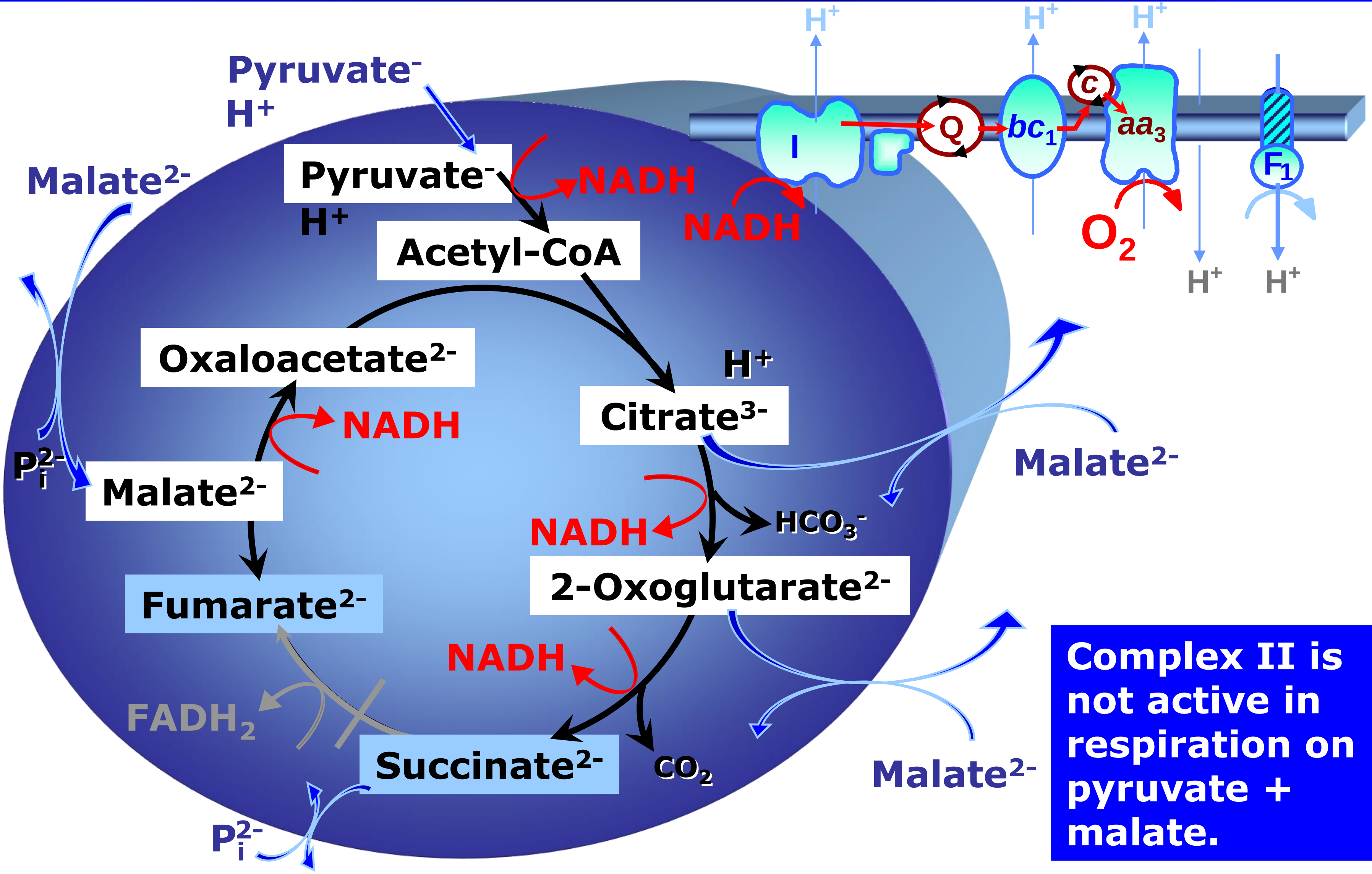
MitoPathways
Succinate + Rotenone



MitoPathways
Pyruvate+Malate+Succinate, PMS

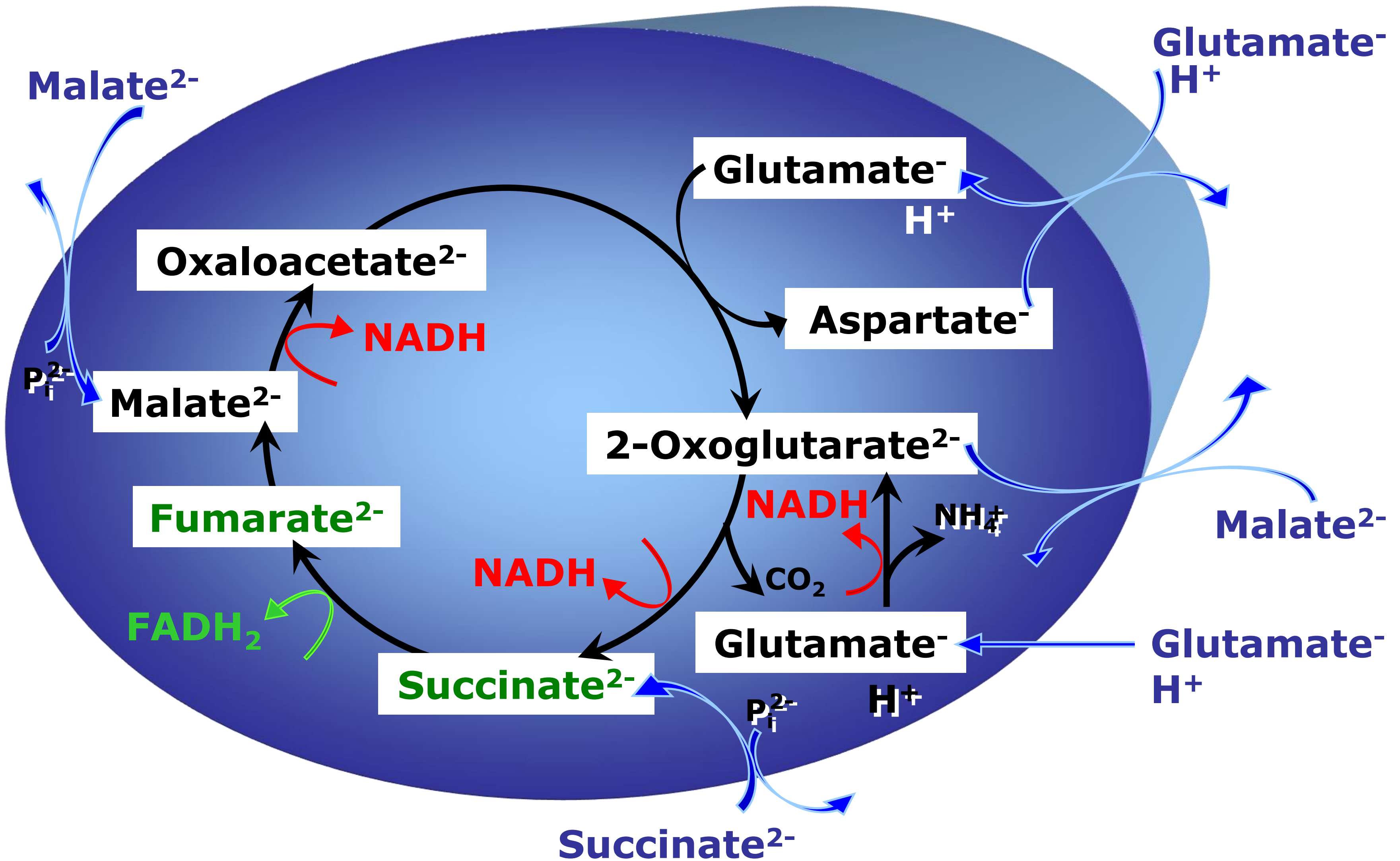


MitoPathways
Pyruvate+Malate, PM



Complex II is not active in respiration on pyruvate + malate.

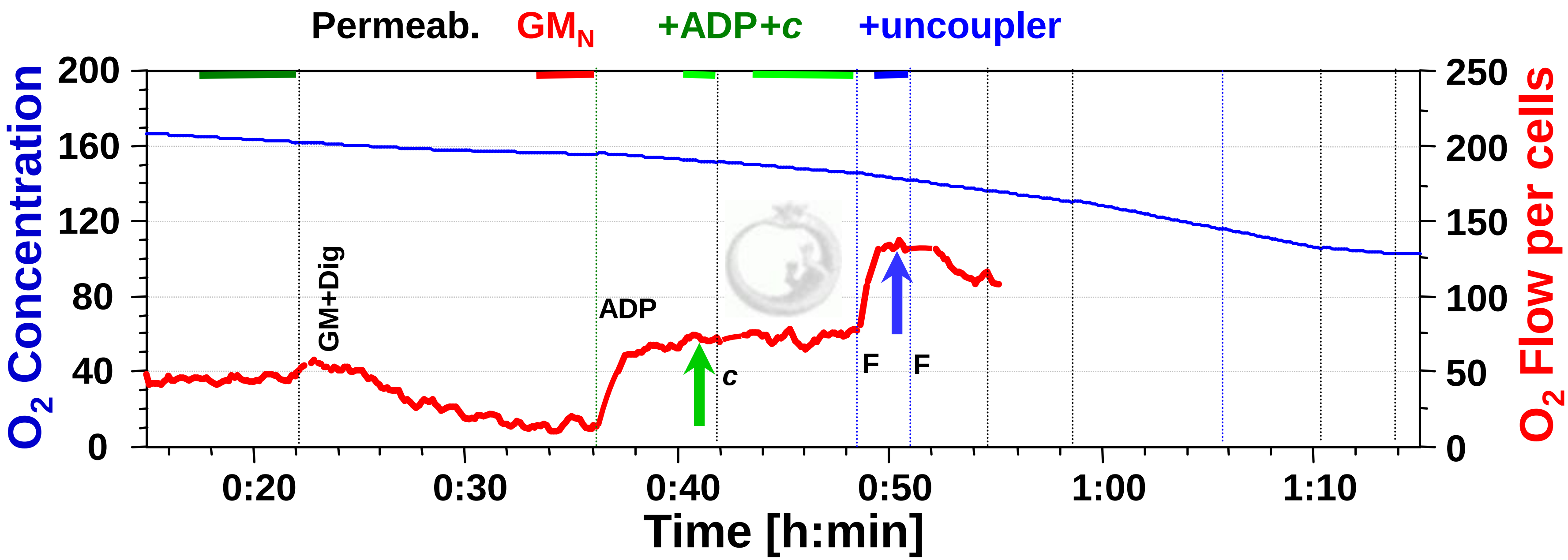
MitoPathways
Glutamate+Malate+Succinate, GMS



High-Resolution Respirometry in Permeabilized Cells

Cytochrome *c* test: Intact mitochondrial outer membrane

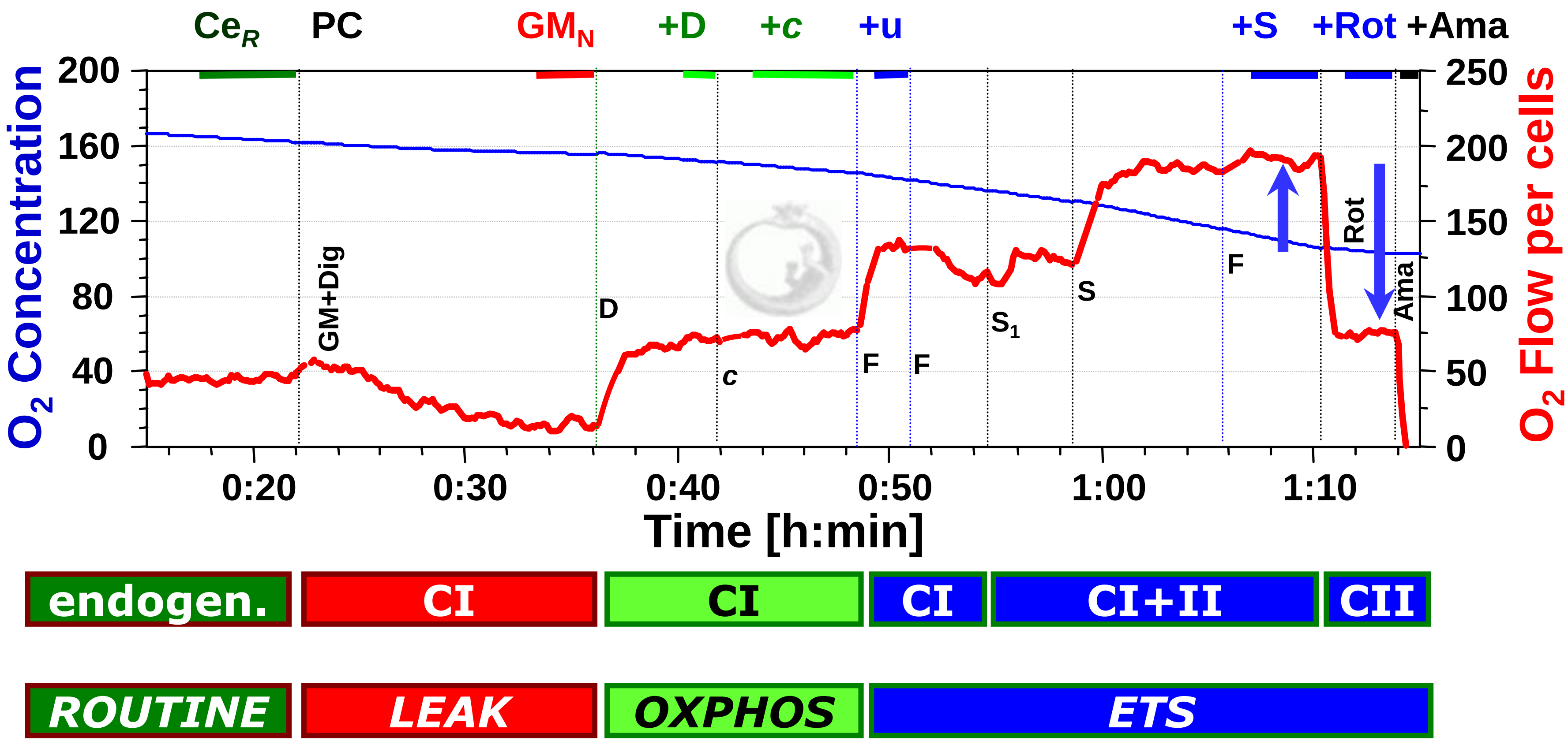
CI Substrates



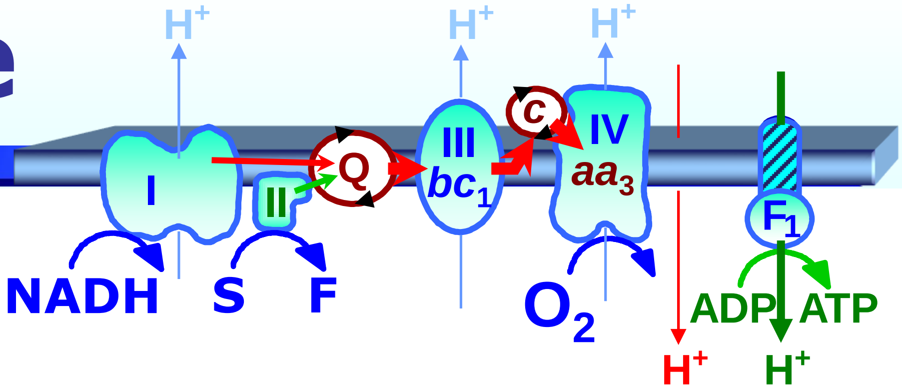
endogen.	CI	CI	CI
ROUTINE	***LEAK***	***OXPHOS***	***ETS***

High-Resolution Respirometry in Permeabilized Cells

ETS capacity with CI+II substrates

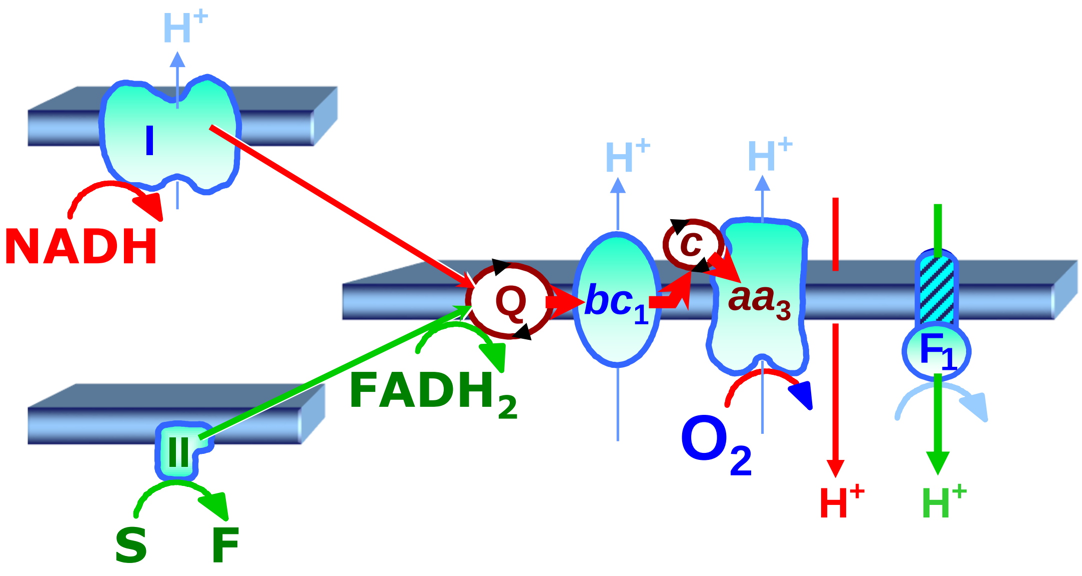


Reference State



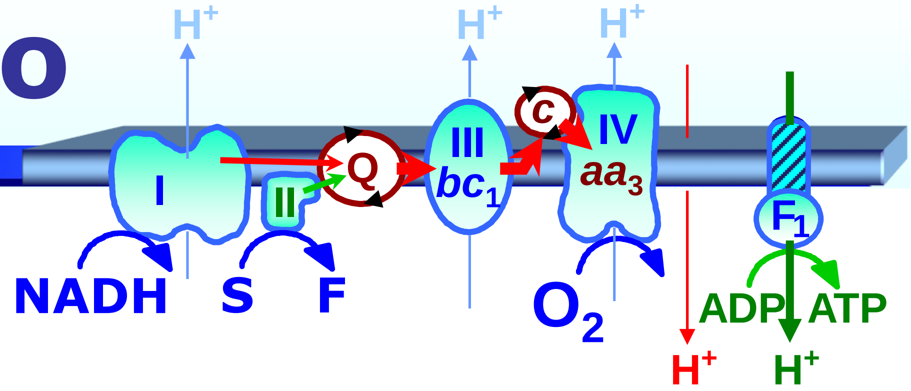
ETS

Maximum electron transport capacity is obtained with convergent CI+II electron input.



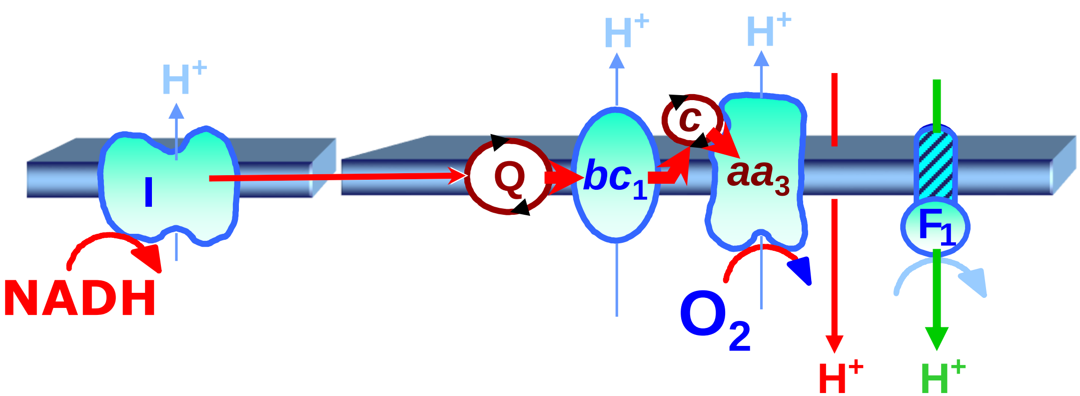
CI+II: 1

Q-Junction Ratio



ETS

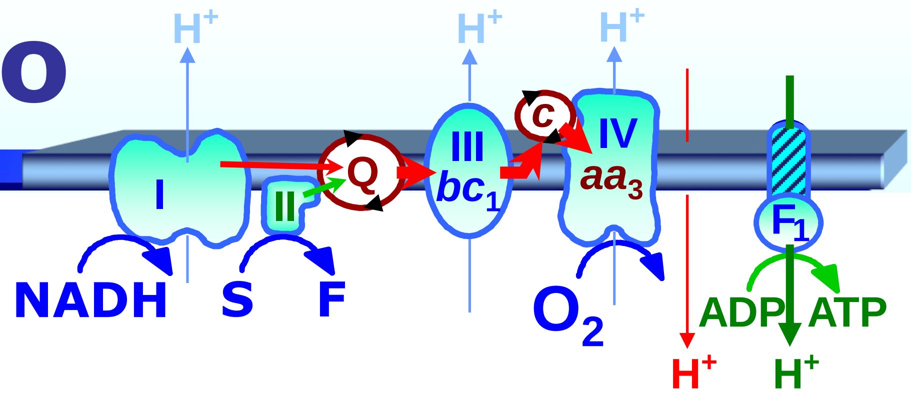
With CI substrates, respiration is limited to 0.70 of *ETS* capacity.



CI: ***0.70***

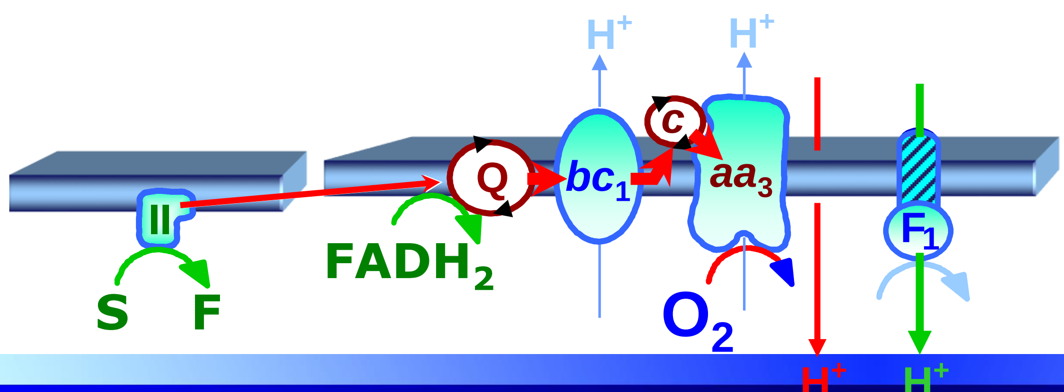
CI+II: **1**

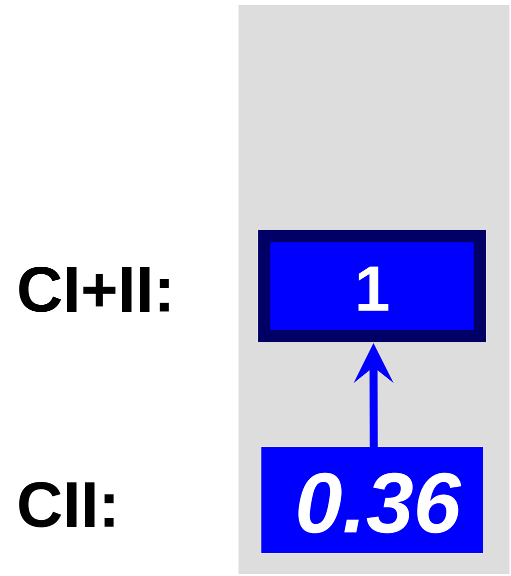
Q-Junction Ratio



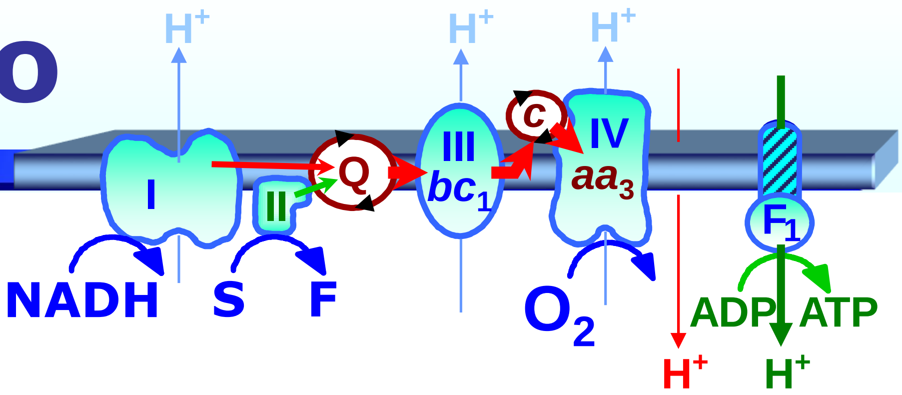
ETS

With CII substrates, respiration is limited to 0.36 of *ETS* capacity.



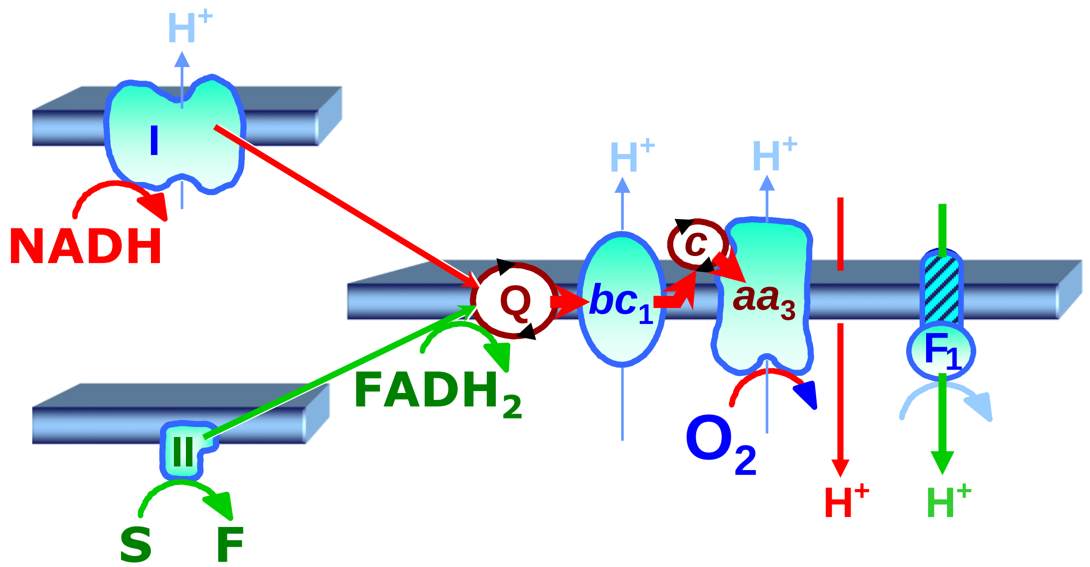


Q-Junction Ratio



ETS

Convergent CI+II electron input exerts an additive effect in human fibroblasts.



CI: *0.70*

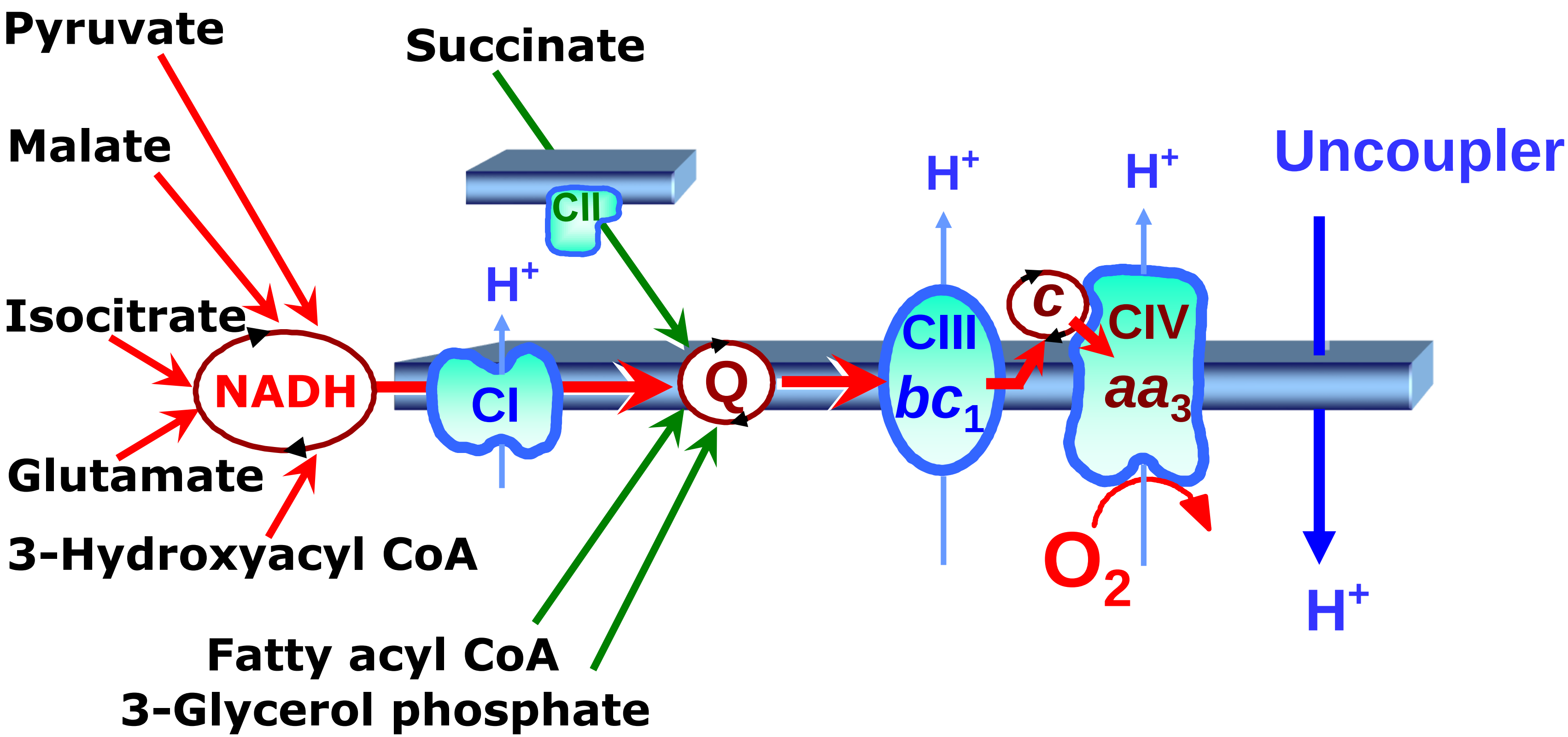
CI+II: 1

CII: *0.36*

Electron Transport: from Chain to System

www.oroboros.at/index.php?id=mipnet-publications

• Electron Transport System, *ETS*



Convergent Electron Flux and the Q-junction

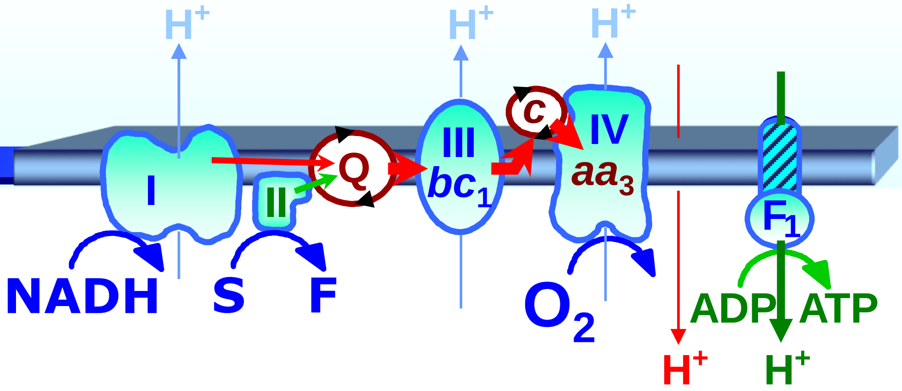
The most frequent

misnomer in bioenergetics:

Electron Transport Chain

ETS

Question 1



ETS

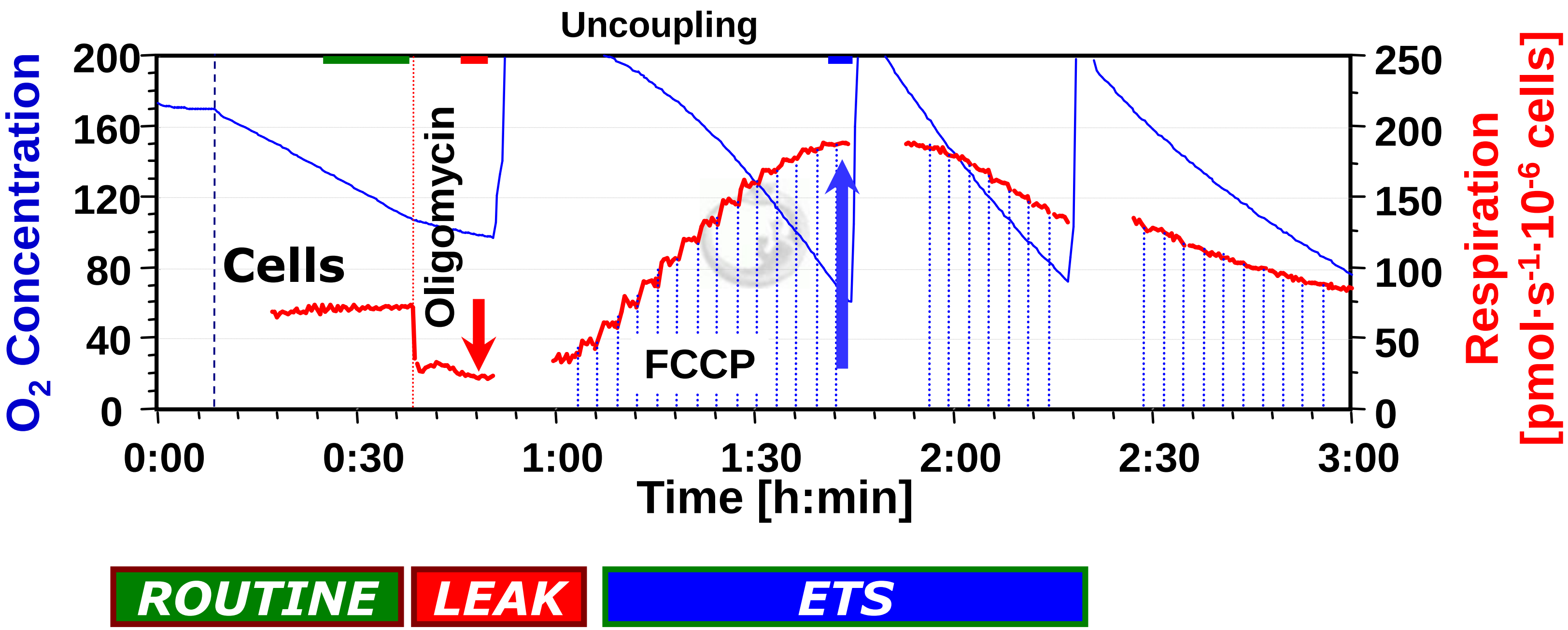
How do we measure mitochondrial electron transport capacity?

A. Mitochondria

B. Intact cells

High-Resolution Respirometry in Intact Cells

Fibroblasts NIH3T3

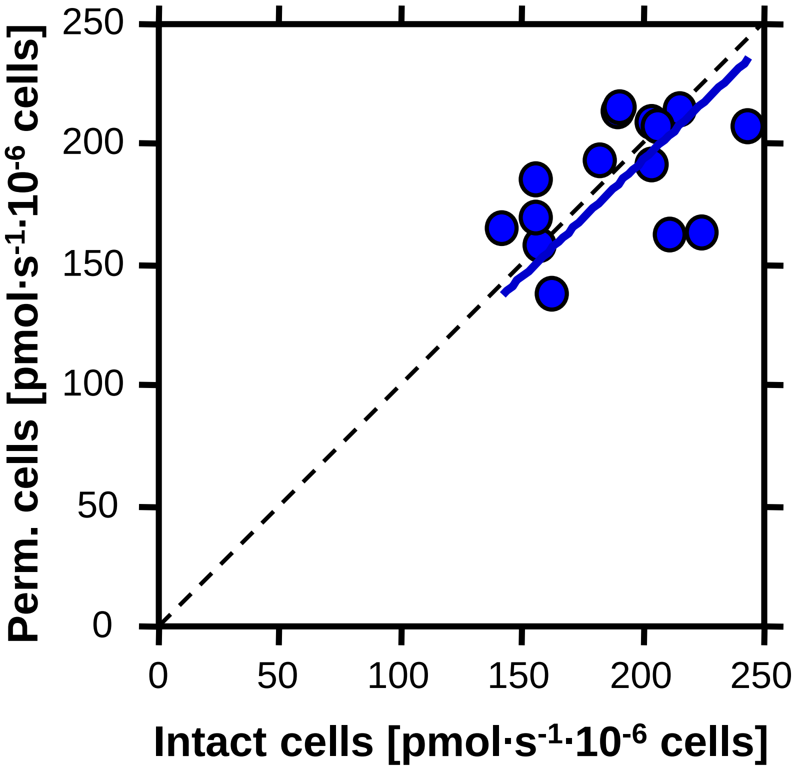


Gnaiger E (2008) In: *Mitochondrial Dysfunction in Drug-Induced Toxicity*. (Dykens JA, Will Y, eds) John Wiley.

Mitochondrial Pathways and Q-Junction

ETS

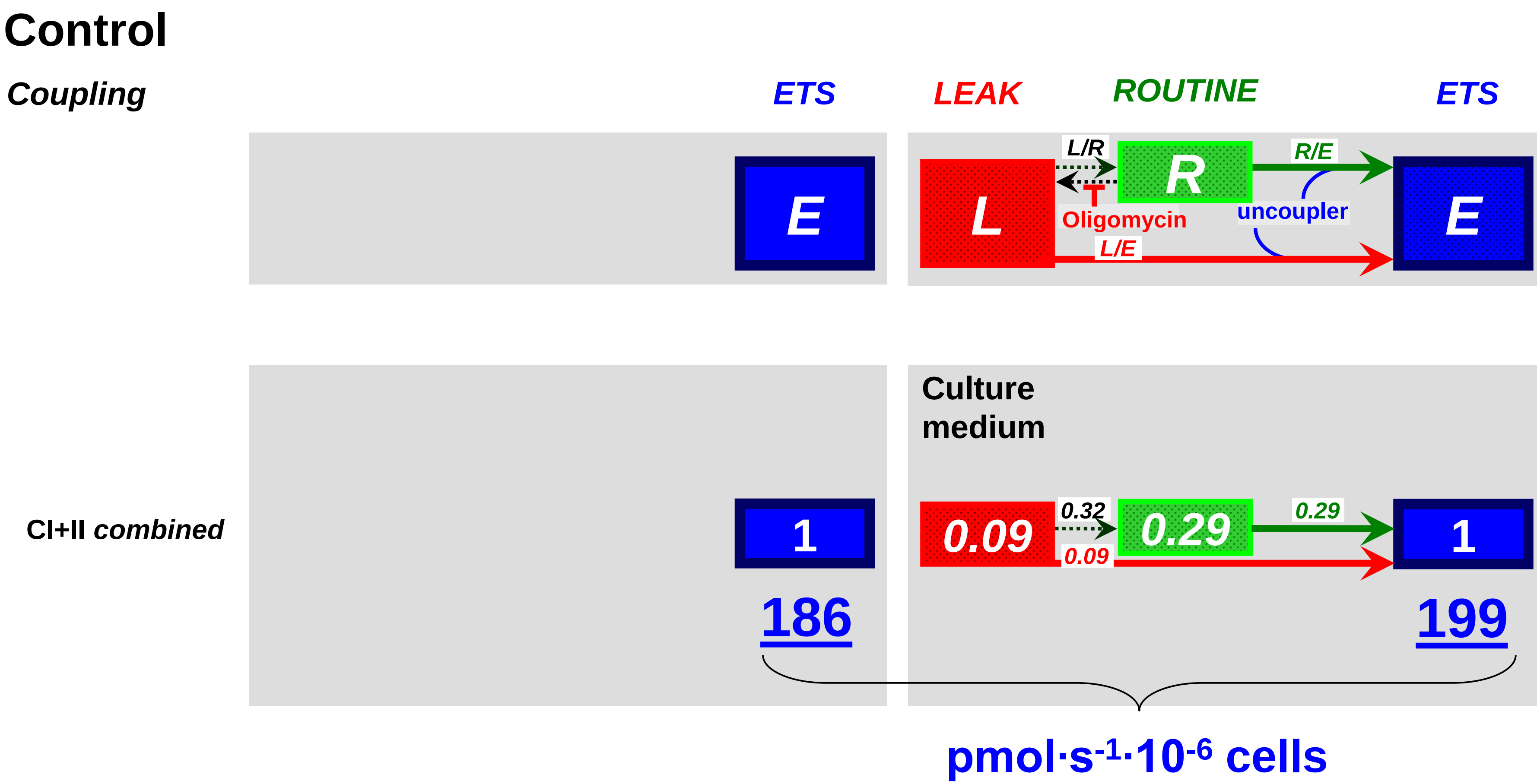
- **CI+II: Glutamate+Malate+Succinate uncoupled**



ETS **capacities were identical in intact and permeabilized cells, with convergent electron flow through Complexes I and II (CI+II e-input).**

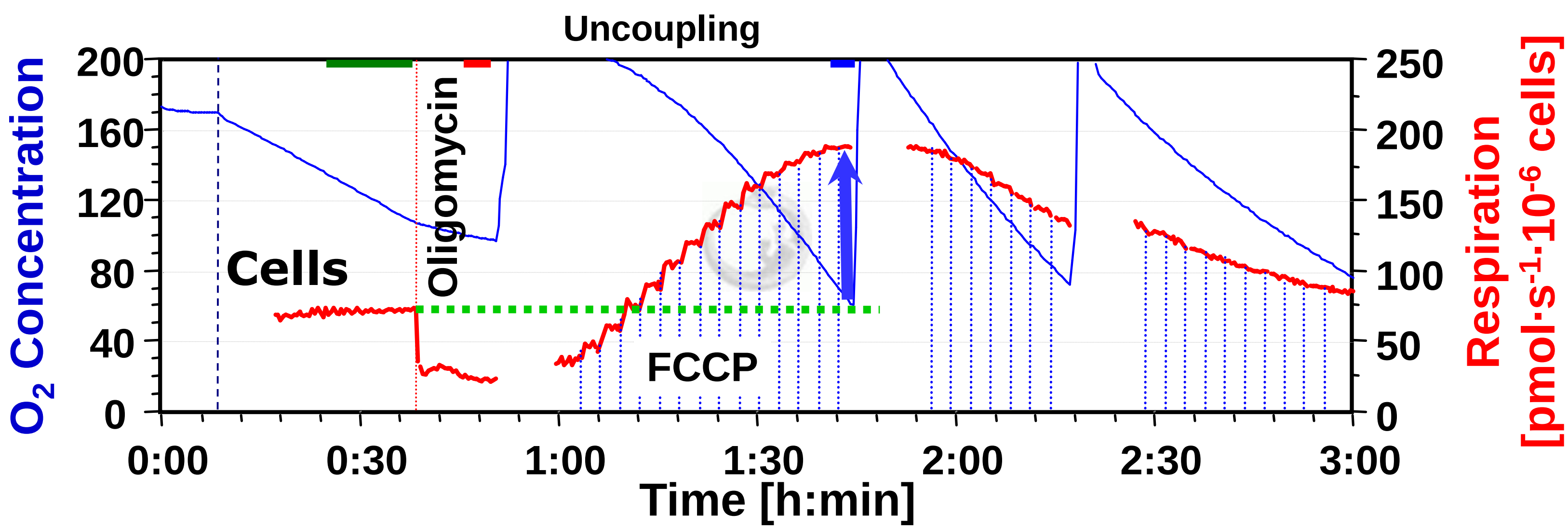
Identical *ETS* Capacity in Permeabilized and Intact Cells

A: Permeabilized Cells   B: Intact Cells



High-Resolution Respirometry in Intact Cells

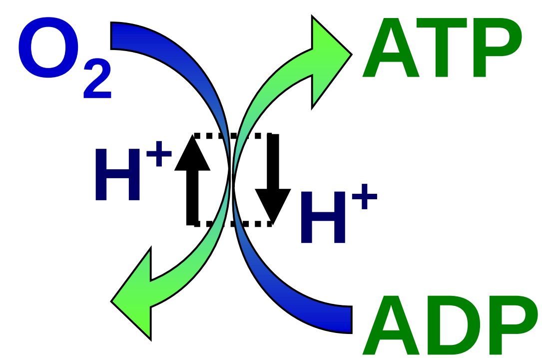
Fibroblasts NIH3T3



ROUTINE **LEAK** **ETS**

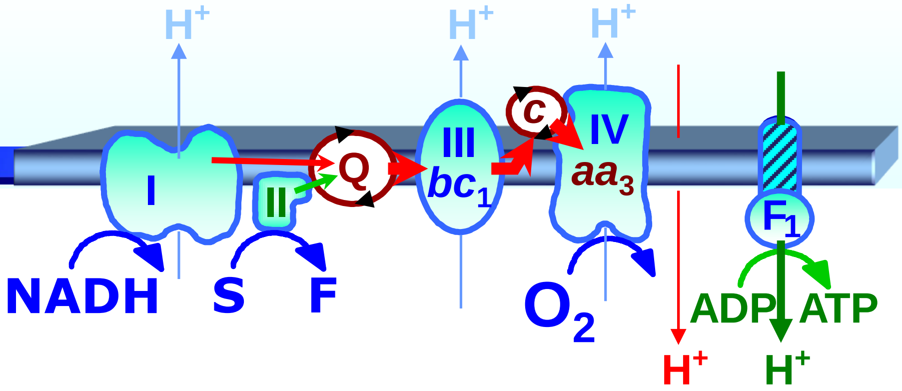
State 3?

***OXPHOS* Capacity ?**



Question 2

OXPHOS



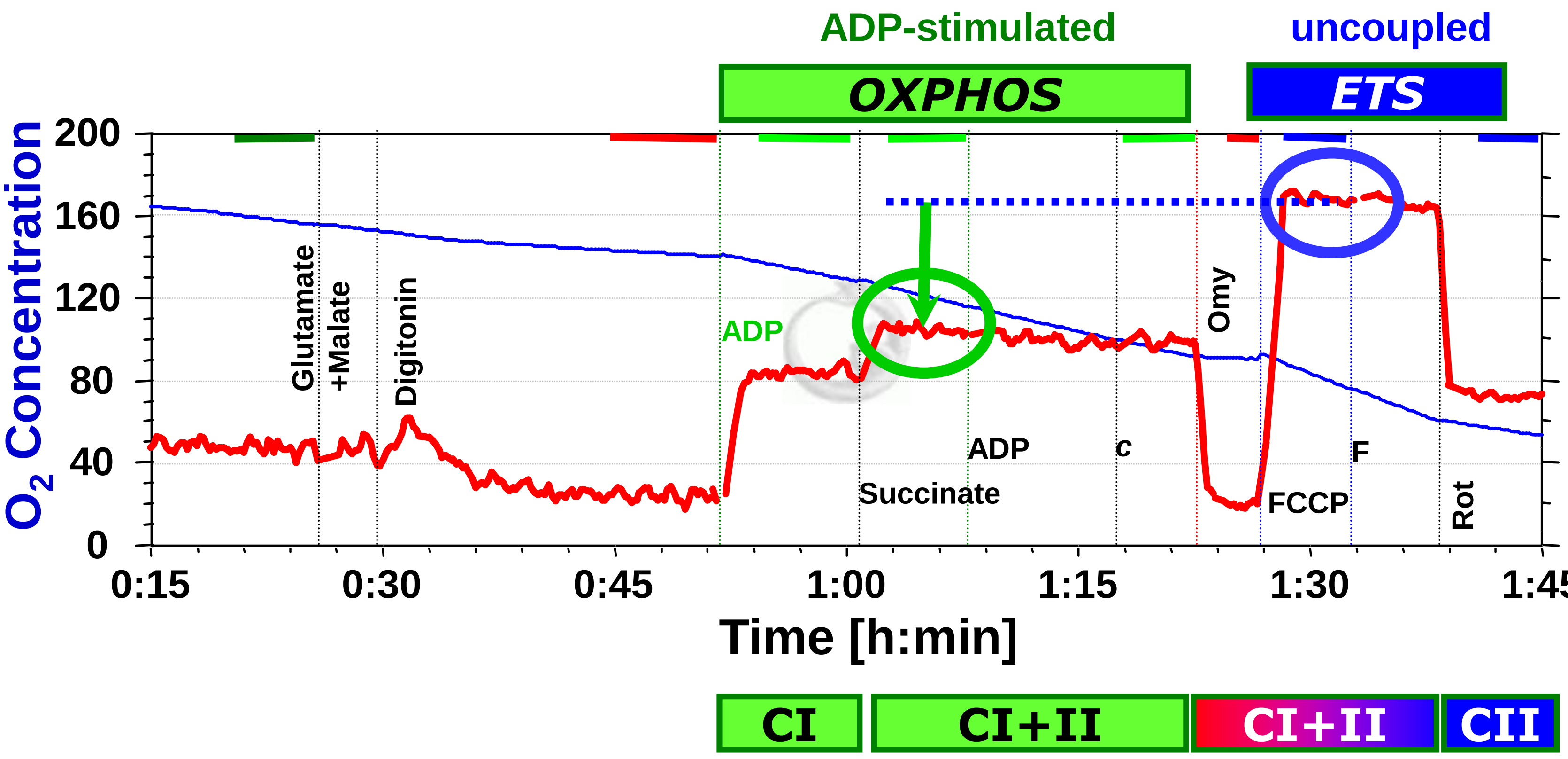
How do we measure *OXPHOS* capacity?

A. Mitochondria

B. In intact cells ?

High-Resolution Respirometry in Permeabilized Cells

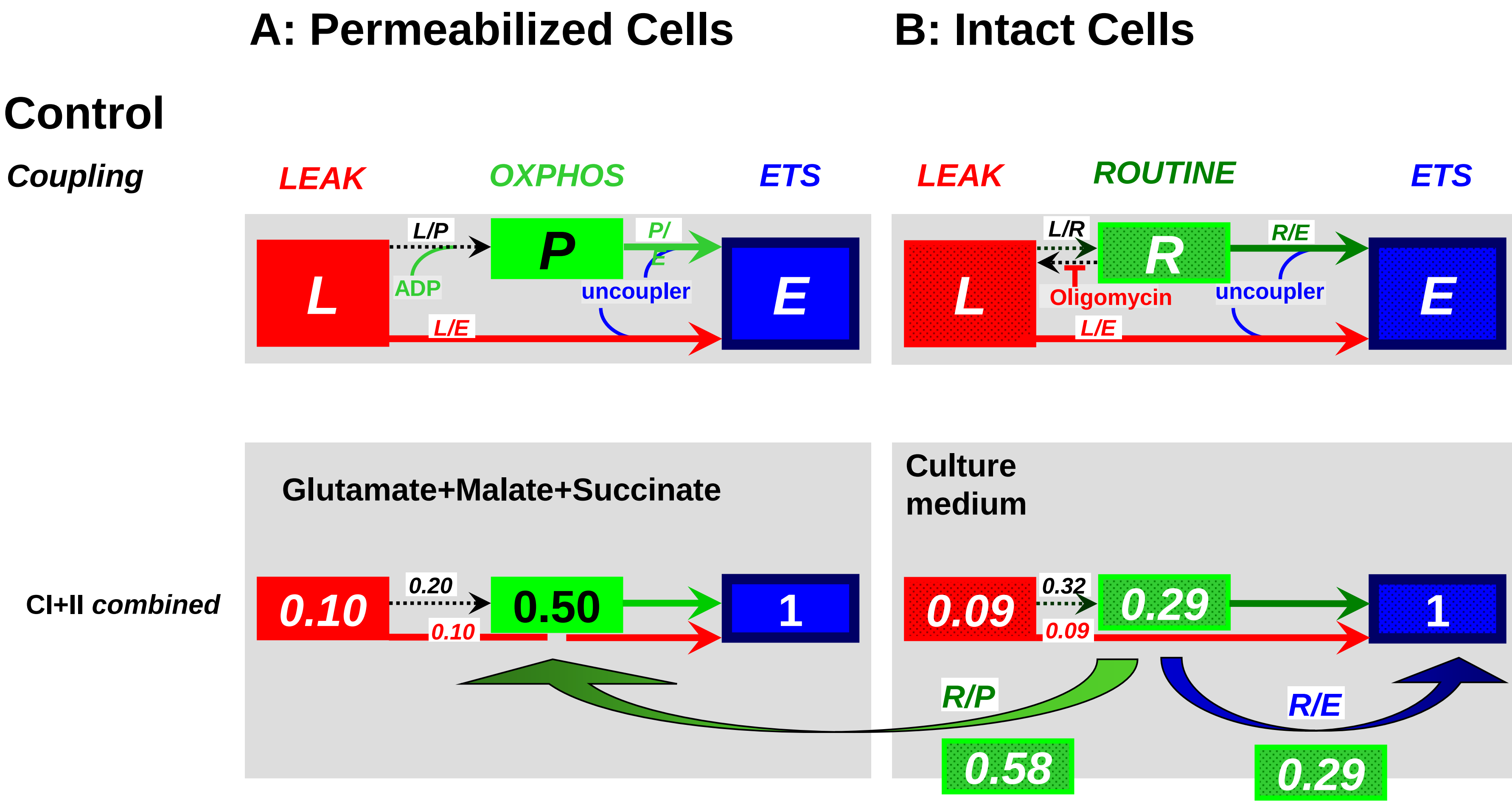
OXPHOS capacity is less than *ETS*



P

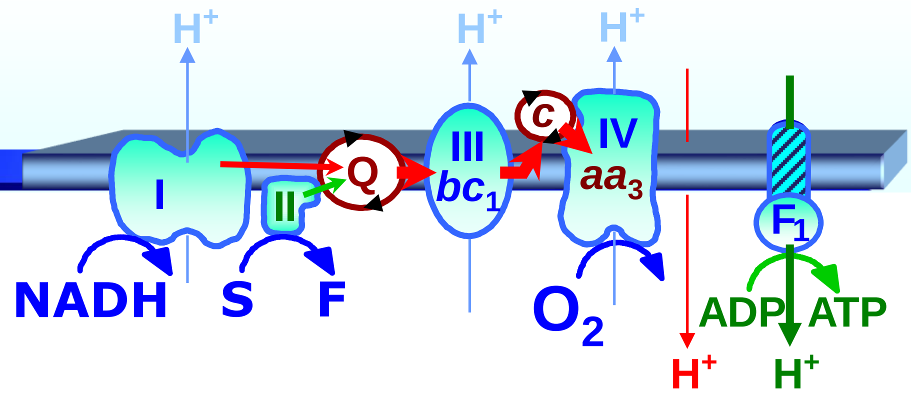
OXPHOS

Flux Control Diagrams for Permeabilized and Intact Cells

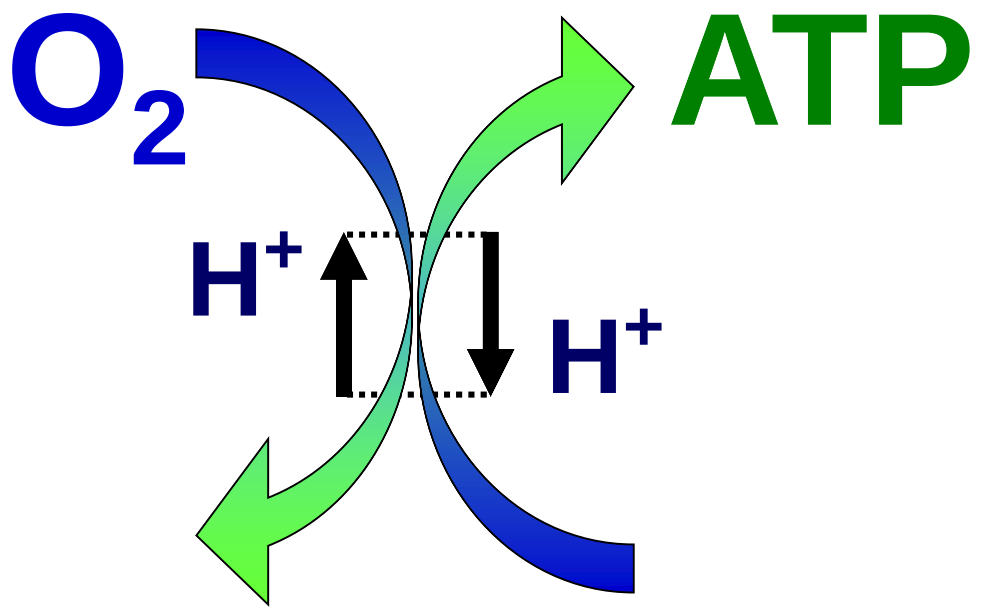


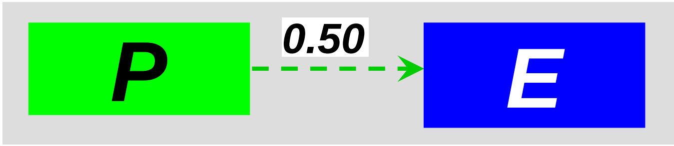
Reserve capacity is overestimated 2-fold

OXPHOS

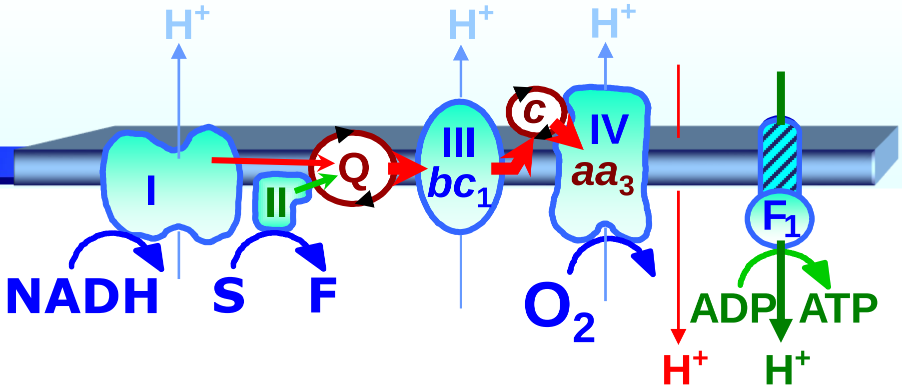


The phosphorylation system exerts strong control over *OXPHOS* in human fibroblasts.





Question 3



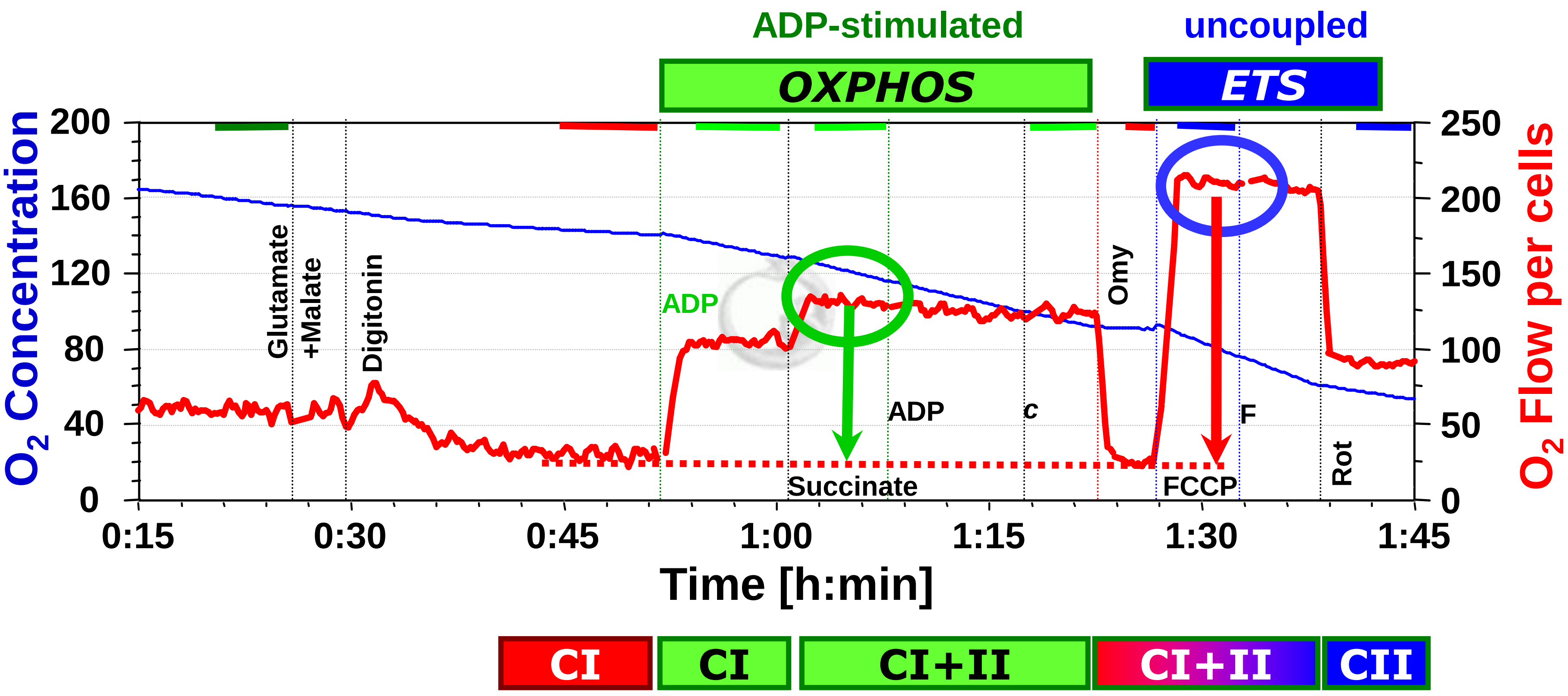
LEAK

How do we express respiratory coupling ratios?

A. Mitochondria
B. Intact cells

High-Resolution Respirometry in Permeabilized Cells

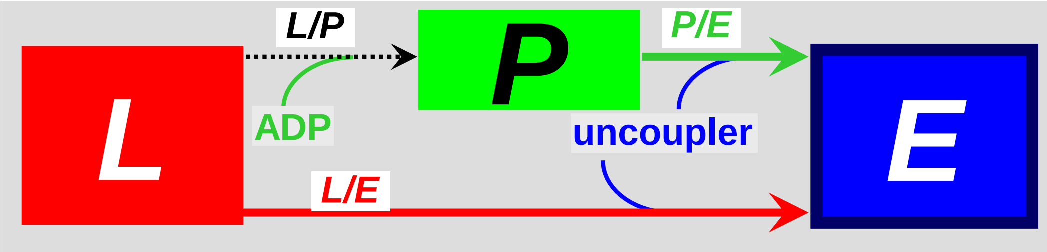
L/*E* ratio but not *L*/*P* ratio reflects the relative *LEAK*.



ETS Capacity versus *OXPHOS* Capacity

Coupling ADP *LEAK* *OXPHOS* *ETS*

Control



Substrate

Glutamate
+Malate

Limitation by the phosphorylation system

4.0

Respiratory Control Ratio (State 3/State 4) is the inverse *L/P* ratio

Permeabilized Cells, NIH3T3 Fibroblasts

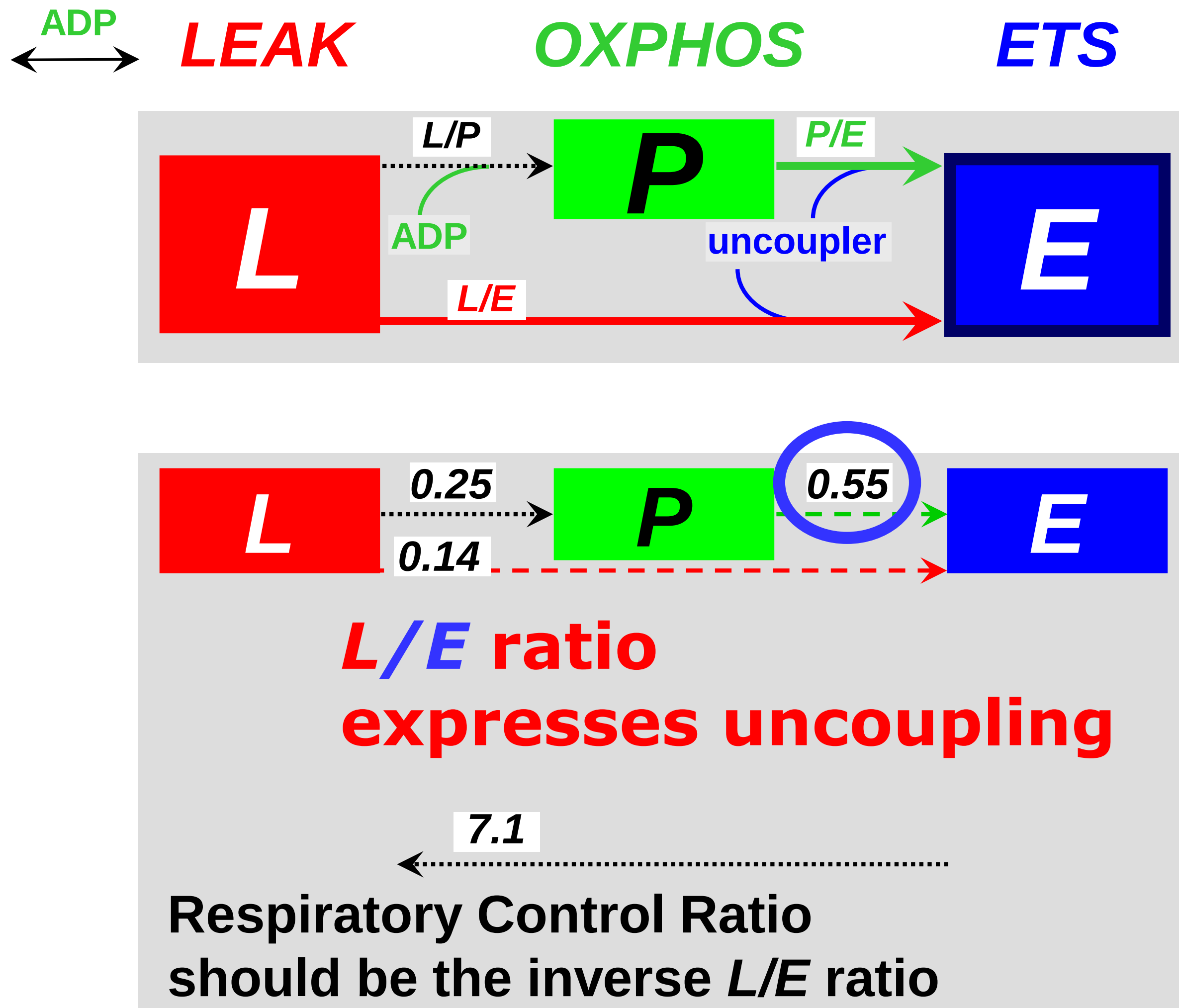
ETS Capacity versus *OXPHOS* Capacity

Coupling

Control

Substrate

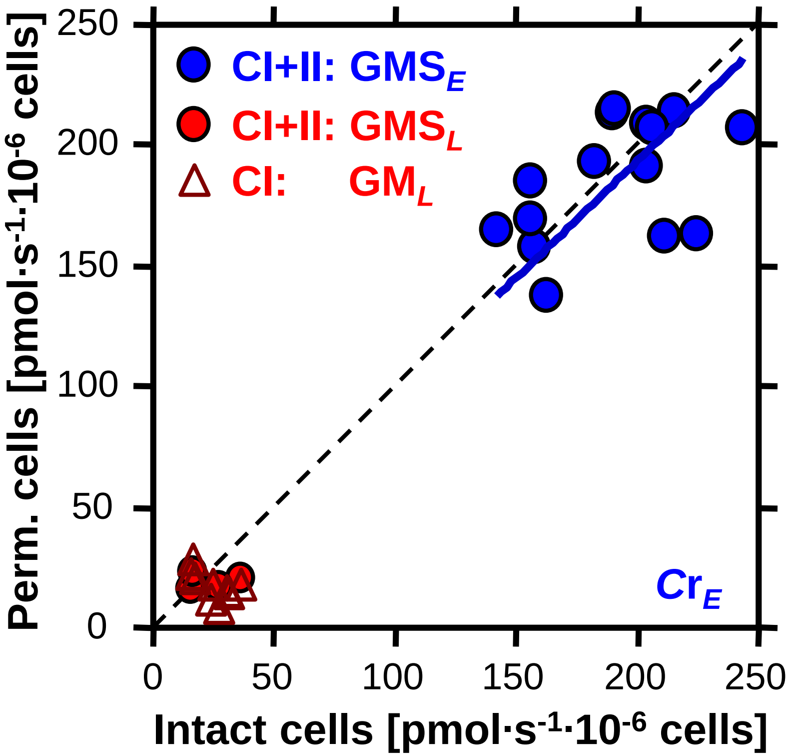
Glutamate
+Malate



Permeabilized Cells, NIH3T3 Fibroblasts

Mitochondrial Pathways and Q-Junction

LEAK



ETS **capacities and** ***LEAK*** **respiration were identical in intact and permeabilized cells, with convergent electron flow through Complexes I and II (CI+II e-input)**

LEAK

Mitochondrial Respiratory Control: The Q-Junction

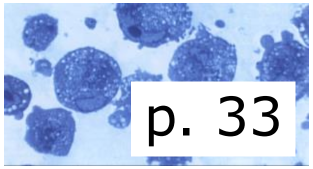
1. Convergent e-input at the Q-junction corresponds to the operation of the citric acid cycle.

2. The additive Q-junction effect and phosphorylation limitation of *OXPHOS* reveal an unexpected diversity of mitochondrial function.

Q-junction ratios: 0.97 to 0.5

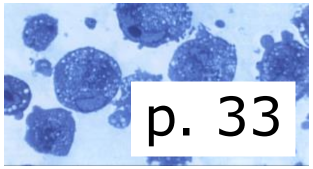
Mitochondrial Respiratory Control: The Q-Junction

3. **Interpretation of apparent excess capacities of ET complexes and of flux control coefficients is largely dependent on the metabolic reference state. Higher capacities with CI+II substrates explain apparent discrepancies between mitochondria and intact cells.**



Mitochondrial Respiratory Control: The Q-Junction

4. **Interpretation of excess capacities of various components of the respiratory chain and of flux control coefficients is largely dependent on the metabolic reference state. Appreciation of the concept of the Q-junction will provide new insights into the functional design of the respiratory chain.**



5. **The relation between membrane potential and flux is reversed when an increase in flux is effected by a change in substrate supply.**

➡ **MultiSensor O2k: $TPMP^+$**

OROBOROS INSTRUMENTS
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Oxygraph-2k

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Faculty Disclosure Statement

O_2

H^+

Ca^{2+}

TPP^+

NO

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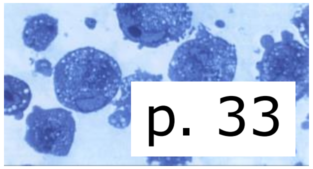
6. **ROS production and reversed electron flow from Complex II to Complex I: Multiple substrate supply plays a key role (Capel et al 2005; Garait et al 2005). The dependence of ROS production on membrane potential and metabolic state will have to be investigated further based on the concept of the Q-junction.**



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Coupling Control
LEAK, *OXPHOS*, *ETS*