

# **O2k-Workshop**

**IOC 162**

## **Substrate-Uncoupler-Inhibitor-Titration (SUIT) protocols: exploring coupling and pathway control**

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# High-resolution respirometry (HRR)

**Oroboros O2k**



**Modular system for HRR for  
mitochondria and cell research**

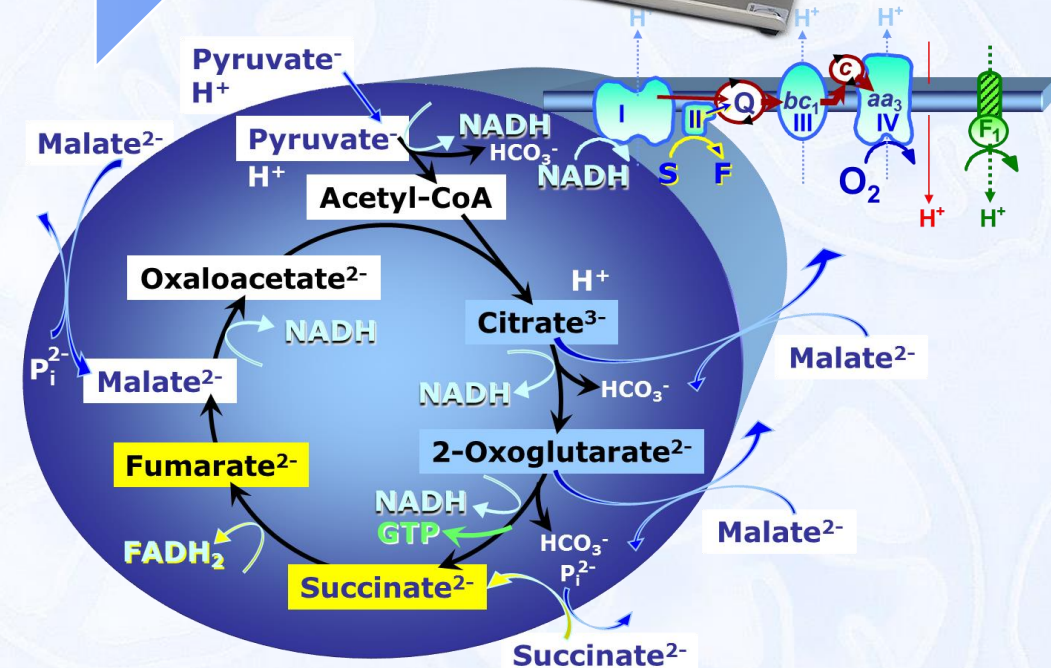
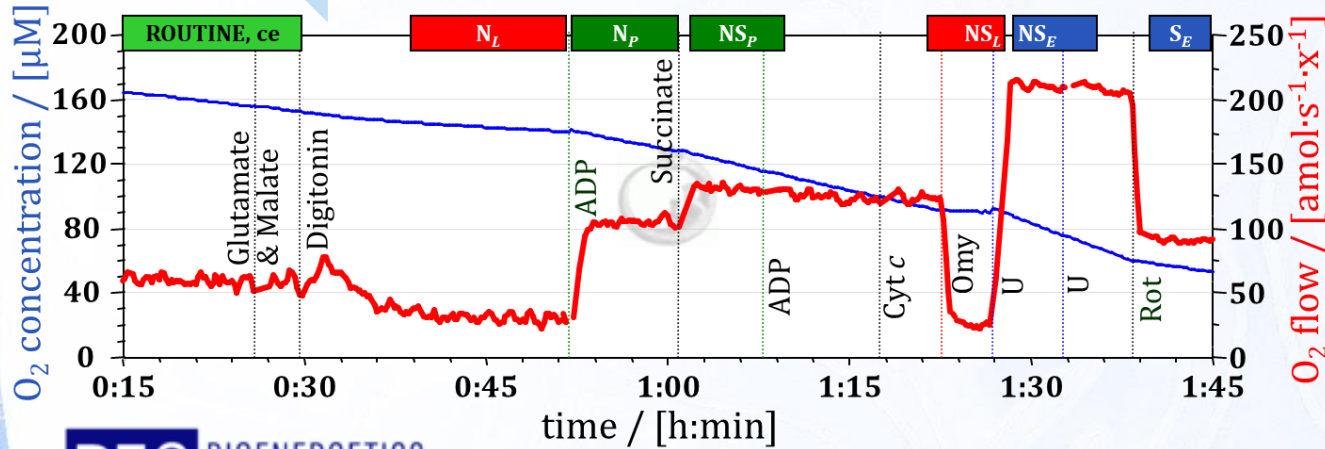
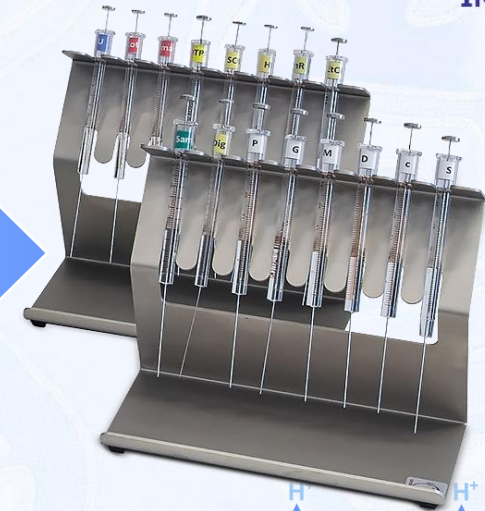
**SUIT: Substrate-Uncoupler-Inhibitor Titration**



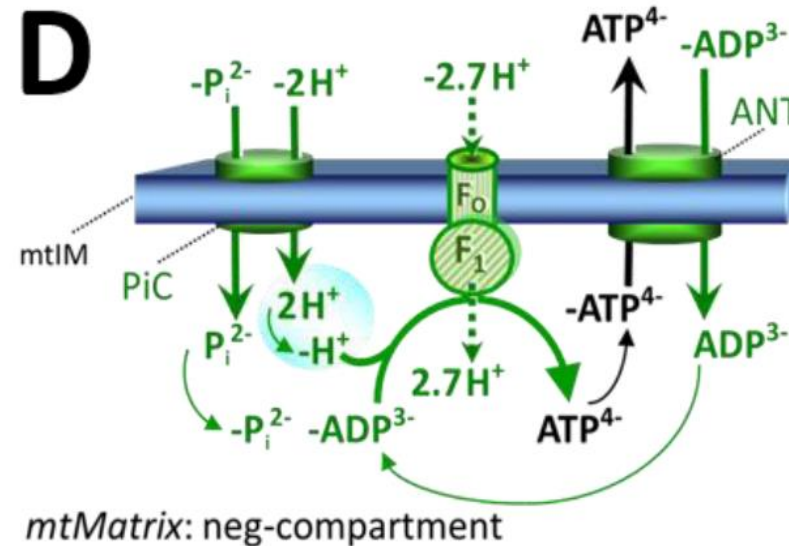
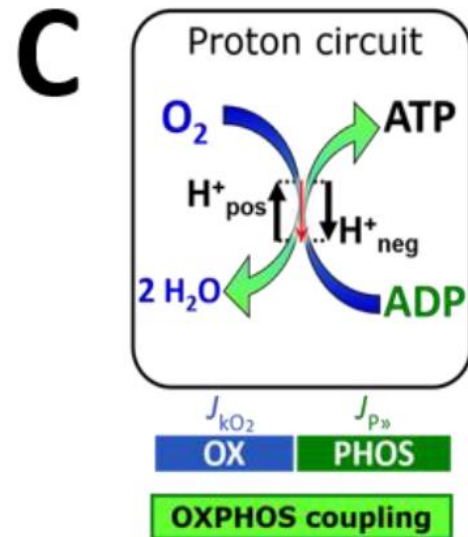
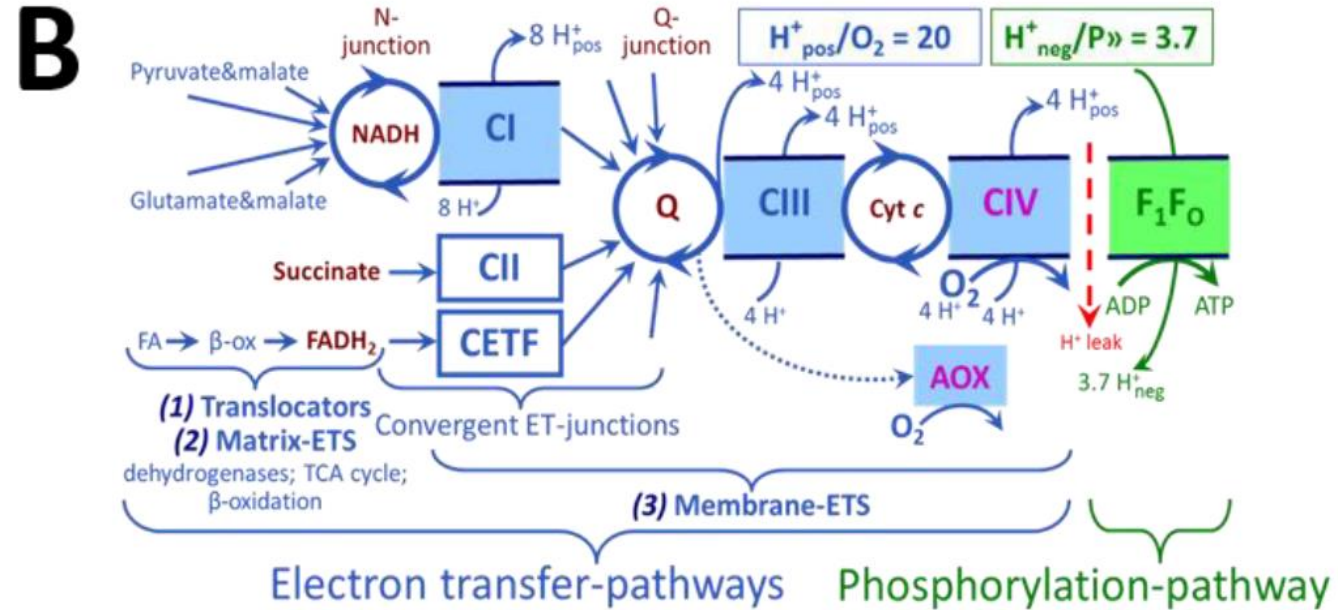
# O2k - unique features: Multiple titrations

Dissect specific branches of metabolic pathways

Multiple titrations in a single assay

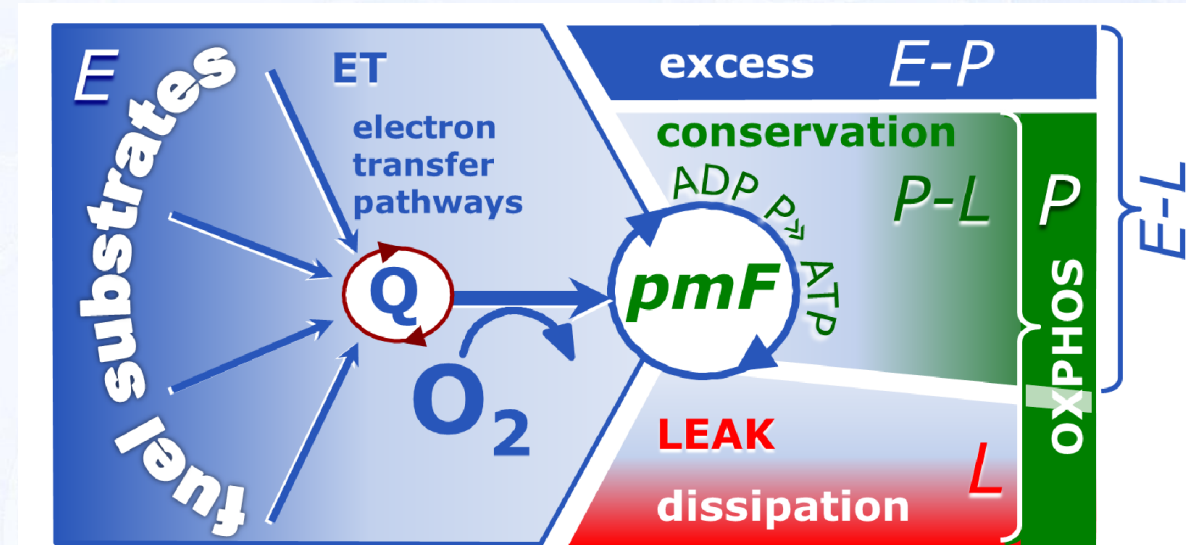
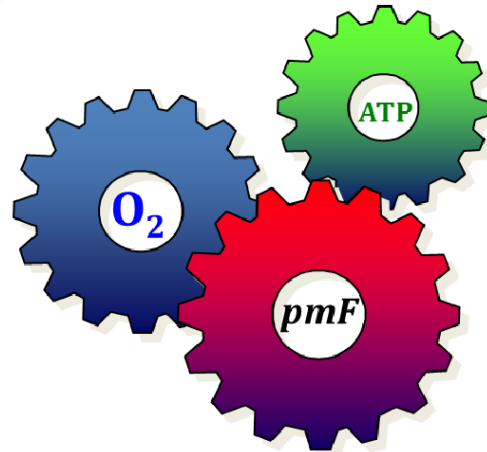
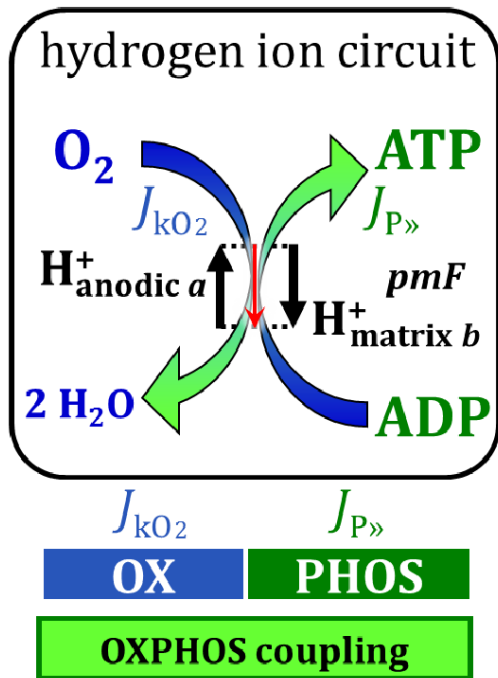


# Coupling of ETS and phosphorylation pathway

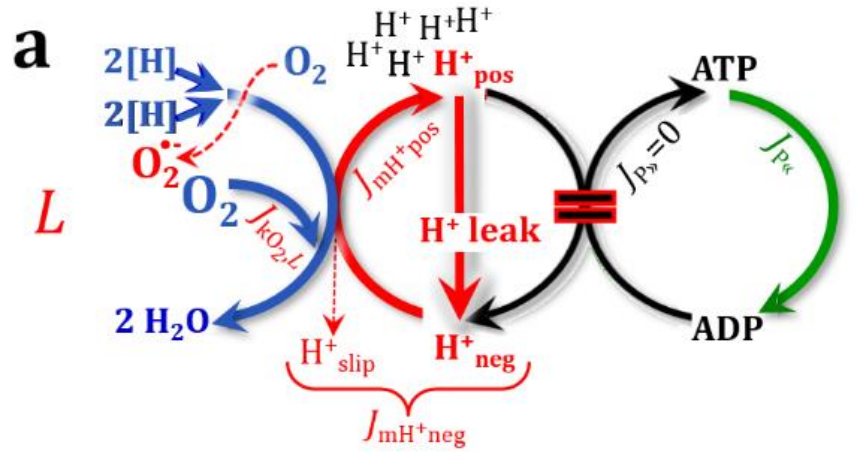




# Coupling control

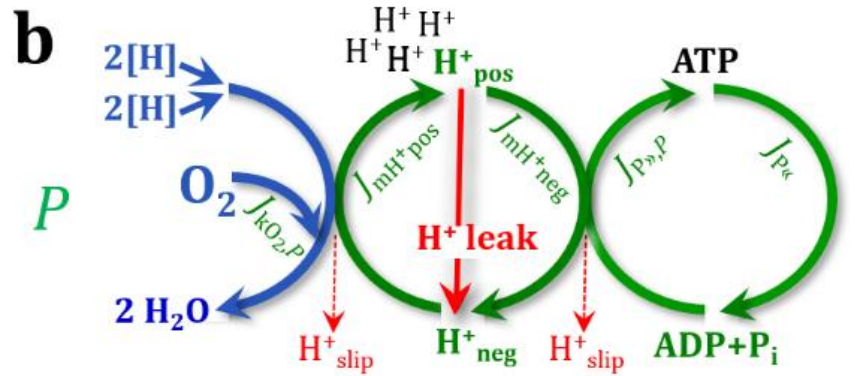


# Coupling control states



## LEAK state and rate *L*:

Phosphorylation is arrested/inhibited  
Proton leak and slip  
Maximum protonmotive force

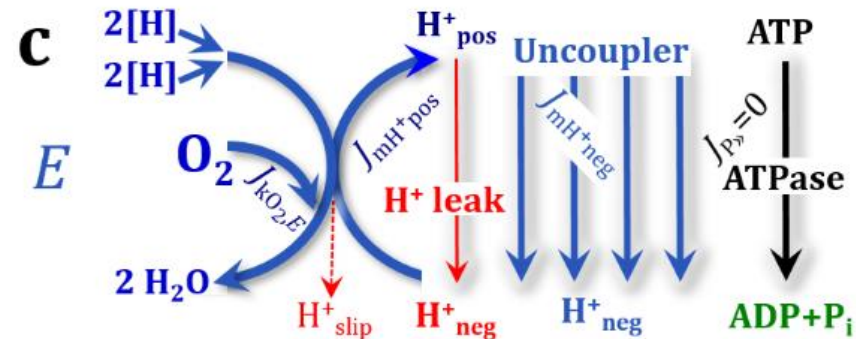


## OXPHOS state and rate *P*:

Oxidation coupled to phosphorylation  
Kinetically-saturating [ADP] and [Pi].

## ROUTINE and rate *R*:

Endogenous substrates only

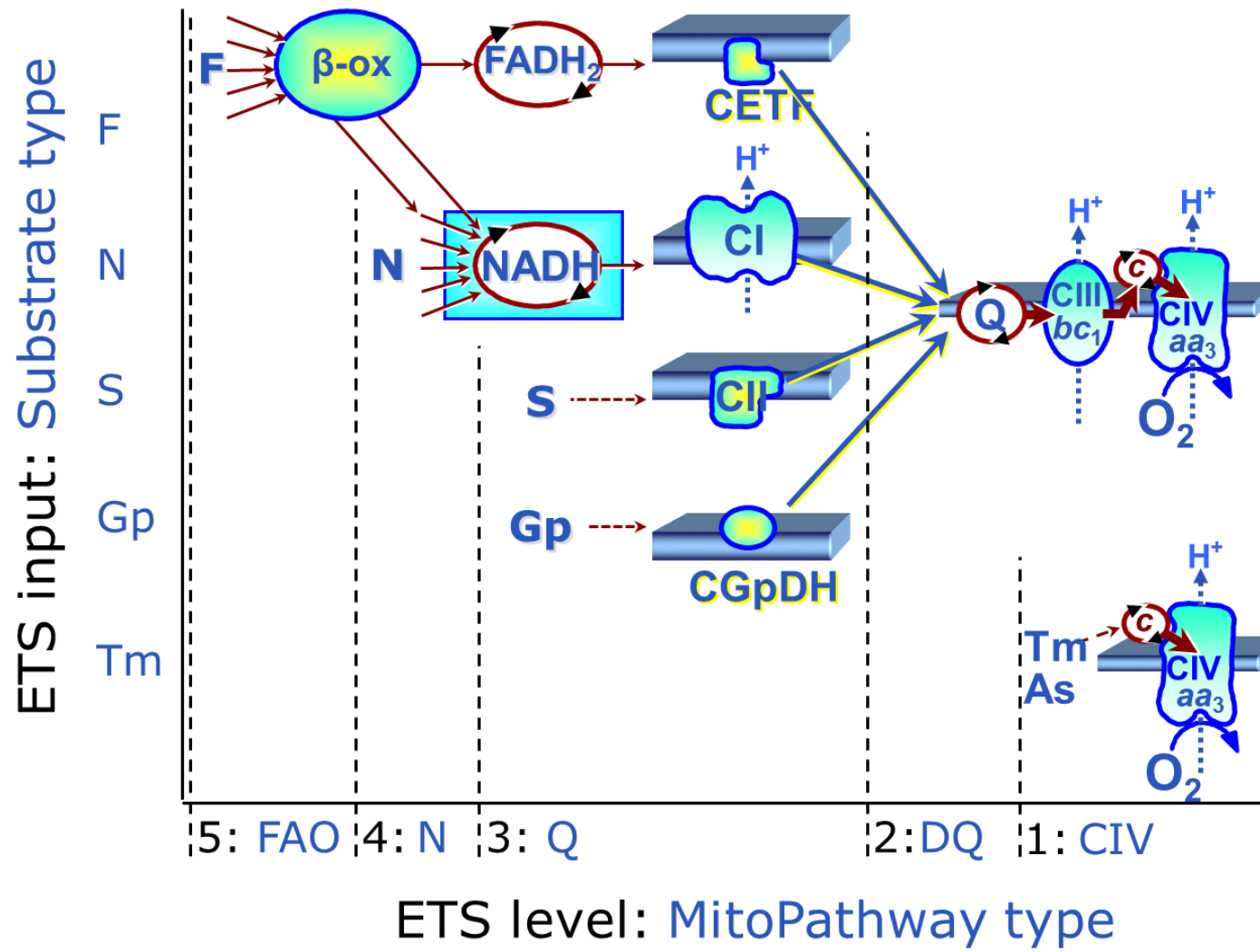


## ET state and rate *E*:

Maximum oxidation/respiration while phosphorylation is zero  
Exogenous uncoupler (protonophore)



# Mitochondrial pathways





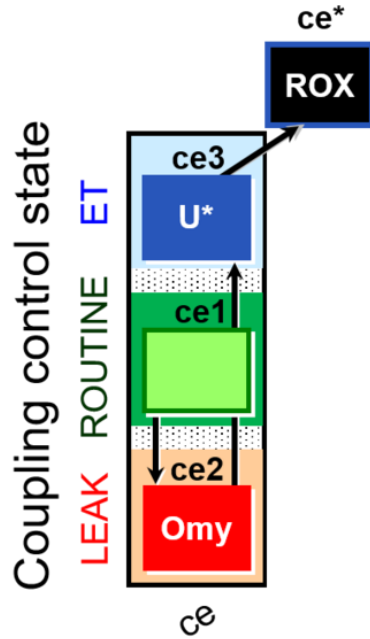
# Which sample preparations can we use?

- Isolated mitochondria (imt)
- Tissue homogenate (thom)
- Cells (ce, living cells and pce, permeabilized cells)
- 3D cell cultures
- Biopsies
- Permeabilized fibers (pfi)
- Brain slices
- Parasites
- *Drosophila*
- *Caenorhabditis elegans*
- Algae (e.g. *Chlamydomonas*)

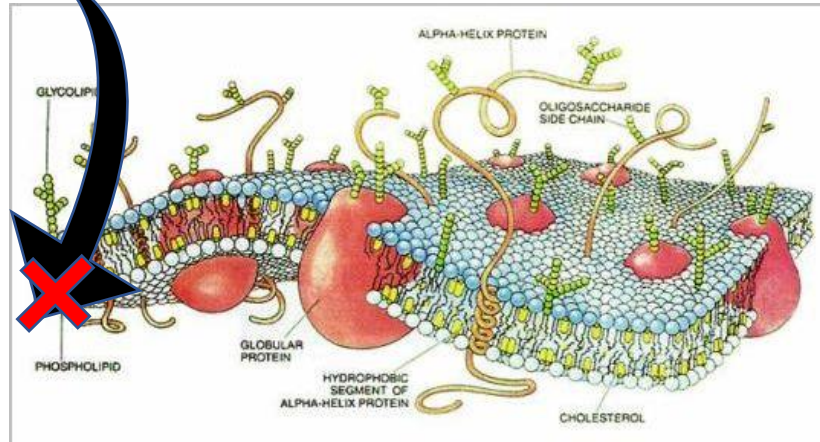
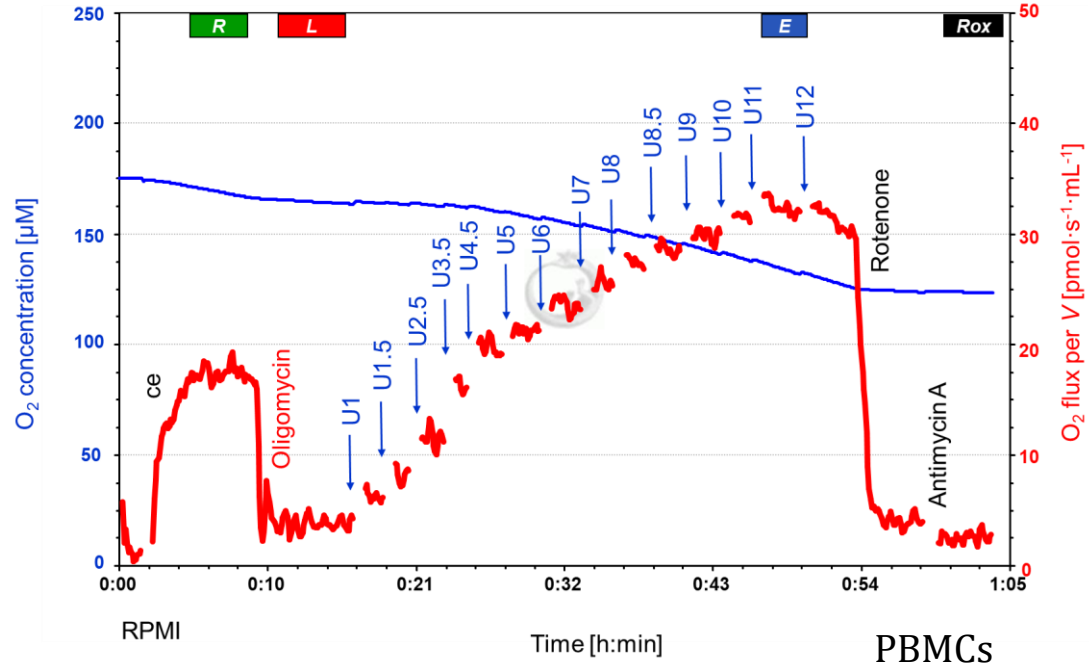


# Coupling Control Protocol (CCP) in living cells

Living cells (ce)

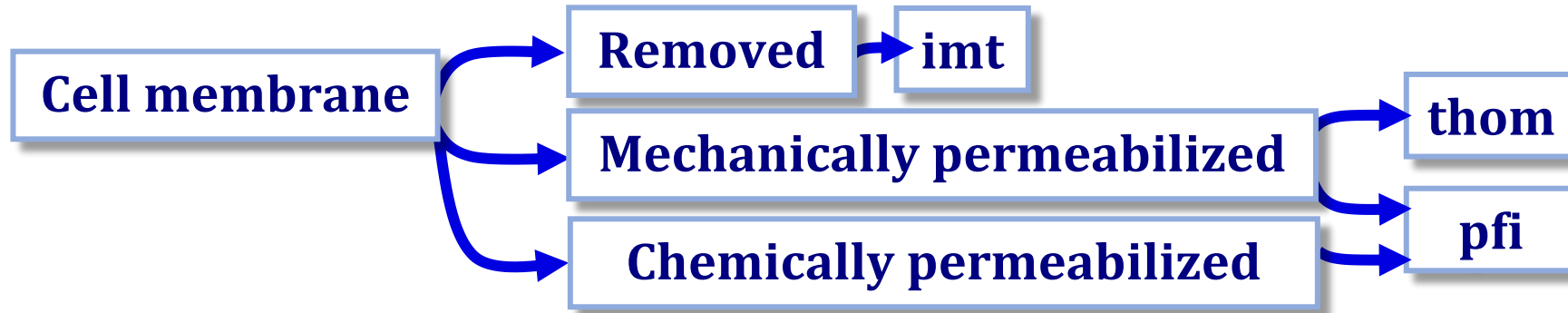


Succinate  
ADP  
Cytochrome c



Cell plasma membrane

# Mitochondrial preparations: mtprep



**Mitochondrial functional integrity is maintained**

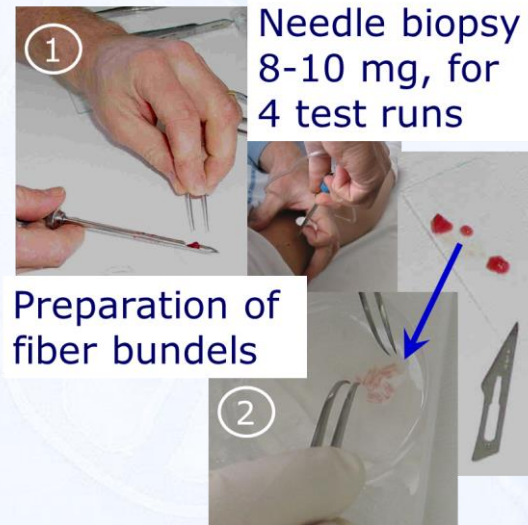
**Mitochondrial preparation types**

- Isolated mitochondria (imt)
- Tissue homogenate (thom)
- Permeabilized cells (pce)
- Permeabilized tissue (pti)
- Permeabilized fibers (pfi)

# Permeabilized muscle fibers

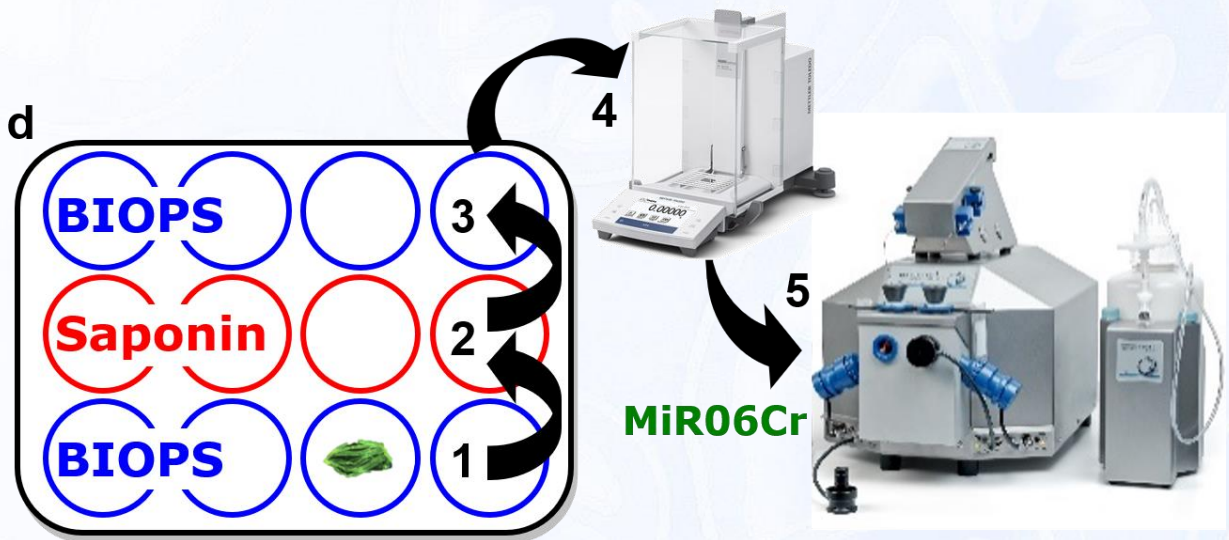
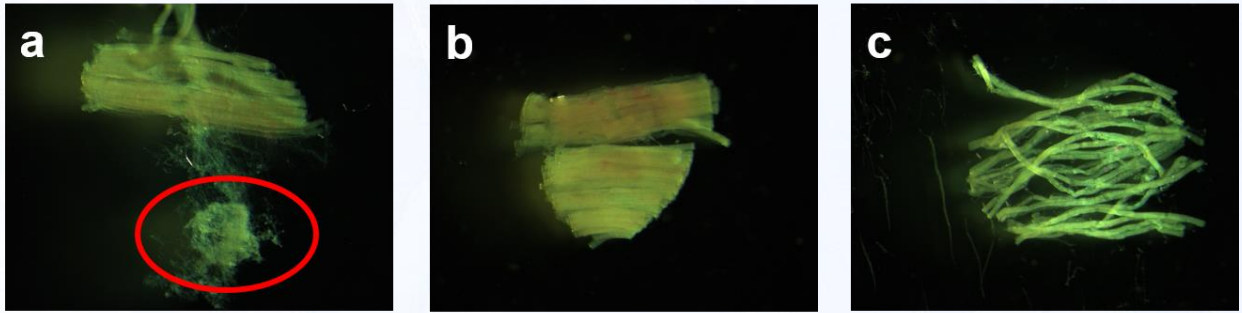
- Less tissue is required
- The mitochondrial morphology is not fragmented due to mechanical homogenization
- All mitochondrial populations are represented

## Muscle biopsy





# Permeabilized muscle fibers

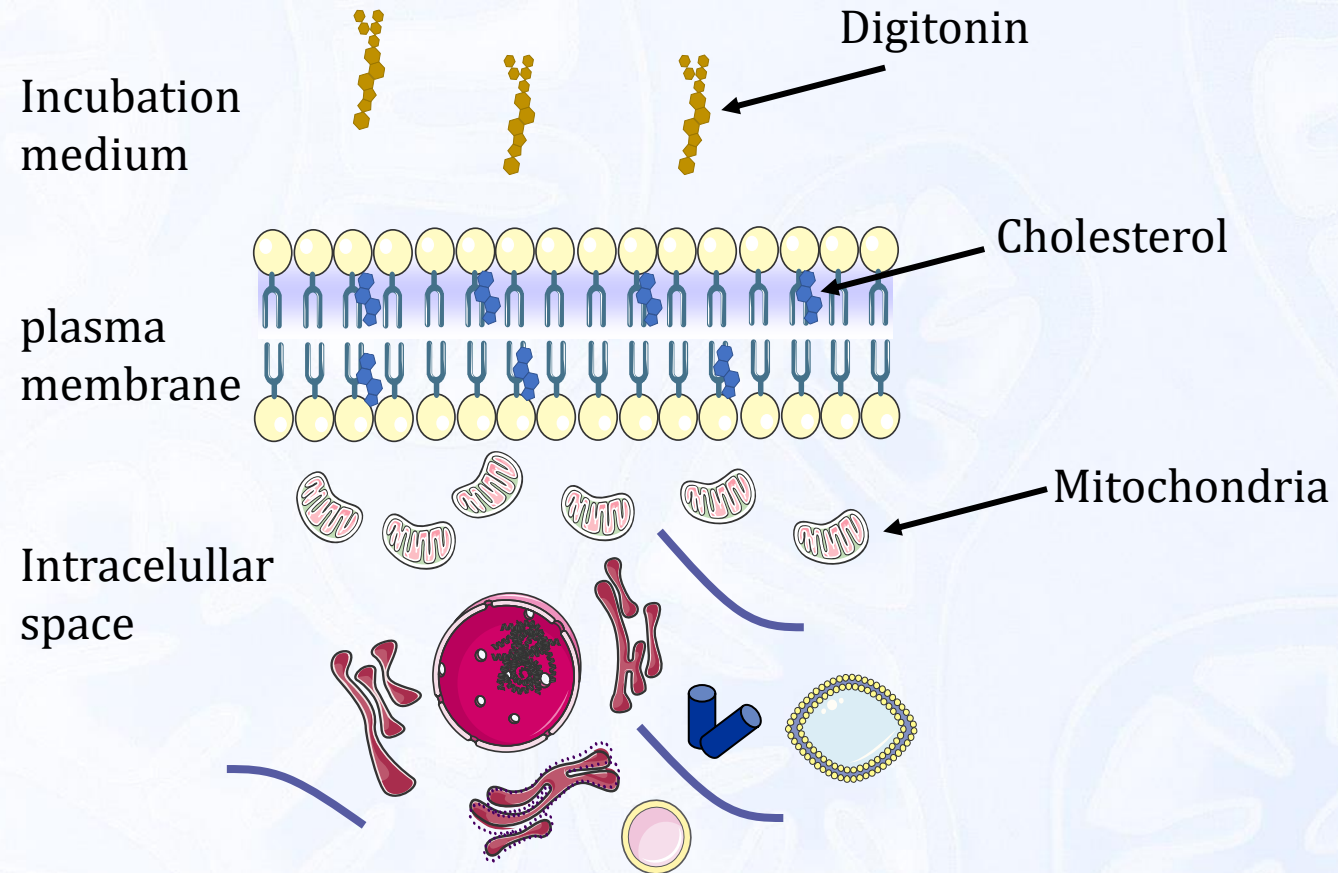




# Tissue homogenate

- The preparation is faster
- Reproducibility
- Tissue homogeneity
- No detergents (i.e. saponin) are required
- Oxygen limitation is reduced in them compared to pfi
- Small amounts of tissue are needed compared to isolated mitochondria

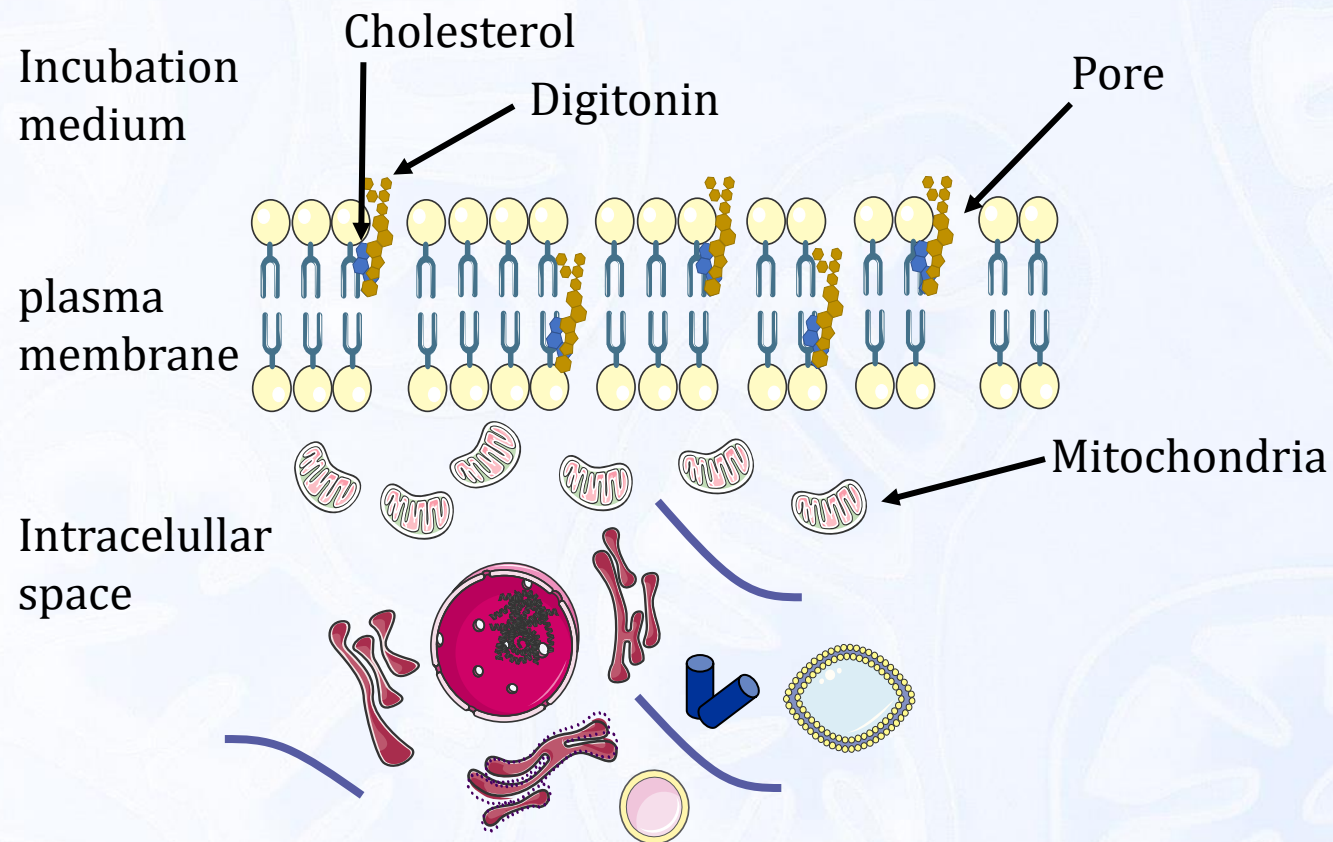
# Plasma membrane permeabilization with digitonin





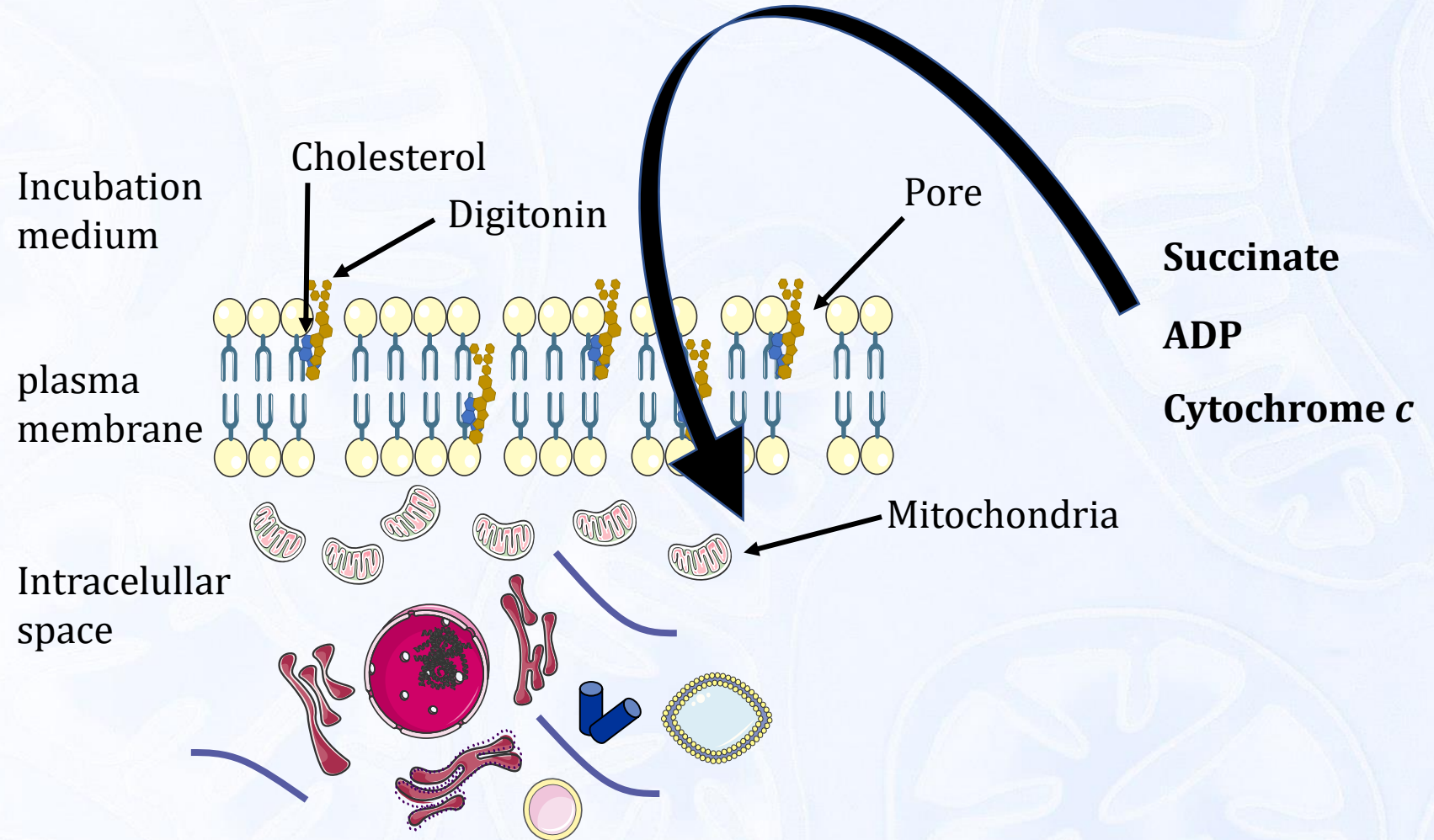
# Plasma membrane permeabilization with digitonin

**Permeabilized  
cells (pce)**



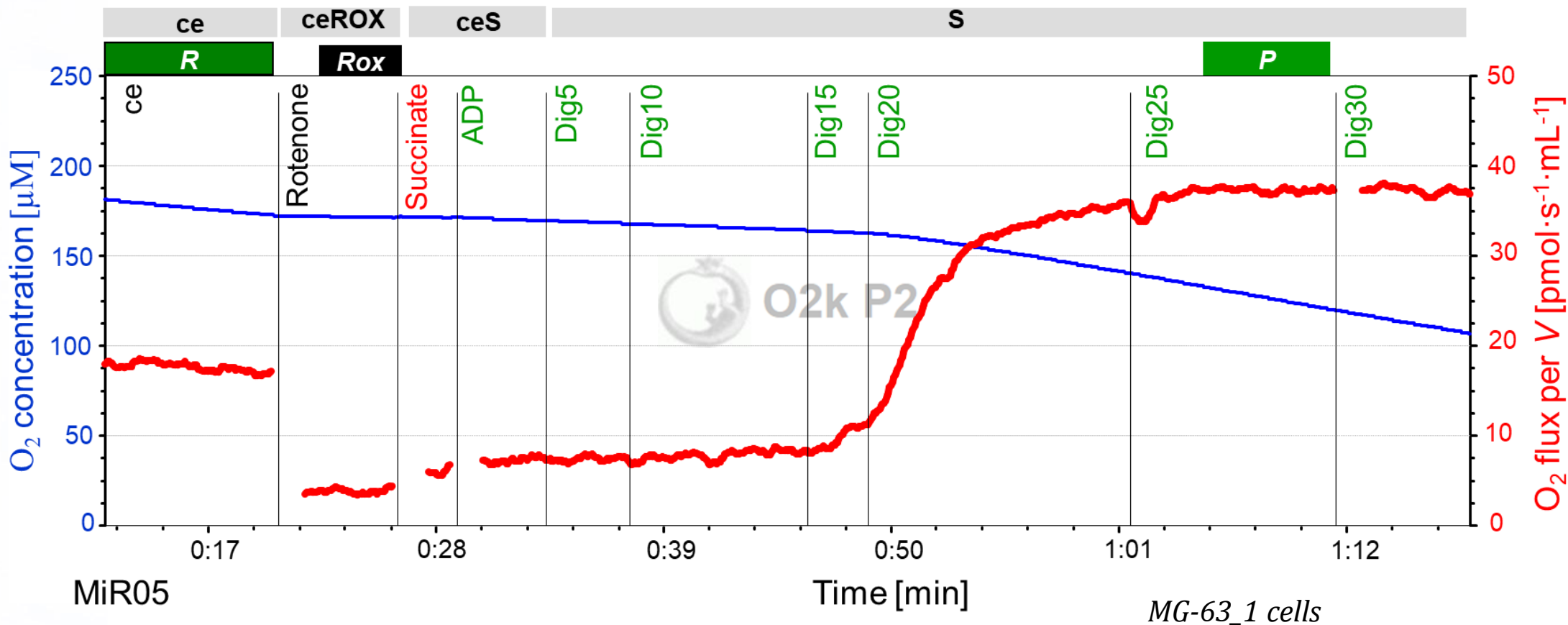
# Plasma membrane permeabilization with digitonin

**Permeabilized  
cells (pce)**



# Digitonin test

## Permeabilization of the plasma membrane



[SUIT-010 O2 ce-pce D008.DLP](#)



# Blood cells as potential diagnostic tool

Sysmex XN-350 hematology analyzer

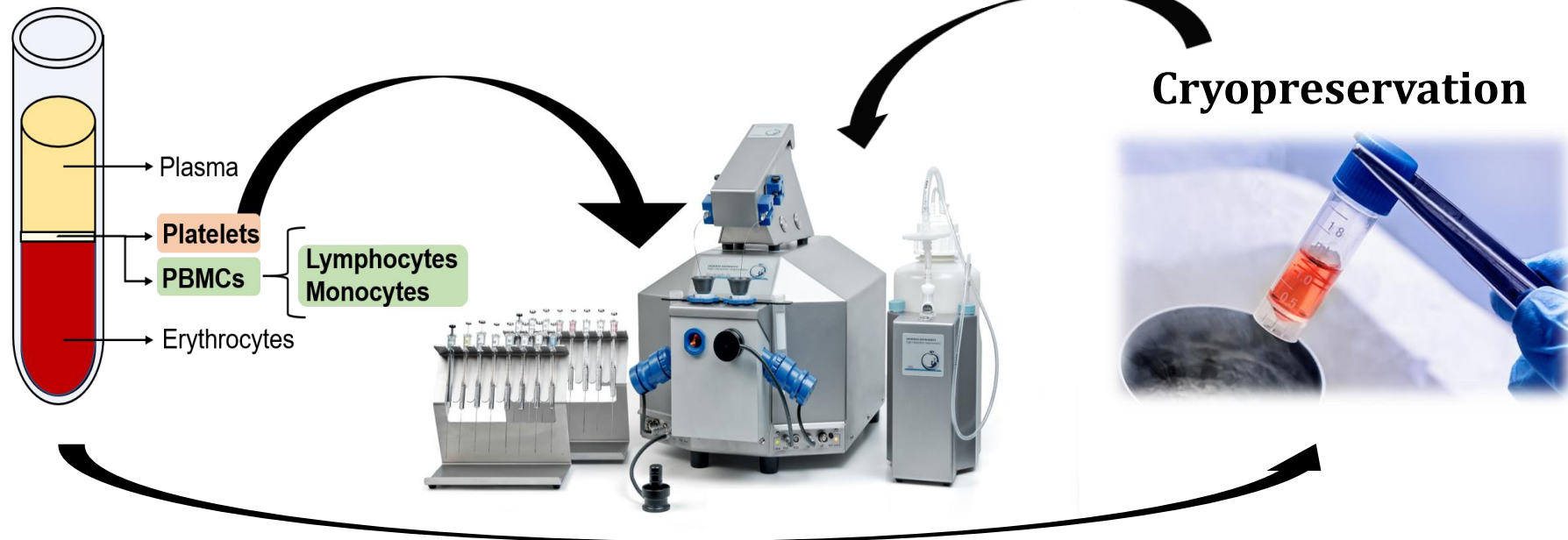


Main			Graph			Q-Flag			Service		
Item	Data	Unit	Item	Data	Unit	Item	Data	Unit	Item	Data	Unit
WBC	6.79	10 <sup>3</sup> /uL	NEUT#	4.15	10 <sup>3</sup> /uL	LYMPH%	4.63	10 <sup>3</sup> /uL			
RBC	6.23	10 <sup>6</sup> /uL	MONO#	0.86	10 <sup>3</sup> /uL						
HGB	16.5	g/dL	EO#	0.19	10 <sup>3</sup> /uL						
HCT	49.1	%	BASO#	0.06	10 <sup>3</sup> /uL						
MCV	78.8	fL	NEUT%	61.1	%	LYMPH%	22.5	%			
MCH	26.5	pg	MONO%	12.7	%	EO%	2.8	%			
MCHC	33.6	g/dL	BASO%	0.9	%	IGM	0.01	10 <sup>3</sup> /uL			
PLT	267	10 <sup>3</sup> /uL	IGM	0.1	%						
RDW-SD	48.0	fL									
RDW-CV	18.0	%									
PDW	12.1	fL									
MPV	10.0	fL									
P-LCR	25.7	%									
PCT	0.27	%									

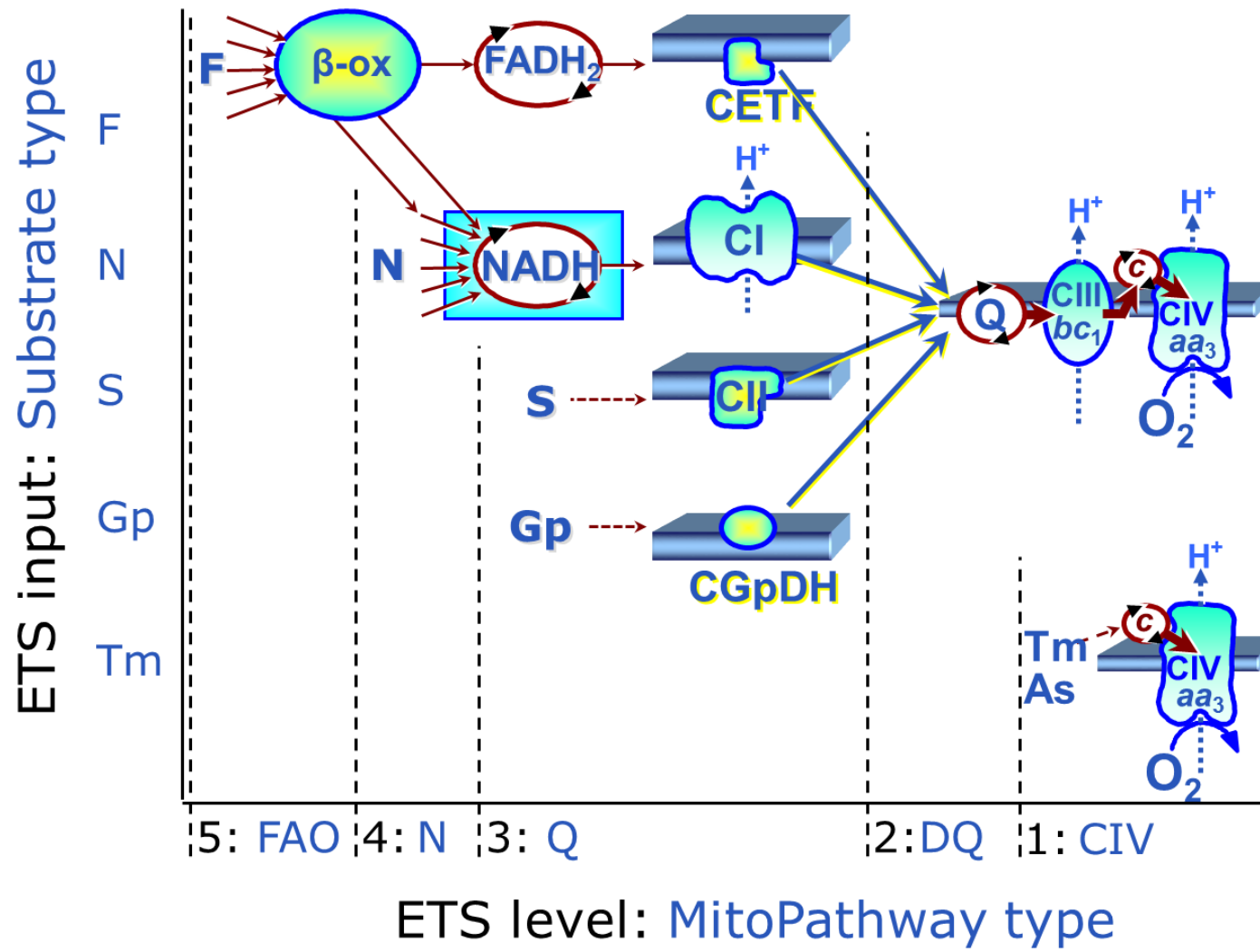
  

Main			Graph			Q-Flag			Service		
RBC			WDF								
PLT			MONO			LYMPH			NEUT		
			PLT			BASO			EO		

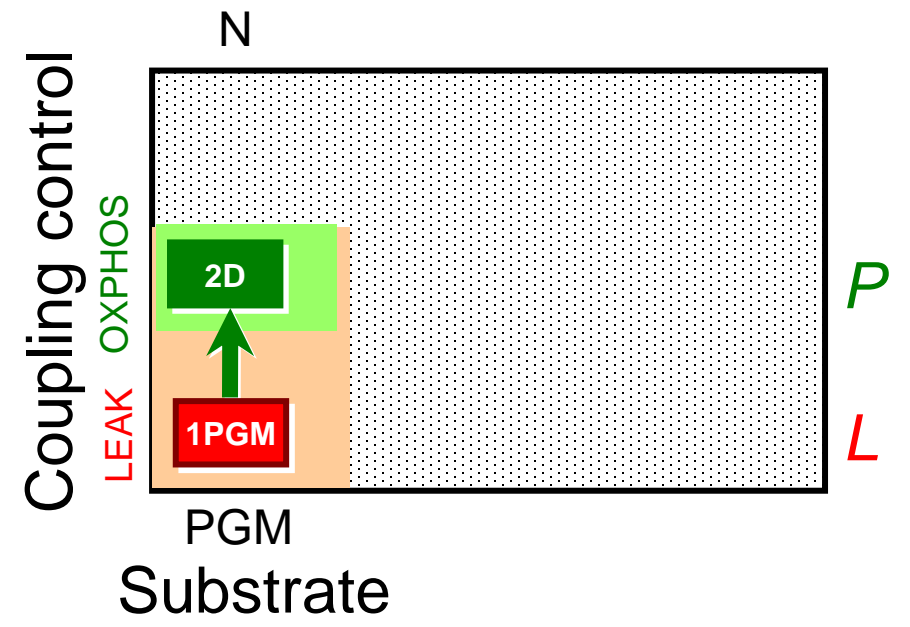
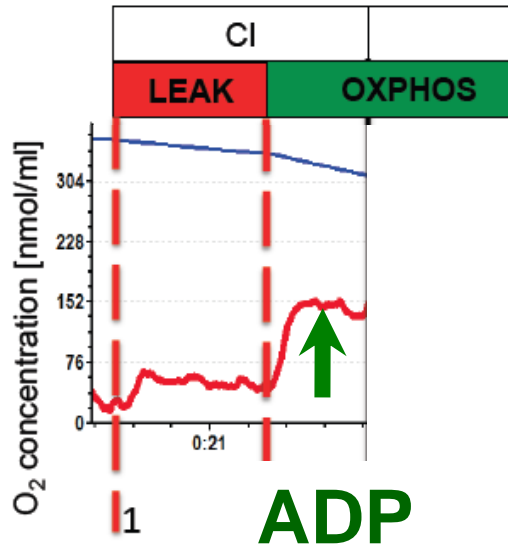
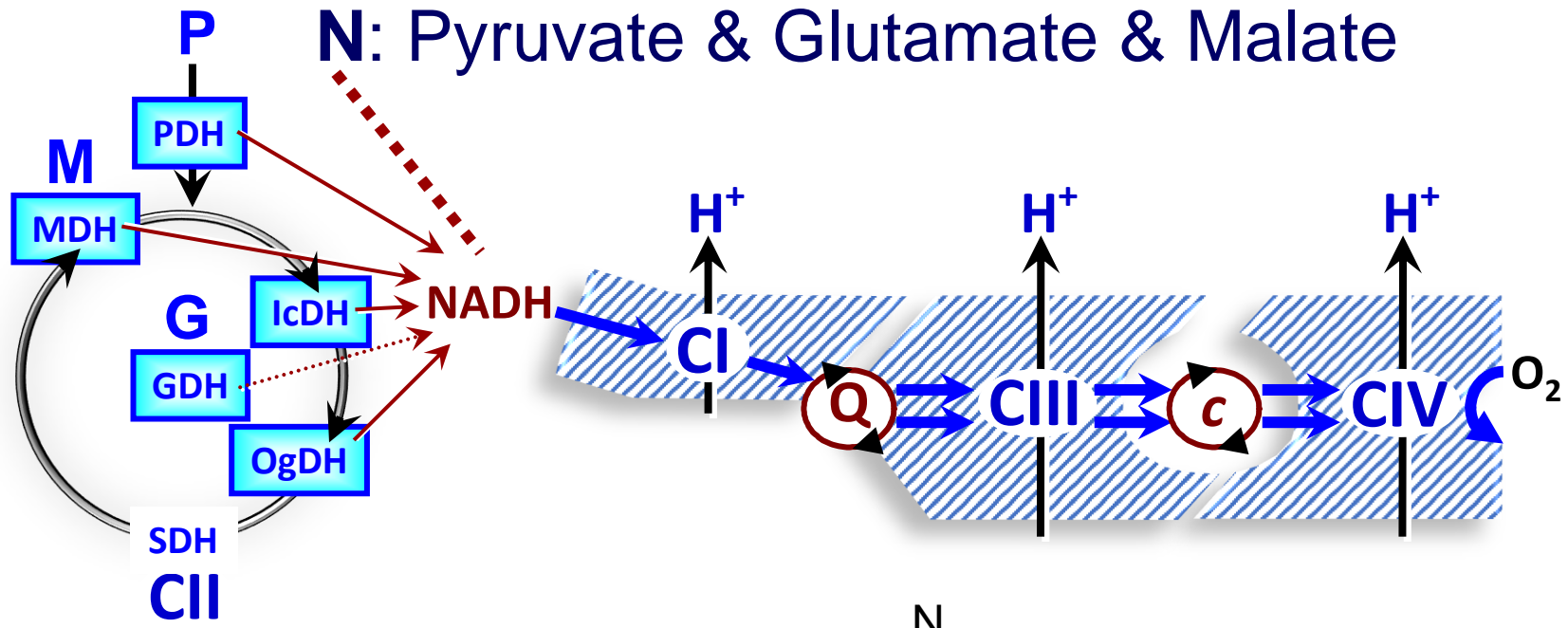
**PBMCs  
and  
platelets**



# Mitochondrial pathways

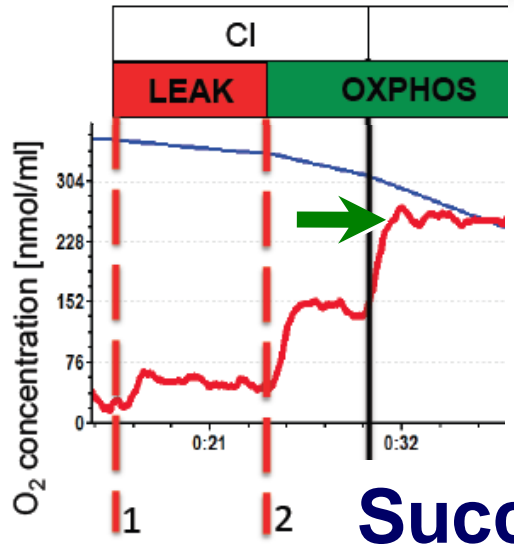
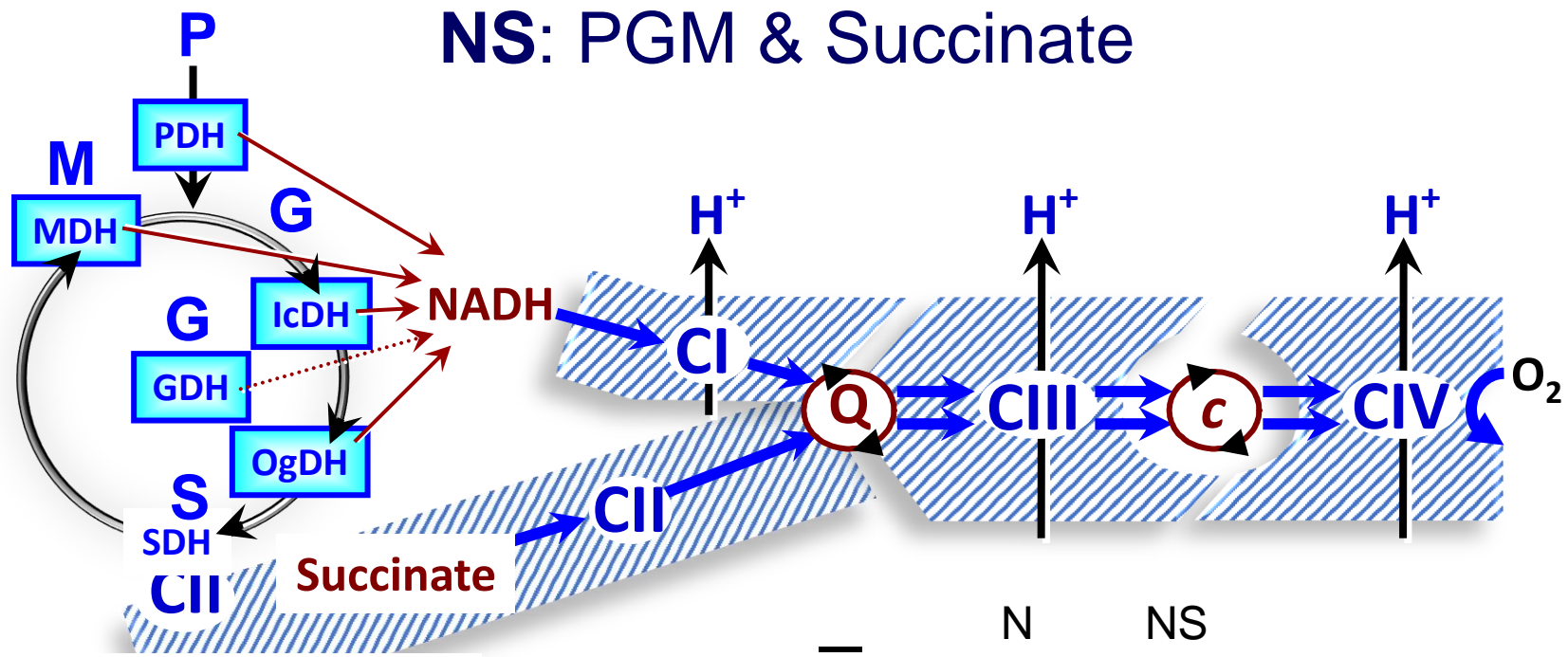


**N:** Pyruvate & Glutamate & Malate

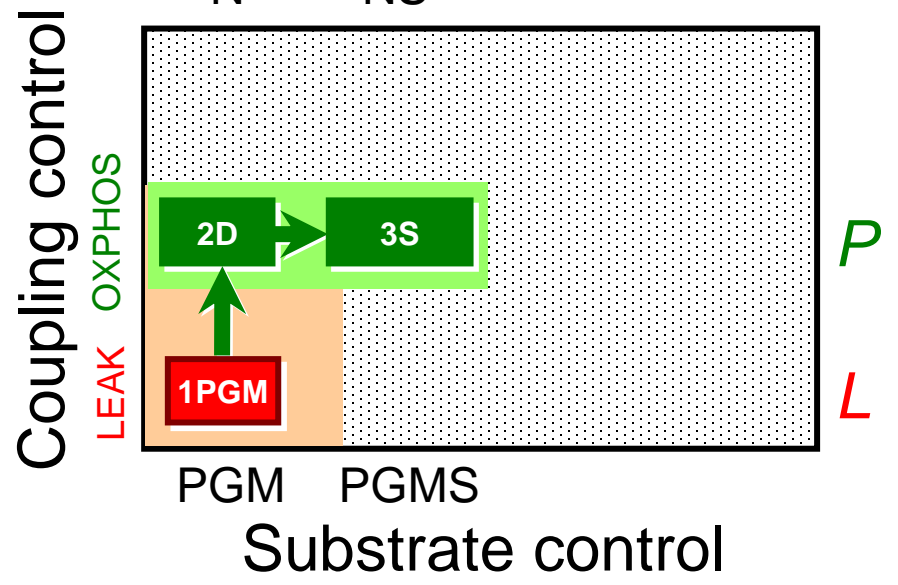


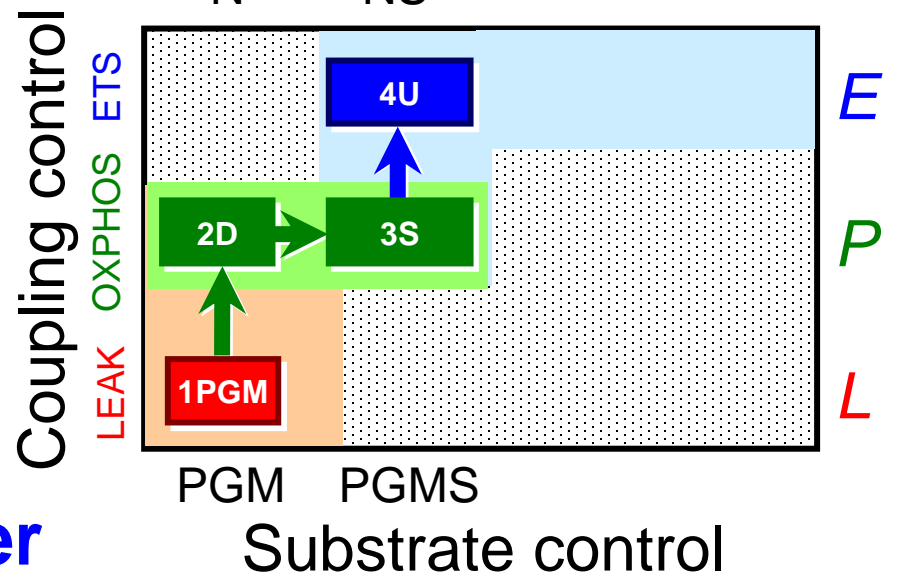
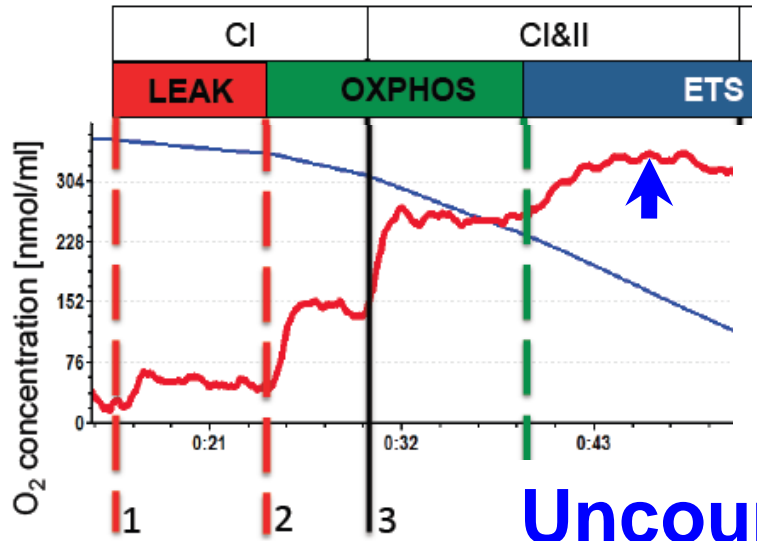
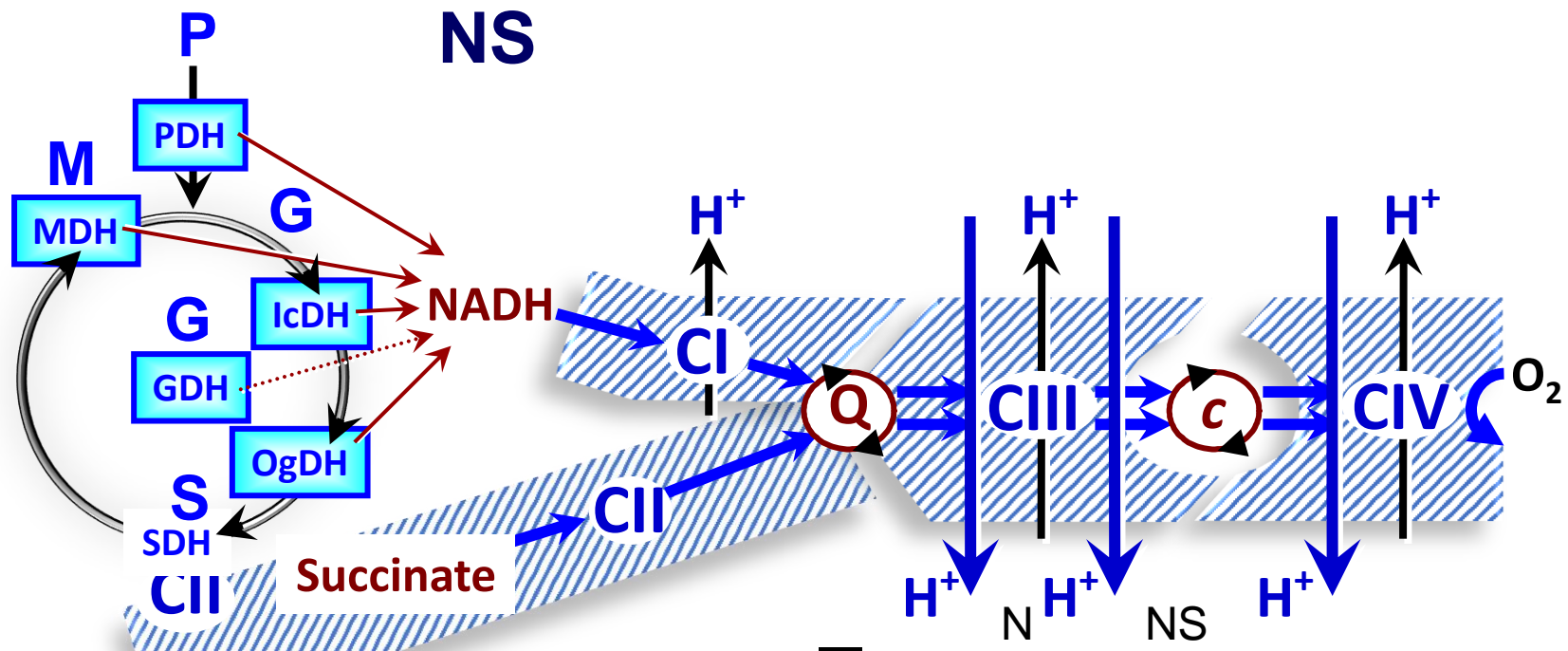


# NS: PGM & Succinate

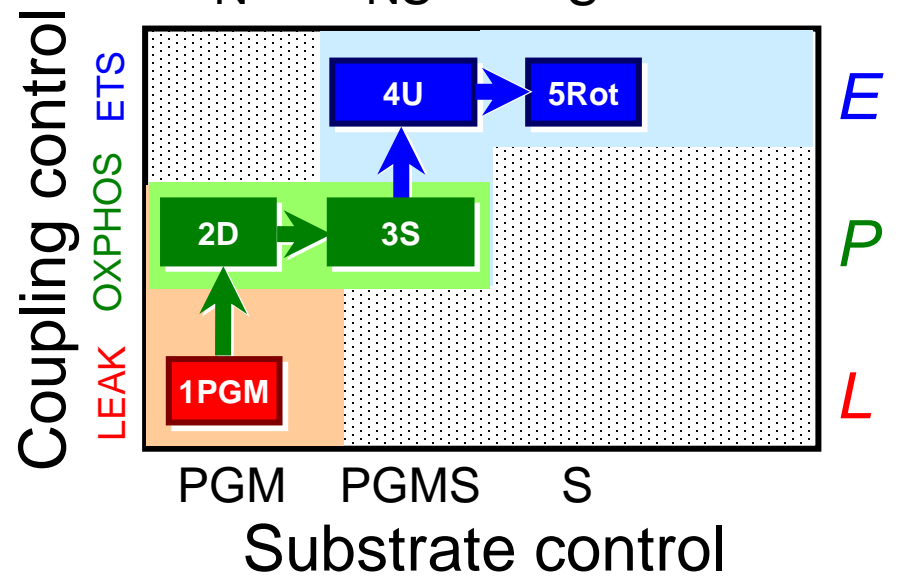
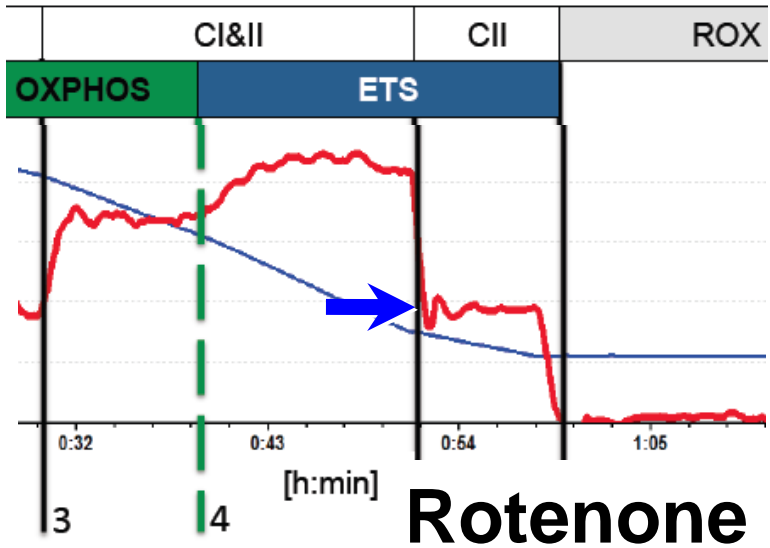
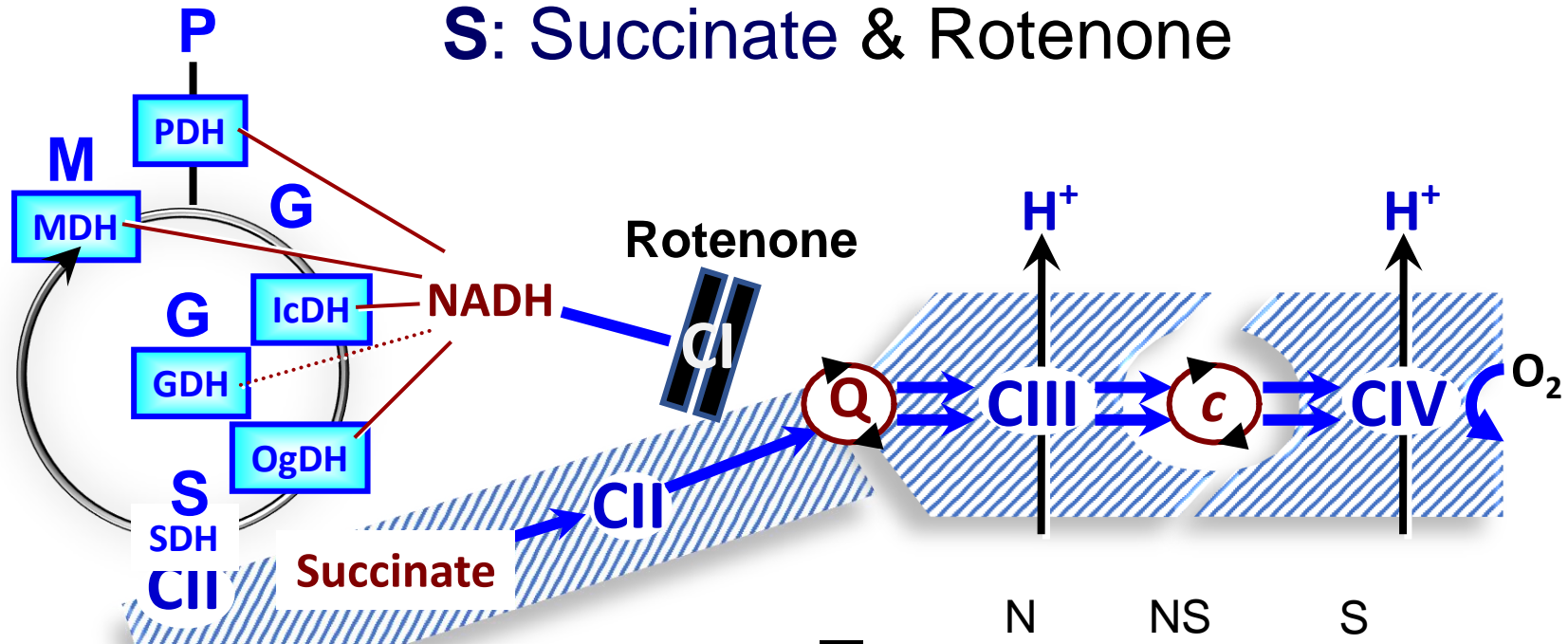


**Succinate**

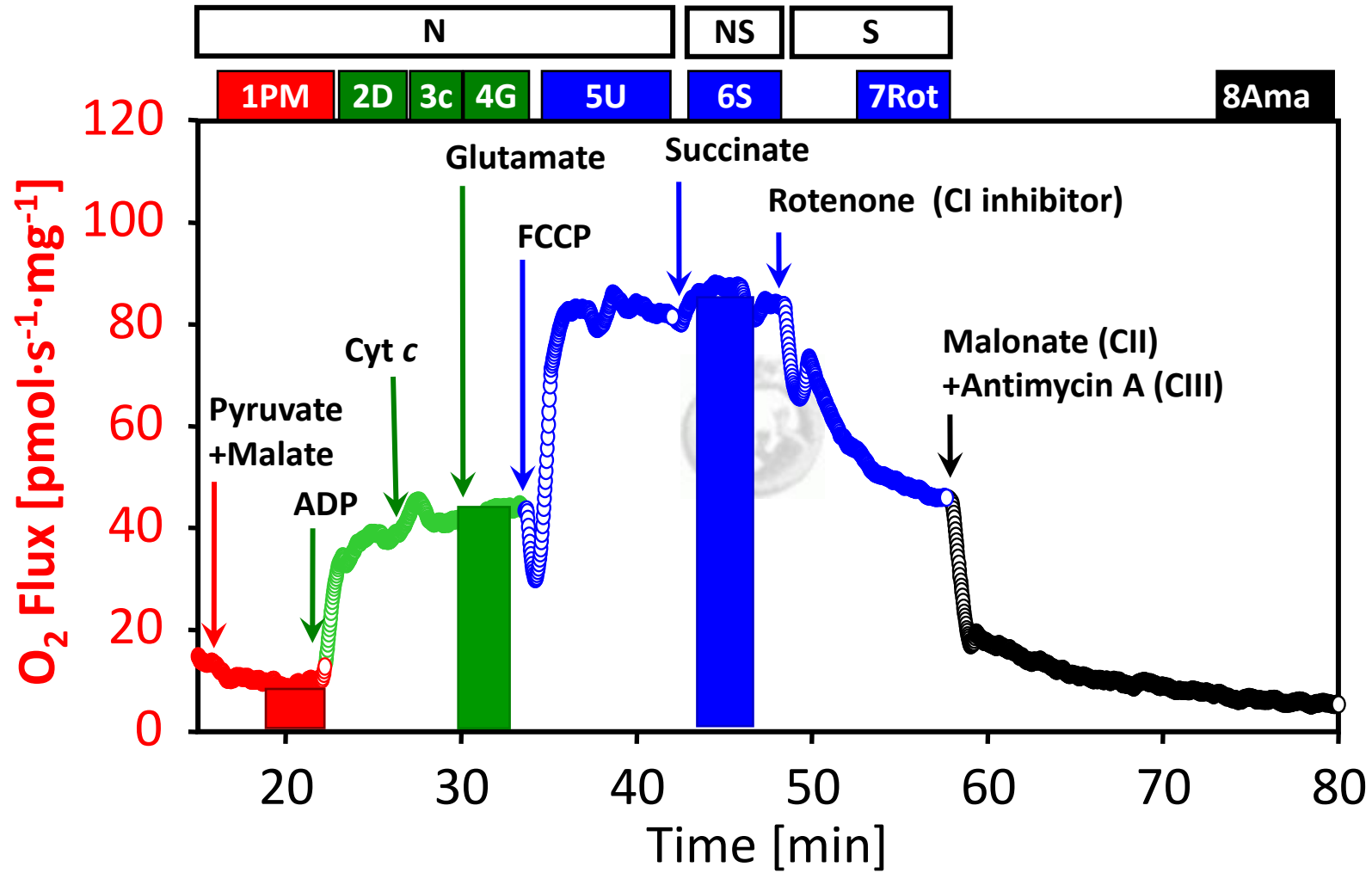




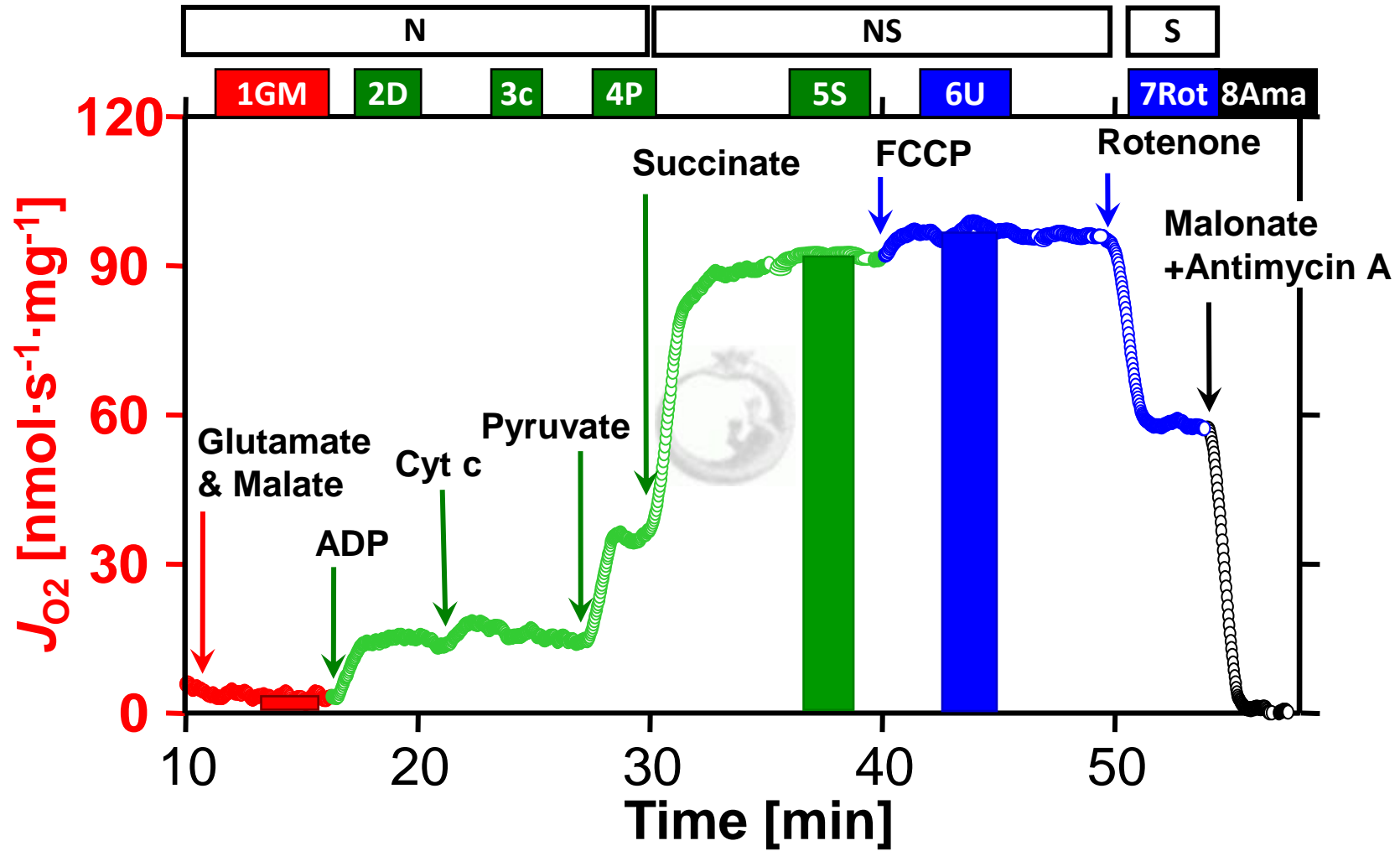
# S: Succinate & Rotenone





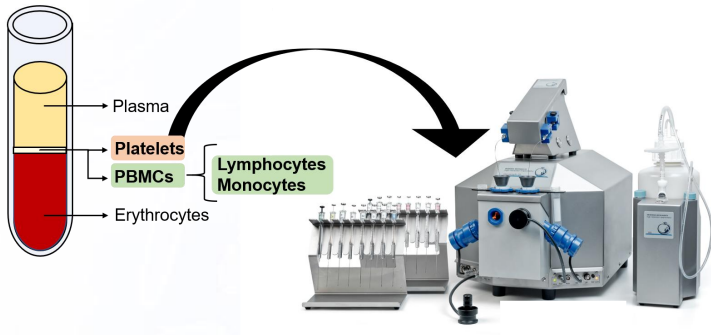
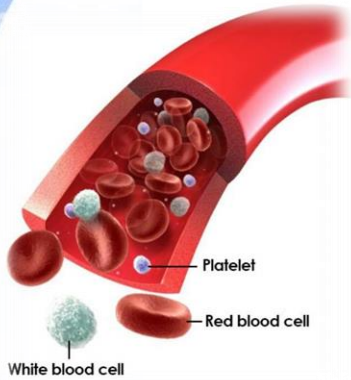


## Human heart, pfi

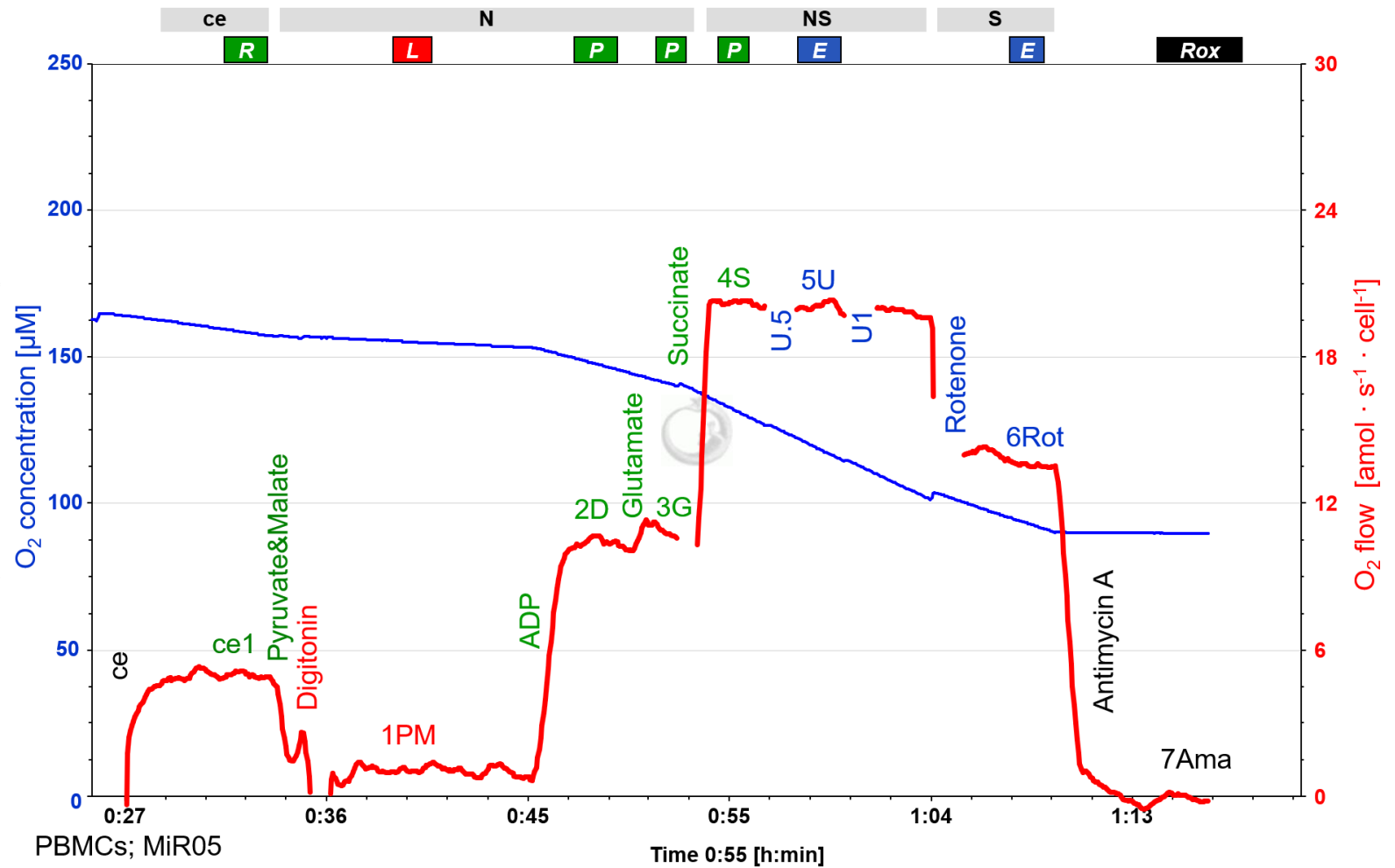


## Mouse heart, pfi

# SUIT protocols: permeabilized cells

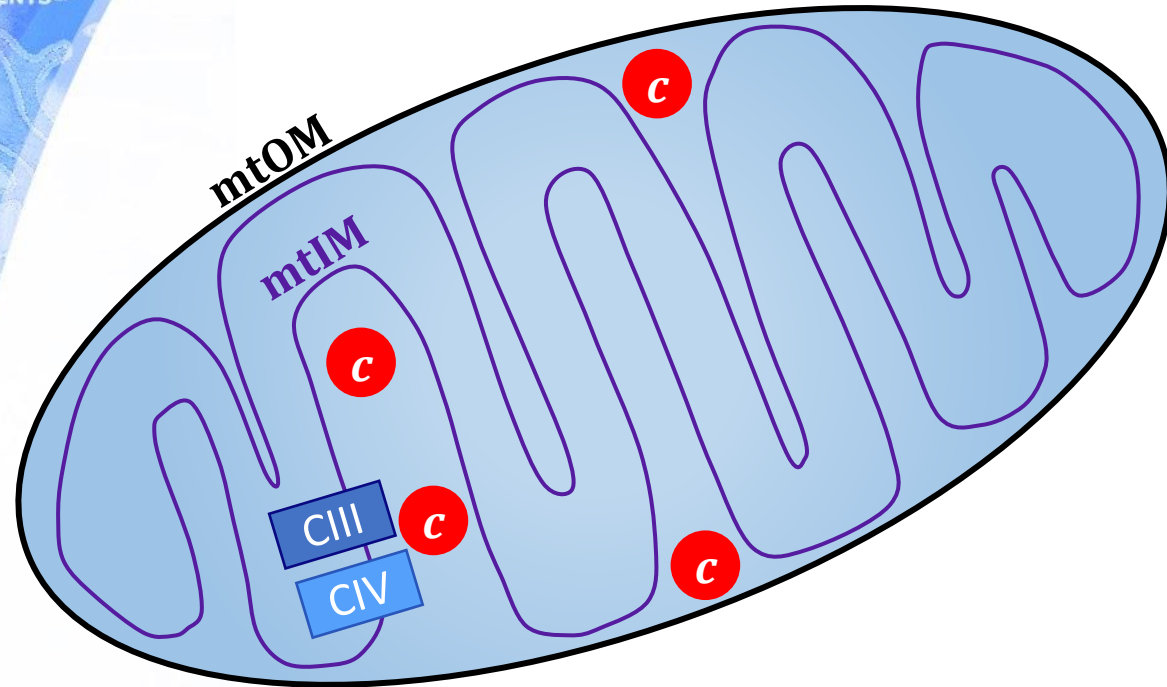


**Permeabilized cells (pce)**



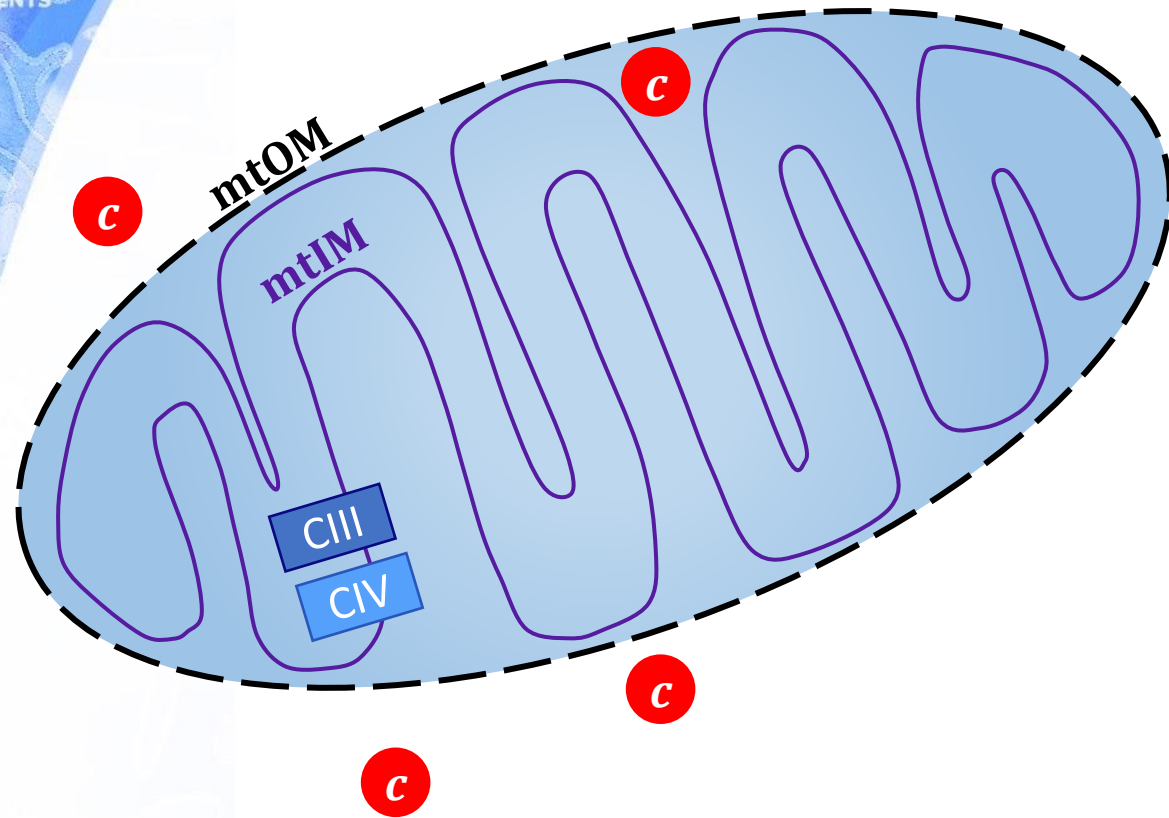


# Cytochrome *c* effect



mtOM integrity evaluation

# Cytochrome *c* effect



# Cytochrome *c* effect

## mtOM integrity evaluation

Sample preparation  
(**exclusion criteria**)

Treatment  
(**relevant result**)

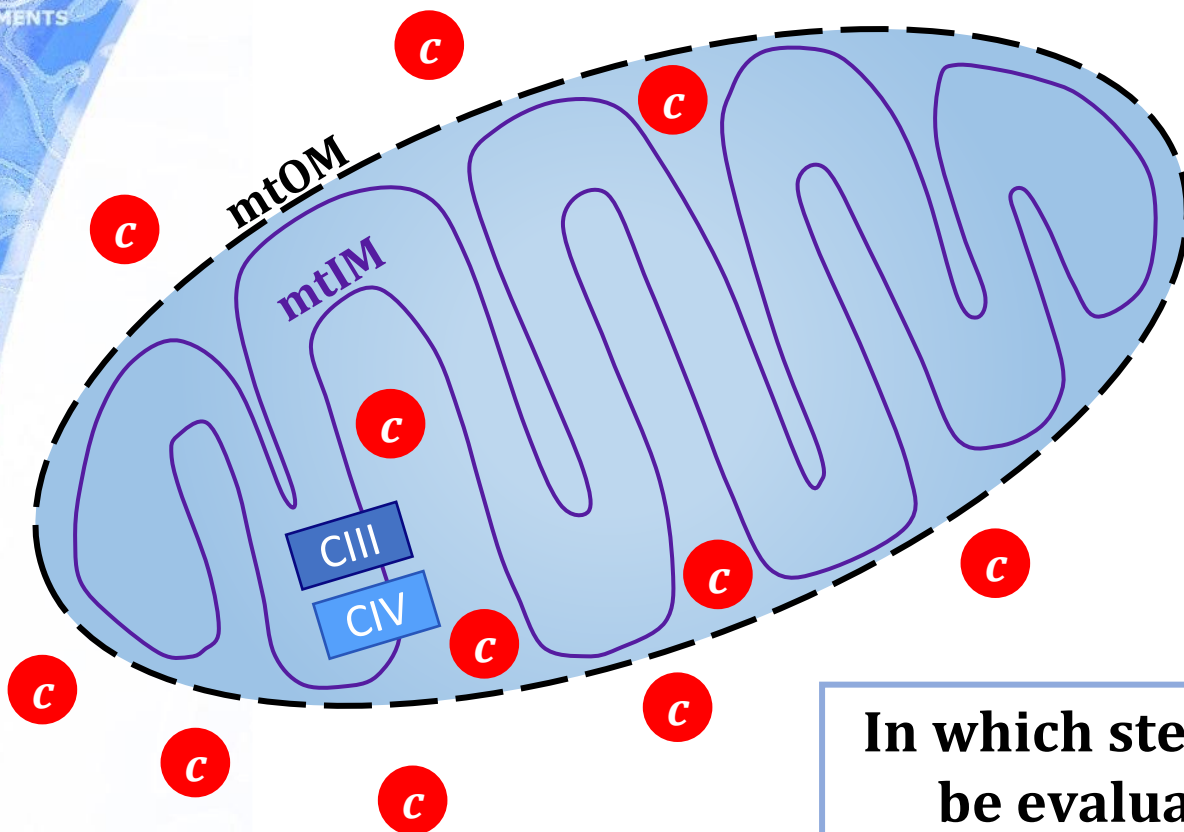
In which step can it  
be evaluated?

Not in LEAK state (after ADP activation)

At early state of the protocol

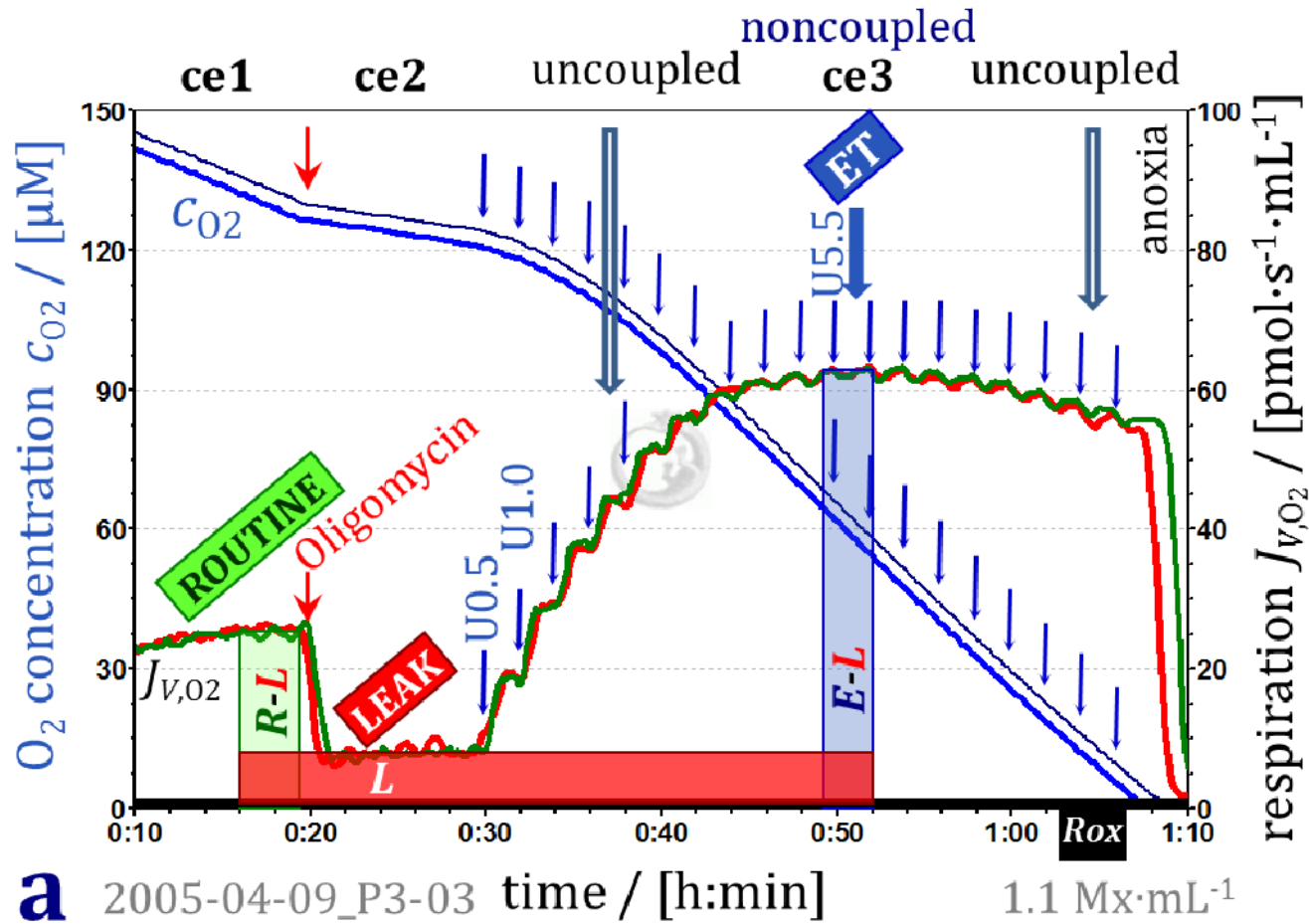
Differences between SUIP  
protocols and sample preparations

$FCF_c > 0.1$





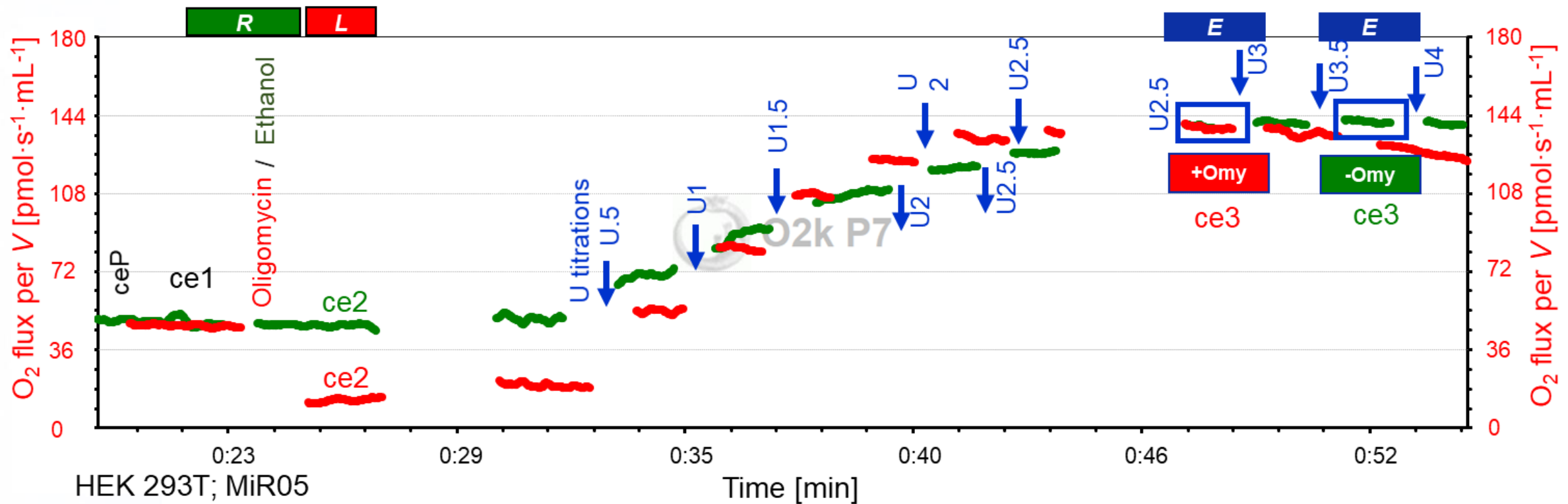
# Uncoupler stepwise titration



parental hematopoietic 32D cells  
RPMI

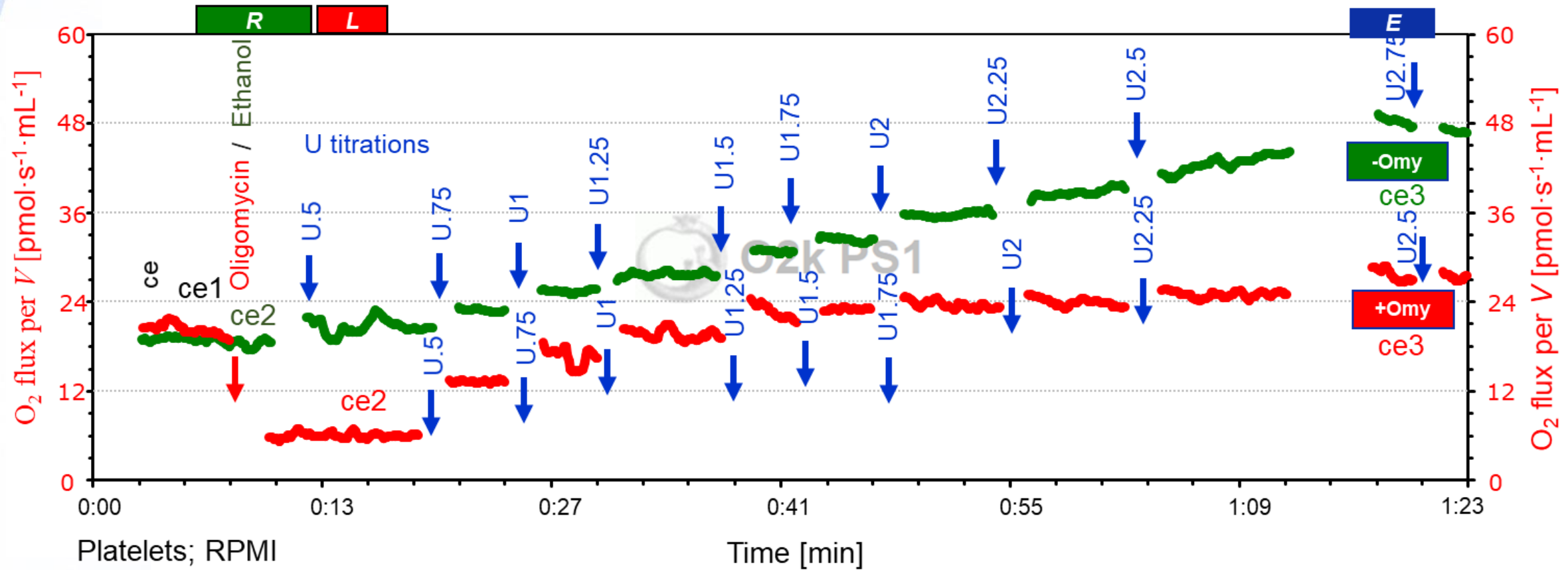
# Oligomycin effect on ET-capacity evaluation

HEK cells



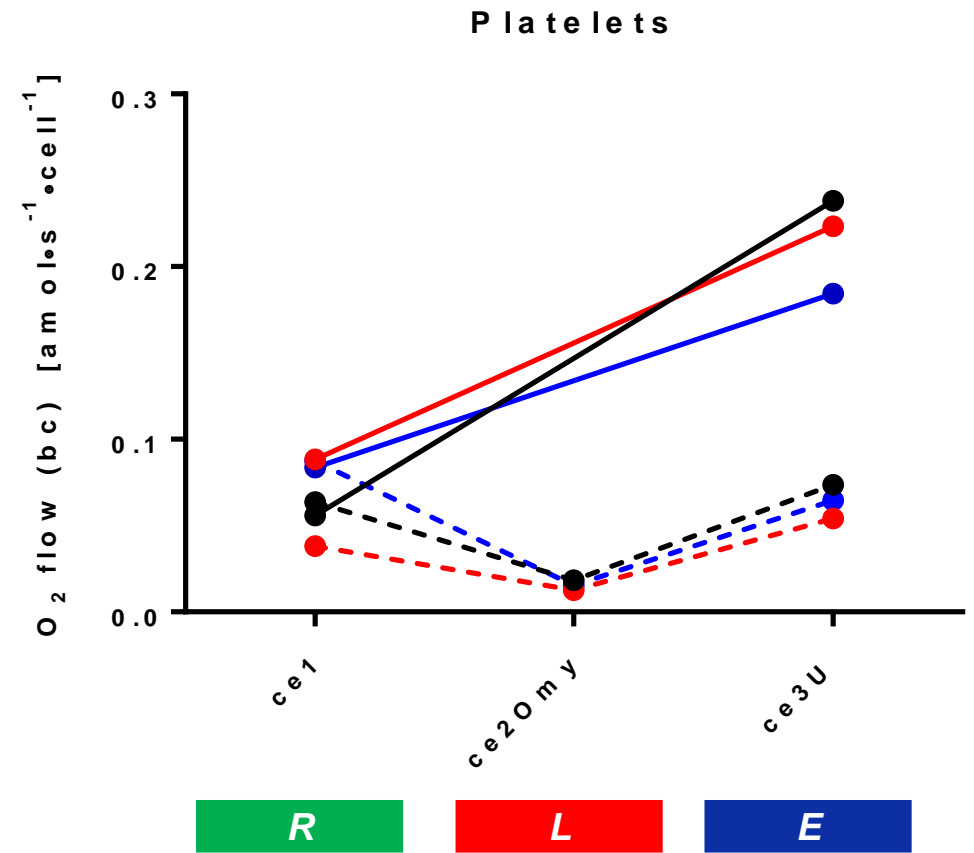
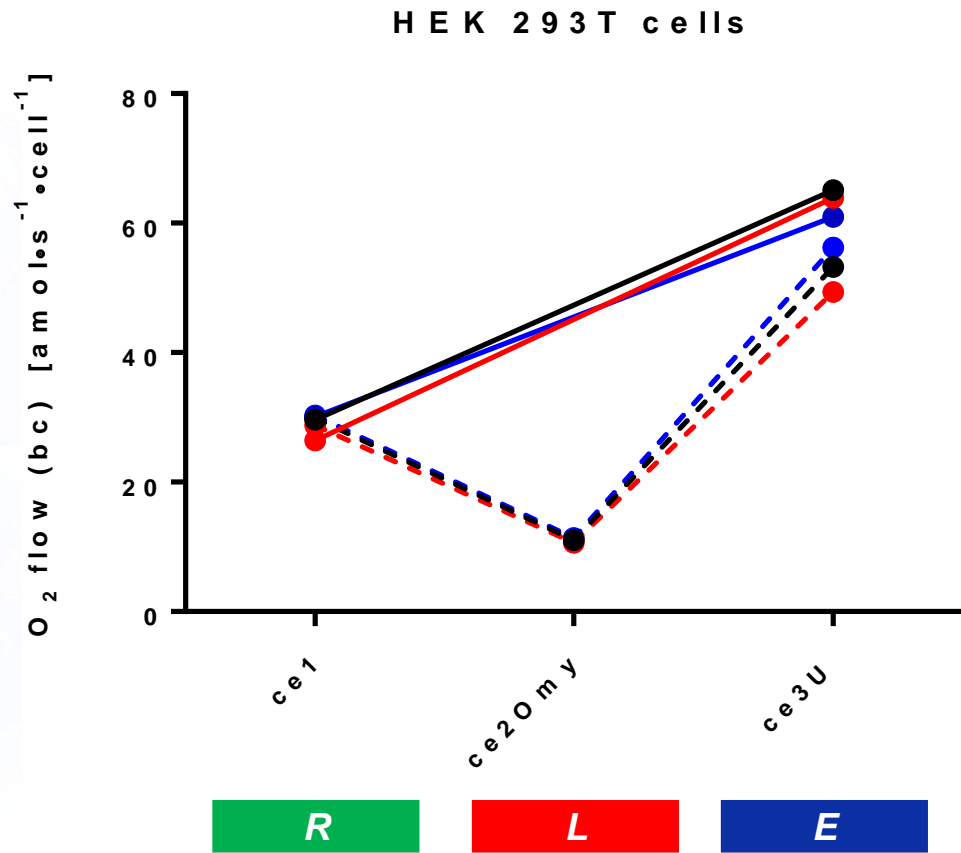
# Oligomycin effect on ET-capacity evaluation

## Platelets

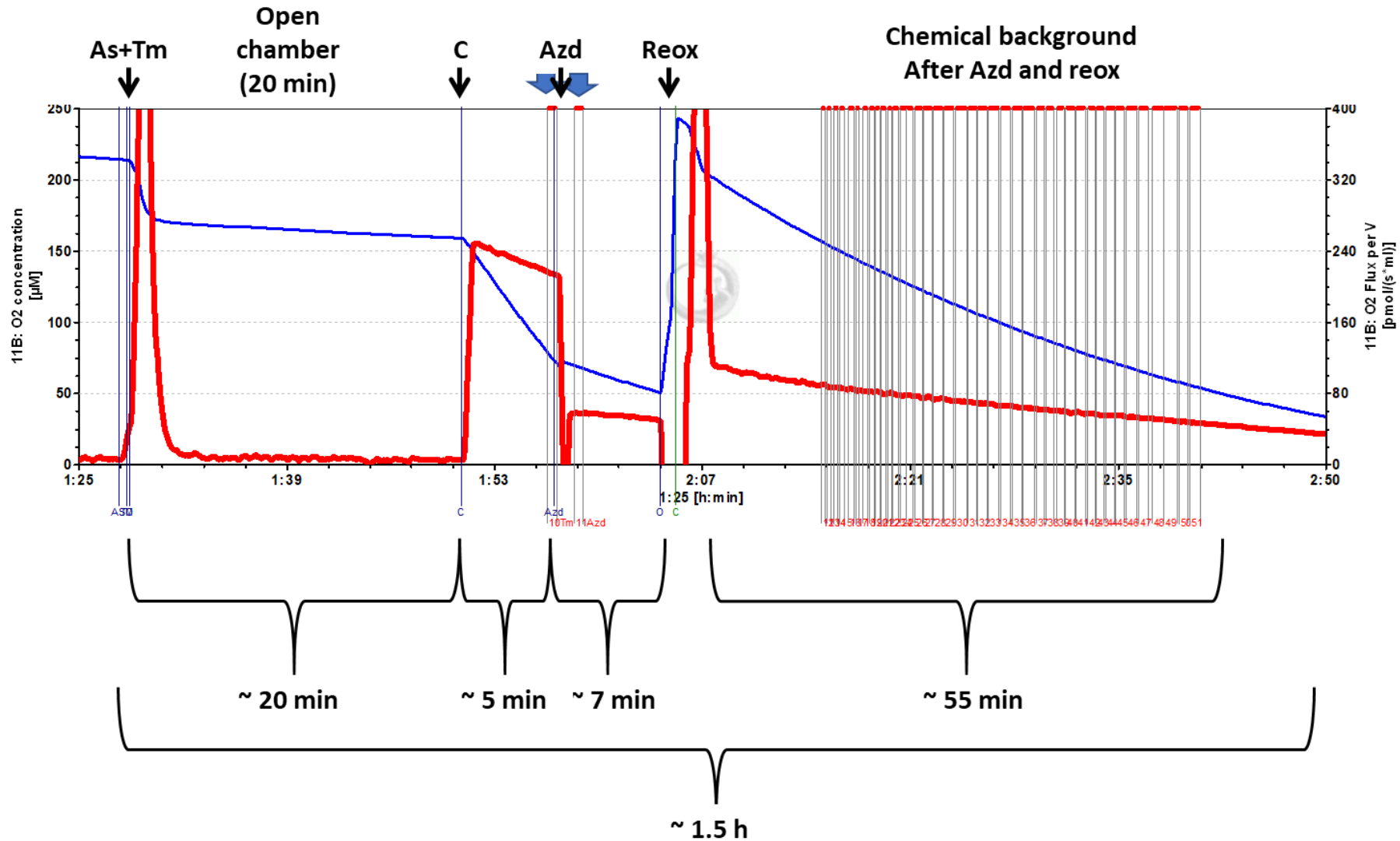




# Oligomycin effect on ET-capacity evaluation

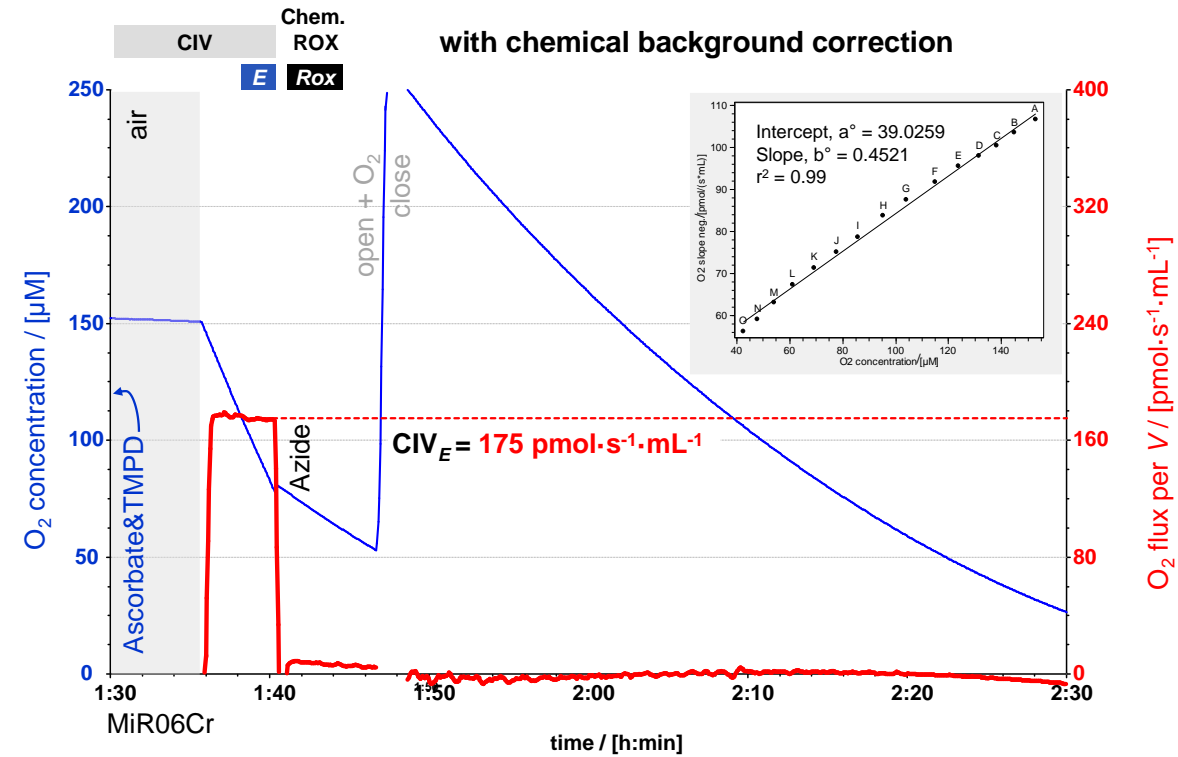
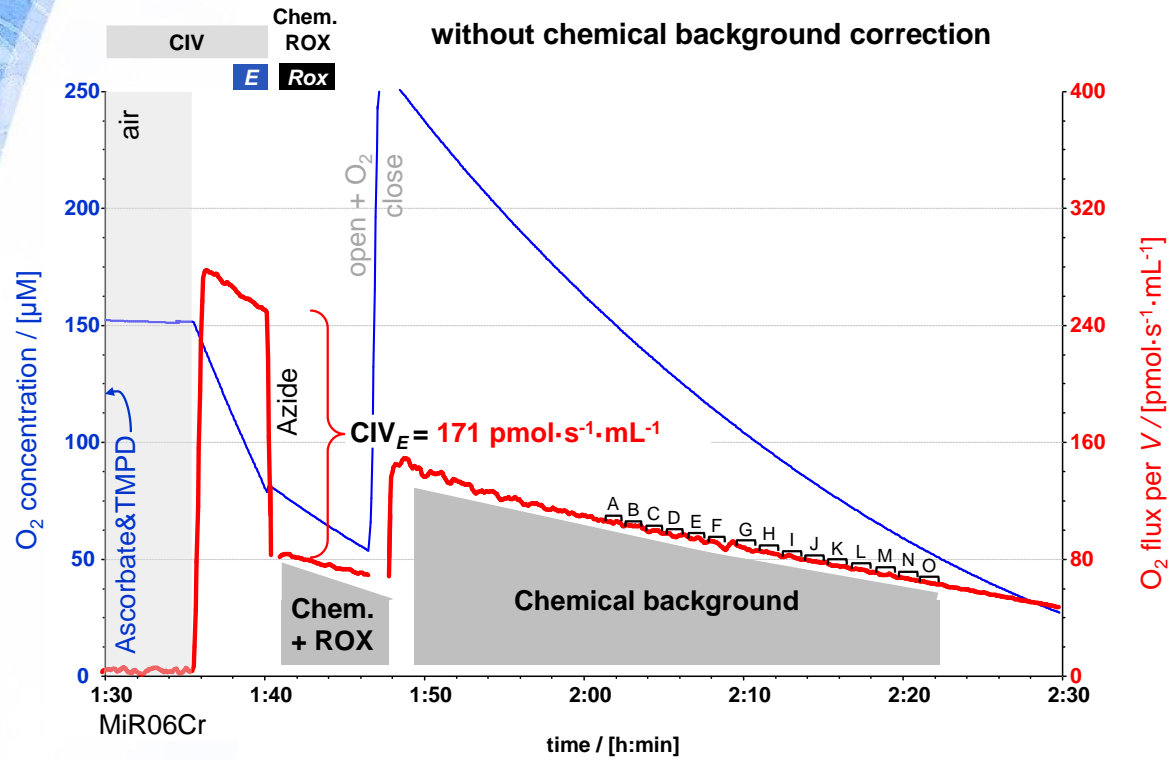


# CIV assay



Additional cleaning: + 20 min with liver thom

# CIV assay



The concentration of O<sub>2</sub> needs to be above 50 µM



# SUIT

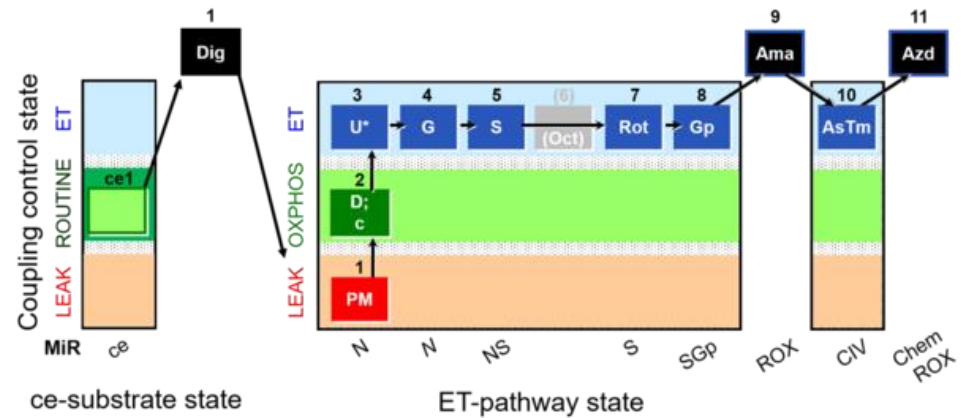
<https://www.bioblast.at/index.php/MitoPedia: SUIT>

SUIT number      O2k-Application      Sample      Unique code number      1 = SUIT sequence  
 O2 = Oxygen      preparation

SUIT code (DLP name) = SUIT-005\_ O2\_ pfi\_ D011

Unique code number      Steps

SUIT technical name = D011\_ 1OctM;2D;3P;4S;5U;6Rot;7Ama



1 = SUIT sequence  
 OctM = substance(s) added

1  
 OctM

\* = Multiple titrations

U\*

(Oct) = Skippable step

(Oct)

\* = option to extend the titrations

ROX

Mitochondrial respiration medium

MiR

Module for living cells (ce)

Module for permeabilized cells (pce)

Module for CIV assay

# Thank you!



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