Quel rôle des microorganismes dans la chimie des nuages ? le cas de H_2O_2

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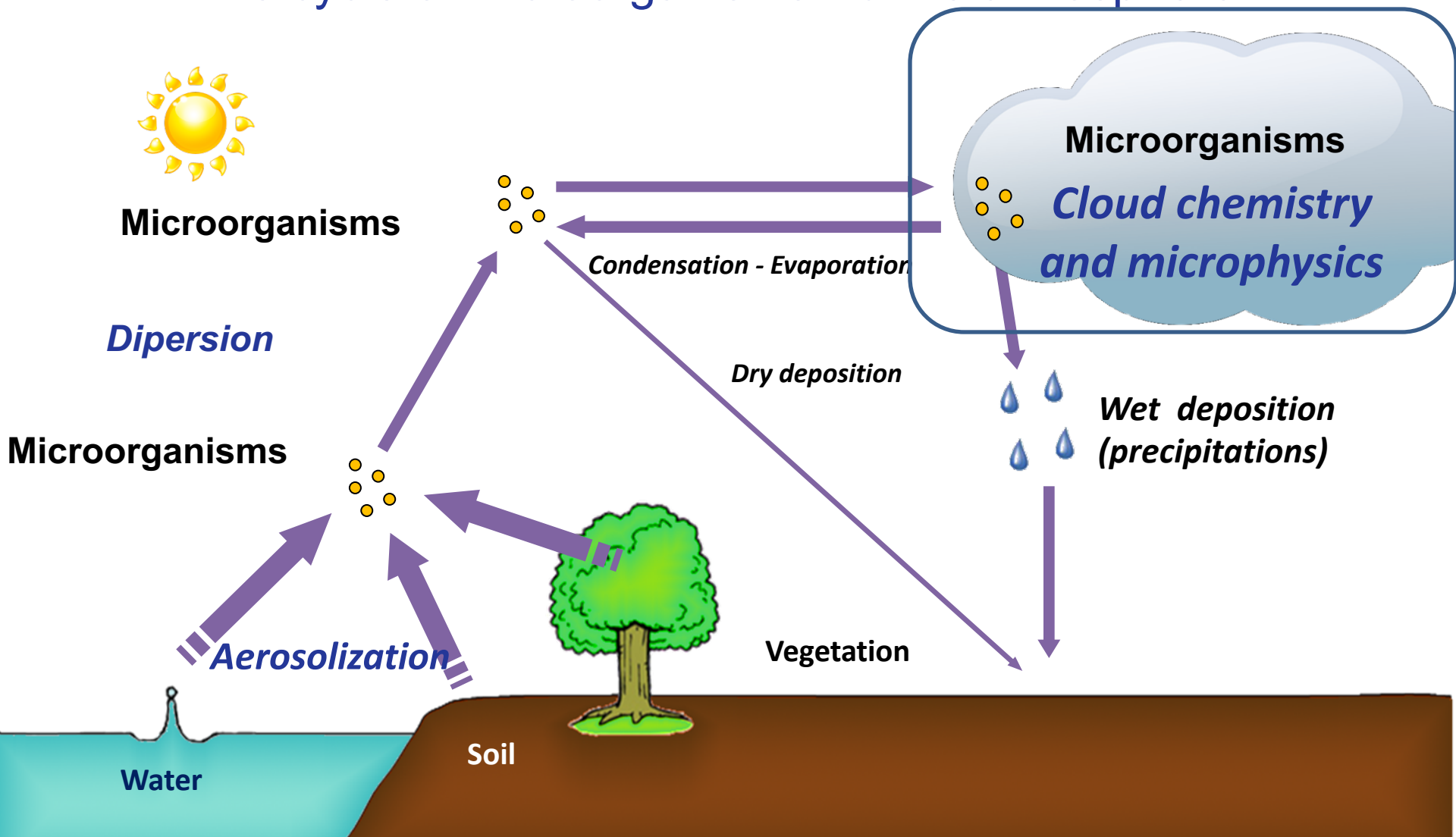
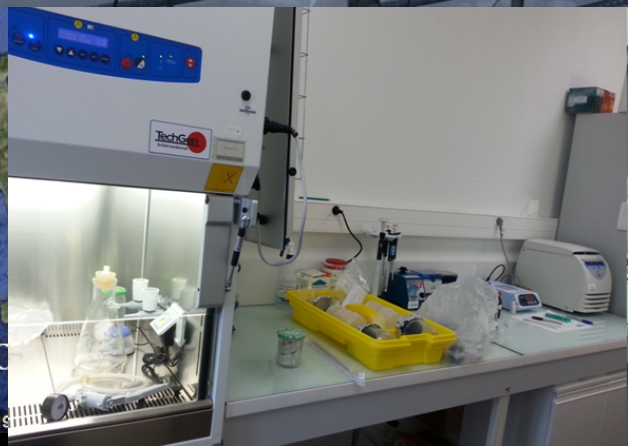
Microorganisms in clouds



Cloud sampling at the puy de Dôme station (1465 m asl)



GAW (Global Atmospheric Watch)
ACTRIS

OPGC

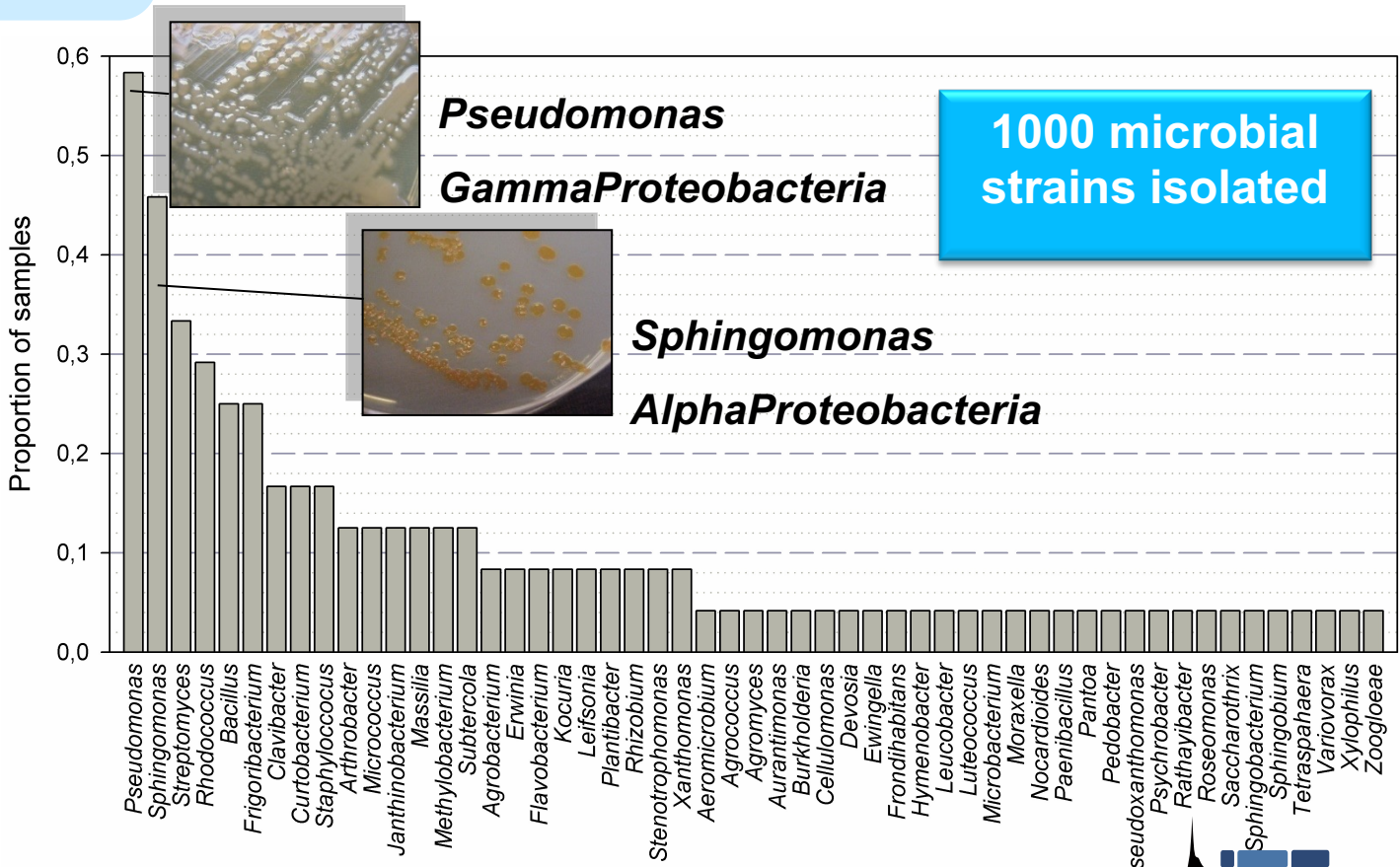
Laboratoire de météorologie physique
LaMP

Microorganisms in clouds

Fungal spores and yeasts:
~10^2 – ~10^4 cells mL^{-1}

Bacteria:
~10^4 – ~10^5 cells mL^{-1}

Frequency of bacterial genera (cultivated)



Vaïtilingom et al., Atmos. Environ. 56:88-100, 2012

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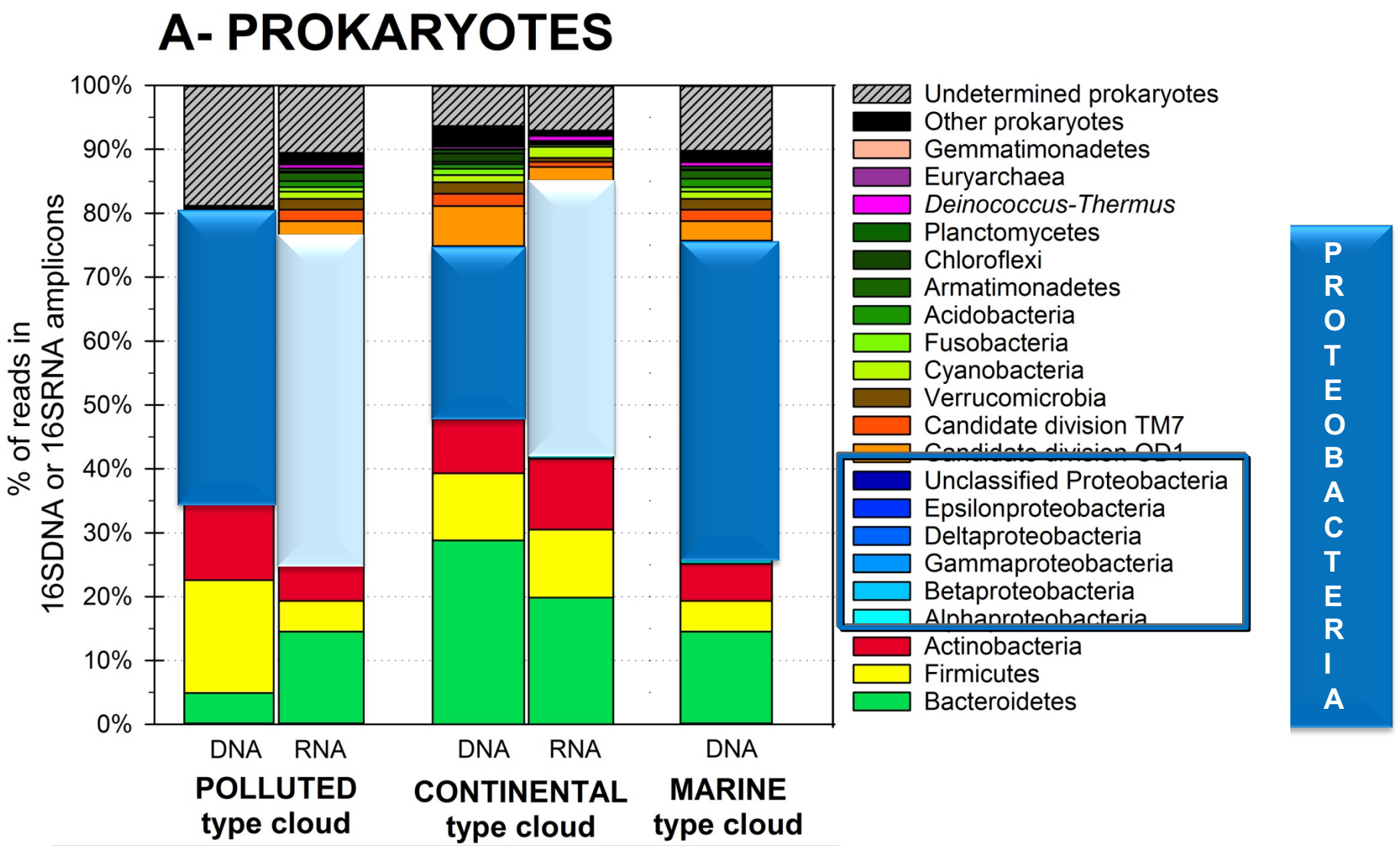
Metabolic activity in clouds

(Metagenomics and Metatranscriptomic)



Proteobacteria are the most active

P. Amato et al., PlosOne , 2017

Cloud chemistry

Photochemistry
(Gaseous / aqueous / Surface)
Photolytic processes

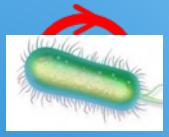
Mass transfer
Gas ⇆ liquid

Soluble chemicals – reactive
(Oxidants , VOCs, …)

CCN

Organic Matter

•OH



?

$NO_3^•$

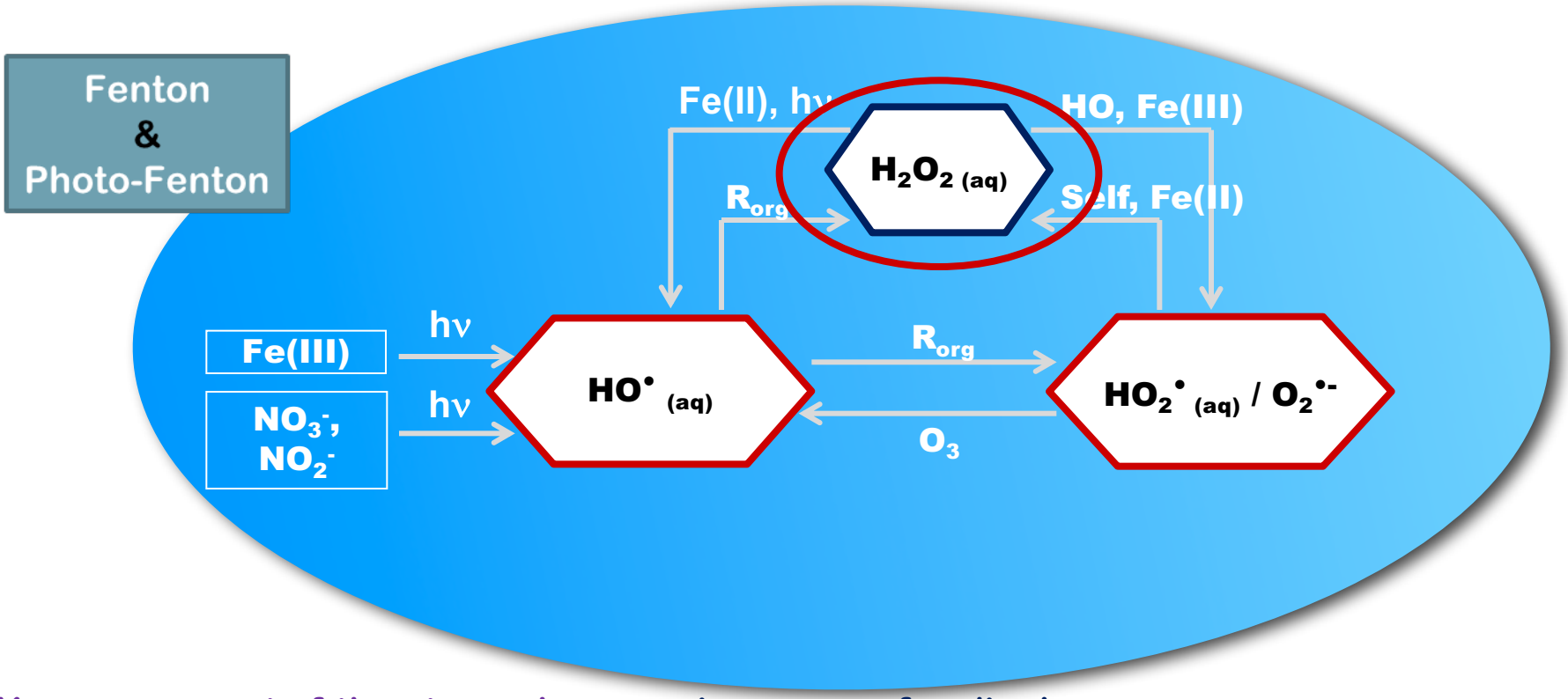
Oxidant species
H_2O_2, Fe

Reactivity

Evaporation of chemical species

Precipitation

Importance of H_2O_2 in the cloud water phase chemistry



- Key component of the atmosphere :main source of radicals
- Reveals the oxidant capacity of the atmosphere
- Concentrations min-max : 0 – 167 µM 0 – 58 µM puy de Dôme
- H_2O_2 is impacted by a wide variety of environmental parameters

(Photolytic activity = daily and seasonal strong variations, Temperature…)

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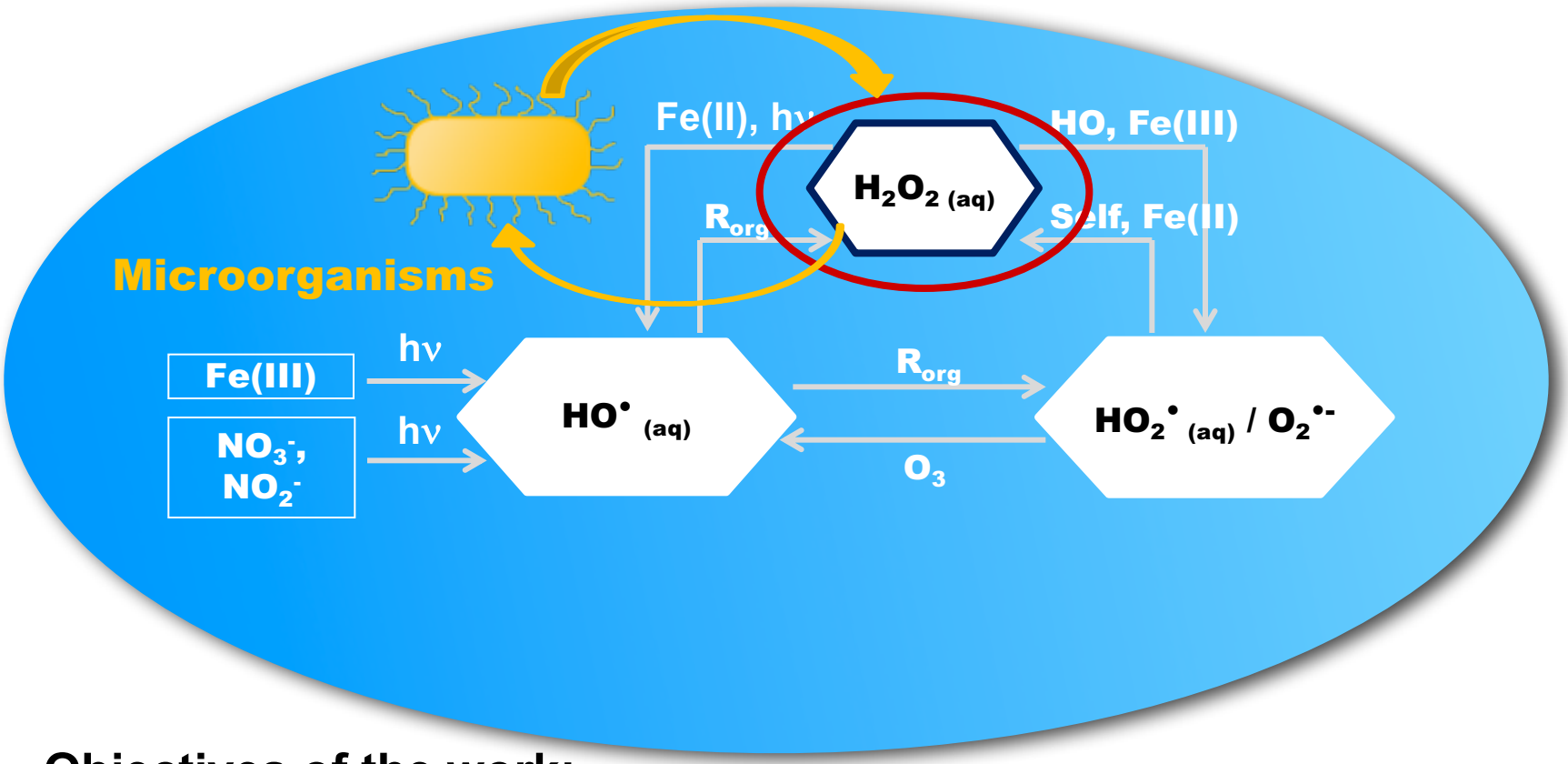
Microorganisms / H_2O_2 Interactions



Objectives of the work:

- **What is the impact of cloud microorganisms on H_2O_2?**
- **What is the impact of H_2O_2 on cloud microorganisms ?**

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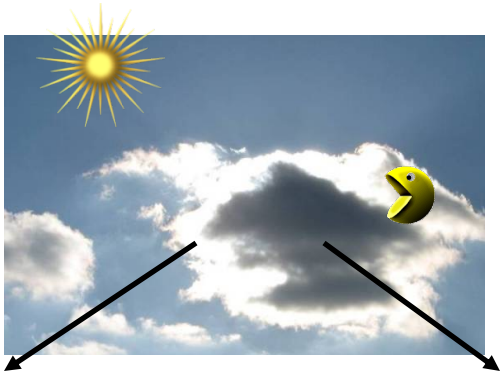
In lab experiments using real cloud samples



Cloud sample

Filter sterilized

Intact

Dark

Exposition to artificial solar light

Dark

Monitoring H_2O_2

Vaïtilingom et al., Atmos. Chem. Phys. 11, 8721-8733, 2011

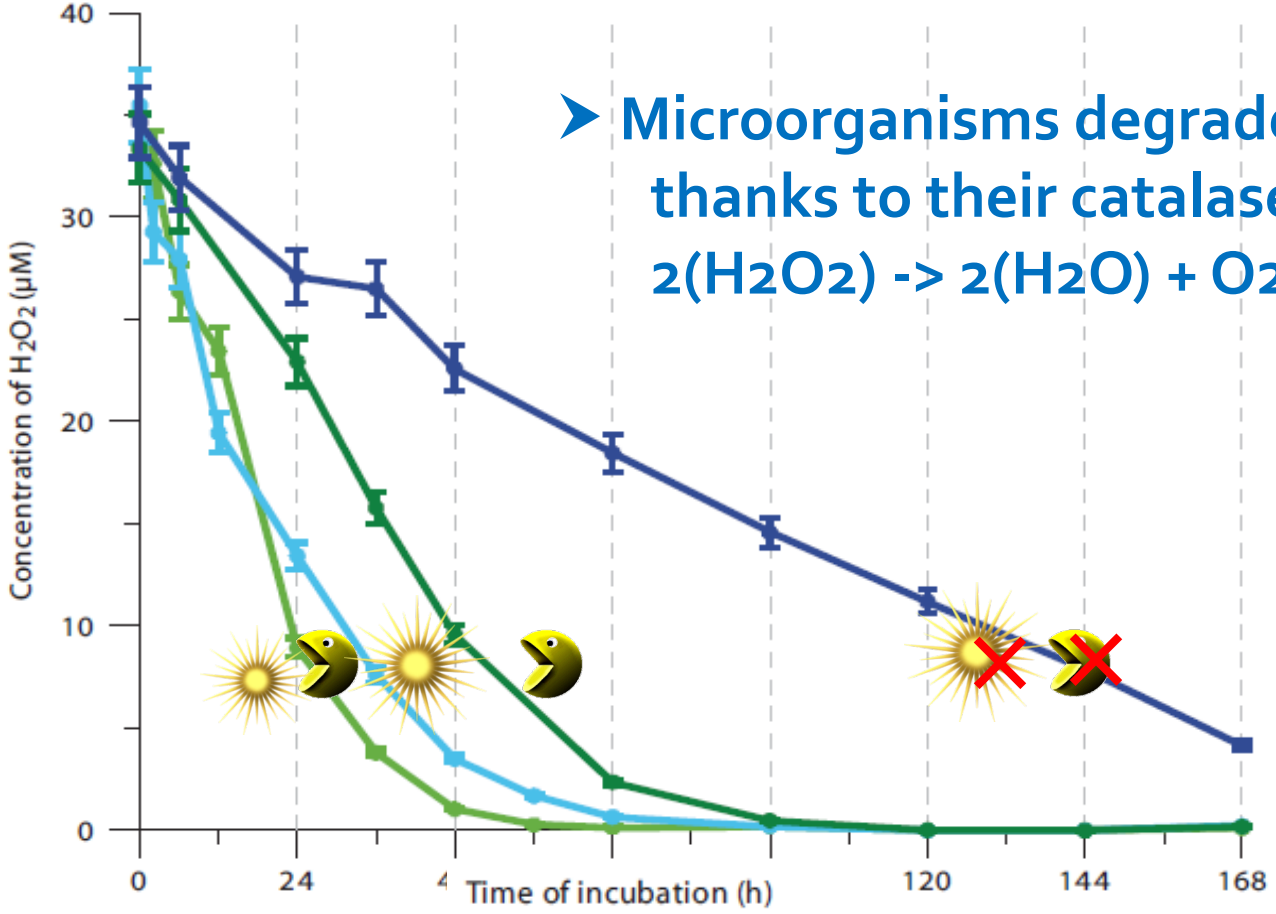
Vaïtilingom et al., Proc. Nat. Acad. Sci. 110 (2) 559-564, 2013

12° C

Photo-Bioreactor

In lab experiments using real cloud samples

Degradation of H_2O_2



- Microorganisms degrade H2O2 thanks to their catalases
  2(H2O2) -> 2(H2O) + O2

Vaïtilingom et al., Proc. Nat. Acad. Sci., 2013

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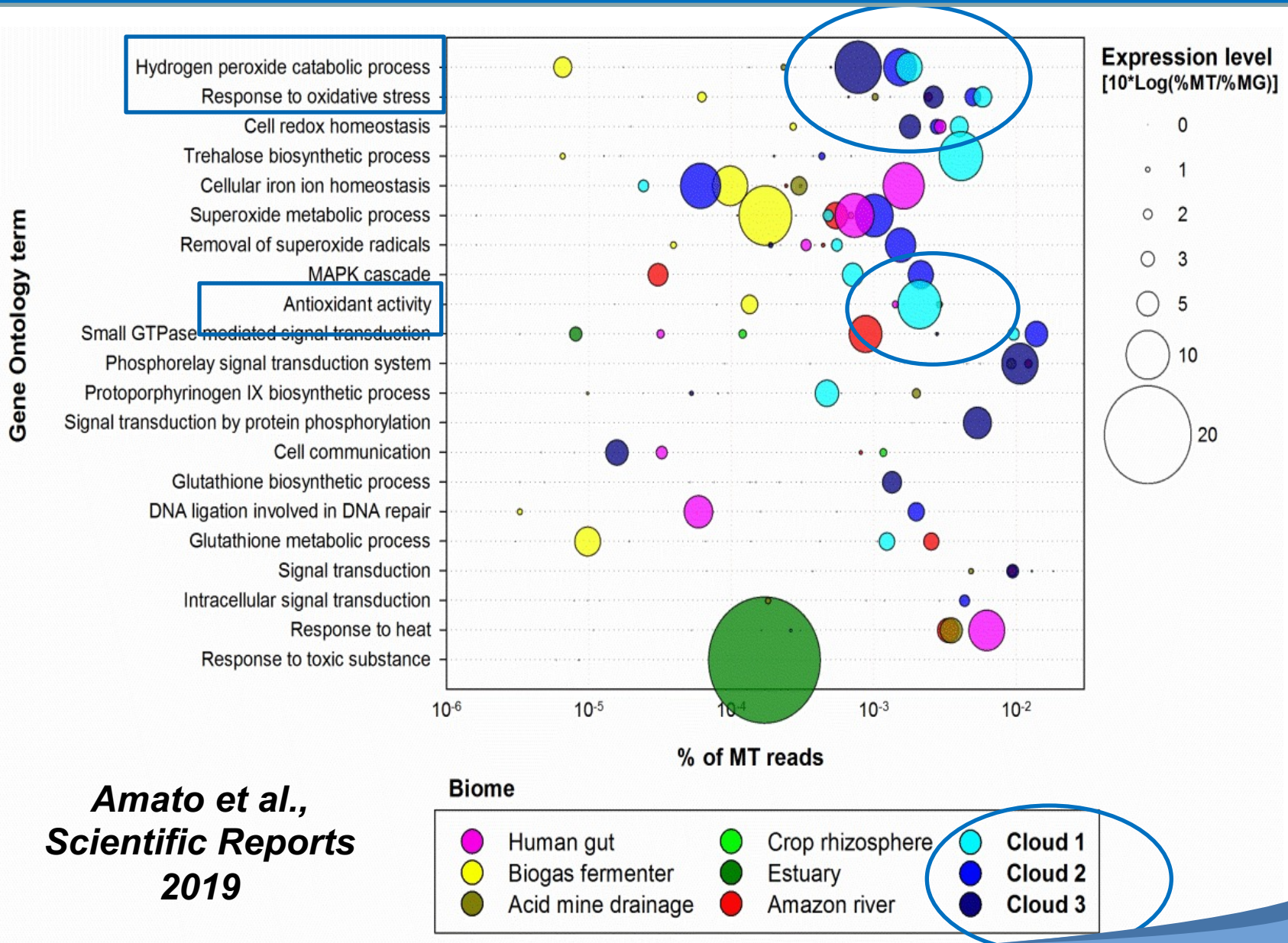
In cloud activity: H_2O_2 and anti-oxydants
(Metatranscriptomics)



Amato et al., Scientific Reports 2019

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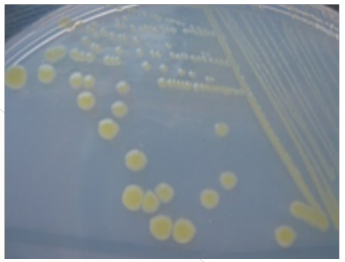
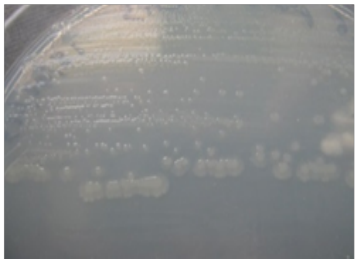
Biotransformation of H_2O_2 by cloud isolates

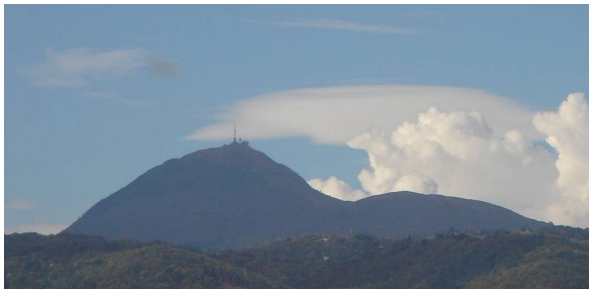
Sphingomonas sp.

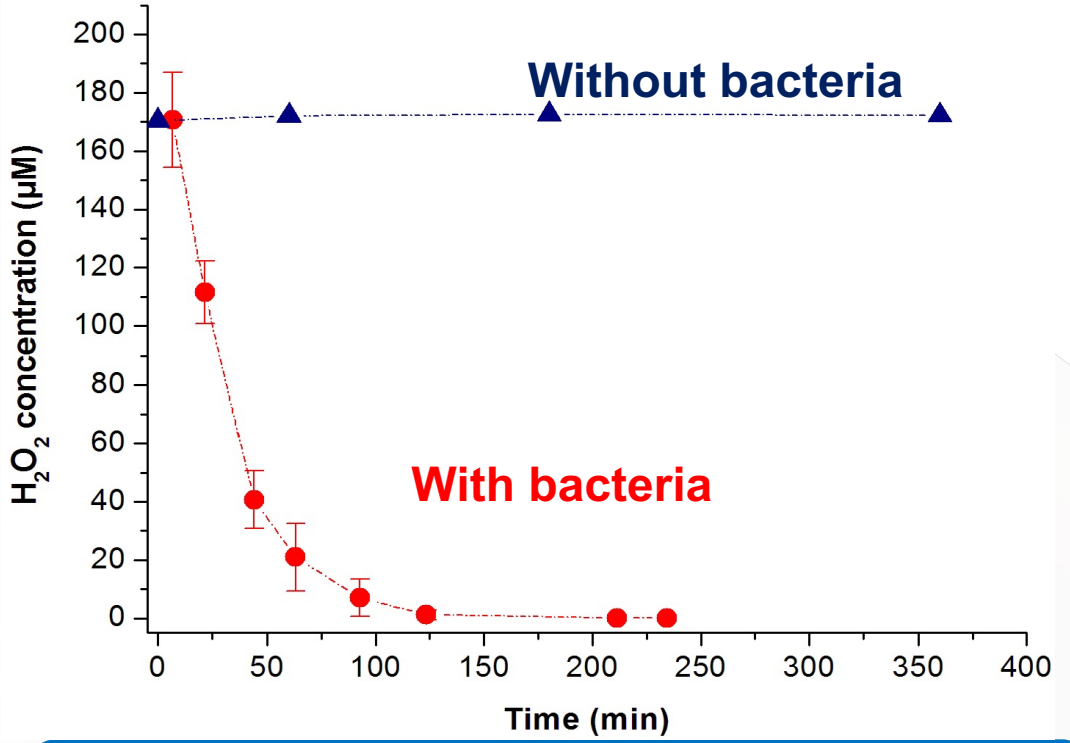
Pseudomonas graminis



Pseudomonas sp.







Bacterial strains isolated from clouds sampled at the puy de Dôme station (1465 m, France)

Wirgot et al.,
Atmos. Chem. Phys. 2017

Cloud bacteria biodegrade H_2O_2 thanks to their catalses

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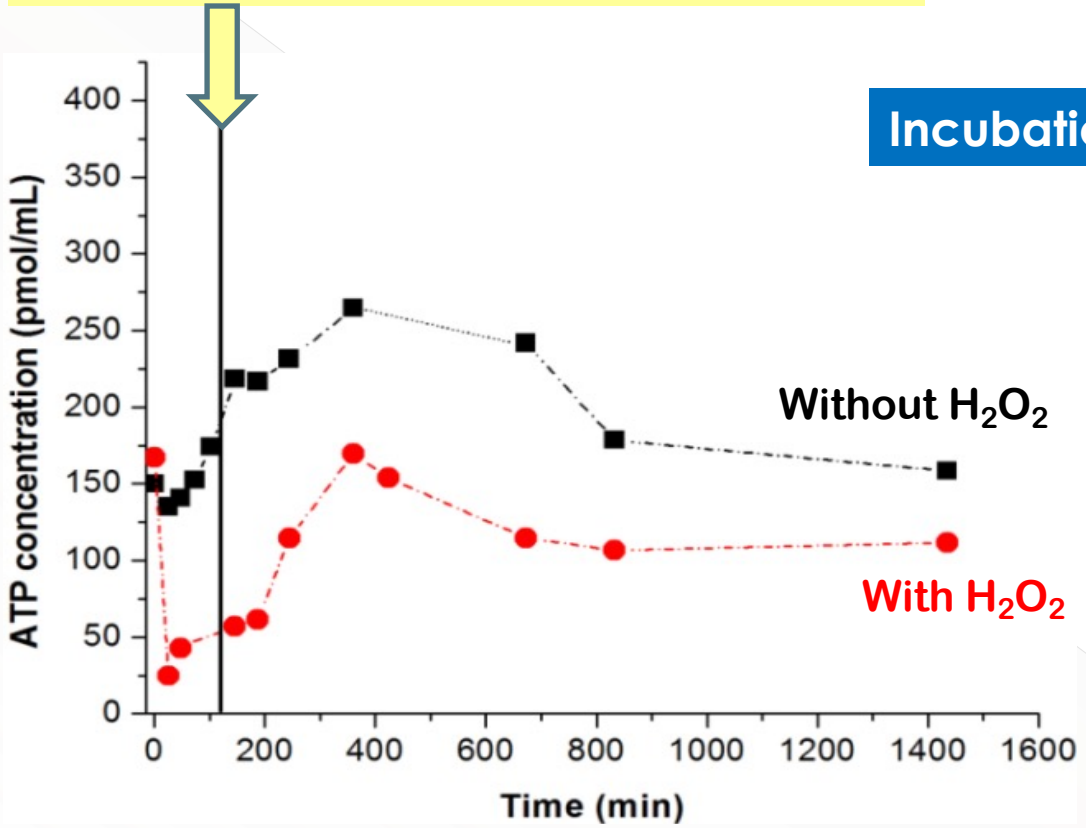
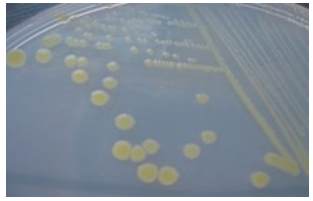
Impact of H_2O_2 on ATP concentration

Time when 100% H_2O_2 is biodegraded



Incubation in artificial cloud water



P. graminis

H_2O_2 has a strong impact on microbial energetic metabolism (ATP depletion)

Wirgot et al., Atmos. Chem. Phys. 2017

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From laboratory to real clouds

Lab Experiments

Cloud system complexity

True in real cloud environment ?

Bulk system

Strong link between

ATP / H_2O_2

Statistical analyses

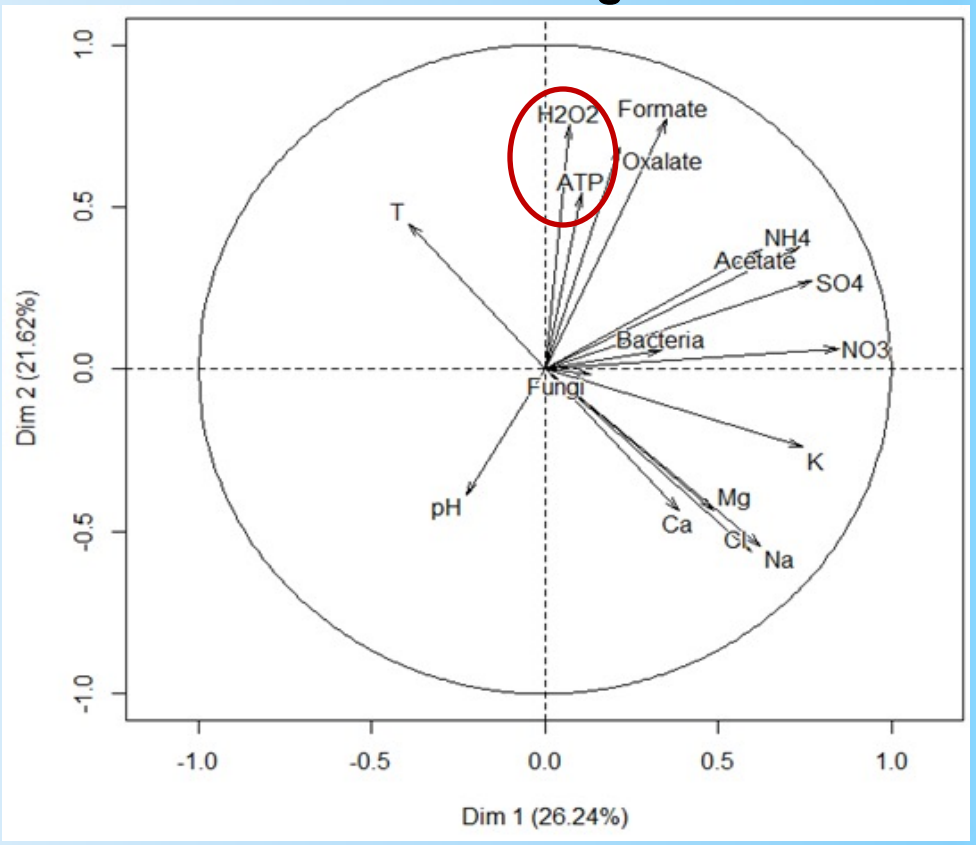
Chemical & microbiological parameters from 37 clouds

Wirgot et al., Atmos. Chem. Phys. 2017

From laboratory to real clouds: Statistical analyses

Multivariate:

Strong correlation



Variables factor map (PCA) of 37 cloud events on the plane PC1-PC2 based on 17 variables

Univariate

Spearman rank correlation test

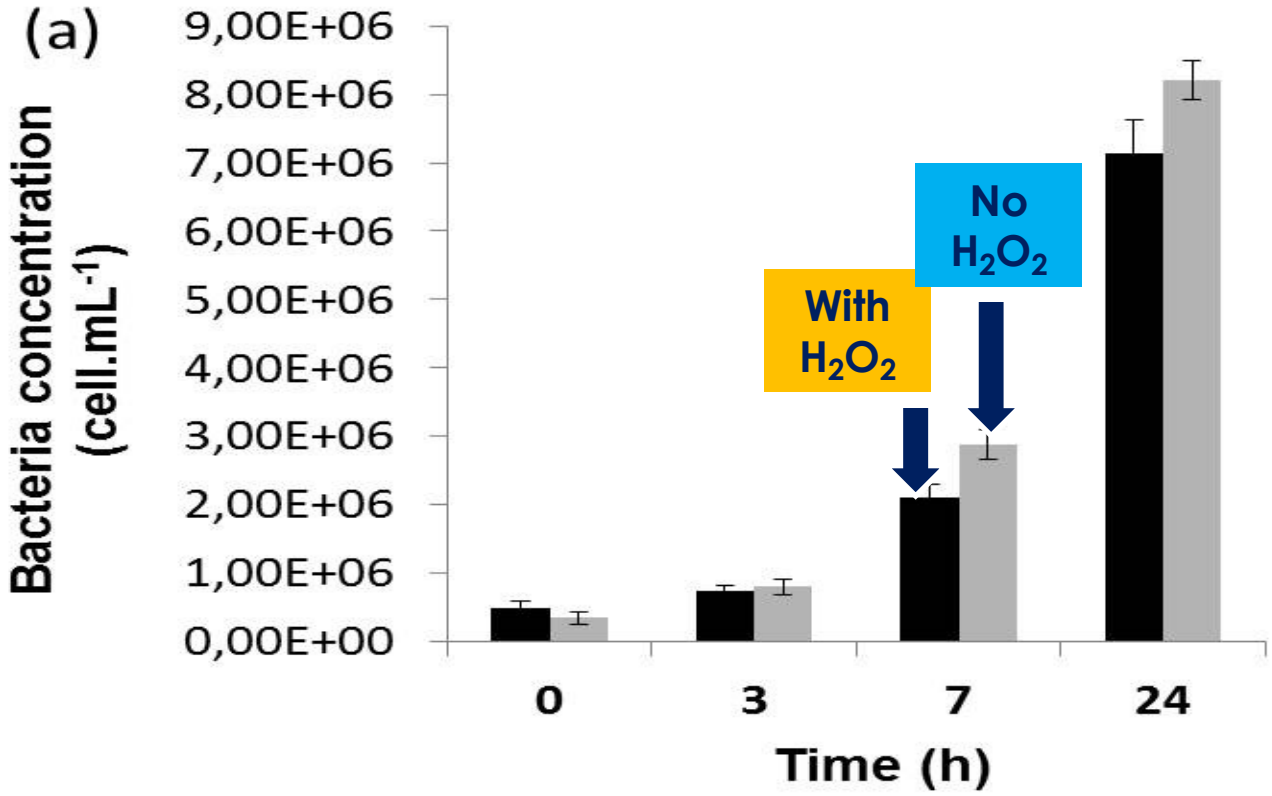
p-value =0.0047
(significance threshold equal to 0.05)

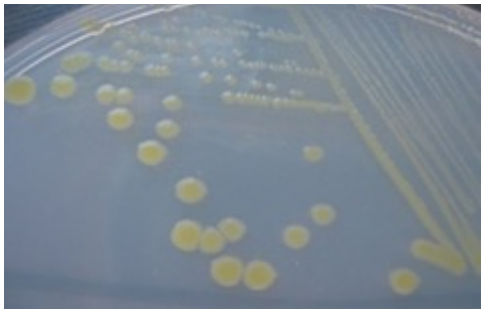
Spearman's coefficient = 0.45
(have to be > 0.27)

Strong correlation between ATP & H_2O_2

Wirgot et al., Atmos. Chem. Phys. 2017

Impact of H_2O_2 on *Pseudomonas graminis survival*





Counts on agar plate

Bacterial cells survive facing H_2O_2 stress and even grow

They modulate their metabolome

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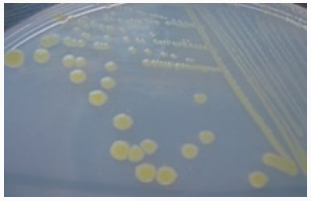
Impact of H_2O_2 : a *Metabolomic study*

***Pseudomonas graminis* 13b3**
Isolated from clouds



Artificial cloud solution

Metabolite Profiling:

UPLC/MS
LC-QTOF, C18 column

1D ^{1}H NMR
500 MHz NMR spectrometer, 5 mm Prodigy

No H_2O_2
Reference

With H_2O_2
Stressed

Three biological batches of 12 samples each

60 identified biomarkers, mapped on *P. graminis* KEGG pathways (sequenced genome)

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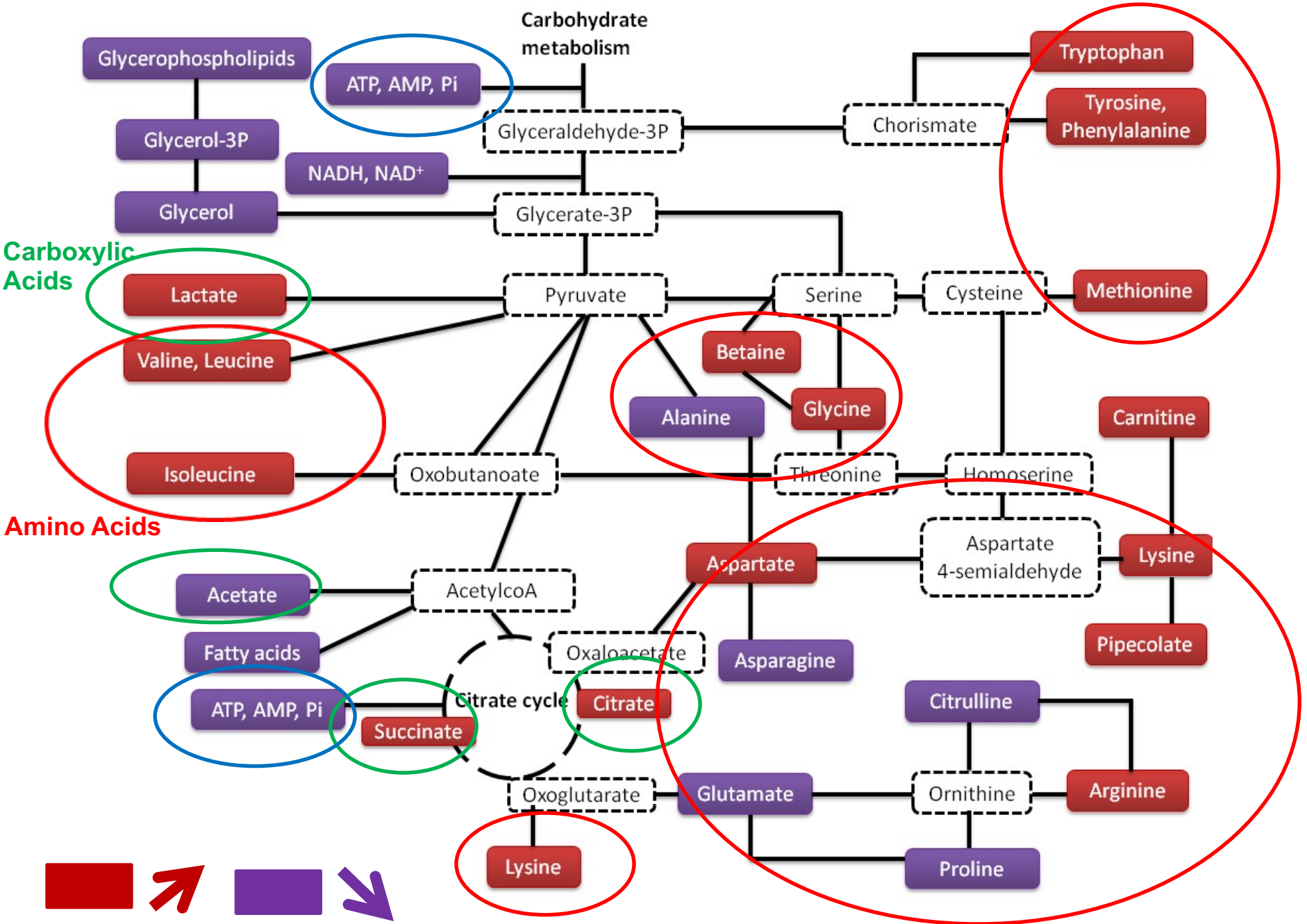
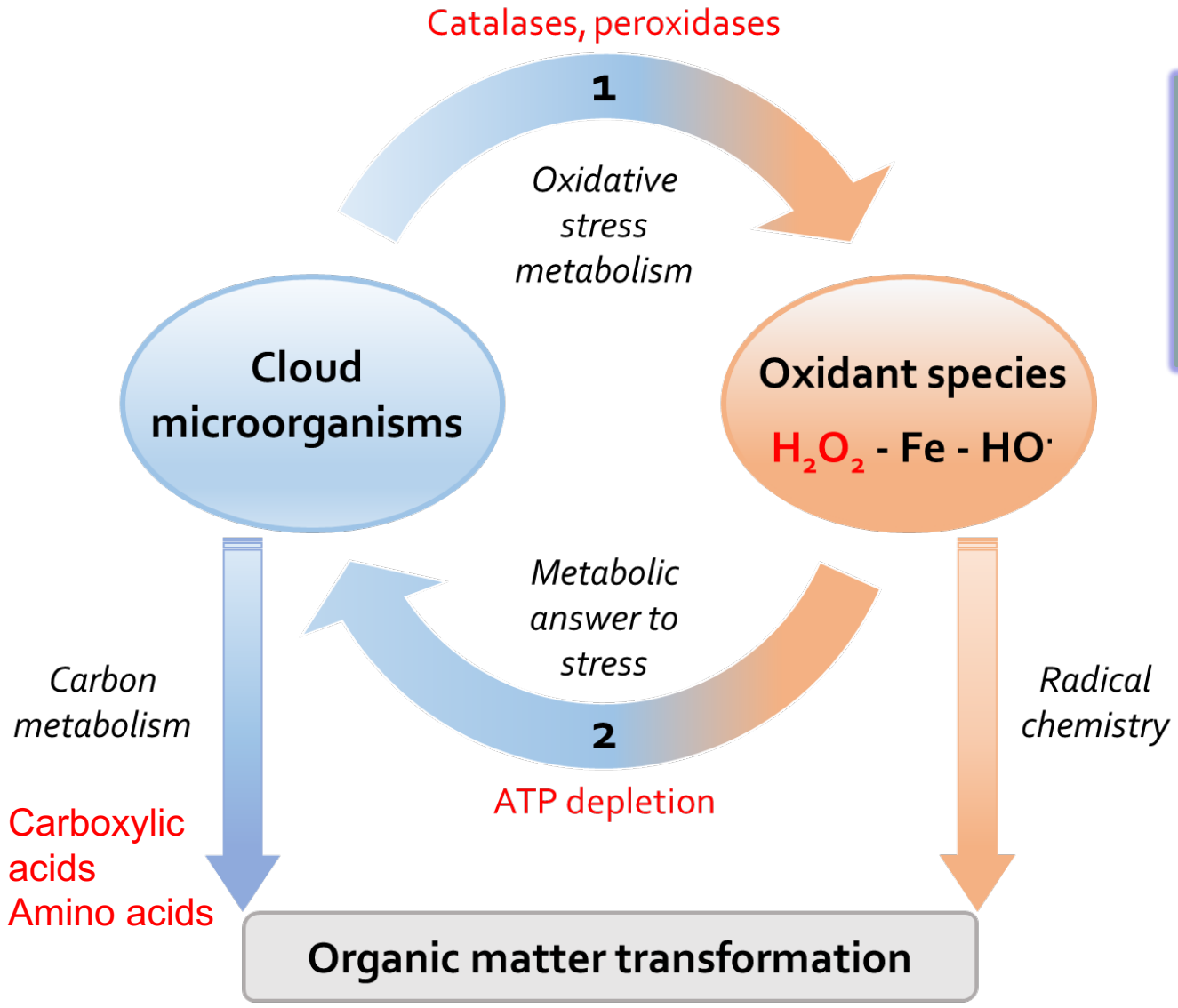
ANR
BIOCAP

Wirgot et al, Sci. Reports, 2019

Example of the modulation of *P. graminis* metabolic pathways

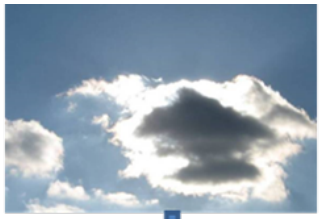


Microorganisms / H_2O_2 Interactions: Conclusions



Modulation by the variations of H_2O_2 concentration

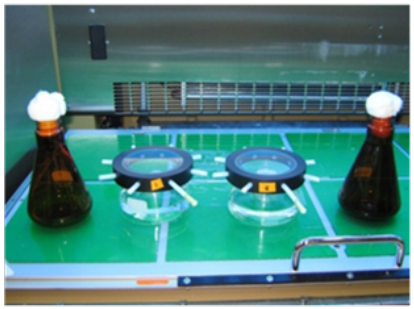
Towards "meta-omics" studies with complex cloud microbiota



Cloud Sample

Tangential filtration

Scenario « Summer day » H_2O_2 +light



Scenario « Winter night » No H_2O_2 +Dark

Incubation in artificial cloud water

Summer day

Winter night

Summer day

Winter night

Extract of metabolites Metabolomics , Fluxomics

Extract of RNA (and DNA) Transcriptomics (genomics)

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METACLOUD

METABOHUB

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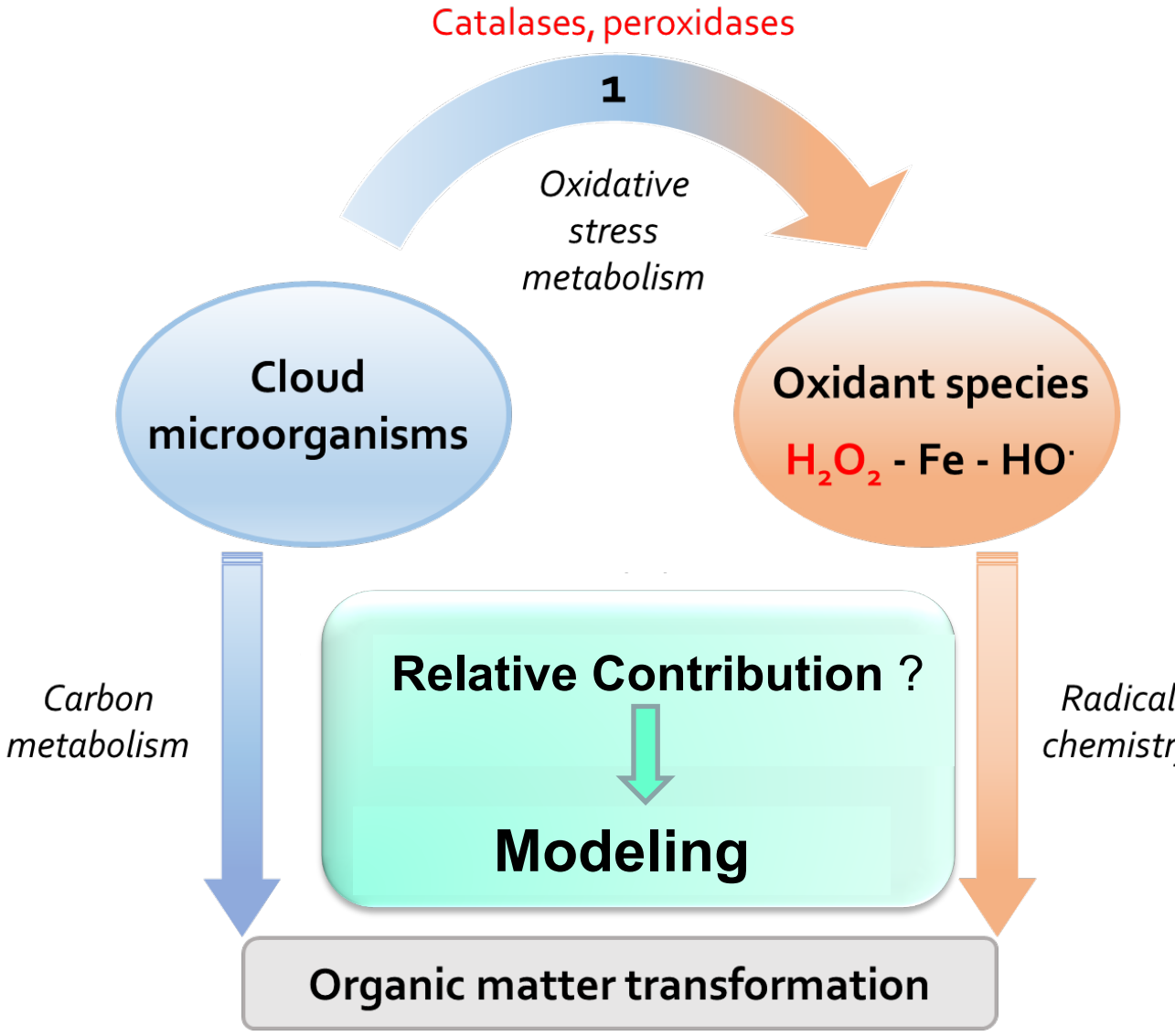
Towards Atmospheric Chemistry Models Integrating Biology



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METACLOUD

(CLEPS model, LaMP)

MAKE OUR PLANET GREAT AGAIN

MOBIDIC

Institut de Chimie de Clermont-Ferrand

Anne-Marie Delort

Pierre Amato

Martine Sancelme

Virginie Vinatier

Cyril Jousse

Muriel Joly

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Florent Rossi (Post-doc)

Laurent Deguillaume

Angelica Bianco

Pascal Renard (post-doc)

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LEFE

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RÉPUBLIQUE FRANÇAISE

MINISTÈRE
DE L'ÉDUCATION NATIONALE,
DE L'ENSEIGNEMENT SUPÉRIEUR
ET DE LA RECHERCHE

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UNIVERSITÉ Clermont Auvergne

CAP 20-25

I-SITE Clermont
Clermont Auvergne Project

Laboratoire de météorologie physique
LaMP

iCCF
Institut de Chimie de Clermont-Ferrand

Méthodes

Extrait ADN (ou ARN)

(RT)

DIVERSITE
DIVERSITE ACTIVE

Amplification gène 16S (PROK) ou 18S (EUK) (PCR)

PROK 16S = 515F-806R → 291 bp
EUK = 18S 960F-1419R → 459 bp

Amplification aléatoire (MDA)

METAGENOME
METATRANSCRIPTOME

Séquençage Illumina MiSeq

Traitement bioinformatique

- Contrôle qualité [FastQC]
- Assemblage [USEARCH]
- Affiliation[PANAM] vs SILVA SSU (100%)
- Suppression des OTUS singletons
- Suppression des OTUs présents dans fraction ARN mais absents de ADN
- Suppression des OTUs présents dans les blancs

3 cloud samples

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	Nuage 1	Nuage 2	Nuage 3
Type de nuage	Marin	Continental	Urban
Température	10 °C	13,5 °C	10 °C
pH	6,1	5,2	3,9
Conductivité ($\mu S\,cm^{-1}$)	3,5	37,6	78,6
TOC (*DOC*) ($mg\,L^{-1}$)	1,1 (1,1)	6,8 (6,7)	6,9 (6,8)
Composé		Concentration (µM)	
Acétate	4,5	25,4	23,2
Formiate	4,9	42,7	33,2
Succinate	-	3,1	3,8
	19 %	25 %	23 %
Oxalate	1	9,7	9,3
Malonate	-	3,1	3,5
Formaldéhyde	1,5	2,7	6,1
Cl^-	3	7,7	11,3
NO_3^-	4,5	70,6	228,7
SO_4^{2-}	1,8	46,1	64
NH_4^+	8,5	100,3	122,3
Na^+	2,2	10,1	8,8
K^+	-	1,5	2,2
Mg^{2+}	1	2,1	2,7
Ca^{2+}	1,7	3,8	3,8
Fe(total)	0,9	1,1	1,3
Fe(II)	0,3	0,5	0,5
H_2O_2	3,6	33,4	57,7
	Concentration cellulaire (cellules mL^{-1})		
Spores fongiques et levures	9×10^3	3×10^3	3×10^3
Bactéries	3×10^4	8×10^4	9×10^4
ATP ($pmol\,mL^{-1}$)	0,2	0,95	0,85
ADP ($pmol\,mL^{-1}$)	0,3	0,3	0,4
Rapport ADP/ATP	1,4	0,3	0,5

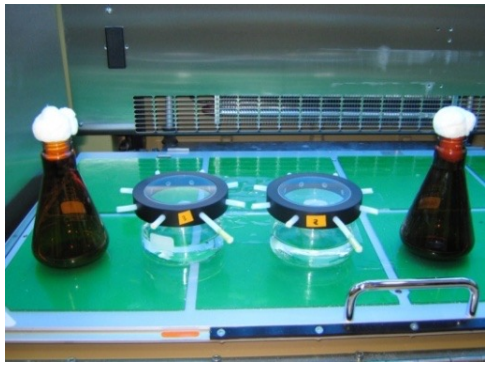
≠ pH

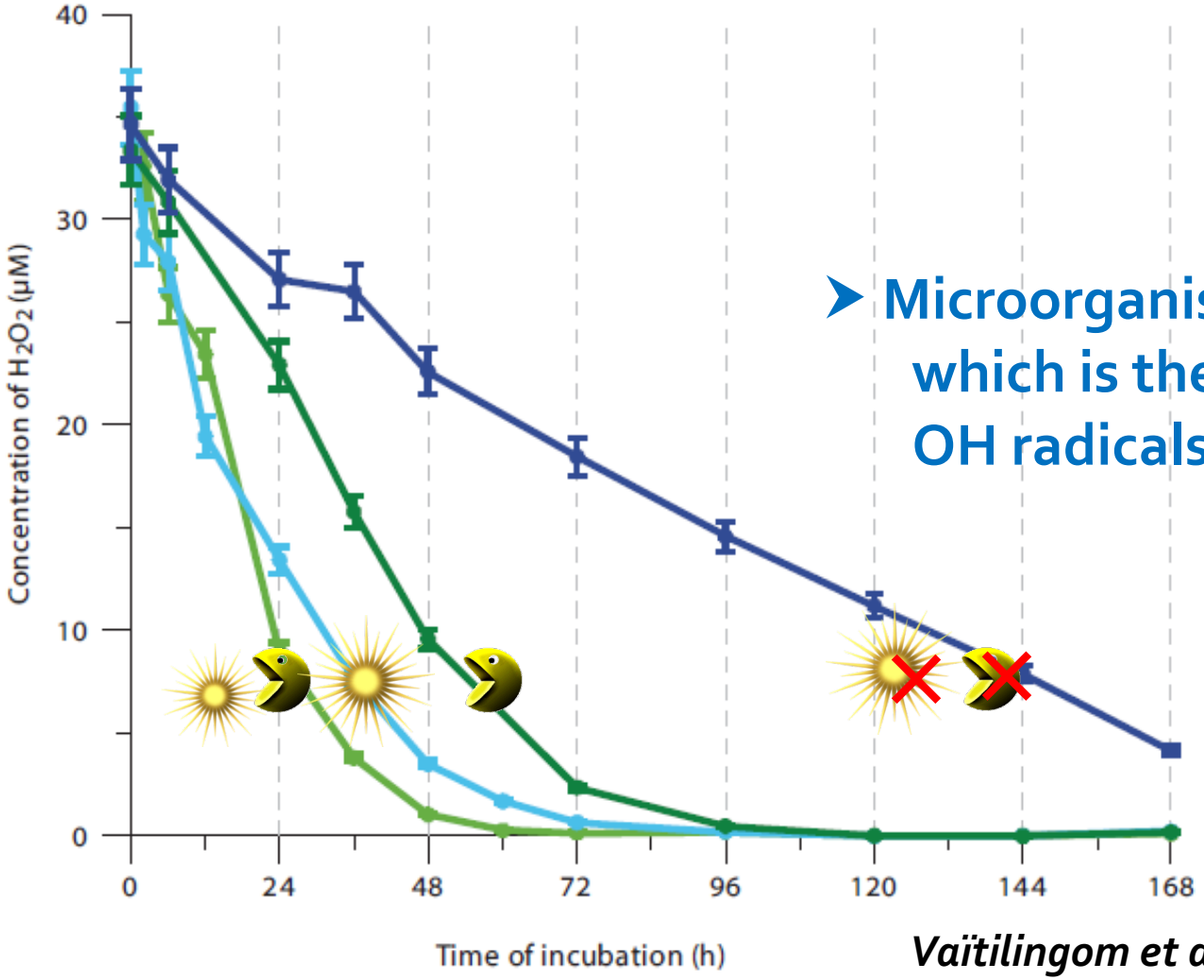
~ 20 % du DOC

≠ Oxidant capacity

Energetic state

Interaction with oxidants (H_2O_2)





- Microorganisms degrade H2O2 which is the major source of OH radicals

Vaïtilingom et al., Proc. Nat. Acad. Sci. 110 (2) 559-564, 2013

Impact of H_2O_2 on Microbial metabolism and ATP concentration

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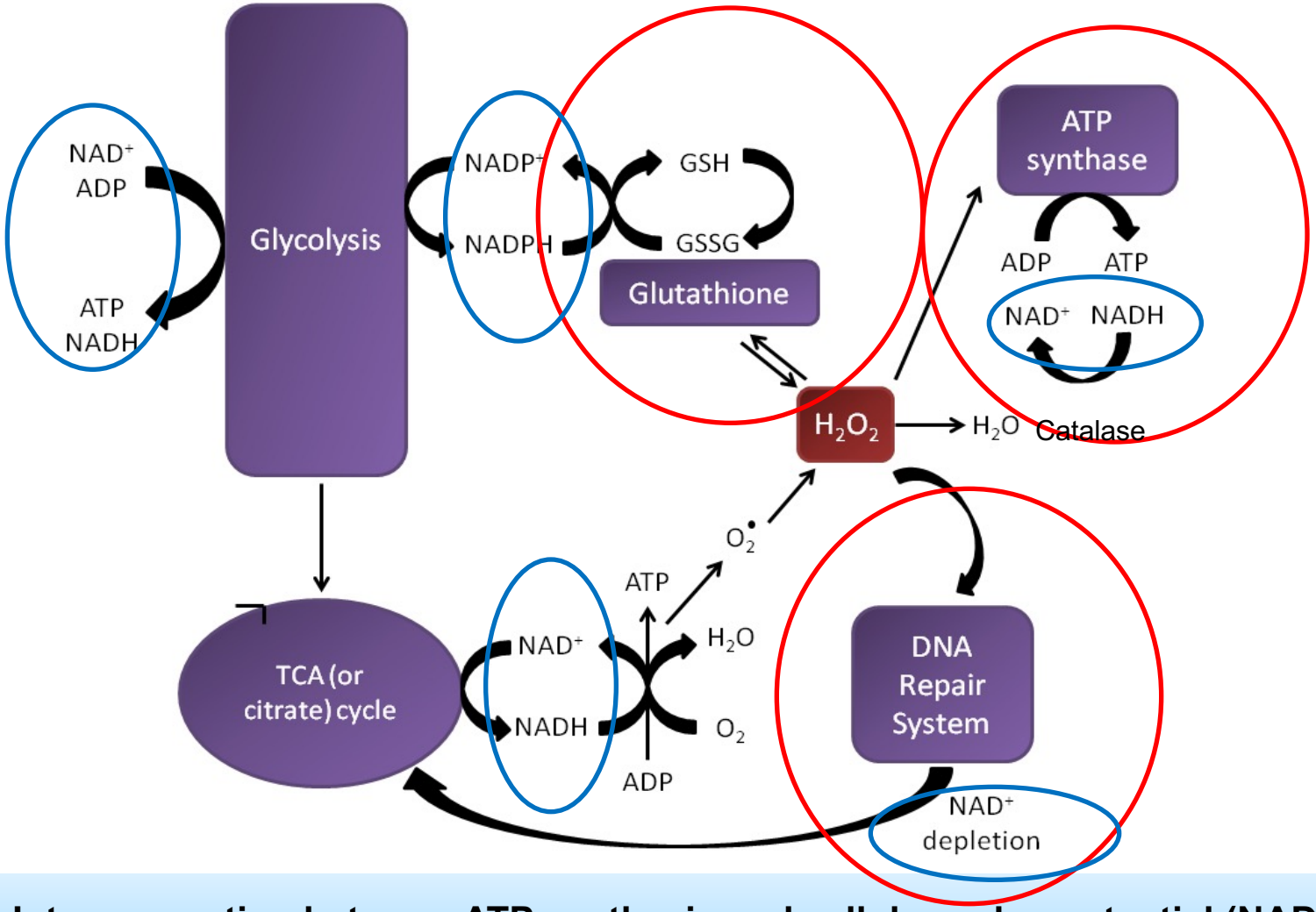
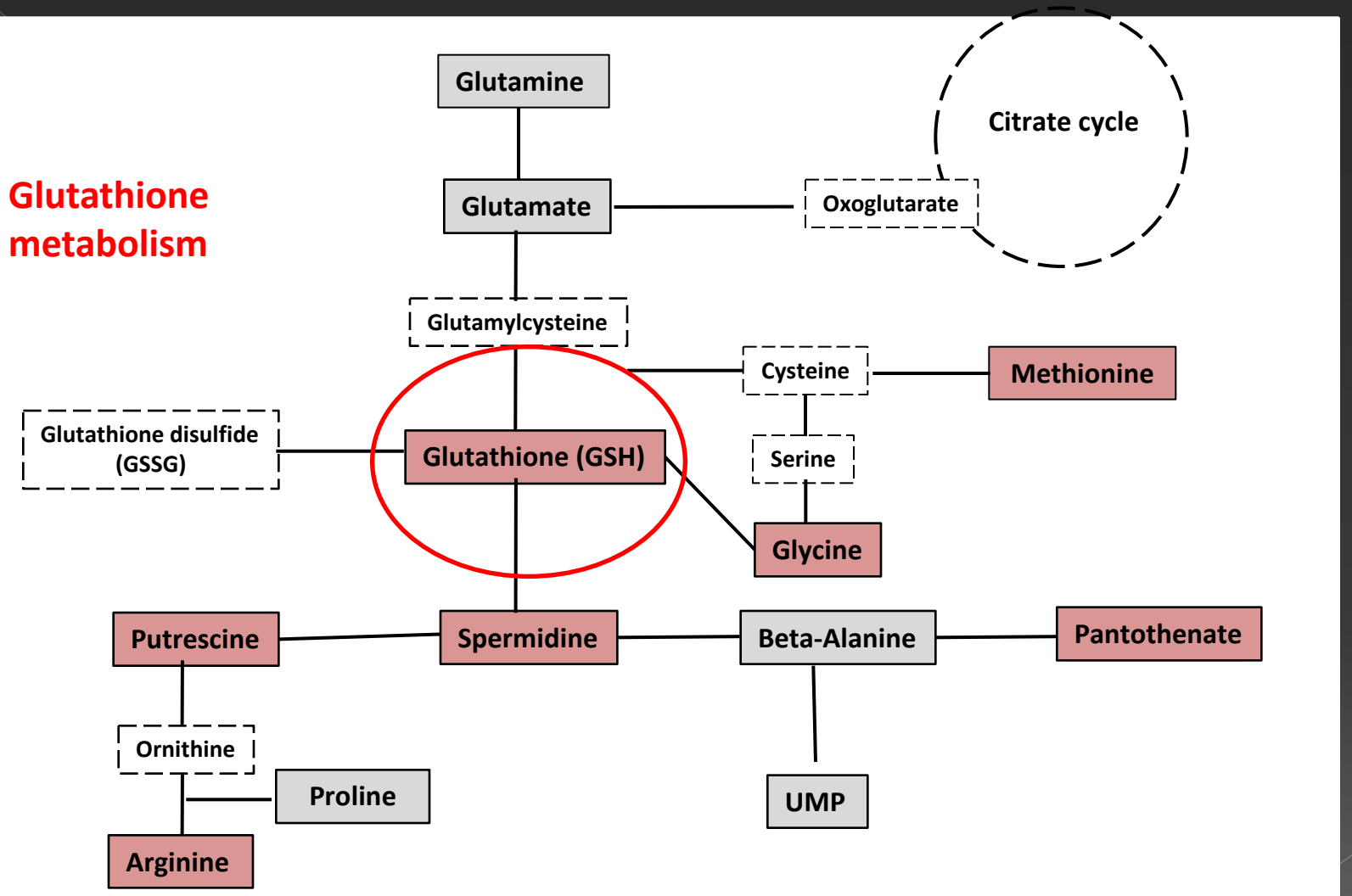


Inhibition

Interconnection between ATP synthesis and cellular redox potential (NAD^+/NADH, $NADP^+$/NADPH ratios). NAD^+ depletion related to DNA repair system

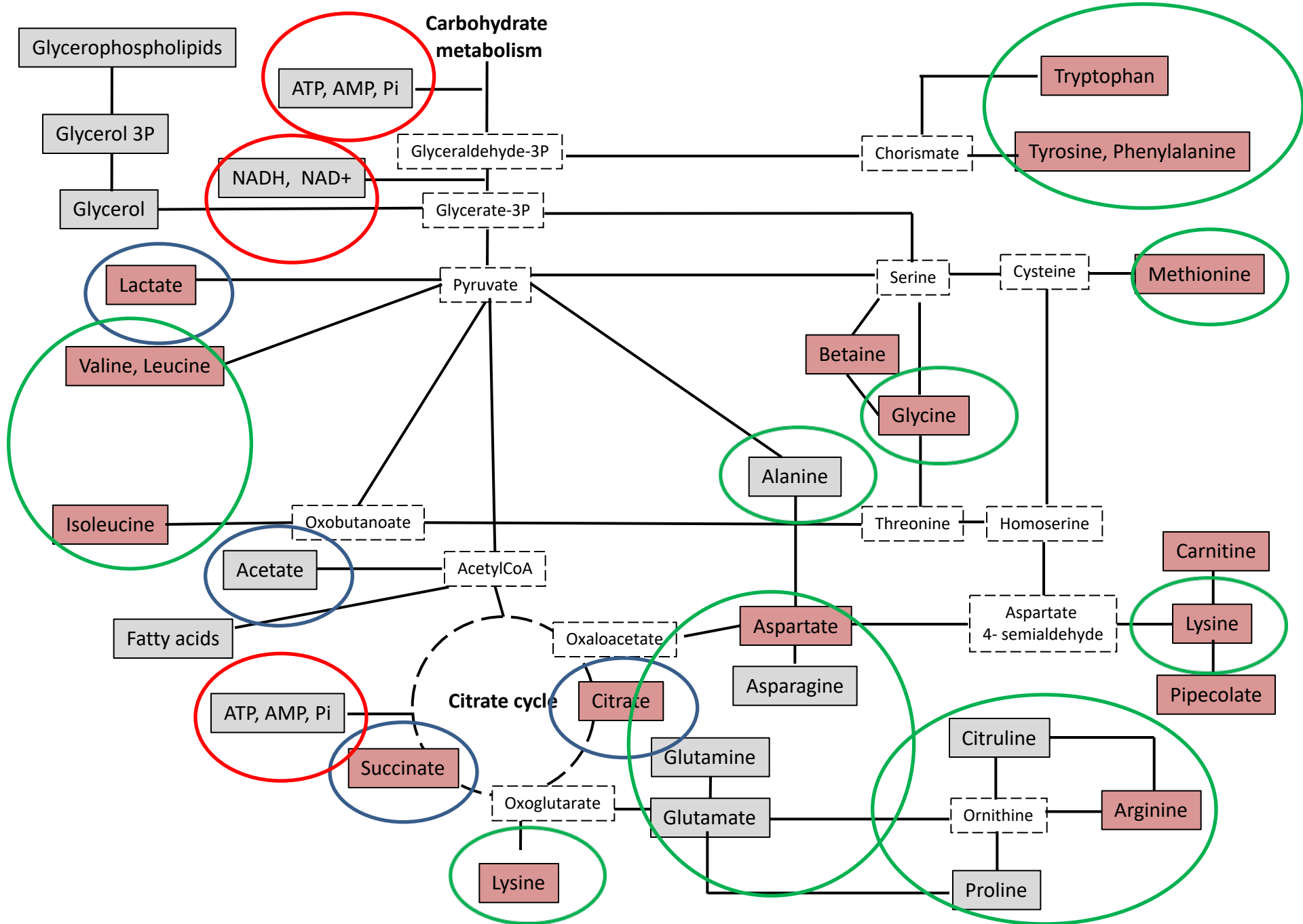
Impact of H_2O_2 : a *Metabolomic approach*

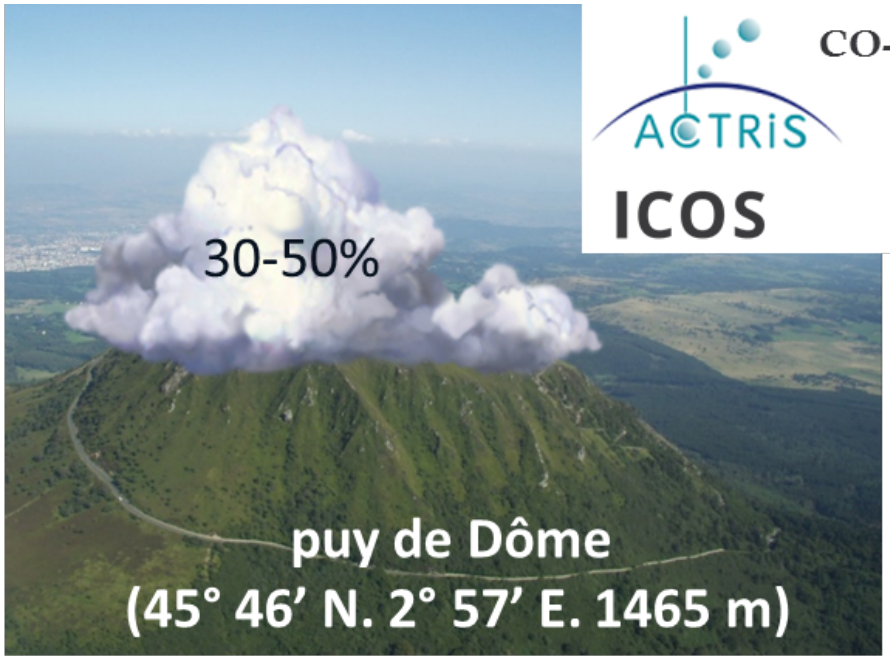


Overproduced

Underproduced

Central metabolism (Carboxylic Acids, Amino acids, lipids) +ATP/NAD



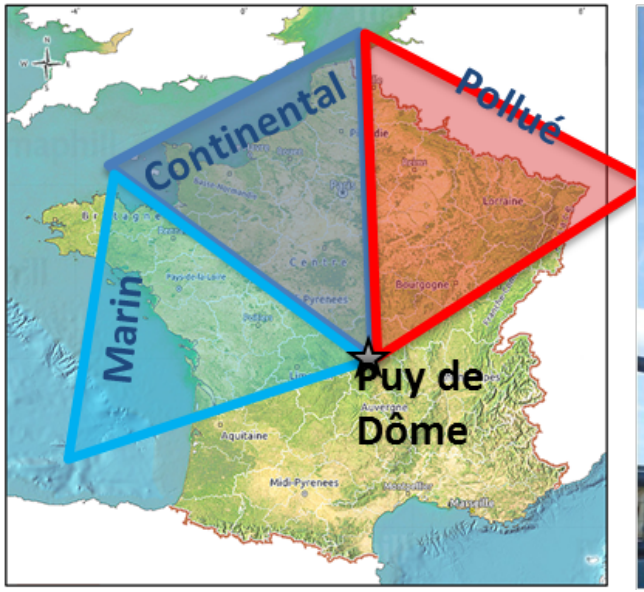


ACTRiS

ICOS

CO-PDD CNRS

GAW



Impact of atmospheric scenarios on the functioning of the cloud microbiota

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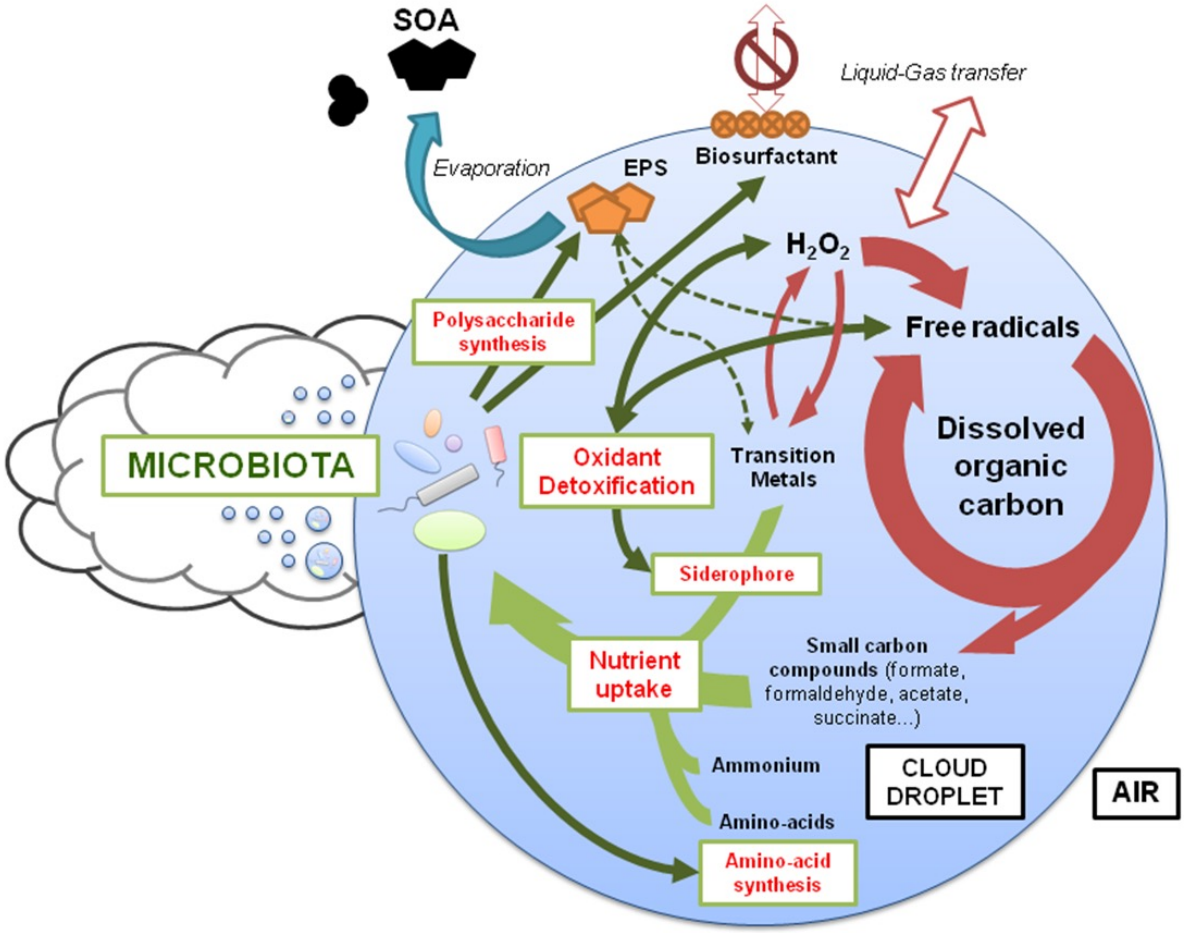

Example of scenarios:

- ❑ **Winter night (No H_2O_2, No light, 5°C)**
- ❑ **Summer day (H_2O_2, Light,17°C)**

First Meta-Transcriptomics study of clouds

Amato et al, Sci. Reports, 2019

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METACLOUD (under review)

Perspectives: Towards « Meta-omics »

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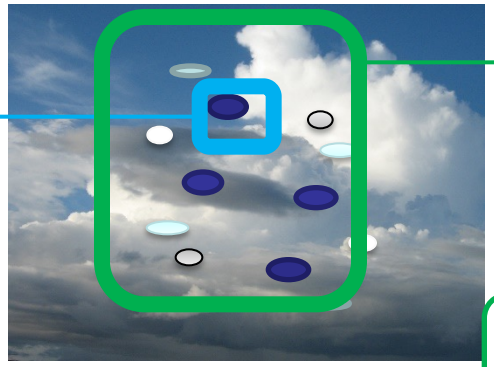
Metabolomics

Cloud Microbial strains (culture)

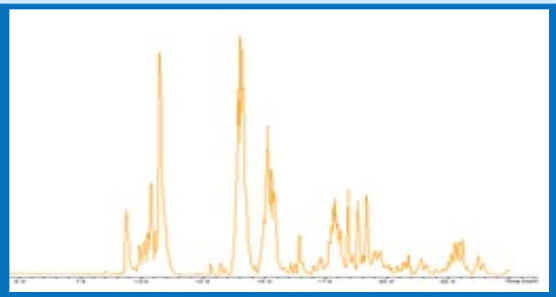


Meta-
Metabolomics
Transcriptomics
Fluxomics

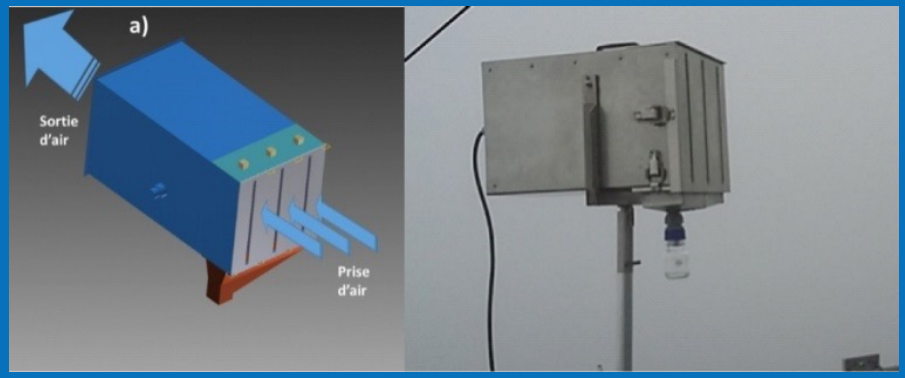
Cloud Ecosystem (cloud water sample)

Technical barriers

Bacteria:
~10^4 – ~10^5 cells mL^{-1}

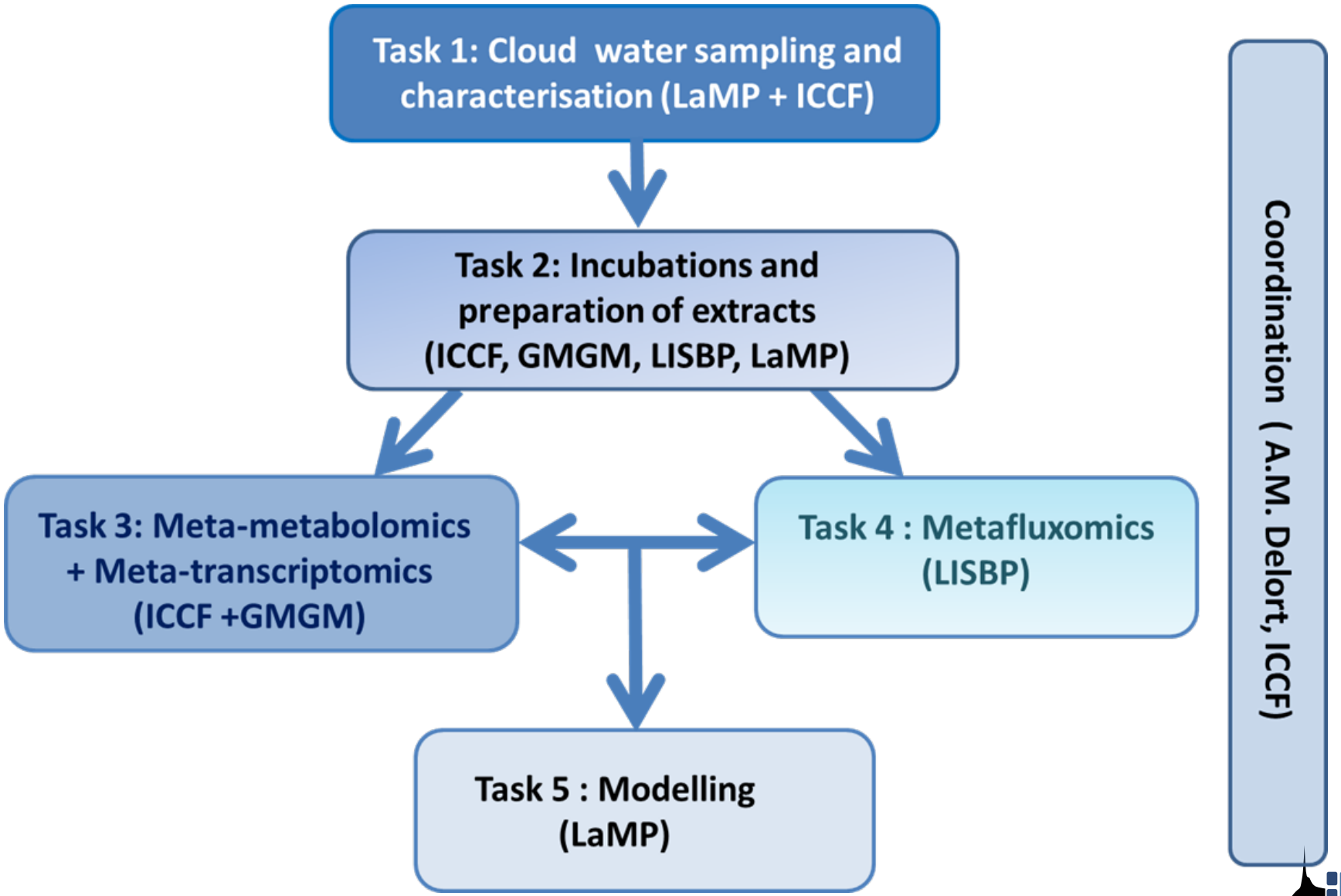


LC-MS 300 ml cloud water
Meta-transcriptomics



Construction of high volume cloud impactors → up to 700 ml /day

METACLOUD organisation



Consequence for atmospheric chemistry

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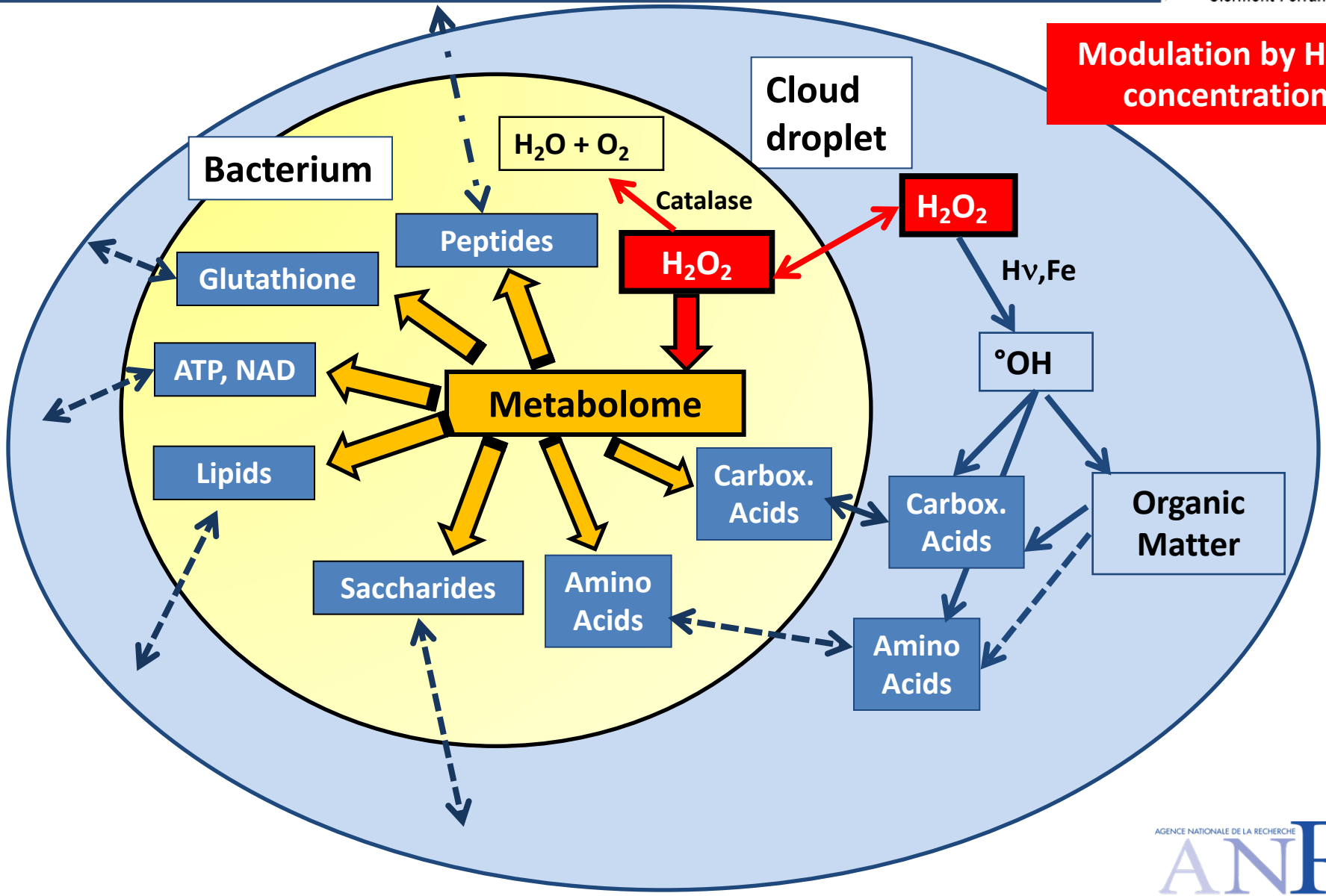

Wirgot et al, Sci. Reports, 2019

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