Diversity of novel viruses infecting methanogenic archaea, discovered through coupling of stable isotope probing and metagenomics

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To cite this version:

Hoang Ngo, François Enault, Cédric Midoux, Mahendra Mariadassou, Olivier Chapleur, et al.. Diversity of novel viruses infecting methanogenic archaea, discovered through coupling of stable isotope probing and metagenomics. Phages in Paris, Phages.fr, Oct 2022, Paris, France. hal-04357012

HAL Id: hal-04357012
https://hal.inrae.fr/hal-04357012v1

Submitted on 20 Dec 2023

PHAGES IN PARIS
2022

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Diversity of novel viruses infecting methanogenic archaea, discovered through coupling of stable isotope probing and metagenomics

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ariane.bize@inrae.fr

université PARIS-SACLAY

PROSE PRocédés biOtechnologiques au Service de l'Environnement

MaIAGE Mathématiques et Informatique Appliquées du Génome à l'Environnement

INSTITUT PASTEUR

cnrs

UCA UNIVERSITÉ Clermont Auvergne

ANR-17-CE05-0011

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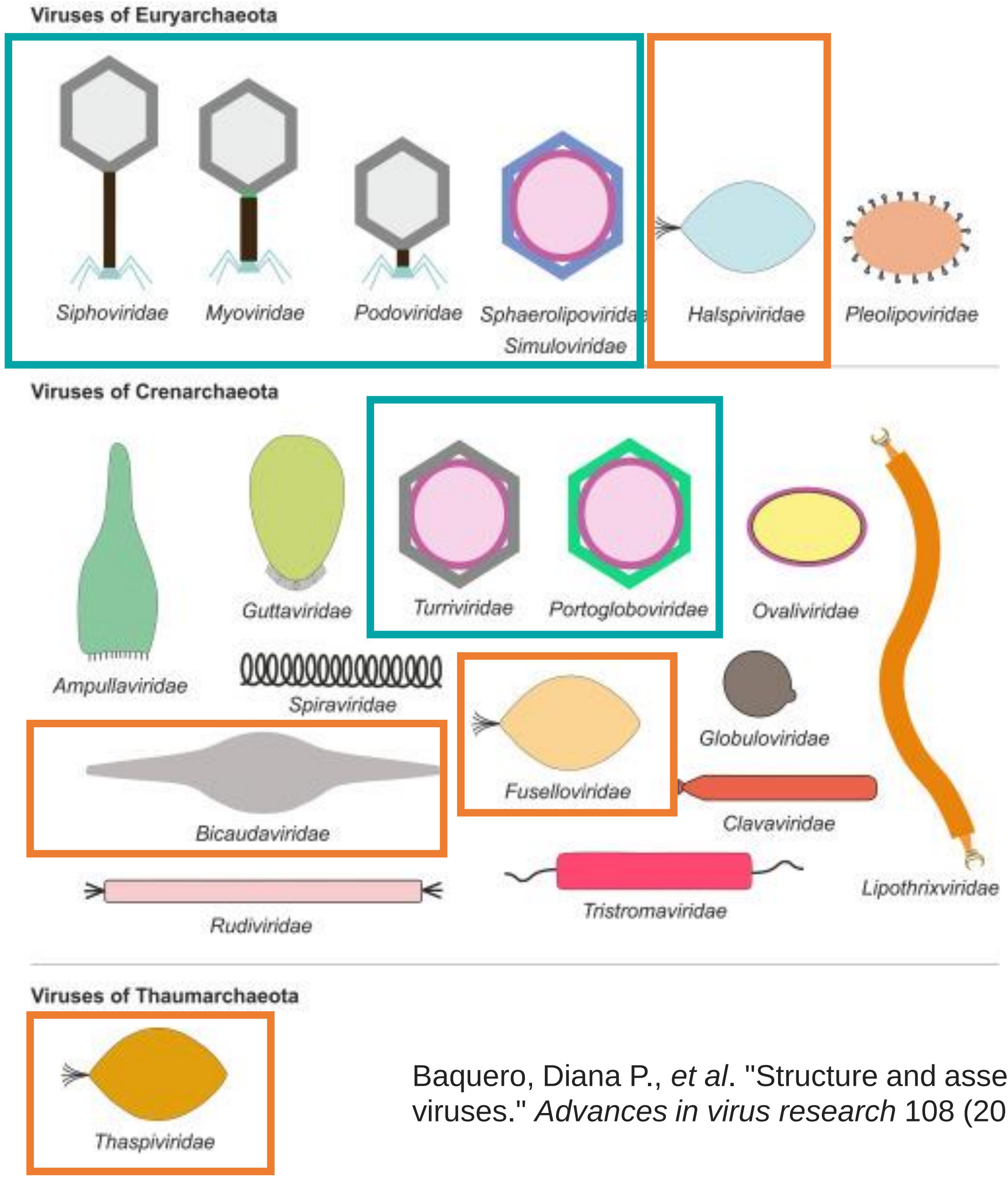
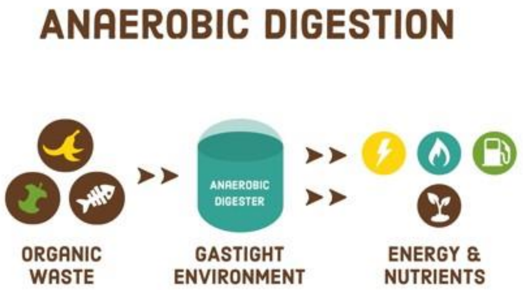
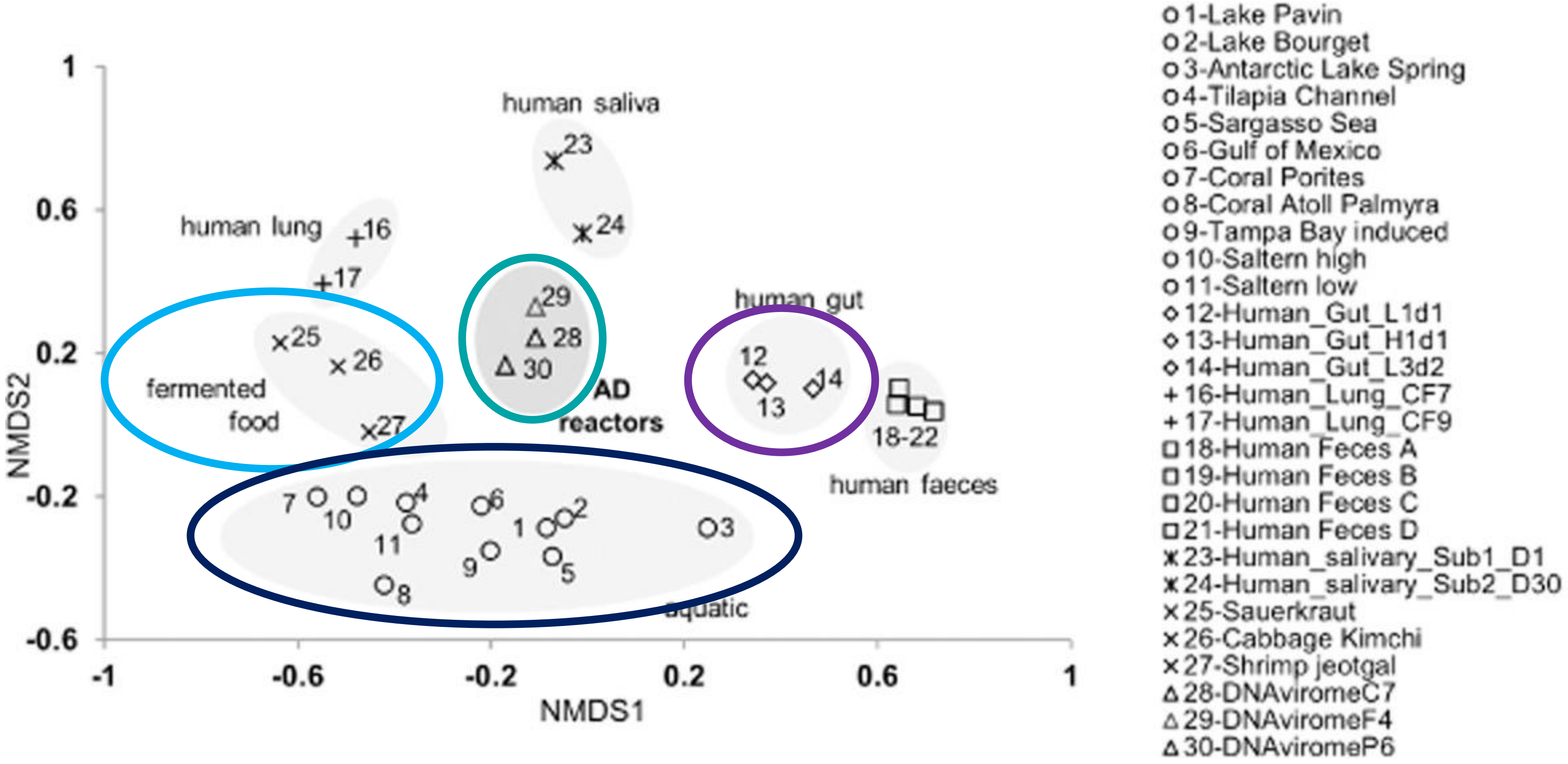
The great diversity of archaeal viruses is only partly characterized



Baquero, Diana P., *et al*. "Structure and assembly of archaeal viruses." *Advances in virus research* 108 (2020): 127-164.

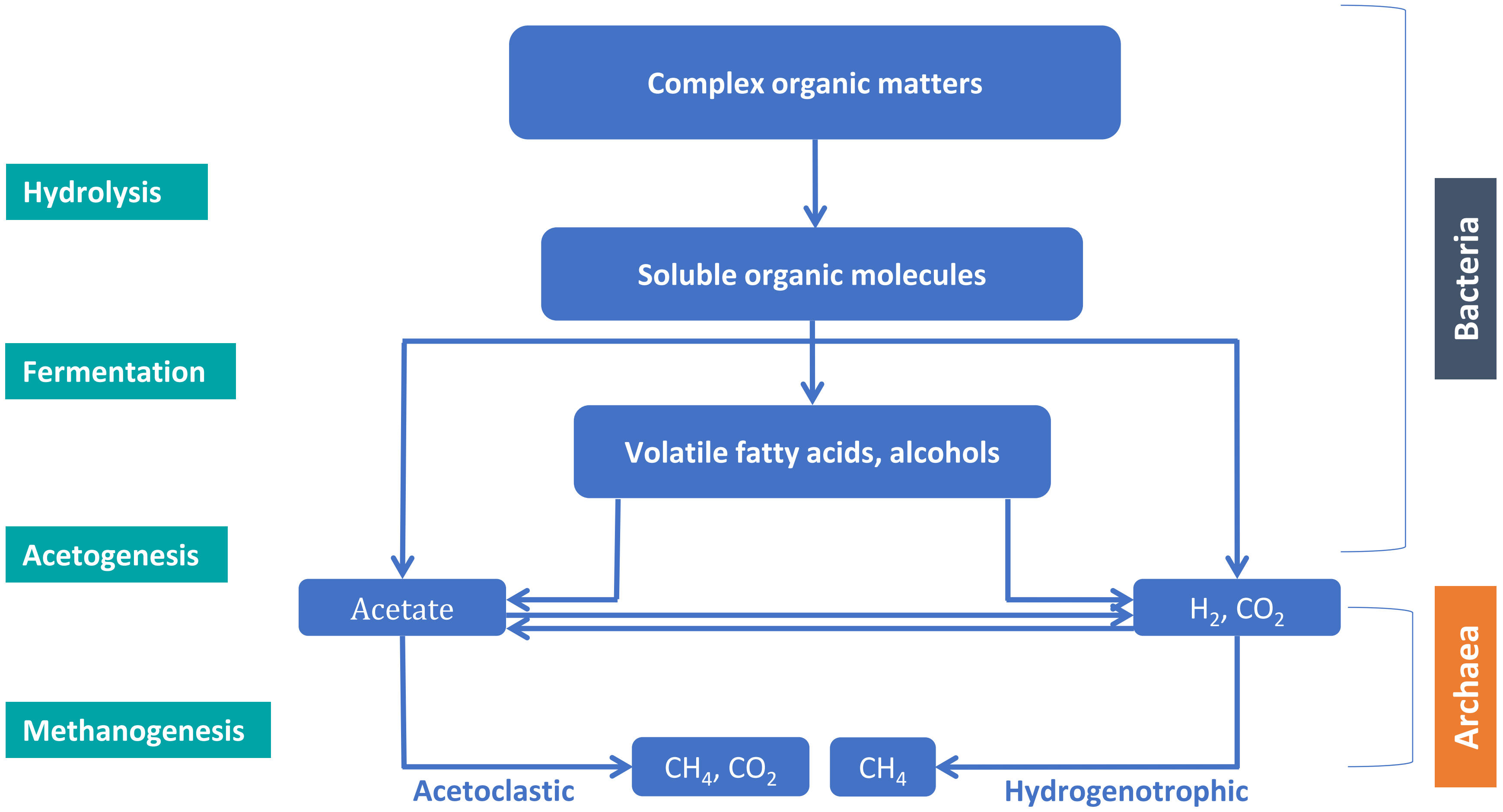
A great potential for virus discovery in anaerobic digesters…



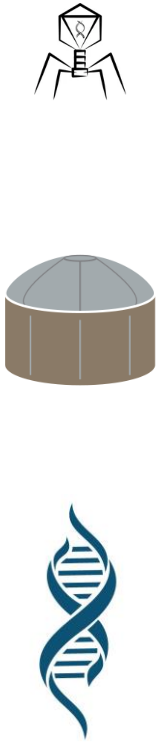


Calusinska, et al *Environmental microbiology* 18.4 (**2016**): 1162-1175.

… in particular viruses of methanogenic archaea



Objective of our study



- Characterize the diversity of viruses infecting methanogenic archaea

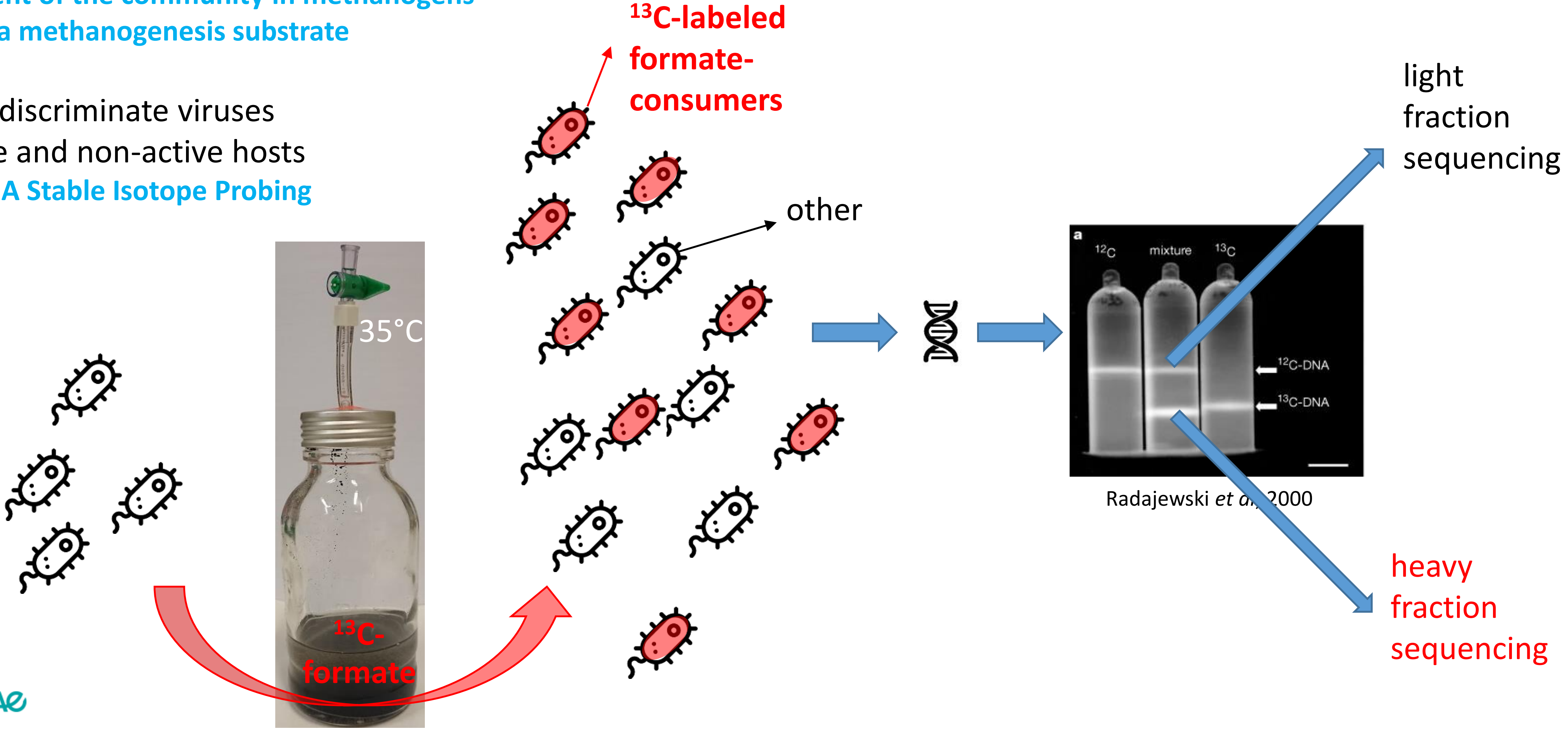
- in anaerobic digesters

- using metagenomics

How to study viruses of methanogenic archaea in anaerobic digesters?

① ~5-10% of methanogens in digesters
→ **Enrichment of the community in methanogens by using a methanogenesis substrate**

② Be able to discriminate viruses from active and non-active hosts
→ **DNA Stable Isotope Probing**



^{13}C-labeled formate-consumers

other

35°C

^{13}C-formate

Radajewski *et al.* 2000

light fraction sequencing

heavy fraction sequencing

How to study viruses of methanogenic archaea in anaerobic digesters?

① ~5-10% of methanogens in digesters

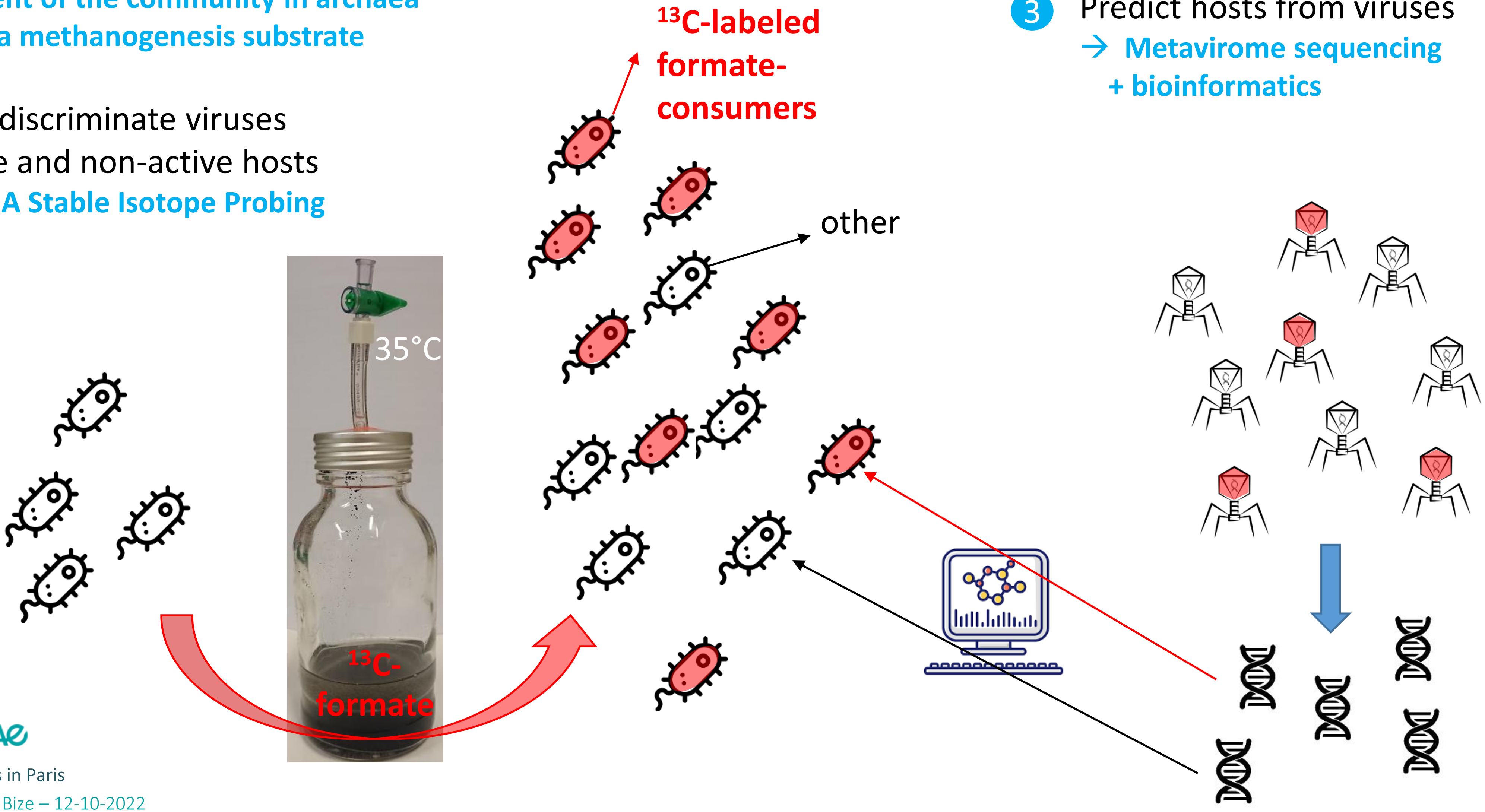
→ **Enrichment of the community in archaea by using a methanogenesis substrate**

② Be able to discriminate viruses from active and non-active hosts

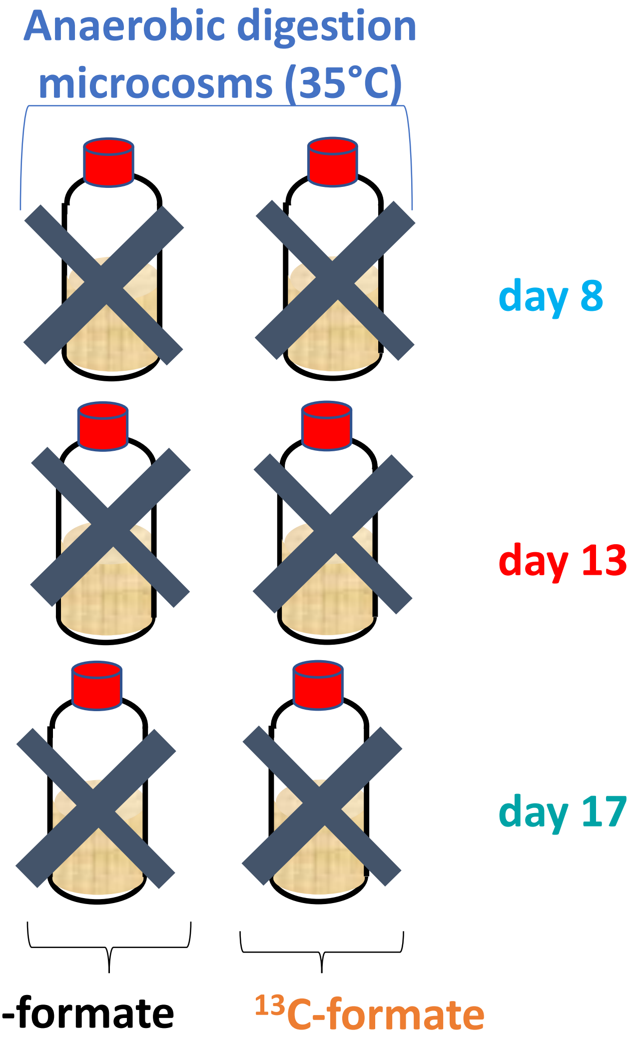
→ **DNA Stable Isotope Probing**

③ Predict hosts from viruses

→ **Metavirome sequencing + bioinformatics**

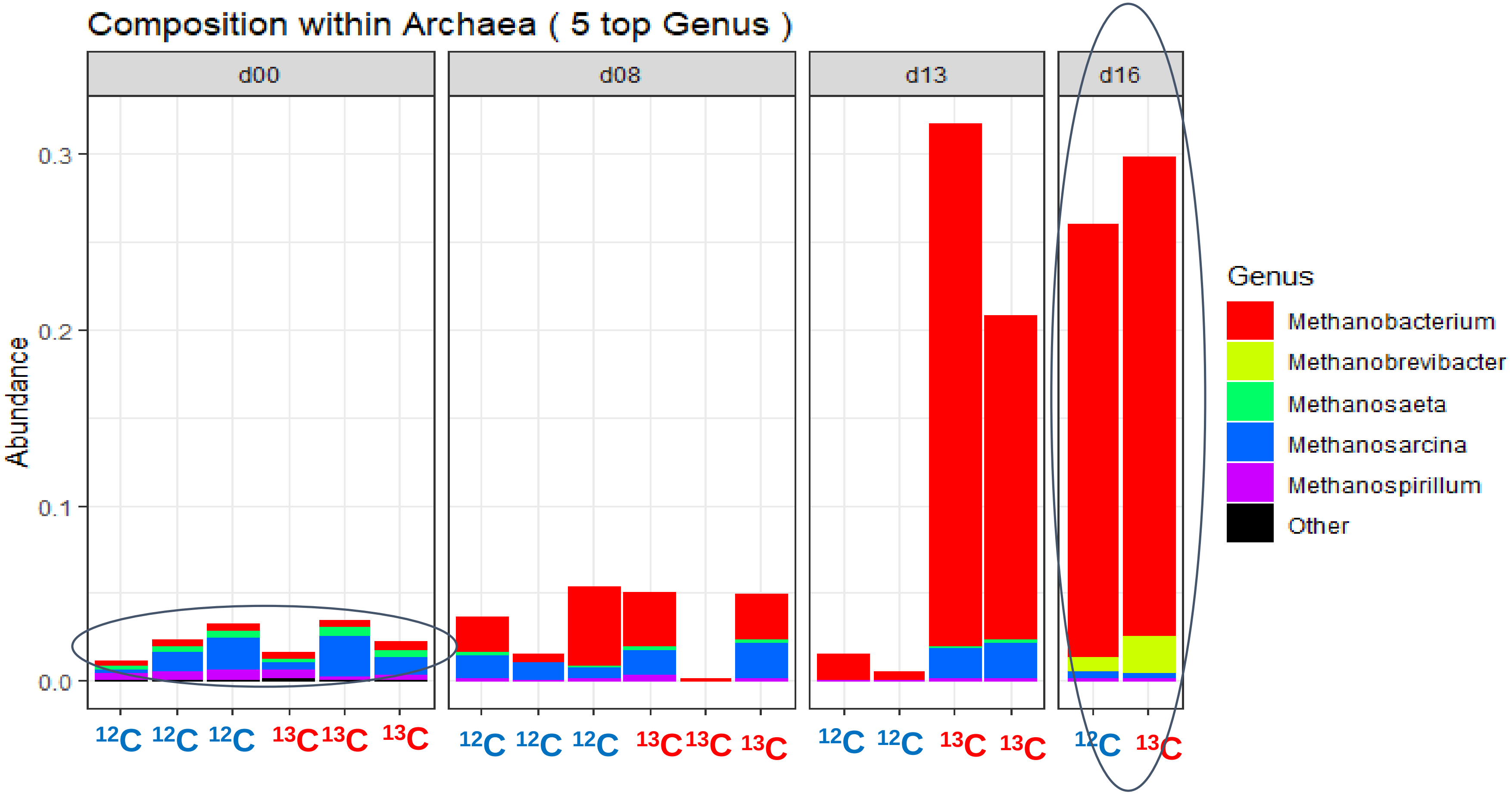


Experimental design



Enrichment of the community in methanogens?

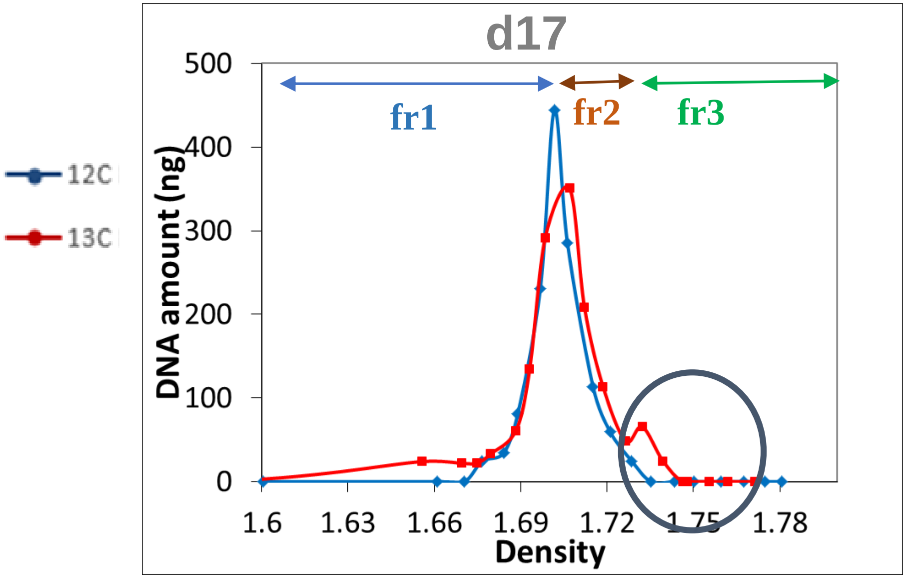
16S metabarcoding of cellular DNA



- ➢ The proportion of archaea increased over time
- ➢ Reaching ~25-30% of the community at day 17

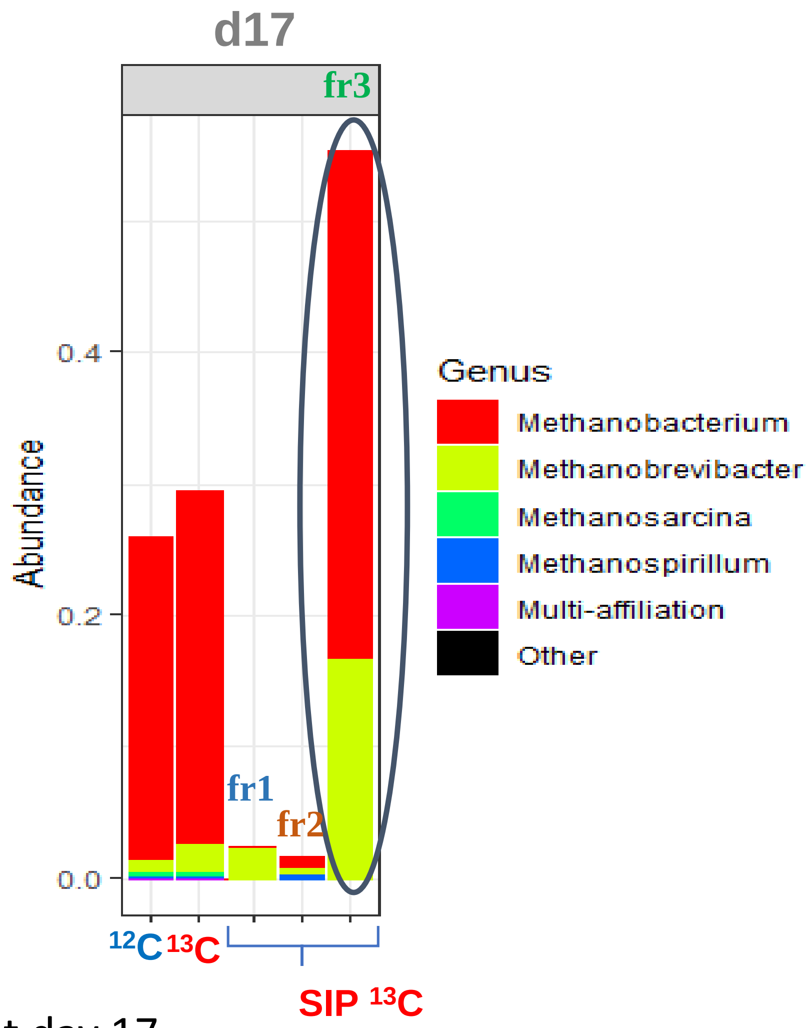
Have methanogens assimilated ^{13}C?

Density profiles of cellular DNA



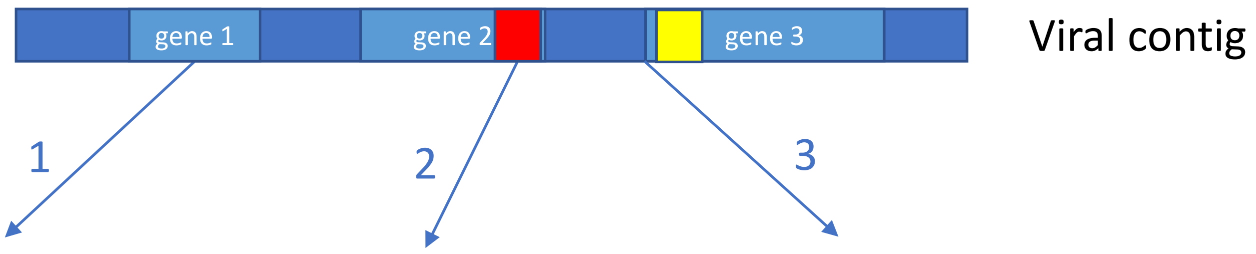
fr1: **^{12}C DNA**
fr2: Overlap ^{12}C -^{13}C DNA
fr3: **^{13}C DNA**

16S metabarcoding of cellular DNA



- ➢ 55% of archaea in the heavy DNA fraction at day 17
- ➢ *Methanobacterium* is dominant among them, followed by *Methanobrevibacter*

How many viral contigs may originate from archaeal viruses?



Gene annotation
(taxonomic and functional)

CRISPR-Cas systems
Presence of protospacers
Public spacer database + Local spacer database (detected in the cellular contigs)

🟧 🟥 🟨 ⬜

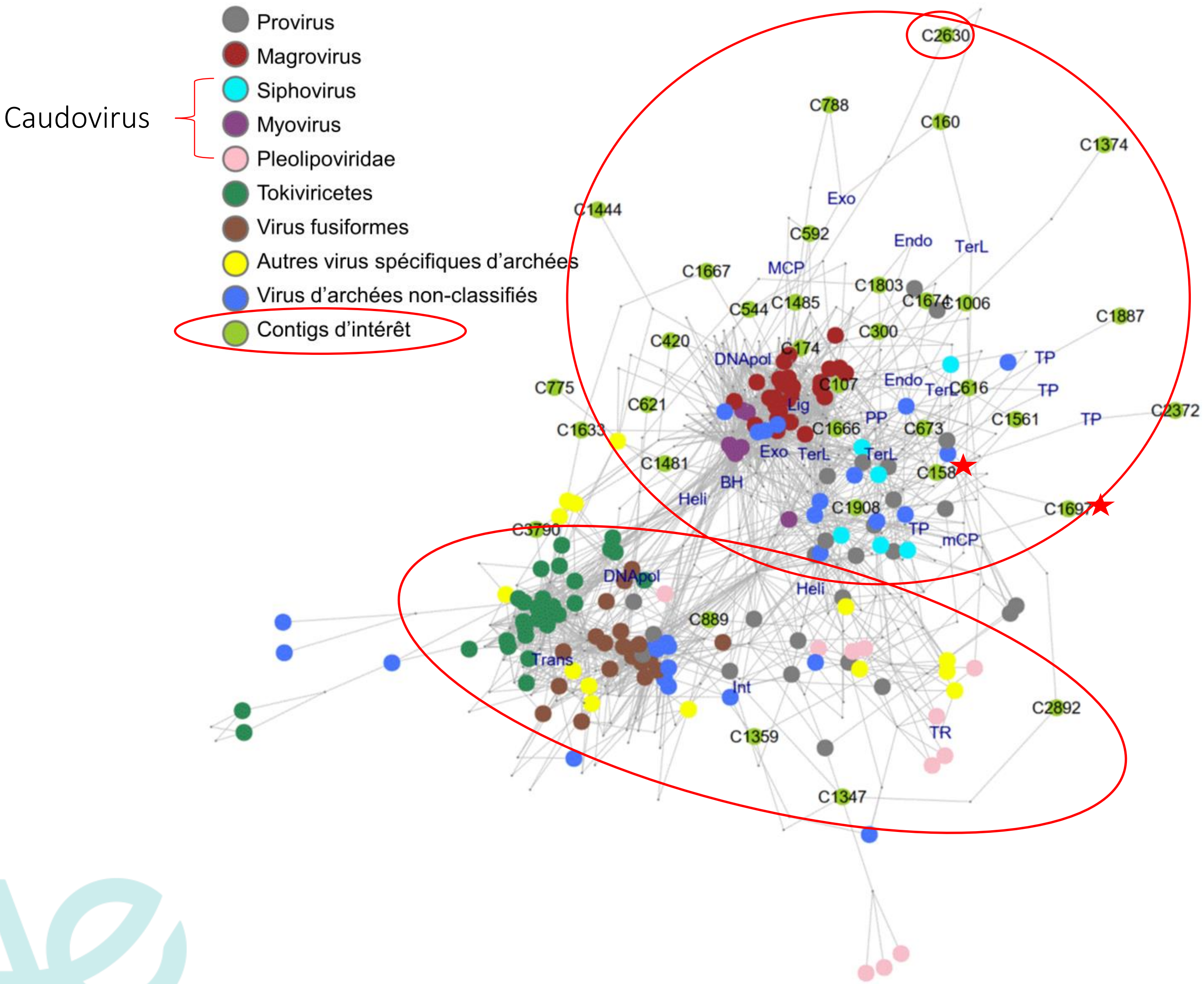
k-mer composition (WiSH) of viral contigs similar to that of the host genome:
Use of Metagenome-Assembled Genomes (MAGs) built by binning of cellular contigs

k-mer	MAG1	MAG2	MAG3
AAAA	2	8	7
AAAC	10	1	4
AAAG	5	3	8
…	…	…	…

- **39 viral contigs likely originating from viruses of archaea**

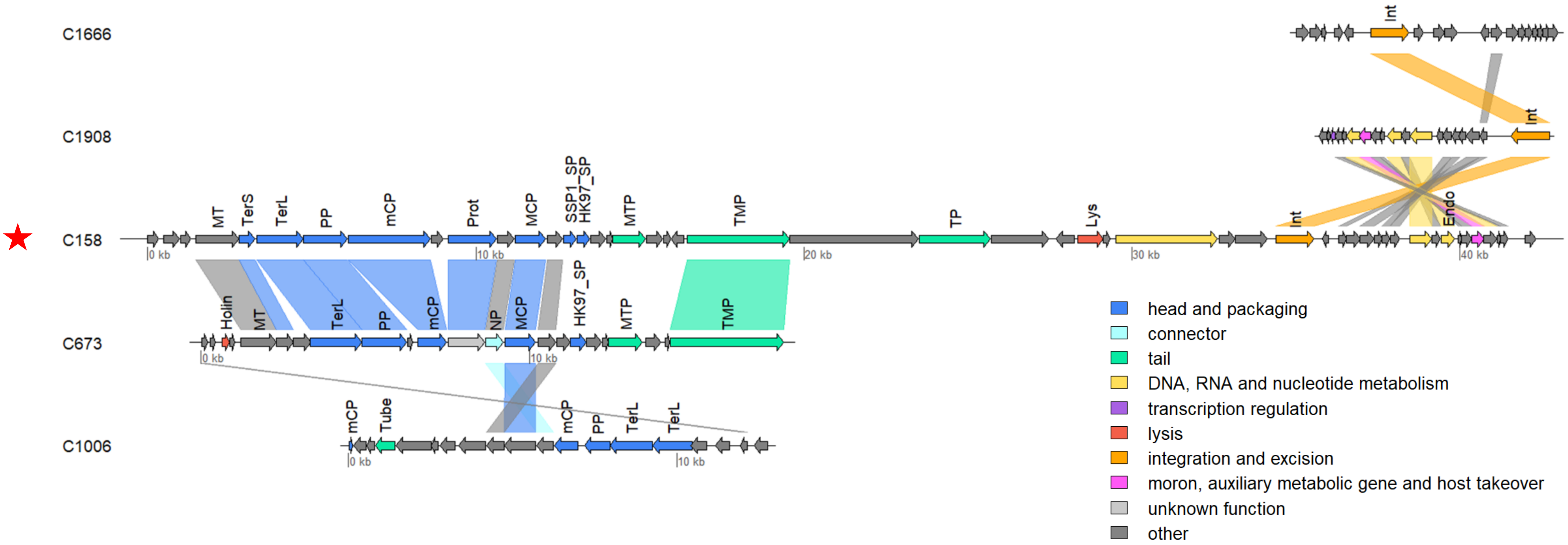
How diverse are these viral contigs?

Bipartite network of known archaeal viruses and the 39 selected viral contigs



- Most of these viral contigs share genes with archaeal caudoviruses
- Some contigs share genes with viruses having an archaea-specific morphotype
- Several contigs share very few genes with previously known viruses

Can we identify a viral contig associated to ^{13}C-labeled methanogens?

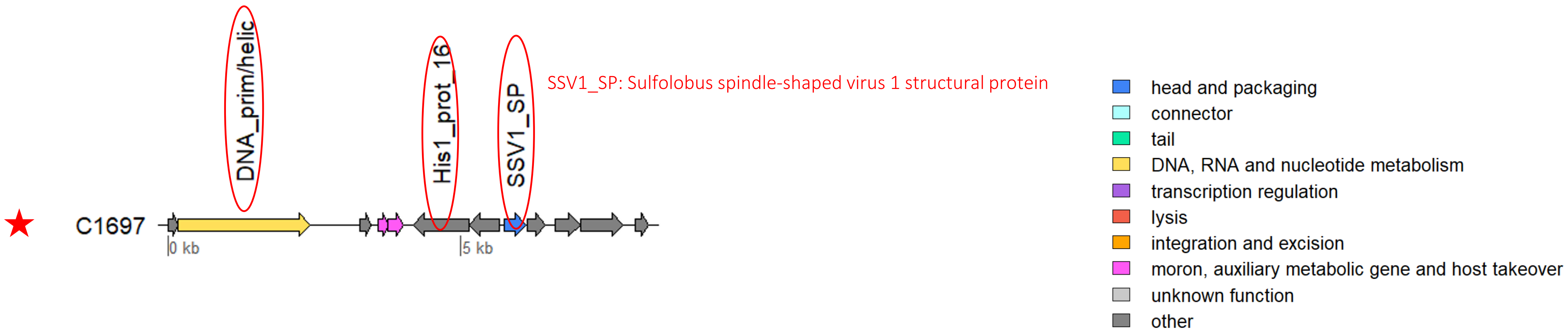


Length (kb)	Predicted host	RPKM*	
		d13	d17
42.49	Archaea; Euryarchaeota; Methanobacteriales; Methanobacteria; Methanobacteriaceae; **Methanobacterium**; *Methanobacterium formicicum*;	**468**	**229**

- Complete circular genome of a caudovirus, siphovirus-like
- New proposed viral family: *Speroviridae*

* Reads Per Kilobase, per Million mapped reads

Can we identify a viral contig associated to unlabeled methanogens?



SSV1_SP: Sulfolobus spindle-shaped virus 1 structural protein

Length (kb)	Predicted host	RPKM	
		d13	d17
8.21	Archaea; Euryarchaeota; Methanosarcinales; Methanomicrobia; Methanosarcinaceae; **Methanosarcina**; *Methanosarcina mazei*;	4	**8**

- ➢ Likely a partial or nearly-complete genome of a spindle-shaped virus
- ➢ The genome may be linear (protein-primed family B DNA polymerase)
- ➢ Could represent a new family
- ➢ One of the first spindle-shaped viruses detected for methanogenic archaea

Viruses of methanogens isolated so far?

Name	Morphotype	Host genus	Origin	Genome length	References
ΨM1	Head-tailed	*Methanothermobacter*	Thermophilic digester	30.4 kb	(Meile *et al.*, 1989; Liu *et al.*, 2021)
ΨM2	Head-tailed	*Methanothermobacter*	Thermophilic digester	26.1 kb	(Pfister *et al.*, 1998; Liu *et al.*, 2021)
ΦF1	Head-tailed	*Methanobacterium*	Thermophilic digester	85 kb	(Nölling *et al.*, 1993)
ΦF3	Head-tailed	*Methanobacterium*	Thermophilic digester	36 kb	(Nölling *et al.*, 1993)
Drs3	Head-tailed	*Methanobacterium*	Mesophilic digester	37 kb	(Wolf *et al.*, 2019; Liu *et al.*, 2021)
Blf4	Head-tailed	*Methanoculleus*	Mesophilic digester	37 kb	(Weidenbach *et al.*, 2021)
MetSV	**Spherical**	*Methanosarcina*	Mesophilic digester	11 kb	(Weidenbach *et al.*, 2017)
A3-VLP	**Oblate**	*Methanococcus*	Mesophilic digester	23 kb	(Wood *et al.*, 1989)
MFTV1	Head-tail	*Methanocaldococcus*	Hydrothermal vent	31 kb	(Thiroux *et al.*, 2021)

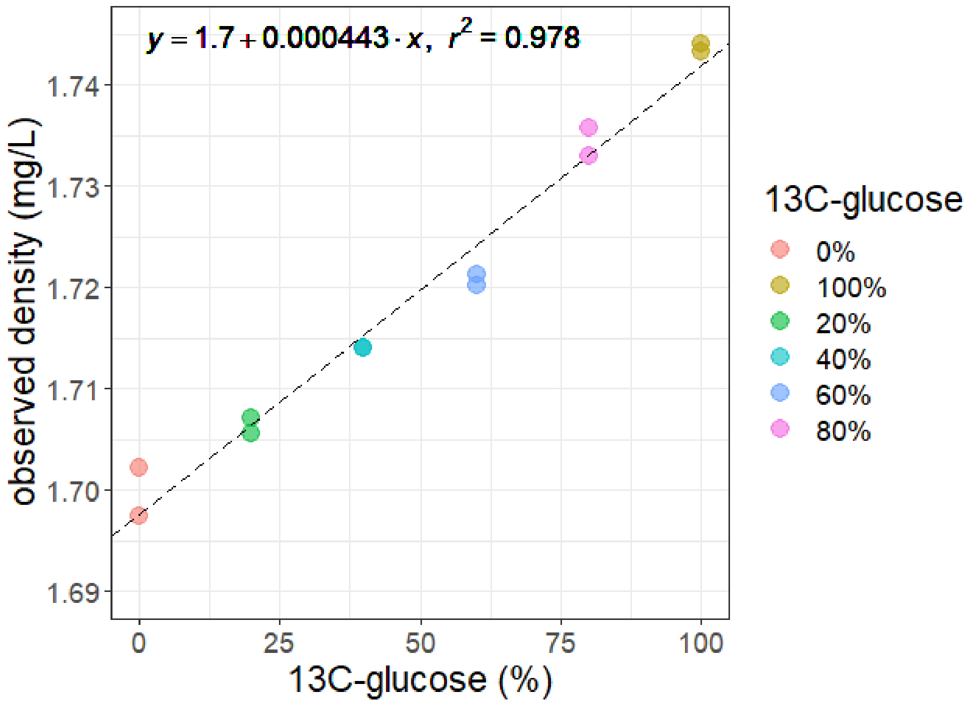
Summary

- Discovery of viruses of methanogens
  - Limited similarity with previously characterized archaeal viruses
  - In particular, discovery of spindle-shaped viruses infecting methanogenic archaea
  - It further supports the widespread phylogenetic and environmental distribution of spindle-shaped archaeal viruses
- Shows the potential of Stable Isotope Probing to identify viruses infecting hosts with a specific metabolism, as previously shown by Sungeun Lee *et al*, 2021
- Work recently published in *Environmental Microbiology* https://doi.org/10.1111/1462-2920.16120

Perspectives

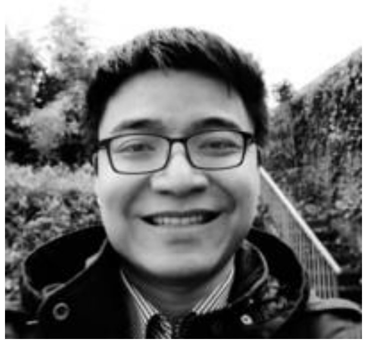
Viral DNA SIP

T4 DNA



Acknowledgements

Hoang Ngo



Laboratoire
Microorganismes :
Génome
et Environnement

Université Clermont Auvergne – LMGE

François Enault

PROSE
PRocédés biOtechnologiques
au Service de l'Environnement

Université Paris Saclay
INRAE – PROSE

Institut Pasteur

Institut Pasteur – Archaeal Virology

Mart Krupovic

Cédric Midoux
Chrystelle Bureau
Angeline Guenne
Nadine Derlet
Laurent Mazéas
Olivier Chapleur
Théodore Bouchez

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Université Paris Saclay – INRAE – MaIAGE
Plate-forme de bioinformatique MIGALE

Mahendra Mariadassou
Valentin Loux

Poster 32 from Marion Covès

Characterization of virus-host dynamics
in anaerobic digesters under abiotic stress