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

Hoang Ngo, François Enault, Cédric Midoux, Mahendra Mariadassou, Olivier Chapleur, Laurent Mazéas, Valentin Loux, Théodore Bouchez, Mart Krupovic, Ariane Bize

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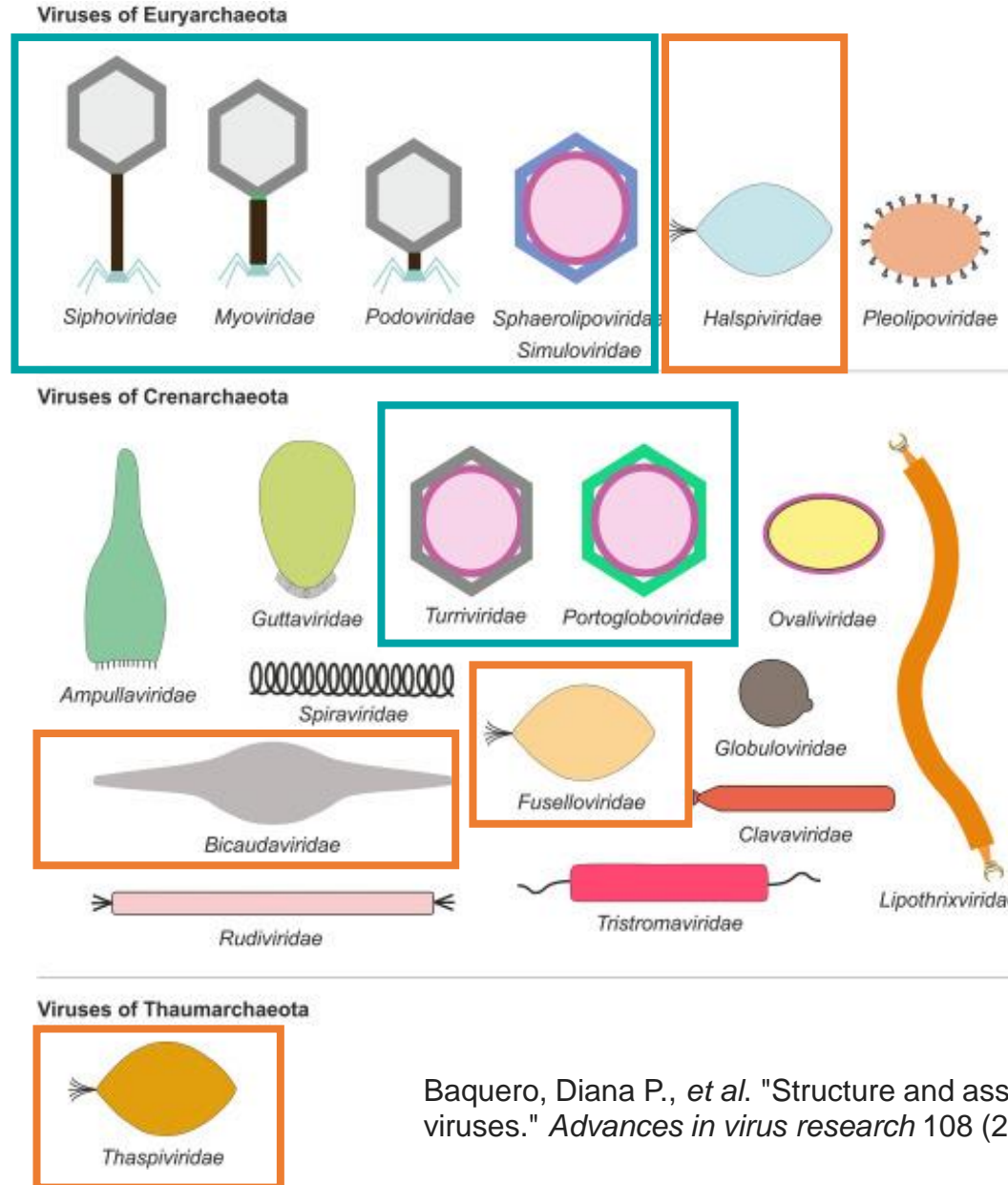
INRAE

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

Hoang Ngo, François Enault, Cédric Midoux, Mahendra Mariadassou, Olivier Chapleur, Laurent Mazéas, Valentin Loux, Théodore Bouchez, Mart Krupovic, Ariane Bize

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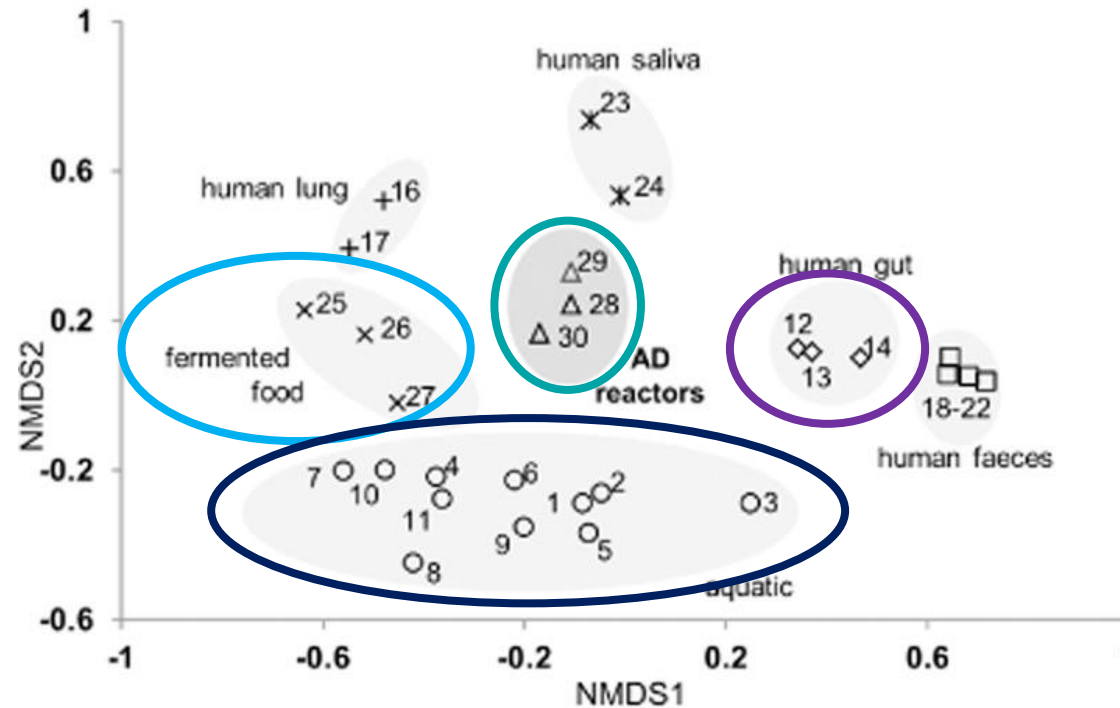
➤ The great diversity of archaeal viruses is only partly characterized



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...

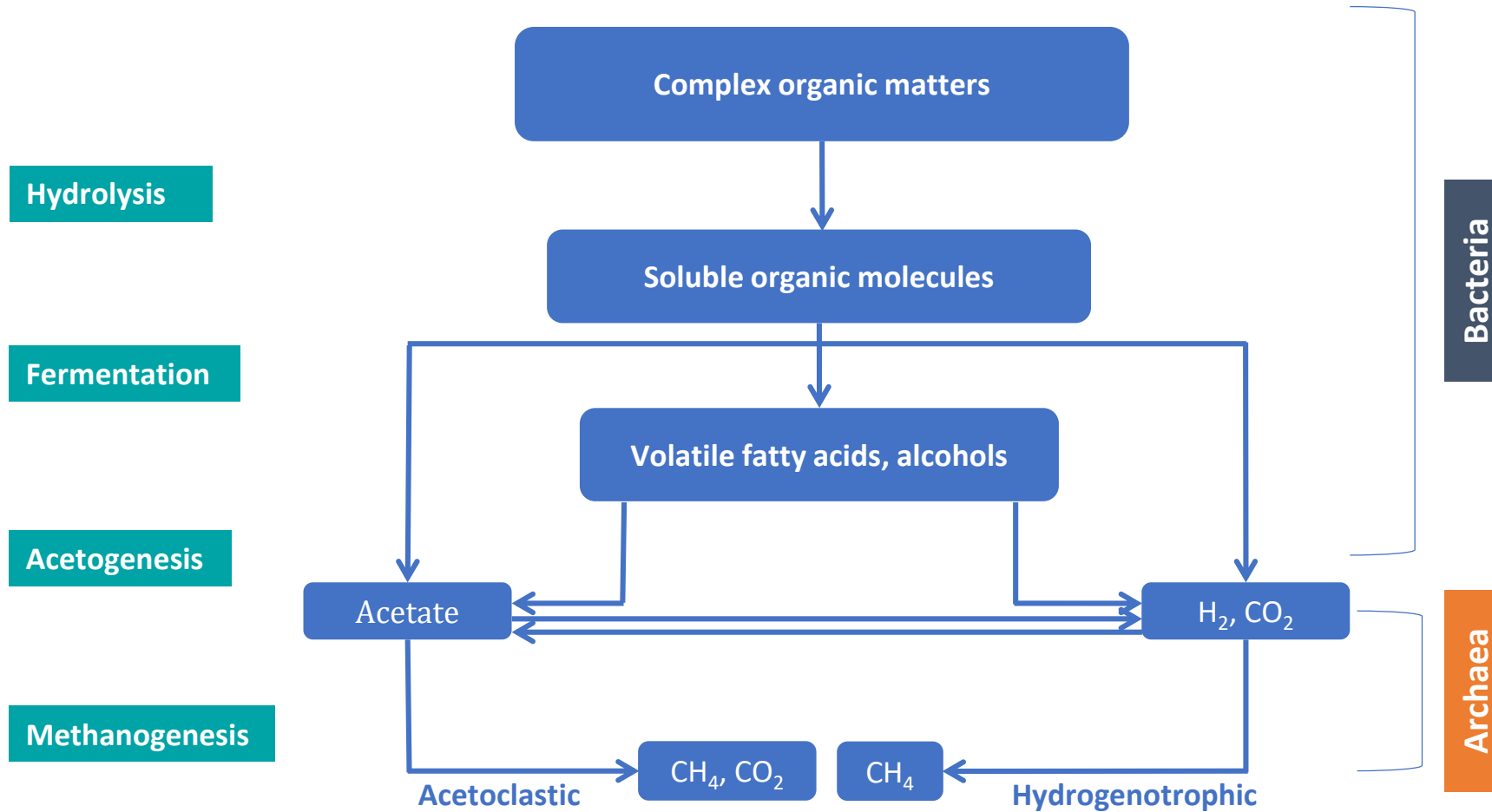
## ANAEROBIC DIGESTION



- 01-Lake Pavin
- 02-Lake Bourget
- 03-Antarctic Lake Spring
- 04-Tilapia Channel
- 05-Sargasso Sea
- 06-Gulf of Mexico
- 07-Coral Porites
- 08-Coral Atoll Palmyra
- 09-Tampa Bay induced
- 10-Salterm high
- 11-Salterm low
- ◇ 12-Human\_Gut\_L1d1
- ◇ 13-Human\_Gut\_H1d1
- ◇ 14-Human\_Gut\_L3d2
- + 16-Human\_Lung\_CF7
- + 17-Human\_Lung\_CF9
- 18-Human Feces A
- 19-Human Feces B
- 20-Human Feces C
- 21-Human Feces D
- x 23-Human\_salivary\_Sub1\_D1
- x 24-Human\_salivary\_Sub2\_D30
- x 25-Sauerkraut
- x 26-Cabbage Kimchi
- x 27-Shrimp jeotgal
- △ 28-DNAviromeC7
- △ 29-DNAviromeF4
- △ 30-DNAviromeP6

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

# ➤ ... in particular viruses of methanogenic archaea

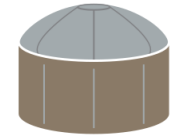


## ➤ Objective of our study

- Characterize the diversity of viruses infecting methanogenic archaea



- in anaerobic digesters

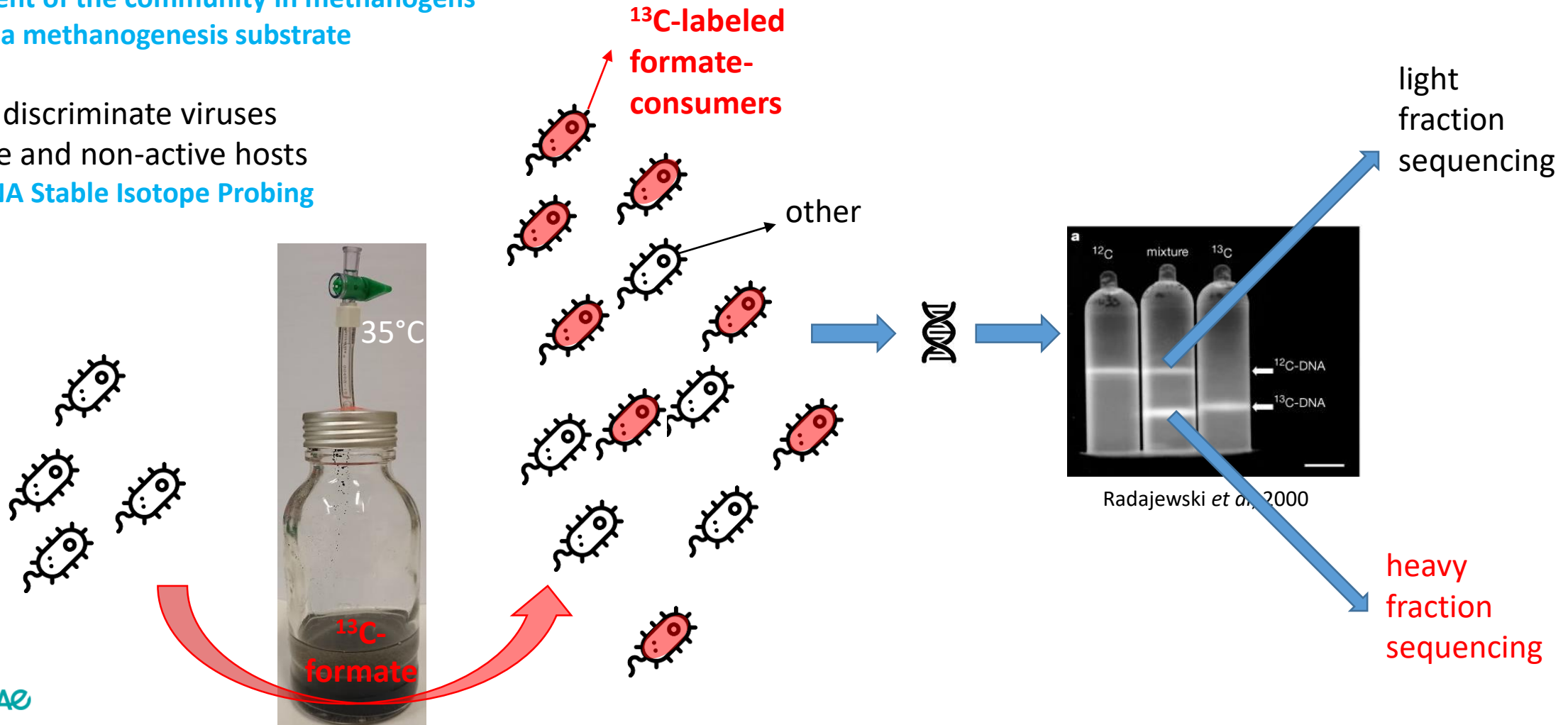


- using metagenomics



# ➤ 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

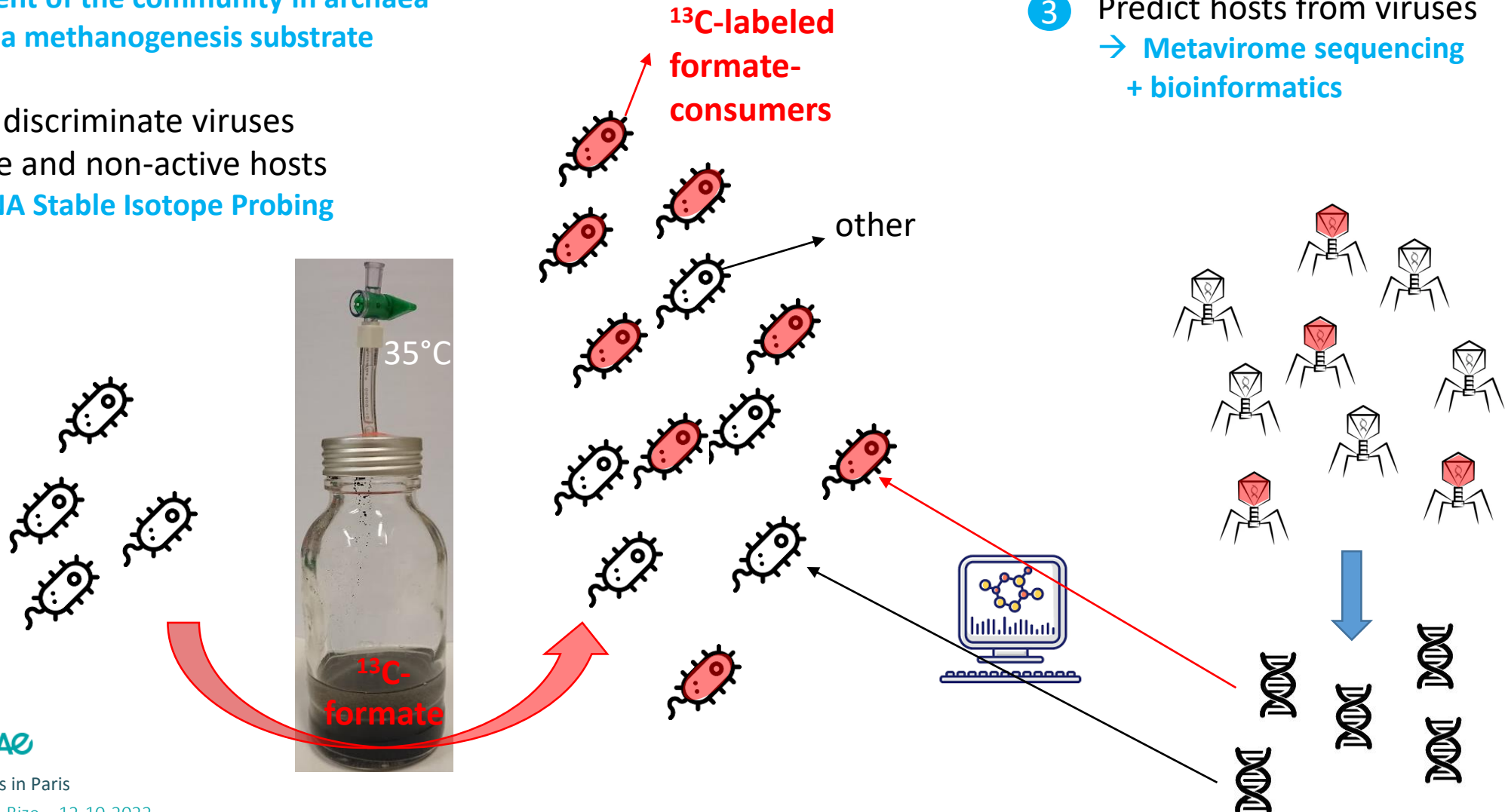


# ➤ How to study viruses of methanogenic archaea in anaerobic digesters?

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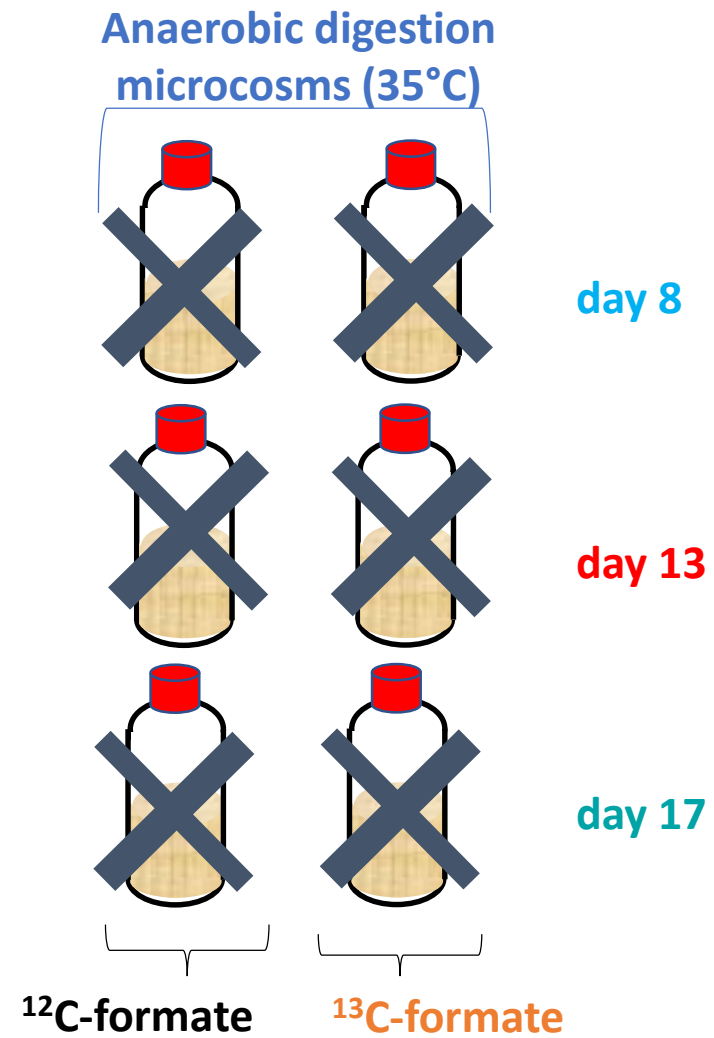
2 Be able to discriminate viruses from active and non-active hosts  
→ DNA Stable Isotope Probing

3 Predict hosts from viruses  
→ Metavirome sequencing  
+ bioinformatics



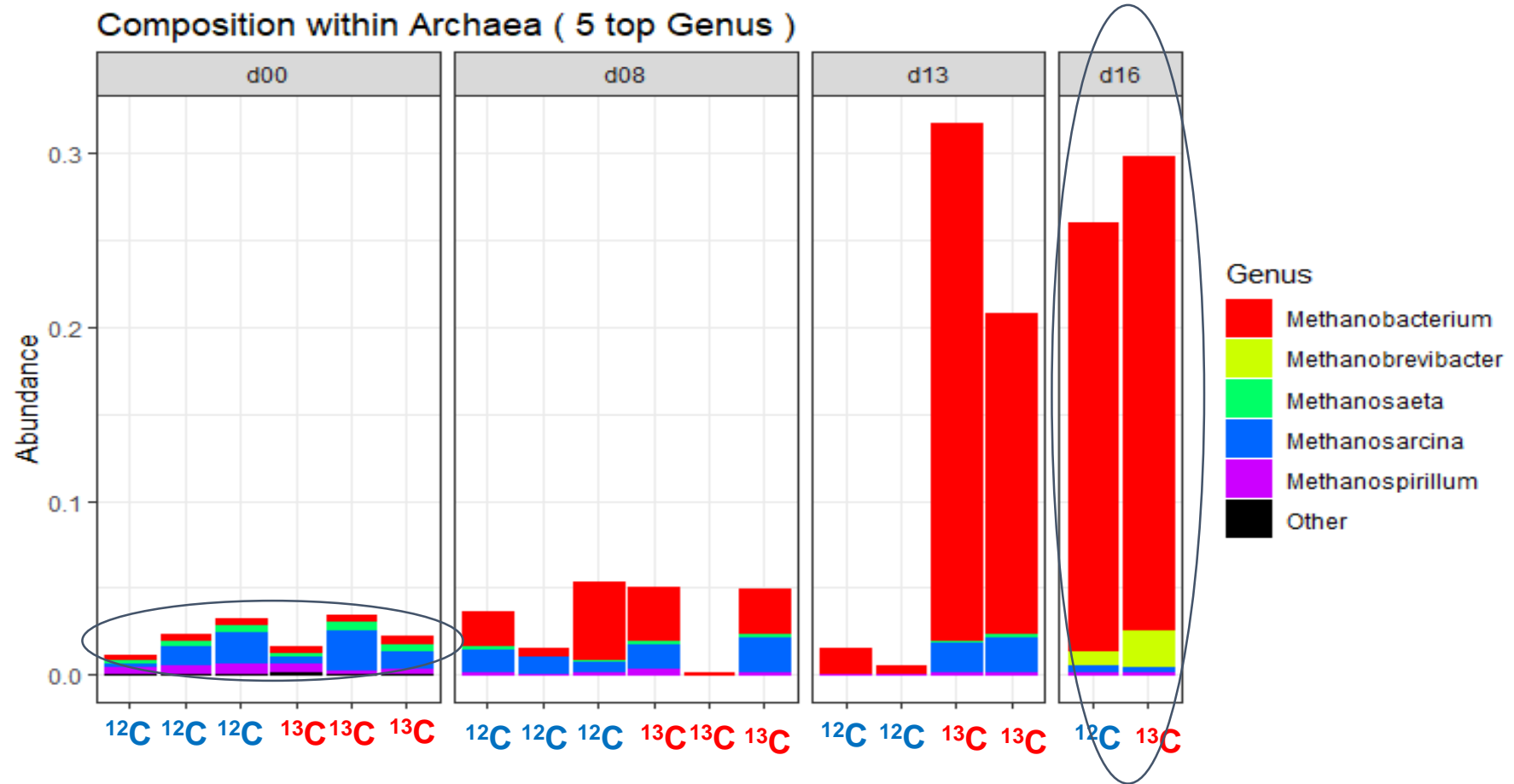


## ➤ Experimental design



# ➤ Enrichment of the community in methanogens?

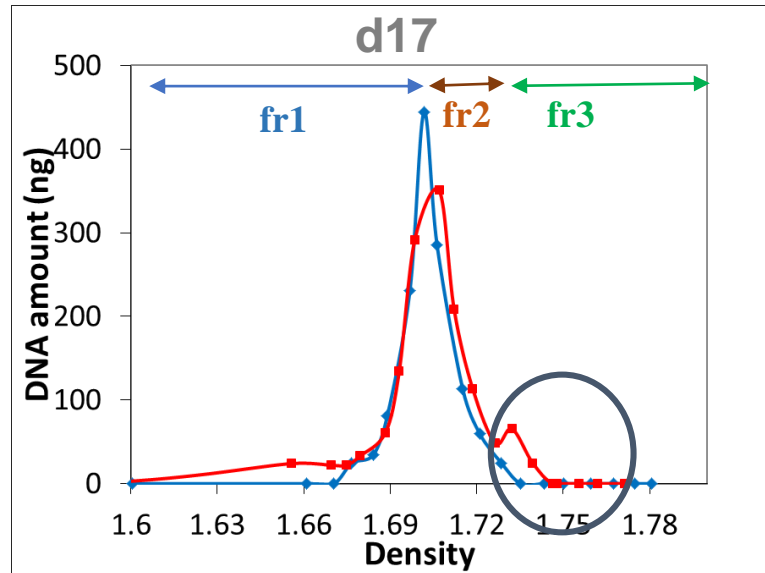
16S metabarcoding of cellular DNA



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

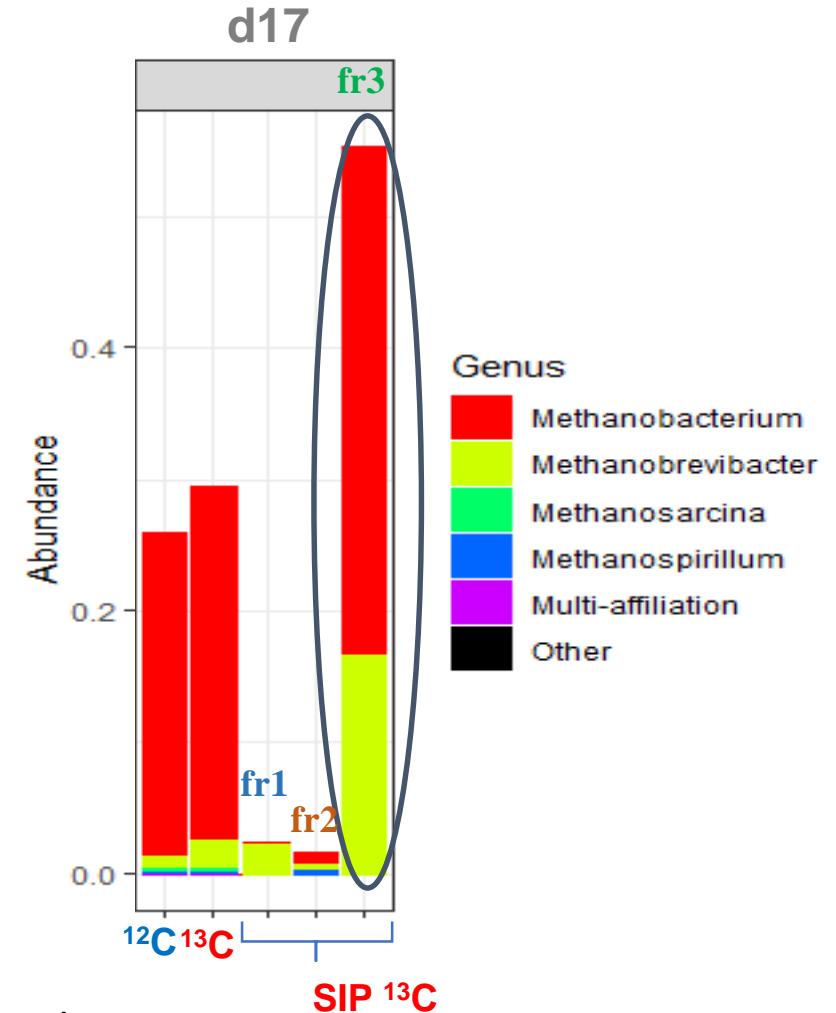
# ➤ Have methanogens assimilated $^{13}\text{C}$ ?

Density profiles of cellular DNA



fr1:  $^{12}\text{C}$  DNA  
 fr2: Overlap  $^{12}\text{C}$  -  $^{13}\text{C}$  DNA  
 fr3:  $^{13}\text{C}$  DNA

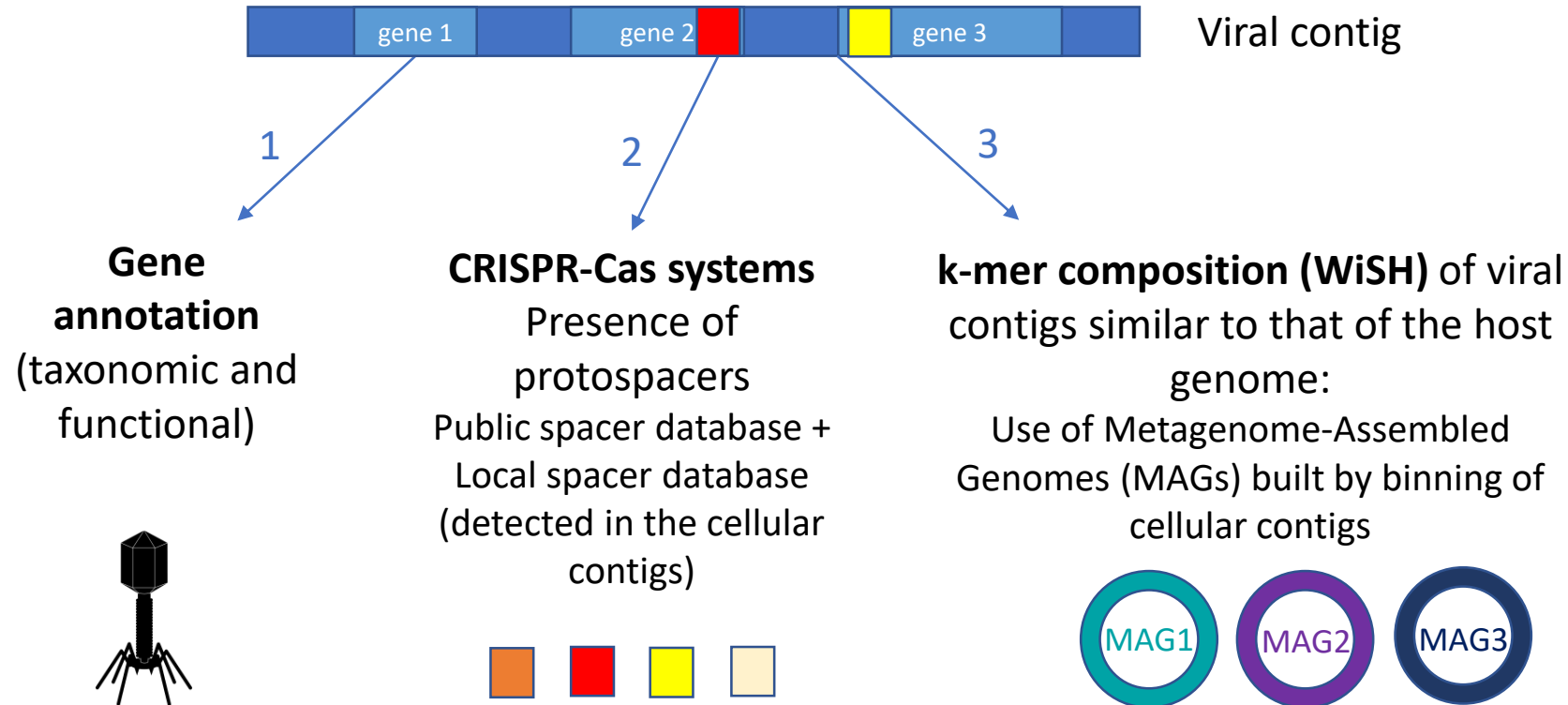
16S metabarcoding of cellular DNA



- 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?

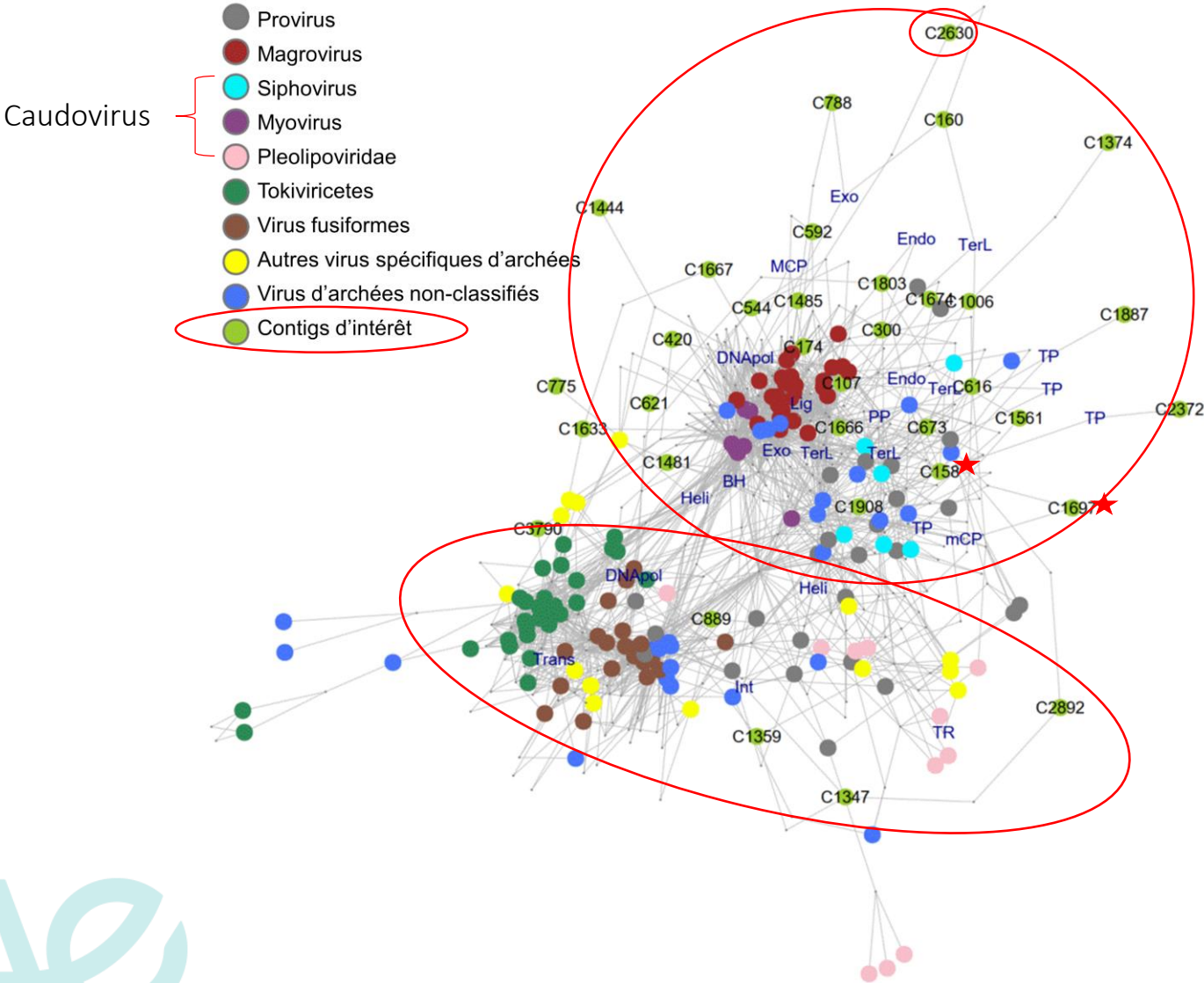


➤ **39 viral contigs likely originating from viruses of archaea**

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

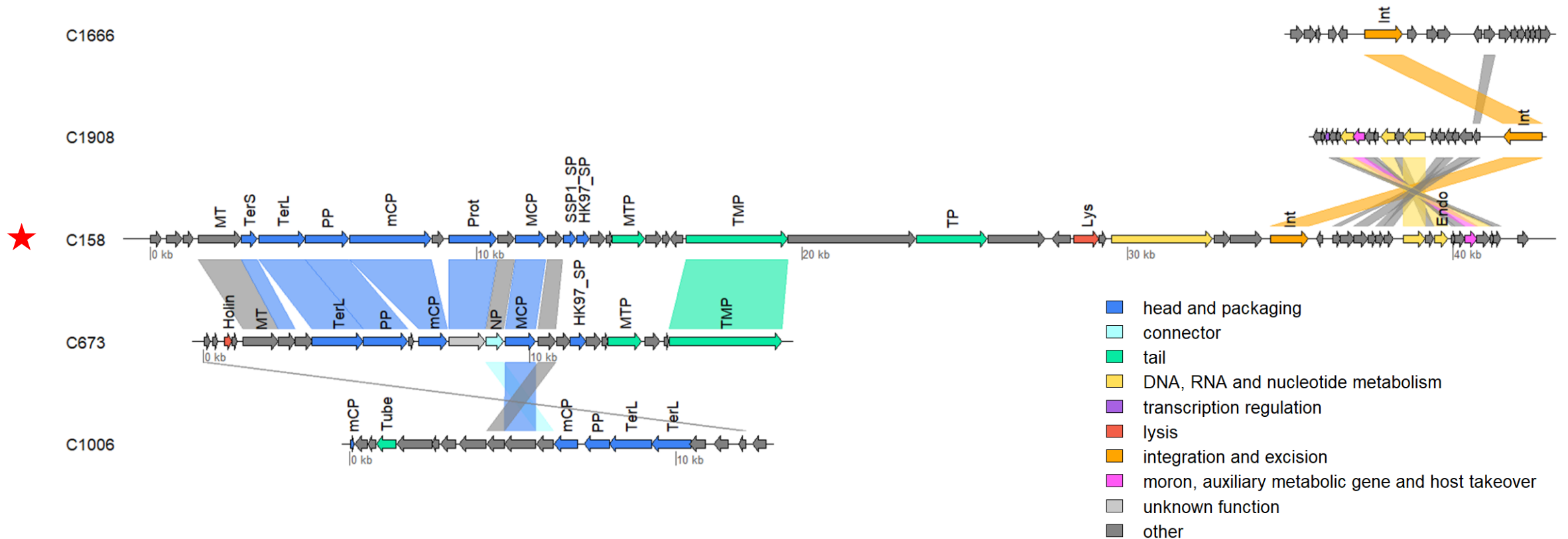
# ➤ How diverse are these viral contigs?

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 <sup>13</sup>C-labeled methanogens?

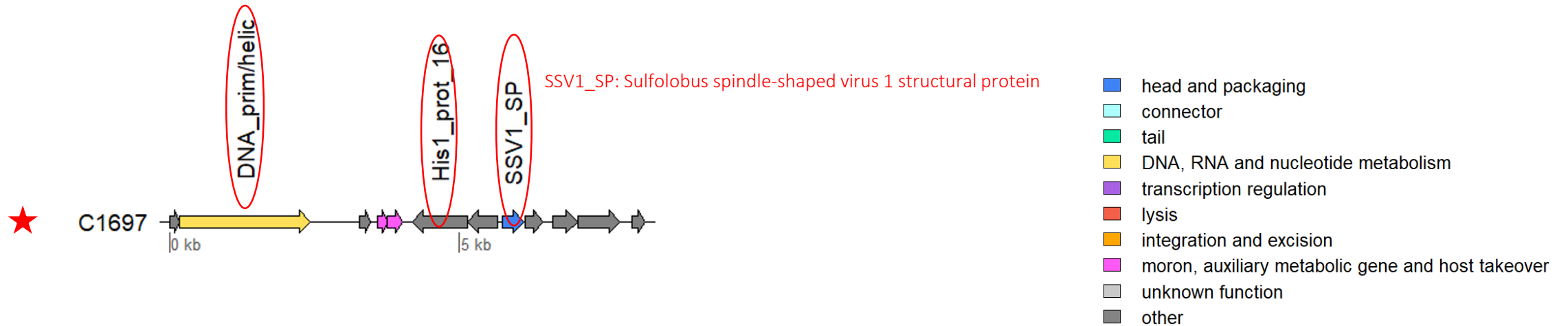


Length (kb)	Predicted host	RPKM*	
		d13	d17
42.49	Archaea; Euryarchaeota; Methanobacteriales; Methanobacteria; Methanobacteriaceae; <b>Methanobacterium</b> ; <i>Methanobacterium formicicum</i> ;	<b>468</b>	<b>229</b>

- 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?



Length (kb)	Predicted host	RPKM	
		d13	d17
8.21	Archaea; Euryarchaeota; Methanosarcinales; Methanomicrobia; Methanosarcinaceae; <b>Methanosarcina</b> ; <i>Methanosarcina mazei</i> ;	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	<i>Methanothermobacter</i>	Thermophilic digester	30.4 kb	(Meile <i>et al.</i> , 1989; Liu <i>et al.</i> , 2021)
ΨM2	Head-tailed	<i>Methanothermobacter</i>	Thermophilic digester	26.1 kb	(Pfister <i>et al.</i> , 1998; Liu <i>et al.</i> , 2021)
ΦF1	Head-tailed	<i>Methanobacterium</i>	Thermophilic digester	85 kb	(Nölling <i>et al.</i> , 1993)
ΦF3	Head-tailed	<i>Methanobacterium</i>	Thermophilic digester	36 kb	(Nölling <i>et al.</i> , 1993)
Drs3	Head-tailed	<i>Methanobacterium</i>	Mesophilic digester	37 kb	(Wolf <i>et al.</i> , 2019; Liu <i>et al.</i> , 2021)
BIf4	Head-tailed	<i>Methanoculleus</i>	Mesophilic digester	37 kb	(Weidenbach <i>et al.</i> , 2021)
MetSV	<b>Spherical</b>	<i>Methanosarcina</i>	Mesophilic digester	11 kb	(Weidenbach <i>et al.</i> , 2017)
A3-VLP	<b>Oblate</b>	<i>Methanococcus</i>	Mesophilic digester	23 kb	(Wood <i>et al.</i> , 1989)
MFTV1	Head-tail	<i>Methanocaldococcus</i>	Hydrothermal vent	31 kb	(Thiroux <i>et al.</i> , 2021)



## ➤ Summary

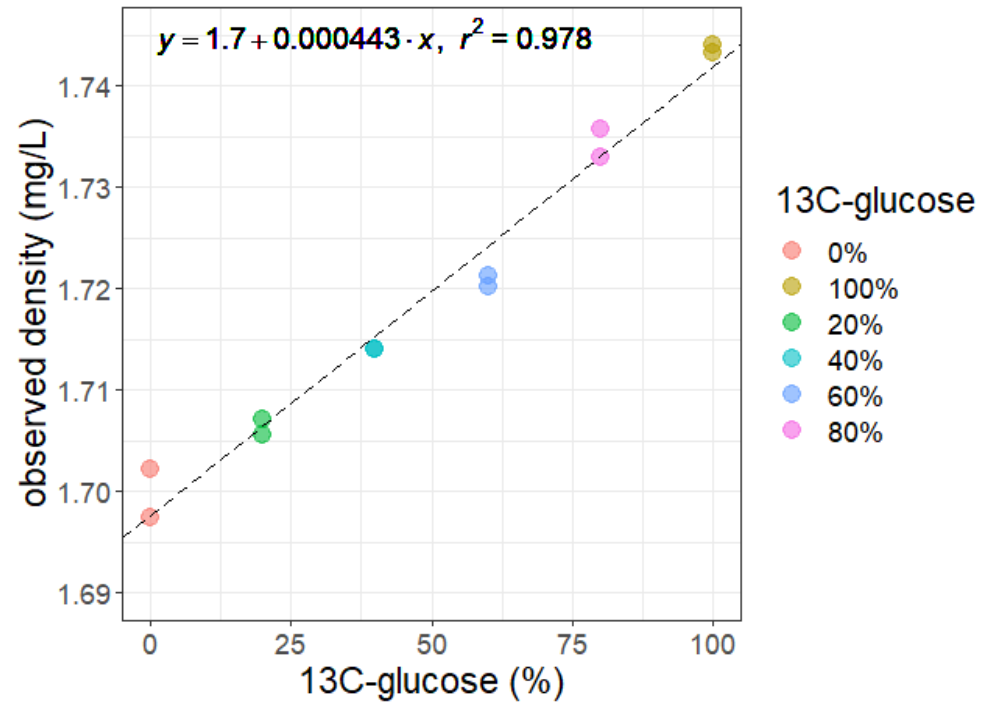
- Discovery of viruses of methanogens
  - Limited similarity with previously characterized archaeal viruses
  - In particular, discovery of spindle-shaped viruses infecting methanogenic archaea
  - It further supports the widespread phylogenetic and environmental distribution of spindle-shaped archaeal 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



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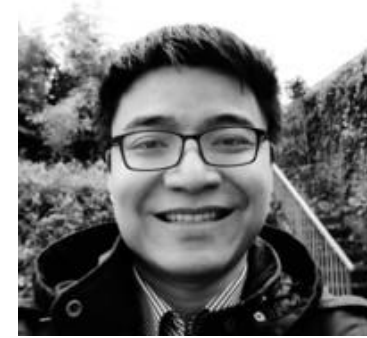
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Phages in Paris

Ariane Bize – 12-10-2022

➤ Poster 32 from Marion Covès

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

