3-nitrooxypropanol:
an inhibitor of methanogenesis that could reduce GHG emissions by ruminants

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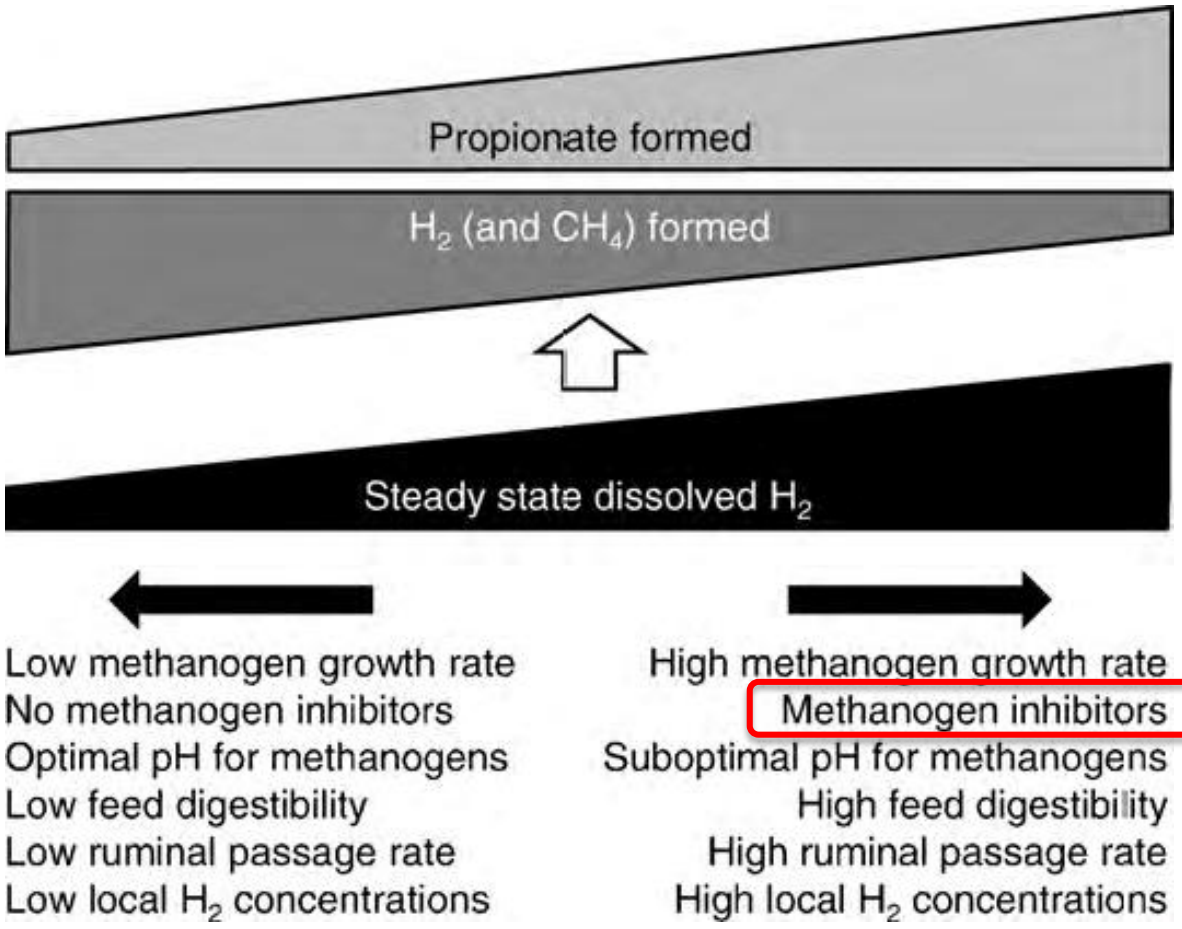
CSIC

DSM

H_2 balance in the rumen – CH_4 production



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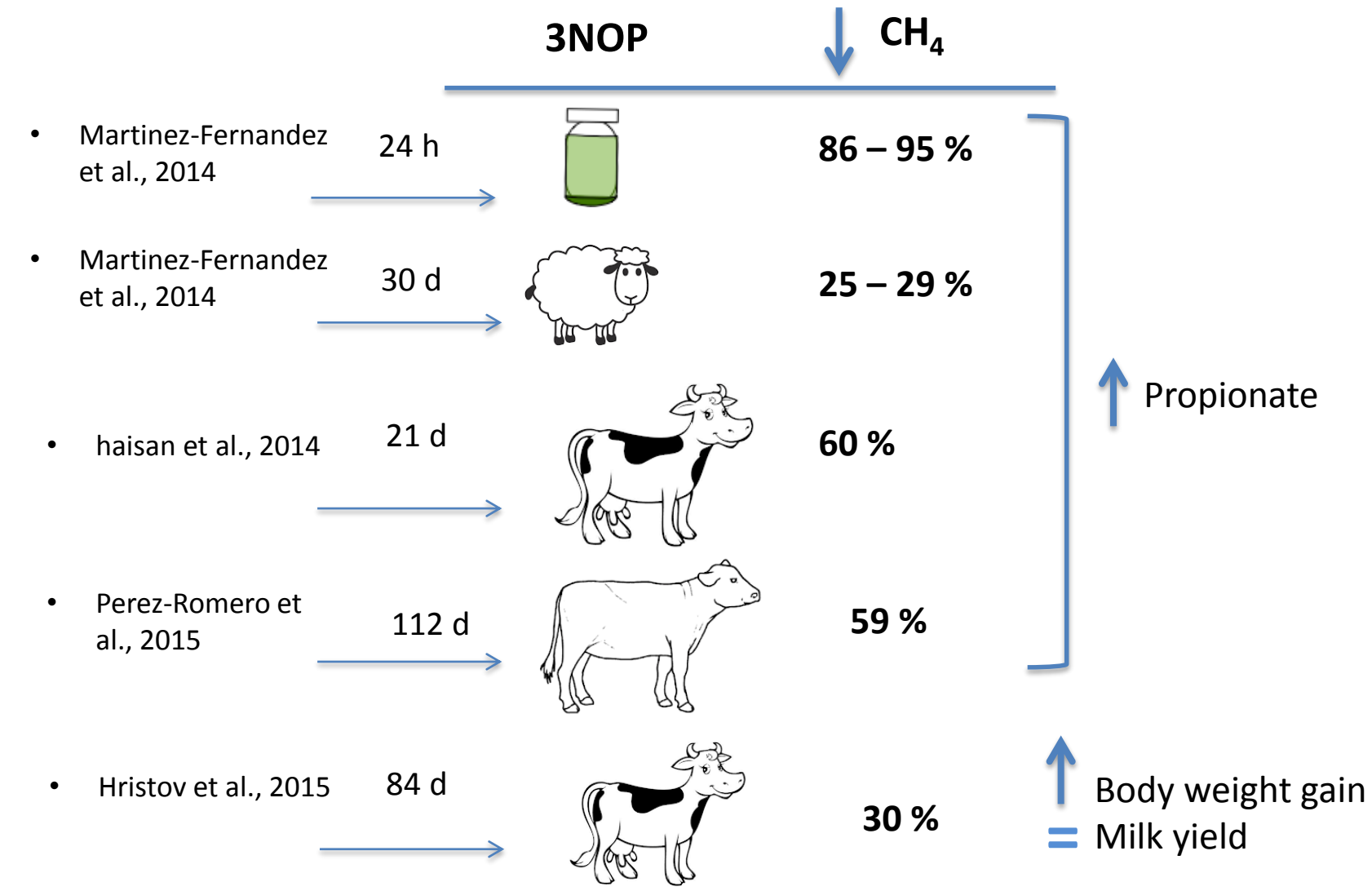
Janssen et al., 2010

3-nitrooxypropanol (3-NOP)

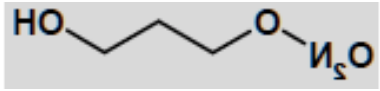


3-nitrooxypropanol (3-NOP)

Acetate

CO_2 MethylH4SPT CoM

3-NOP



- Similar shape
- oxidation potential

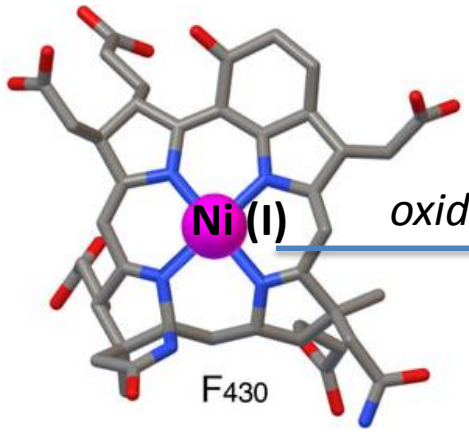
Methyl-coenzyme M reductase (MCR)

Methyl-coenzyme M → **CH_4**

Coenzyme B

(di-tri) Methylamines

Methanol



oxidation →

Ni (II)
inactive

Objective and methodology

Study the specific **mode of action** of 3-NOP to inihibit MCR:

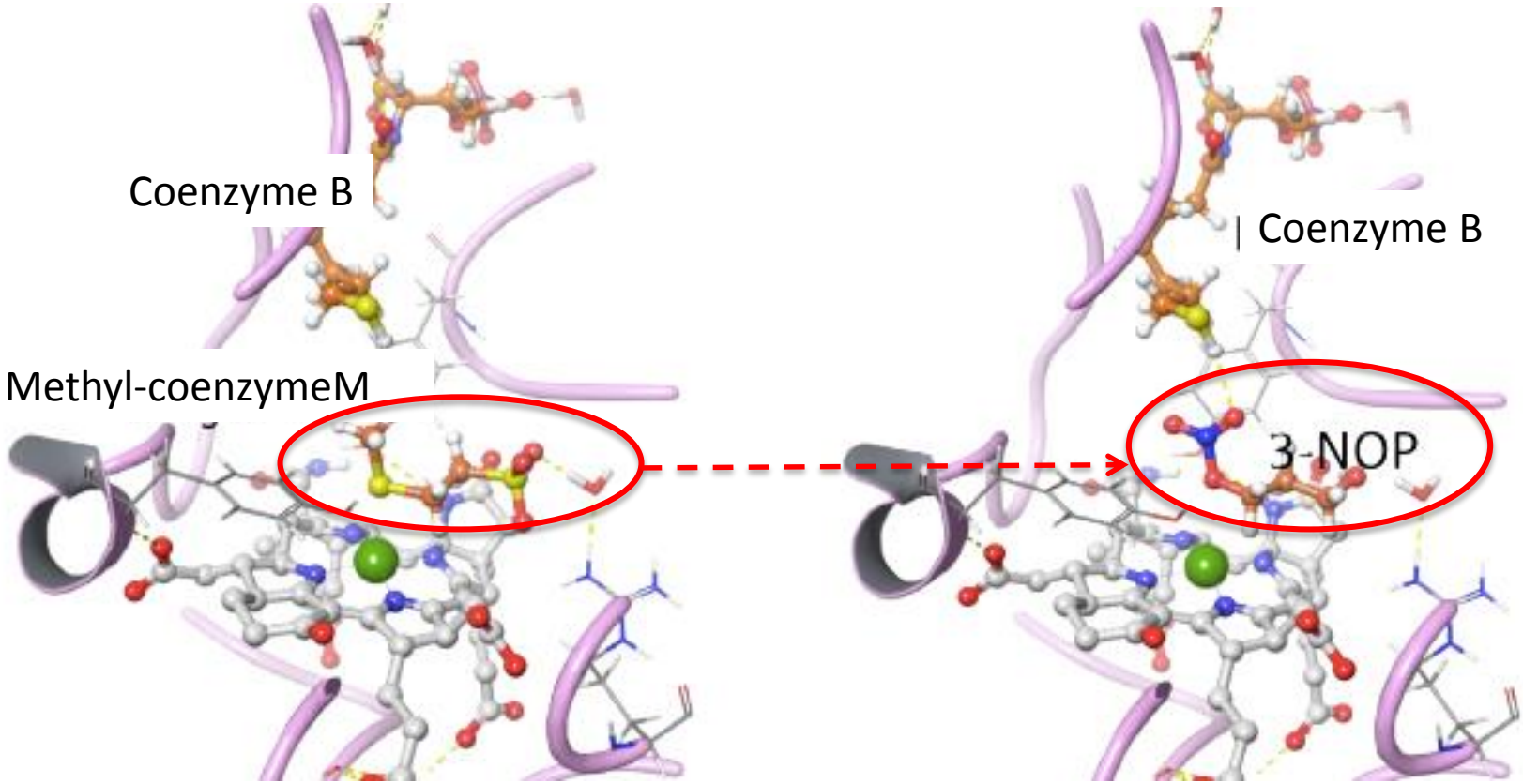
- ***In silico***: - Molecular modelling

- ***In vitro***: - MCR purification (*Methanothermobacter marburgensis*)
  - Activity assay,
  - EPR signal determination
  - X-ray analysis
  - NO_2^- NO_3^- production

- ***In vivo***: - Anaerobic culture of methanogens and bacteria

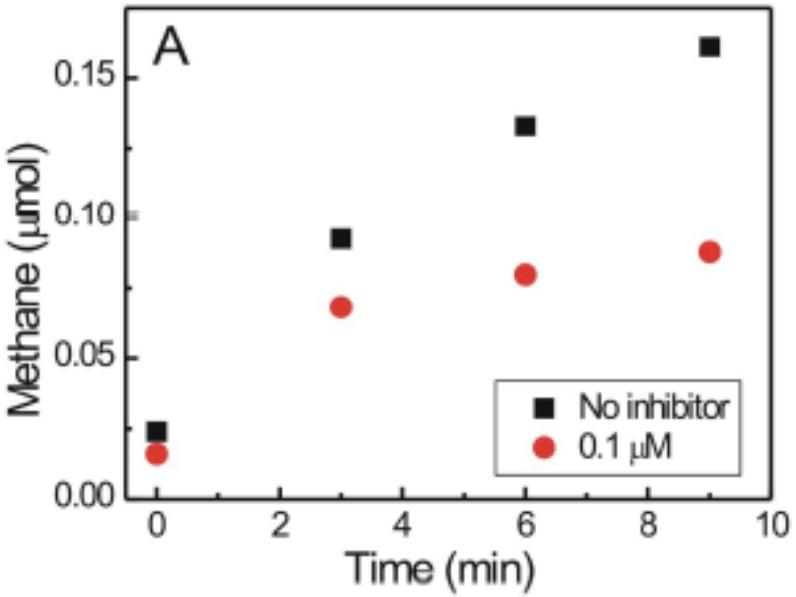
Results: *In silico*

3-NOP was developed by using 3D pharmacophore-based virtual screening and molecular docking – analogue of methyl-coM

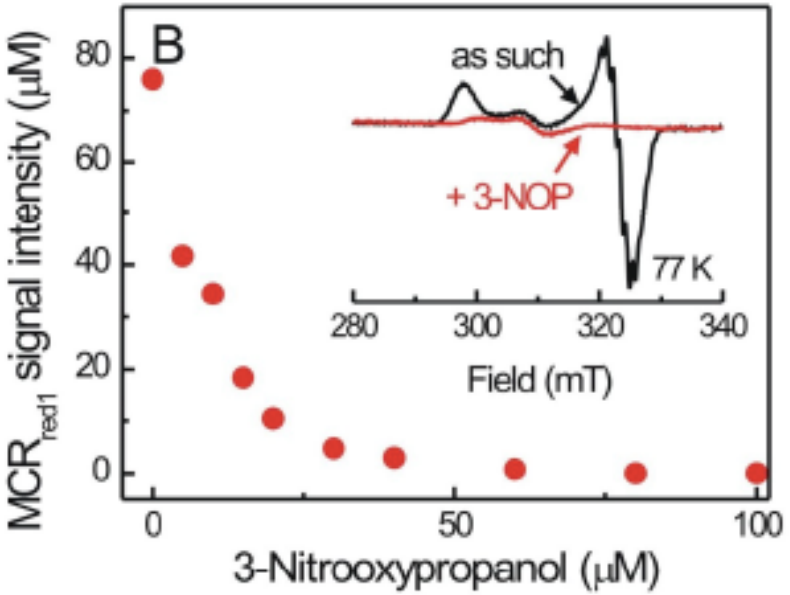


Results: *In vitro I*

- Purified MCR was quickly inactivated by 3-NOP



- Detected by EPR signal
  - MCR $_{red}$ ------ (Ni I)
  - MCR $_{ox1}$ ------ (Ni III)

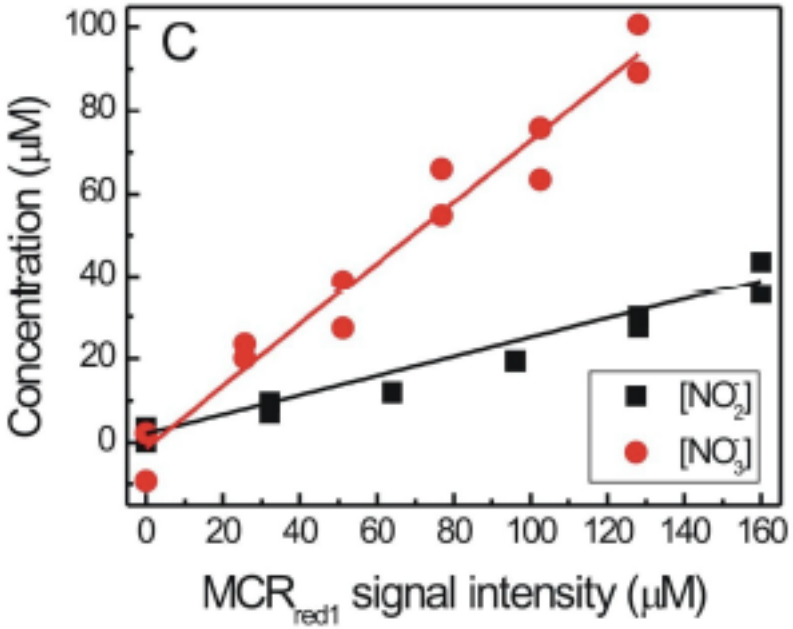


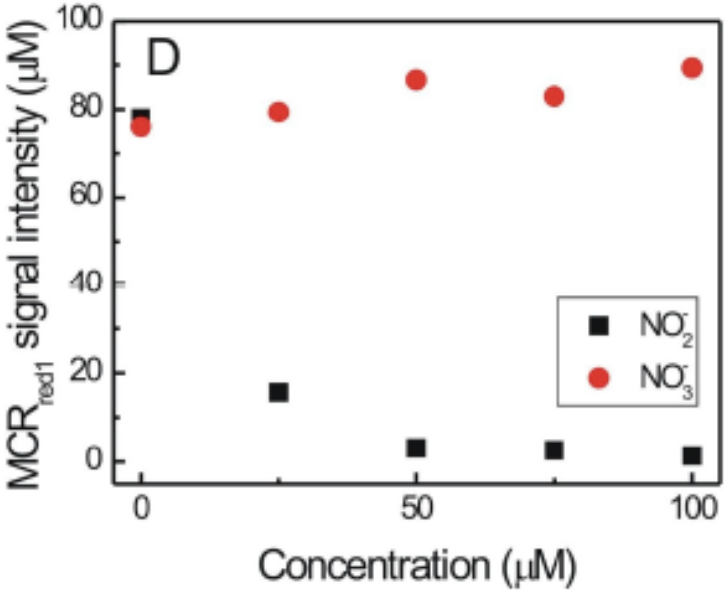
Results: *In vitro II*

- NO_2^- and NO_3^- were formed upon inactivation of MCR

- NO_2^- also inactivated MCR

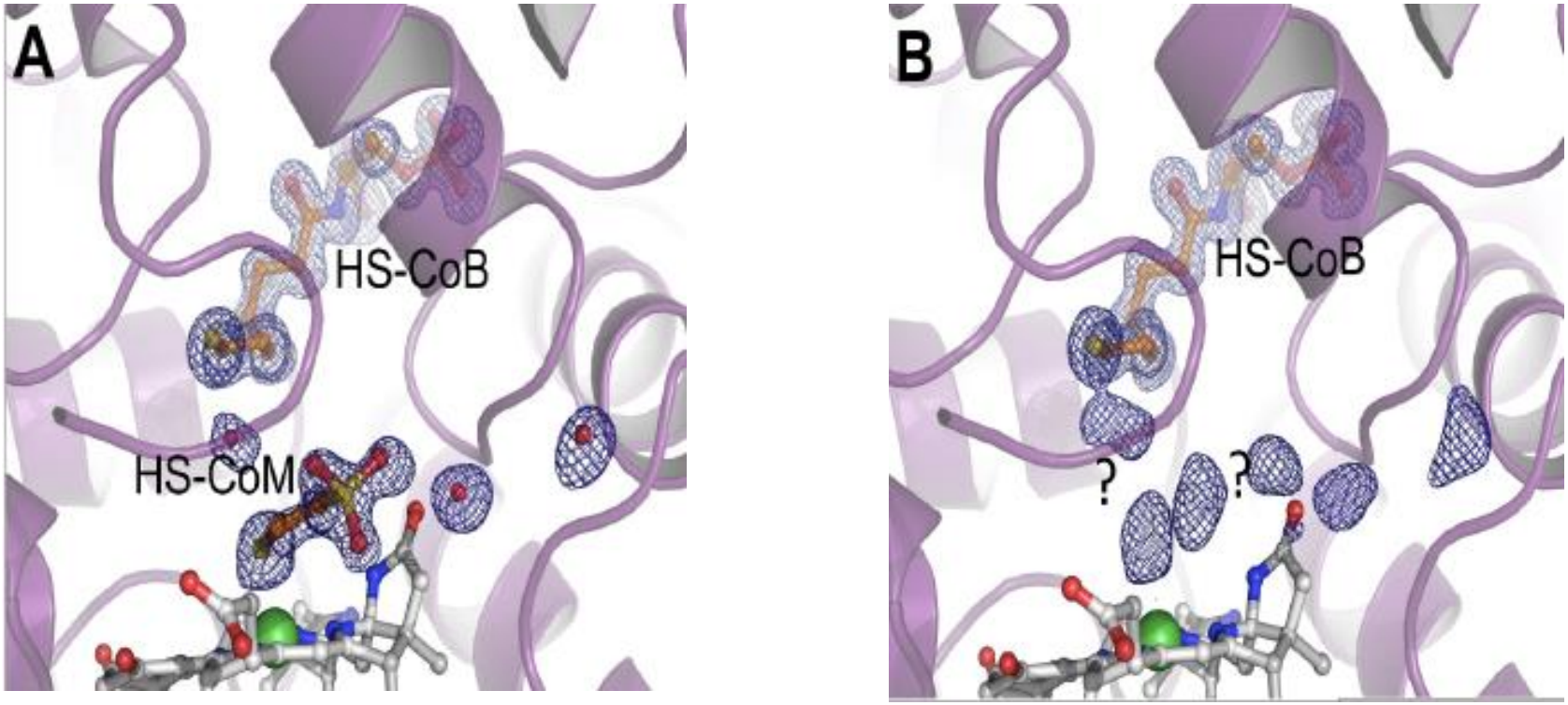
Unique double tandem-charge warhead inhibitor
- 3-NOP
- NO_2^-





Results: *In vitro III*

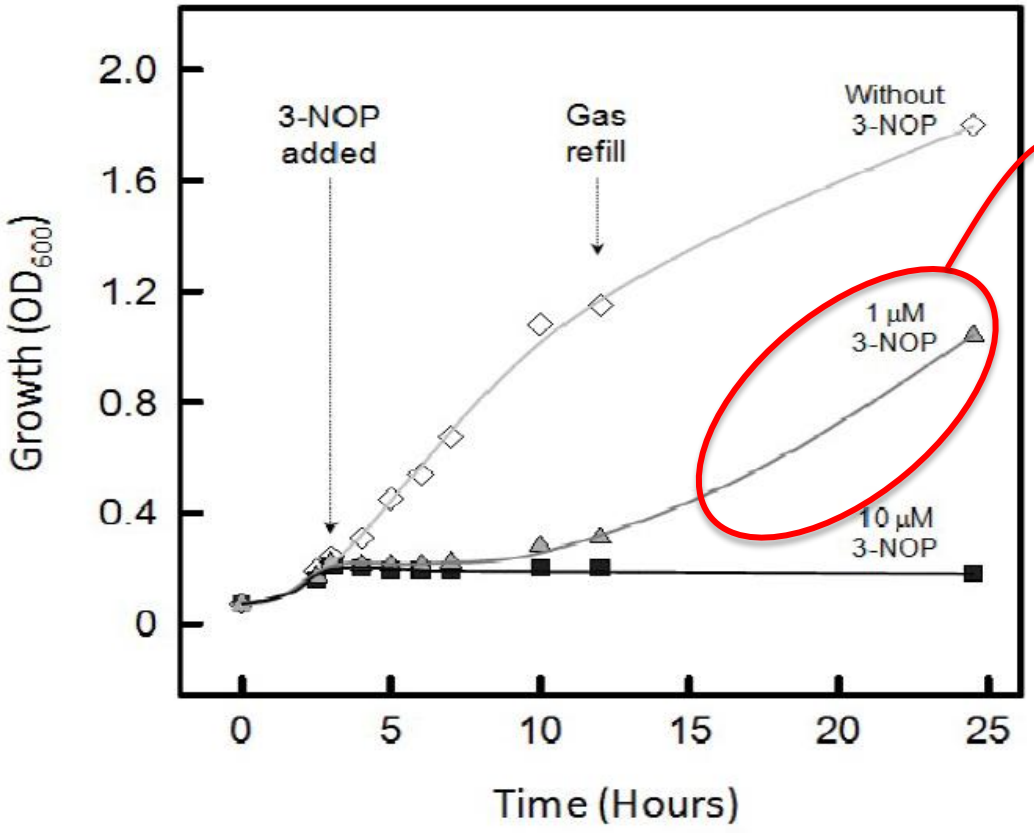
Active site of MCR crystallized before (A) and after (B) inactivation by 3-NOP



Entrapment of reduction products of 3-NOP in the active site

Results: *In vivo I*

Growth of *Methanothermobacter marburgensis* was inihibited by 3-NOP

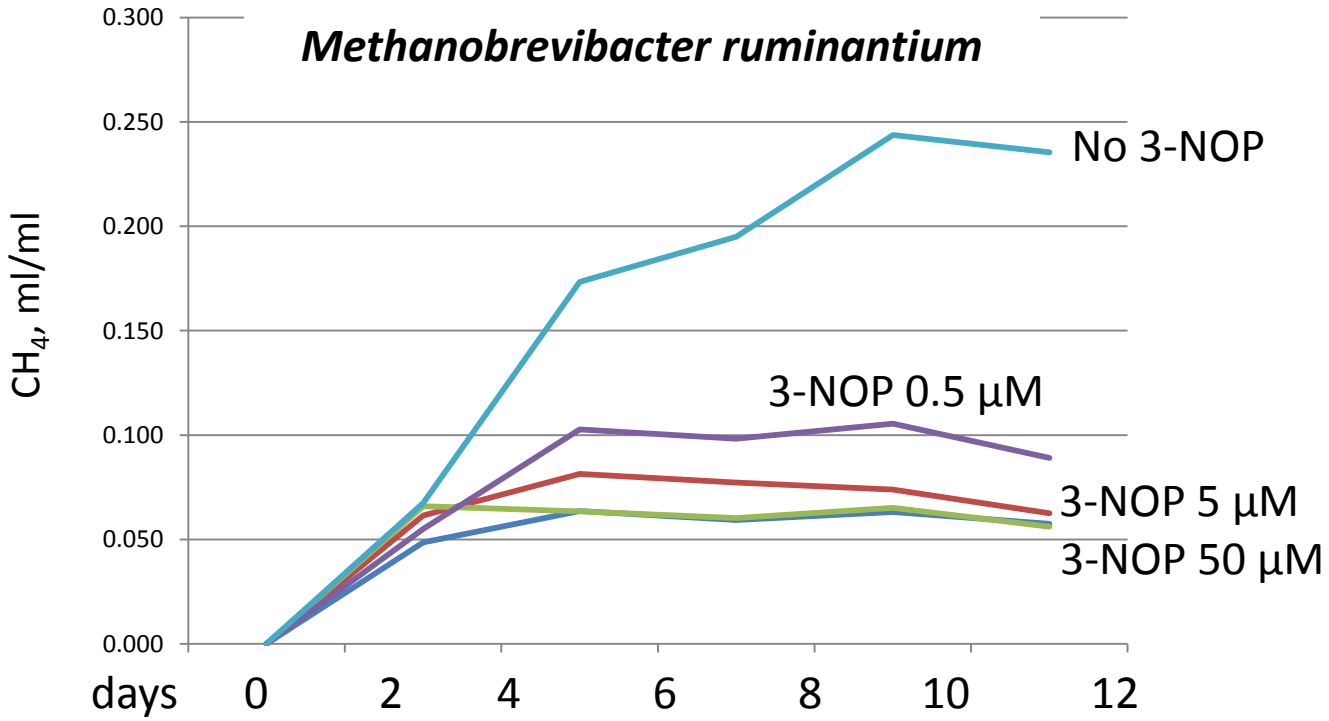


- Growth and CH_4 production recovered
- Methanogens contain a repair system, inhibition is reversible

Results: *In vivo II*

- Pure culture test on 7 rumen methanogenic **archaea** species

		3-NOP inhibition
1.	*Methanobrevibacter ruminantium*	< 1 µM
2.	*Methanobrevibacter smithii*	1 µM
3.	*Methanobrevibacter millerae*	1 µM
4.	*Methanosphaera stadtmanae*	5 µM
5.	*Methanobacterium bryantii*	1 µM
6.	*Methanomicrobium mobile*	5 µM
7.	*Methanosarcina barkeri*	250 µM

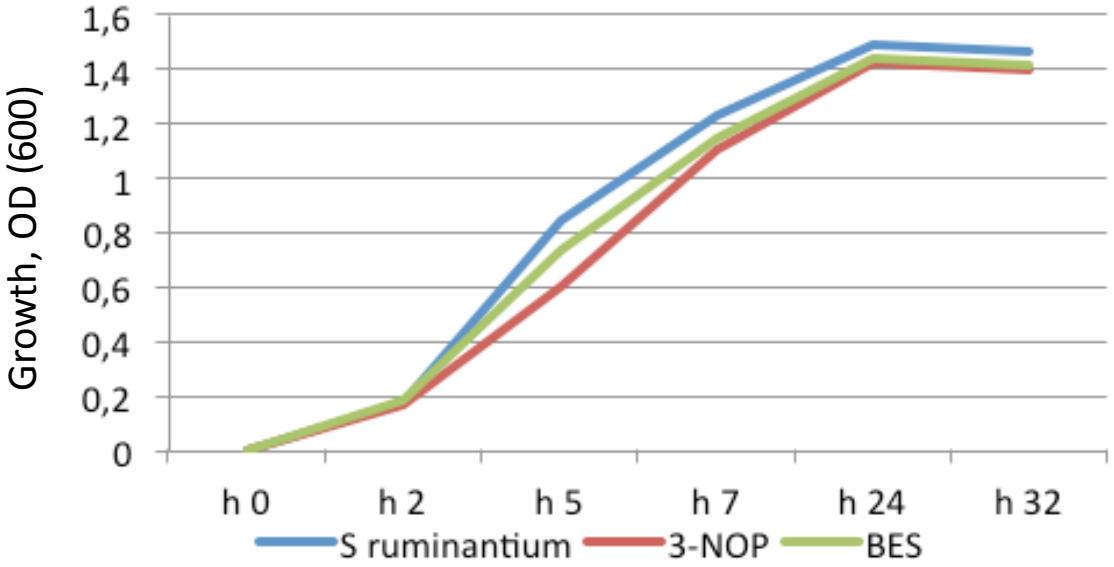


Results: *In vivo III*

- Pure culture test on 10 rumen **bacteria** species

1. *Fibrobacter succinogenes*
2. *Ruminococcus albus*
3. *Ruminococcus flavefaciens*
4. *Butyrivibrio fibrisolvens*
5. *Selenomonas ruminantium*
6. *Prevotella ruminicola*
7. *Prevotella bryantii*
8. *Streptococcus bovis*
9. *Megasphaera elsdenii*
10. *Anaerovibrio lipolytica*

NO 3-NOP Inhibition



100 µM

Conclusions

- 3-NOP inhibits enteric methane emissions from ruminants by specific inactivation of enzyme MCR
- Dual tandem-charge warhead inhibition mechanism
- Potential as feed additive and research tool

Thank you

Work funded by DSM Nutritional Products