# Application of Molecular Docking and Virtual Screening in Developing Methane Inhibitors

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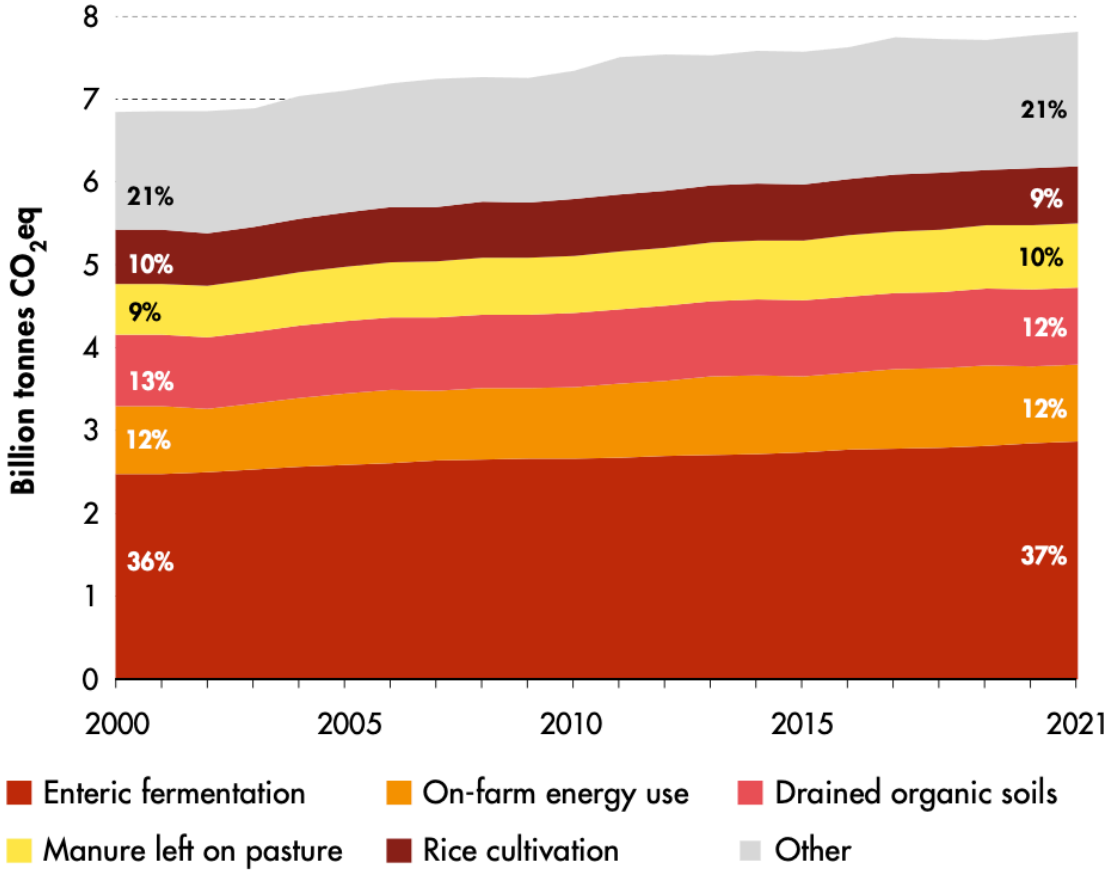
**Sep 4, 2024**

# Overview of world farm-gate GHG sources

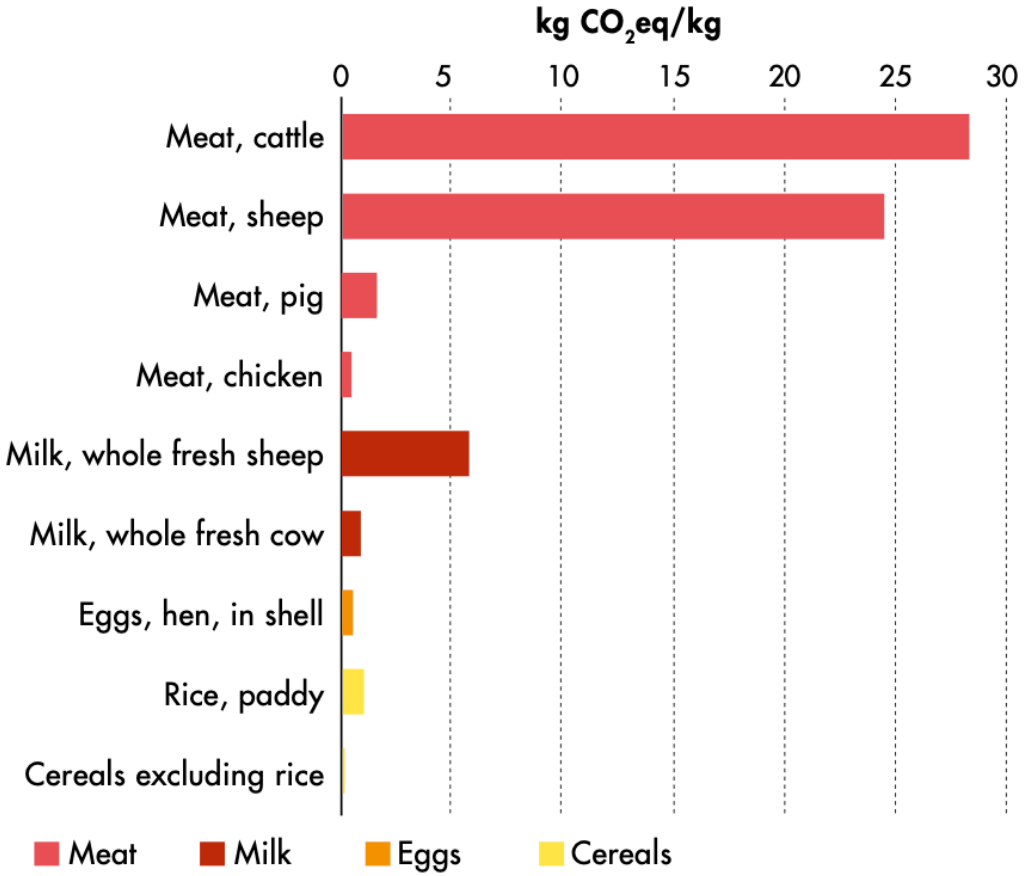
## WORLD FARM-GATE GREENHOUSE GAS EMISSIONS BY ACTIVITY




Note: Percentages on the figure indicate the shares in the total; they may not tally due to rounding.

Source: FAO. 2023. Emissions totals. In: *FAOSTAT*. Rome. [Cited October 2023].
https://www.fao.org/faostat/en/#data/GT
Download: https://doi.org/10.4060/cc8166en-fig67

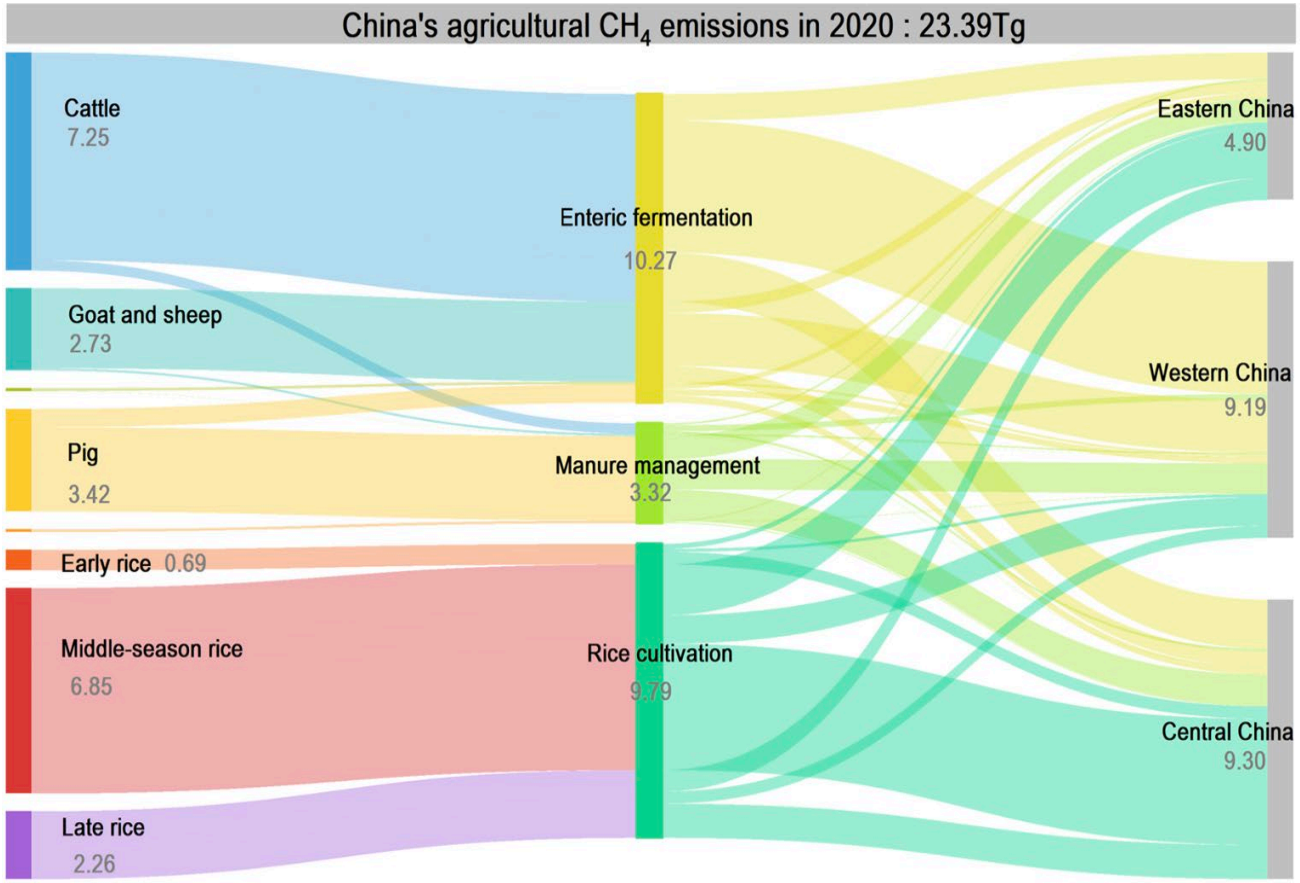
## WORLD EMISSIONS INTENSITY OF AGRICULTURAL COMMODITIES (2021)



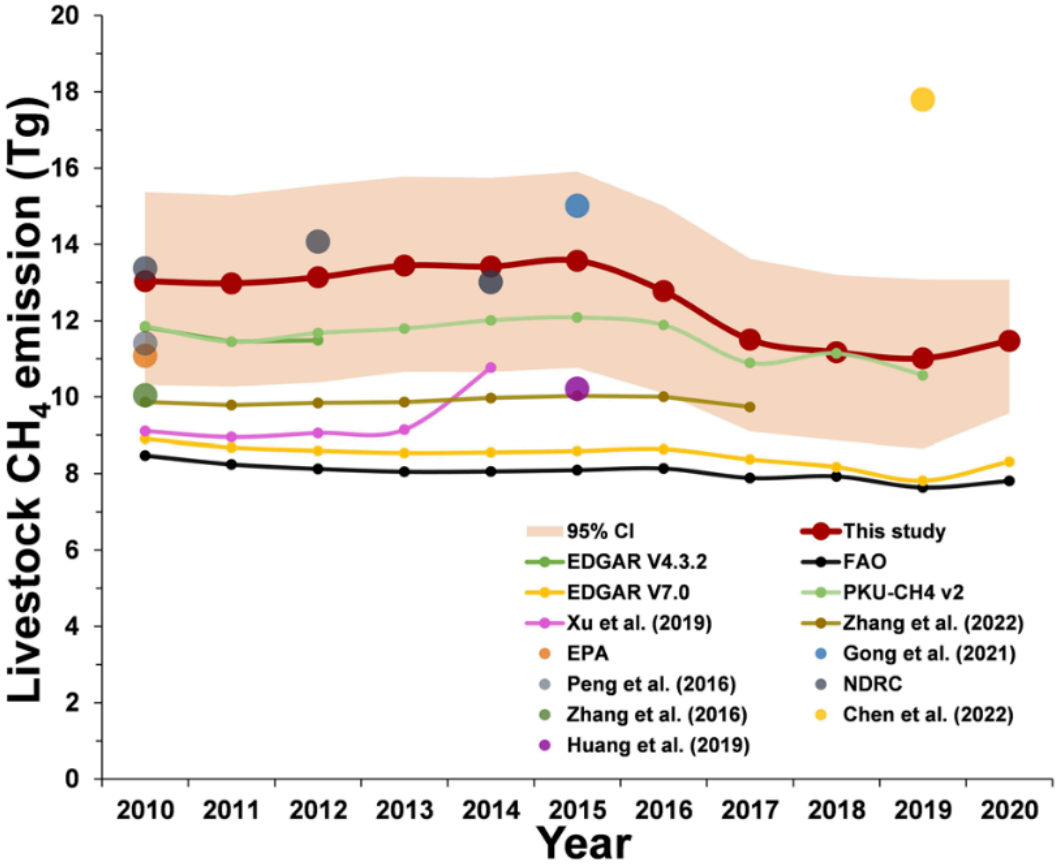

Source: FAO. 2023. Emissions intensities. In: *FAOSTAT*. Rome. [Cited October 2023].
https://www.fao.org/faostat/en/#data/EI
Download: https://doi.org/10.4060/cc8166en-fig68

# Livestock $CH_4$ emissions in China

Enteric fermentation contributes to 10.27 Tg of the total 23.39Tg $CH_4$ emission



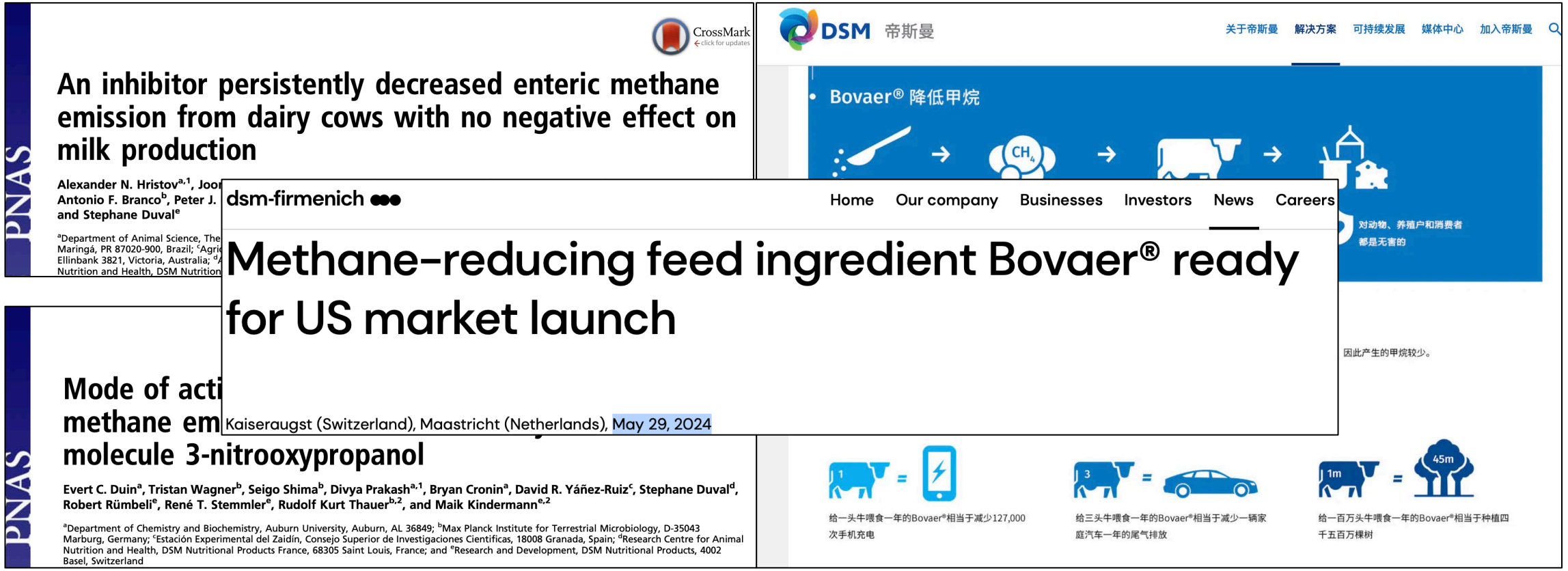

Duan et al., 2023




Du et al., 2024

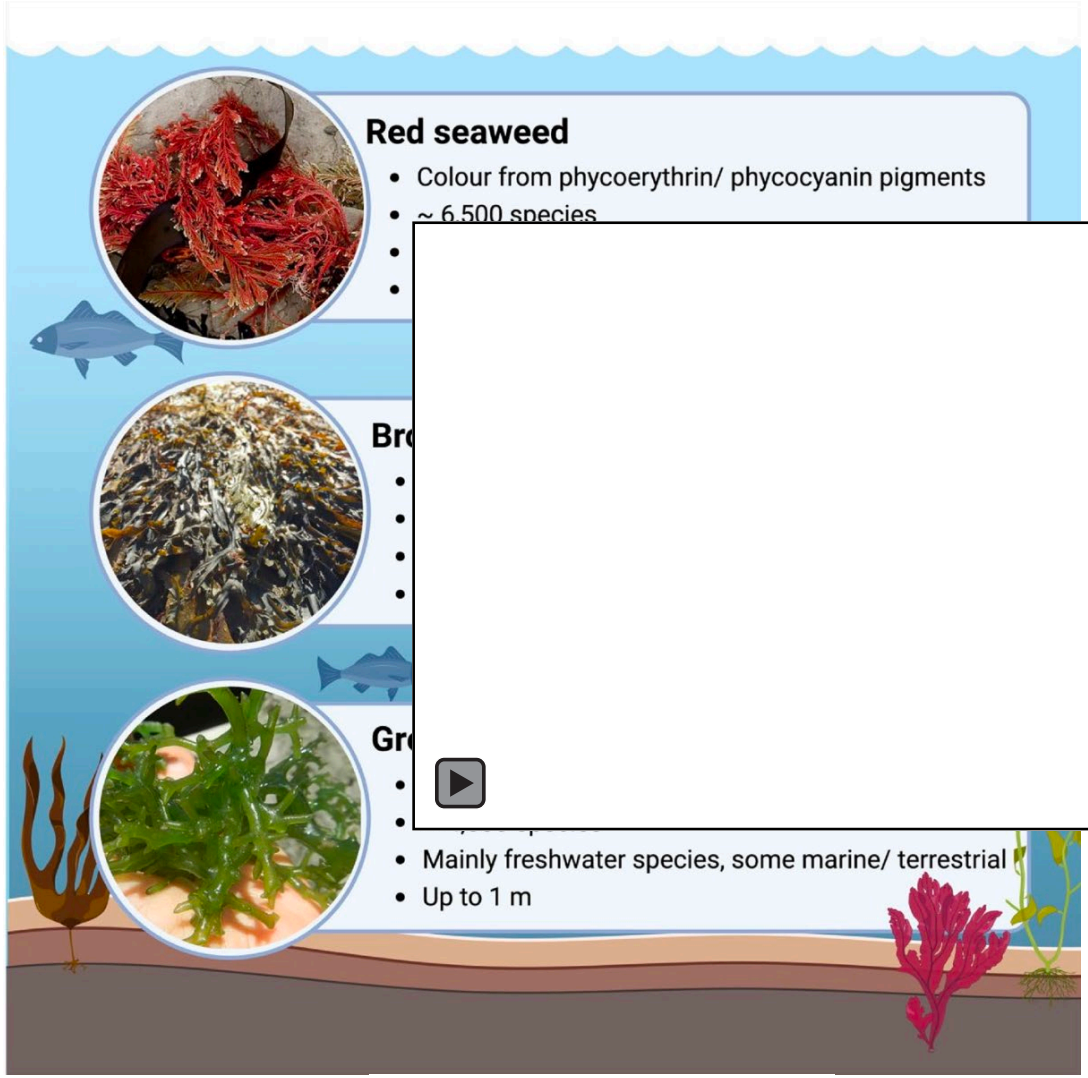
# Advances in developing methane inhibitors

Bovaer, a 3-NOP-based product, has been approved by EU, South America, Canada, and recently by US market.



# Advances in developing methane inhibitors

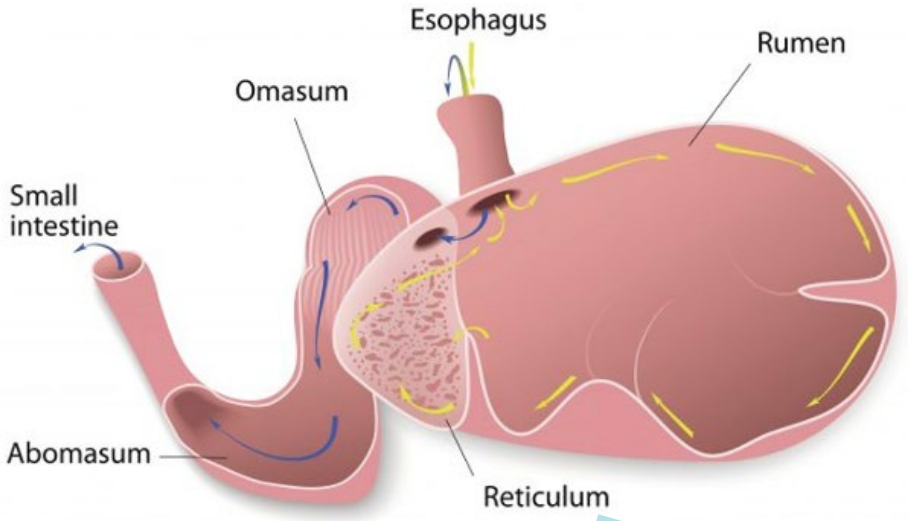
## Seaweed-based methane inhibitor products



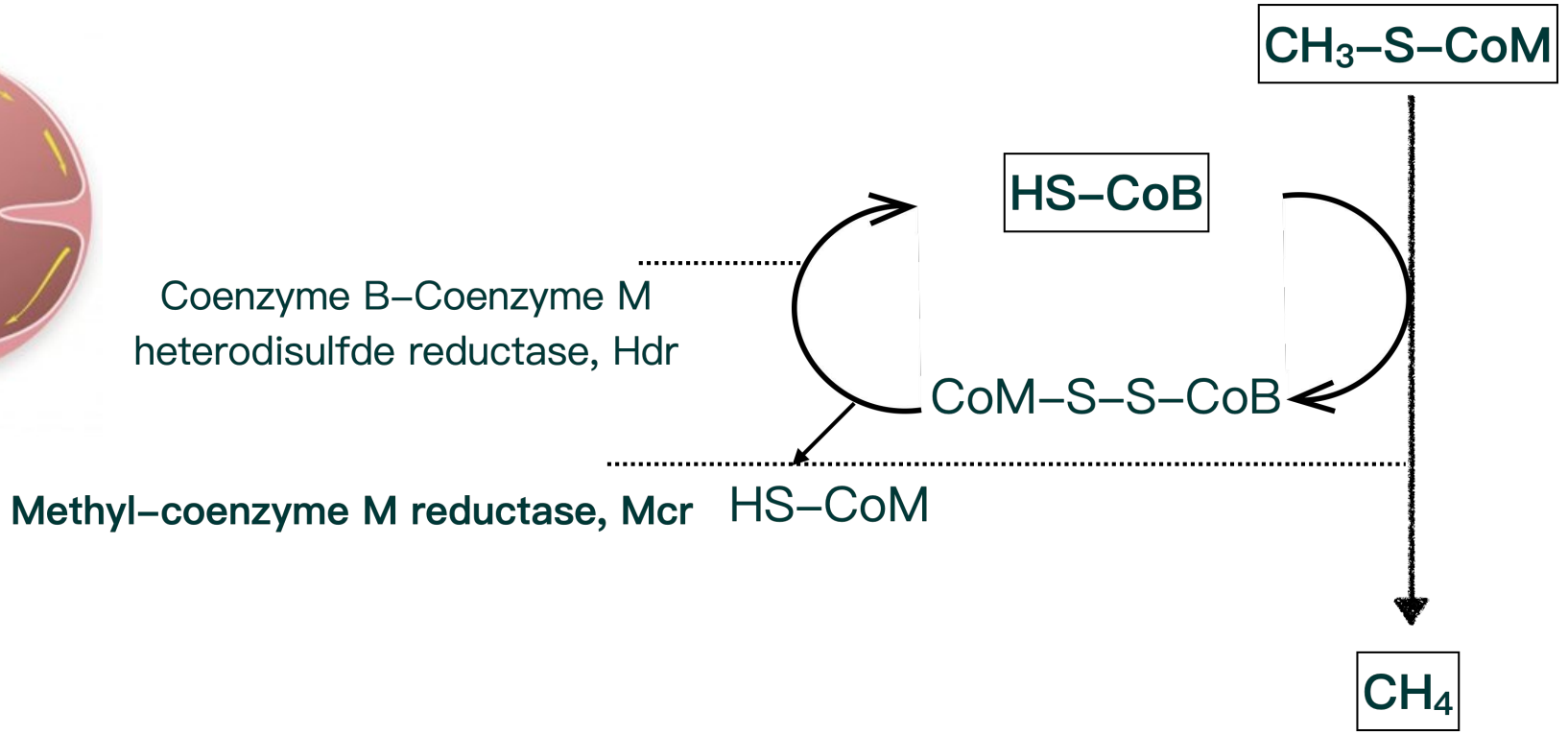


McGurrin et al., 2023

# Principles of methanogenesis in rumen



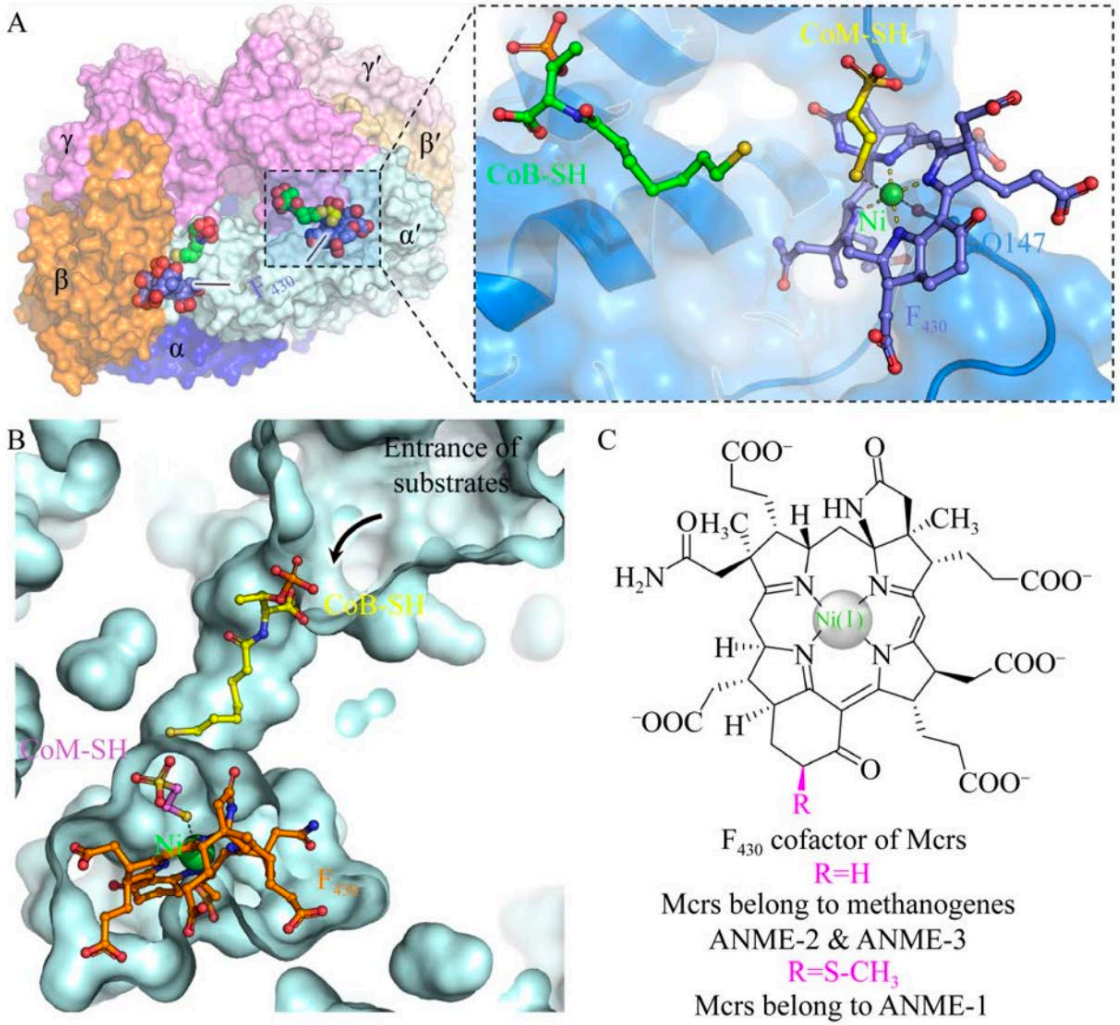

Substrates： $H_2$, $CH_3$-, acetate



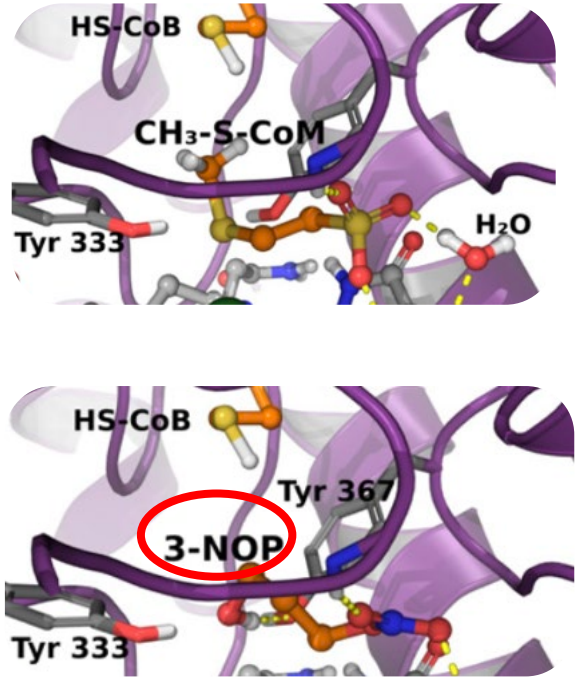

EC: 1.5.98.2

# Structure of MCR

- The MCR contains (αβγ)2 complex, hydrophobic channel, $F_{430}$



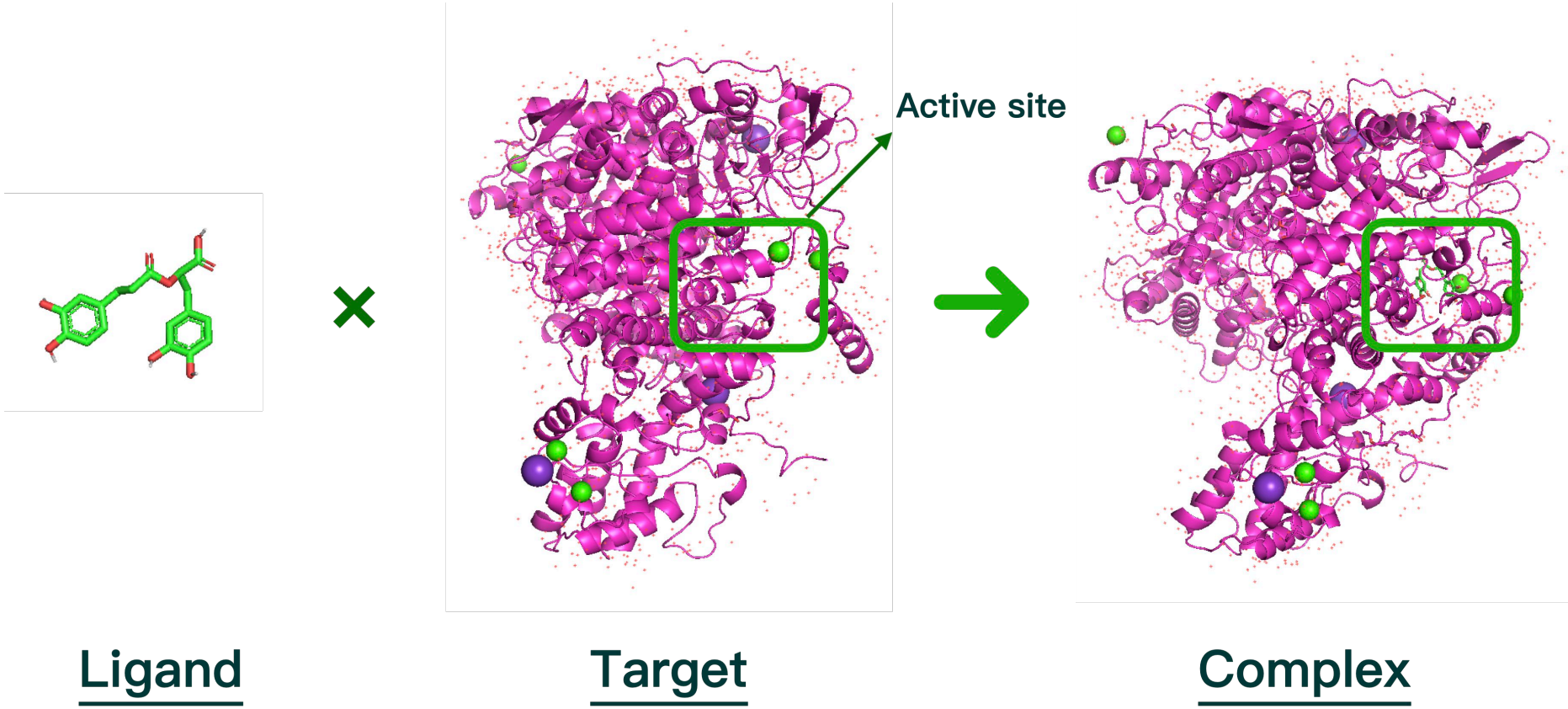

Lai et al，2021




3-NOP prevents $CH_3$-S-CoM from binding to MCR

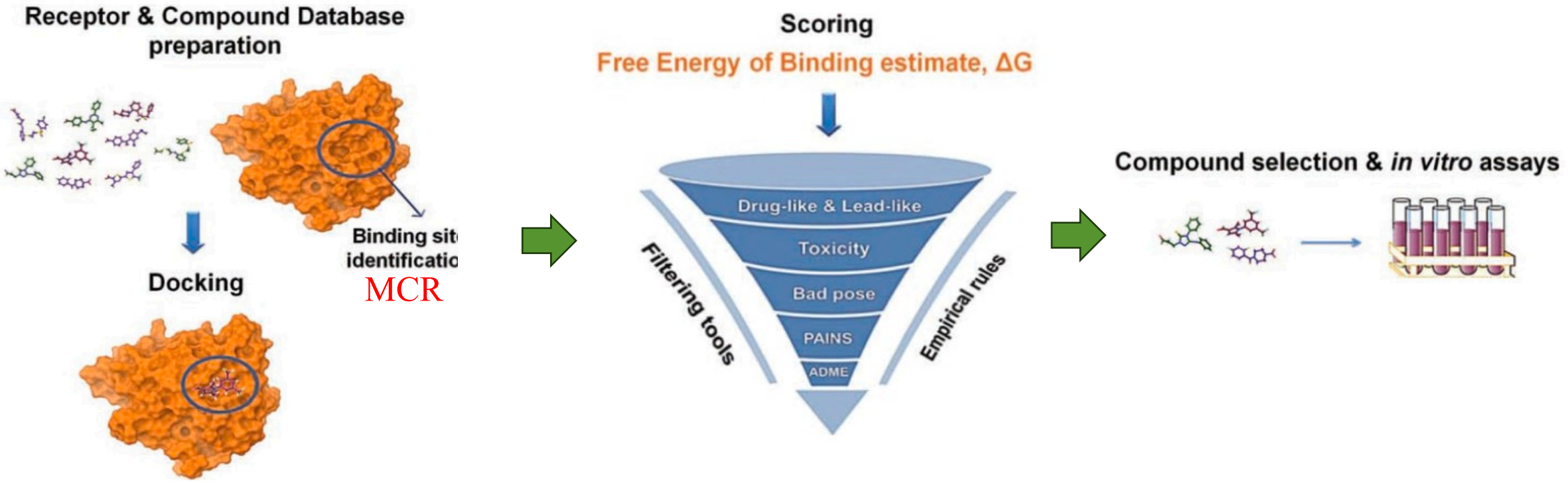
# Molecular docking

- Key-Lock theory-The protein can be thought of as the “lock” and the ligand can be thought of as a “key”



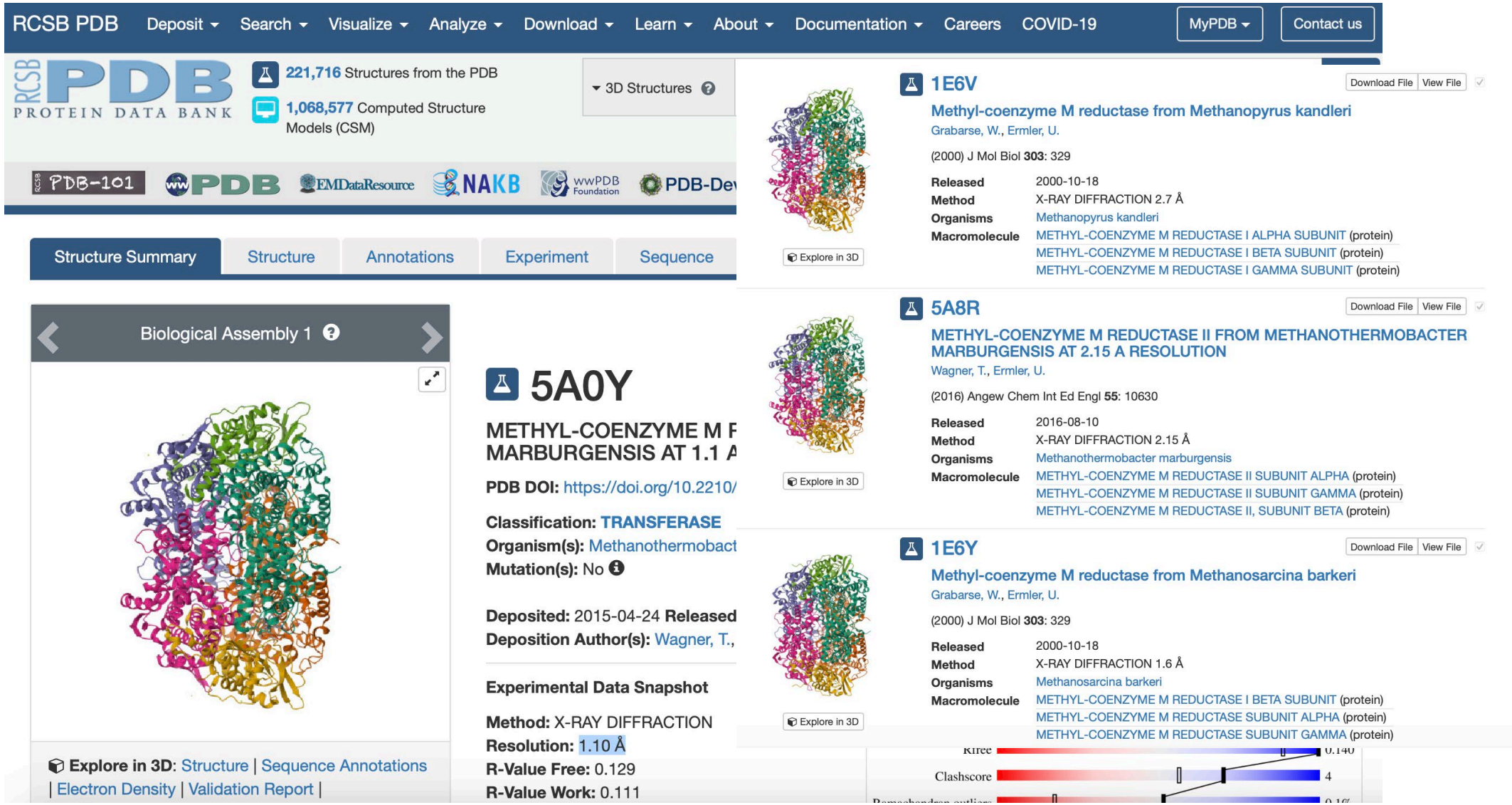
# Virtual screening

- A computational technique used to search libraries of small molecules in order to identify those structures which are most likely to bind to a drug target



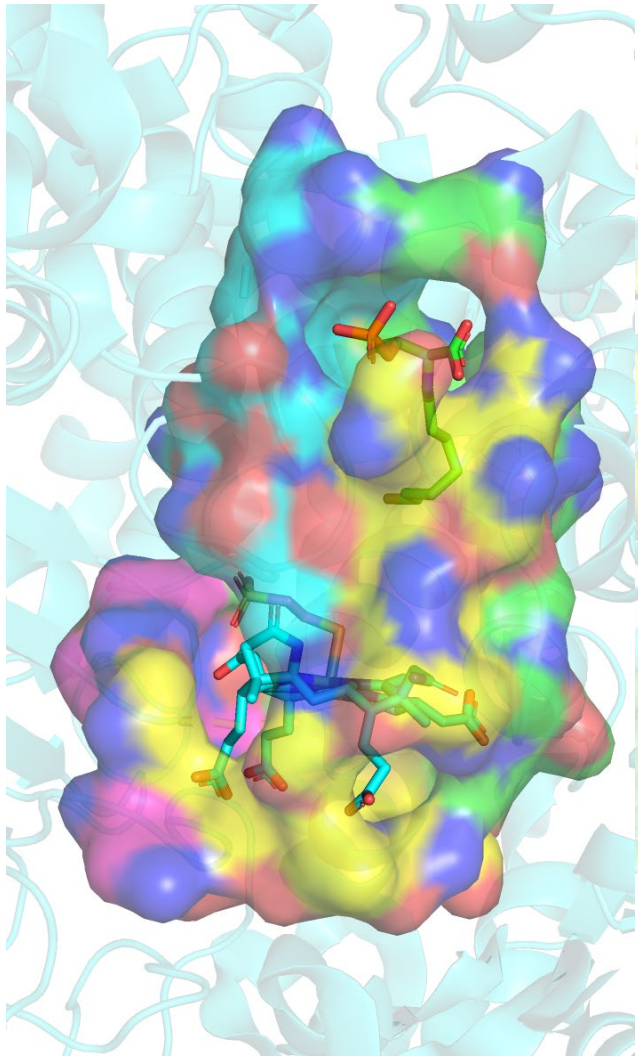
# Application of molecular docking and virtual screening

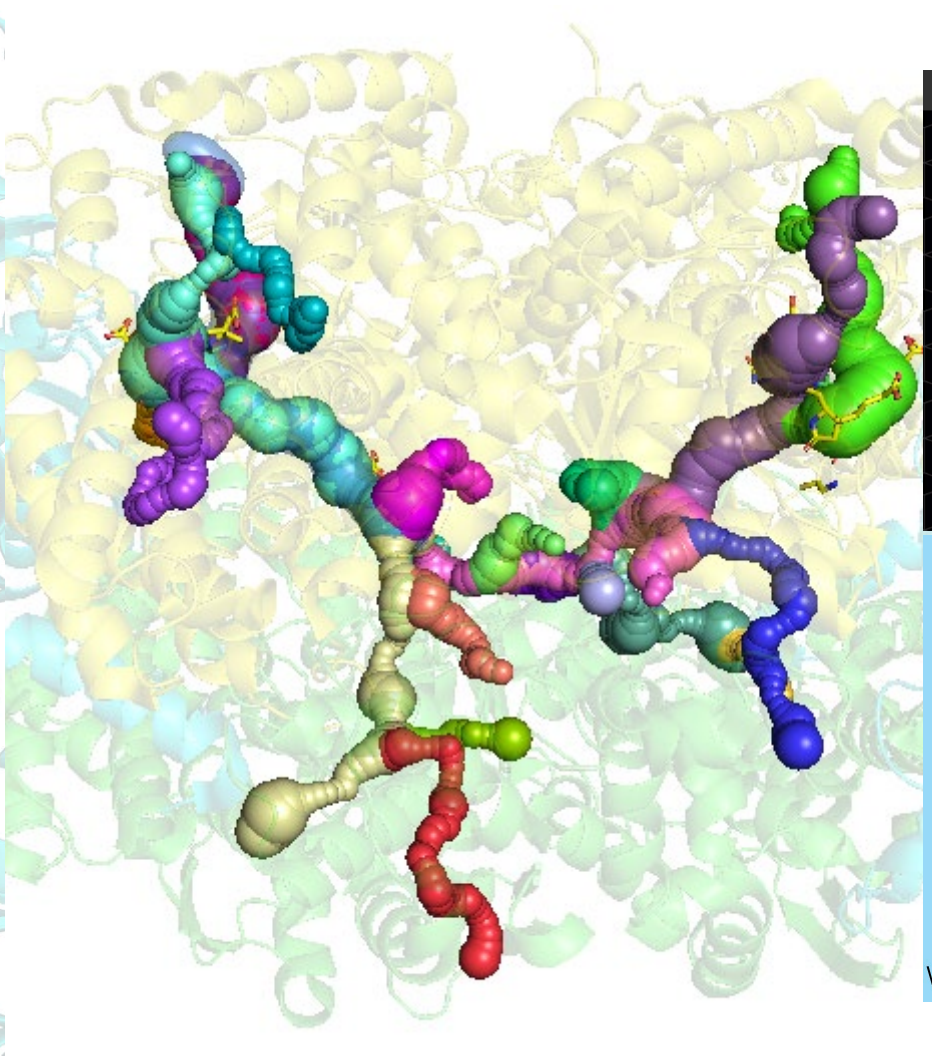
**Step 1**: Searching 'Protein Data Bank' for MCR with the lowest resolution

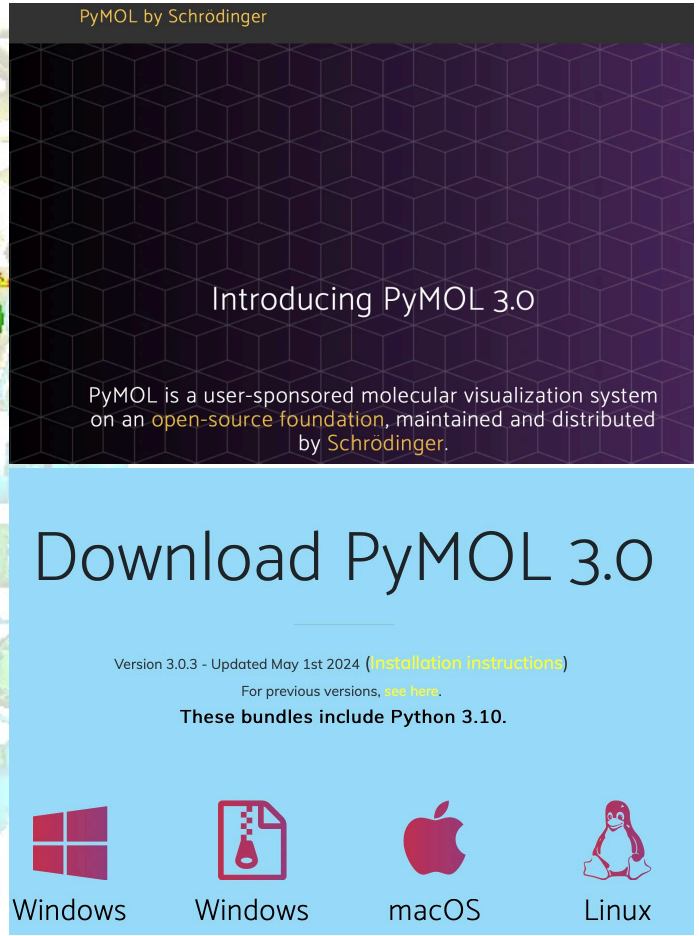


# Application of molecular docking and virtual screening

**Step 2**: Determining the hydrophobic channel in MCR

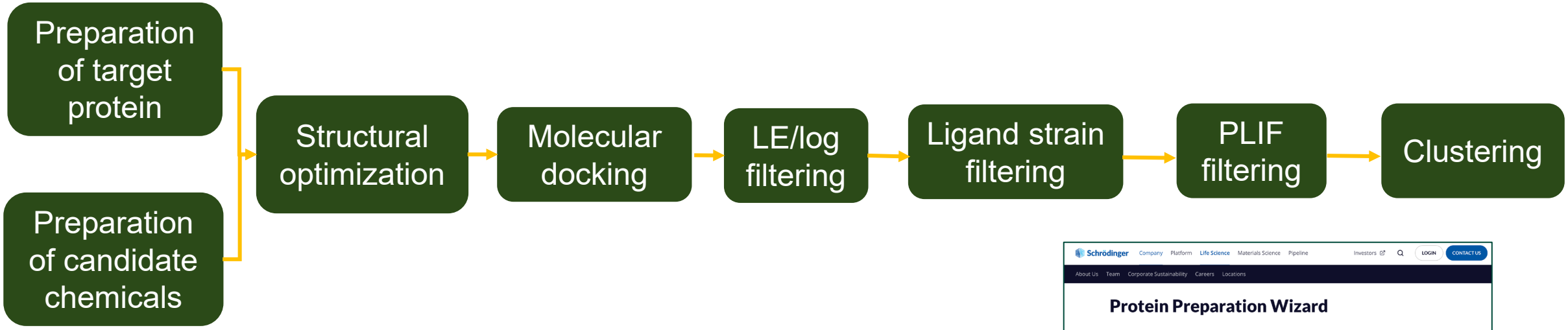






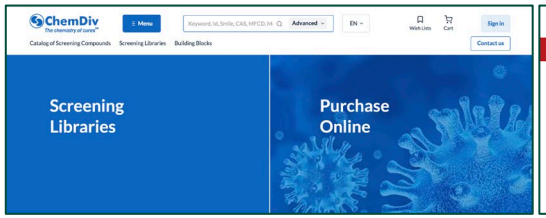
# Application of molecular docking and virtual screening

**Step 3**: Virtual screening

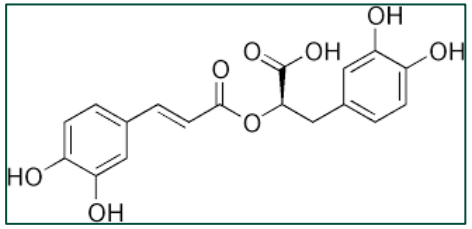



- Preparation of target protein: Schrödinger
- Preparation of chemicals： ChemDiv + L4000
- **52 molecules to be validated**
- Rosmarinic acid









Immune function, thyroid, heart health

# Application of molecular docking and virtual screening

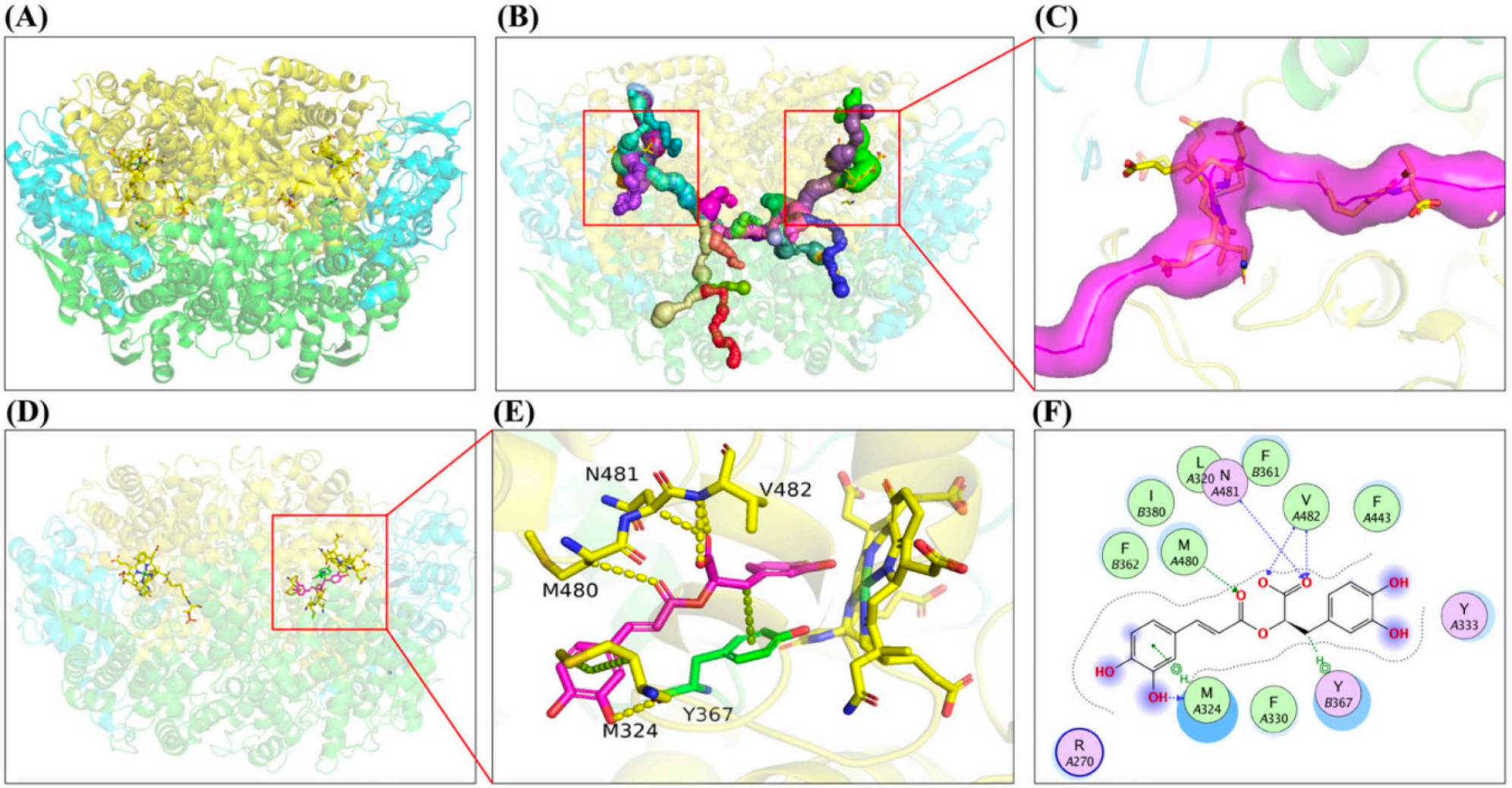
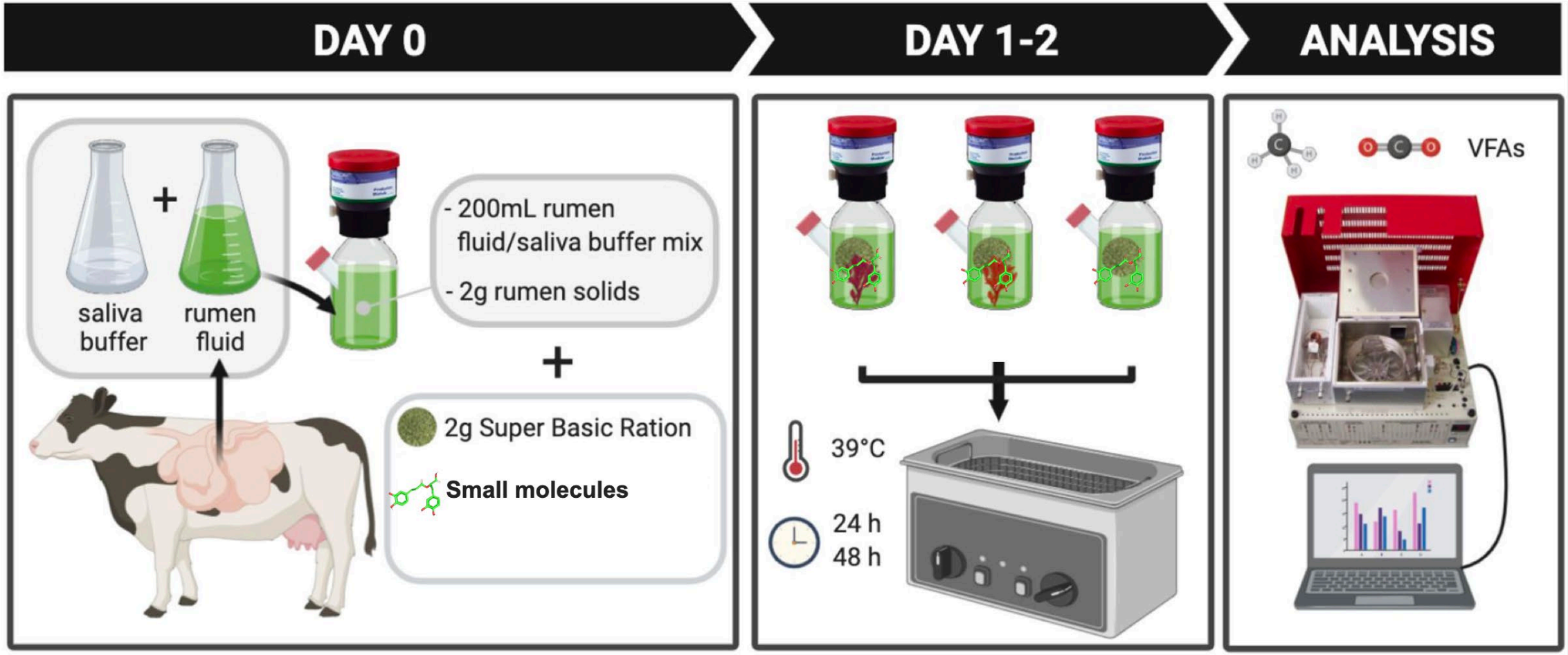



**Figure 1.** **(A)** shows the crystal structure of MCR, which consists of 3 subunits: α (McrA, Yellow), β (McrB, Green), and gamma (McrG, Blue). **(B)** and **(C)** shows the MCR's internal channel and activated sites. **(D)** exhibit the binding position of RA in the MCR. **(E)** and **(F)** shows the 3D and 2D binding patterns of the interaction between RA and MCR, respectively.

# Evaluating the methane inhibitory effect of candidates

*In vitro* gas production was assessed using an ANKOM RF Gas Production




Liu et al. 2024
*J Dairy Sci*

# Effect of RA levels on 24 h *in vitro* gas production (mg/mL OM)

- Total gas production was not affected by RA levels.
- Hydrogen and methane production was decreased.
- Carbon dioxide production was increased.

| Item | Treatments | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | 1% RA | 2% RA | 3% RA | | C vs. T | L | Q |
| **Total gas production** | 170.6 | 170.9 | 172.1 | 173.1 | 2.40 | 0.723 | 0.276 | 0.837 |
| **Hydrogen** | $0.06^{a}$ | $0.05^{ab}$ | $0.05^{b}$ | $0.04^{b}$ | 0.007 | 0.049 | 0.017 | 0.126 |
| **Methane** | $26.6^{a}$ | $22.9^{b}$ | $23.1^{b}$ | $22.5^{b}$ | 0.37 | <0.001 | <0.001 | <0.001 |
| **Carbon dioxide** | $100.5^{b}$ | $103.0^{ab}$ | $103.1^{ab}$ | $104.7^{a}$ | 1.30 | 0.034 | 0.006 | 0.621 |

# Effect of RA levels on rumen fermentation parameters

- Ammonia was decreased while MCP was not affected.
- TVFA was higher at 1%RA.
- Molar proportion of acetate and propionate was increased, butyrate was decreased.

| Item | Treatments | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | 1% RA | 2% RA | 3% RA | | C vs. T | L | Q |
| pH | 6.16$^{a}$ | 6.12$^{b}$ | 6.12$^{b}$ | 6.11$^{b}$ | 0.005 | <0.001 | <0.001 | 0.003 |
| $NH_3$-N (mg/dL) | 27.3$^{a}$ | 3.93$^{b}$ | 2.00$^{c}$ | 1.96$^{c}$ | 0.53 | <0.001 | <0.001 | <0.001 |
| MCP (mg/dL) | 32.5 | 31.4 | 30.5 | 32.2 | 1.55 | 0.589 | 0.714 | 0.222 |
| TVFA (mmol/L) | 131.9$^{ab}$ | 144.2$^{a}$ | 130.7$^{ab}$ | 127.8$^{b}$ | 5.63 | 0.042 | 0.160 | 0.071 |
| Molar proportion (mmol/100 mmol) | | | | | | | | |
| Acetate | 62.7$^{b}$ | 61.9$^{b}$ | 67.0$^{a}$ | 68.8$^{a}$ | 0.012 | <0.001 | 0.152 | 0.033 |
| Propionate | 19.8$^{c}$ | 19.4$^{bc}$ | 20.7$^{ab}$ | 21.1$^{a}$ | 0.004 | 0.004 | 0.001 | 0.211 |
| Isobutyrate | 0.46$^{c}$ | 0.09$^{b}$ | 1.48$^{a}$ | 1.48$^{a}$ | 0.001 | <0.001 | <0.001 | 0.028 |
| Butyrate | 14.3$^{a}$ | 14.4$^{a}$ | 7.48$^{b}$ | 3.92$^{b}$ | 0.015 | <0.001 | <0.001 | 0.100 |
| Isovalerate | 1.62$^{b}$ | 2.02$^{ab}$ | 2.43$^{a}$ | 2.06$^{ab}$ | 0.002 | 0.002 | 0.005 | 0.164 |
| Valerate | 1.10$^{b}$ | 1.29$^{a}$ | 1.38$^{a}$ | 1.41$^{a}$ | 0.001 | 0.005 | <0.001 | 0.200 |
| A / P | 3.16$^{b}$ | 3.19$^{ab}$ | 3.23$^{ab}$ | 3.26$^{a}$ | 0.031 | 0.028 | 0.003 | 0.962 |
| (A + B) / P | 3.88$^{a}$ | 3.93$^{a}$ | 3.60$^{b}$ | 3.44$^{b}$ | 0.094 | <0.001 | 0.142 | 0.088 |

# Effect of RA levels on the changes in prokaryotic communities

| Item | Treatments | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | CON | 1% RA | 2% RA | 3% RA | | |
| **Bacteria** | | | | | | |
| *Rikenellaceae* RC9 gut group | 19.65$^{a}$ | 12.17$^{b}$ | 13.11$^{b}$ | 16.19$^{ab}$ | 0.933 | 0.019 |
| *Christensenellaceae* R7 group | 8.09$^{a}$ | 6.43$^{ab}$ | 5.79$^{b}$ | 6.27$^{b}$ | 0.296 | 0.026 |
| *Ruminococcus* | 5.52$^{a}$ | 5.18$^{a}$ | 3.51$^{b}$ | 3.72$^{b}$ | 0.298 | 0.012 |
| *Eggerthellaceae* DNF00809 | 0.75$^{b}$ | 3.66$^{a}$ | 3.35$^{a}$ | 3.69$^{a}$ | 0.384 | 0.007 |
| *Candidatus Saccharimonas* | 1.50$^{a}$ | 1.38$^{a}$ | 0.99$^{b}$ | 1.02$^{b}$ | 0.077 | 0.038 |
| *Denitrobacterium* | 0.02$^{b}$ | 1.68$^{a}$ | 1.00$^{a}$ | 1.24$^{a}$ | 0.181 | 0.003 |
| *Anaerovorax* | 0.60$^{a}$ | 0.36$^{b}$ | 0.48$^{ab}$ | 0.44$^{ab}$ | 0.030 | 0.039 |
| *Treponema* | 0.28$^{ab}$ | 0.56$^{a}$ | 0.59$^{a}$ | 0.19$^{b}$ | 0.060 | 0.036 |
| *Enterorhabdus* | 0.08$^{b}$ | 0.34$^{a}$ | 0.37$^{a}$ | 0.34$^{a}$ | 0.033 | 0.005 |
| *Desulfovibrio* | 0.50$^{a}$ | 0.26$^{ab}$ | 0.15$^{b}$ | 0.11$^{b}$ | 0.052 | 0.026 |
| *Lachnospiraceae* FE2018 group | 0.37$^{a}$ | 0.20$^{b}$ | 0.22$^{b}$ | 0.20$^{b}$ | 0.020 | 0.016 |
| unclassified *Atopobiaeae* genus | 0.02$^{b}$ | 0.30$^{a}$ | 0.31$^{a}$ | 0.31$^{a}$ | 0.039 | 0.004 |
| **Archaea** | | | | | | |
| *Methanobrevibacter* | 78.66$^{a}$ | 70.46$^{ab}$ | 67.44$^{b}$ | 63.27$^{b}$ | 0.016 | 0.009 |
| *Methanosphaera* | 21.09$^{b}$ | 29.33$^{ab}$ | 31.87$^{a}$ | 35.32$^{a}$ | 0.016 | 0.015 |

→ Lipid metabolism, hemicellulose

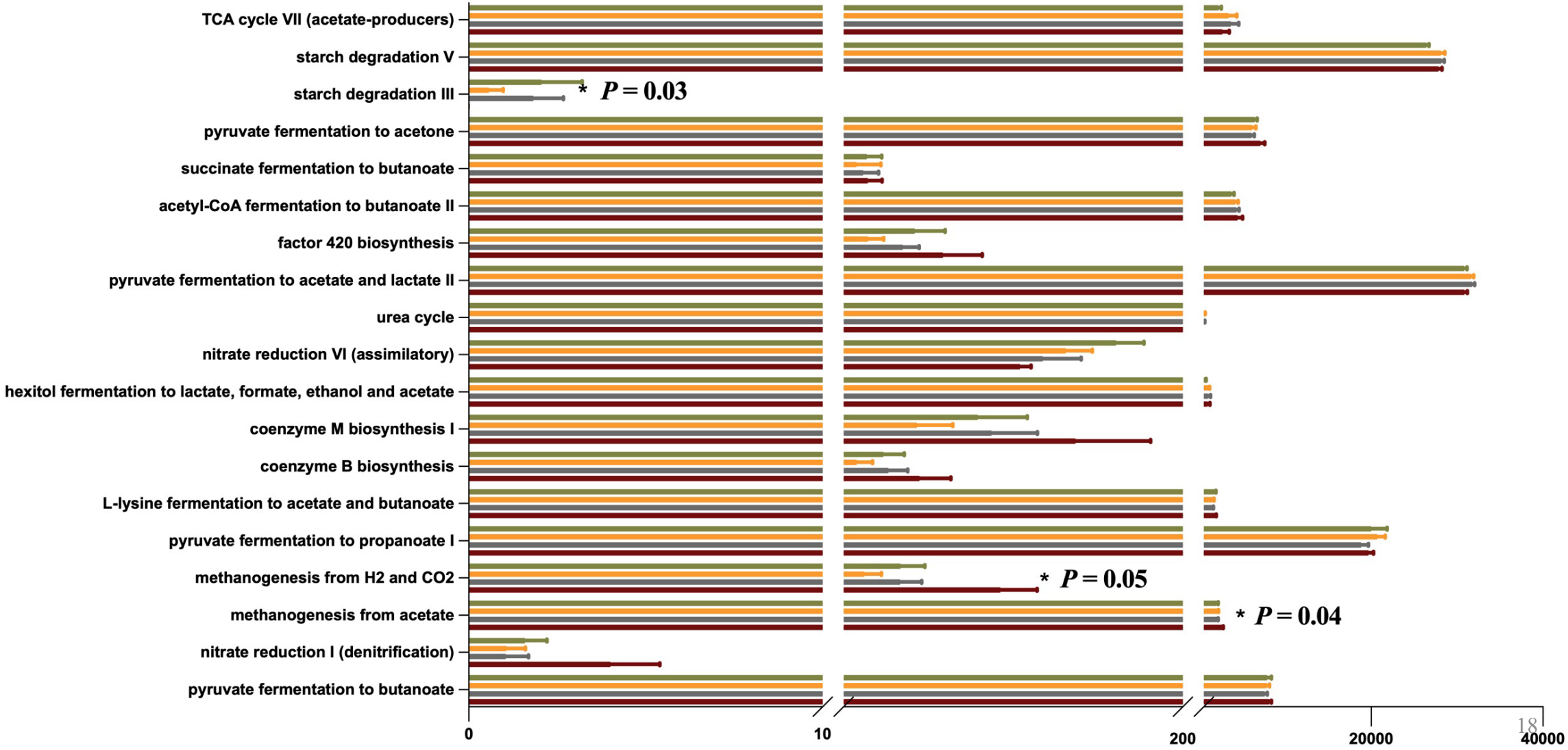
→ Negatively correlated with feed efficiency Hegarty et al., 2007

→ Degradation of polyphenols and maintaining homeostasis Rodríguez-Daza M-C et al., 2020

→ NeKittelmann et al., 2014

# Effect of different RA level on predicted functions

- Pathways associated with starch degradation was up-regulated, and that with methanogenesis was down-regulated by RA supplementation.



# Summary

- A total of 52 candidate compounds were obtained through molecular docking and virtual screening technique. Rosmarinic acid (RA) was one of the compounds that could traverse a narrow channel and bind to the active sites of methyl-coenzyme M reductase.

- Supplementation of RA at 1% of OM could decrease ruminal methane emission by about 15% without increasing hydrogen production. Its mode of action could be mainly by inhibiting the relative abundance of *Methanobrevibacter* and pathways associated with methanogenesis.

# THANK YOU FOR YOUR ATTENTION

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