



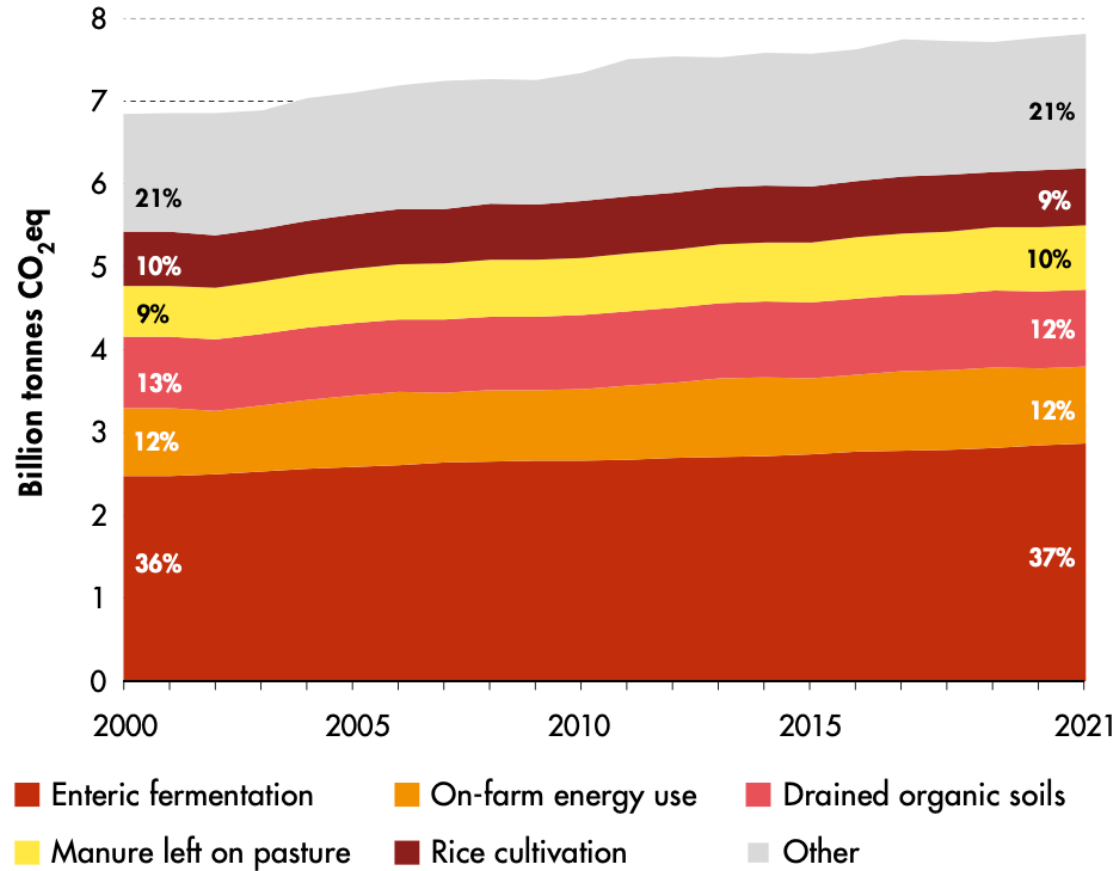
Application of Molecular Docking and Virtual Screening in Developing Methane Inhibitors

Yunlong Liu, Xiaopeng Li, Yan Tu, and **Tao Ma**
Sep 4, 2024

Overview of world farm-gate GHG sources

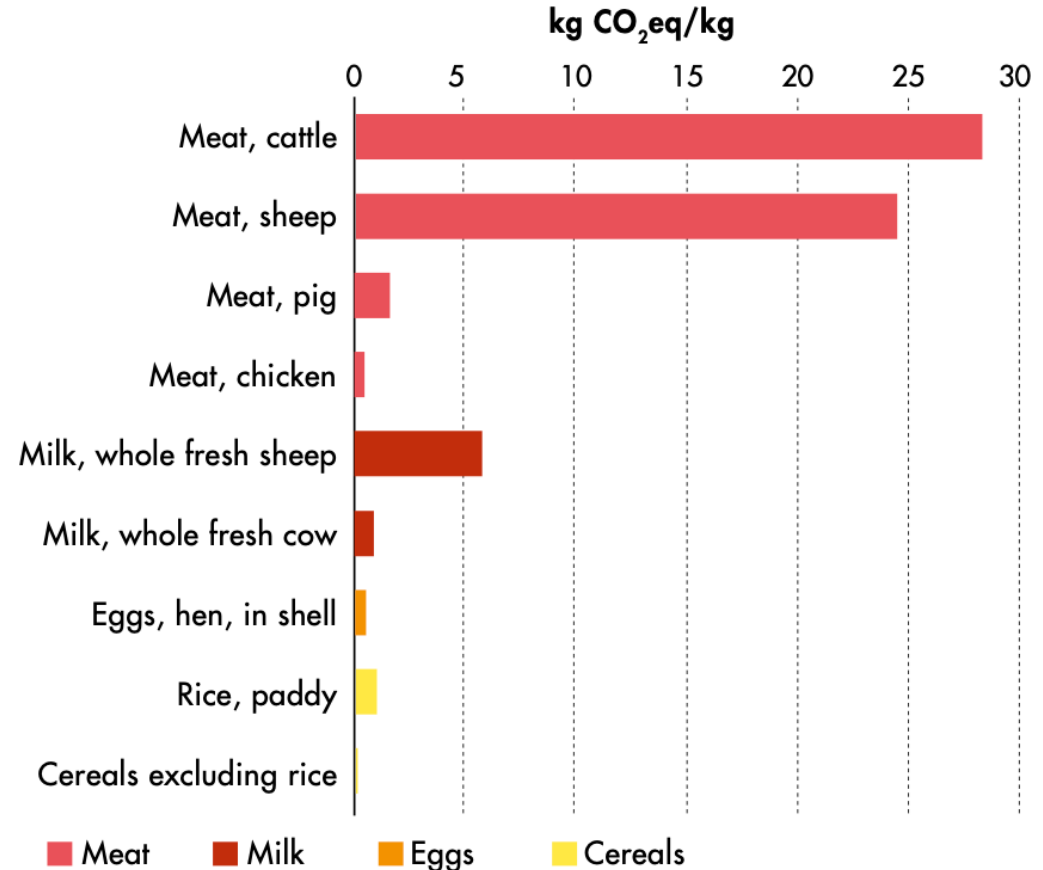


WORLD FARM-GATE GREENHOUSE GAS EMISSIONS BY ACTIVITY



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)

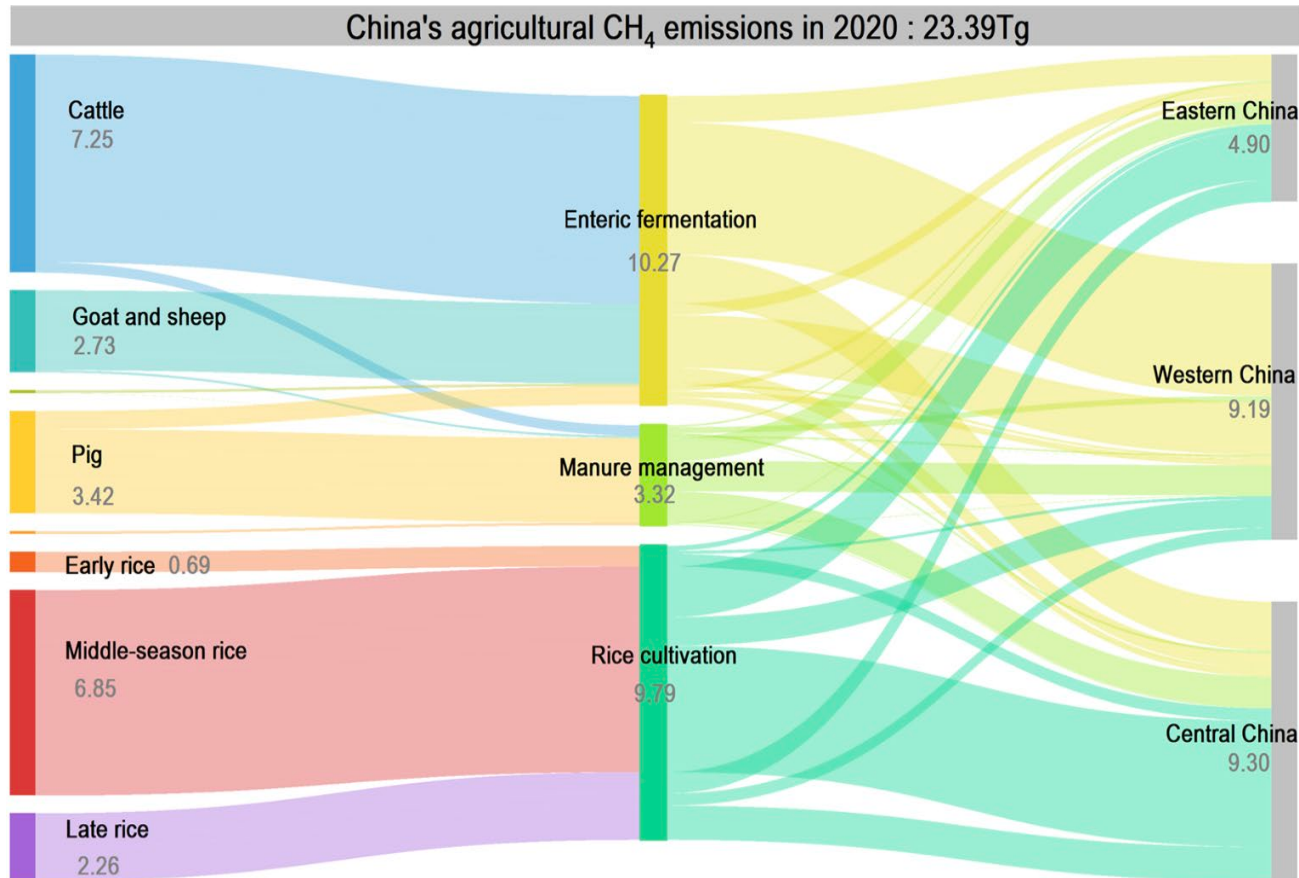


Source: FAO. 2023. Emissions intensities. In: FAOSTAT. Rome. [Cited October 2023].
<https://www.fao.org/faostat/en/#data/EI>
 Download: <https://doi.org/10.4060/cc8166en-fig68>

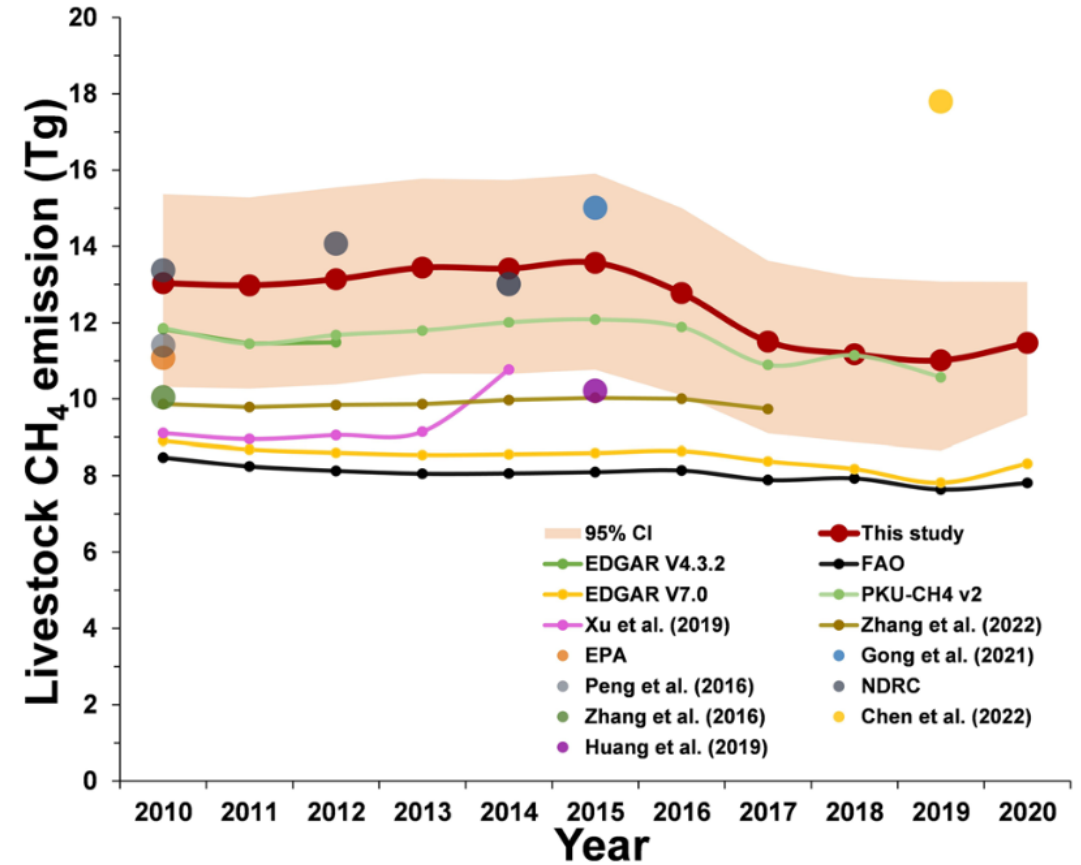
Livestock CH₄ emissions in China



Enteric fermentation contributes to 10.27 Tg of the total 23.39Tg CH₄ 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.

PNAS

An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production

Alexander N. Hristov^{a,1}, Joao Antonio F. Branco^b, Peter J. and Stephane Duval^c

^aDepartment of Animal Science, The Maringá, PR 87020-900, Brazil; ^bAgriculture and Food, University of Melbourne, Ellinbank 3821, Victoria, Australia; ^cDSM Nutrition and Health, DSM Nutritional Products, CH-4002 Basel, Switzerland

PNAS

Mode of action of the methane emission-reducing molecule 3-nitrooxypropanol

Evert C. Duin^a, Tristan Wagner^b, Seigo Shima^b, Divya Prakash^{a,1}, Bryan Cronin^a, David R. Yáñez-Ruiz^c, Stephane Duval^d, Robert Rumbeli^e, René T. Stemmler^e, Rudolf Kurt Thauer^{b,2}, and Maik Kindermann^{e,2}

^aDepartment of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849; ^bMax Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany; ^cEstación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, 18008 Granada, Spain; ^dResearch Centre for Animal Nutrition and Health, DSM Nutritional Products France, 68305 Saint Louis, France; and ^eResearch and Development, DSM Nutritional Products, 4002 Basel, Switzerland

CrossMark

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dsM-firmenich

Methane-reducing feed ingredient Bovaer[®] ready for US market launch

Kaiseraugst (Switzerland), Maastricht (Netherlands), May 29, 2024

DSM 帝斯曼

关于帝斯曼 解决方案 可持续发展 媒体中心 加入帝斯曼

Bovaer[®] 降低甲烷

Home Our company Businesses Investors News Careers

对动物、养殖户和消费者都是无害的

因此产生的甲烷较少。

给一头牛喂食一年的Bovaer[®]相当于减少127,000次手机充电

给三头牛喂食一年的Bovaer[®]相当于减少一辆家庭汽车一年的尾气排放

给一百万头牛喂食一年的Bovaer[®]相当于种植四千五百万棵树



Seaweed-based methane inhibitor products

Red seaweed

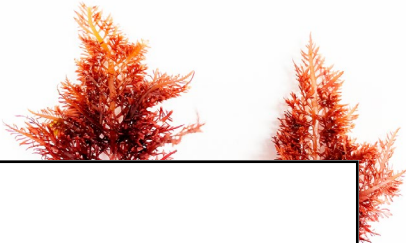
- Colour from phycoerythrin/ phycocyanin pigments
- ~ 6,500 species

Brown seaweed

- Mainly freshwater species, some marine/ terrestrial
- Up to 1 m

Green seaweed

- Mainly freshwater species, some marine/ terrestrial
- Up to 1 m



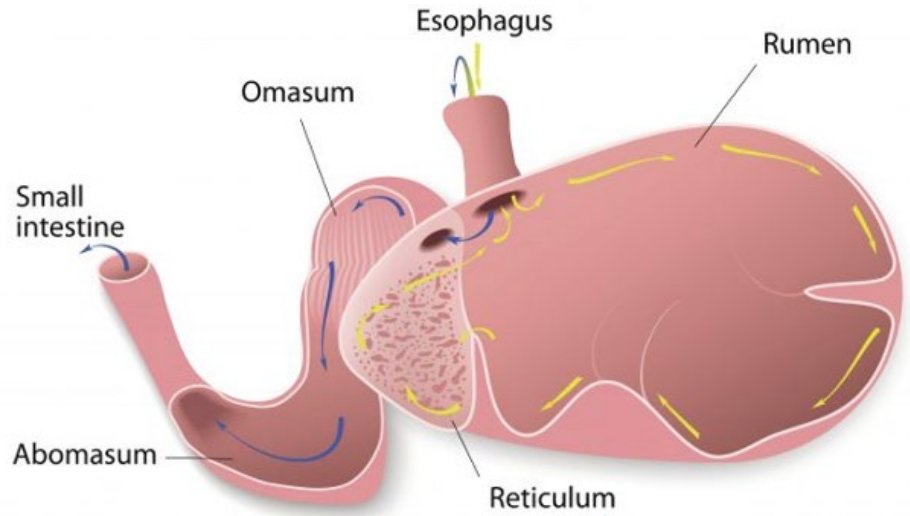
马尾藻科 叶囊藻属
Hormophysa



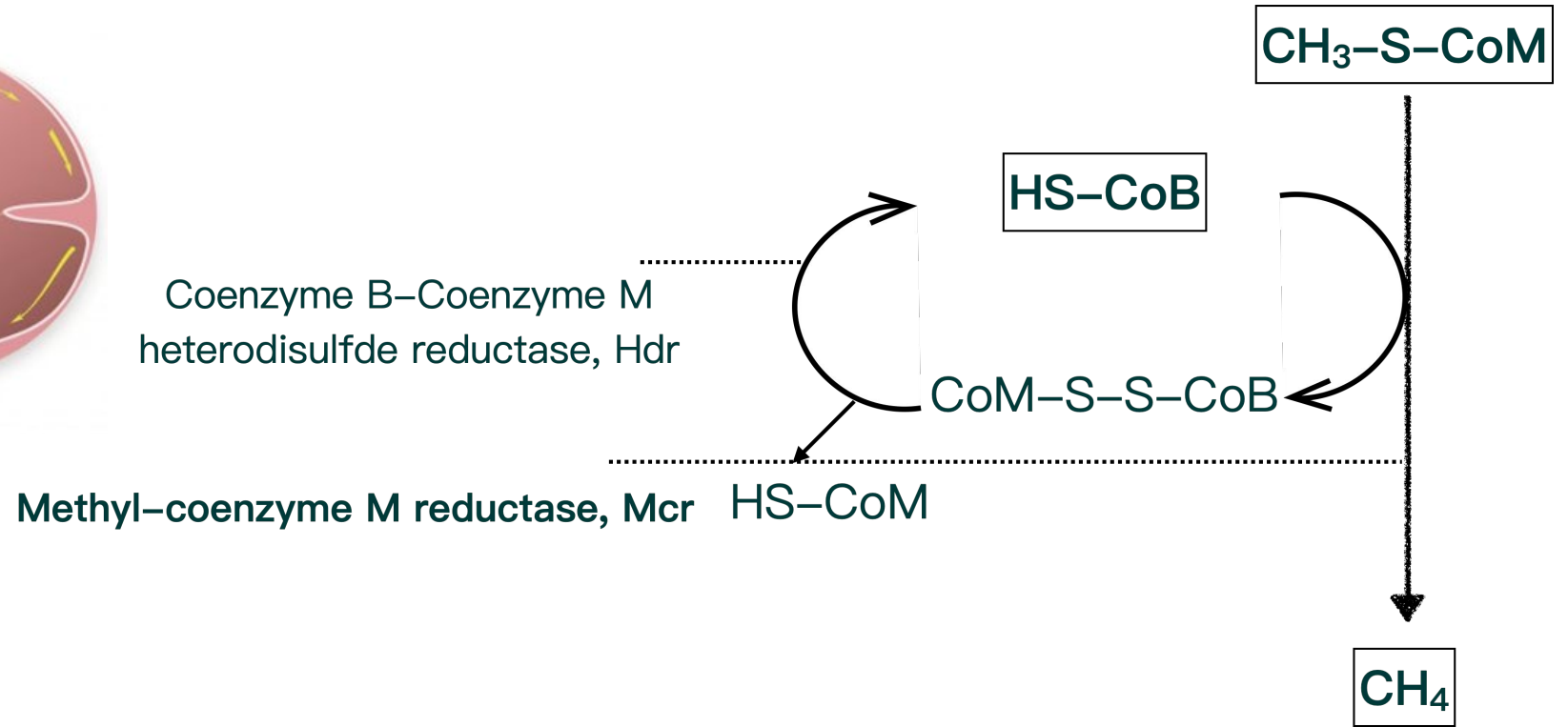
网地藻科 团扇藻属
Padina

水云目萱藻科 囊藻属
Colpomenia

Principles of methanogenesis in rumen



Substrates: H_2 , CH_3 -, acetate

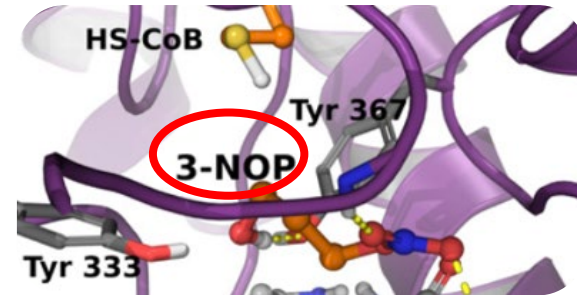
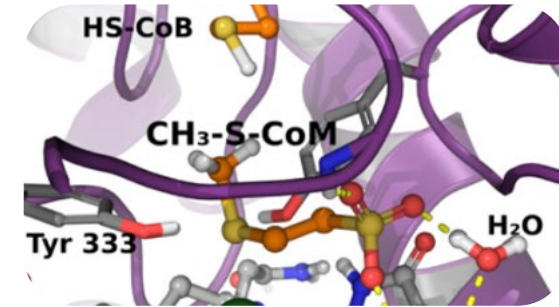
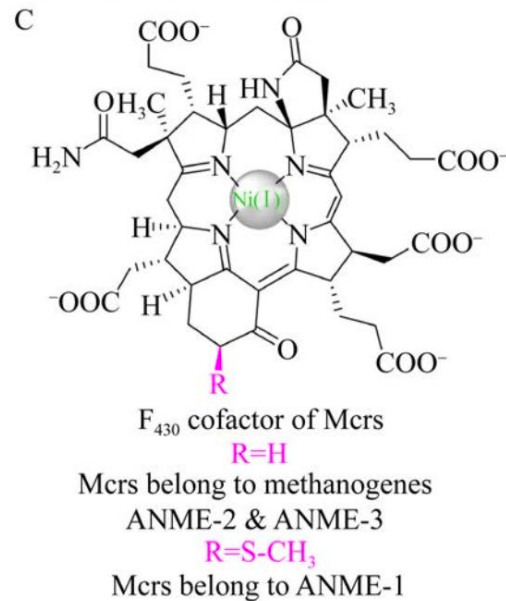
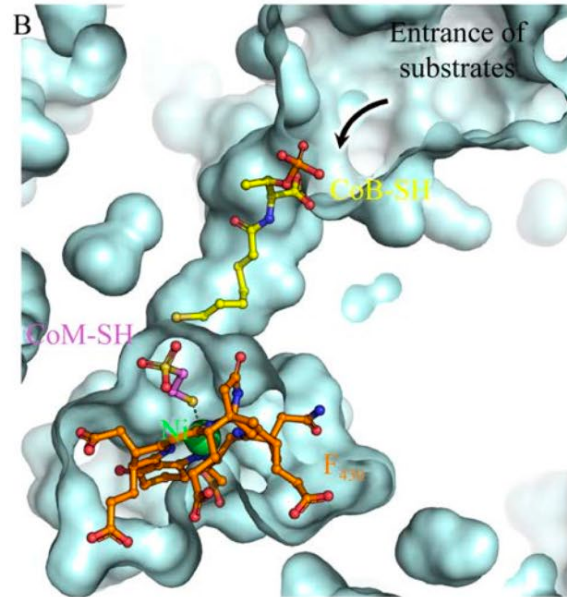
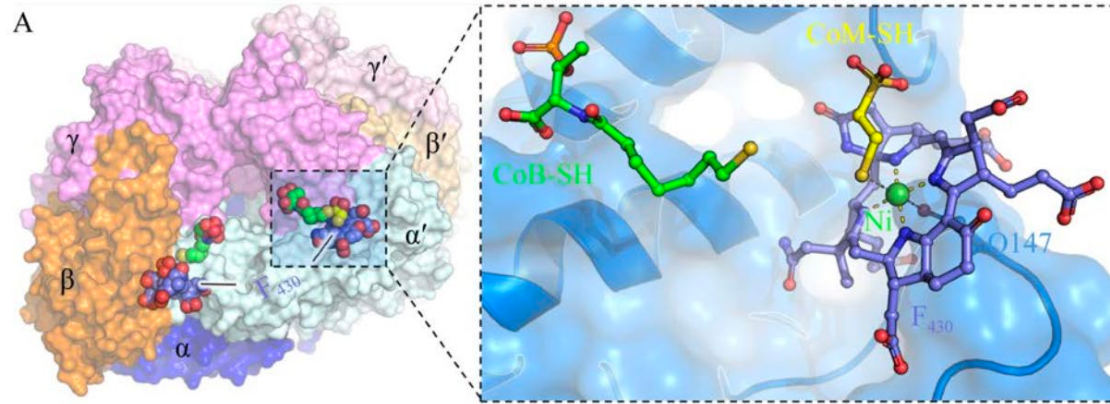


EC: 1.5.98.2

Structure of MCR

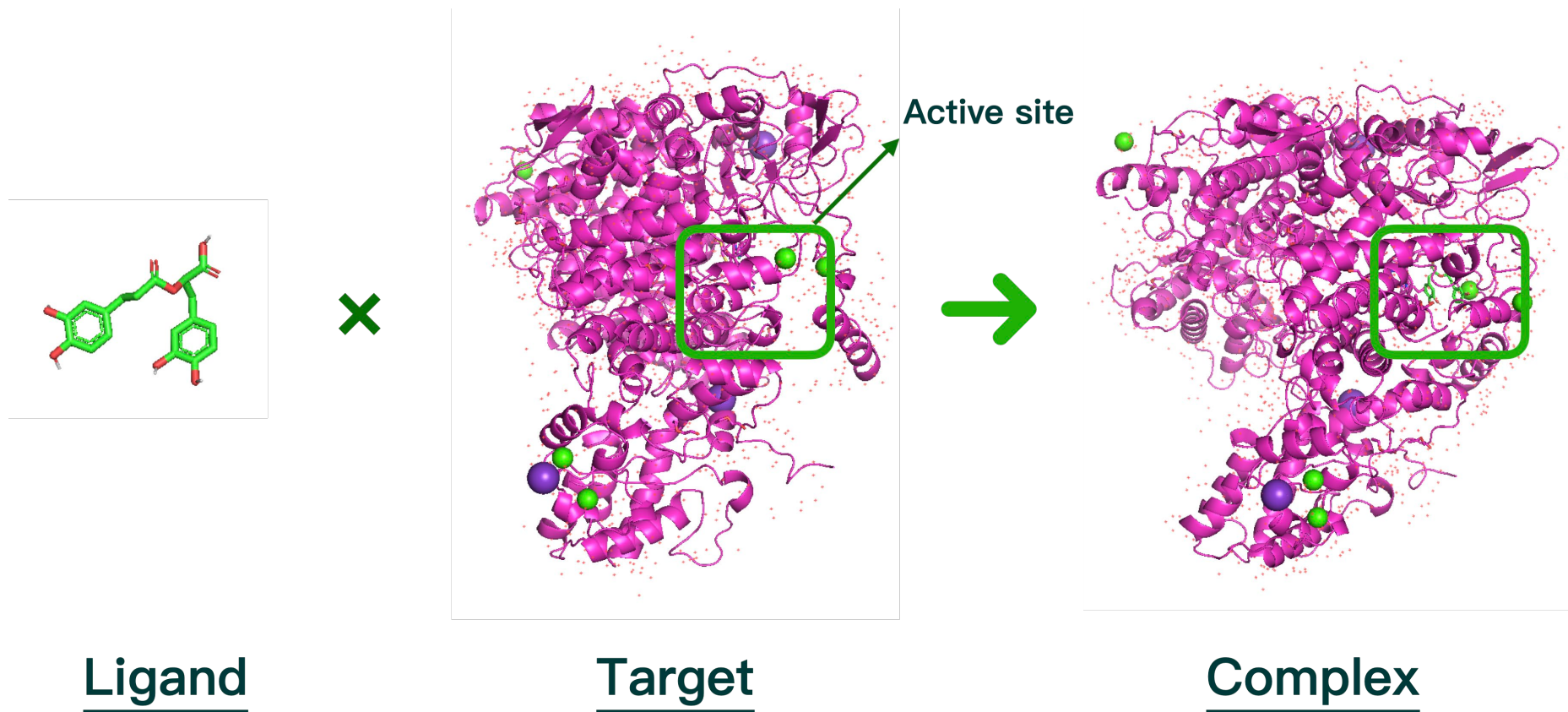


- The MCR contains $(\alpha\beta\gamma)_2$ complex, hydrophobic channel, F₄₃₀



3-NOP prevents CH₃-S-CoM from binding to MCR

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

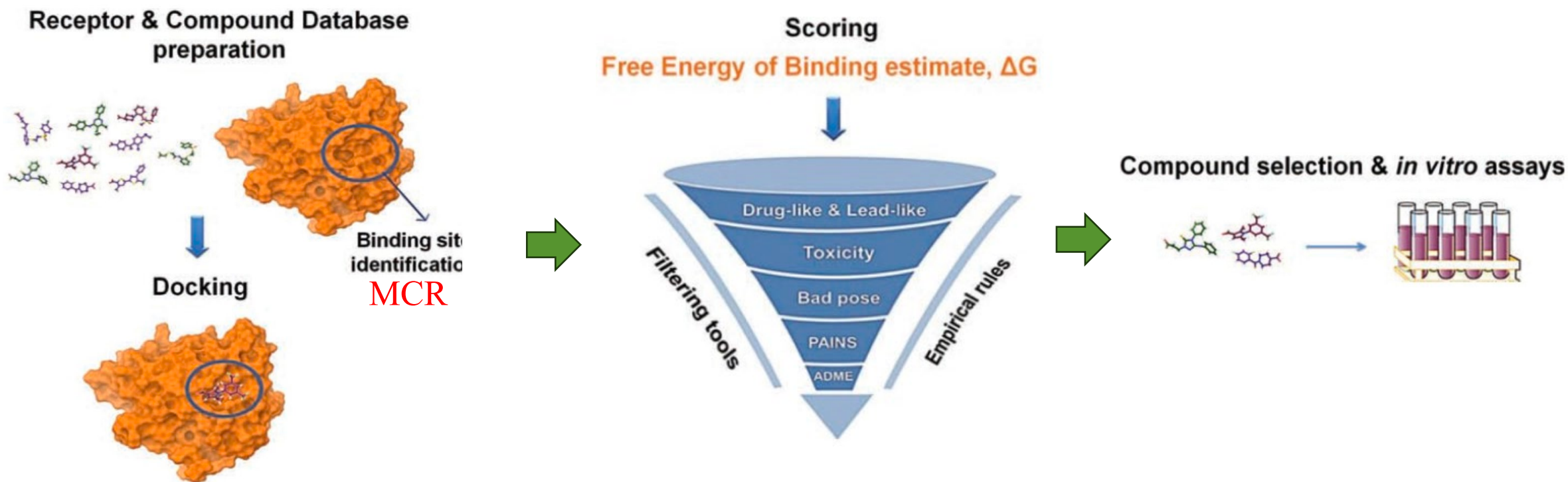




Virtual screening



- 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

RCSB PDB Deposit Search Visualize Analyze Download Learn About Documentation Careers COVID-19 MyPDB Contact us

RCSB PDB PROTEIN DATA BANK 221,716 Structures from the PDB 1,068,577 Computed Structure Models (CSM) 3D Structures

PDB-101 PDB wwEMDataResource NAKB wwPDB Foundation PDB-Dev

Structure Summary Structure Annotations Experiment Sequence

Biological Assembly 1

5A0Y
METHYL-COENZYME M F MARBURGENSIS AT 1.1 Å
PDB DOI: <https://doi.org/10.2210/>
Classification: **TRANSFERASE**
Organism(s): **Methanothermobacter**
Mutation(s): No
Deposited: 2015-04-24 Released
Deposition Author(s): **Wagner, T.,**
Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: **1.10 Å**
R-Value Free: 0.129
R-Value Work: 0.111

1E6V
Methyl-coenzyme M reductase from *Methanopyrus kandleri*
Grabarse, W., Ermier, U.
(2000) J Mol Biol **303**: 329
Released: 2000-10-18
Method: X-RAY DIFFRACTION 2.7 Å
Organisms: *Methanopyrus kandleri*
Macromolecule: METHYL-COENZYME M REDUCTASE I ALPHA SUBUNIT (protein)
METHYL-COENZYME M REDUCTASE I BETA SUBUNIT (protein)
METHYL-COENZYME M REDUCTASE I GAMMA SUBUNIT (protein)

5A8R
METHYL-COENZYME M REDUCTASE II FROM METHANOTHERMOBACTER MARBURGENSIS AT 2.15 Å RESOLUTION
Wagner, T., Ermier, U.
(2016) Angew Chem Int Ed Engl **55**: 10630
Released: 2016-08-10
Method: X-RAY DIFFRACTION 2.15 Å
Organisms: *Methanothermobacter marburgensis*
Macromolecule: METHYL-COENZYME M REDUCTASE II SUBUNIT ALPHA (protein)
METHYL-COENZYME M REDUCTASE II SUBUNIT GAMMA (protein)
METHYL-COENZYME M REDUCTASE II, SUBUNIT BETA (protein)

1E6Y
Methyl-coenzyme M reductase from *Methanosarcina barkeri*
Grabarse, W., Ermier, U.
(2000) J Mol Biol **303**: 329
Released: 2000-10-18
Method: X-RAY DIFFRACTION 1.6 Å
Organisms: *Methanosarcina barkeri*
Macromolecule: METHYL-COENZYME M REDUCTASE I BETA SUBUNIT (protein)
METHYL-COENZYME M REDUCTASE SUBUNIT ALPHA (protein)
METHYL-COENZYME M REDUCTASE SUBUNIT GAMMA (protein)

Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report |

Klee Clashscore

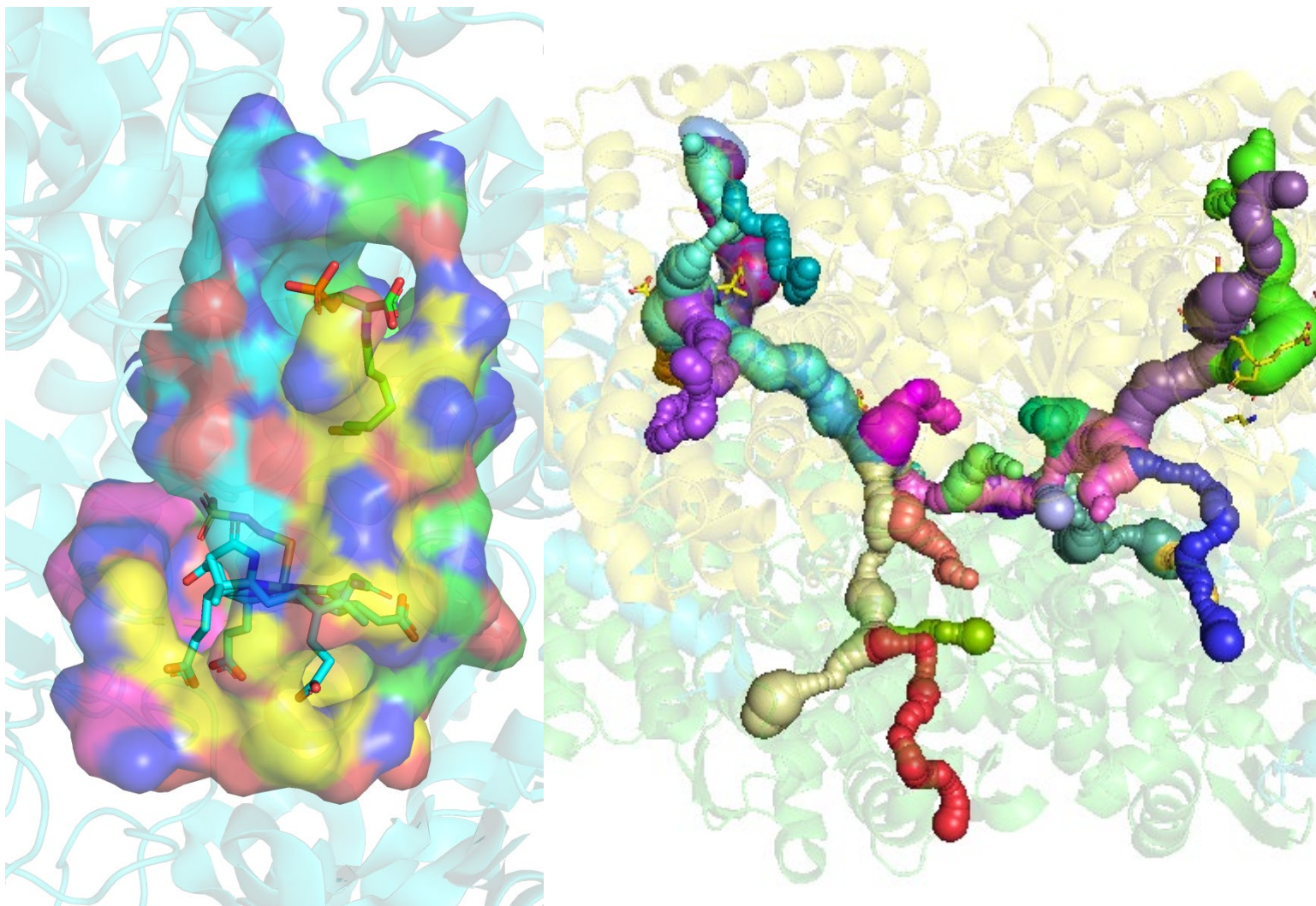
0.140 4 0.16



Application of molecular docking and virtual screening



Step 2: Determining the hydrophobic channel in MCR



PyMOL by Schrödinger

Introducing PyMOL 3.0

PyMOL is a user-sponsored molecular visualization system on an [open-source foundation](#), maintained and distributed by [Schrödinger](#).

Download PyMOL 3.0

Version 3.0.3 - Updated May 1st 2024 ([Installation instructions](#))
For previous versions, [see here](#).

These bundles include Python 3.10.


Windows


Windows

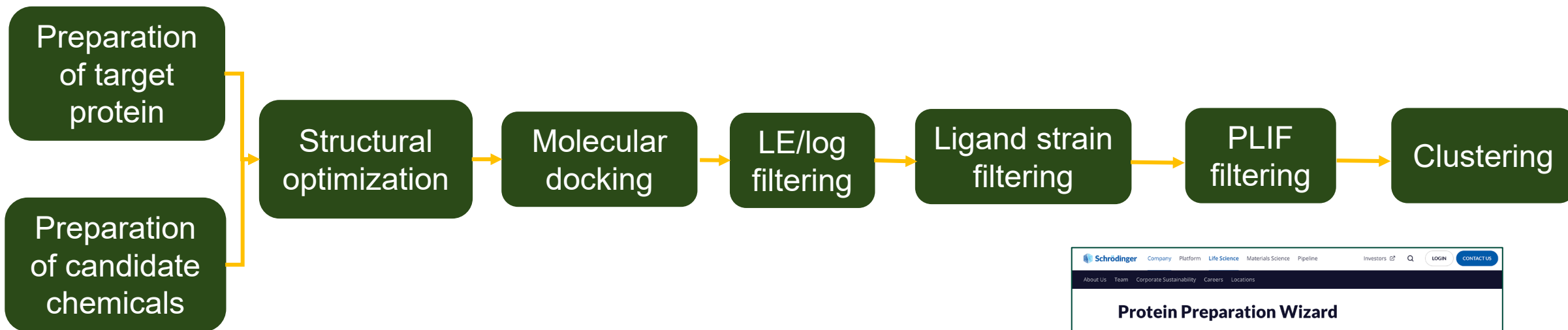

macOS


Linux

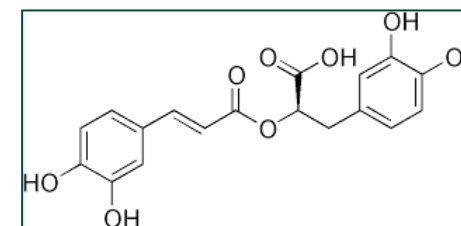
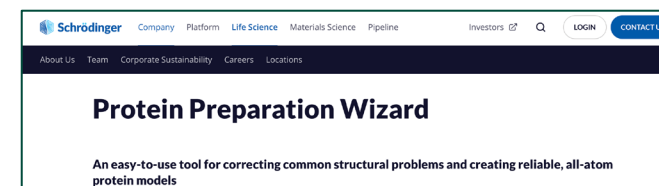
Application of molecular docking and virtual screening



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

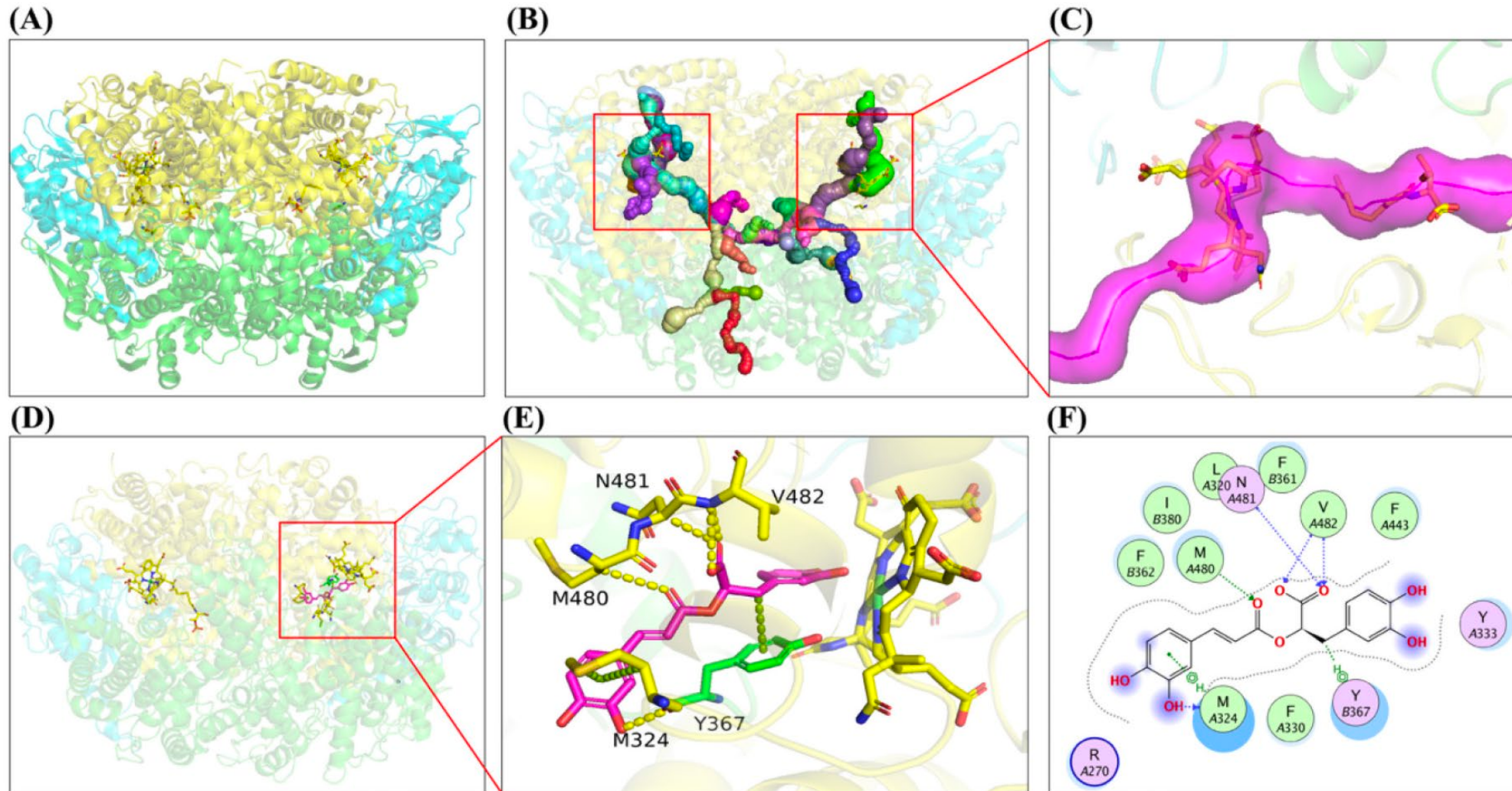
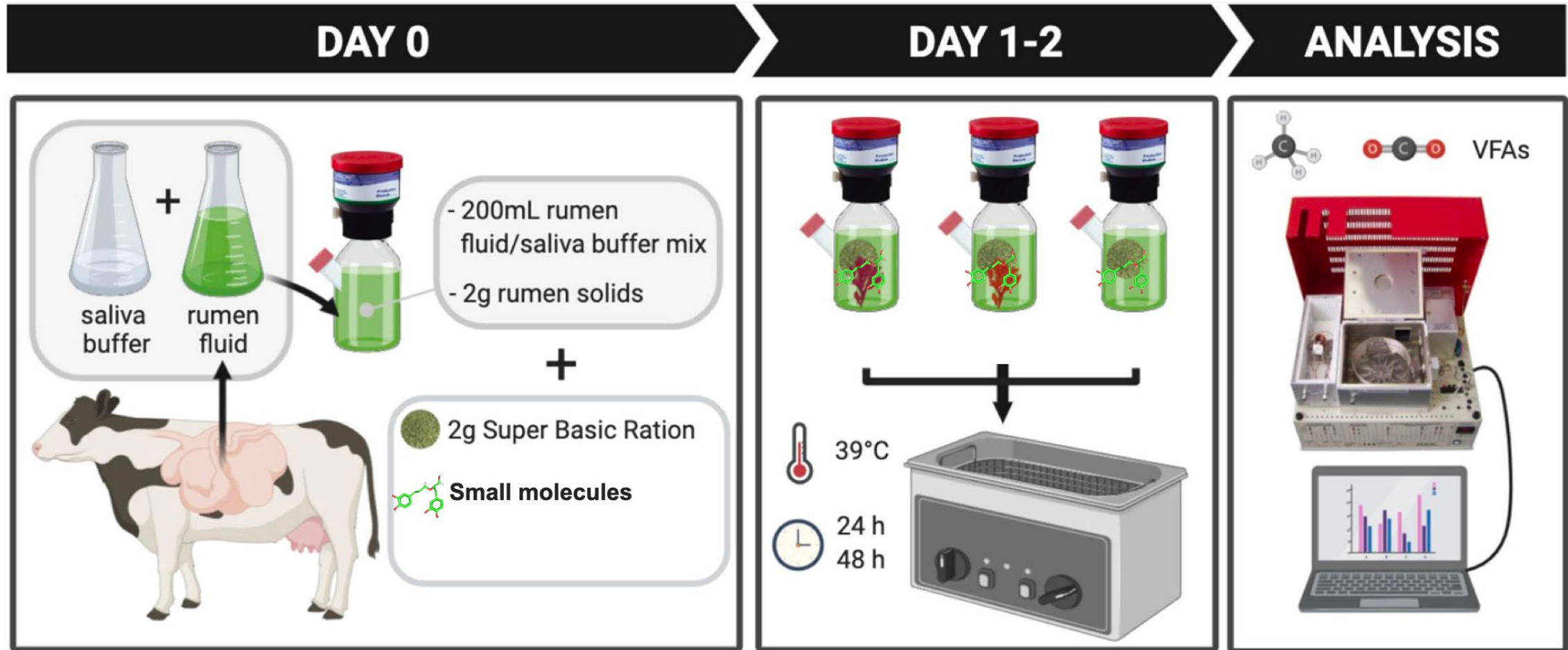


Figure 1. (A) shows the crystal structure of MCR, which consists of 3 subunits: α (McrA, Yellow), β (McrB, Green), and gamma (McrG, Blue). (B) and (C) shows the MCR's internal channel and activated sites. (D) exhibit the binding position of RA in the MCR. (E) and (F) shows the 3D and 2D binding patterns 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					P-value		
	CON	1% RA	2% RA	3% RA	SEM	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						
<i>Rikenellaceae</i> RC9 gut group	19.65 ^a	12.17 ^b	13.11 ^b	16.19 ^{ab}	0.933	0.019
<i>Christensenellaceae</i> R7 group	8.09 ^a	6.43 ^{ab}	5.79 ^b	6.27 ^b	0.296	0.026
<i>Ruminococcus</i>	5.52 ^a	5.18 ^a	3.51 ^b	3.72 ^b	0.298	0.012
<i>Eggerthellaceae</i> DNF00809	0.75 ^b	3.66 ^a	3.35 ^a	3.69 ^a	0.384	0.007
<i>Candidatus Saccharimonas</i>	1.50 ^a	1.38 ^a	0.99 ^b	1.02 ^b	0.077	0.038
<i>Denitrobacterium</i>	0.02 ^b	1.68 ^a	1.00 ^a	1.24 ^a	0.181	0.003
<i>Anaerovorax</i>	0.60 ^a	0.36 ^b	0.48 ^{ab}	0.44 ^{ab}	0.030	0.039
<i>Treponema</i>	0.28 ^{ab}	0.56 ^a	0.59 ^a	0.19 ^b	0.060	0.036
<i>Enterorhabdus</i>	0.08 ^b	0.34 ^a	0.37 ^a	0.34 ^a	0.033	0.005
<i>Desulfovibrio</i>	0.50 ^a	0.26 ^{ab}	0.15 ^b	0.11 ^b	0.052	0.026
<i>Lachnospiraceae</i> FE2018 group	0.37 ^a	0.20 ^b	0.22 ^b	0.20 ^b	0.020	0.016
unclassified <i>Atopobiaeae</i> genus	0.02 ^b	0.30 ^a	0.31 ^a	0.31 ^a	0.039	0.004
Archaea						
<i>Methanobrevibacter</i>	78.66 ^a	70.46 ^{ab}	67.44 ^b	63.27 ^b	0.016	0.009
<i>Methanosphaera</i>	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

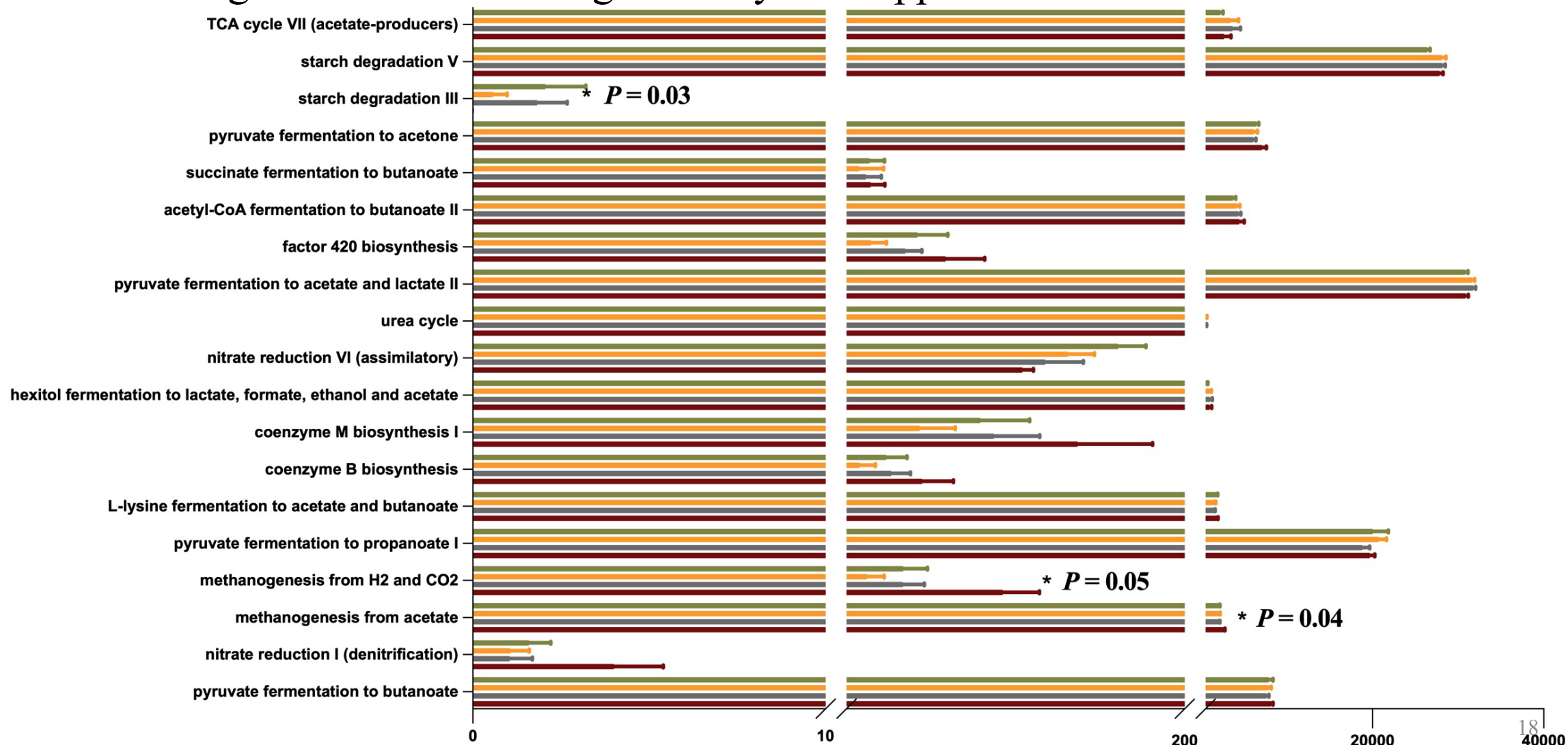
NeKittelmann et al., 2014



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.



- 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



中国农业科学院饲料研究所
INSTITUTE OF FEED RESEARCH OF CHINESE ACADEMY OF AGRICULTURAL SCIENCES