NEW ZEALAND
AGRICULTURAL GREENHOUSE GAS
Research Centre

State of Science: Methane

Methane inhibitors for pastoral grazing systems

Ron Ronimus

Introducing the team

- **AgResearch**: Dr Ron Ronimus, Dr Vince Carbone, Dr Linley Schofield, Dr Peter Janssen and Dr Stefan Muetzel
- **University of Auckland**: Dr David Rennison, Dame Dist. Prof Margaret Brimble (plus postgrads)
- **DairyNZ**: Dr Elena Minnee
- **PGgRc/RGP**: Mark Aspin
- **Commercialisation team**: Dr Ian Boddy, Dr Jane Calvert and Katherine Kemshall
- **Argenta**: Capsule development: Dr Desmond Morrow

Developing slow-release capsules for pasture-fed animals

- Aiming for 30% inhibition using slow-release capsules lasting >200 days (maximum of ~320 days).
- Slow-release capsules require highly potent and stable inhibitors that can be formulated.
- Any compound suitable for capsule use could also be used as a feed supplement (or other delivery system).
- Capsules allow the tailoring of dosing levels (e.g. slower at start to aid adaptation).
- For capsules lasting 200 days: sheep (7 g payload, 34 mg/day); cattle (52 g, 260 mg/day).
- Data suggests that about two times as much may be needed using capsules to achieve the same 24 hour level of inhibition compared to when a compound is added to twice daily feeds.
- An earlier lead at 30 mg/day in sheep trials (dosed in feed) caused ~30% inhibition. A capsule was tested using sheep (39 mg/day) caused ~7% inhibition using an *ad lib* pelletised lucerne diet.

Methane inhibitor development pipeline

Discovery

- Methanogen genome analyses
- Enzyme purification and assays
- Methanogen pure culture assays
- Rumen fluid *in vitro* assays (0.2 and 50 ml)
- Enzyme *in silico* screening
- Enzyme substrate analogues

Hits

Optimisation

- Medicinal chemistry
- Commercial sources

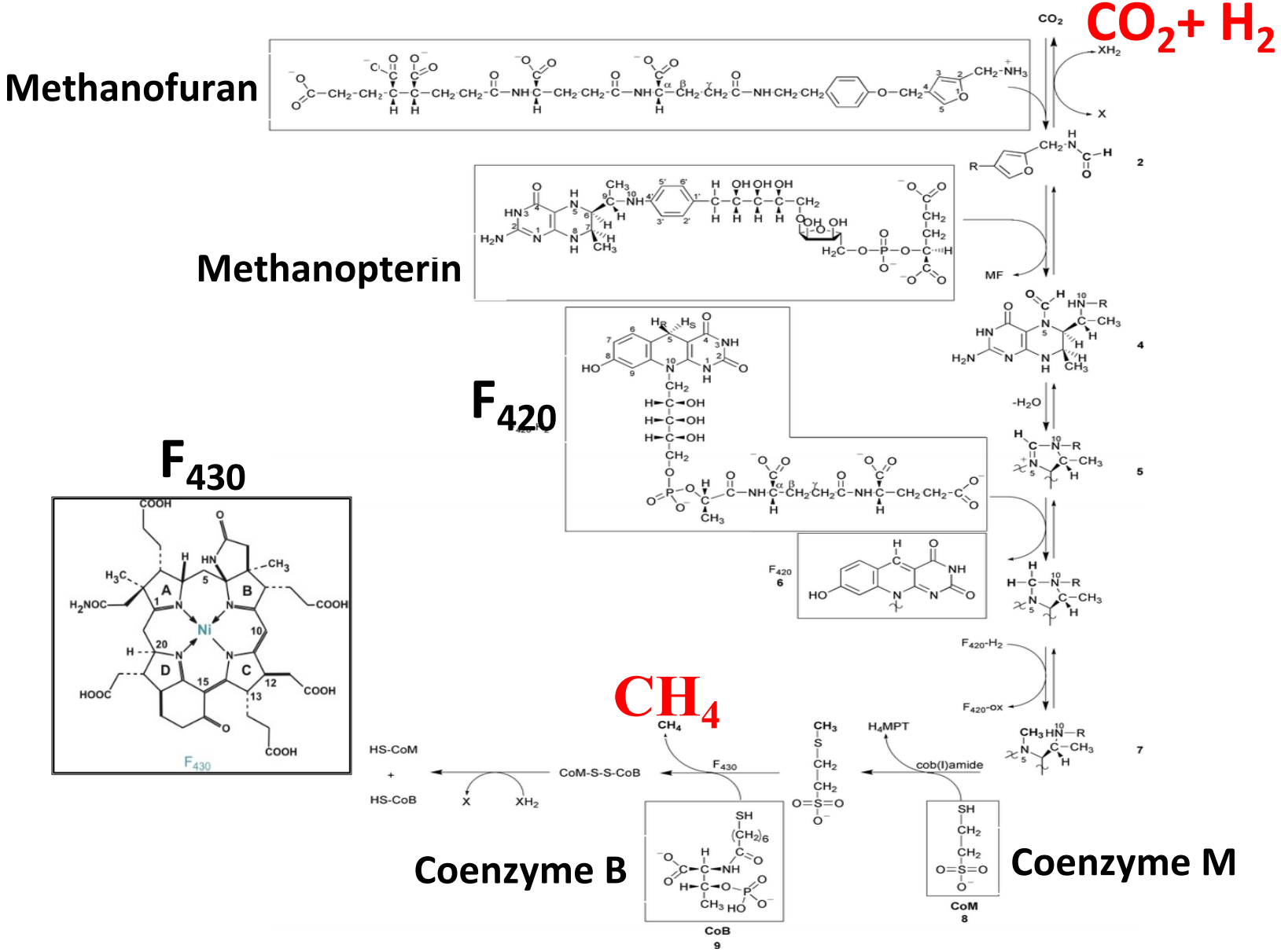
- Enzyme assays
- Culture assays
- Rumen *in vitro* assays

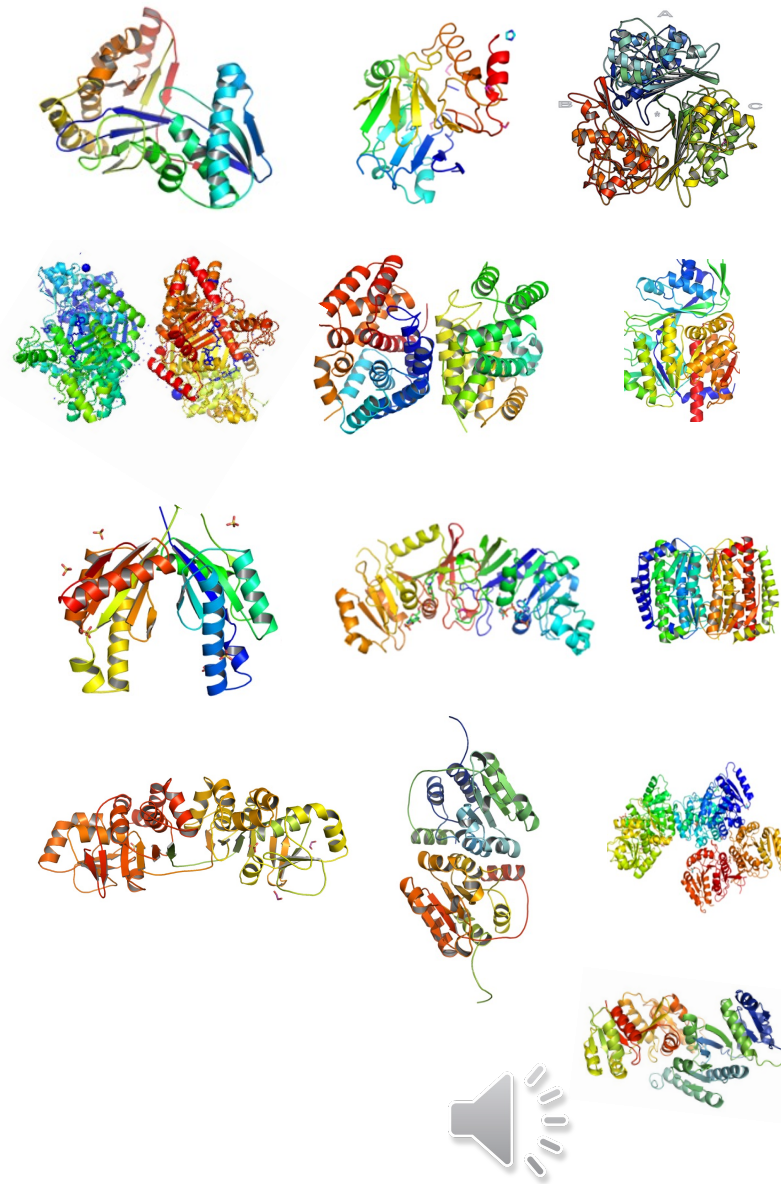
Derivatives

In vivo testing

- Mouse toxicity testing
- Short term sheep chamber trial (3 days)
- Mid-term sheep trial (16-28 days)
- Short term cattle trial (chambers)
- Mid-term cattle (chambers/GreenFeed)
- Long-term trials (~90 days)
- Delivery method development (grazing)
- Regulatory approval studies

Methanogen cofactor synthesis pathways and methane formation





Graham and White 2002 Natural Product Reports 19:133-147

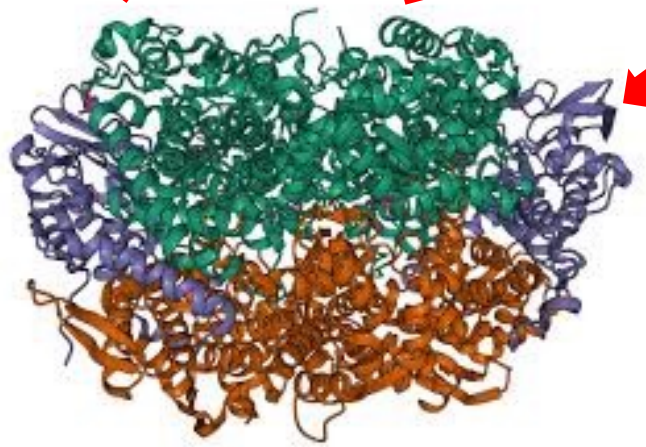
Targeting enzymes required to obtain fully active methane forming enzyme (MCR)

F_{430} biosynthesis (4 enzymes)

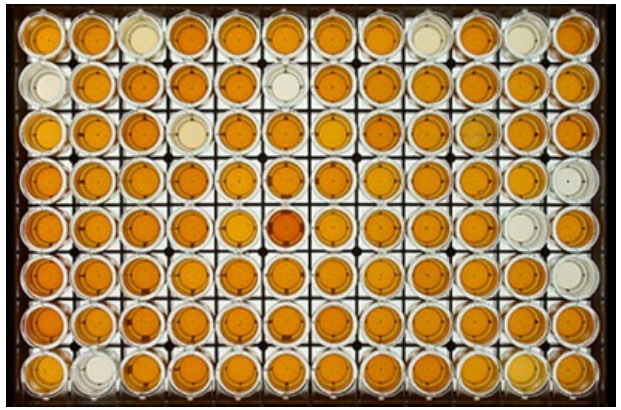
Coenzyme M biosynthesis (6 enzymes)

Coenzyme B biosynthesis (6 enzymes?)

16 enzymes for the activation, assembly and modification of MCR



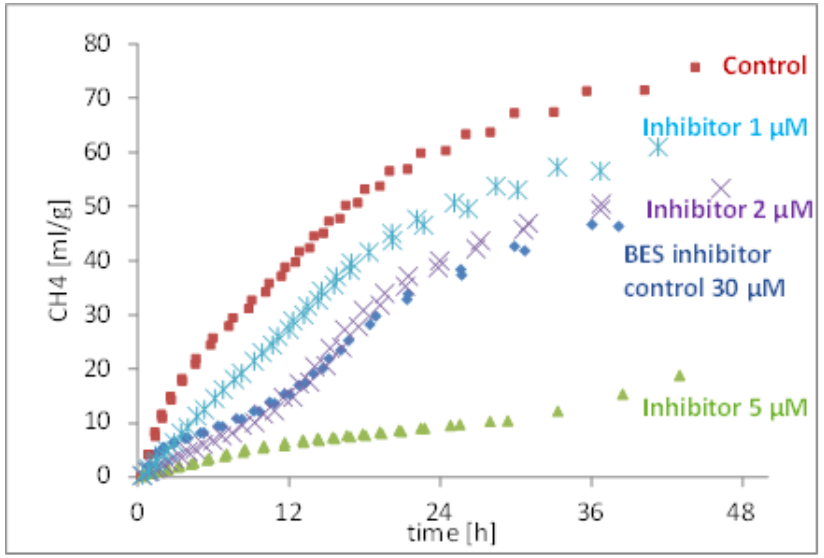
Summary of enzyme, pure culture and rumen *in vitro* assay screenings



96-well culture assay (4 days)

Measure CH_4, H_2 and total gas over 24-48 hours

CH_4



Hours

- >140,000 compounds screened against various enzymes (8 enzymes).
- >40,000 compounds screened against methanogen cultures (= 1.5 ×10^7 interactions).
- >15 million compounds screened against enzymes *in silico*
- ~13,000 compounds (in duplicate) checked in rumen fluid-based assays.
- All strategies have produced hits (>1,000 overall), validating the approaches.

Overall summary of discoveries of the pipeline: current work

- 12 classes of compounds, about half with >100 analogues, and 40+ compounds tested in animals.
- Our current lead class has >140 analogues; more details on next slide.
- Hits from the screening of a 10,000 compound proprietary library are showing strong inhibition in pure culture assays. These could lead to additional new classes.
- Drug discovery requires an ongoing pipeline approach with a steady flow of compounds for testing and discovery of new hits, followed up with medicinal chemistry optimisation.

Current focus: developing a new class of inhibitor

- The first compound in the class was found in one of our screening assays.
- An early derivative has been tested in sheep and led to 11% inhibition.
- Our latest derivatives are up to >30-times more active *in vitro* than the first hit discovered. Some are active at low nanomolar levels in *in vitro* pure culture assays.
- Four examples of these compounds have recently been tested in sheep.
- There is likely to still be some room for further improvement within the class (e.g., medicinal chemistry) to help achieve the target dose for capsule delivery.

Other research ideas for finding and delivering inhibitors

- Expanding work on ‘backup’ classes from the pipeline.
- Systematic targeting of the six methanogen cofactors using substrate analogues.
- Continued screening of large compound libraries using methanogen pure culture assays.
- Expanding the use of in-house methanogen enzyme crystal structures for identifying new inhibitors by using computer-based screening.
- Evaluating confirmed actives for application through different delivery mechanisms, e.g. feed additives, capsule, water delivery, etc.
- Scaling these studies up to industry impact size.

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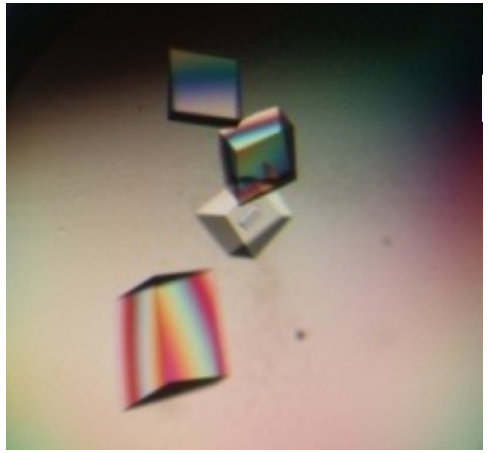
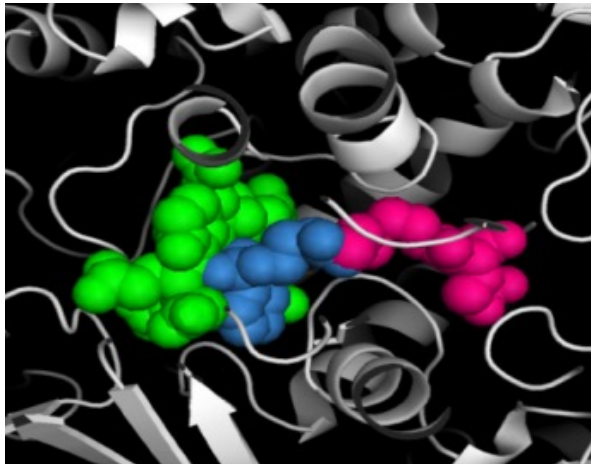
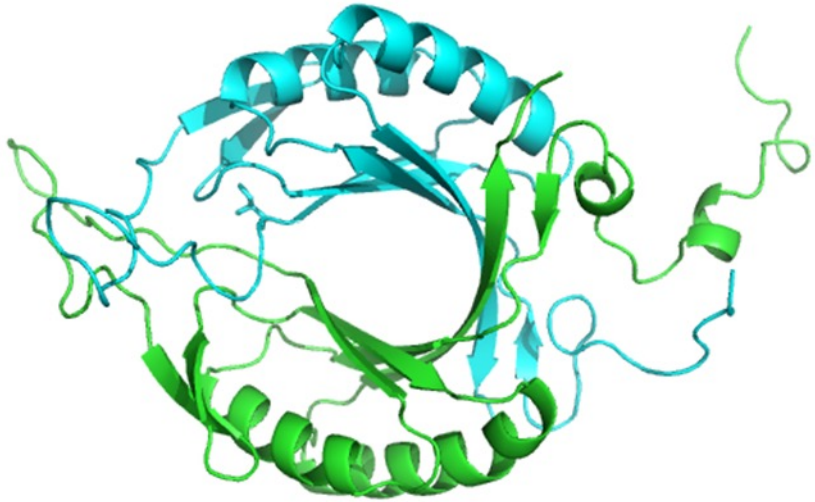


Methanogen enzyme crystals



Example of an inhibitor docked into the active site of a methanogen enzyme



Newly solved enzyme structure Involved in methane formation (first discovered 36 years ago)

Thank you for your attention