# Methanogenic Enzymes

# Cofactor Biosynetheses

# Amino Acid Biosynthesis

# Anaerobic Oxidation of Methane (AOM)

**Methane oxidation by anaerobic archaea for conversion to liquid fuels (Mueller et al, 2014)**

Natural gas isn’t ideal because it has low energy density and we don’t have infrastructure setup to use compressed natural gas, so we’re looking towards gas-to-liquid (GTL) processes. The top process is Fischer-Tropsch, which can only recover 25-45% of the carbon and aren’t economically attractive. Biological processes have potential to do much better at GTL and come in two flavors: AOM and aerobic methane oxidation. AOM has an advantage over aerobic methane oxidation in that it can potentially achieve 100% carbon efficiency, whereas the latter process has 66.7% maximum efficiency because one carbon is lost as CO2 instead of converted to useful things (e.g. methanol, ethanol, butanol). AOM is really attractive, but we still haven’t achieved a pure culture of an ANME organism because they grow very slowly and need a syntrophic partner, plus the slow growth makes them poor production candidates. Additionally, we don’t understand the pathways, regulatory structures, and possible interacting partners.

ANME organisms eat methane by doing reverse methanogenesis, but this process is energetically unfavorable on its own, so they typically form consortia with sulfate reducing bacteria. Theoretically the methanogens could go backwards, but the MCR acts a little differently (more on this later). Back to the energy problem; to achieve reverse methanogenesis, you need to pair with an electron acceptor (e.g. sulfate, nitrate) that makes the overall conversion to alcohols favorable. There are 4 potential acceptors referenced here: sulfate, nitrate, manganese, and iron. Sulfate is considered most promising, likely because we know of the consortia with ANME organisms and sulfate reducing bacteria, so it’s the one we know most about. The nitrate reductase reaction is also a possibility, and might actually be needed for the alcohol-based biofuel processes. The metals aren’t really as promising because they’re mostly insoluble solids, so they’re not as accessible.

The MCR is the important step, it has to break the C-H bond in methane and creates methyl-CoM without breaking other bonds. Likely F430 is involved with the MCR. We don’t know much about the activity of MCR or the exact reaction mechanism.

*Overall, the ANME organisms are promising because you can get the most out of each carbon in theory, but we don’t understand them or their pathways well because they’re hard to culture. We know the MCR is important and we know there must be a coupling to exergonic reactions, like sulfate/nitrate reduction. We’re still figuring out the mechanisms, but if we can there’s lots of advantages over aerobic processes. If we can figure it out, this may become the GTL method of choice*

**A reversed genetic approach reveals the coenzyme specificity and other catalytic properties of three enzymes putatively involved in anaerobic oxidation of methane with sulfate (Kojima et al 2014)**

Up to this point, we know that MCR is in the ANME organisms, but what about the other enzymes in the methanogenic pathway? If we’re truly running methanogenesis in reverse, we should be able to find and characterize those enzymes in methanotrophs. This paper does so, characterizing the first step enzymes in methanogenesis (MFR, H4MPT), thus lending credence to the idea that we’re simply running the same pathway in reverse.

**Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage (Haroon et al 2013)**

This new organism is called *Can. Methanoperedens nitroreducens* and can reduce nitrate to nitrite, giving it the energy to make carbon dioxide from methane. They also characterized the entire reverse methanogenesis pathway and nitrate reduction/translocation enzymes. In nitrate-rich environments, they hypothesize that in ammonium-rich environments, these ANME organisms live with bacteria that take in the nitrite and ammonia to produce nitrogen gas and a bit of nitrate. It couples them like the sulfate case described in Milucka et al (below). Without ammonium, the bacterium isn’t there, you just get this organism and another ANME organism I think. And with lots of nitrite only, you just get the other ANME organism.

*Importantly, they demonstrate that this new organism can couple nitrate reduction to AOM.*

**Zero-valent sulphur is a key intermediate in marine methane oxidation (Milucka et al 2012)**

Generally we characterize the ANME organisms as living in syntrophy with the sulfur reducers to couple to sulfate reduction (sulfate to HS-). However, this group asserts that “zero-valent sulfur” (S0) is an important intermediate; this arises from the fact that we don’t know what the syntrophic intermediates are between the bacteria and the archaea. Their theory: the ANME organisms take in methane and sulfate (a bit from the bacteria) to produce CO2 and HS2-, which the bacteria use to produce HS- and a sulfate ion that cycles back. In doing so, the organisms couple together via sulfur-based ions and achieve the basic sulfate-to-sulfide reaction (sulfur goes from +6 to -2 in the reduction) that has been hypothesized, but they must create zero-valent sulfur in the AMNE organisms along the way. This mechanism suggests that in environments with high sulfate and methane concentrations, the ANME organisms might not even need the bacteria, because their sulfate partial reduction is highly favorable in and of itself. Of course, if the disulfide product of the ANME is scavenged (as by the bacteria), the process becomes more favorable.

# Metabolic Engineering

**Large-Scale Bi-Level Strain Design Approaches and Mixed-Integer Programming Solution Techniques (Kim et al 2011)**

This is the Reed group’s SimOptStrain paper. SimOptStrain improves on OptStrain because Optstrain has 2 steps: 1) Add non-native reactions to produce something, sometimes to make more of it; 2) Delete reactions to improve production, a la OptKnock. SimOptStrain does both at once and deletes genes instead of reactions. This is a bi-level optimization problem with the inner problem being FBA and the outer one being max production of a byproduct. They use a universal database made from KEGG that was altered and note that they had to make lots of changes to the database so that it worked. It’s worth noting that some of their results suggest a simple NAD-NADP swap. You can also use SimOptStrain to refine models where FBA isn’t predicting correct by-product secretion. They still need to improve the database, both the reactions in the database and the nomenclature, so that it’s more universal.

*Bottom line: SimOptStrain looks like the best tool to do strain design for non-native reaction additions.*