Big idea: Our model of M. maripaludis is qualitatively correct and this is particularly important as it has numerous biological implications

* As discussed in depth in the introduction, the electron bifurcation mechanism is a vital difference between hydrogenotrophic and methylotrophic methanogenesis. Our model accurately depicts this phenomenon based on recent literature, setting it apart from other methanogen models with their linear metabolisms.
* The results of including bifurcation are quantitatively difficult to differentiate from those of linear models. It would be trivial to, for example, compile a model of M. maripaludis with a linear methanogenesis pathway capable of consuming H2 and CO2, particularly because this path has been rigorously curated in its entirely in two models of Methanosarcina barkeri. Though Thauer has demonstrated that this linear pathway is capable of much greater cell yields than is the corresponding circular pathway, model yield predictions could easily be tuned by altering the model’s ATP maintenance parameters.
* In the case of the other group’s model, our inspection of their model reveals a linear pathway of methanogenesis, essentially identical to the M. barkeri pathway. It is thus somewhat surprising to find that their predicted cell yields are quantitatively consistent with those expected from a hydrogenotrophic methanogen. It serves to highlight the fact that matching these quantitative predictions doesn’t guarantee a high quality model.
* The inclusion of electron bifurcation opens up some really interesting questions we can ask with our model. A particularly pressing question revolves around ferredoxin specificity, namely as it applies to energy conservation in M. maripaludis. We generally assume in our model that all ferredoxins in the model are functionally equivalent and can seamlessly substitute for one another; however, recent experiments by Costa et al have suggested that this is not the case. Our alternative treatment of ferredoxins, grouping those involved in the electron bifurcation into their own specific type, allows us to probe at the consequences of bifurcation.
* We can also use our model to probe metabolic questions about other methanogens outside of the general methylotrophic/hydrogenotrophic split. In Thauer’s review, he describes methanogenesis as performed by M. stadtmaneae, whereby methanol is reduced to methane using electrons from hydrogen. The energy conservation mechanism in M. stadtmaneae is actually similar to that in M. maripaludis in that it lacks a membrane-bound HdrDE. The major difference is that methanol is directly converted to methyl-CoM by methanol methyltransferase rather than proceeding through the CO2 reduction steps, thus it skips the ion gradient-generating Mtr step. Instead, the ion gradient is generated by Ehb, which oxidizes ferredoxin to translocate ions for ATP synthesis. Using our hydrogenotrophic model with bifurcation as a starting point, we were able to easily simulate this with a few small changes. First, we restricted flux through Fwd, the first step of hydrogenotrophic methanogenesis, thus disallowing the use of CO2 as the methanogenic carbon source. Then we added the methanol methyltransferase reaction and allowed uptake and transport of methanol to supply this reaction. These additions, coupled with supplying methanol in our *in silico* medium, enabled us to simulate methanogenesis from methanol and H2. Notably, many methylotrophs can also use H2 to reduce methanol to methane, as illustrated by Welander et al. However, their use of the membrane-bound HdrDE complex facilitates direct build-up of an ion gradient, versus M. stadtmaneae metabolism, which requires Ehb hydrogenase to create the gradient. Hence, by eschewing the linear methanogenic pathway in favor of the accurate circular hydrogenotrophic pathway, we have created a bridge to correctly describe alternative methanogenesis mechanisms. Further, our model provides a manually-curated starting point for creating metabolic models for other methanogens lacking HdrDE, including but not limited to M. stadtmaneae