**Supplemental File 4: Demonstrating our Free Energy Estimation Capabilities**

By including available free energy of formation values for exchange metabolites, we have equipped our model to quickly estimate overall free energy generation. To demonstrate this functionality, we consider the case of hydrogen concentrations in methanogenic environments. In our growth simulations we accept a list of exchange metabolites and a vector of their concentrations in units of mM (see https://github.com/marichards/methanococcus). By default, we assume that all aqueous concentrations for these metabolites are 1 mM, thus if concentrations are not supplied we calculate overall free energy as follows:

>> solution = maxGrowthOnH2Only(model);

Biomass flux: 0.096861

Formate flux: 0.000000

CO2 flux: -52.579347

H2 flux: -205.169846

H2O flux: 103.908440

CH4 flux: 50.000000

NH3 flux: -0.752865

PO4 flux: 0.009705

Acetate flux: 0.000000

Overall reaction:

CO2 + 4 H2 --> 2 H2O + CH4

Model overall reaction (per mole CH4)

1.05 CO2 + 4.10 H2 --> 2.08 H2O + CH4

Predicted Yield Coefficient: 2.79 gDCW/mol CH4

Expected ATP/CH4 Yield: 0.5

Predicted ATP/CH4 Yield: 0.475

Warning: All external metabolite concentrations set to 1 mM

> In maxGrowthOnH2Only at 99

Predicted Free Energy Generation: -6.457393 kJ/gDCW

A key feature of hydrogenotrophic methanogens is their ability to thrive in conditions with low H2 partial pressure (~10 Pa). Converting to aqueous concentration via Henry’s Law coefficient (1), 10 Pa corresponds to 7.7 x 10-5 mM. We can estimate overall free energy for this hydrogen concentration by specifying this parameter:

>> solution = maxGrowthOnH2Only(model,{'EX\_cpd11640[e0]'},[7.7e-5]);

Biomass flux: 0.096861

Formate flux: 0.000000

CO2 flux: -52.579347

H2 flux: -205.169846

H2O flux: 103.908440

CH4 flux: 50.000000

NH3 flux: -0.752865

PO4 flux: 0.009705

Acetate flux: 0.000000

Overall reaction:

CO2 + 4 H2 --> 2 H2O + CH4

Model overall reaction (per mole CH4)

1.05 CO2 + 4.10 H2 --> 2.08 H2O + CH4

Predicted Yield Coefficient: 2.79 gDCW/mol CH4

Expected ATP/CH4 Yield: 0.5

Predicted ATP/CH4 Yield: 0.475

Predicted Free Energy Generation: -1.448827 kJ/gDCW

As illustrated by this quick calculation, we predict overall free energy of the system to still be favorable for methane generation from H2, though of much smaller magnitude than at higher H2 concentrations. Assuming other external metabolite concentrations remain at 1 mM, we can also conduct a short sensitivity analysis of H2 concentration on overall free energy. Setting H2 concentrations from (10-10 - 100), we can calculating the overall free energy for methanogenesis in each case produces Figure S5. This figure shows the expected logarithmic relationship between H2 concentration and overall free energy, with ΔG = 0 at [H2] ≈ 5 x 10-6 mM (PH2 ≈ 0.65 Pa).

## References

1. **Sander R**. 2015. Compilation of Henry’s law constants (version 4.0) for water as solvent. Atmos Chem Phys **15**:4399–4981.



Figure S5: Semi-log plot showing our sensitivity analysis of the effects of [H2] on overall free energy generation(ΔG).