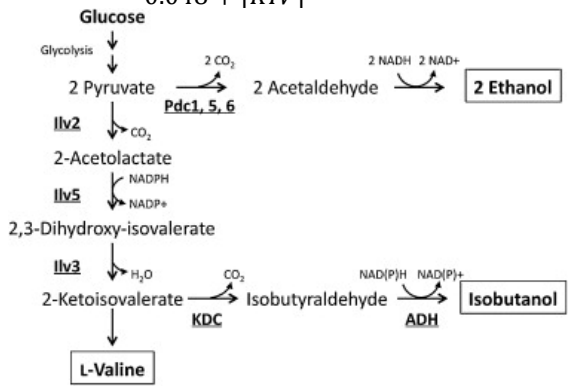
ABE591 Principles of Systems & Synthetic Biology

HOMEWORK #5 **Due: Tues Nov 27, 2018**

1) Isobutanol, an advanced biofuel suitable for direct replacement of gasoline, may be produced via the Ehrlich pathway below. To keep costs low, you express this pathway from cells grown in cheaper minimal media where they must synthesize all needed nutrients.

   a) You perform a metabolomics experiment to profile key metabolite concentrations and note you only produce 1 µg/ml of isobutanol, but 5 g/L of valine and 0.05 mM of 2-ketoisovalerate (KIV). If the activity of KDC is given by the expression below, how might you engineer this pathway for better productivity. Explain your reasoning (**5 points**)

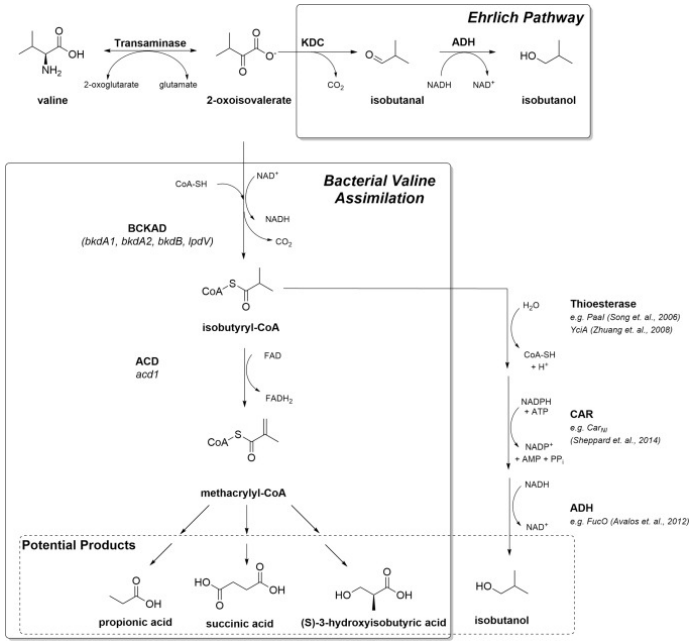
$$r_{KDC} = \frac{0.82[KIV]}{0.048 + [KIV]}[=]mM/s$$



Kondo et al. J. Biotechnol, 2012

   b) Does your answer change if you measured the KIV concentration as 1 mM? If so, how? (**3 points**)

   c) You also consider the alternative bacterial valine assimilation route to isobutanol. Given the pathways, which option would you expect to be more efficient. i.e produce more isobutanol? Why? (**2 points**)



Solomon, et al ME Comm, 2016.

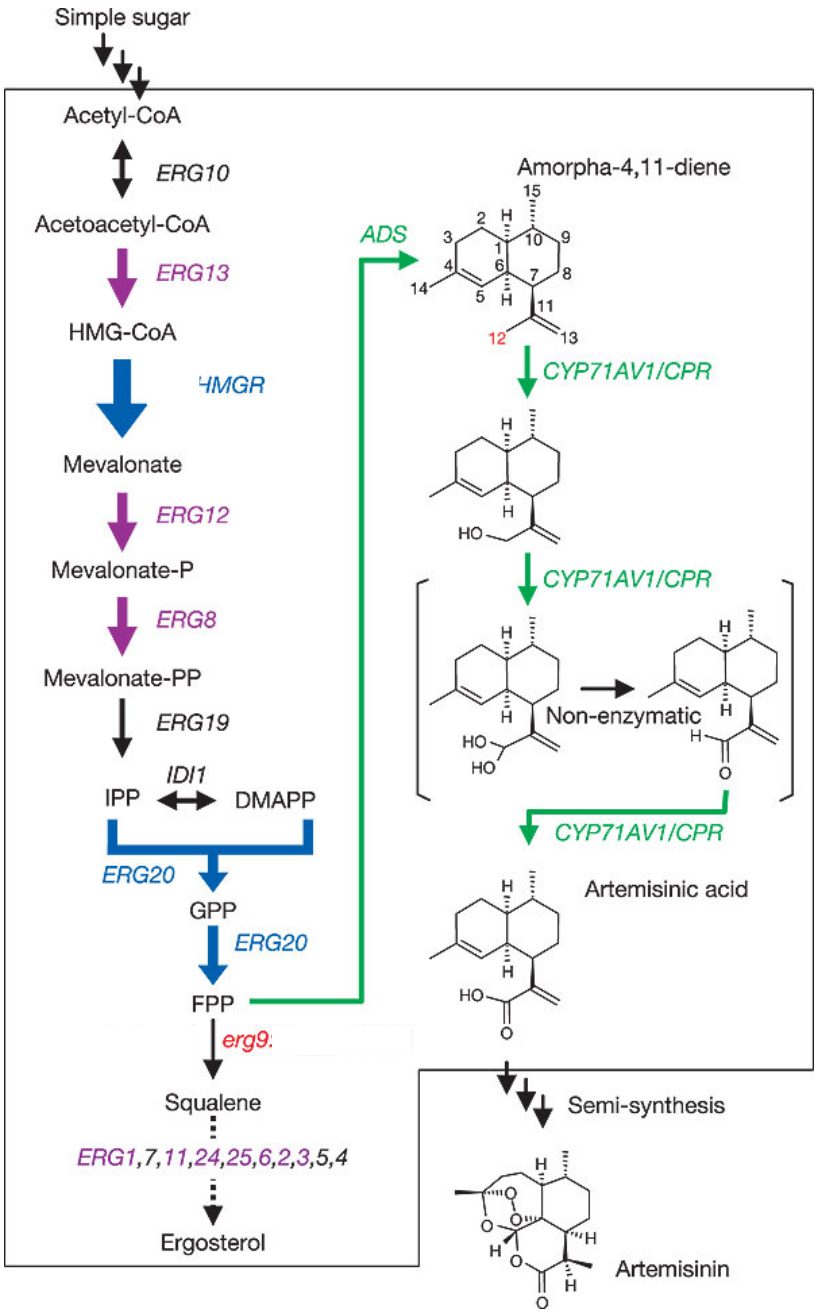
2) You are working on the pathway to produce artemisinc acid (an antimalarial drug precursor) in yeast. The intermediate HMG-CoA in the pathway (right) for artemesinic acid, an antimalarial precursor, is a toxic metabolite that quickly kills your drug producing strain. Thus, it cannot be allowed to accumulate.

   a) Which enzymes might you choose to regulate to prevent pathway toxicity? Would you upregulate or downregulate them? (**4 points**)

   b) Discuss 2 strategies, other than static gene regulation, that you would consider to alleviate the toxicity. (**4 points**)

   c) You build the pathway and discover your yeast is actually producing lots of organic acids and alcohol biofuels instead. Analyze the pathway to identify where you are losing carbon flux and propose a strategy to circumvent this. (2 **points**).

   d) You realize that the pathway requires many enzymatic steps, which may cause undue burden on producing cells. Thus, you decide to distribute specific portions of the pathway across a community of cells. What strategies must you develop to ensure that pathway production is stable, with enzyme production matched at appropriate levels across the community? (**3 points**).



Ro et al, Nature, 2006.

3) In building a pathway to create a new biofuel, you find various homologs (variants) of a key enzymatic step that is subject to feedback inhibition. Each homolog has several 'mutations' relative to your base strain, however, the specific function of each mutation is unknown. Some of your mutants are feedback resistant, while others have superior $k_{cat,}$ broader substrate specificity, and so on. Using this library of variants, how would you create the 'best' enzyme for your pathway? Describe the approach taken. **(5 points)**

4) **Design Project**. What key properties (Hill equations, MM kinetics parameters, etc) do you need to estimate system performance? List strategies/resources you will use to identify these numbers. **(2 points**)