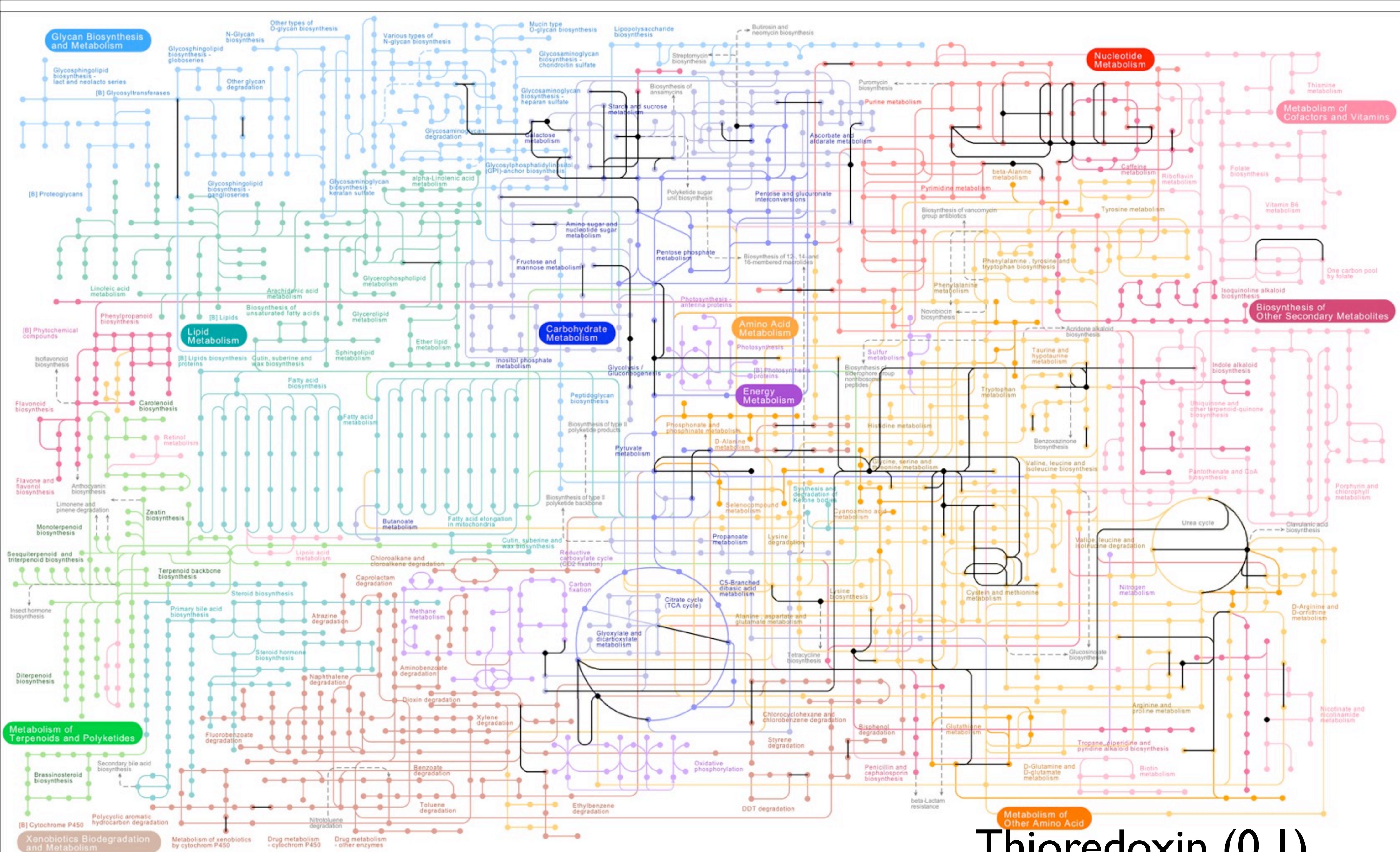


Dimensions 05-10-12 Update

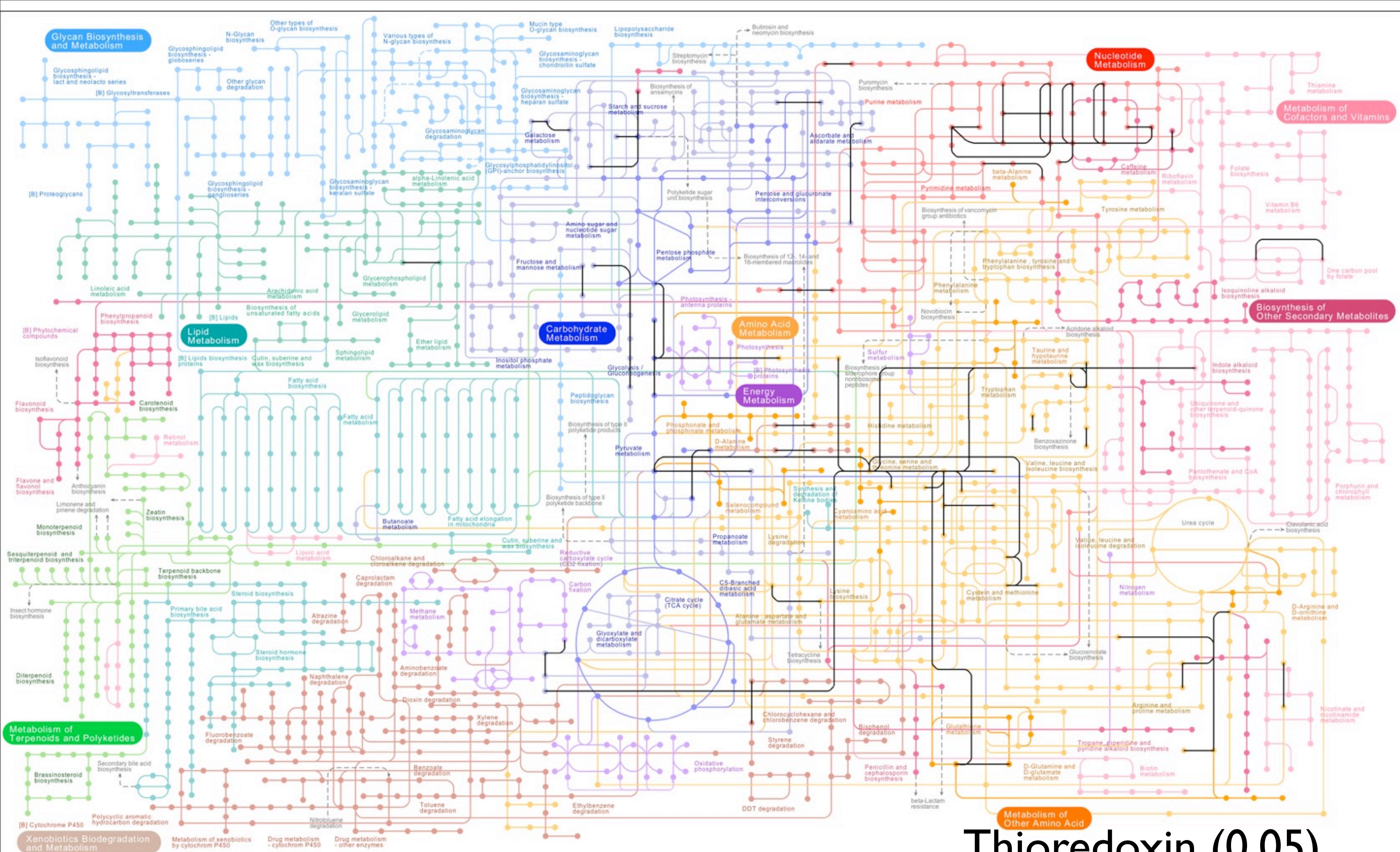
Jeremy Frank

Analysis of Metabolomics Data

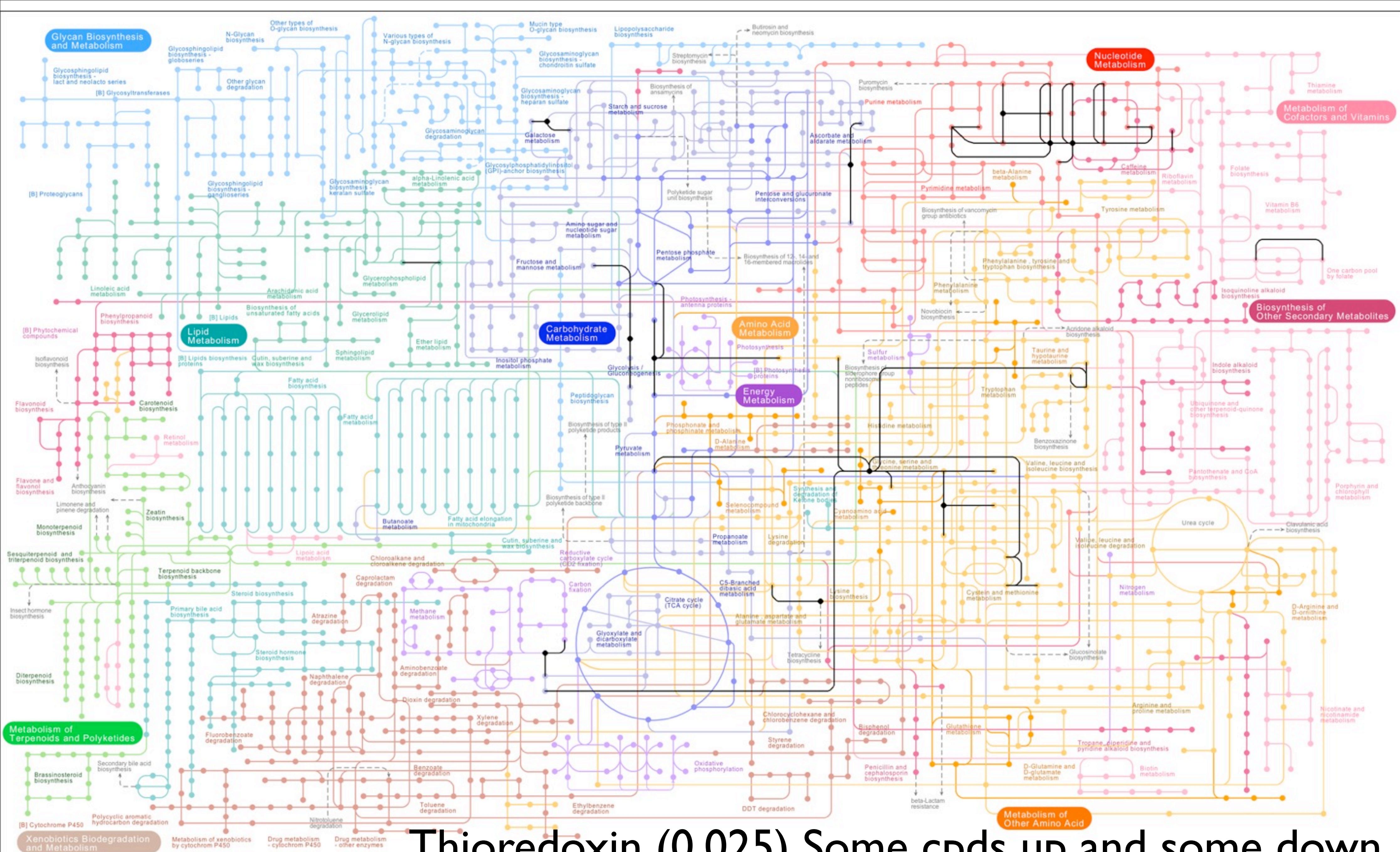
- Generated overall metabolic maps for seven proteins with conserved functional domains
- Compounds considered significantly affected if the difference between mean abundances had a p-value < 0.05
- Is the stringency of 0.05 too high? Too low? Tried 0.025 and 0.1
- Proteins chosen were PhoH, thioredoxin, peptide deformylase, 3 peptide chain release factors and another PhoH-like protein



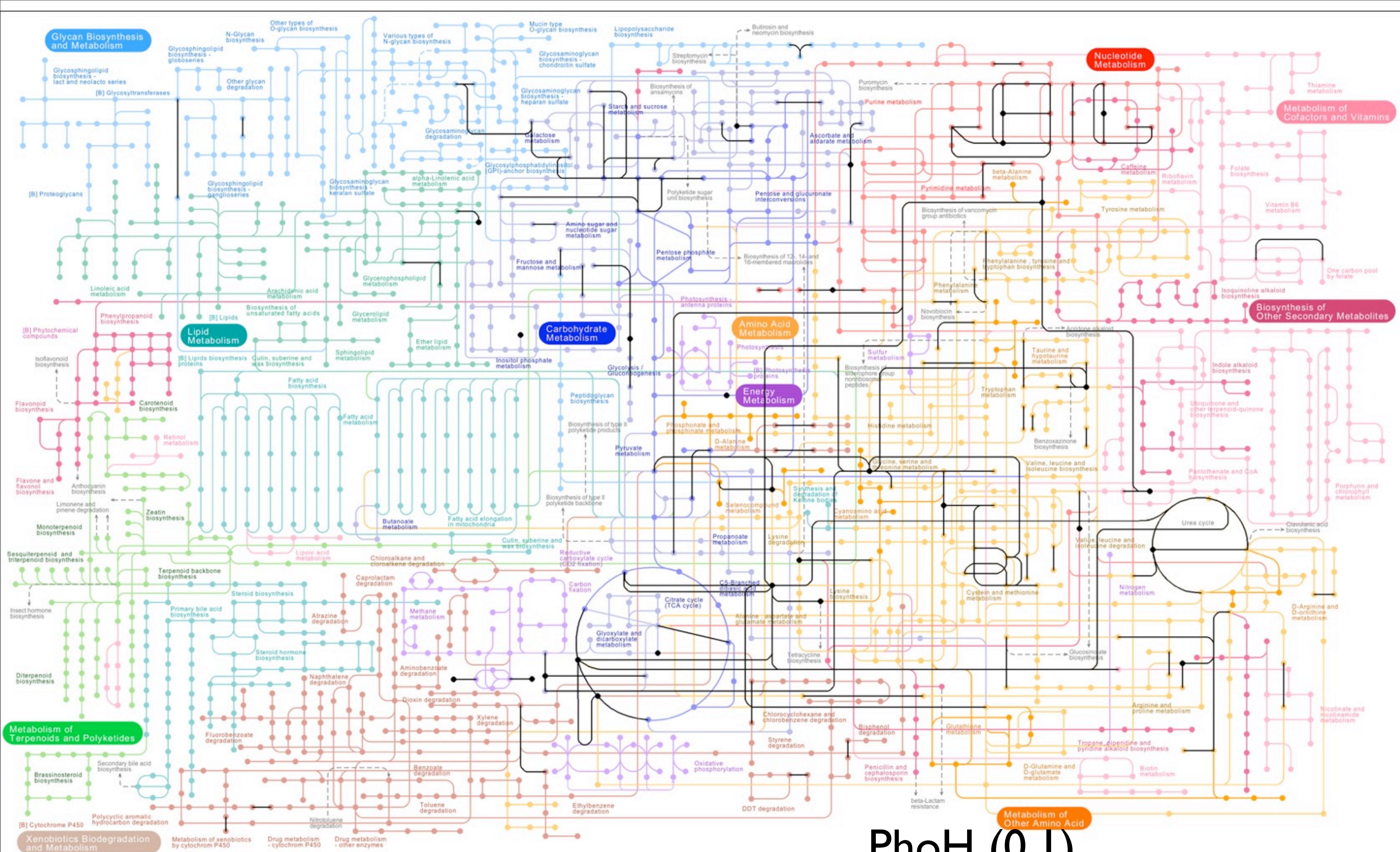
Thioredoxin (0.1)



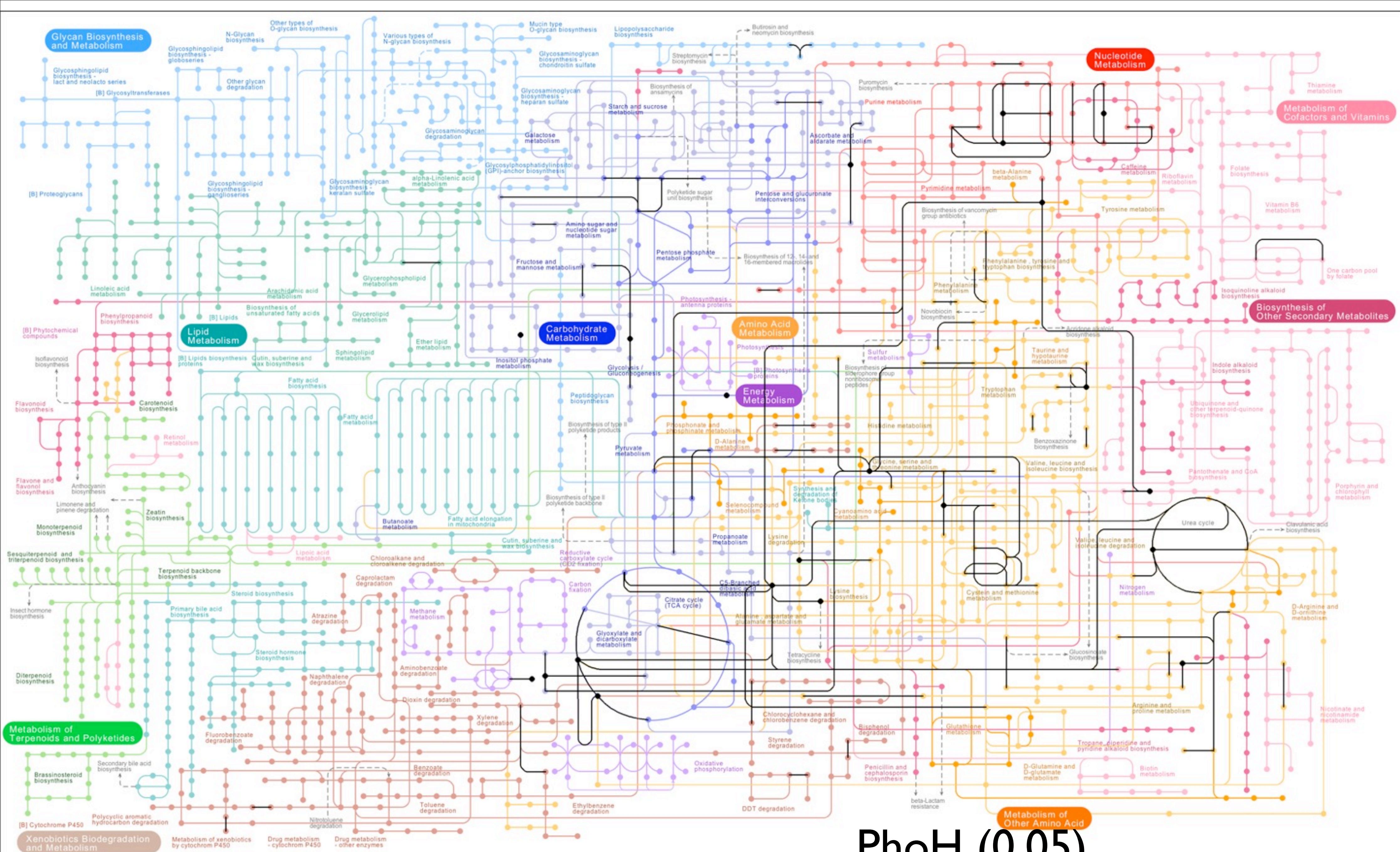
Thioredoxin (0.05)



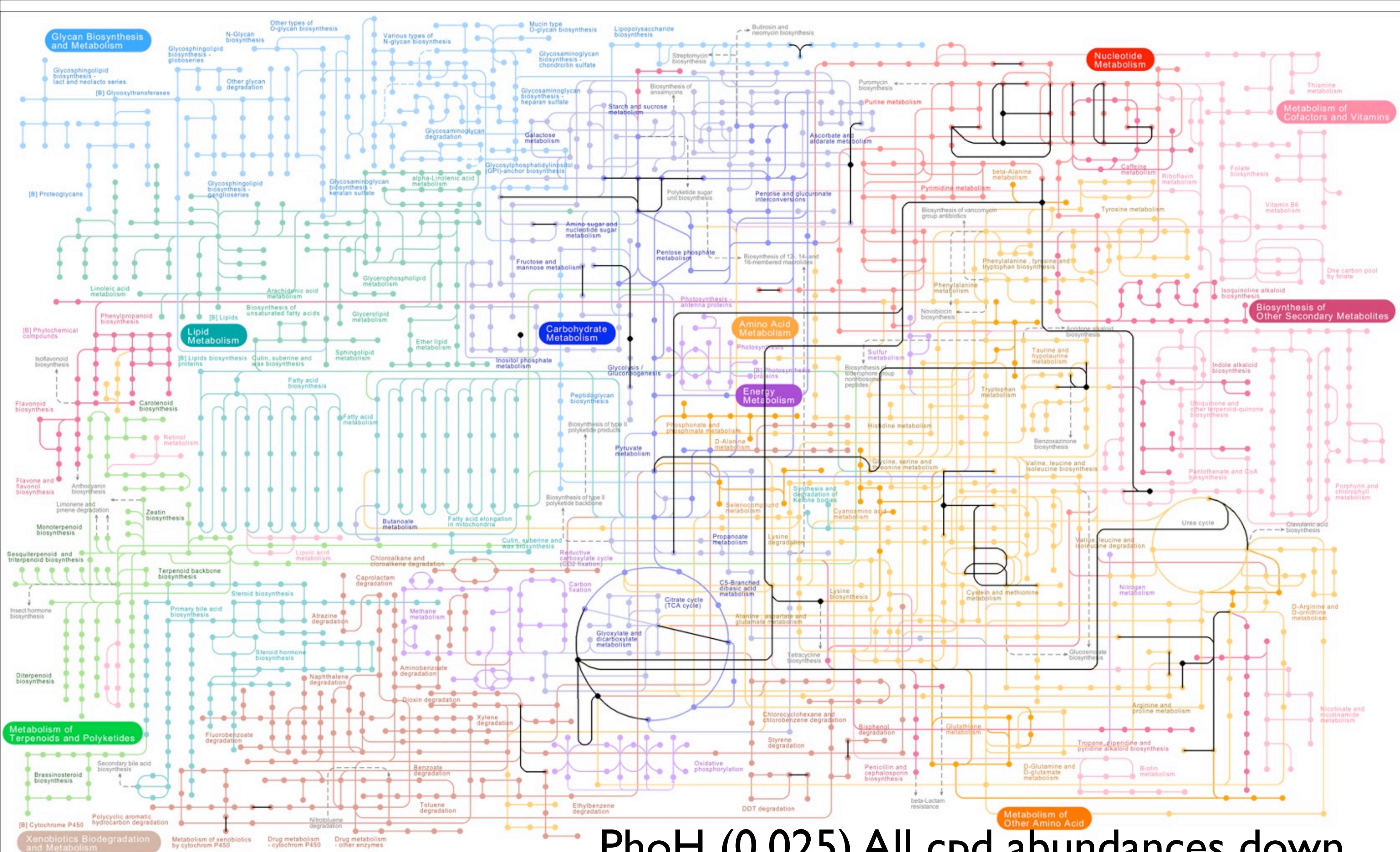
Thioredoxin (0.025) Some cpds up and some down



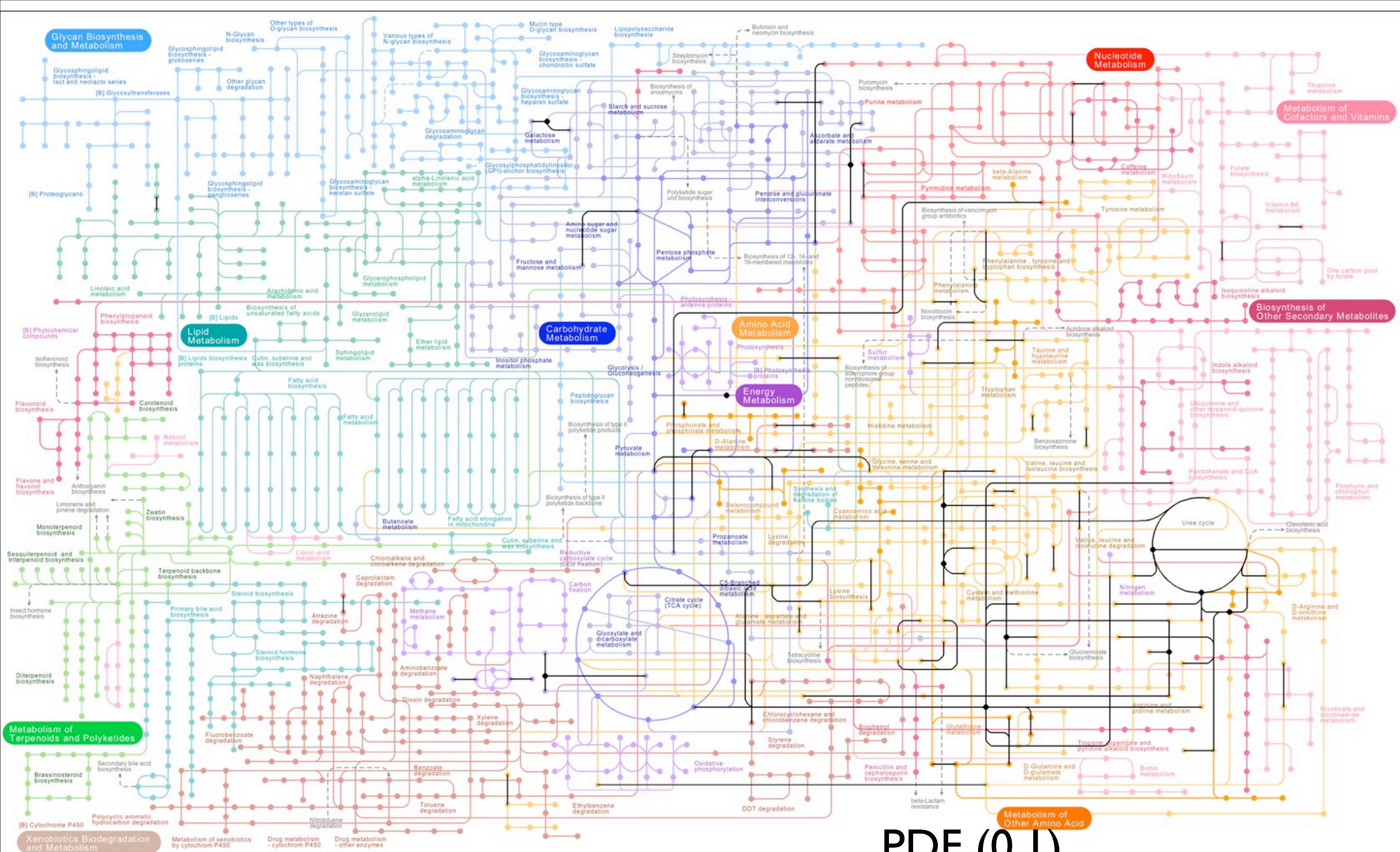
PhoH (0.1)



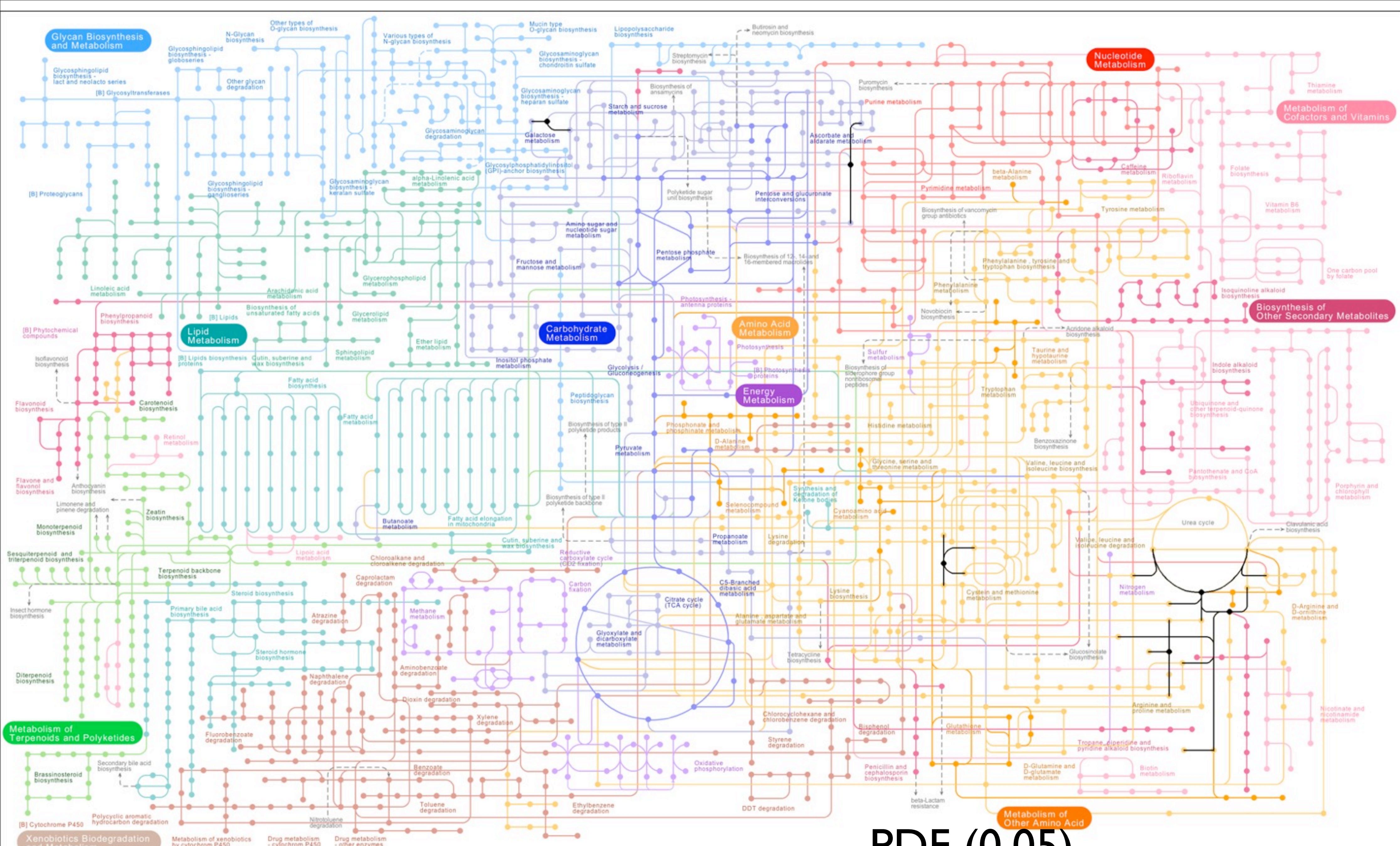
PhoH (0.05)



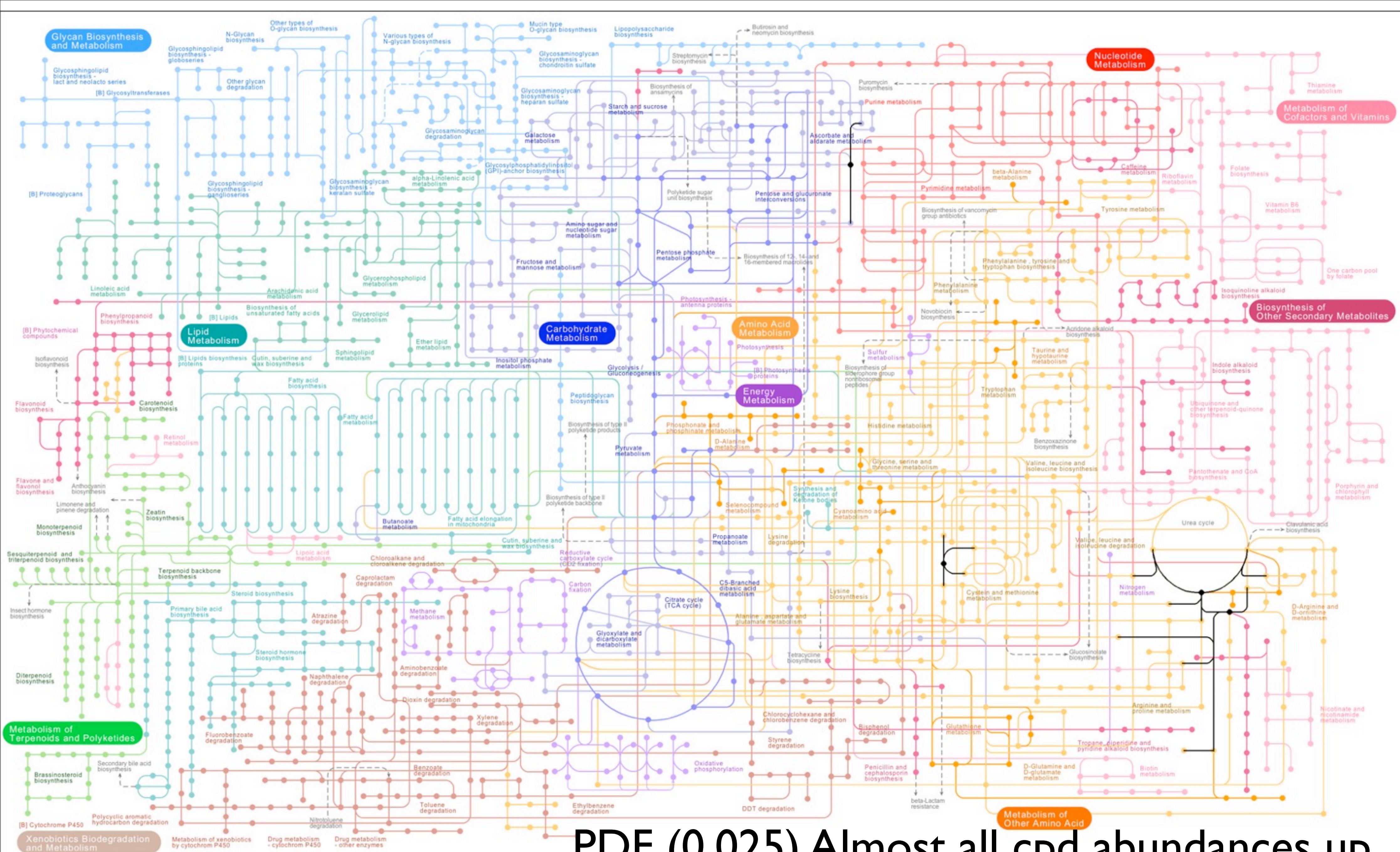
PhoH (0.025) All cpd abundances down



PDF (0.1)



PDF (0.05)



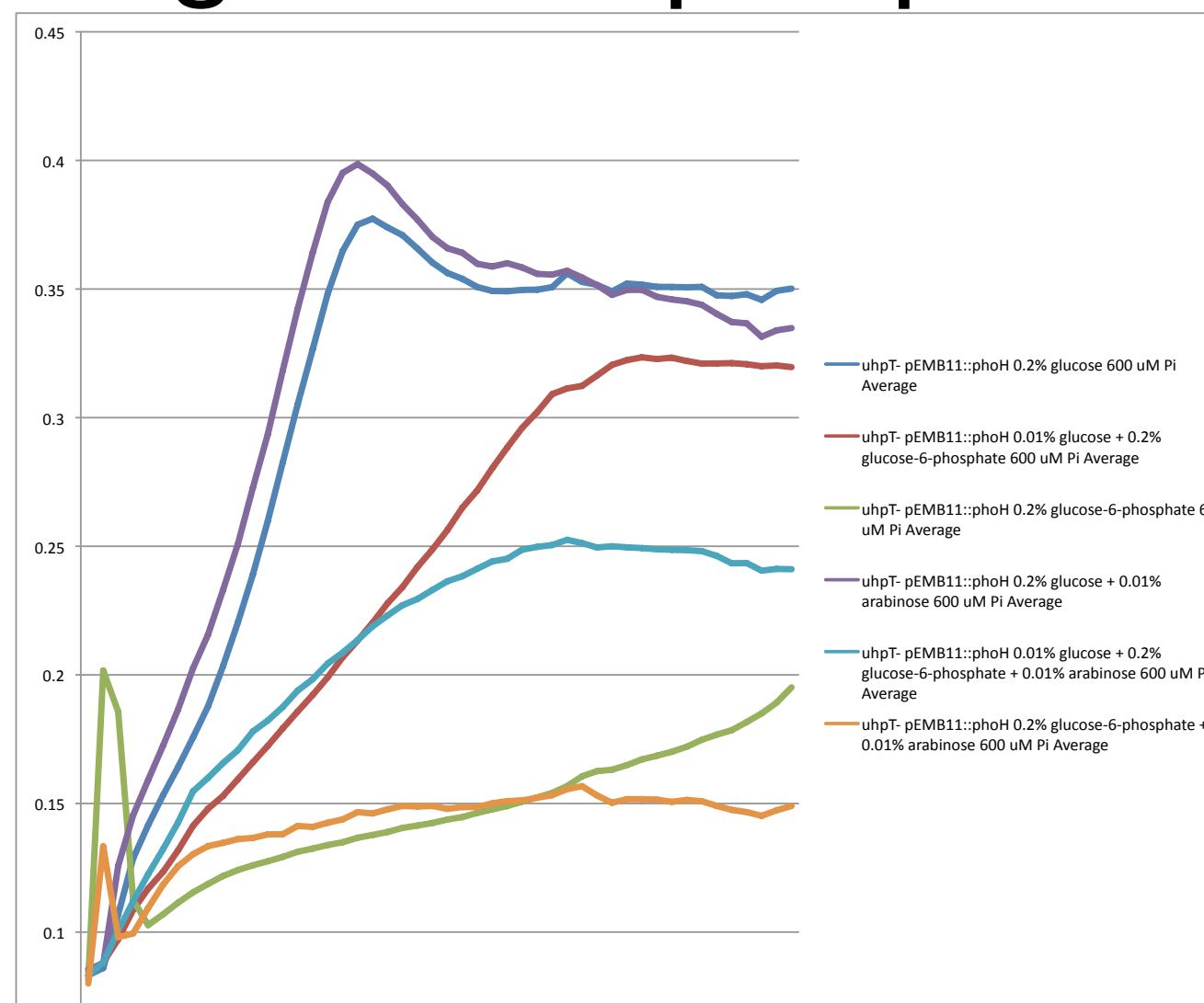
PDF (0.025) Almost all cpd abundances up

Preliminary Metabolomics Data Conclusions

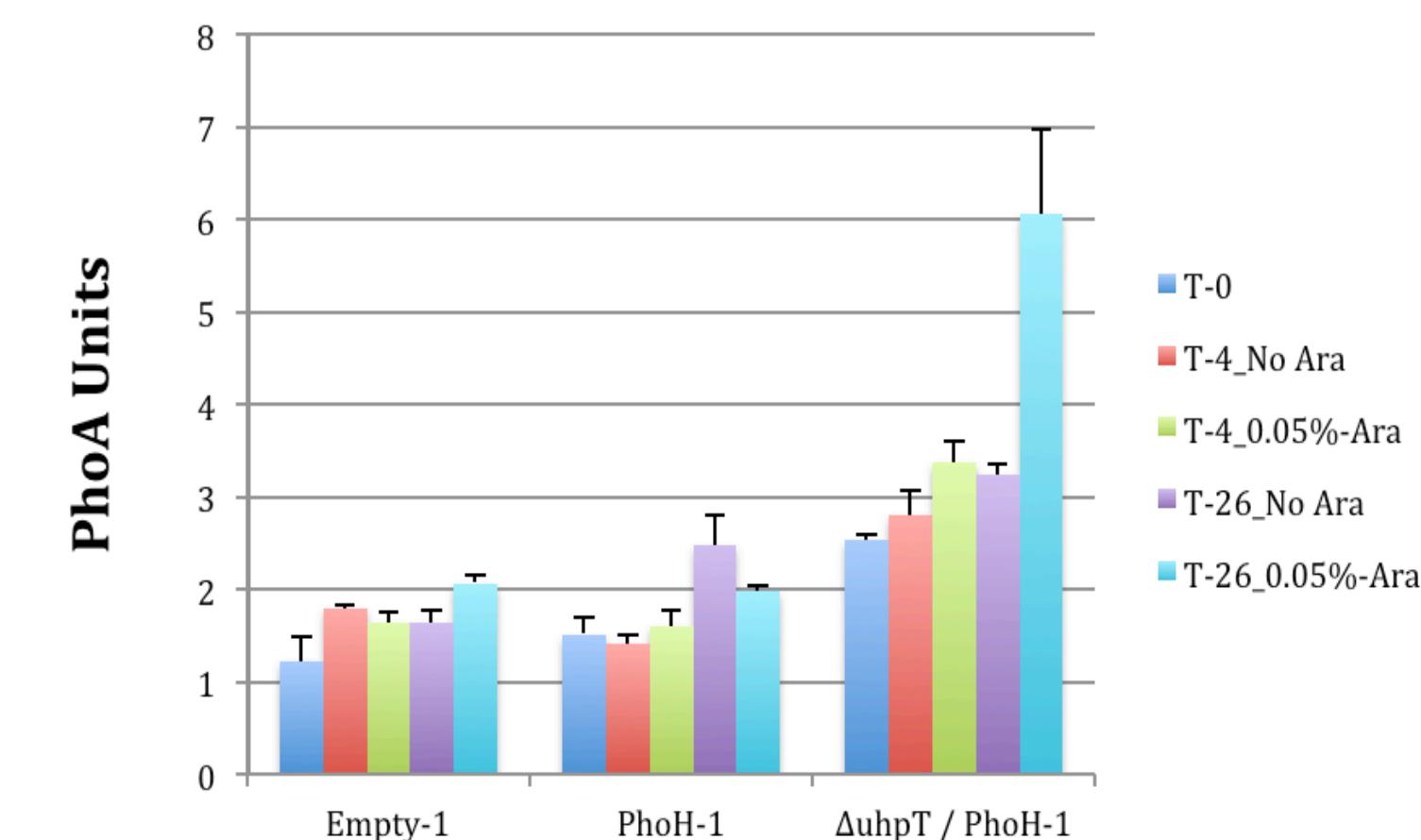
- The good news:
 - The affected metabolisms appear to fall in line with predicted protein function (I need to evaluate this in more detail)
 - In most cases, proteins of the same type generate similar data (in the case of RFs, there are different classes)
- The bad news:
 - A majority of the phage proteins will not produce phenotypes because of our expression host (metabolic genes carried will reflect selective pressures affecting both host and phage)
 - Many of the phenotypes we do see will be similar (because of the relatively limited number of functions required by phage that are generally necessary)

PhoH

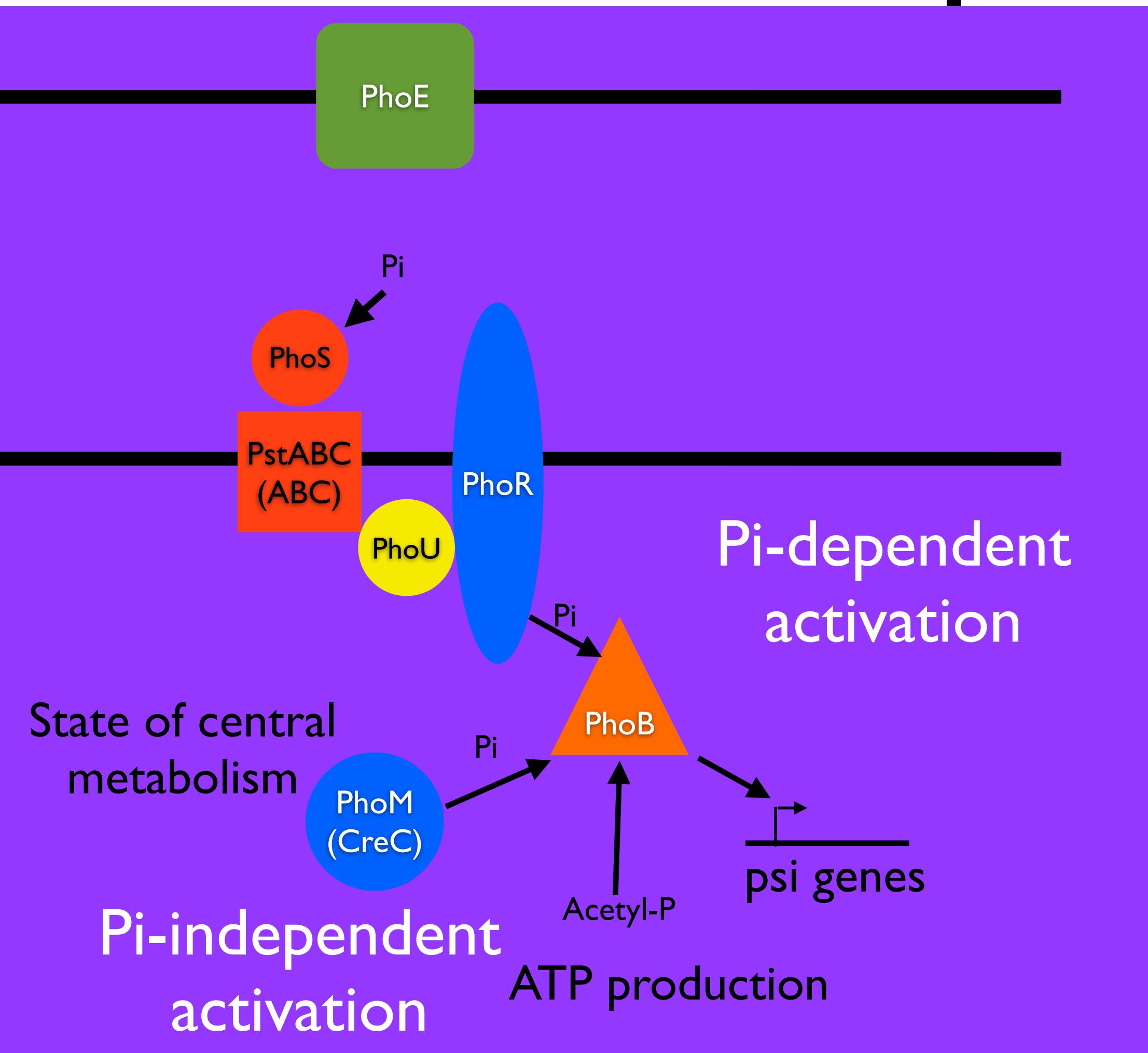
- Based on metabolomics data, wondered if PhoH is a transporter
- The answer is no BUT it gave us better insight and a strain which produced phenotypes (Δ uhpT, hexose phosphate transporter knock out)
- With the over expression of PhoH, Δ uhpT had a decrease in biomass production and an increase in PhoA activity when grown on glucose-6-phosphate + low glucose



PhoA activity
0.2% G-6-P, 0.01% Glu, 200uM Pi

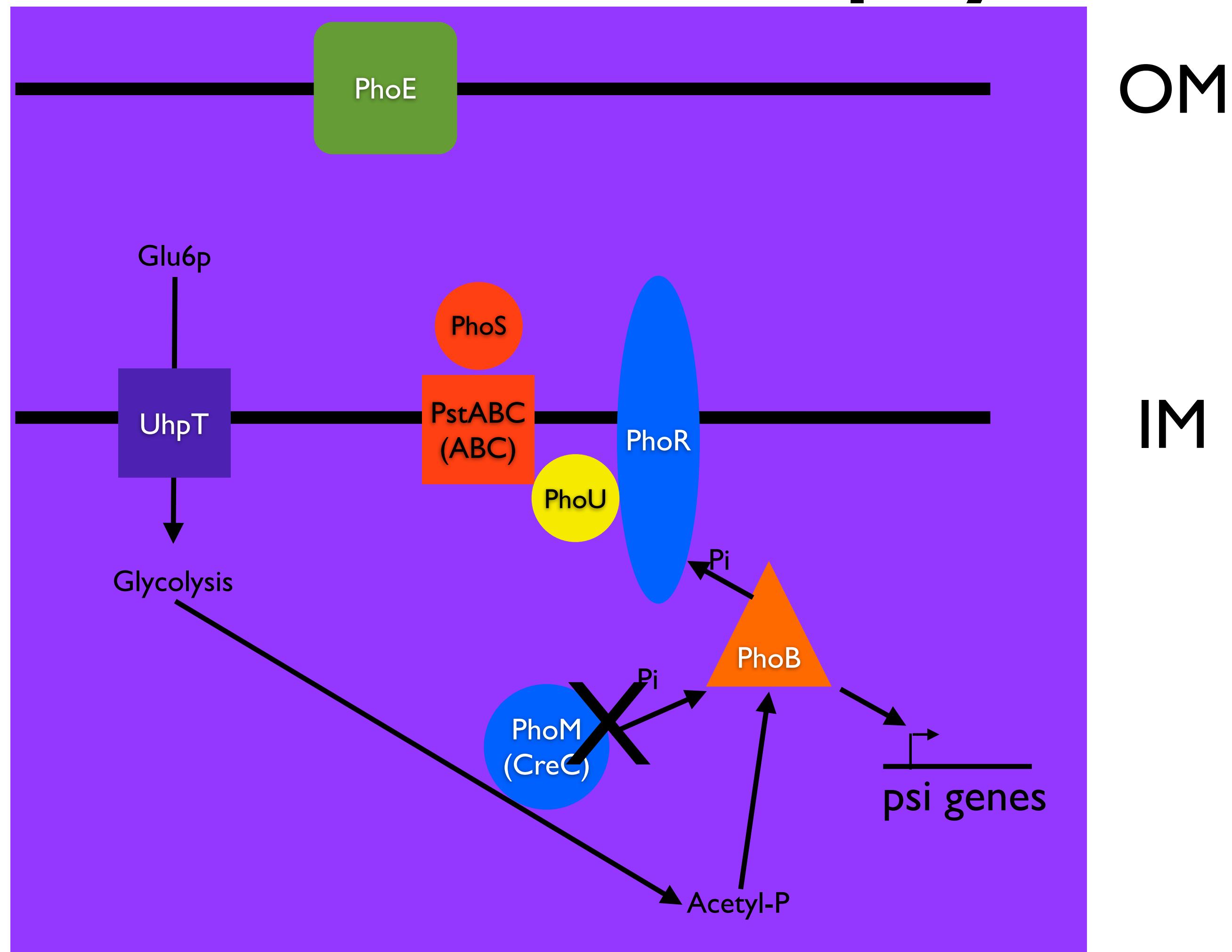


Regulation of PhoA Expression

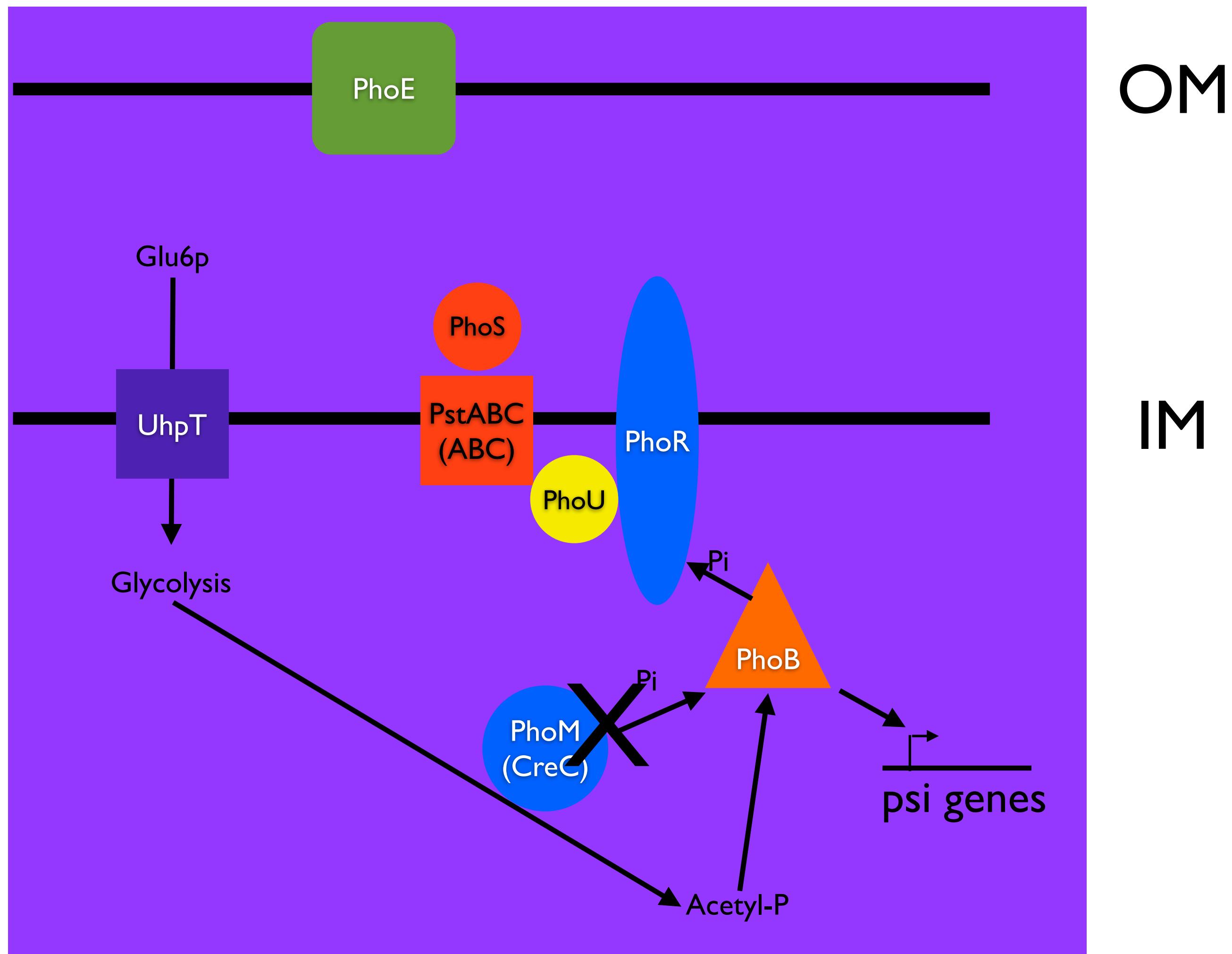


- OM
 - **Pi-dependent:** senses extracellular $[\text{Pi}]$, if low **PhoR** phosphorylates **PhoB** (if high $[\text{Pi}]$, **PhoR** dephosphorylates **PhoB**)
- IM
 - **Pi-independent:**
 - Acetyl-P (global P source for RR autophosphorylation...leads to basal level of activation)
 - **CreC** (HK that senses central metabolism)

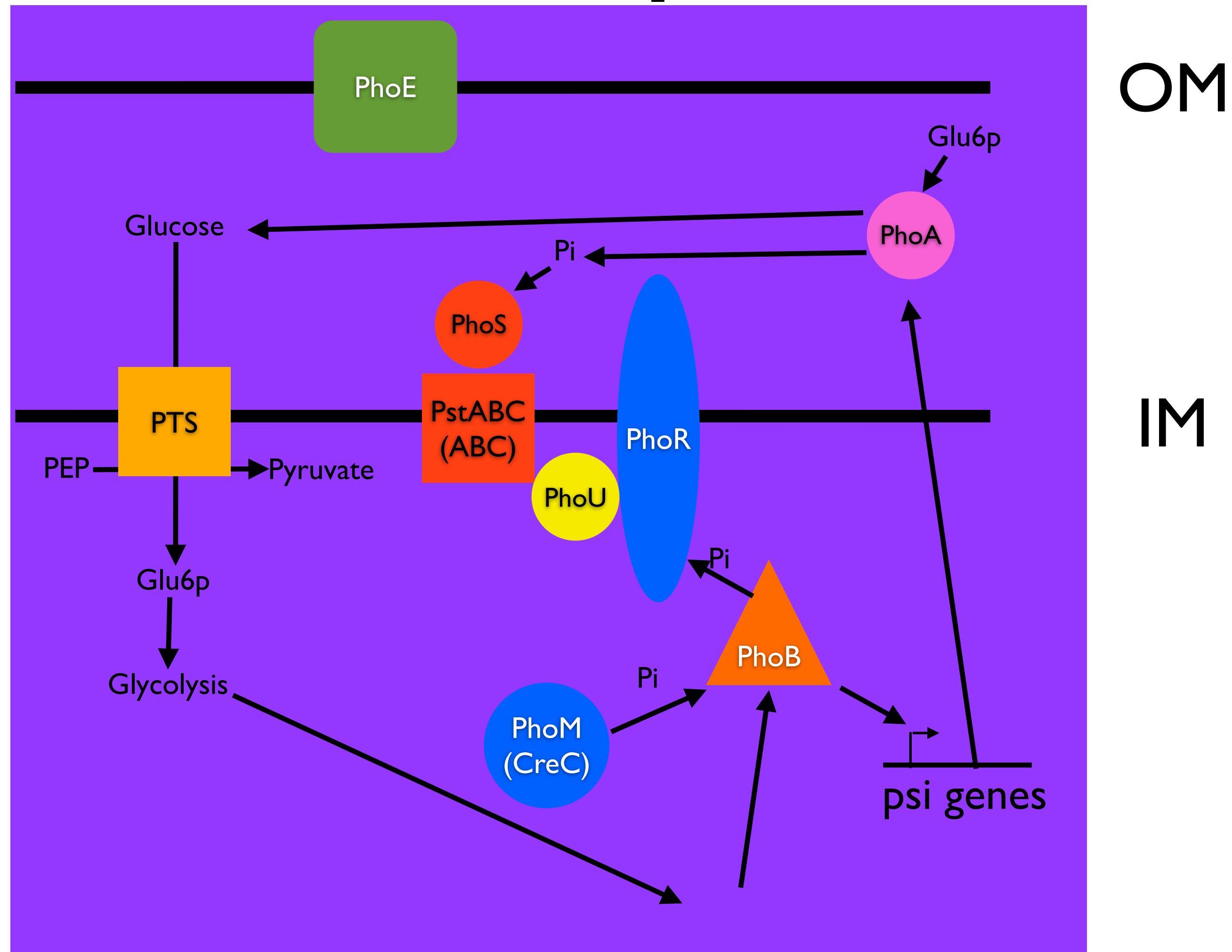
Model for Differences in PhoA Levels: G786 + Empty



Model for Differences in PhoA Levels: G786 + PhoH



Model for Differences in PhoA Levels: Δu_{hpT}



Model for Differences in PhoA Levels: $\Delta u_{hpt} + \text{PhoH}$

- Ligand for CreC unknown (we may be able to shed light on it)
- PhoH can bind ATP
 - Predicted ATPase
- Still working on where PhoH fits in
- Probably something to do with glycolytic flux and [ATP]/[ADP]

