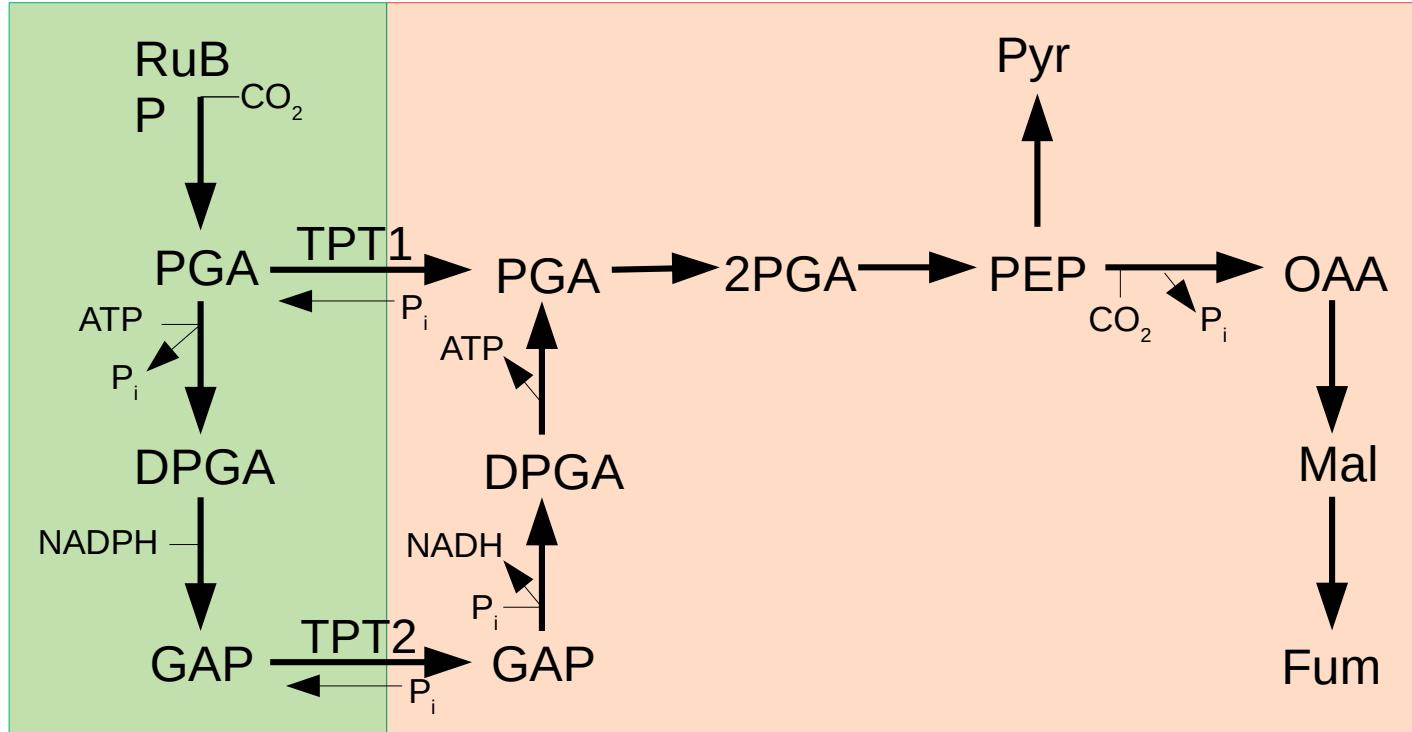


How do we get from carbon assimilation to fumarate accumulation?

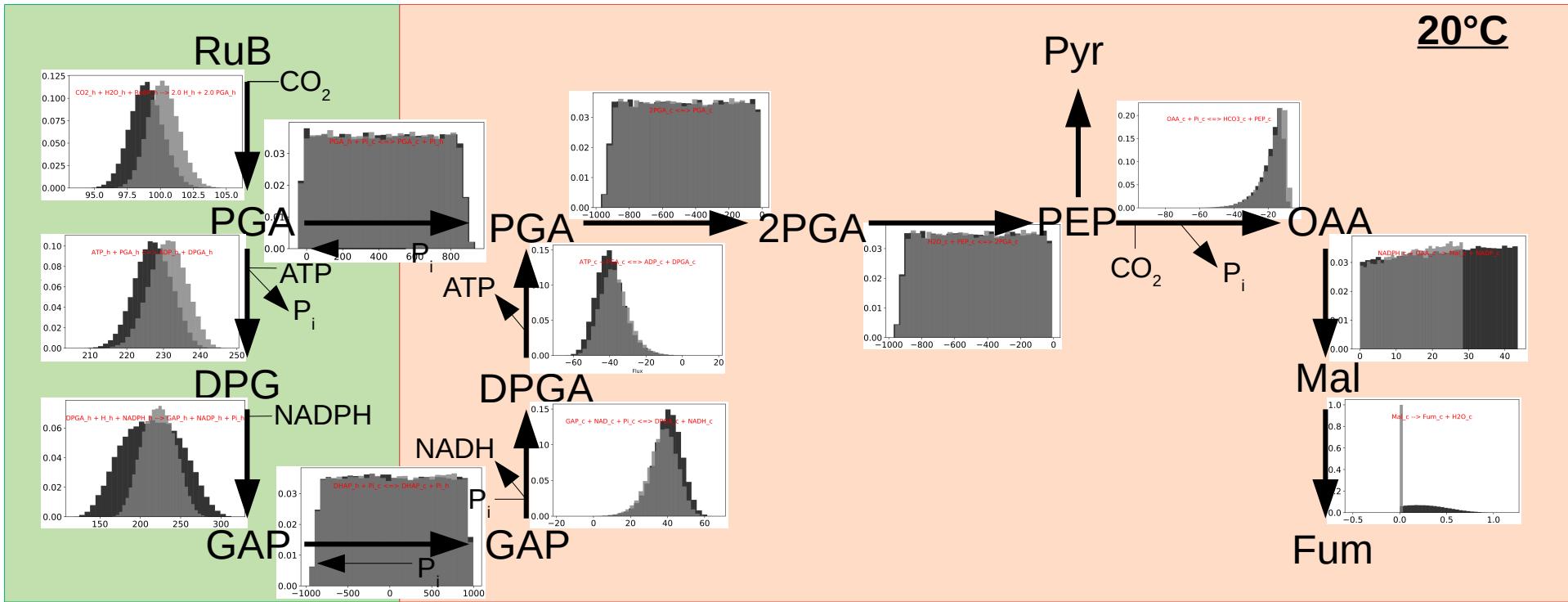
Propose two possible pathways.

Pathways 2 appears to be the defaults pathway.

Pathway 1 may be preferred in NADPH-limiting conditions.



Using the min-path methods we identified all shortest possible paths from carbon assimilation to cytosolic fumarate storage. We verified the possible paths against the flux sampling results in order to ensure that they were able to carry a flux significant enough to contribute substantially to fumarate accumulation and were left the two possible pathways shown (figure above).



Black = Wild-type

Gray = Mutant

Not much of an obvious difference between mutant and wild-type in control conditions, other than that the flux from OAA to Fum has a higher capacity.

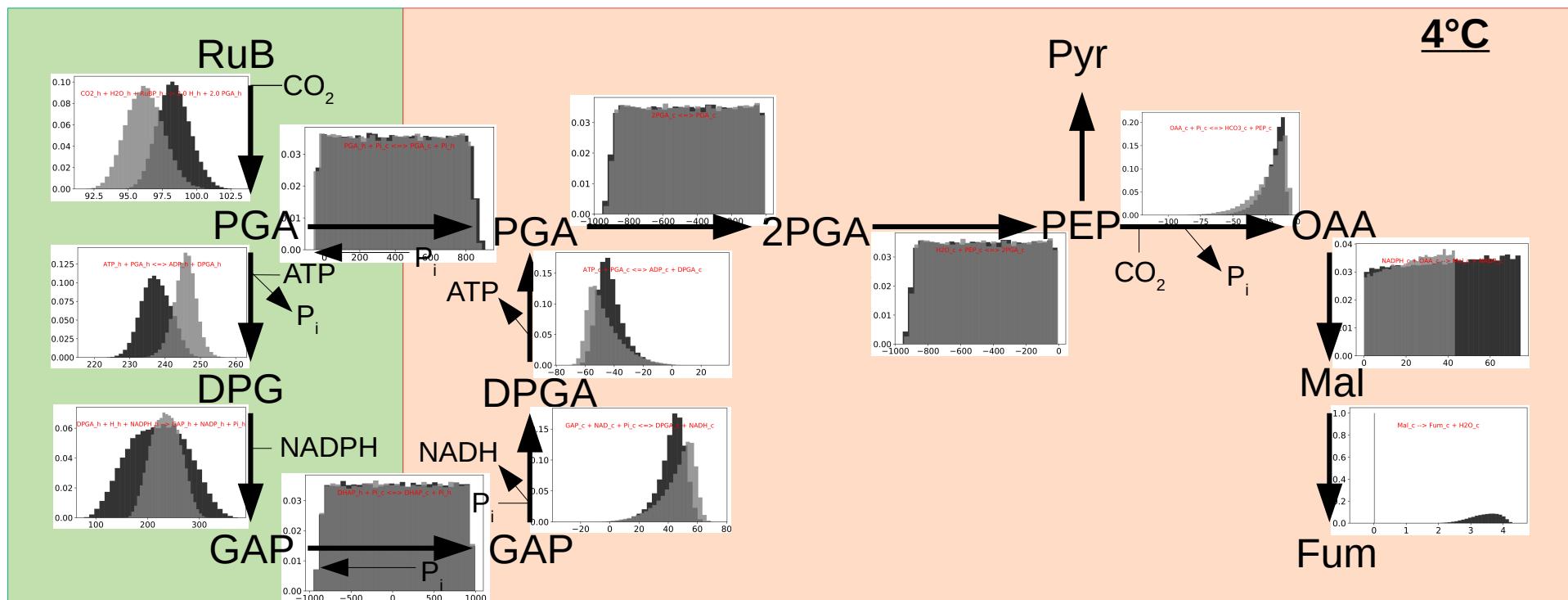
Model constraints are too unspecific to really tell which one of the two pathways is preferred but does demonstrate that pathway 2 must carry a flux from GAP to PGA in the cytosol

Black = Wild-type
 Gray = Mutant

Capacity for carbon uptake is higher in the wild-type than in the mutant in cold conditions.

Fluxes show that cytosolic fumarase is essential for fumarate production in the cold.

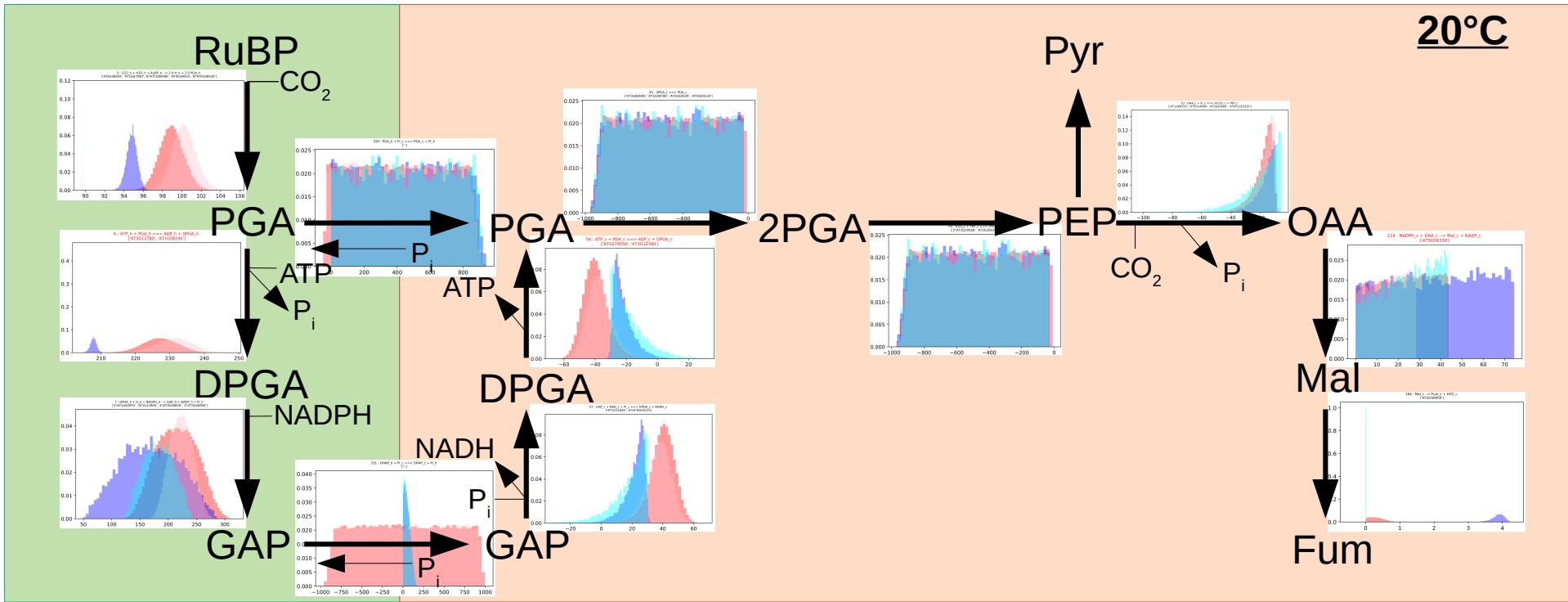
Capacity for Pathway 2 is increase in both mutant and wild-type equally in response to cold but given that total flux to fumarate and malate is higher in the wild-type suggests that Pathway 1 is also increased in the wild-type in response to cold.



	<i>tpt1</i>		<i>tpt2</i>	
	ATPase	Fd_NADPH	ATPase	Fd_NADPH
Col0 20°C	[82 , 107]	[93 , 250]	[90 , 107]	[68 , 241]
Col0 4°C	[90 , 107]	[110 , 250]	[100 , 107]	[105 , 230]
fum2 20°C	[87 , 107]	[127 , 250]	[95 , 107]	[107 , 235]
fum2 4°C	[90 , 107]	[166 , 250]	NA	NA

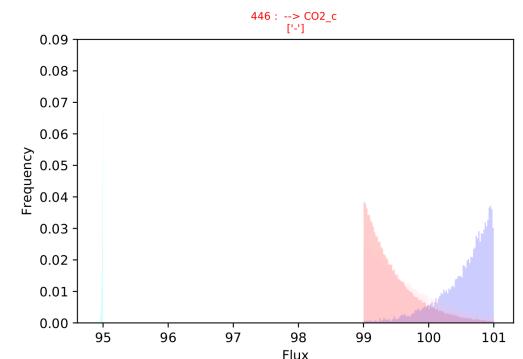
We simulated knockout mutants of *tpt1* and *tpt2* under the proteomics and metabolic constraints set for Col0 and *fum2* at 4°C and 20°C. The FVA results (table above) show that the TPT2 pathway is more NADPH-demanding, whereas the TPT1 pathway is more ATP-demanding.

The constraints set for the *fum2* mutant in 4°C cannot be met when knocking out TPT2 as this is an essential reaction in the cold.



Comparing mutant and wild-type in cold and control conditions where we have set an NADPH-limitation in cold conditions.

Pathway 2 has less capacity in NADPH-limited conditions (i.e. in the cold) but overall flux from OAA to Mal and Fum is increased in the cold in the wild-type, therefore likely to be using Pathway 1 more in the cold.



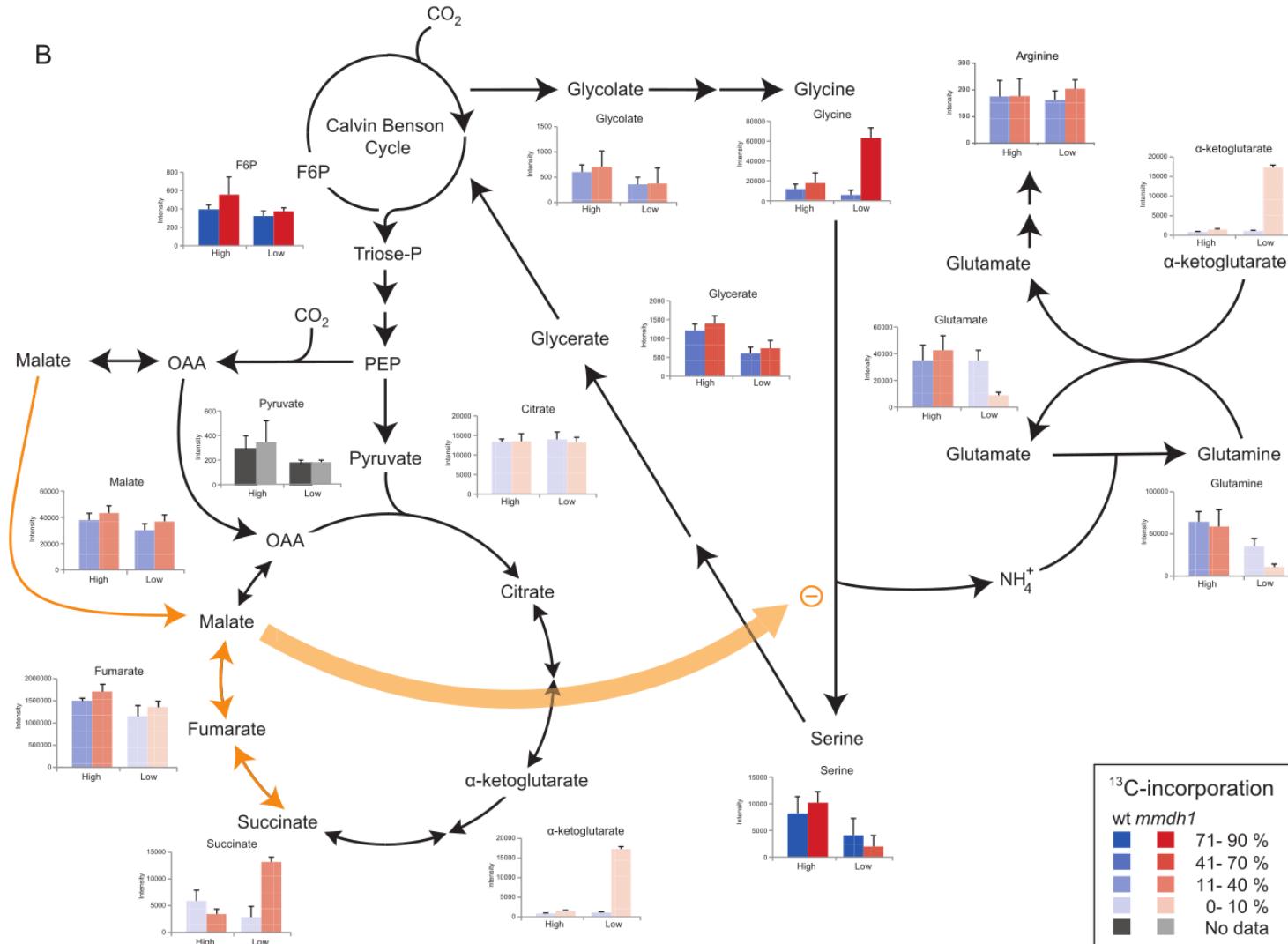
Why put carbon into fumarate and not malate?

To better control the level of malate

Why do malate levels need to remain constant?

- malate suppresses NR (evidence in tobacco)
- malate affects glycine to serine ratio and subsequent photorespiration (evidence in Arabidopsis)

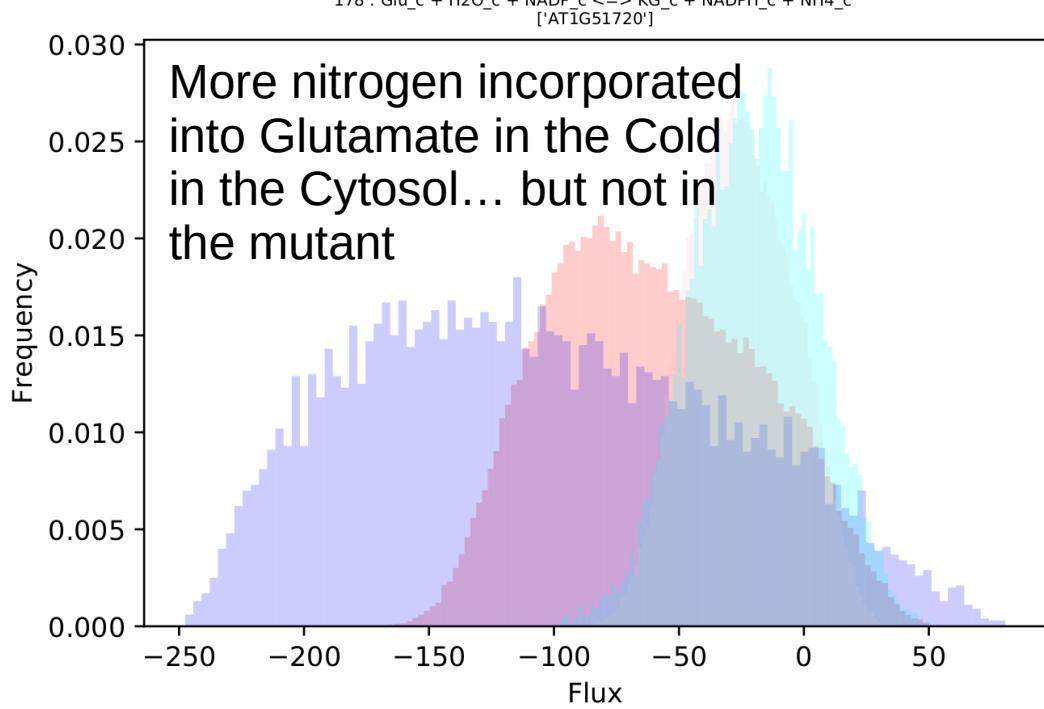
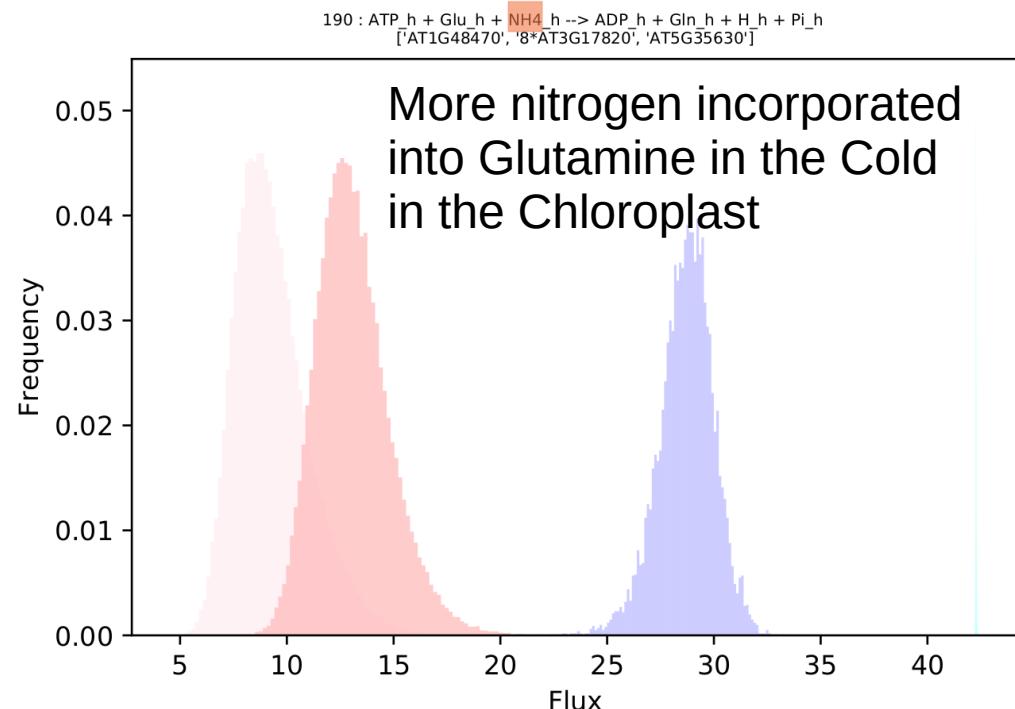
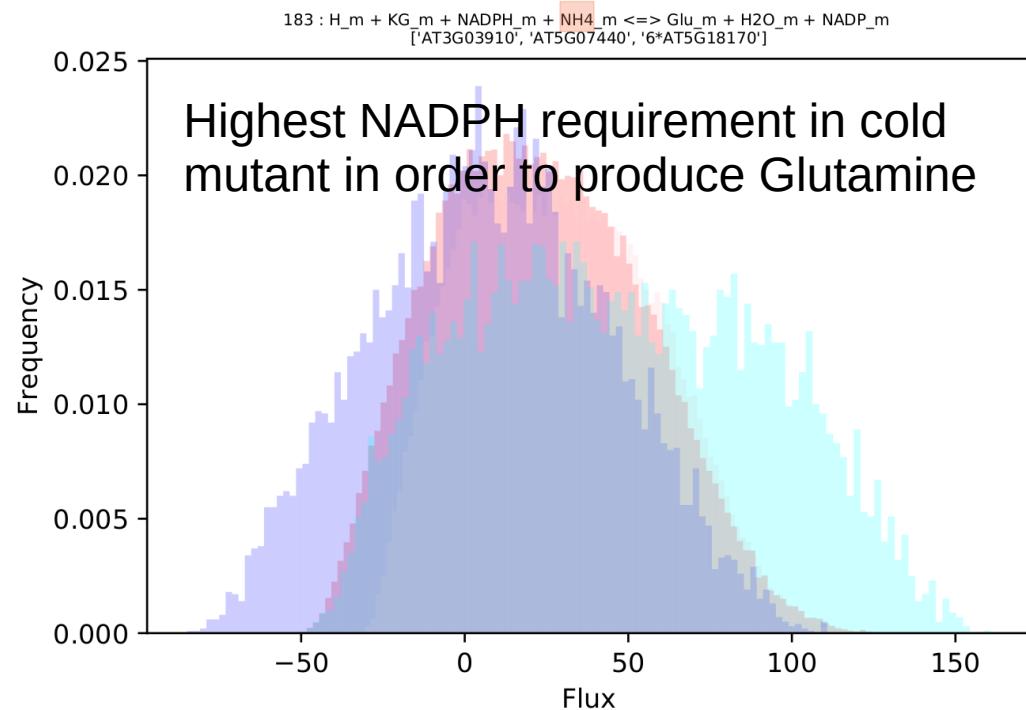
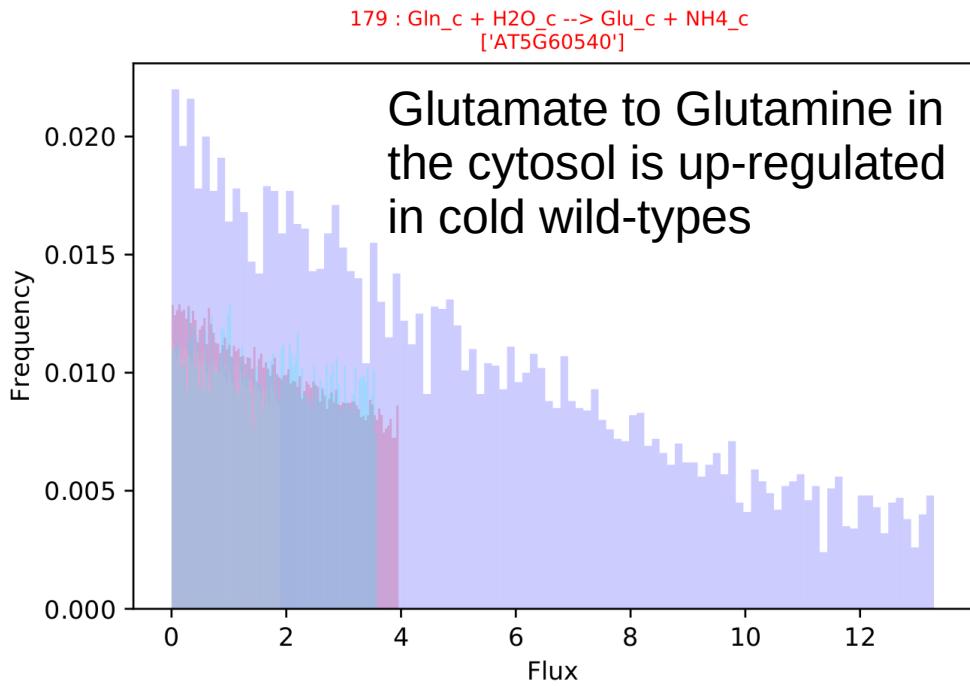
B



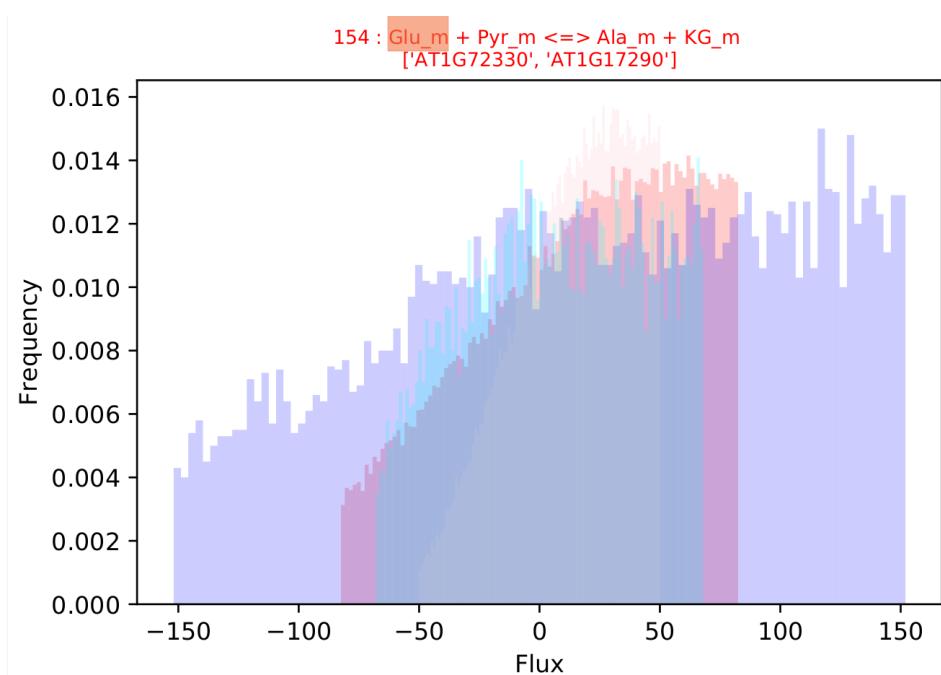
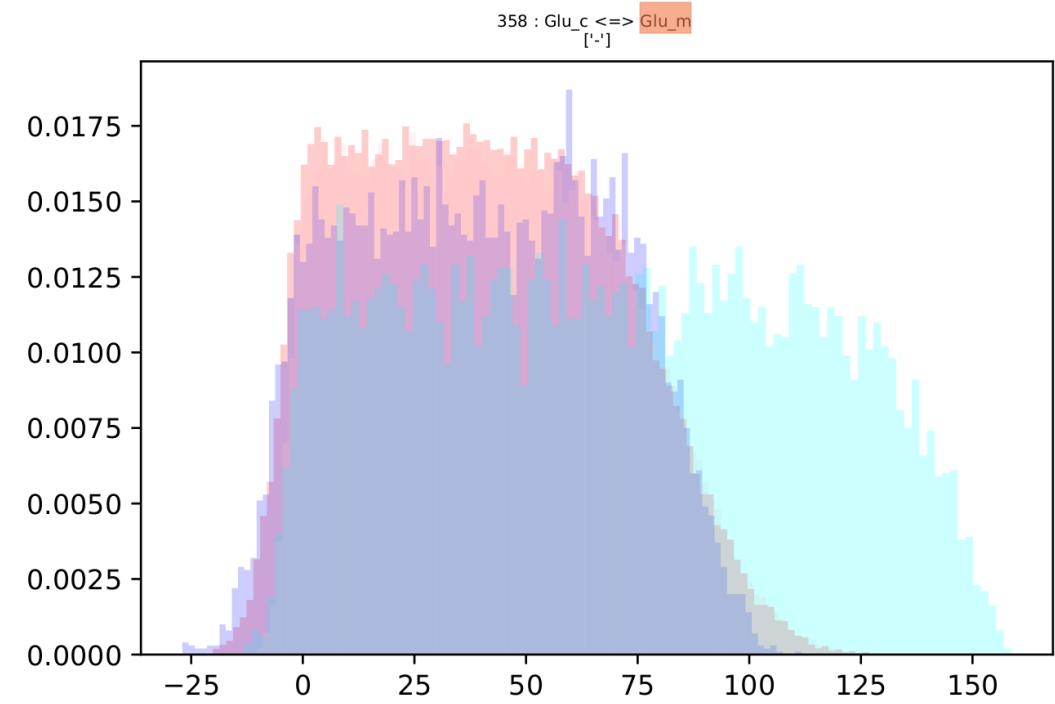
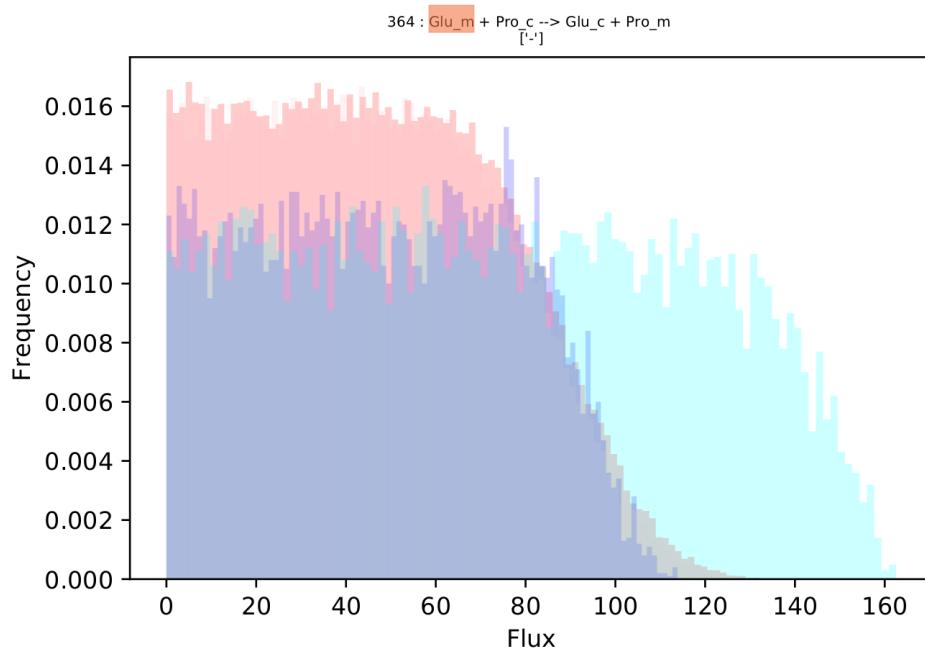
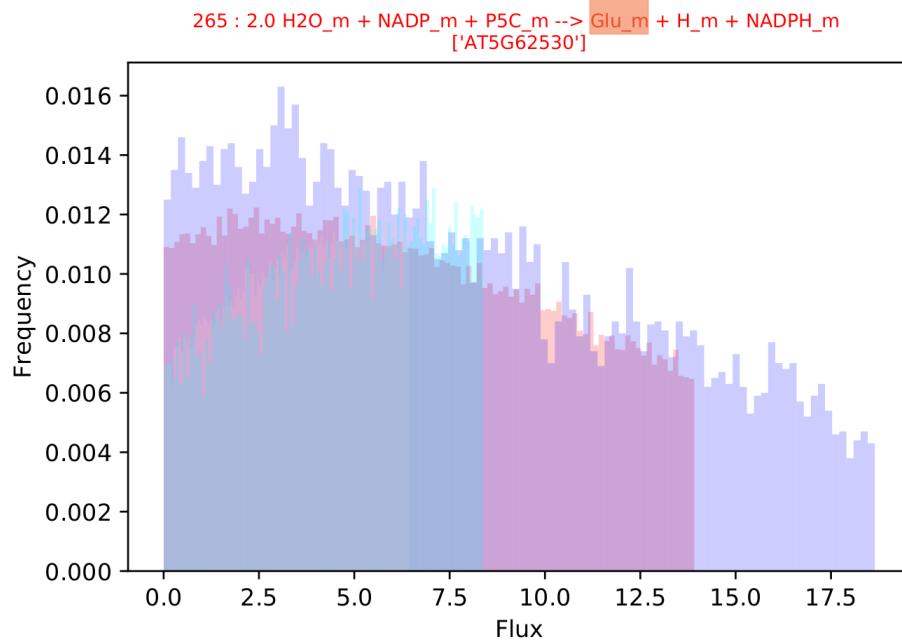
Linden et al. 2016, JXB

- greater flux from OAA_m to Mal_m means greater flux from Gly to Ser
- greater export of malate from cytosol to mitochondria means less flux from Gly to Ser

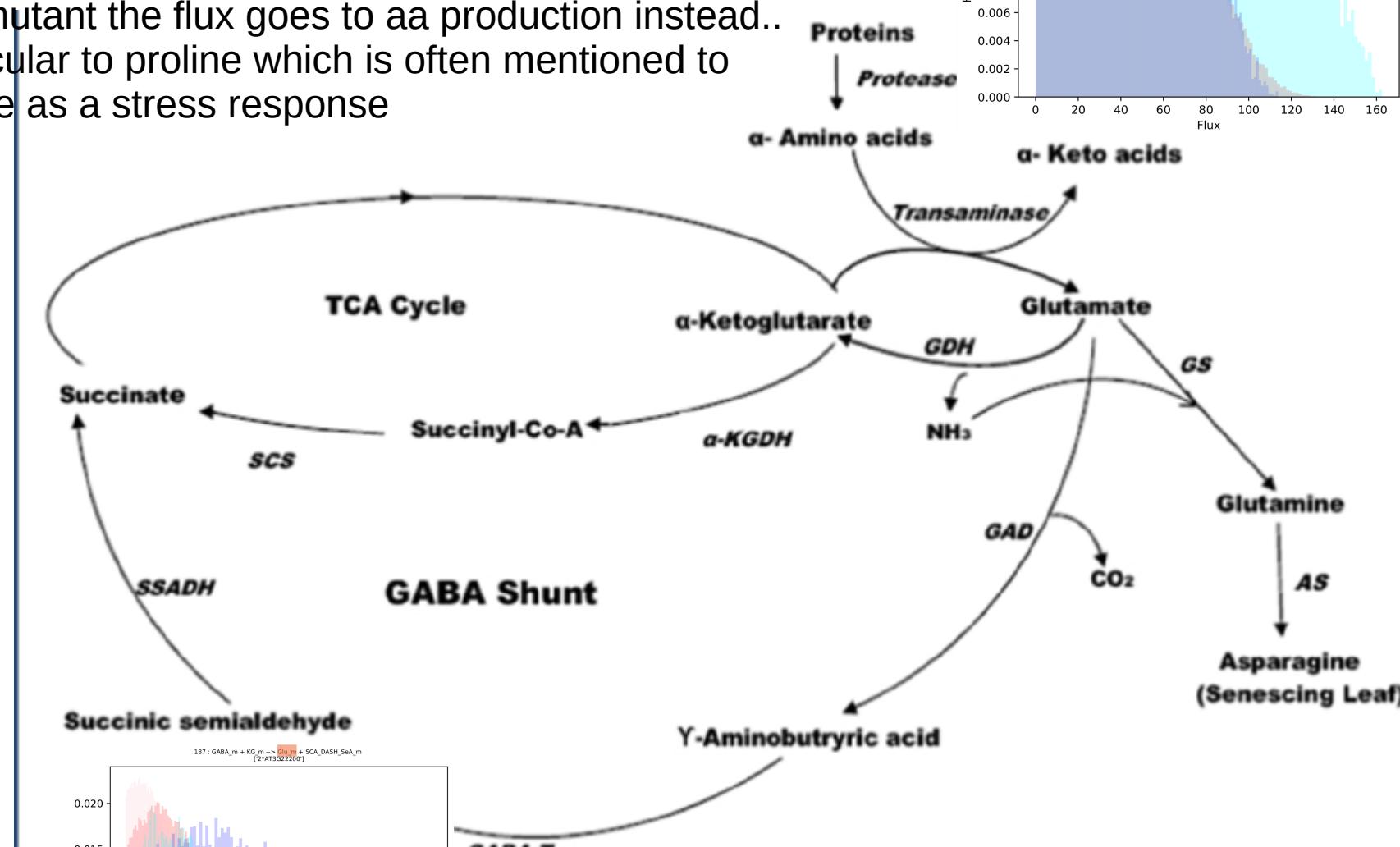
The increase in glycine/serine ratio could indicate that in mmdh1 the limitation in OAA to malate conversion directly influences the glycine to serine conversion although the increased ratio could also reflect an adjustment to maintain the flux through the GDC. However, the significant reduced growth of mutant plants in strong photorespiratory conditions and the effects on glutamate/glutamine/ α -ketoglutarate (see below) support a direct limitation in the reaction. A reduced capacity to shuttle NADH produced in glycine decarboxylation from the mitochondria out to the peroxisomes is likely to result in an increased NADH/NAD⁺ ratio in the mitochondrial matrix. This could in turn inhibit the glycine decarboxylase complex, which is inhibited by NADH with a K_i of 15 μM (Bykova et al., 2014). Furthermore, the reductions in glutamate and glutamine pools together with the increase in α -ketoglutarate are most likely related to the reduced rates of ammonium production, from mitochondrial glycine oxidation, which would limit its re-fixation via the GS/GOGAT system.



Glutamine Production



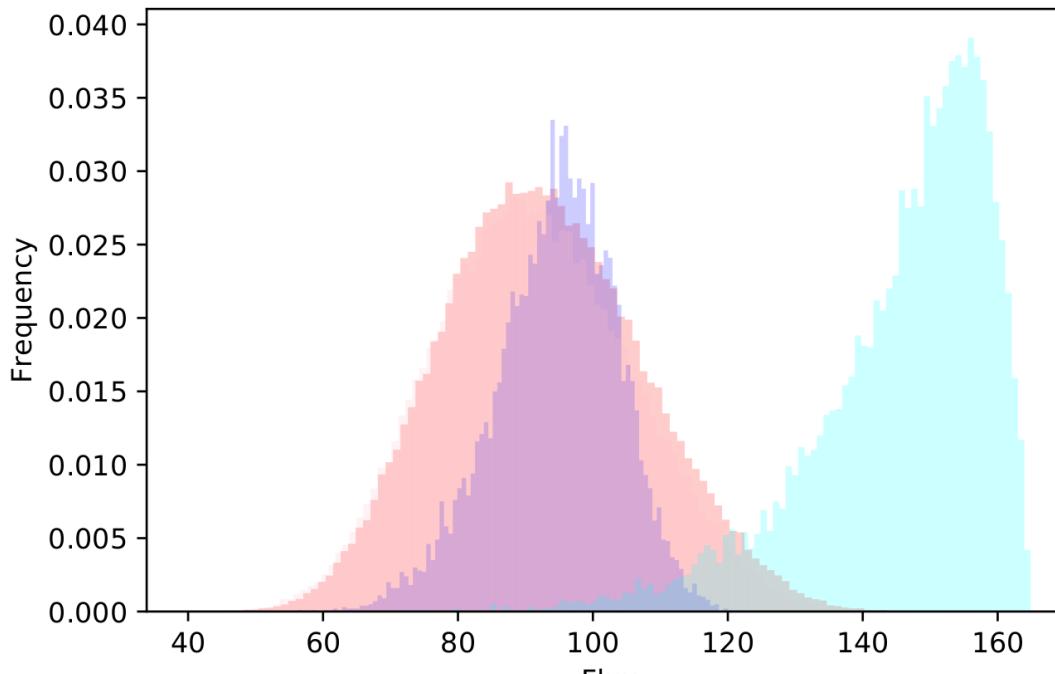
Both mutant and wild-type plants have increased glutamine production in the cold. In the wild-type a large part of this seems to be re-directed through the GABA shunt back into the TCA cycle via succinate. In the mutant the flux goes to aa production instead.. in particular to proline which is often mentioned to increase as a stress response



I kind of lost track how this all links back to malate but that's what got me here in the first place... see Slide 8.
Also all of this clearly links to Nitrogen metabolism...

2-Oxoglutarate (KG) participates in a range of reactions in distinct plant cell compartments (Weber and Flügge, 2002; Foyer et al., 2003), also being a key metabolite at the crossroads of carbon/nitrogen metabolism as it is required for ammonia assimilation (Hodges, 2002).

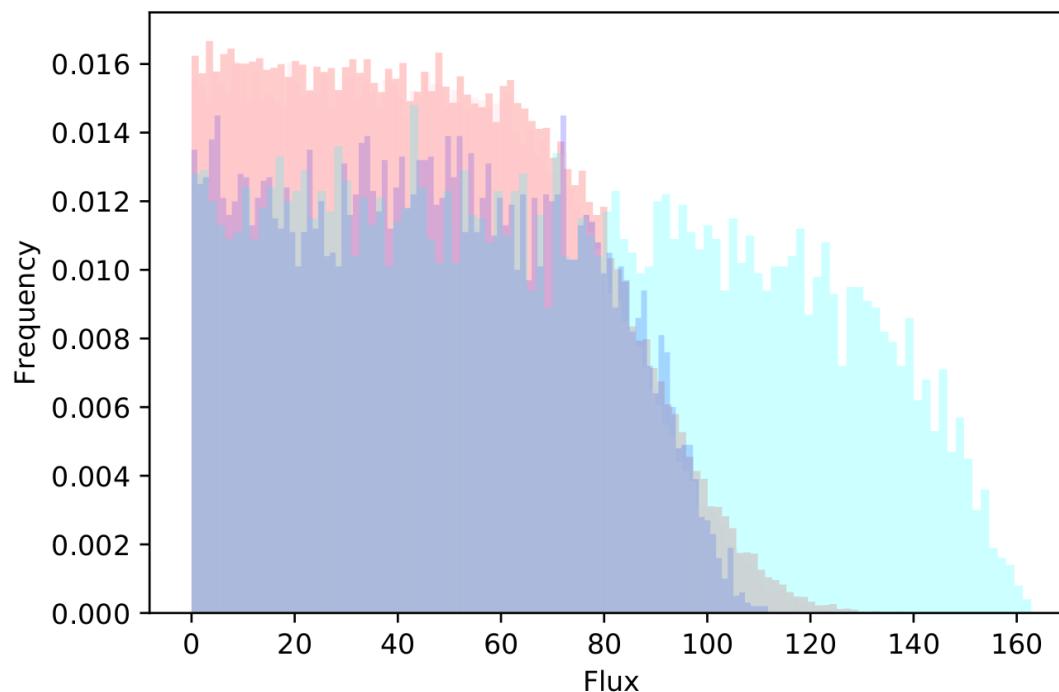
260 : H_c + NADH_c + P5C_c --> NAD_c + Pro_c
['AT5G14800']



Proline accumulates as a stress response

Lots in the literature about this....

262 : NADP_m + Pro_m --> 2.0 H_m + NADPH_m + P5C_m
['AT5G38710', 'AT3G30775']



From the flux sampling paper:

The sampling distributions suggest a trade off between increased carbon compound accumulation and decreased amino acid production (Fig 4), linking nitrogen and carbon metabolism. Synthesis of γ -aminobutyric acid (GABA), however, is predicted to increase in the cold. GABA has previously been reported to accumulate in response to energetically demanding stresses, including cold treatment [58]. GABA has been suggested as a signalling molecule of the carbon to nitrogen status in plant leaves and evidence for its role in regulating nitrate uptake exists for both rapeseed and *A. thaliana* [59–61]. *A. thaliana* plants in the cold show increased nitrogen assimilation compared to those in control conditions [62].

Increased levels of malate have previously been shown to suppress nitrate reductase expression and activity in tobacco leaves [63]. Malate levels in Col-0 may be kept below a certain threshold, by redirecting carbon to fumarate, thereby supporting adequate nitrogen assimilation and an increased photosynthetic capacity. This hypothesis is supported by the observation that *A. thaliana* mutants, which show increased levels of malate and decreased levels of fumarate, grow significantly less well in high-nitrogen conditions than Col-0 [21]. Fumarate has few known metabolic functions in *A. thaliana* leaves [25], and may thus serve the purpose of a carbon storage buffer in changing environmental conditions.