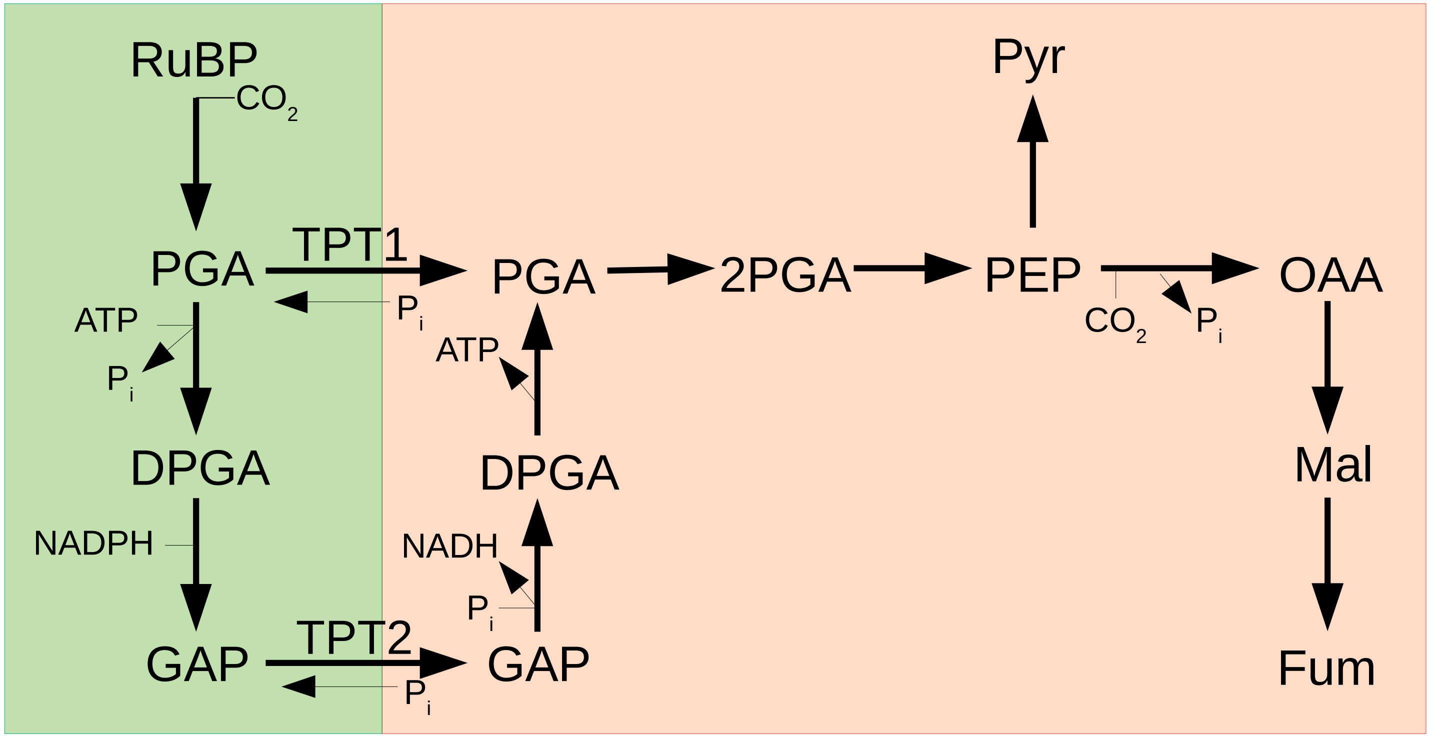
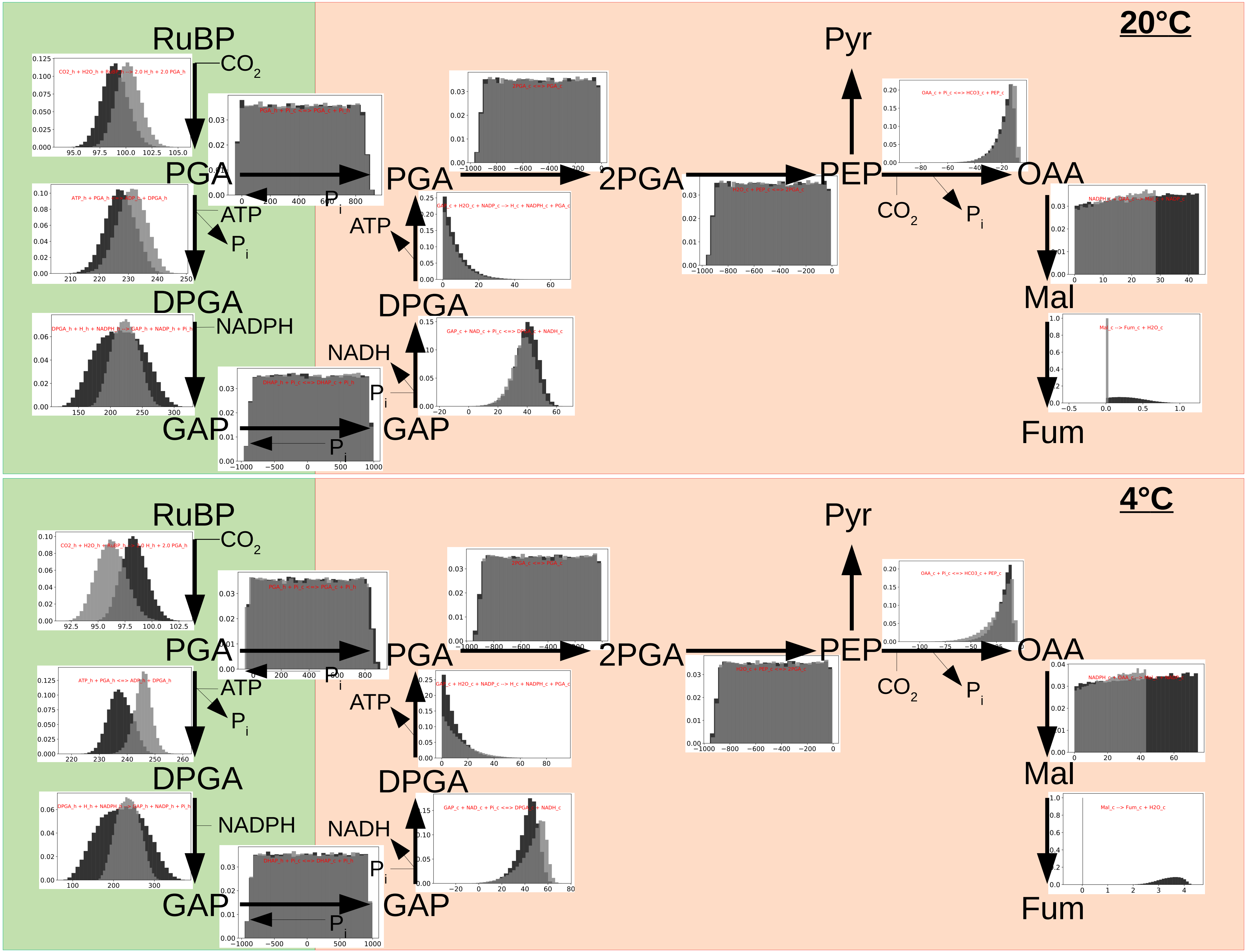


Pathway 1: via TPT1

Pathway 2: via TPT2

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).


20°C
RuBP
CO_2
PGA
PGA
ATP
P_i
DPGA
NADPH
GAP
GAP
NADH
DPGA
2PGA
PEP
Pyr
OAA
CO_2
Mal
Fum
4°C
RuBP
CO_2
PGA
PGA
ATP
P_i
DPGA
NADPH
GAP
GAP
NADH
DPGA
2PGA
PEP
Pyr
OAA
CO_2
Mal
Fum

	tpt1		*tpt2*	
	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.