

Central Hypothesis for ME/CFS

There are one or more **Metabolic Traps**

These are the existence of two stable metabolic states:
a healthy state and a Disease state

ME/CFS Patients rarely if ever become healthy
However, the very few that do become healthy are totally normal
What is blocking the return to a healthy state?

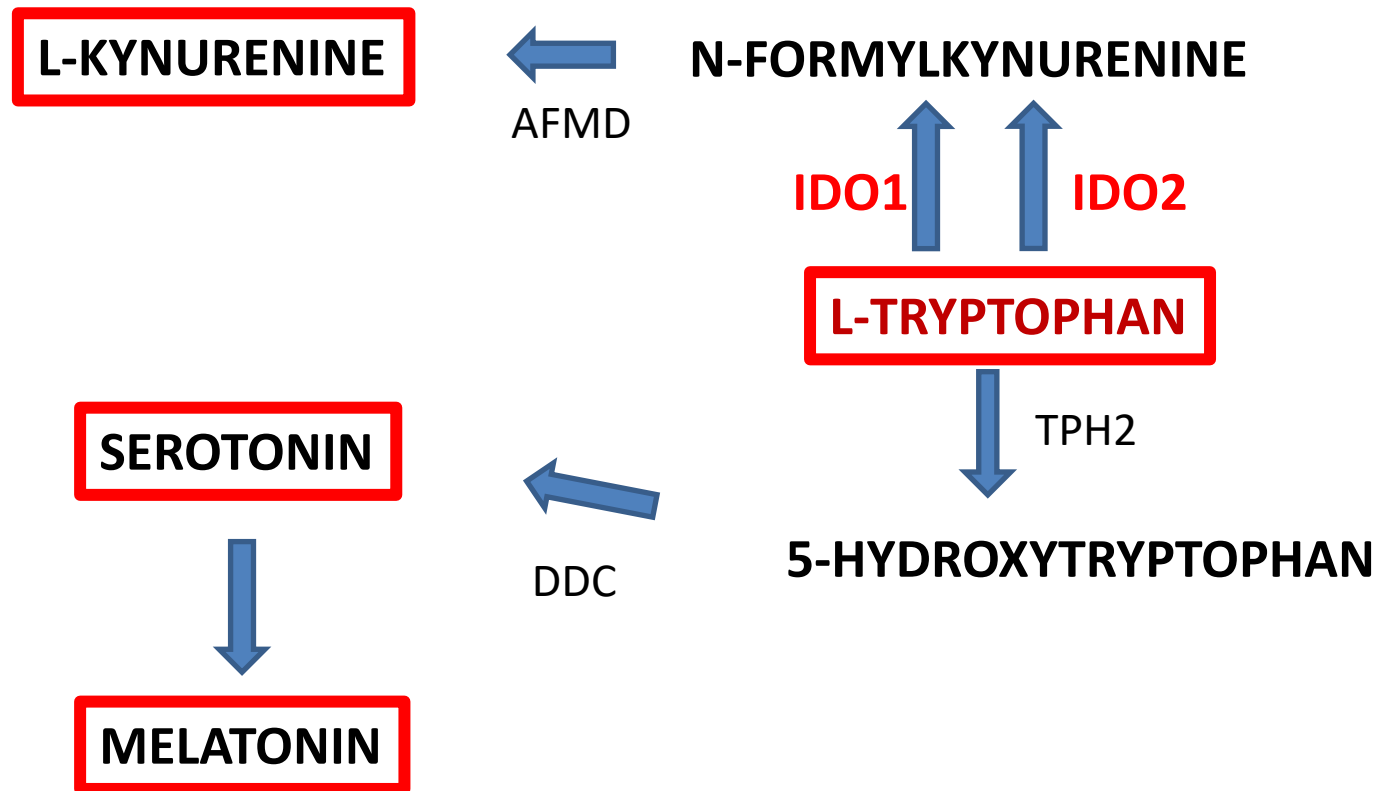
Metabolic Trap by Substrate inhibition

There are 80 enzymes that show substrate inhibition

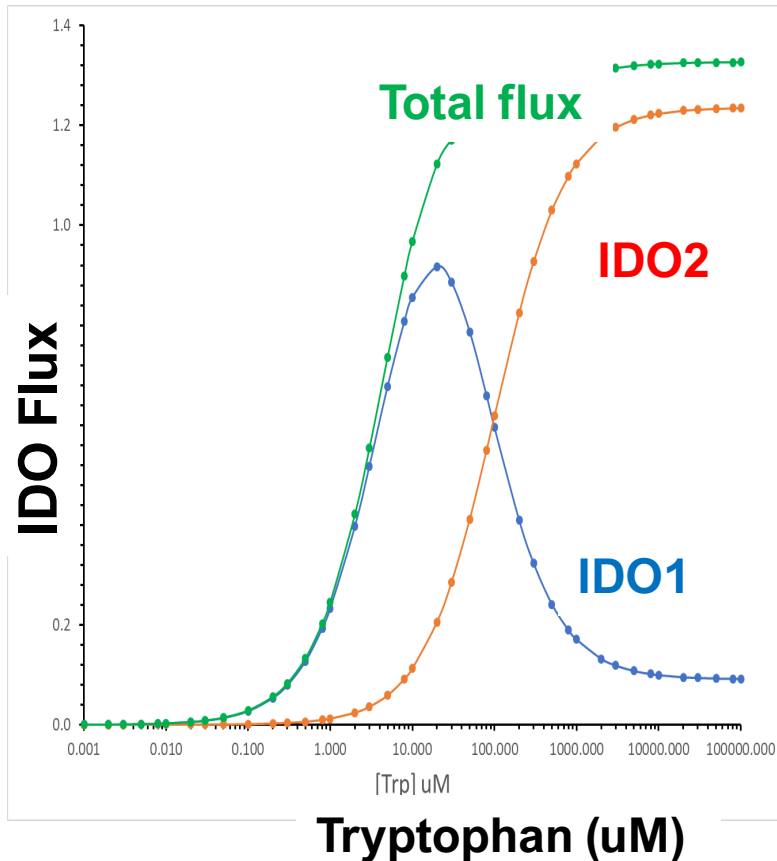
The kynurenic pathway shows substrate inhibition and
could form a metabolic trap

The kynurenic pathway is important for immune regulation

IDO1&2 catalyzes the first step of the kynurenic pathway

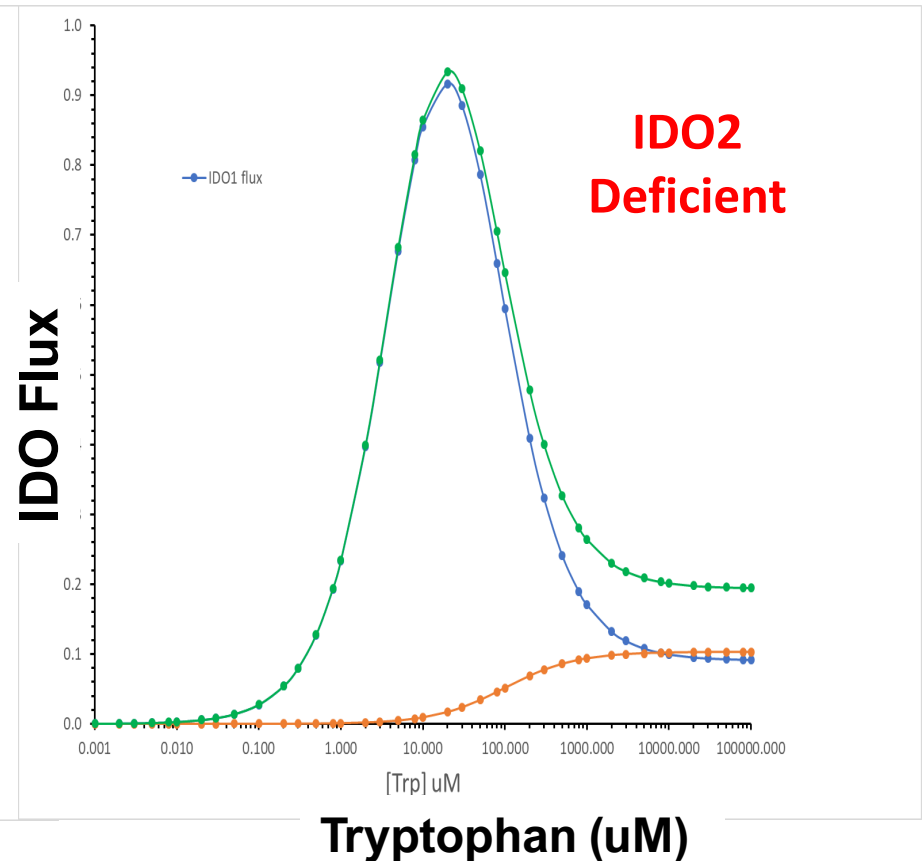


IDO1 is substrate inhibited.



Biochemistry

Yamamoto JBC 1967



Nonlinear Systems theory

Robert Phair

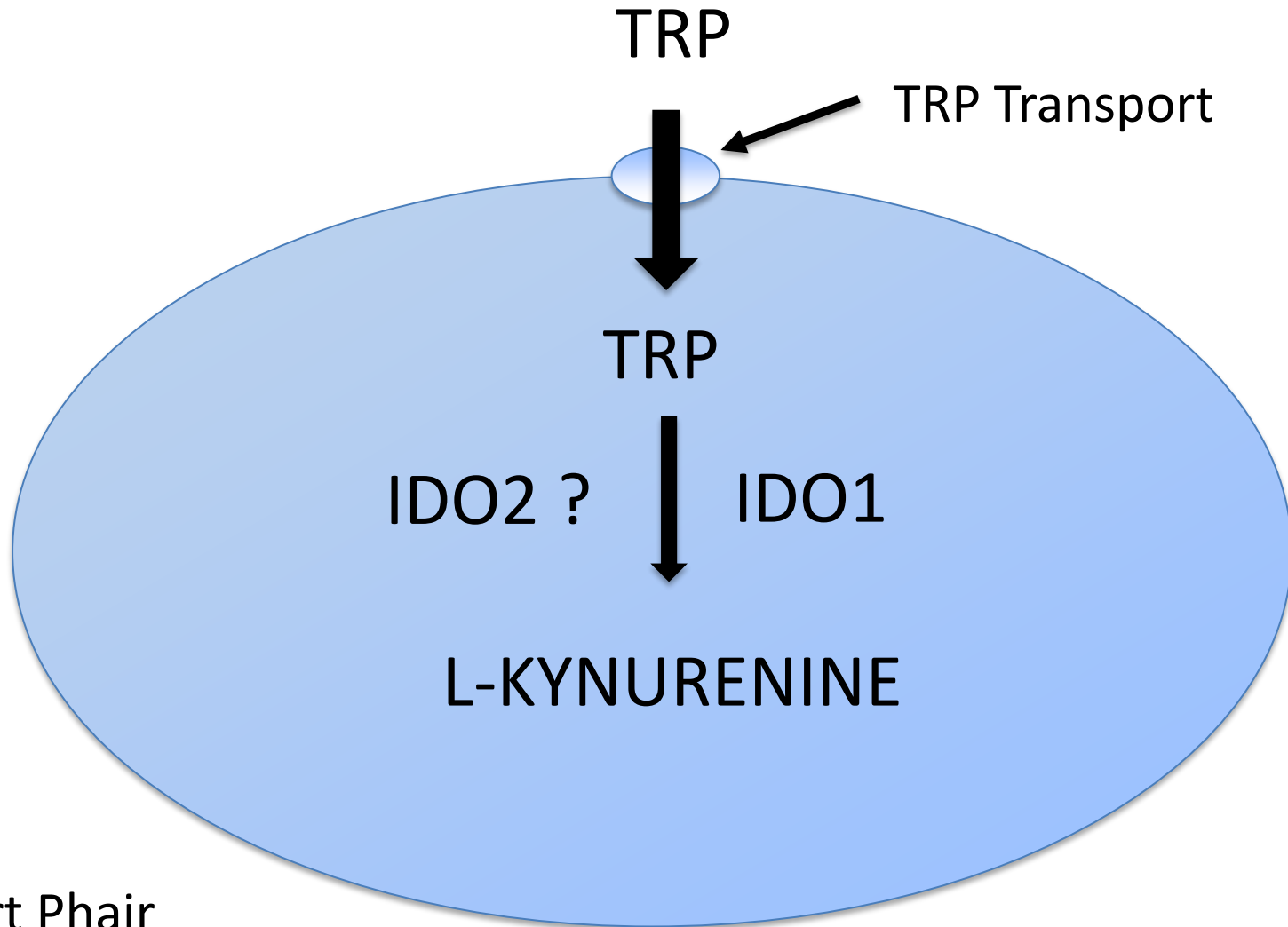
Genetic

IDO2 Sequencing

- All 20 sever patients have (1.7) non-functional mutations in IDO2
- 46 additional ME/CFS patients
All have non-functional mutations

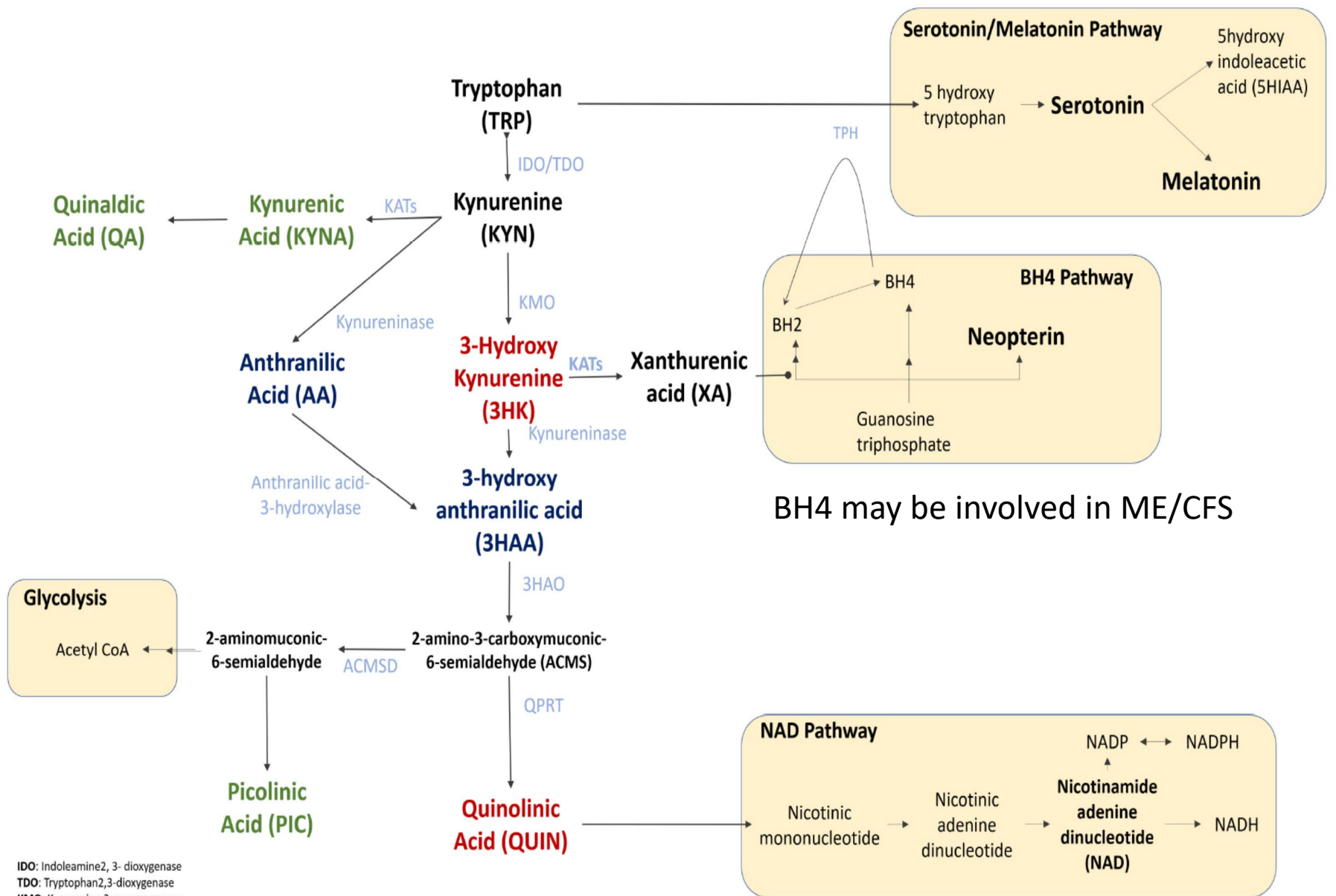
About 75% of population have IDO2 non-functional mutations

Race Between TRP import and TRP Degradation



Can Cells be Traped

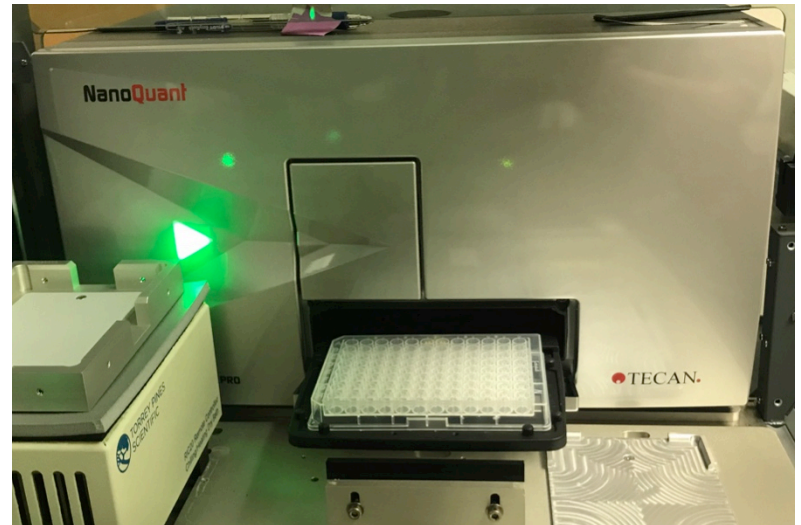
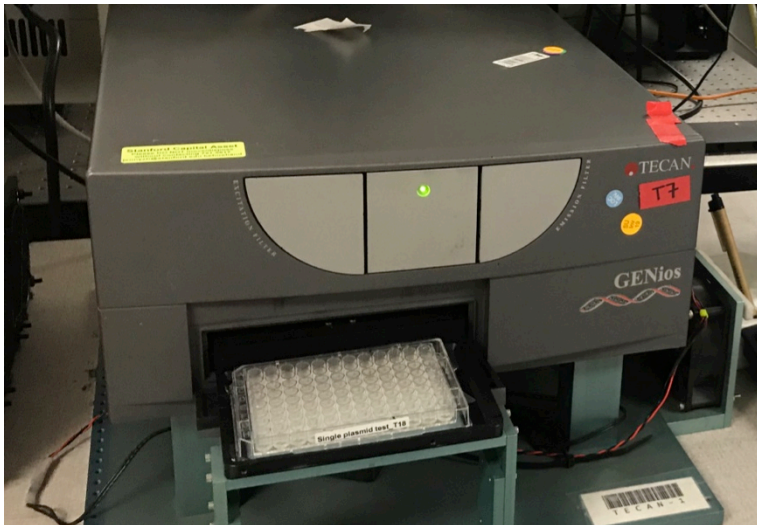
- Substrate inhibition only demonstrated *In Vitro*
- Can yeast cells be trapped using Human IDO1?
No Kynurenine to NAD = No Growth
- Can Human Immune cells be trapped?
No depletion of TRP and No Kynurenine



BH4 may be involved in ME/CFS

IDO: Indoleamine2, 3- dioxygenase
TDO: Tryptophan2,3-dioxygenase
KMO: Kynurenine 3- monooxygenase
KAT: Kynurenine amino transferase
3HAO: 3-hydroxyanthranilic acid oxygenase
QPRT: Quinolinic acid phosphoribosyl transferase
ACMSD: ACMS decarboxylase
TPH: Tryptophan hydroxylase

Tecan Grower Systems for YEAST

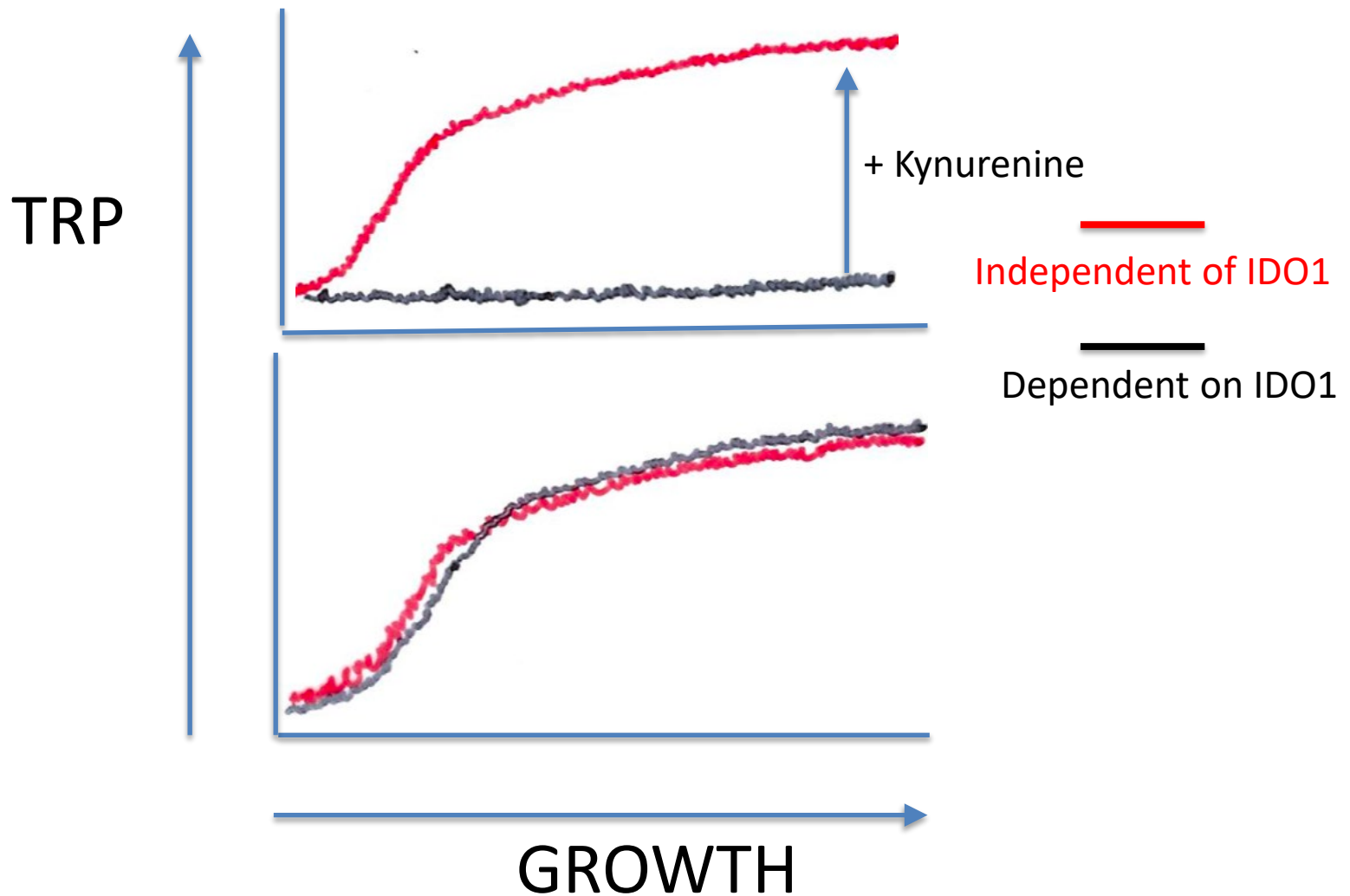


96 well multiwell plate shaking incubators with timed OD measurements

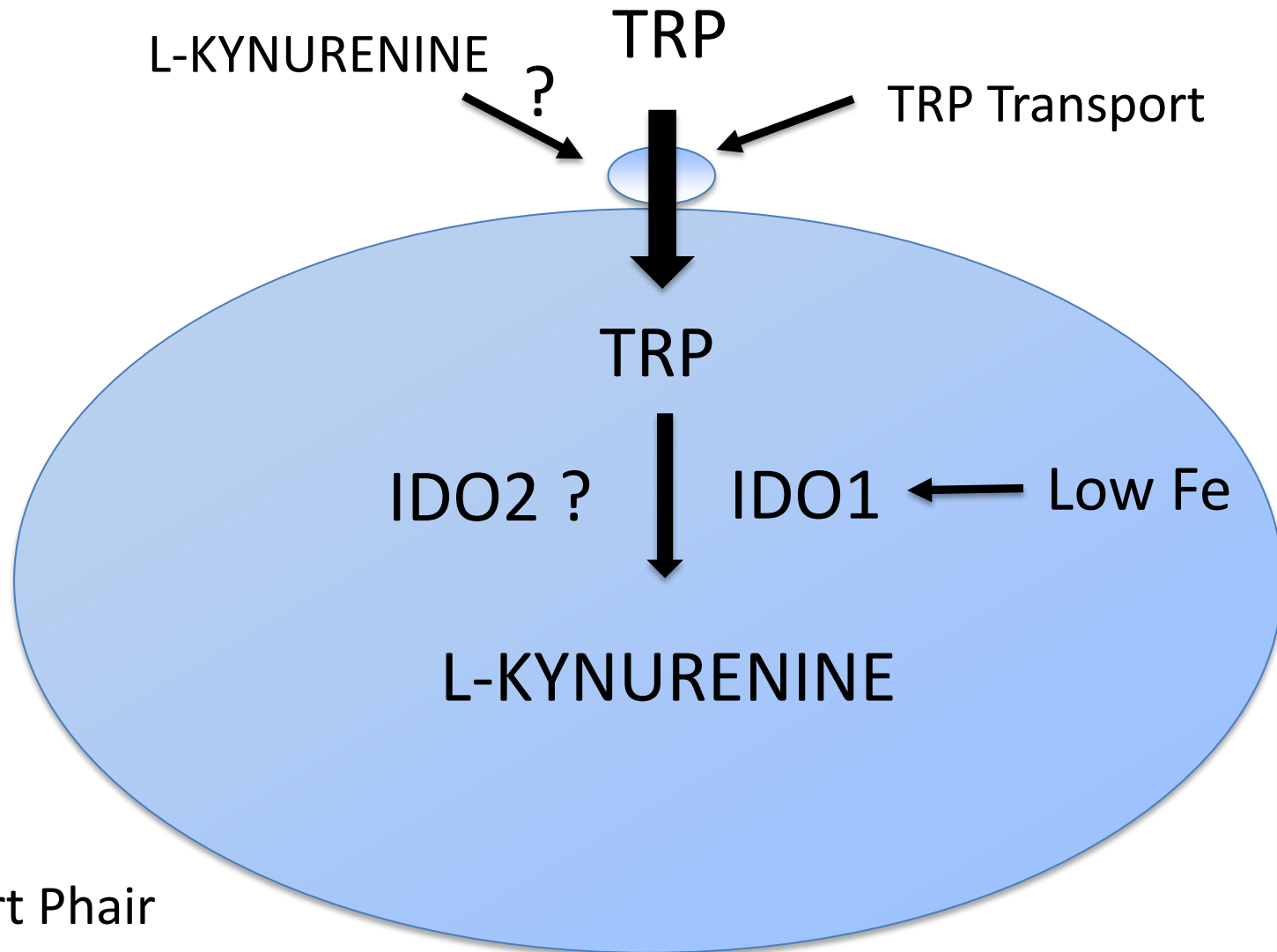
Yeast Model for Metabolic Trap

- Yeast: Added Human IDO1 Gene under yeast control. Removed all other genes that make NAD and all other genes that consume TRP. Yeast growth now dependent upon IDO1 function (NAD needed to make ATP).
- Modified Yeast grow normally at low TRP but **stop** growing at high TRP. They are **trapped**
- This shows that the trap can function in a cell
- Can now screen for a drug that reactivates IDO1 that would consume the TRP and get them out of the **trap**

Yeast in Metabolic Trap



Race Between TRP import and TRP Degradation



Preliminary Results

- It appears Human Immune Cells can be trapped
- In High TRP Human cells stop metabolizing TRP to produce Kynurenine.
- Our first experiment shows IDO2 does not block the trap. But much more needs to be done

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