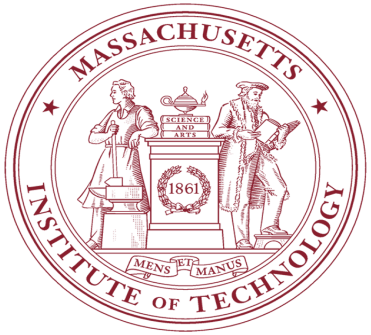


BROAD INSTITUTE

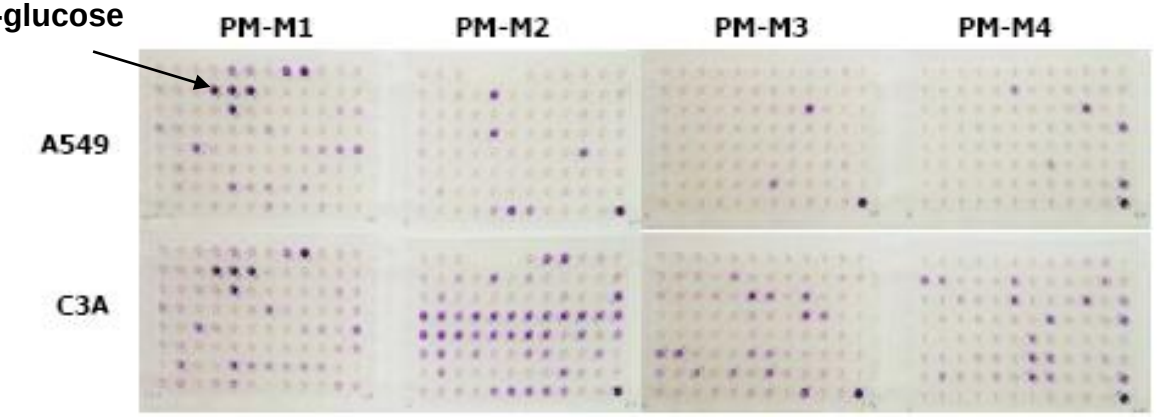
Effects of microtubule inhibitors on cancer cell metabolism

Kristin Rose

August 7, 2009

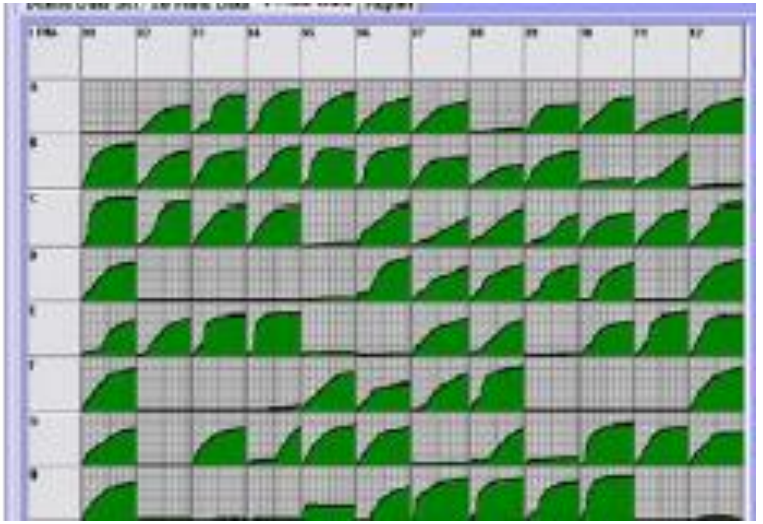
Biolog's Phenotype MicroArrays:
A system for nutrient metabolism and chemosensitivity profiling

***e.g.*, α-D-glucose**



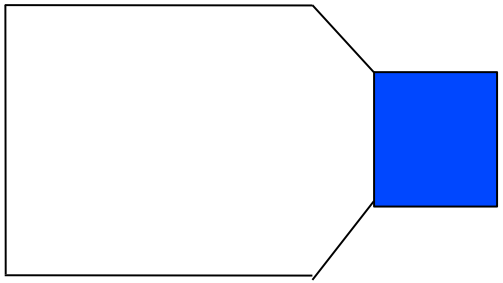
one nutrient per well

OmniLog® Incubator-Reader

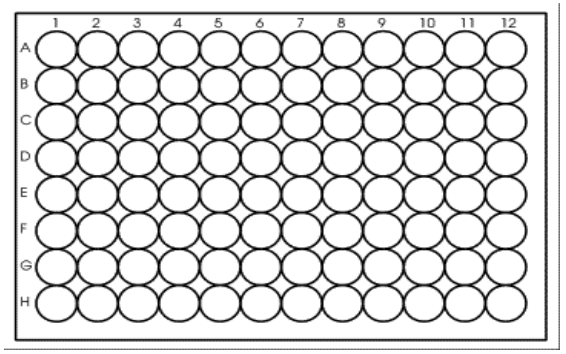


kinetic readout per nutrient

Timeline for preparing Biolog Phenotype MicroArray assays

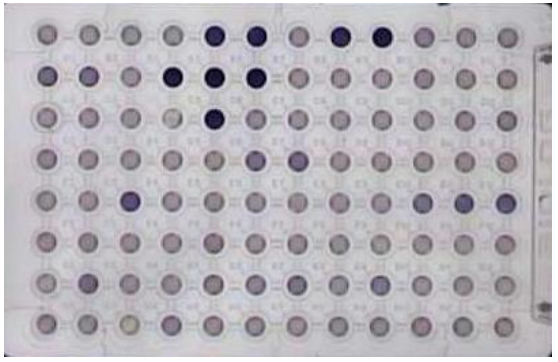


Culture cells to confluence in flask



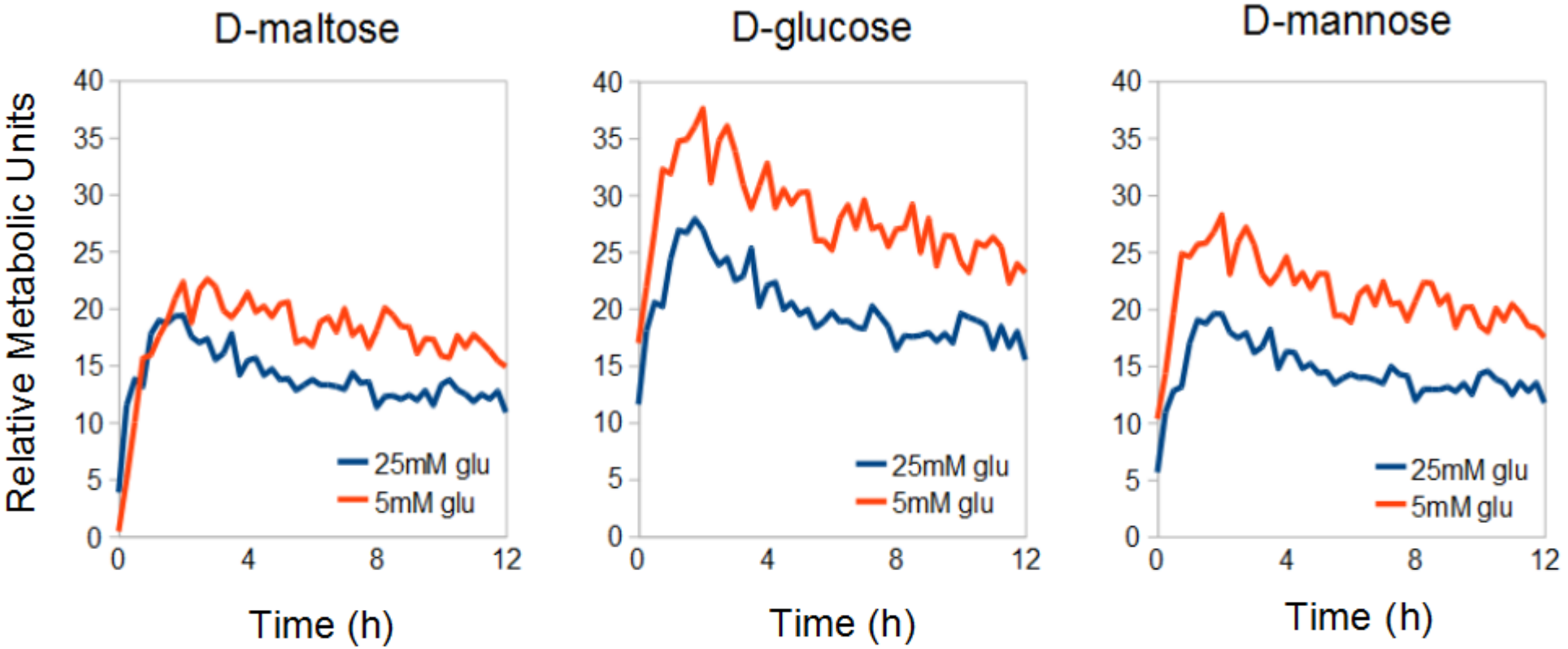
Seed 96-well Biolog plate (minimal media)

40 h

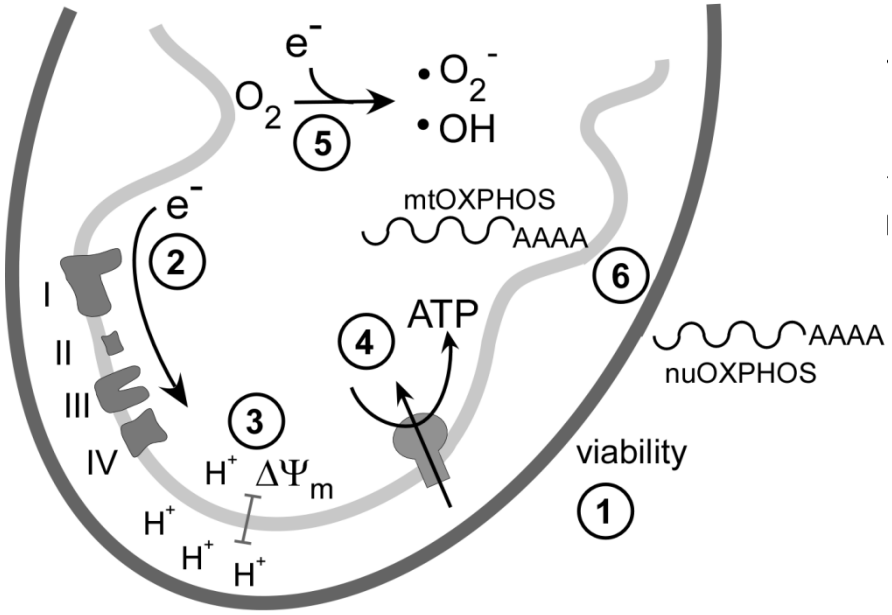


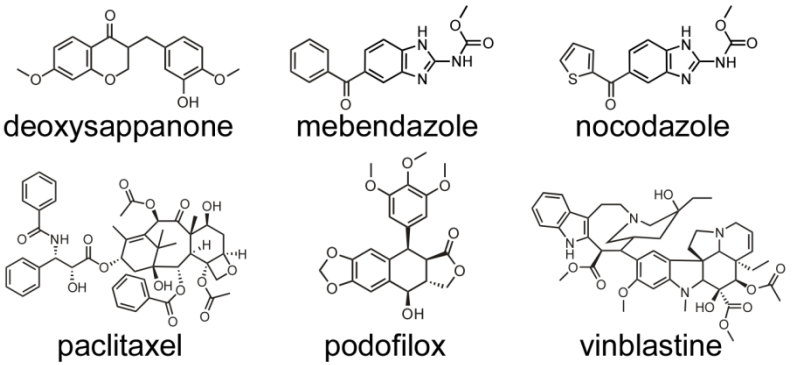
Add metabolic dye

A549 cells cultured in 5mM glucose media have higher rates of simple sugar metabolism (vs. 25mM glucose)



High-throughput screening for mitochondrial effects in muscle revealed a role for microtubule modulators





<u>Mitochondrial assays</u>
- viability
- electron transport
- membrane potential
- ATP production
- reactive oxygen species
- gene expression

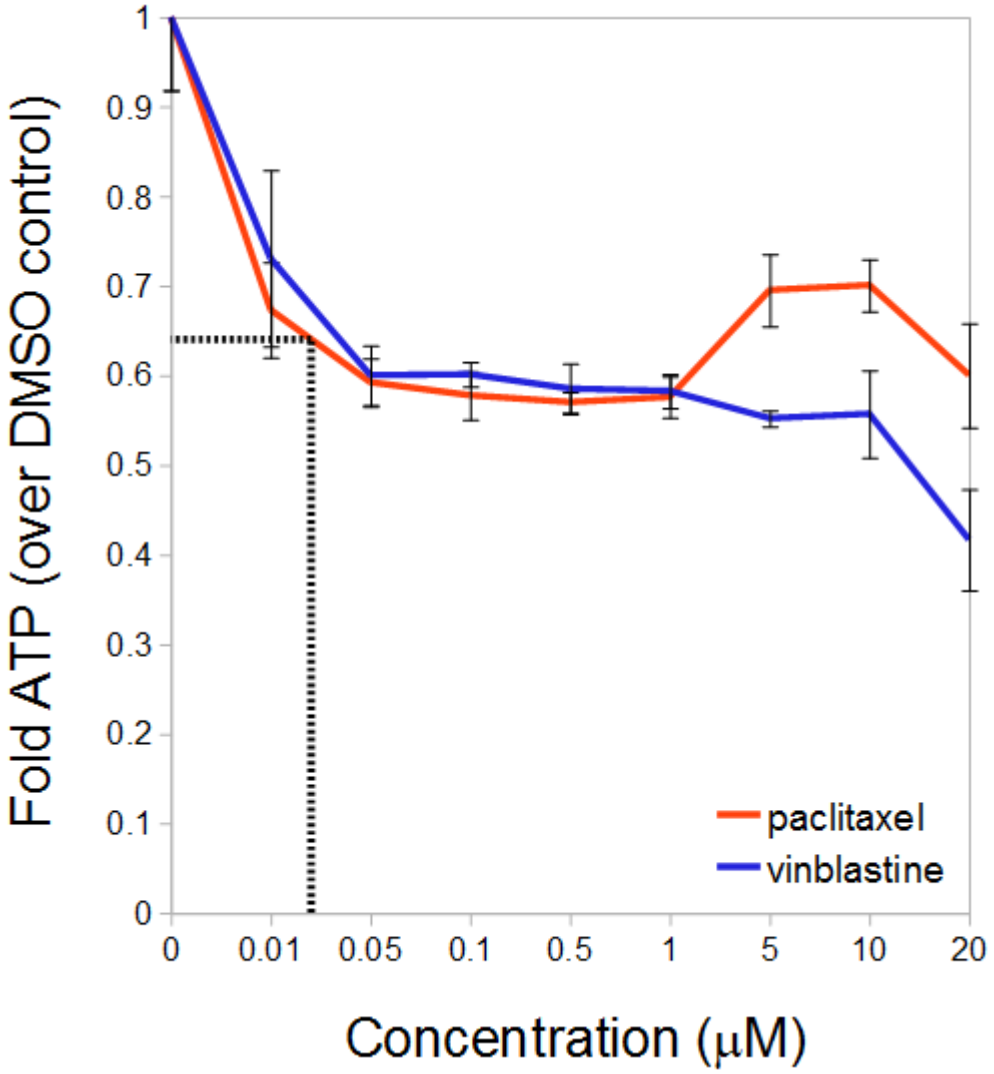
In muscle cells, they:

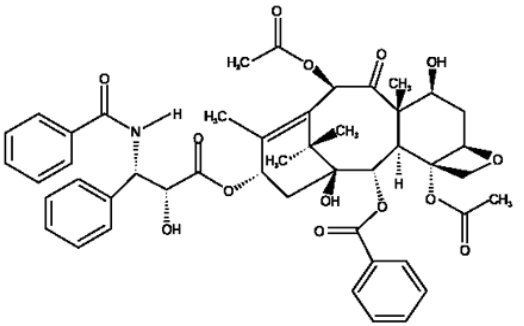
- increased OXPHOS gene expression
- decreased cellular ROS

Wagner *et al.* (2008) *Nat Biotech*

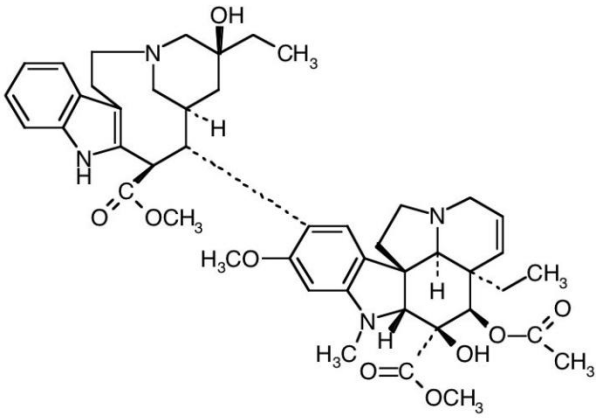
Do microtubule modulators have an effect on mitochondrial biology in *cancer* cells?

Selecting appropriate concentrations of microtubule inhibitors





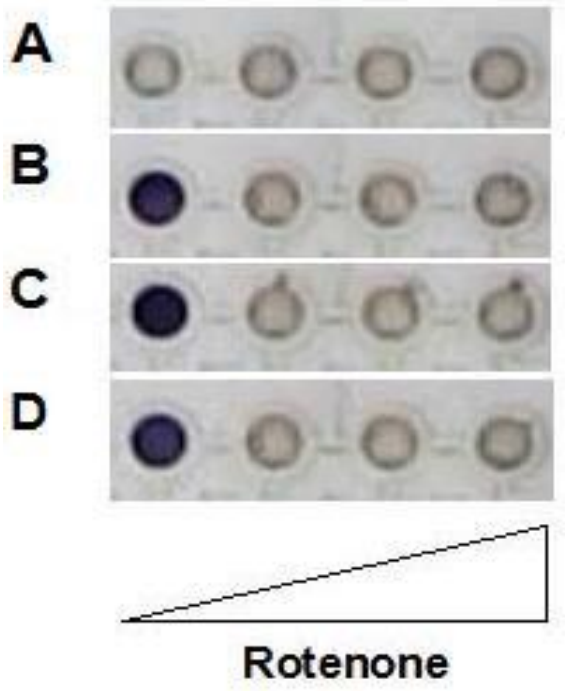
Paclitaxel = microtubule stabilizer



Vinblastine = microtubule destabilizer

A549 lung cancer cells treated 48h

Chemosensitivity plates reveal cases of increased resistance to rotenone



25mM glucose (glu)

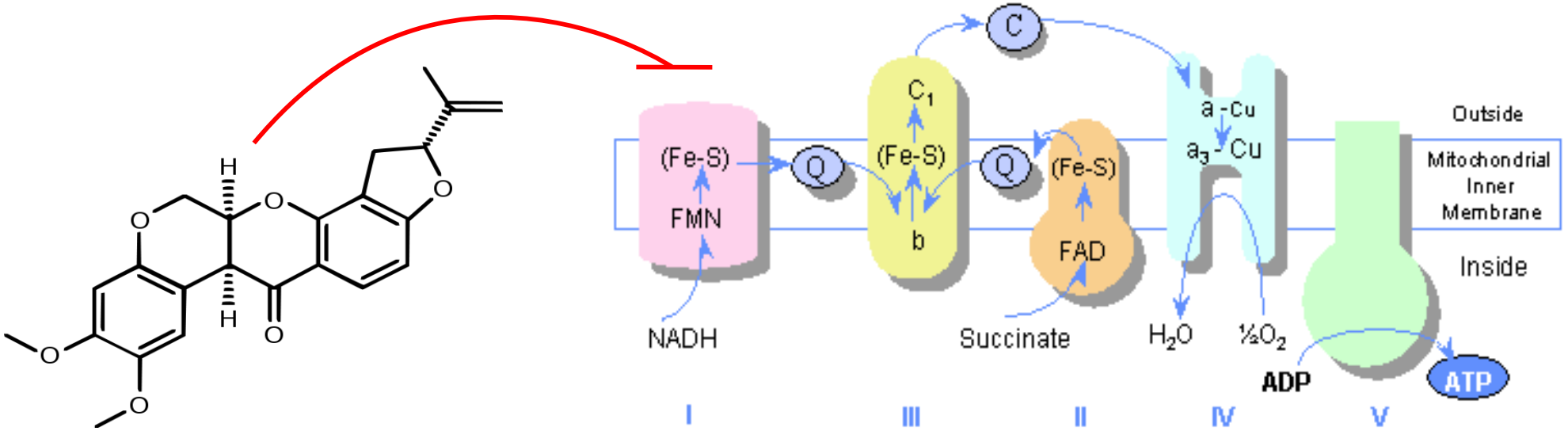
5mM glucose

25mM glucose + paclitaxel (pac)

25mM glucose + vinblastine (vin)

cell death

metabolism



Cell viability assays (ATP) confirm chemosensitivity results

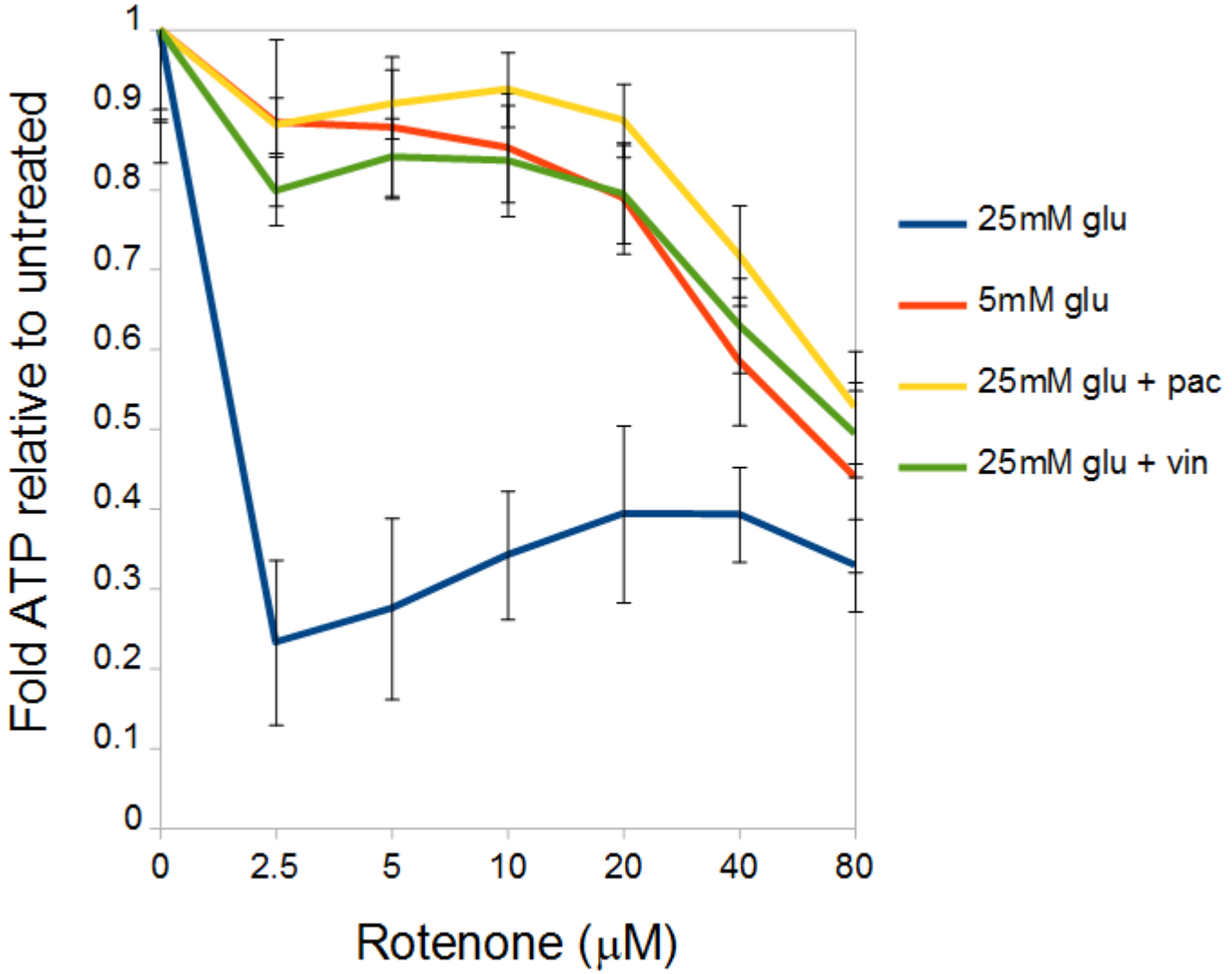
25nM pac or vin

48h

rotenone

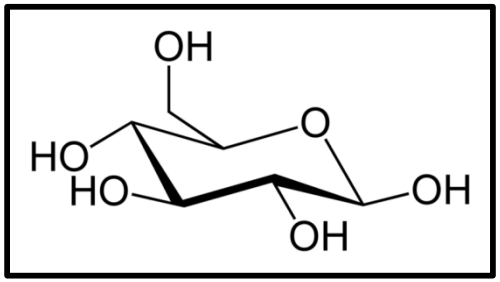
24h

measure ATP



Alternate energy metabolism pathways with measurable factors

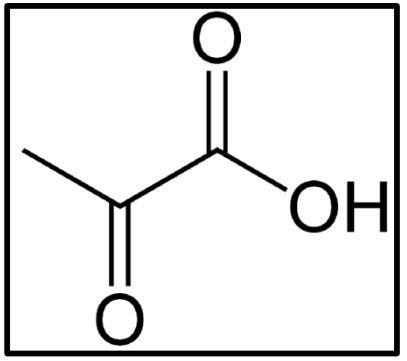
Glycolysis (cancer **Warburg effect**)



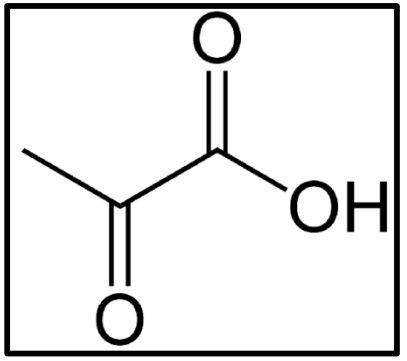
Glucose

+ $6O_2$

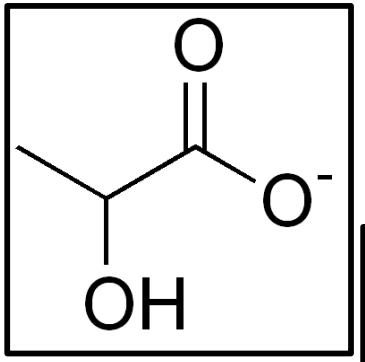
Glycolysis



Pyruvic Acid



Pyruvic Acid



Lactate

2 ATP

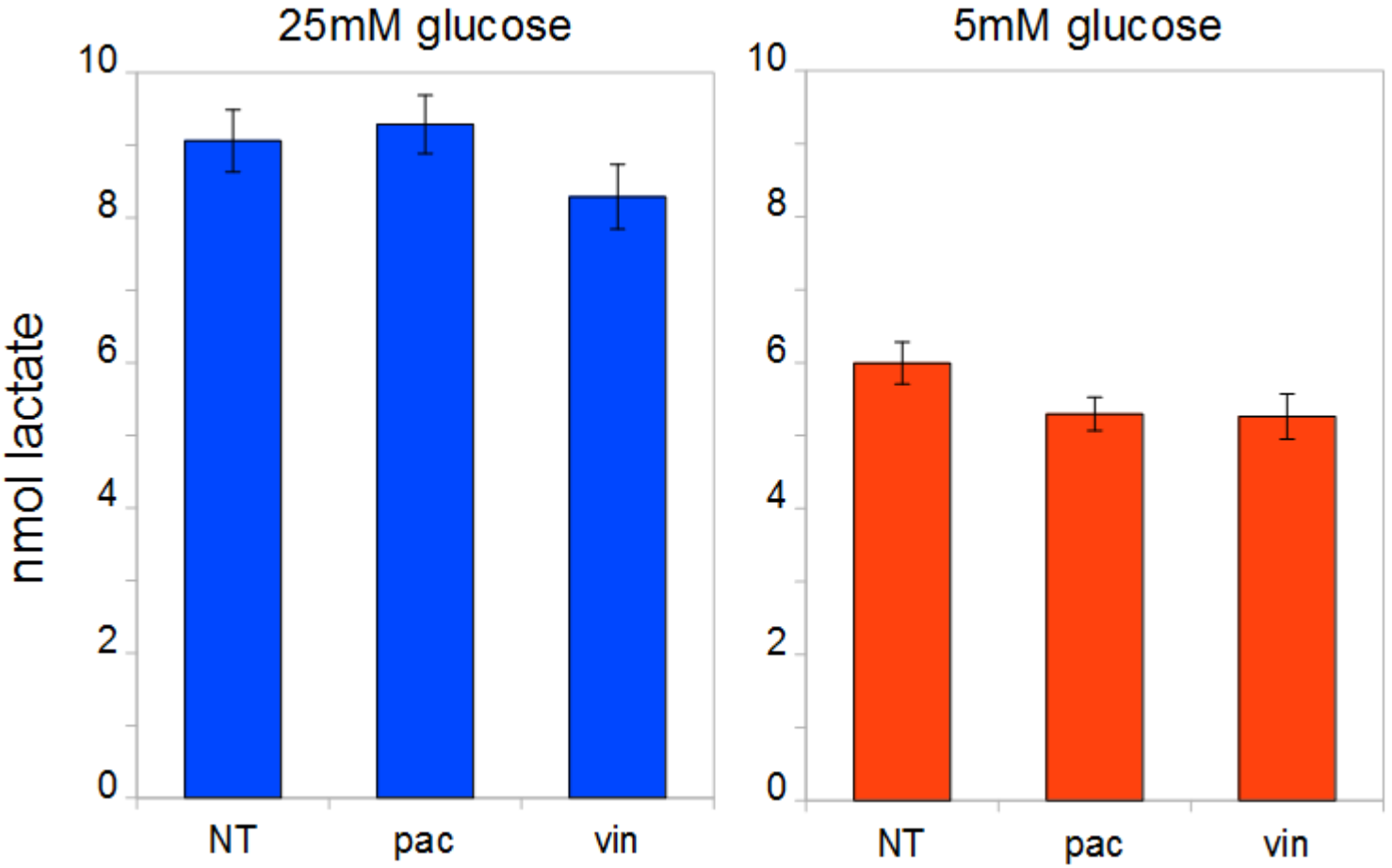
GLYCOLYTIC PHENOTYPE

Krebs Cycle (Respiration)

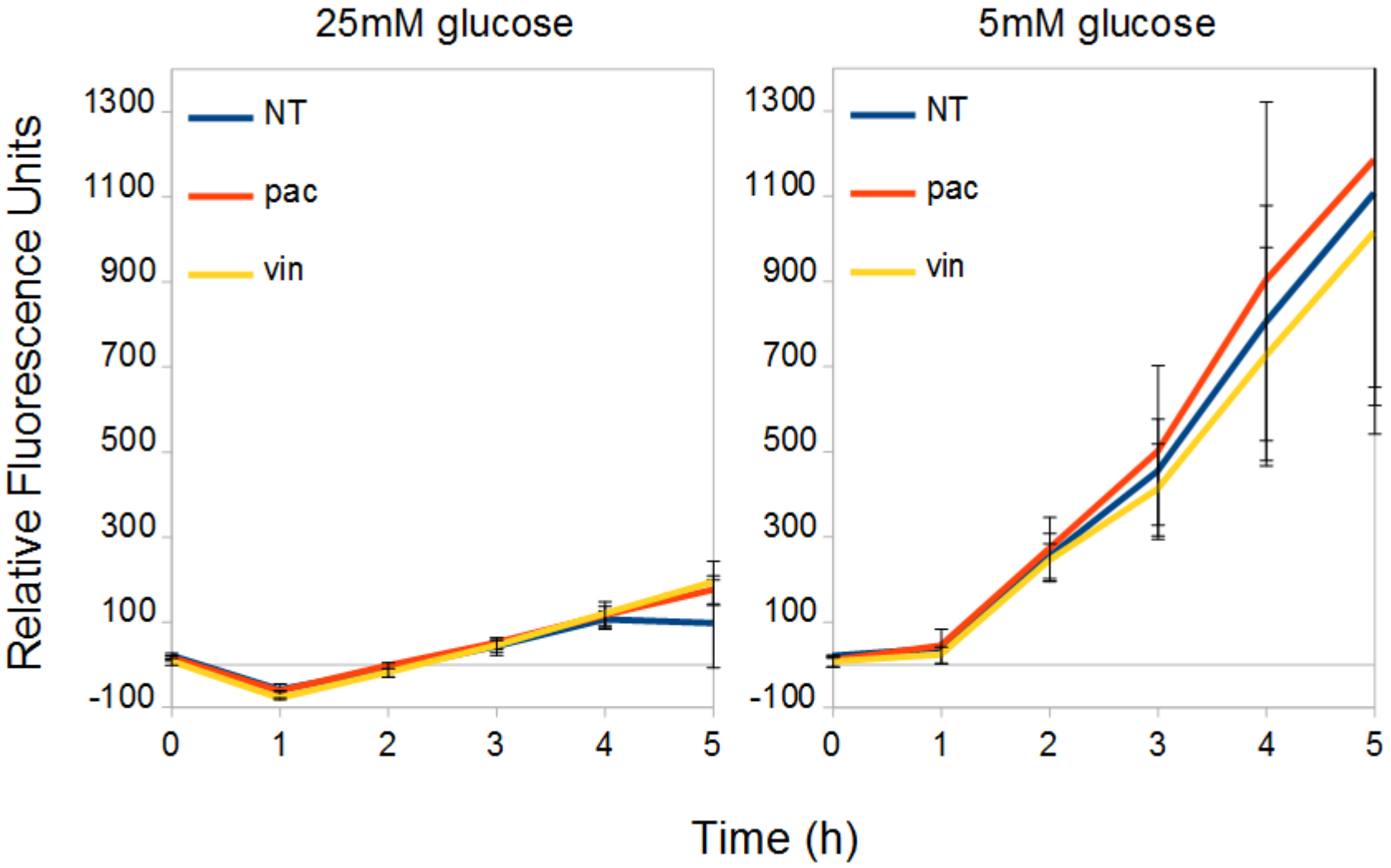
36 ATP

RESPIRING PHENOTYPE

Lactate production in response to glucose content and microtubule modulator treatment



Oxygen consumption in response to glucose content and microtubule modulator treatment



Conclusions

- **Hypothesis**: Do microtubule modulators have an effect on mitochondrial biology in cancer cells?

- **Conclusions**:
  - A549 cells in 25mM glucose appear more glycolytic.
  - A549 cells in 5mM glucose appear to be respiring.
  - Paclitaxel and vinblastine increase resistance to rotenone.
  - Further experimentation is required to clarify the role of paclitaxel and vinblastine in mitochondrial metabolism.

Future directions

- Do mitochondria-targeting drugs sensitize A549 cells to microtubule modulators?
- Do microtubule modulators induce gene expression changes of interest?
- Can we recapitulate these results with other cancer drugs?

Acknowledgments

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