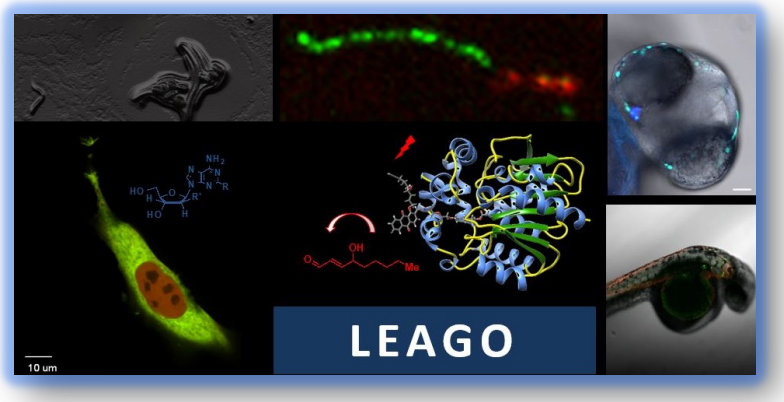
Welcome to CH-313: Chemical Biology

Prof. Yimon Aye https://leago.epfl.ch/



Laboratory of Electrophiles And Genome Operation

Lecture Week 14:

Tools to monitor

cell energy status (continued):

Case study examples

2023 Dec 19th (Room: BS 270): 10:15 am – noon

https://moodle.epfl.ch/course/view.php?id=15521

Part II: Targeting metabolic regulation

3 stages of cellular respiration

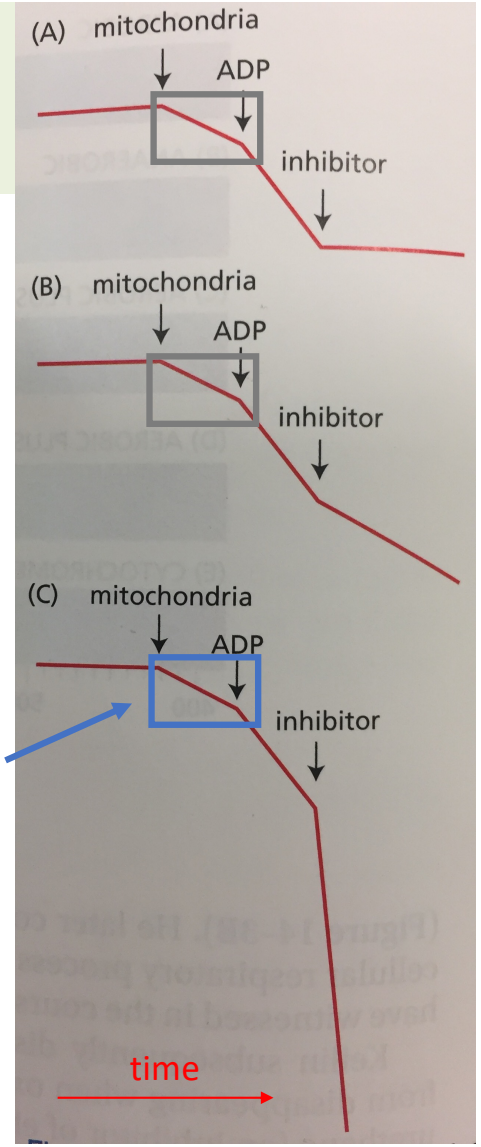
Week	Date	Topic	Notes
9	14th Nov	**Glycolysis**	
10	21st Nov	**Isotopic tracing / pathway flux analyses**	**PSet 5**
11	28th Nov	**TCA cycle and ETC**	
12	5th Dec	**ETC and Oxphos**	**PSet 6**
13	12th Dec	**OCAR / ECAR mitochondrial functional analyses**	
14	19th Dec	Integrated metabolomics & proteomics tools and applications	**PSet 7**

Glycolytic Signaling Switches in Cancer (the Warburg Effect aka aerobic glycolysis)

Anaerobic vs. Aerobic Metabolism (lactate production vs. TCA cycle)

Electron transport chain (ETC) and Oxidative phosphorylation (Oxphos)

A problem on mitochondria function



Question: What is this background rate (before addition of ADP) attributable to ?

Q: Based on the question, were these experiments conducted in live cells or with isolated mitochondria?

Q: what is the purpose of adding ADP?

Figure shows oxygen electrode traces showing 3 different patterns of effects of inhibitors on **oxygen consumption** by mitochondria.
In all experiments, mitochondria were added to a phosphate-buffered solution containing succinate as the sole source of electrons for the ETC. After a short interval, ADP was added followed by an inhibitor. The rates of O_2 consumption were measured (the steeper the slope → the faster the rate).

Question:
Match each inhibitor in table below to one of the traces.

Additional question: Sketch the oxygen traces you would expect for the following sequential addition of the pairs of inhibitors: (i) FCCP followed by cyanide; (ii) FCCP followed by oligomycin; (iii) oligomycin followed by FCCP

Inhibitor		Function
1. FCCP	→ C	Makes membranes permeable to protons
2. Malonate	→ A	Prevents oxidation of succinate
3. Cyanide	→ A	Inhibits the cytochrome *c* oxidase complex (aka Complex IV)
4. Atractyloside	→ B	Inhibits the ADP/ATP transporter
5. Oligomycin	→ B	Inhibits ATP synthase
6. Butylmalonate	→ A	Blocks mitochondrial uptake of succinate

Case study: Integrated Quantitative Proteomics and Metabolomics Interrogations

Breast tumor aggressiveness

- **10A**: Parental
- **T1K**: Pre-neoplastic
- **CA1h**: Low-grade tumorigenic (Mortality within 6 wks)
- **CA1a**: High-grade tumorigenic (Mortality within 3 wks)

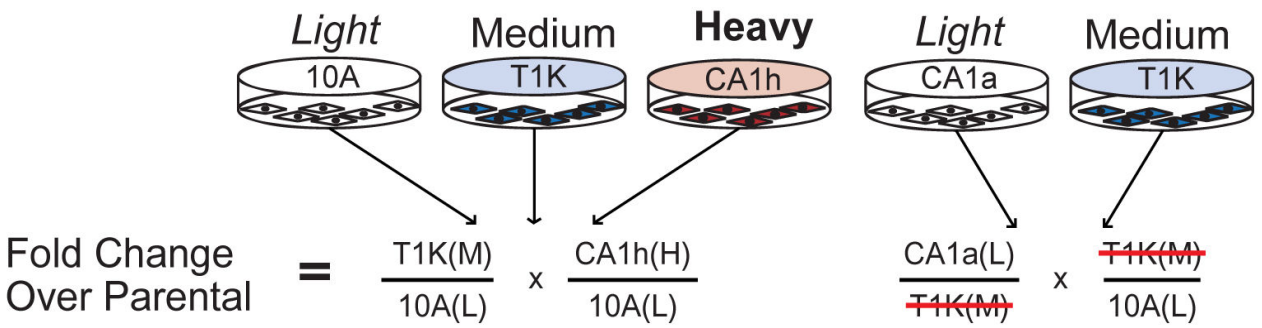
Q: Propose an experiment/method that you have learned in Part I that allows you to quantitatively assess relative expression levels on specific proteins across different cell lines

Q: Propose a strategy that you have learned in Part I that allows you to identify subcellular localization of *proteins*. (As an aside, how about for mRNA?). **[Compare your answer to the method the authors used (next slide) and comment on pros/cons].**

Q: SILAC is practically limited in terms of multi-plexing (it only allows a maximum of triplex: light AA-, medium AA-, and heavy AA-labeled cells). The authors need to get relative (and quantitative) comparison against 4 cell lines. How should they set up these SILAC assays (using Parental line as normalization)

(I) Measurement of relative expression and subcellular localization of proteins

Multiplexed SILAC experiment



→ Bridging sample
(Q: what is the bridging sample in the set up shown?)

continued: **Case study**: Integrated Quantitative Proteomics and Metabolomics Interrogations

Breast tumor aggressiveness

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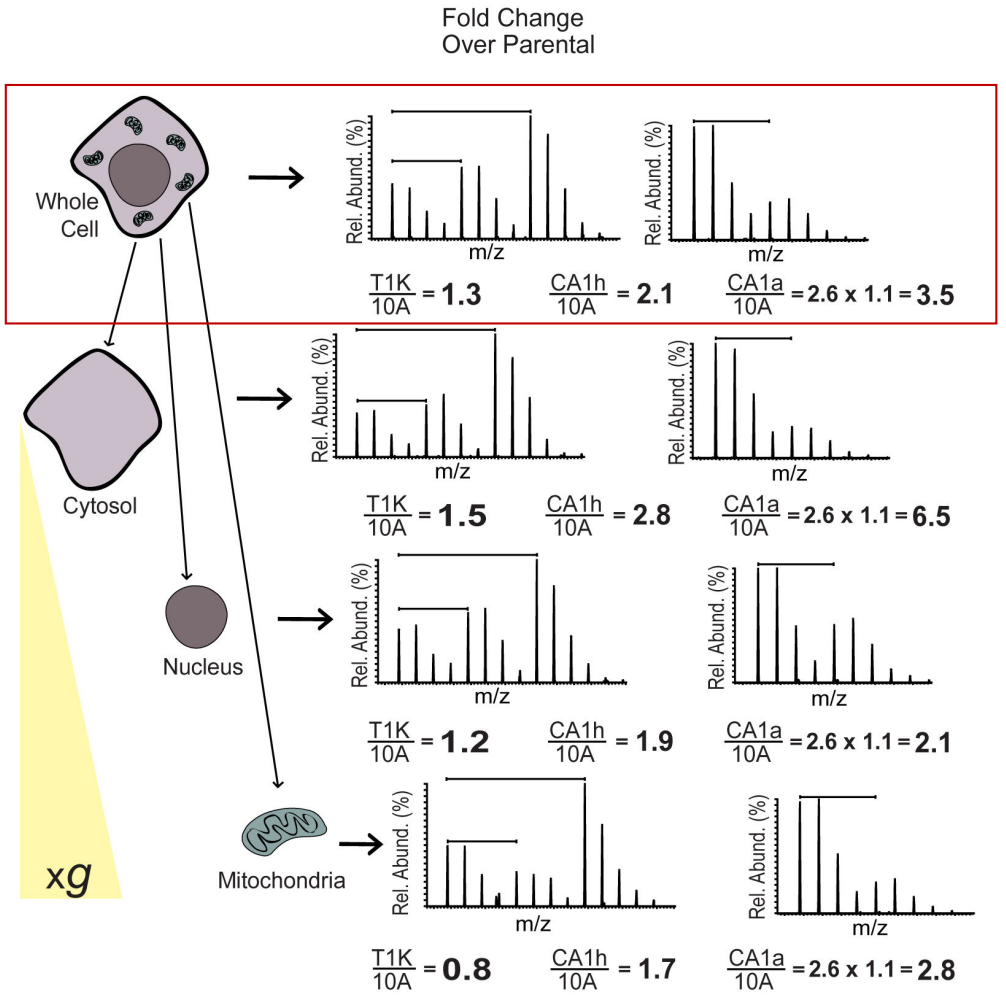

Q: Mass Spectra (from the peptide NPDDITQEEYGEFYK) are shown on the left, to illustrate the fold change calculation for heat shock protein 90 (Hsp90), which was identified in every cellular fraction.

Comment on the nucleocytoplasmic distribution of Hsp90 against breast cancer progression

Case study, continued: (II) Measurement of OCR and ECAR (mitochondrial bioenergetics)

Dynamic expression and subcellular localization of metabolic enzymes were recorded in the breast cancer progression cell lines. These observations reflect a correlation with, and possibly a dependency upon, metabolic dysfunction with cancer progression. To further investigate the differences in metabolic capacity with cancer progression, a Seahorse X24 extracellular flux analyzer was used to measure the cellular oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR). **The capacity for or dependency upon OXPHOS and glycolysis for energy metabolism was assessed using the established pharmacological profiling approach**:

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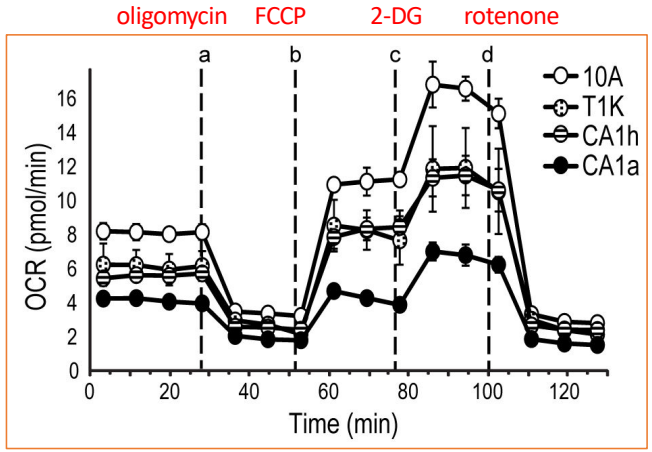

Basal OCR, a measure of OXPHOS, was observed to (increase or decrease?) with cancer progression with the lowest initial rate of mitochondrial respiration observed in ---?---- cells.
CA1a

Upon treatment with **a** (ATP-synthesis inhibitor), a shift to ----?---- occurs: why? Because OXPHOS is inhibited...
glycolysis

Upon treatment with **b** (ETC and ATP-synthesis uncoupling reagent), ---?---- cells show poorest recovery: any implication?

CA1a --> a greater degree of mito-dysfunction ..

Q: why can TCA cycle in the cell still run without glycolysis?

Addition of **c** (hexokinase inhibitor) led to an increase in OCR that represents cellular reserve capacity for -----?-----, and this capacity is lowest for --?– cells.
OXPHOS
CA1a

Treatment with **d** (complex I inhibitor) suppresses mitochondrial ----?----
and OCR drops below baseline.
electron transport

→ do these observations show that the capacity for OXPHOS decreases with breast cancer progression in this cell line model? YES or NO?

a, 1 ug/ml oligomycin; **b**, 300 nM FCCP; **c**, 100 mM 2-DG; **d**, 1 uM rotenone

Each data-point represents the mean of five independent samples measured in triplicate. Error bars indicate standard deviation of the mean

Case study, continued: (II) Measurement of OCR and ECAR (mitochondrial bioenergetics)

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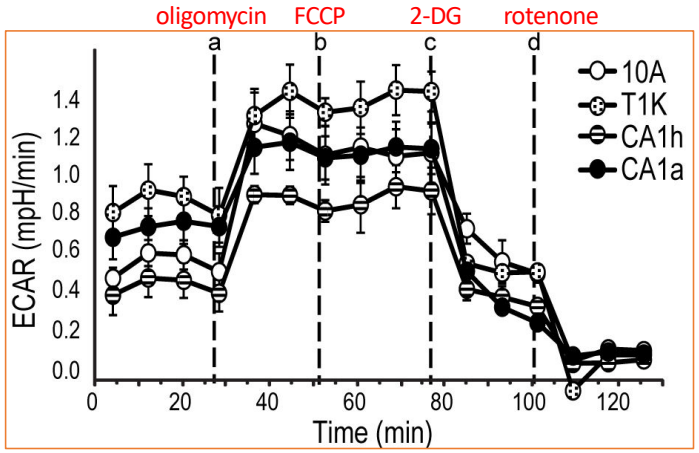

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Basal ECAR, a measure of lactate production from glycolysis ---?---, was highest in T1K ----?--- cells and lowest in CA1h ---?--- cells.

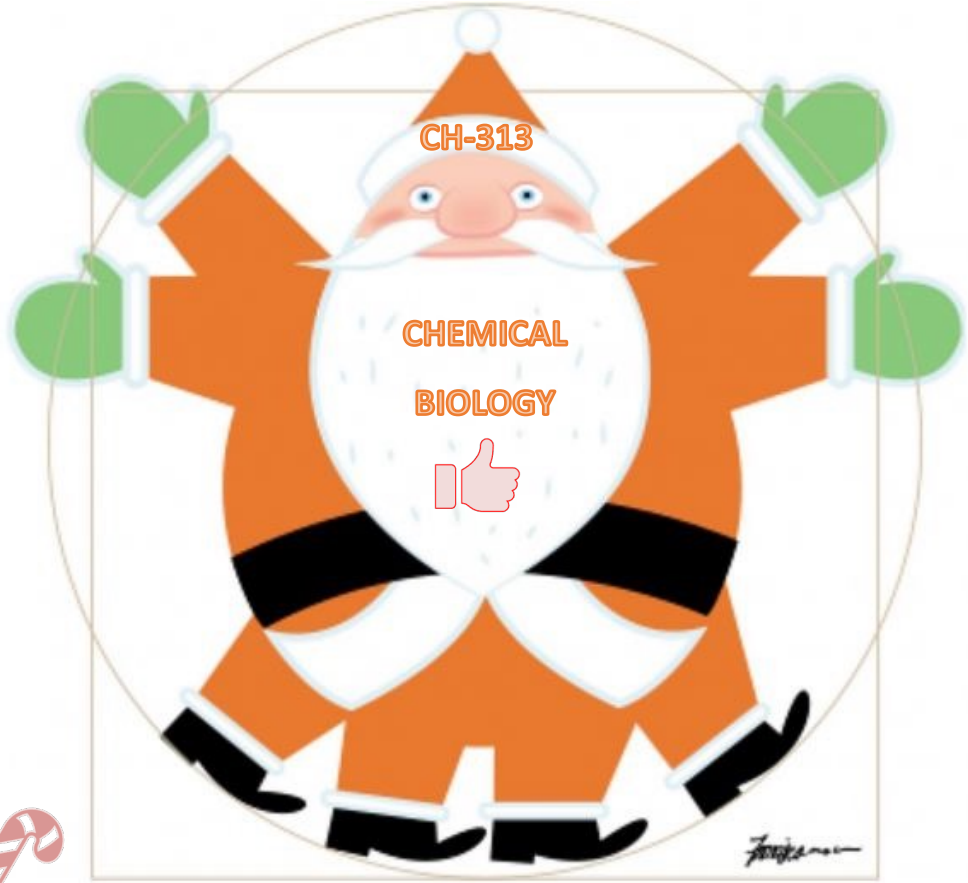
ECAR rates remain elevated for all 4 cell lines even after electron transport is restored with FCCP ---?----treatment. This is because ATP-synthesis remained shut-down from treatment with oligomycin ---?----. However, the parental 10A cell line showed the greatest proportional increase relative to basal ECAR in response to inhibition of OXPHOS.

As expected, ECAR values dropped in response to treatment with 2-DG ----?------ for all cell lines.

Learning outcomes (Week 14: CH-313 Chemical Biology - Synopsis)

- Integrating tools and concepts from both Part I and II of the course

  (Case study example: combining quantitative proteomics and metabolic investigations to interrogate disease-stage-specific changes in biology)



Happy Holidays and Good Luck with the Final!