


Prof. Dr. Markus Jeschek, LSAM

# General Course Information

- Moodle: https://go.epfl.ch/CH-313
  - Lecture slides (evening before the lecture)
  - Distributed presentation topics (assignments)
  - Forum (for questions and announcements)

- Examination (written, graded, detailed information will follow)
- Contact:
  - Moodle forum (for questions)
  - markus.jeschek@epfl.ch

- **“Concepts over details!”**
- **Interact! Ask! Discuss! Anytime!**



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# Group Presentations

- Critical discussion of primary literature
- Illustrative examples for topics from the lecture

- Why?
  - Repetition of core concepts, techniques etc.
  - Presentation skills and critical discussion of research
  - Insight into current research topics

- How?
  - Two students per group
  - Assignments distributed one week before delivery of presentation (via Moodle)
  - **Send slides: markus.jeschek@epfl.ch (Mon evening before presentation)**
  - **15 min presentation (both group members should present!)** + Q&A

# Tipps for Group Presentations

- Rough structure
  - Short intro on general topic
  - Main presentation according to assignment
  - Brief outlook incl. points of criticism/open questions/personal opinion as kick-starter for the discussion
- Everybody should participate in the discussion, incl. constructive(!) feedback on presentation style
- Questionnaires with different points, feedback by peers
- Typical assignment:
  - You will receive a certain topic including a related publication
  - Introduce the topic using the publication
  - present the motivation behind the research, methodology, key results (not every graph!)
  - Additional questions will be provided hinting towards central points
  - Be encouraged to look/present beyond the questions and the provided paper

# Group Presentations – Schedule

| # | Name1 | Name2 | Presentation on… | Assignment on… |
|---|---|---|---|---|
| 1 | Wininger Quentin | Jeremy | **Sep 23, 2025** | Sep 16, 2025 |
| 2 | Ema | Ariane | **Sep 30, 2025** | Sep 23, 2025 |
| 3 | Benjamin | Matthieu | **Oct 7, 2025** | Sep 30, 2025 |
| 4 | Ivana | Ipek | **Oct 14, 2025** | Oct 7, 2025 |
| 5 | Mridhula | Elodie | **Oct 28, 2025** | Oct 21, 2025 |
| 6 | Abigail | Robin | **Nov 4, 2025** | Oct 28, 2025 |
| 7 | Eva | Florian | **Nov 11, 2025** | Nov 4, 2025 |
| 8 | Bastien | Axel | **Nov 18, 2025** | Nov 11, 2025 |
| 9 | Melodie | Siolène | **Nov 25, 2025** | Nov 18, 2025 |
| 10 | Nicole | Maria | **Dec 2, 2025** | Nov 25, 2025 |

# Course Topics – Overview

- Week 1 | Introduction + DNA
- Week 2 | DNA
- Week 3 | DNA
- Week 4 | DNA
- Week 5 | DNA/RNA
- Week 6 | RNA/Translation
- Week 7 | Translation
- Week 8 | Enzymes (Zoom)
- Week 9 | Enzymes (Zoom)
- Week 10 | Enzymes (Zoom)
- Week 11 | Enzymes (Zoom)
- Week 12 | Enzymes
- **Week 13 | Metabolism**
- Week 14 | LSAM Intro + Exam Preparation + Evaluation

[tentative schedule]



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# Protein – Enzymes
(Kinetics)



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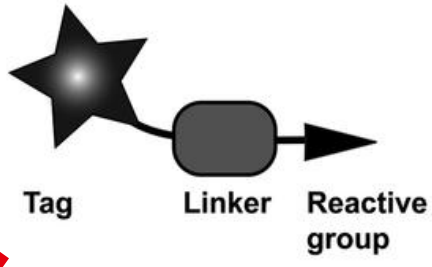
# Activity-Based Protein Profiling (ABPP)

- Methods to identify drug targets, druggable sites etc.
- Testing for functional (catalytic) state of enzymes in complex proteomic mixtures
- Via activity-based probes (ABPs) that modify the active site




| Tag | Linker | Reactive group |
|---|---|---|
| Fluorophore | Alkyl | Electrophile |
| Biotin | Peptide | Latent electrophile |
| Alkyne | Cleavable | Photocrosslinker |

detection or enrichment

covalently binds to conserved active-site residue



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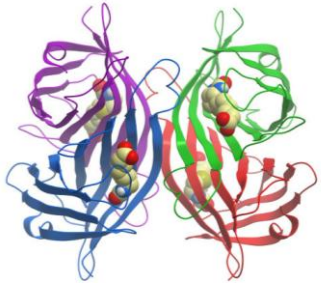
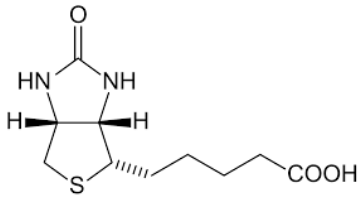
# The Biotin-Streptavidin Technology

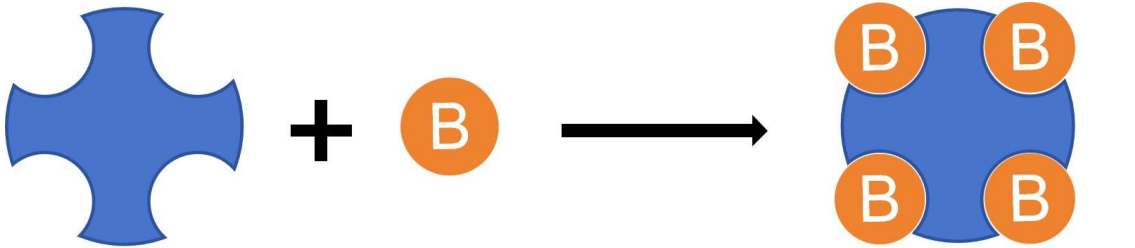
- Streptavidin: bacterial protein (4-mer) with high, non-covalent affinity to biotin
- Avidin = eukaryotic homologue
- $K_D \sim 10^{-15}$ M
- Extremely stable (T, solvents, pH etc.)
- Detection, labelling, purification, conjugation etc. etc.



**streptavidin**
(4-mer)




**D-biotin**
("vitamin H")



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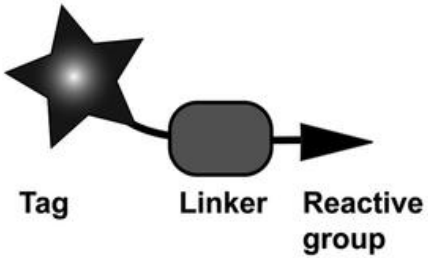


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**e.g.:**
- **fluorophosphonates → Ser hydrolases**
- **epoxides/vinyl sulfones → Cys proteases**
- **photoreactive groups**



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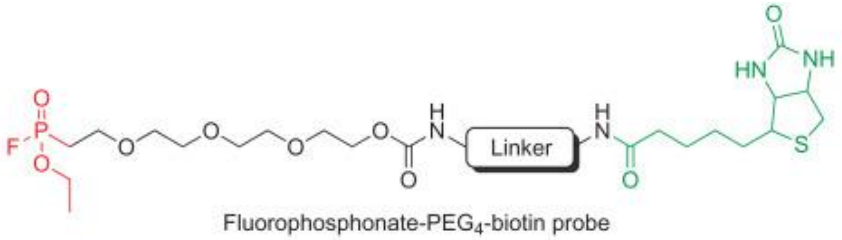
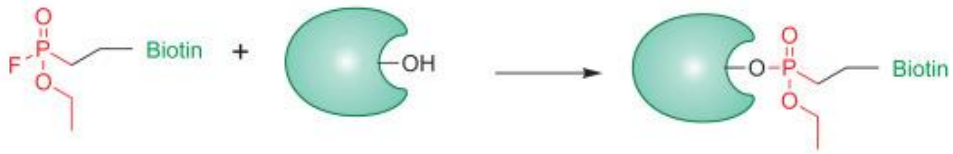
# Fluorophosphonate ABPs





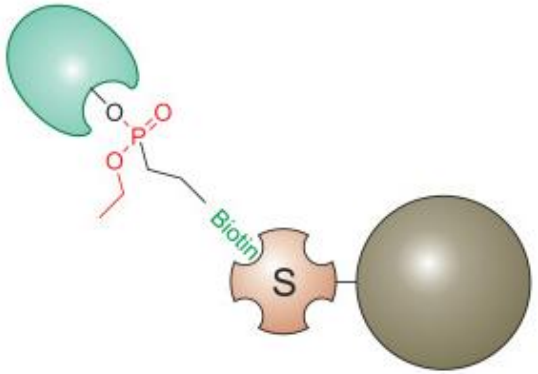

Fluorophosphonate-$PEG_4$-biotin probe




Fluorophosphonate-biotin probe

Serine hydrolase with hydroxyl in active site

Biotin-labeled serine hydrolase




Isolation of serine hydrolases on immobilized streptavidin



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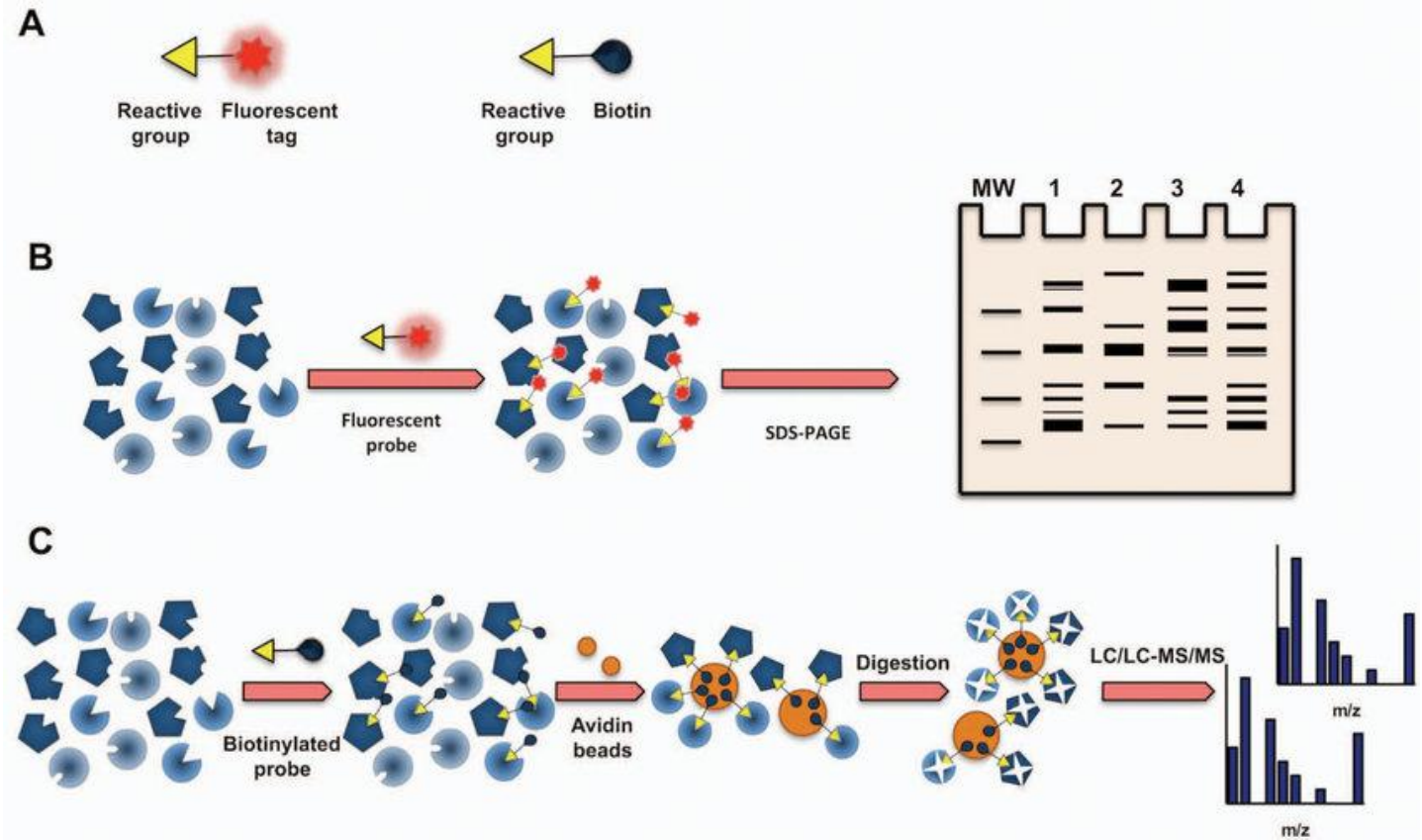
# ABPP – Direct Target Capture



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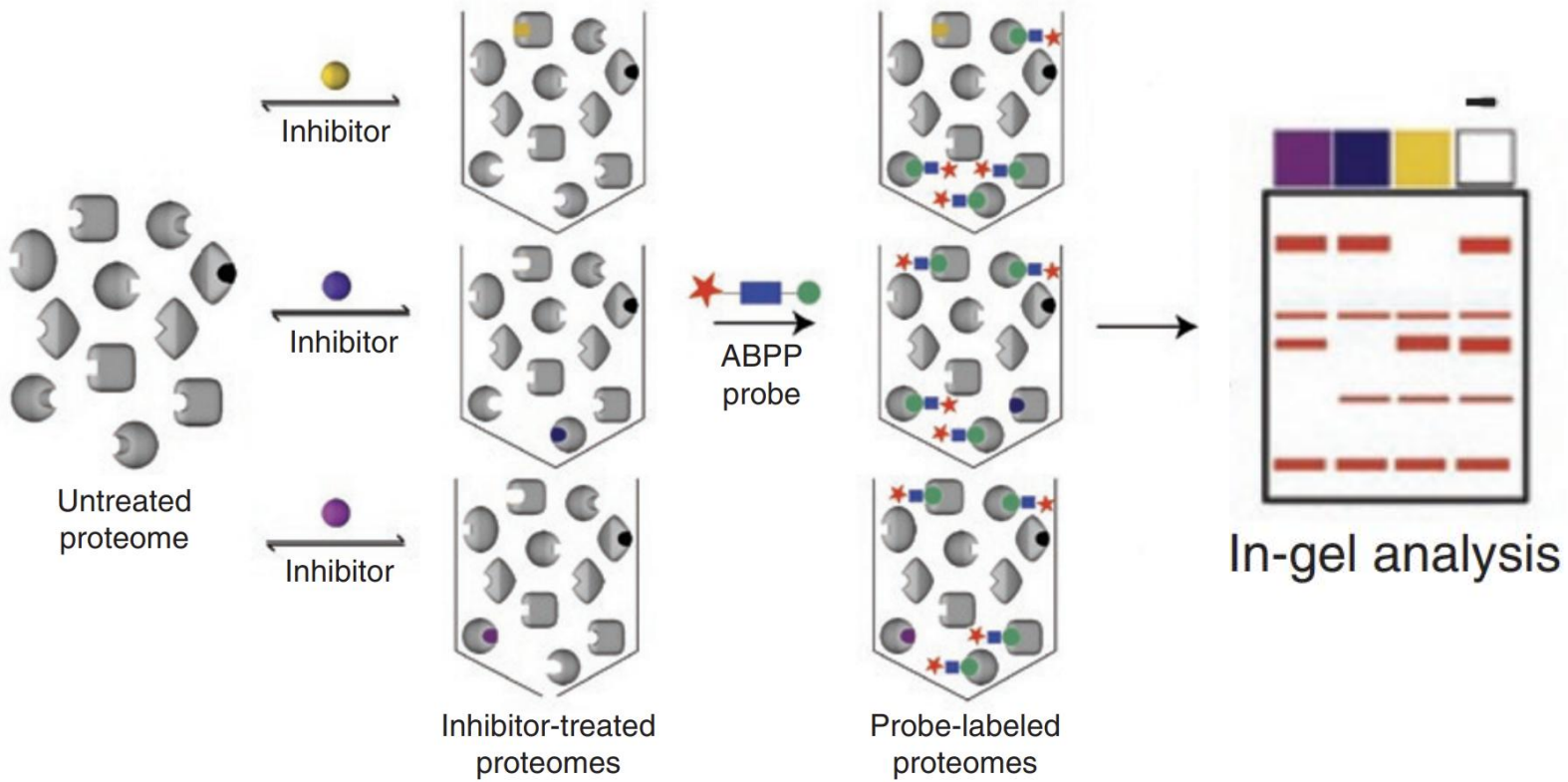
# Competitive ABPP




Q: How would this look like for an enrichment-based probe (e.g. biotinylated)?

Q: What are advantages of the competitive format (over direct capture)?



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# Metabolism



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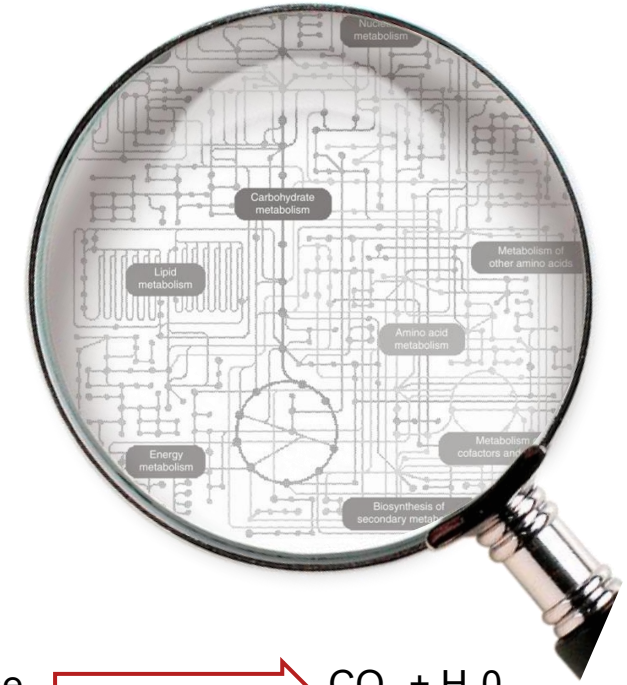
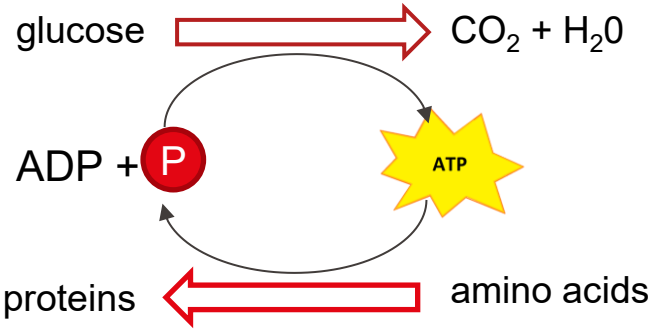
# Metabolism - Overview

- "Sum of all (bio-)chemical reactions in a living organism."
- E.g. *E. coli*:
  - 600-800 enzymes
  - approx. 1,000 metabolites
- Two main types:
  - Catabolism: Breakdown of larger molecules to "generate" chemical energy (ATP)
  - Anabolism: Building of larger molecules at the expense of chemical energy (ATP)



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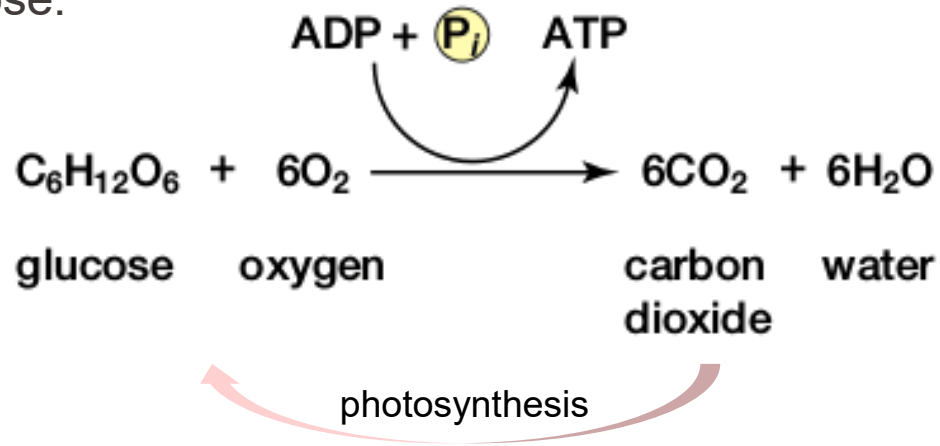
# Cellular Respiration

- = aerobic catabolism of sugars, amino acids, lipids etc.
- For glucose:




- Aerobic respiration: using molecular oxygen as terminal electron acceptor
- Complete oxidation of glucose: approx. 686 kcal/mol
- Energy stored in ATP: approx. 7 kcal/mol

Q: How much ATP is generated per molecule glucose through respiration?



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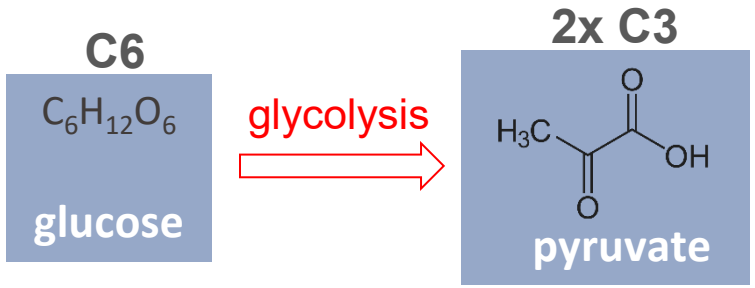
# Glycolysis

- Net reaction:

**glucose** + 2 $NAD^+$ + 2 $ADP/P_i$ → **2 pyruvate** + **2 NADH** + 2 $H^+$ + **2 ATP** + 2 $H_2O$

- 10 enzymatic steps in the cytosol
- no oxygen required
- product: pyruvate
  - further processed of respiration
  - alternative: fermentation (anaerobic catabolism)
  - important anabolic precursor (e.g. amino acids etc.)



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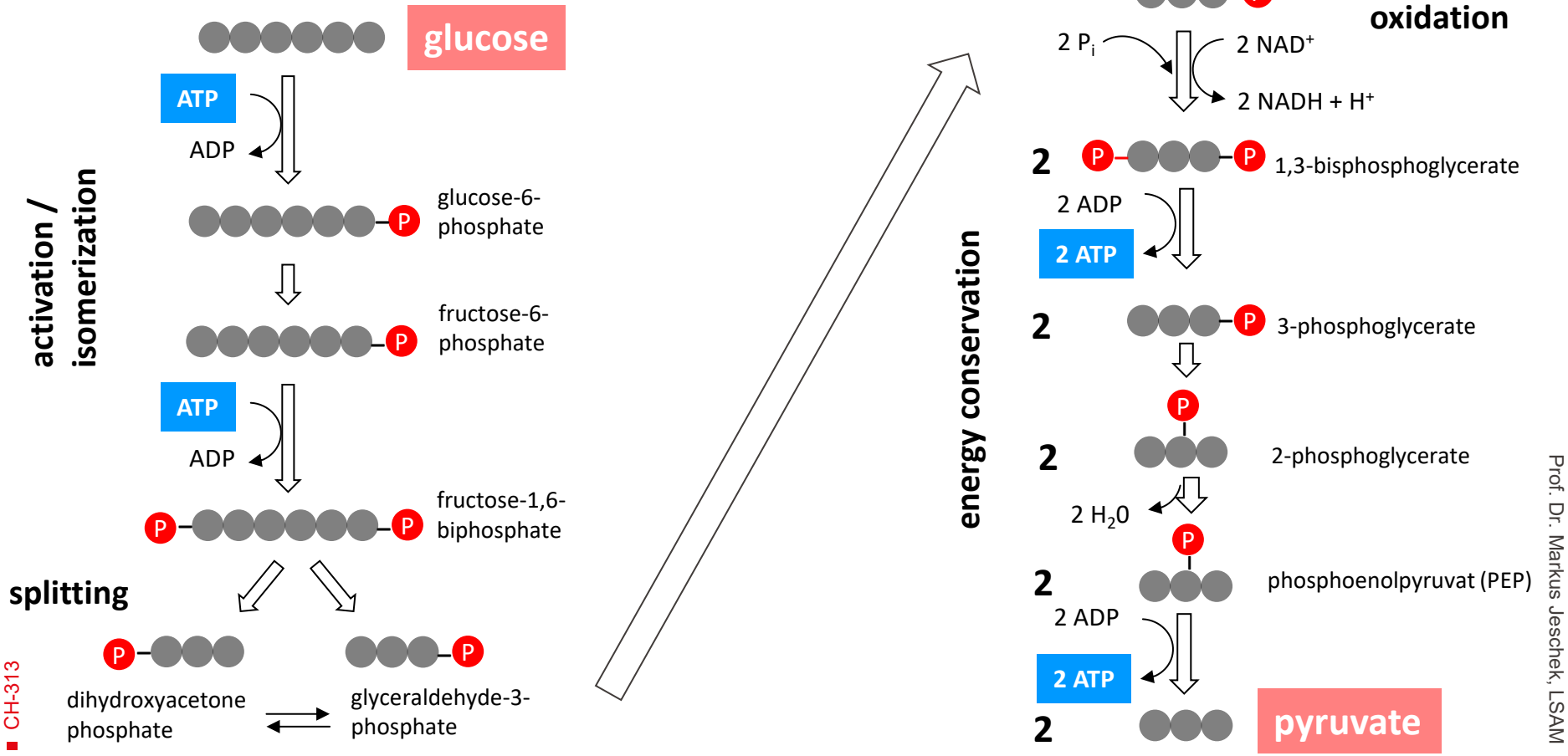
# Glycolysis – Conceptual Steps



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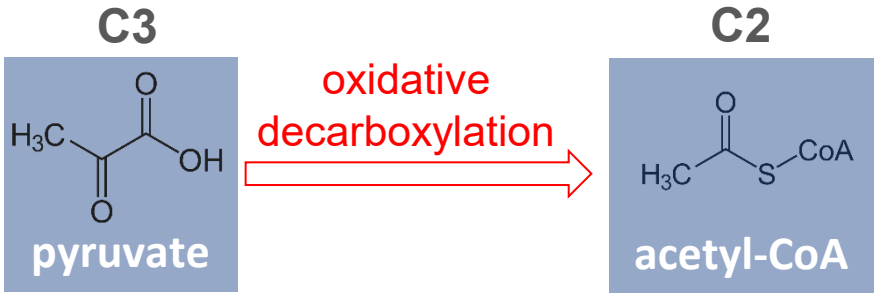
# Oxidative Decarboxylation

- Net reaction (2x per glucose molecule):

**pyruvate** + CoA-SH + $NAD^+$ → **acetyl-CoA** + **NADH** + $H^+$ + **$CO_2$**



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# Citric Acid/Krebs/TCA Cycle

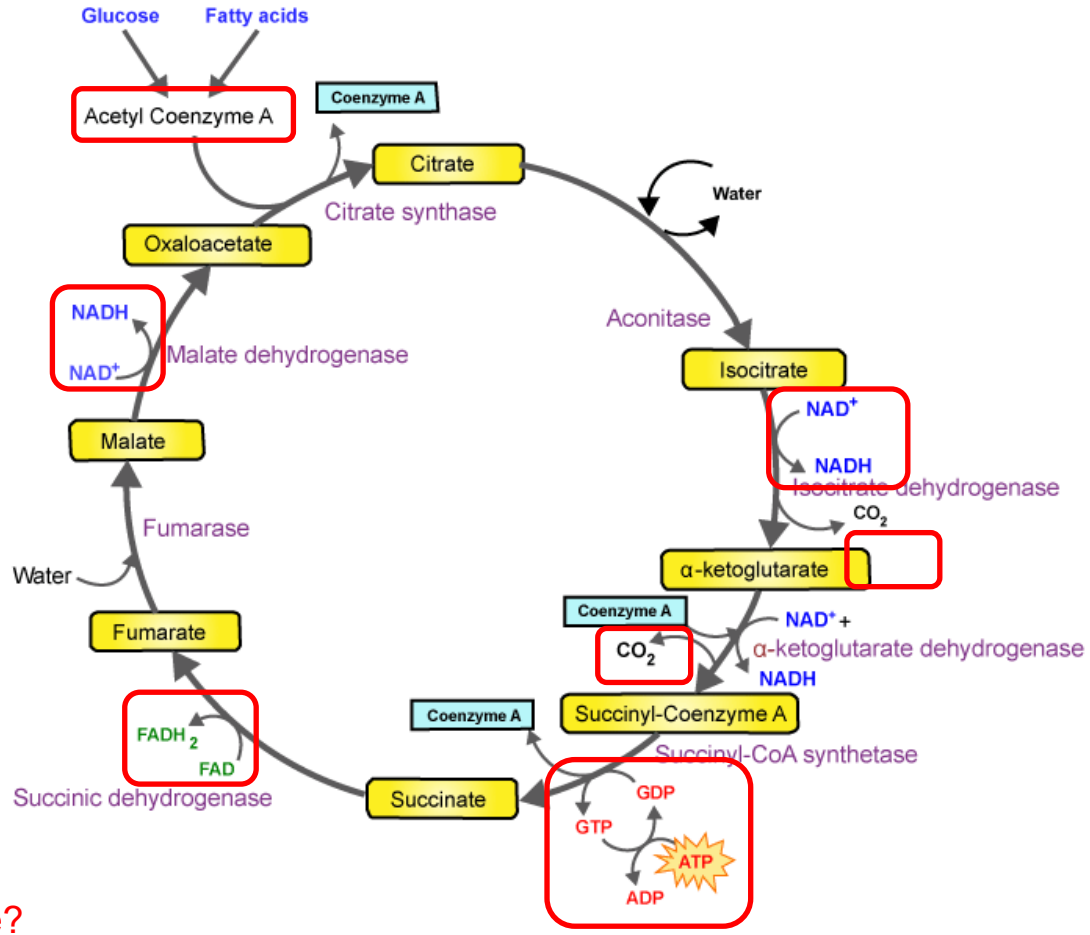
- Net reaction (2x per glucose molecule):

**acetyl-CoA** + 2 $H_2O$ + 3 $NAD^+$ + FAD + $ADP/P_i$

→ **2 $CO_2$** + **3 NADH** + 3 $H^+$ + **$FADH_2$** + **ATP** + CoA-SH

- Delivers various precursors for anabolism (amino acids, fatty acids, purins, pyrimidins etc.)

Q: Where does the TCA cycle take place?



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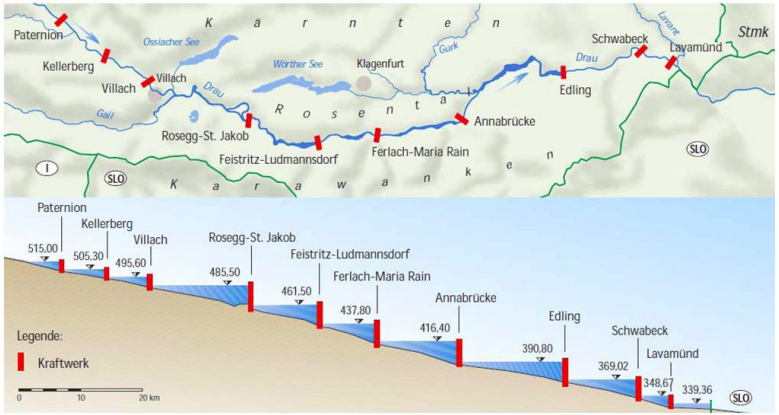
# Electron Transport Chain (ETC)

- Energy and redox balance after TCA cycle (per glucose molecule)
  - 4 ATP (2x glycolysis, 2x TCA)
  - **10 NADH** (2x glycolysis, 2x oxid. decarb., 6x TCA)
  - **2 $FADH_2$** (TCA)

- ETC: controlled, step-wise transfer of electrons to the terminal acceptor $O_2$
  - regeneration of $NAD^+$/$FAD^+$
  - establishment of trans-membrane proton gradient

  (→ later: ATP generation)
  - $\Delta G$ (NADH→NAD+) ~ -218 kJ/mol



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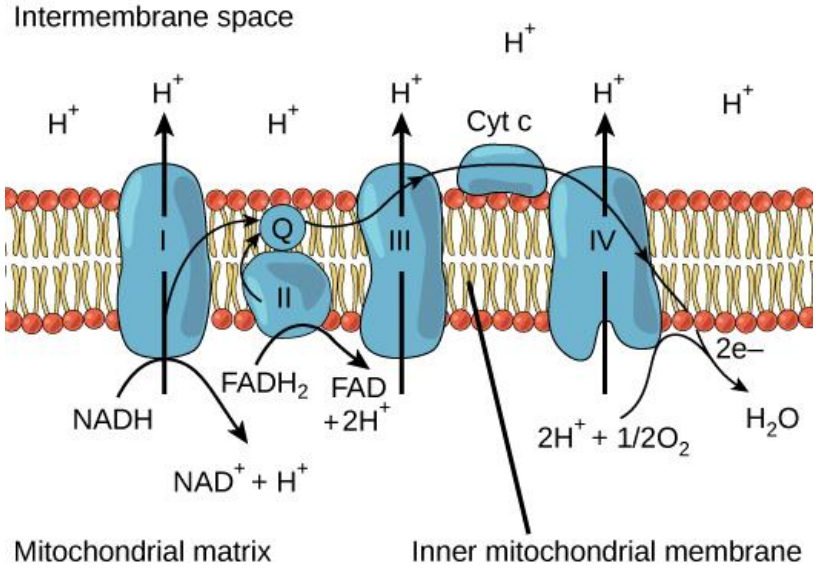
# Electron Transport Chain (ETC)

- Intricate, membrane-bound system of redox enzymes, cofactors and other electron carriers (quinones)
- Four complexes (I – IV)
  - I: NADH dehydrogenase:
  - II: succinate dehydrogenase
  - III: cytochrome c reductase
  - IV: cytochrome c oxidase
- Gradual export of protons

→ proton motive force (PMV) across the membrane



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# Electron Transport Chain (ETC)

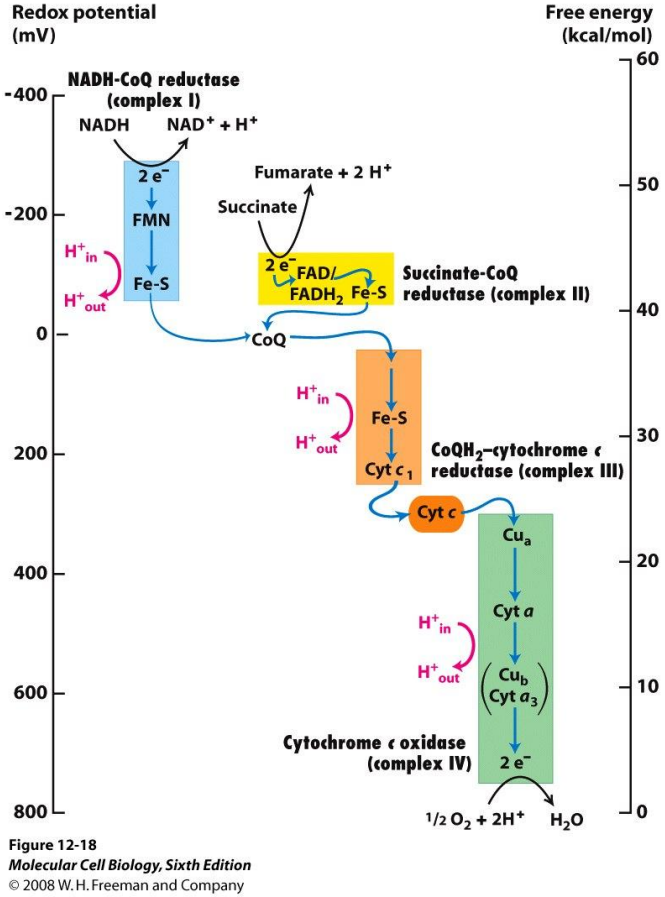
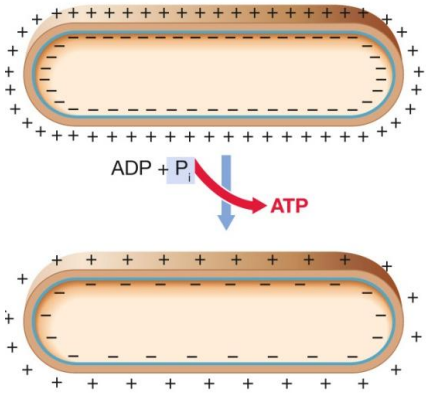



Figure 12-18
*Molecular Cell Biology, Sixth Edition*


- Complex I: 4 $H^+$
- Complex III: 4 $H^+$
- Complex IV: 2 $H^+$

→ 10 $H^+$ per NADH

→ 6 $H^+$ per $FADH_2$



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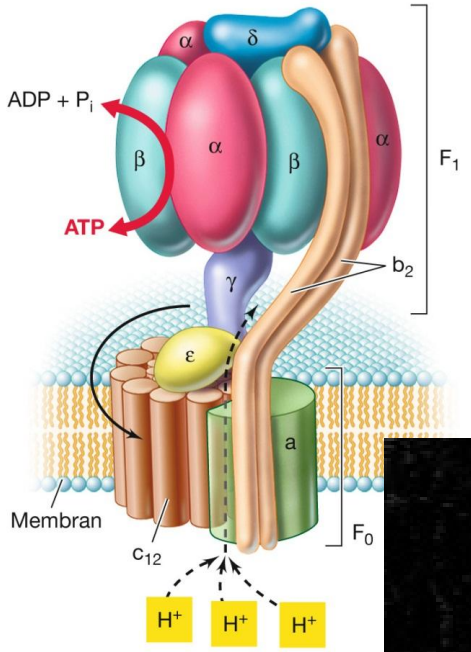
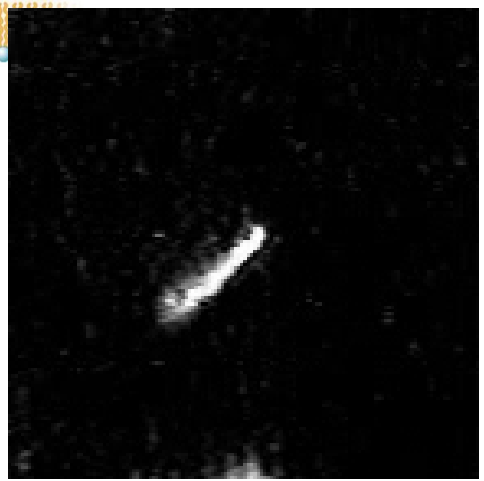
# Oxidative Phosphorylation

- PMV is used to generate ATP
- ATP-synthase (“ATPase”) complex
  - $F_0$: protons are channelled to the inside leading to rotation of c subunits
  - $F_1$: rotation leads to conformational changes that lead to ATP formation
- 3-4 protons per ATP
- ATPase reaction is reversible!



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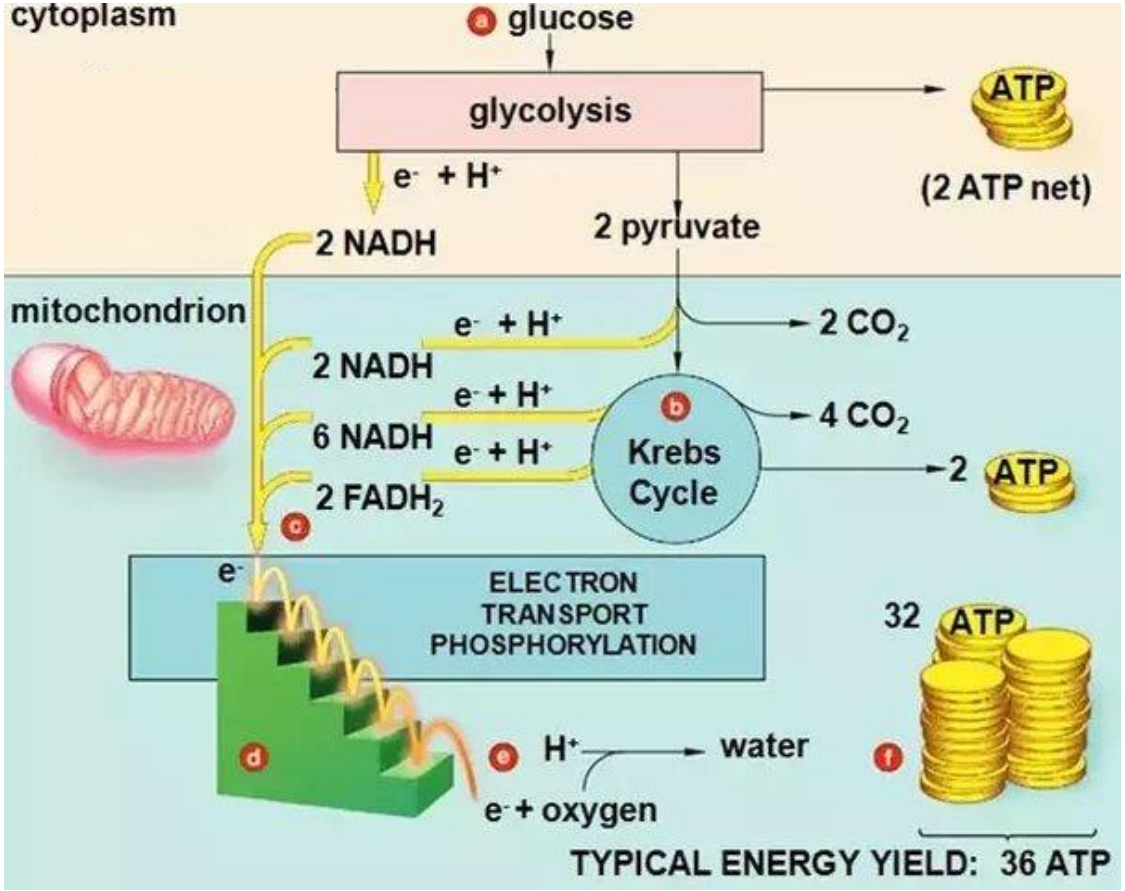
Q: What could the reverse reaction of the ATPase be used for in nature?

# Balance of Aerobic ATP Generation



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# Fermentation

- Catabolism of organic substrates (e.g. sugars) without using oxygen
- Terminal electron acceptors are organic metabolites (substrate-derived)
- Goals:
  - Regeneration of $NAD^+$!
  - ATP generation (in addition to glycolysis) → not all types of fermentation

Q: Which types of fermentations do you know? Which are associated with these pictures?



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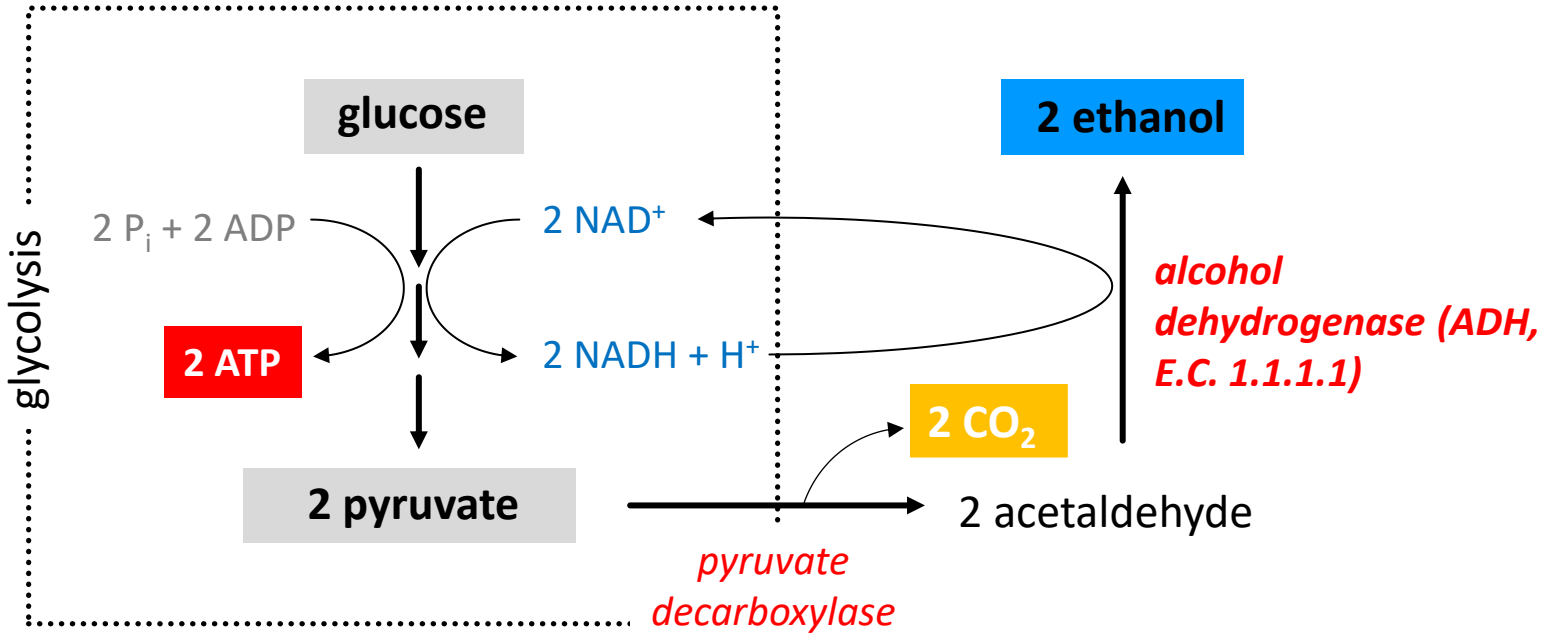
# Alcoholic/Ethanol Fermentation




- different yeasts (facultative anaerobes), few bacteria
- no additional ATP production

Q: What are facultative anaerobes?



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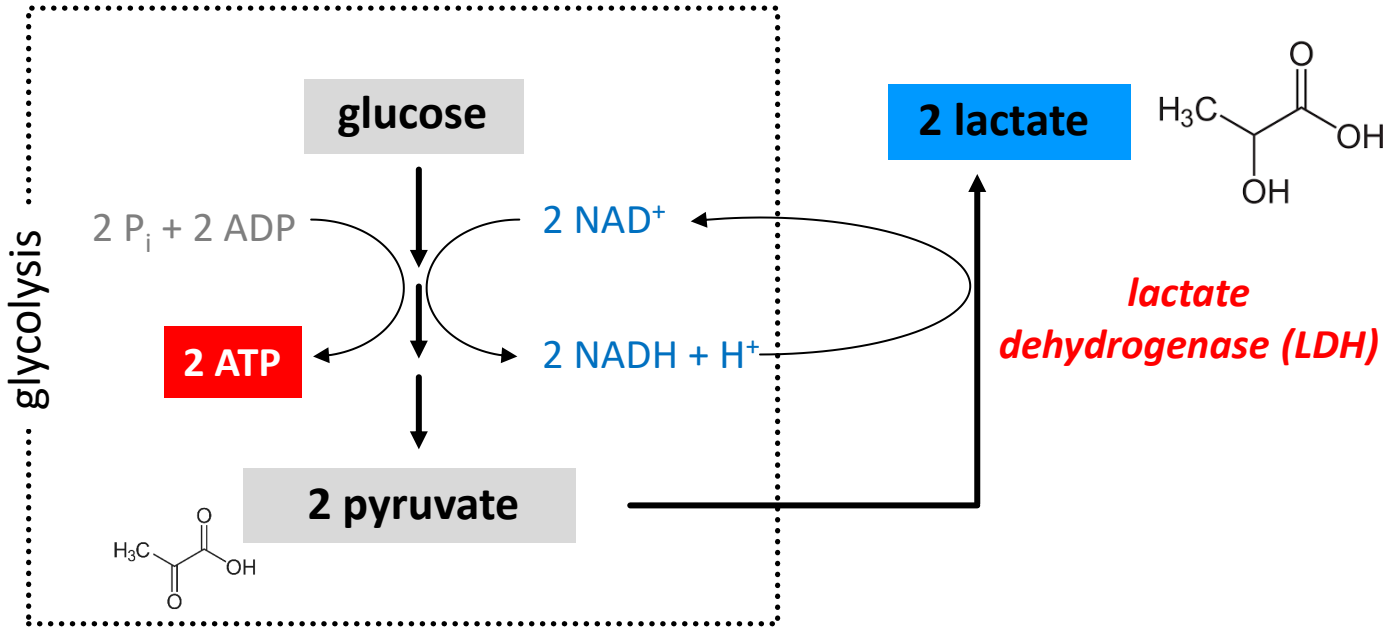
# Lactic Acid Fermentation (Homolactic)




- different bacteria, human cells (e.g. muscles)
- no additional ATP production



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# The Warburg Effect

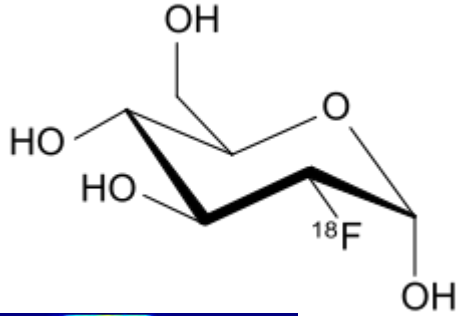
- Otto Heinrich Warburg, Nobel Prize 1931
- most cancers use “aerobic fermentation” and lactic acid fermentation for energy generation (not respiration via TCA!)
  - low ATP yield
  - no complete break down of metabolites to $CO_2$

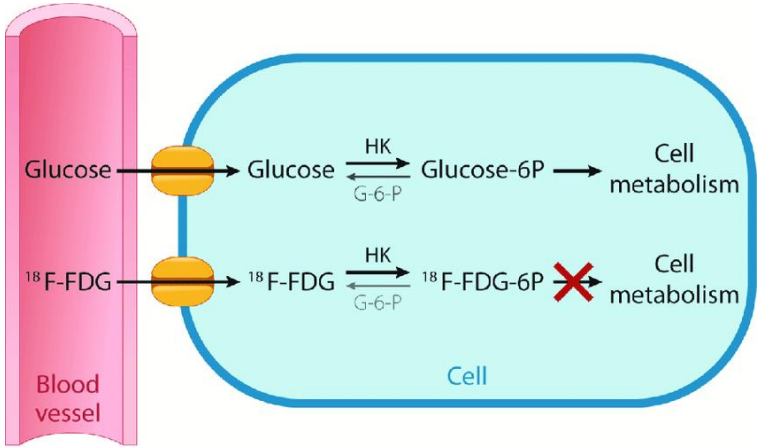
  → increased precursor supply for anabolism/biomass formation
  - fast glucose consumption! (glycolysis rate is approx. 200-fold compared to “normal” cells)
- Related: so-called “Crabtree effect” yeast

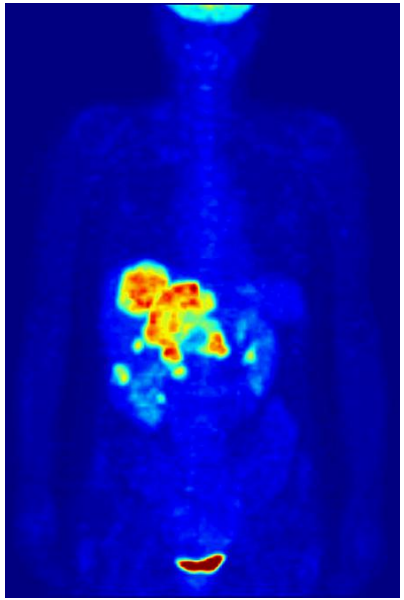
Q: What is the Crabtree effect?

# Visualization of Glucose Consumption

- Fluorodeoxyglucose (18F)
- Radiopharmaceutical for positron emission tomography
- Taken up by cells and phosphorylated by hexokinase
- Not further metabolized → accumulation in glucose-consuming cells (e.g. tumors)





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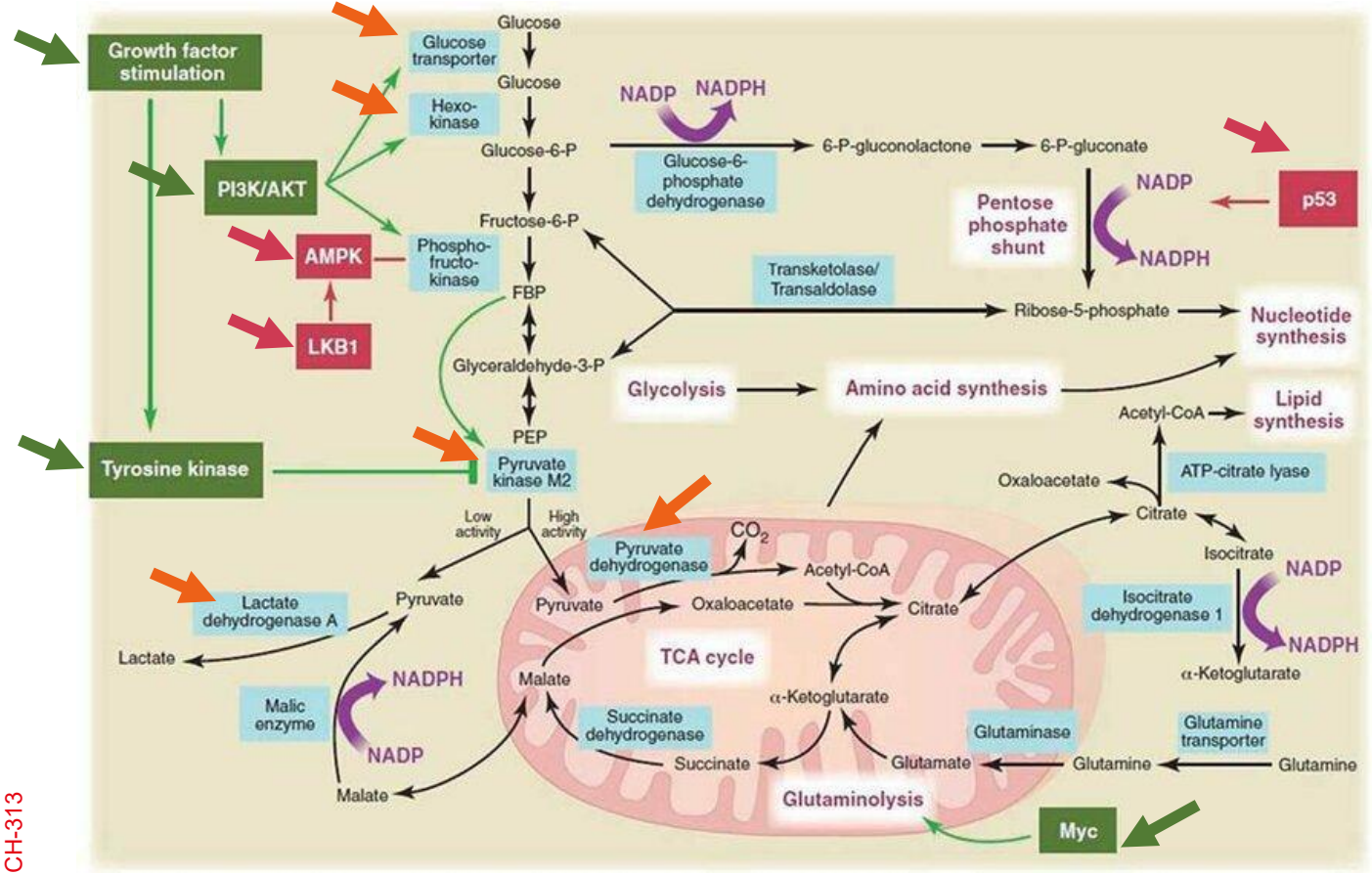
# Cancer Metabolism and Therapy




oncogenes

tumor suppressor genes

potential drug targets



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