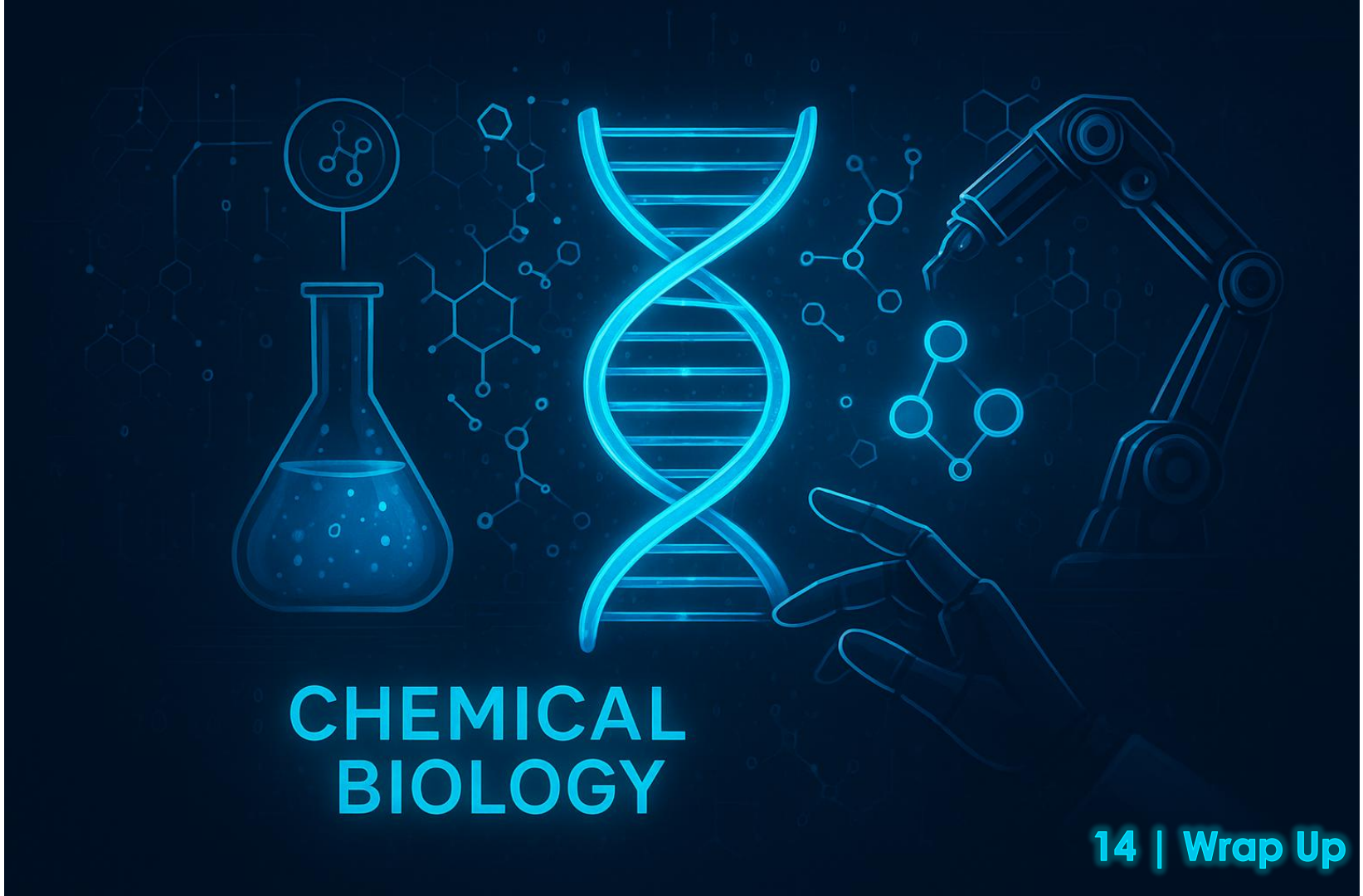

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General Course Information

- 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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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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Information Exam

- Wednesday January 14th , 2026
- 09:15 – 12:15 (actual examination time: 90 minutes)
- Room: AAC 1 32

- Mode: mix between free text, single- and multiple-choice questions (pointing scheme will be explained before the examination)

- Recommendations:
  - keep the answers short and only answer what is asked for (not more)
  - first answer(s) will be counted

Recap

Important Note

This is an overview of the most important topics discussed during the lecture including exemplary questions. It is not comprehensive and questions about other parts will be asked during the examination.

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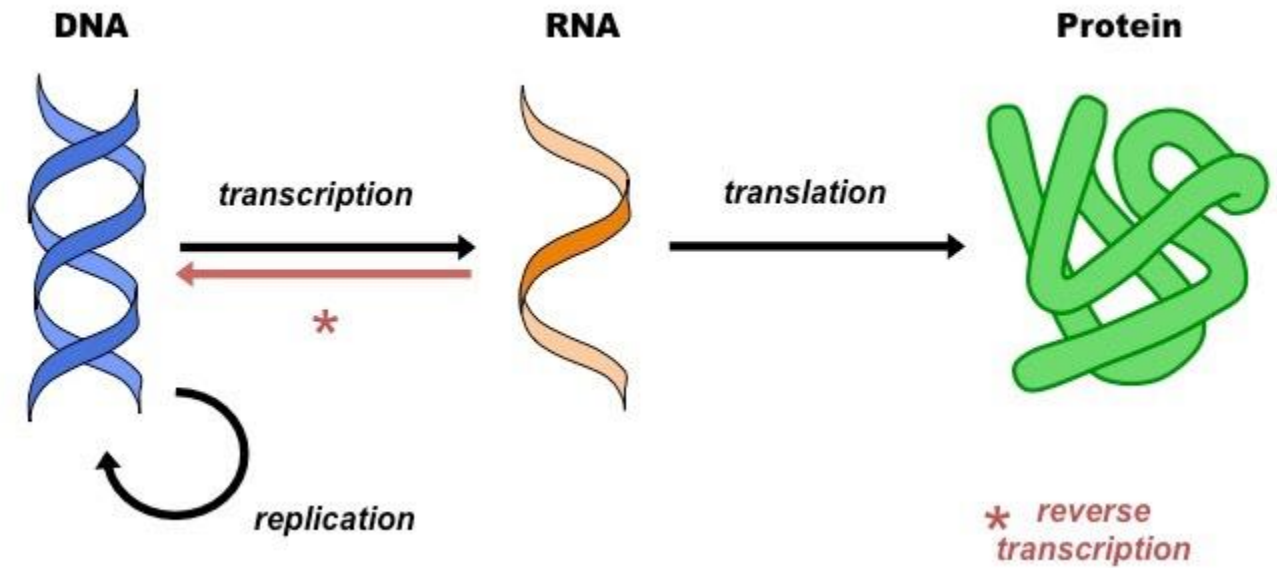
01_Introduction

- Central dogma
- Genetic code
- Basic components and structure of pro- and eukaryotic cells (differences!)
- DNA
  - molecular structure
  - key properties

The Central Dogma

- …of Molecular Biology



Q: What are the “biological machines” responsible for the different processes?

Q: What is the genetic code?

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Example Question 1

- Name five differences between pro- and eukaryotic cells

02_DNA

- Oligonucleotides
  - Chemical synthesis (conceptual steps)
  - HTP oligo synthesis (basic technologies)
  - Synthesis errors
- Typical sizes of genetic parts (approximately)
- Conceptual steps to synthesize larger DNA fragments
- Maxam-Gilbert and Sanger Sequencing
  - Basic principles
  - Sanger: Measures to increase the efficiency of the method
  - Length limitation and strategies to read longer sequences

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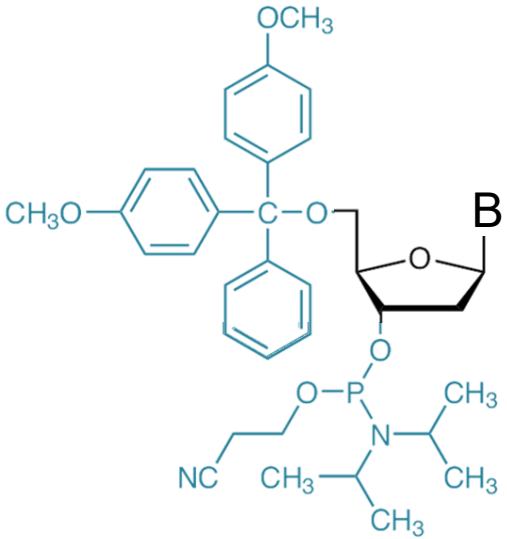
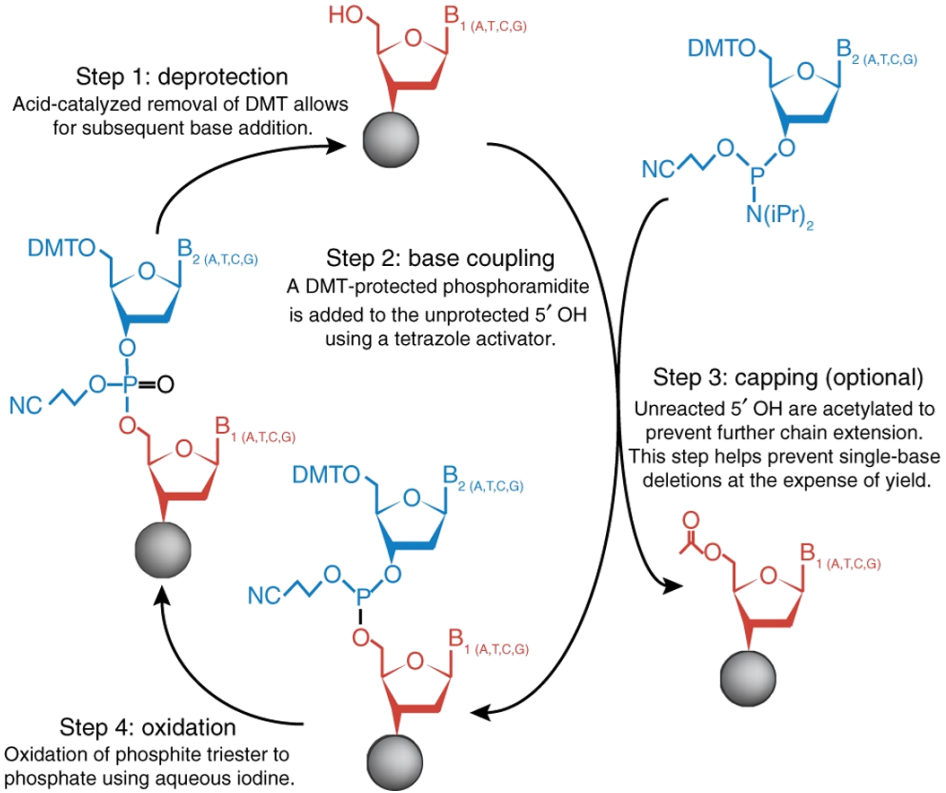
Chemical Oligo Synthesis

dimethoxytrityl (DMT)



phosphoramidite



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Example Question 2

- Which of the following sentences are true (T) or false (F)?
  - Like natural DNA synthesis by polymerases, chemical oligo synthesis occurs in the direction from 3’ to 5’
  - Commonly, the per-nucleotide error rates are higher for microarray synthesis of oligos compared to column-based synthesis
  - In Sanger sequencing, di-deoxynucleotides (ddNTPs) are added instead of normal dNTPs to a PCR reaction
  - Repetitive sequences still represent a major limitation for available sequencing technologies

03/04_DNA(NGS)

- NGS
  - Main methods
    - Conceptual steps that lead to a base- and position-specific signal (“sequencing chemistry”)
    - Hardware setup of the main methods
    - Distinctive feature of 3rd generation methods (vs. 2nd generation)
    - Main pros and cons of methods

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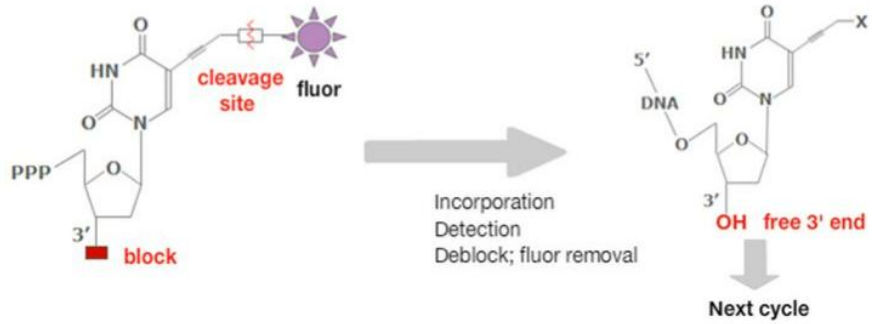
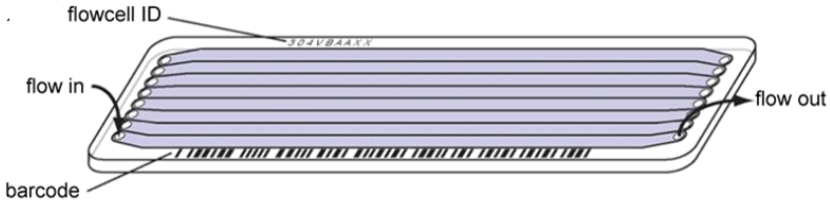
Sequencing by Synthesis (Illumina)

- "Extension of Sanger principle"
- Most widely used NGS technology today
- Sequencing on chip surface ("flow cells")
- **Fluorescently labelled, "blocked" dNTPs**
- **Reversible termination**: blocked dNTPs converted into dNTPs to continue elongation after each nucleotide/"cycle"





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Example Question 3

- Name two advantages of Sequencing by Synthesis (Illumina) over SMRT sequencing (PacBio)

04/05_DNA

- Eukaryotic cell cycle (main phases)
- GFP (structure, chromophore)
- Flow cytometrie (principle, histograms, 2D dot plots)
- DNA intercalators
- Click-chemistry (Azide-Alkyne)
- Nucleoside analogues
    - Labelling
    - Therapeutics

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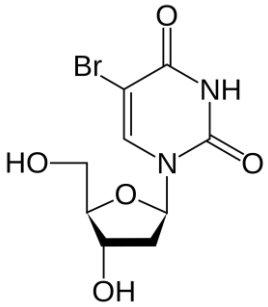
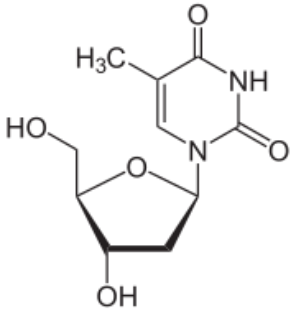
Nucleoside Analogues – Labelling

- BrdU (5-bromo-2'-deoxyuridine, “broxuridine”)
  - incorporates in DNA instead of T
  - detectable with anti-BrdU antibodies (fixed samples!)
  - cancerogenic
  - *in vivo* use possible

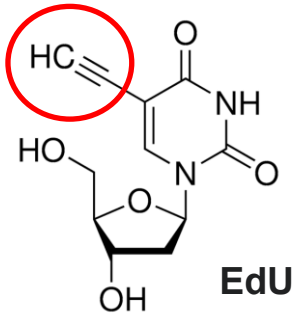


BrdU



thymidine

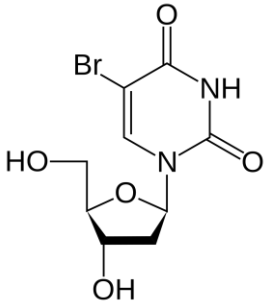
- EdU (5-ethynyl-2’-deoxyuridine)
  - incorporates in DNA instead of T
  - labelling with “clickable” dyes
  - no denaturing required!
  - DNA damage via interstrand crosslinking



EdU

Example Question 4

- Which statements about BrdU (5-bromo-2'-deoxyuridine, “broxuridine”) are true (T) or false (F)?
  - BrdU incorporates in DNA instead of A
  - BrdU can be labelled via alkyne/azide click chemistry
  - Fixing of samples is required upon BrdU labelling to stop the DNA polymerase reaction
  - BrdU is a cancerogenic nucleotide analogue



BrdU

05/06_RNA

- RNA aptamers incl. examples
- Riboswitches
- SELEX (conceptual steps)

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Example Question 5

- Name the two main parts of a riboswitch and their function?

06/07_Translation

- Pro- and eukaryotic mRNAs and ribosomes
- The genetic code
- tRNAs and amino acyl tRNA synthetases
- translation
- Translational blockers
- mRNA display
- ncAA incorporation/amber suppression
- Engineering of orthogonal pairs (positive/negative selection)
- Exemplary ncAAs incl. functionalities

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The Genetic Code and Amino Acids

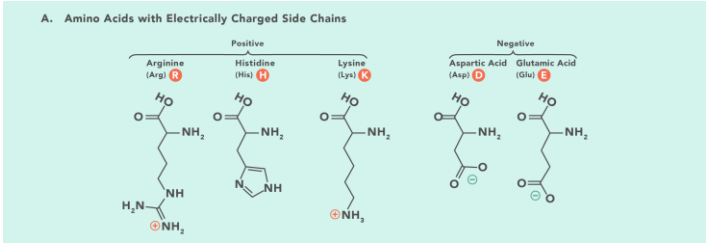
- Universal amongst all forms of life
  - 64 codons
  - 20 amino acids
  - 3x Stop signals
  - Start signals:
    - Bacteria: AUG (also GUG, UUG)

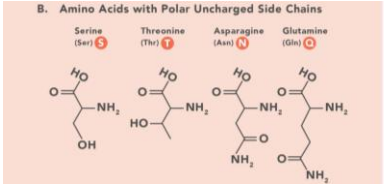
    → N-formylmethionine (fMet)

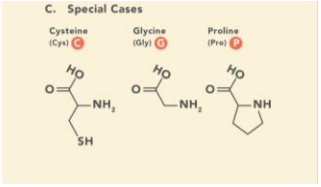
    - Eukaryotes: AUG

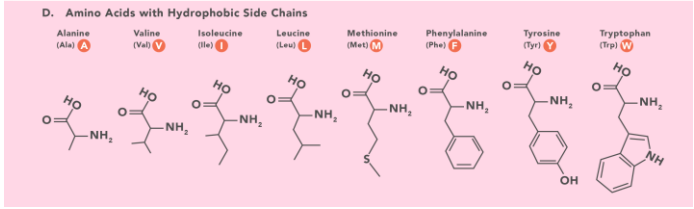
    → Methionine (Met)

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	**UAA Stop**	**UGA Stop**	A
	UUG Leu	UCG Ser	**UAG Stop**	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G









Q: Where is the genetic code encoded?

RECAP

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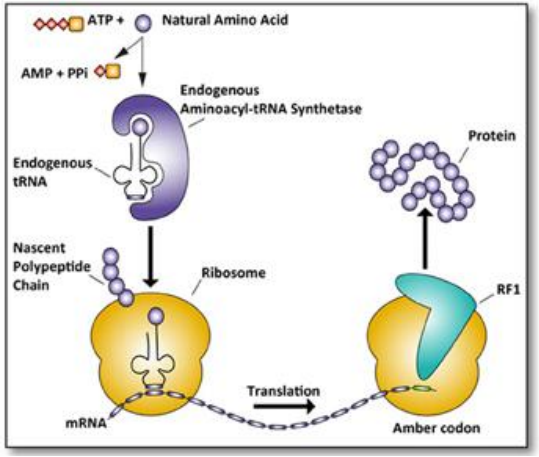
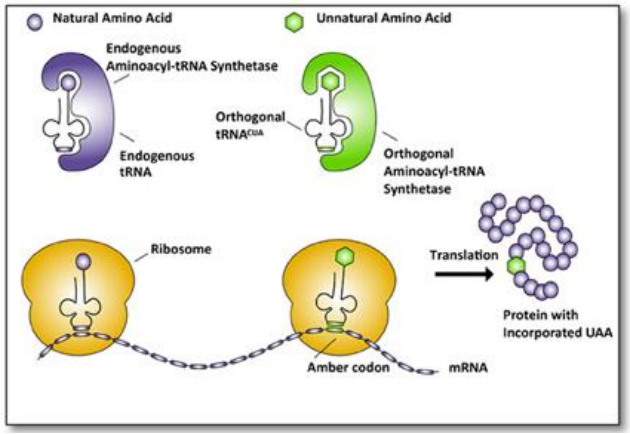
Amber Suppression – Principle

- Natural translation termination: a stop codon is decoded by a release factor (RF) aborting translation
- Amber suppression:
  - (1) a tRNA decoding the amber stop codon (UAG) is loaded with ncAA by engineered tRNA synthetase
  - (2) the ncAA is incorporated upon occurrence of UAG in the mRNA via the ribosome (instead of stopping)
- "orthogonal pair"
  - tRNA + tRNA synthetase
  - Requirements:
    - loading of tRNA with ncAA
    - no loading with other amino acids!





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Example Question 6

- Name two modes of action of translation-blocking antibiotics and one example for one of the two.

08/09_Enzymes

- How do enzymes work?
- Different types of selectivity
- EC classes
- Cofactors (types and examples)
- HFCS process

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Example Question 7

- Which of the following statements about the HFCS process are true (T) or false (F)?
  - In the process, E.C. classes III and VI are involved
  - Oligosaccarides are converted by an isomerase to increase sweetness
  - Fructose has a higher perceived sweetness and thus higher value than glucose
  - Xylose isomerase irreversibly converts glucose into fructose

09/10_Enzymes (Kinetics)

- Reaction rates and rate constants
- Michaelis-Menten model
- Catalytic efficiency
- Inhibitions
- Temperature dependence
- Enzyme inactivation

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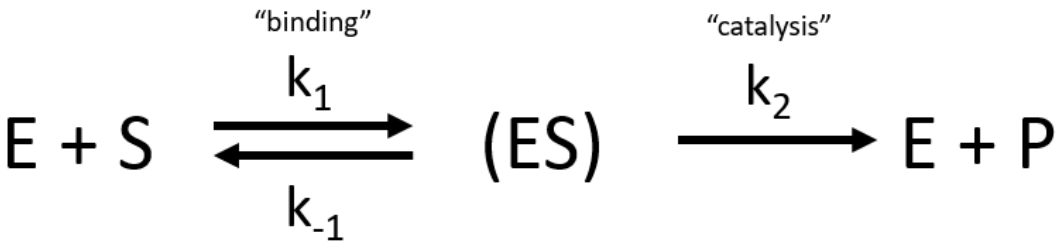
Michaelis-Menten (MM) Model

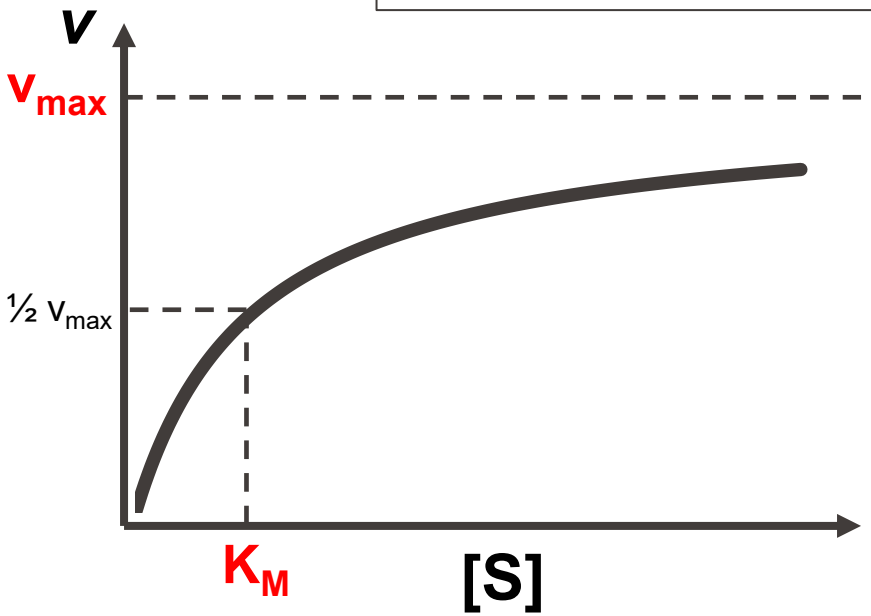


S: substrate
P: product
v: reaction rate
E: enzyme
(ES): enzyme-substrate complex
k_1: rate constant (ES) formation
k_{-1}: rate constant (ES) disintegration
k_2: rate constant P formation

Michaelis-Menten equation:

$$-\frac{d[S]}{dt} = v = \frac{v_{max} * [S]}{K_M + [S]}$$

K_M: [S] at which v = 0.5 v_{max}



Q: How do you get to the Michaelis-Menten plot experimentally?

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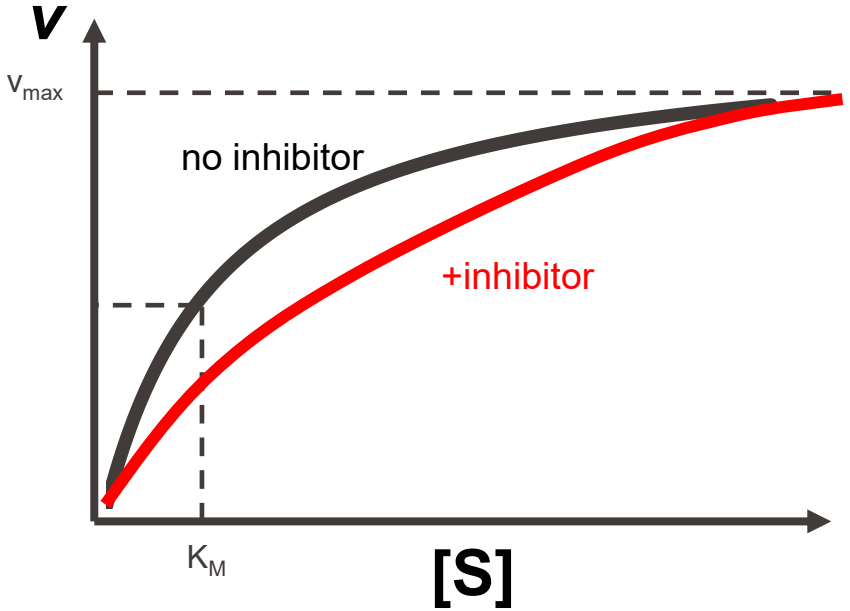
Example Question 8

- Name two assumptions (simplifications) that underlie the basic Michealis-Menten model

Example Question 9

- Which type of inhibition is likely giving rise to the following Michaelis-Menten graph? What would happen if you double the inhibitor concentration (draw curve in)?



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11/12/13_Enzymes (Drugs)

- Competitive inhibitor drugs
- IC_{50}
- Uncompetitive inhibitor drugs
- Transition state analogs
- Covalent drugs (incl. amino acids)
- Activity-Based Protein Profiling (ABPP)
- Biotin-Streptavidin Technology

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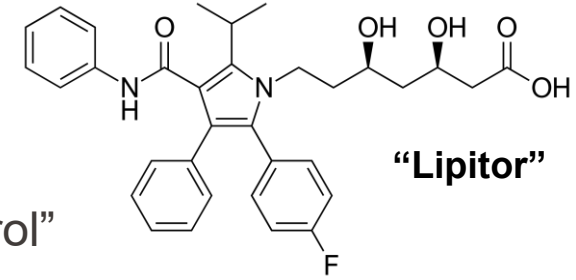
Atorvastatin ("Lipitor")

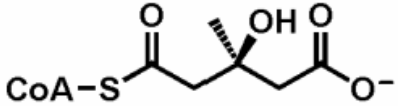
- Statin for oral treatment of cardiovascular diseases
- Reduces cholesterol biosynthesis → reduced

levels of low-density lipoprotein (LDL) = "bad cholesterol"

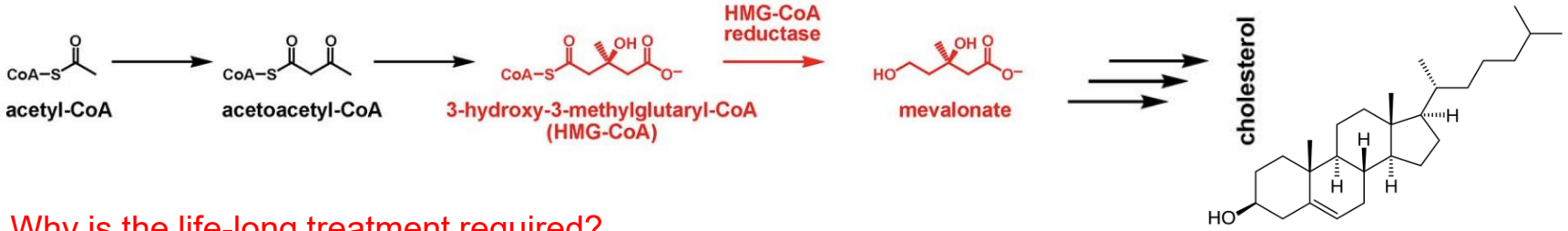
- Inhibitor of HMG-CoA reductase (liver)
- WHO list of "essential medicines"
- Life-long treatment required!



"Lipitor"



HMG-CoA



Q: Why is the life-long treatment required?

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Example Question 10

- What are the three-letter and one-letter codes for the amino acids alanine, tyrosine, glutamine and serine? Draw the structure for two of them indicating also the correct stereochemistry at chiral centers.

13_Metabolism

- Net reactions for different types of metabolism (cofactor balance!)
- Conceptual understanding the different metabolic pathways discussed
- Cellular locations of different metabolic processes
- Fermentations
- Warburg effect

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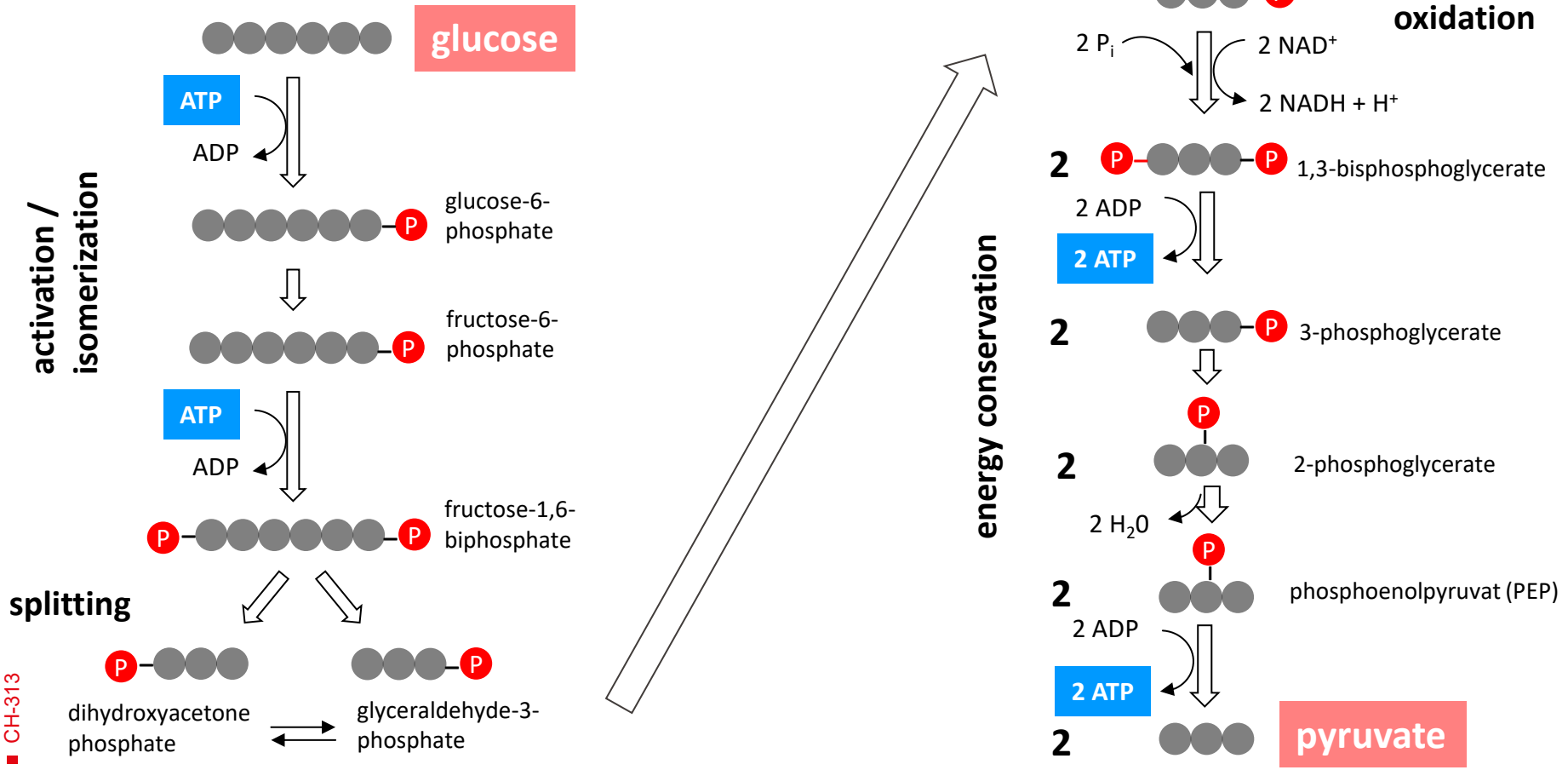
Glycolysis – Conceptual Steps



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Citric Acid/Krebs/TCA Cycle

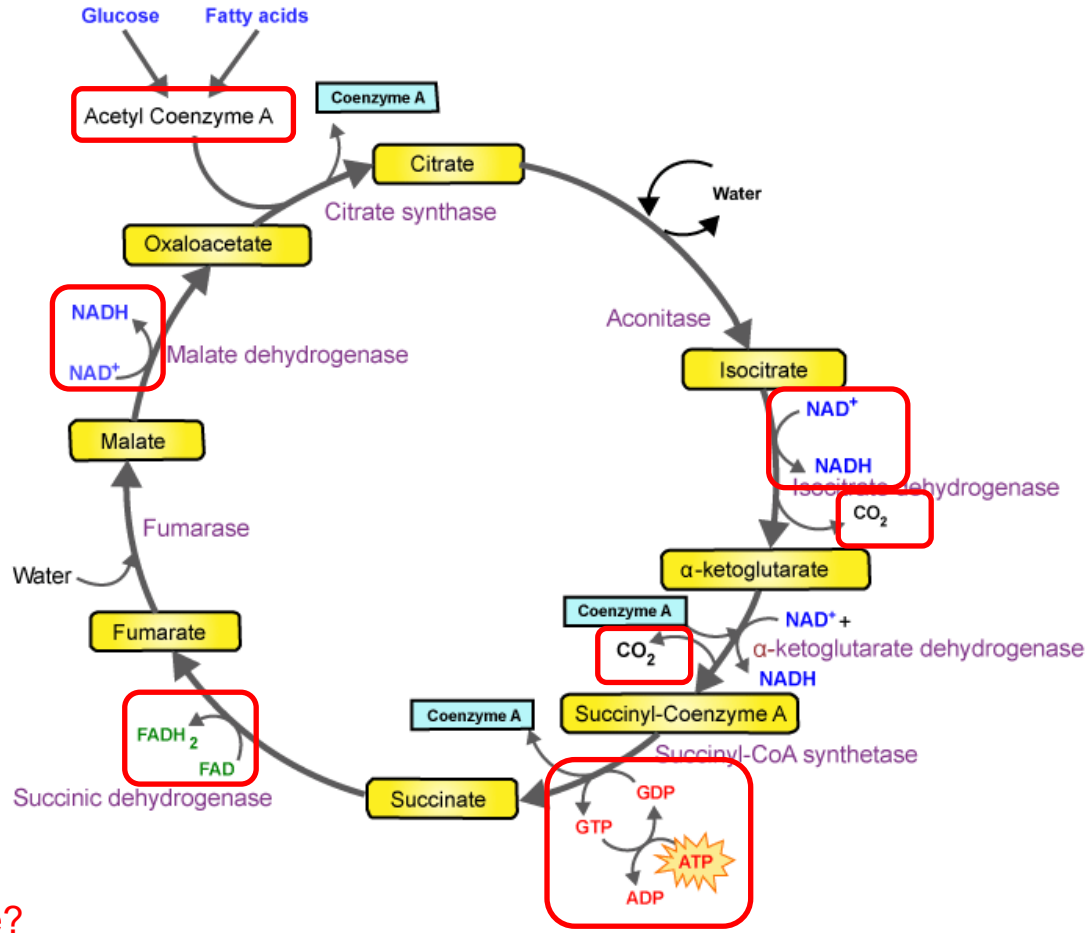
- Net reaction (2x per glucose molecule):

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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Example Question 11

- Which of the following statements about cellular respiration are true (T) or false (F)?
  - Cellular respiration offers a higher ATP yield than fermentative metabolism
  - Glycolysis is a part of cellular respiration, which requires oxygen
  - The ATP synthase complex can pump protons out of bacterial cells
  - In the ETC, Protons are pumped outside while NADH is reduced

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