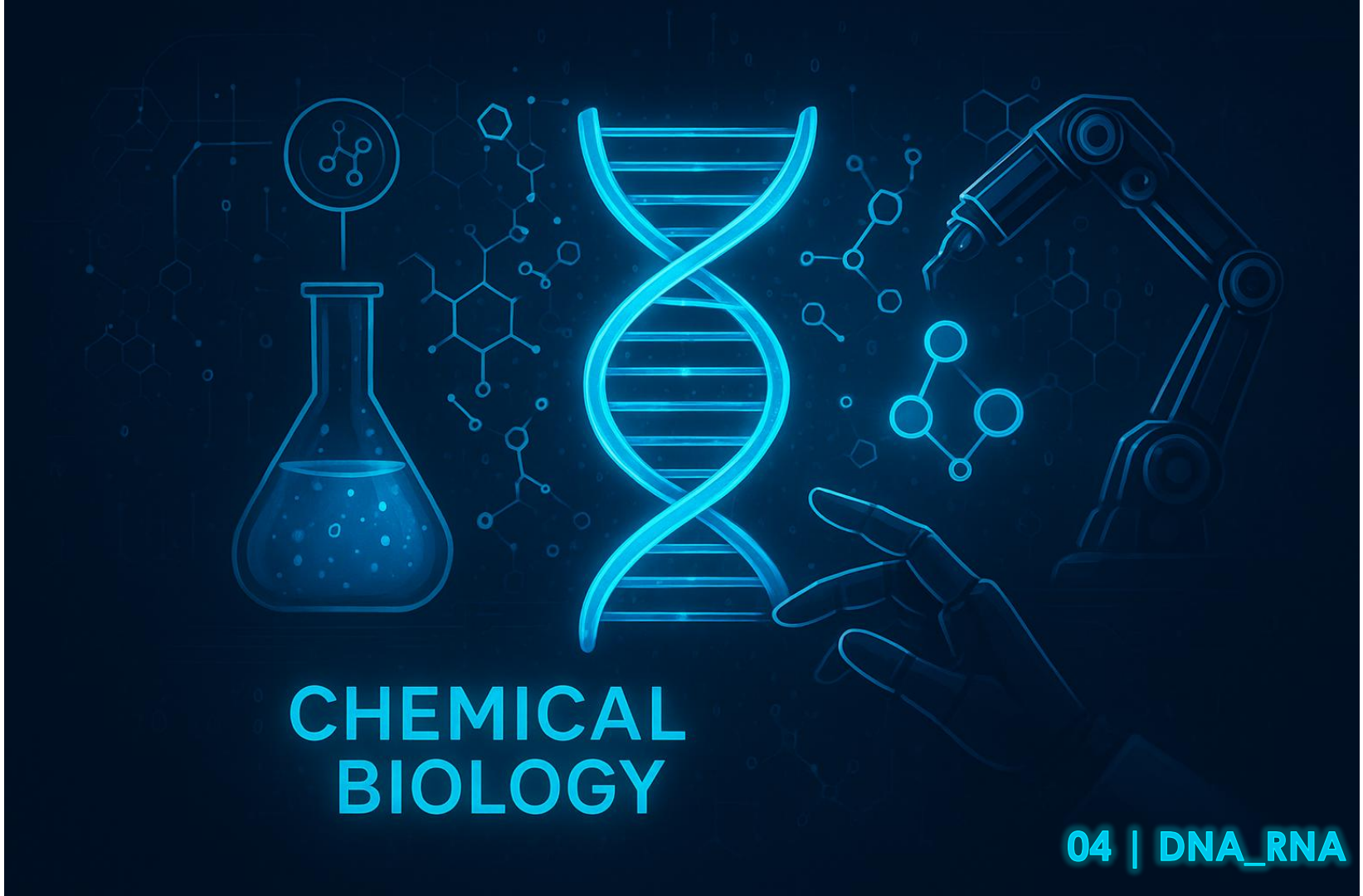

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General Course Information

- 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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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 14, 2025
6	Abigail	Robin	**Nov 4, 2025**	Oct 28, 2025
7	Eva	Florian	**Nov 11, 2025**	Nov 4, 2025
8	Bastien	?	**Nov 18, 2025**	Nov 11, 2025
9	Melodie	**Siolène (E-Mail)**	**Nov 25, 2025**	Nov 18, 2025
10	Nicole	Maria	**Dec 2, 2025**	Nov 25, 2025
11			**Dec 9, 2025**	Dec 2, 2025

Course Topics – Overview

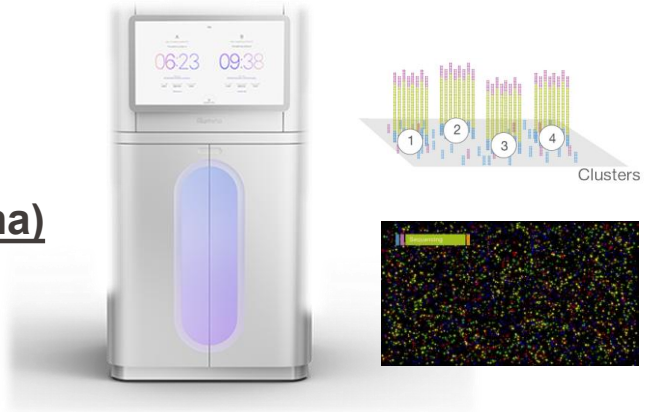
- Week 1 | Introduction + DNA
- Week 2 | DNA
- Week 3 | DNA
- **Week 4 | DNA/RNA**
- Week 5 | Protein/Enzymes
- Week 6 | Enzymes
- Week 7 | Enzymes
- Week 8 | Membranes
- Week 9 | Metabolism
- Week 10 | Metabolism
- Week 11 | Engineering
- Week 12 | Engineering
- Week 13 | Engineering
- Week 14 | LSAM Intro + Exam Preparation

[tentative schedule]

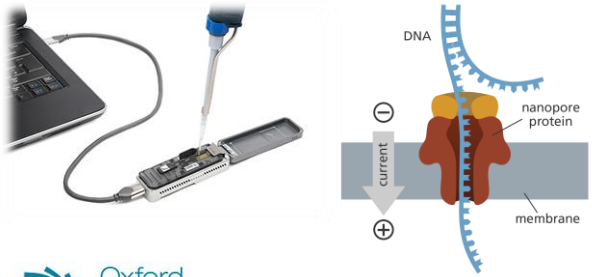
DNA (“read”)

NGS – Main Methods (Overview)

- "Second generation" methods
  - **(1) Pyrosequencing (Roche 454)**
  - **(2) Sequencing by synthesis (Illumina)**
  - (3) Sequencing by ligation
  - (4) Ion semiconductor sequencing



- "Third generation" methods (single molecule)
  - **(1) Single-molecule real-time sequencing (SMRT; Pacific Biosciences)**
  - **(2) Nanopore sequencing (MinION etc.; Oxford Nanopore Technologies)**



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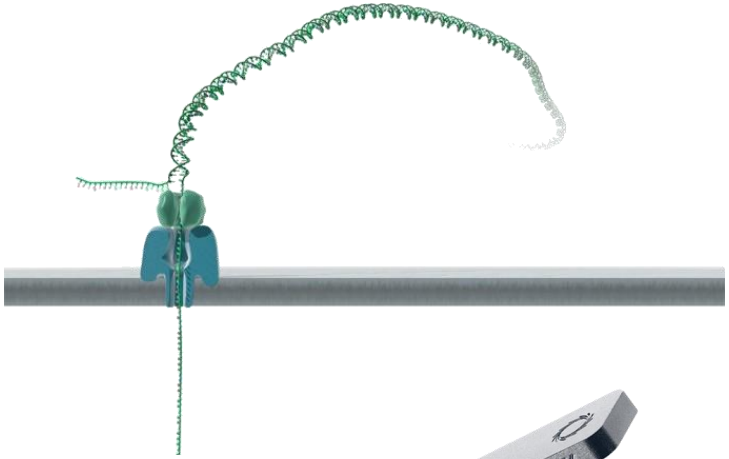
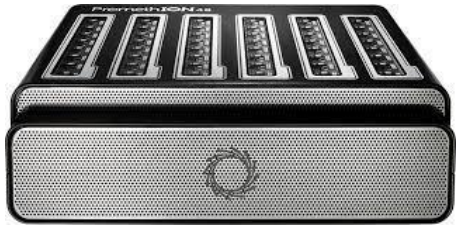
Nanopore Sequencing

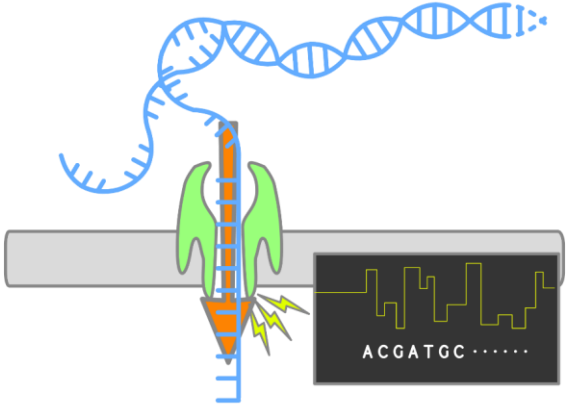
- Oxford Nanopore Technologies (MinION, PromethION etc.)
- Label-free, single-molecule technique
- Portable equipment, real-time results
- Long-read technology (> 4Mbp successfully demonstrated)
- 512 – 2,675 pores per flow cell, repeated passage possible
- Error rates of 3-8% (lately improving drastically)
- Modified bases and other molecules (RNA, proteins) can be directly “sequenced“

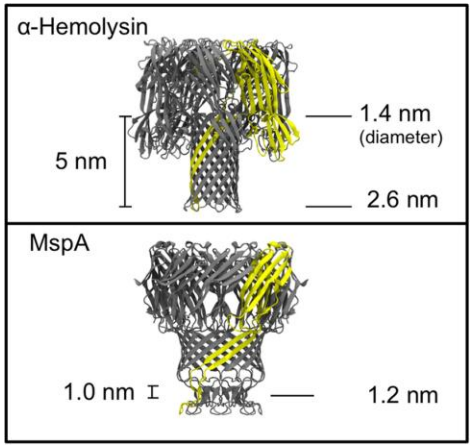




Nanopore Sequencing - Principle

- single-stranded DNA/RNA molecules are “pushed“ through nanopore via processive enzyme (e.g. DNA helicase)
- pore embedded in membrane and surrounded by electrolyte
- electric field across the membrane → electrophoretic motion of ions through pore
- if a larger molecule (e.g. DNA strand) occupies pore, ion flux is disrupted (detectable by voltage change in real time)
- voltage changes are specific for base/molecules





- pores: biological (α-hemolysin, MspA) or solid-state (metal, metal alloy)
- mostly synthetic membranes

https://www.youtube.com/watch?v=E9-Rm5AoZGw

NGS – Comparison of Main Methods (approximate numbers)

Method	max. read length [bp]	error rate [%]	max. reads per run	time per run [h]	single molecule?	costs per Gb [US-$]	Remarks
Pyrosequencing (Roche)	700	0.1-1	1M	24	no	10k	discontinued, expensive, homopolymer errors
Sequencing by synthesis (Illumina)	50-600	**0.1-1**	**52B**	4-48	no	**2-150**	expensive equipment, cheap Gb price, low error rates
Ion semiconductor (Ion Torrent)	600	~0.5	80M	**2**	no	50-1000	cheap equipment, very fast, homopolymer errors
SMRT sequencing (PacBio)	**30k-100k**	5-15	4M	0.5-20	**yes**	5-50	expensive equipment, long reads, fast, methylation
Nanopore Sequencing (Oxford)	**> 4000k**	3-8	dep. on length (~few 100k)	72	**yes**	5-100	handheld, cheap equipment, longest reads, other molecules
Sanger	1200	**0.01**	1	0.2-3	no	2-3M	gold standard, low throughput

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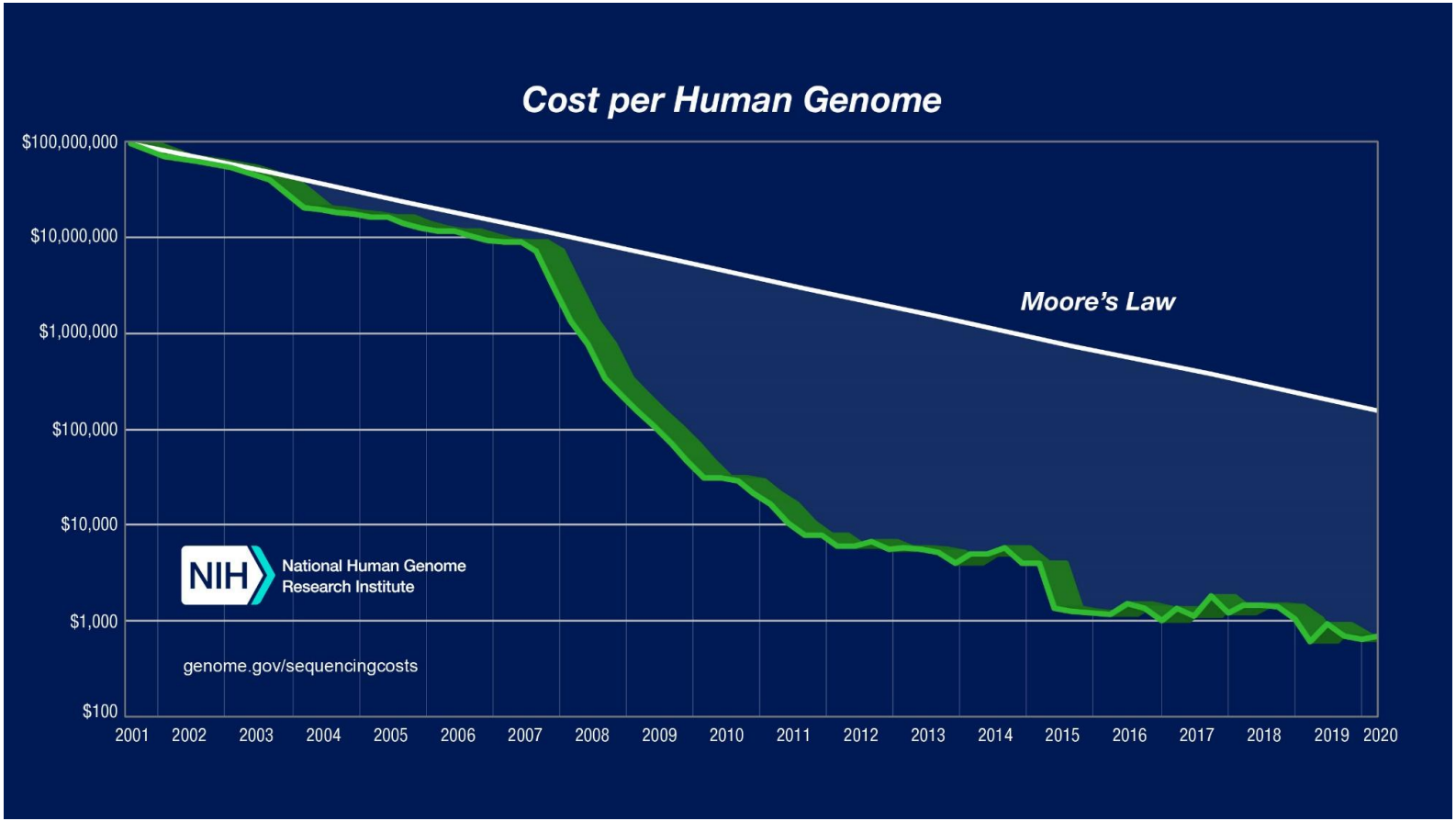
DNA Sequencing – Costs



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DNA

("How to interrogate and manipulate DNA in cells?")

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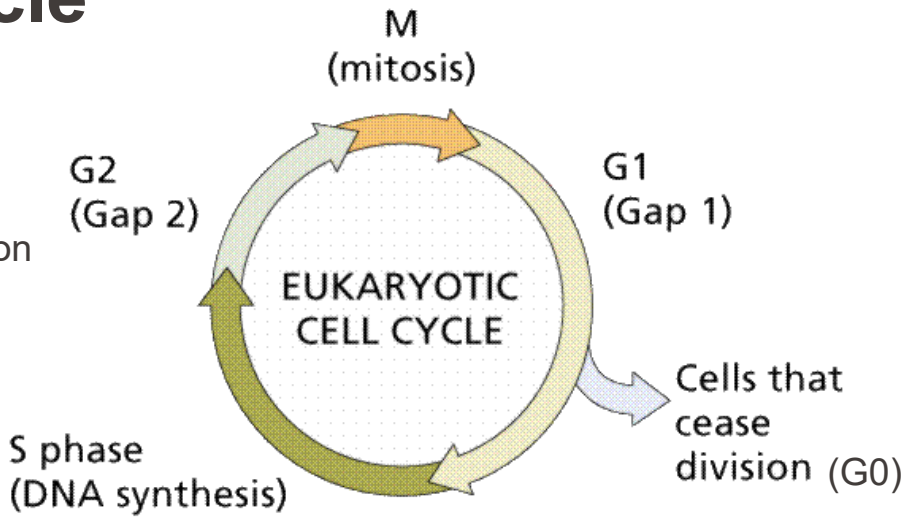
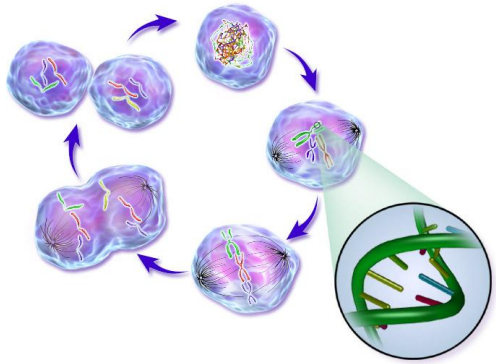
The Eukaryotic Cell Cycle

- Four main phases
  - **G1** (Gap 1)
    - "normal" functions, preparations for division
  - **S** (Synthesis)
    - DNA replication
  - **G2** (Gap 2)
    - continued growth
  - **M** (Mitotic phase)
    - mitosis (nuclear division) & cytokinesis (cytoplasmic division)



- Checkpoints for transition between phases (e.g. G1/S, G2/M)
- Interphase = G1+S+G2
- G0 = resting phase



RECAP

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Green Fluorescent Protein (GFP)

- Fluorescent protein from *Aequorea victoria*
- Important reporter protein
- Excitation: ~488nm / emission: ~510-530nm
- O_2-dependent fluorophore formation (“maturation”)
- Many alternatives (YFP, RFP, mCherry etc.)

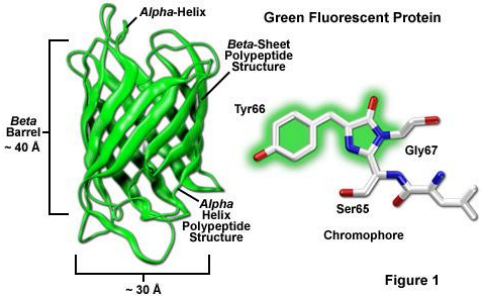
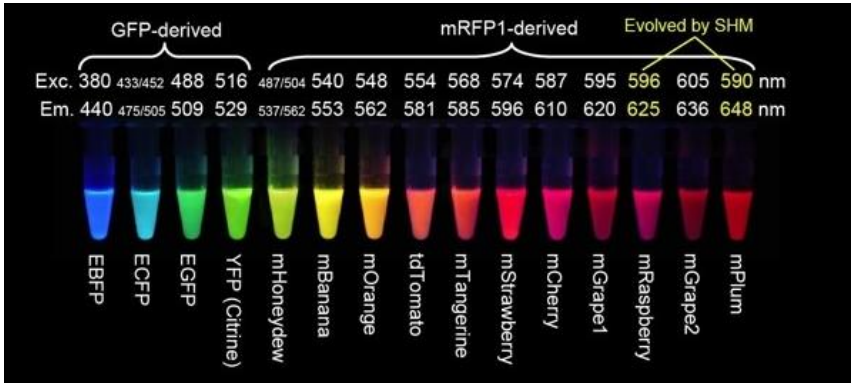
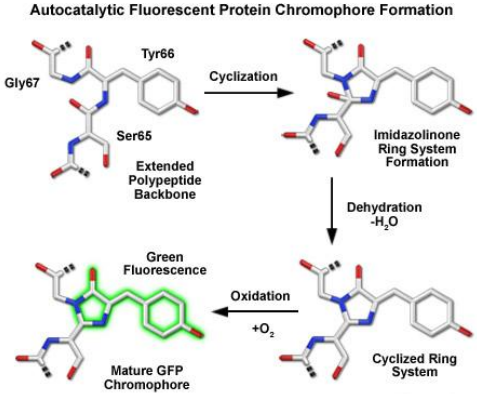


Figure 1





RECAP

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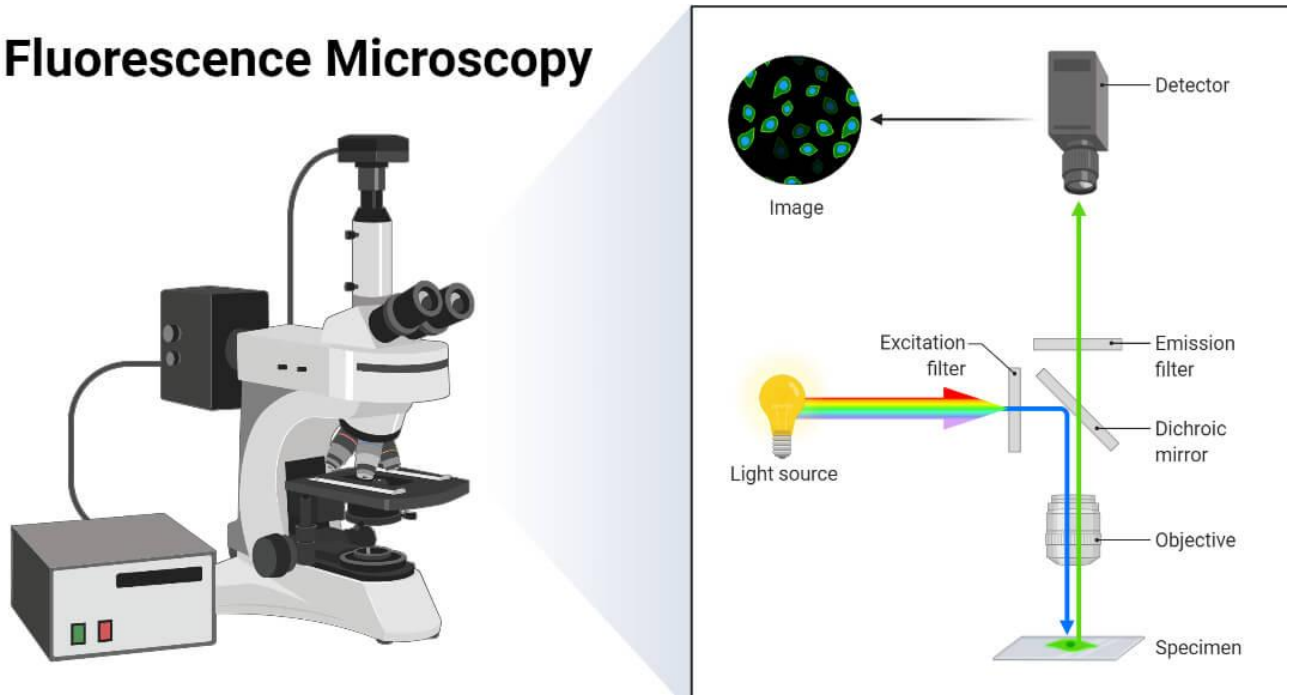
Fluorescence Microscopy



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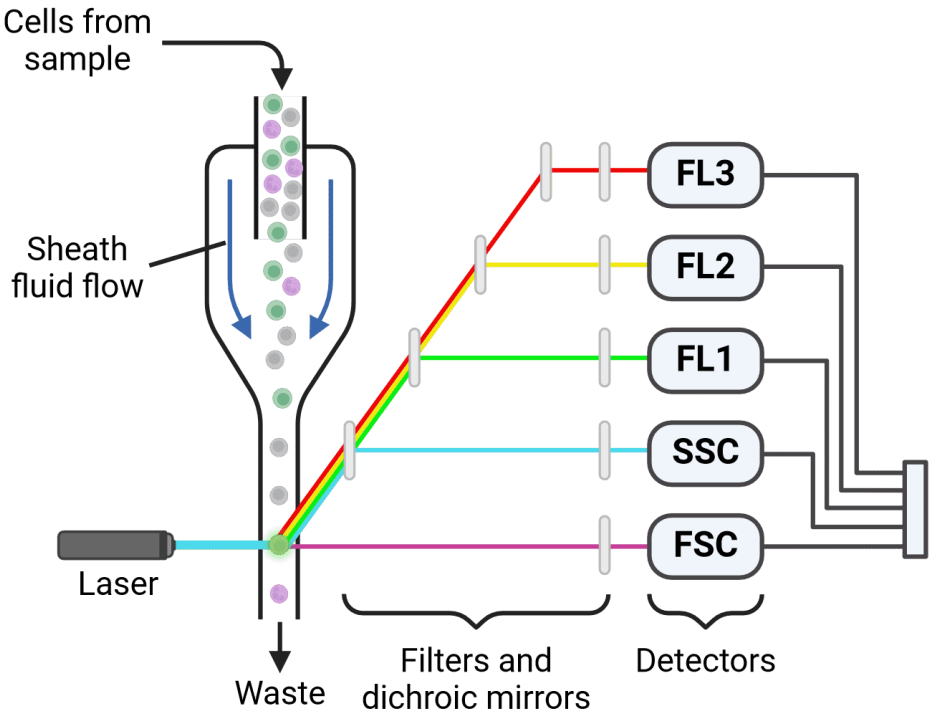
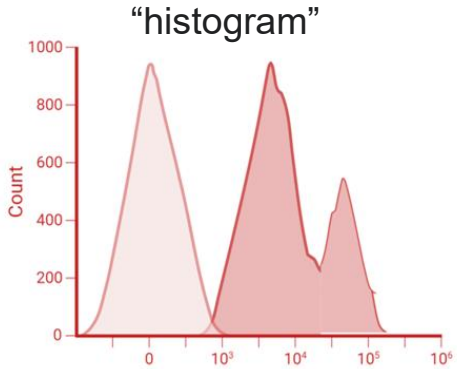
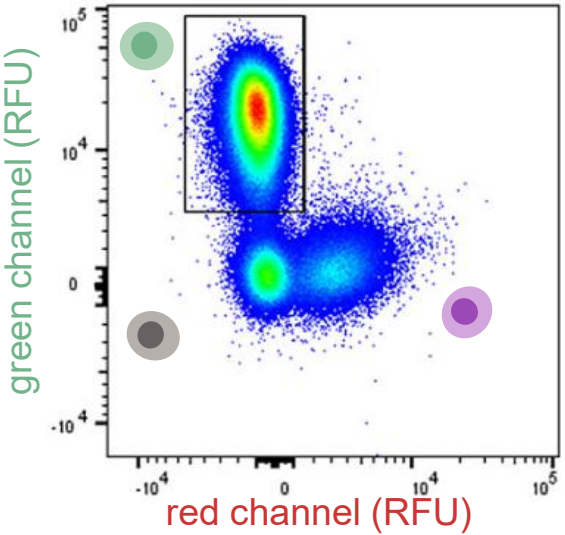
RECAP

Flow Cytometry







RECAP

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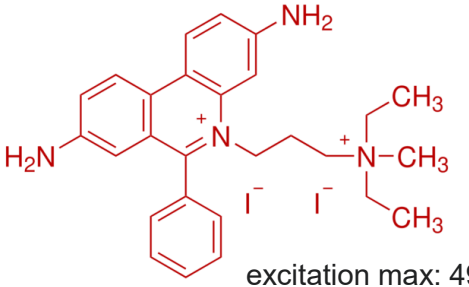
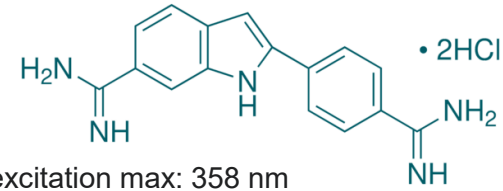
DNA Intercalators

- Propidium iodide (PI)
  - stains DNA (and RNA) intercalating between bases
  - membrane impermeable
  - “dead cell stain”



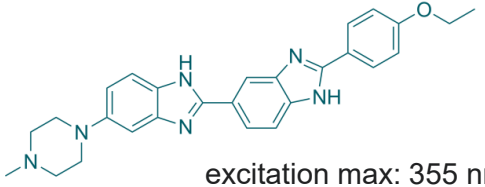
excitation max: 493 nm
emission max: 636 nm (red)

- DAPI (4′,6-diamidino-2-phenylindole)
  - stains dsDNA intercalating at AT-rich regions
  - binds minor groove
  - membrane permeable



excitation max: 358 nm
emission max: 461 nm (blue)

- Hoechst 33342
  - stains dsDNA intercalating at AT-rich regions
  - binds minor groove
  - membrane permeable



excitation max: 355 nm
emission max: 465 nm (blue)

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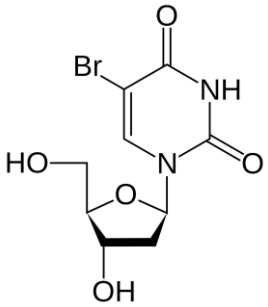
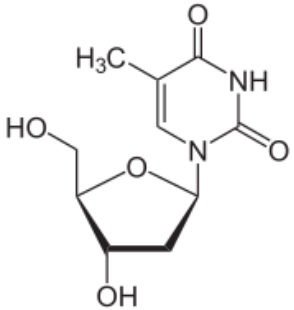
Nucleoside Analogues – Labelling

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

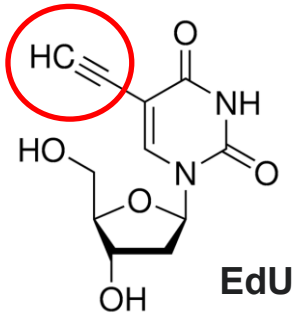


BrdU



thymidine

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



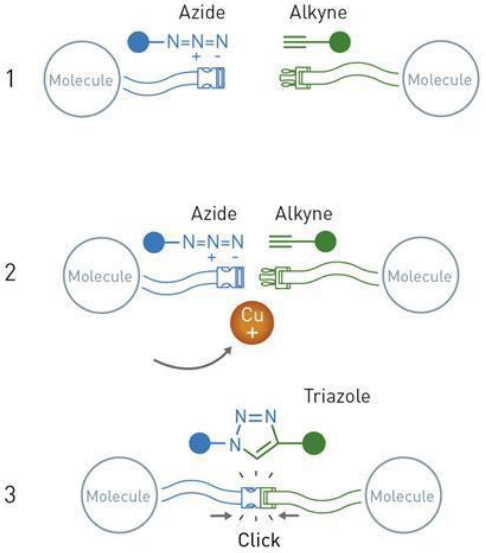
EdU

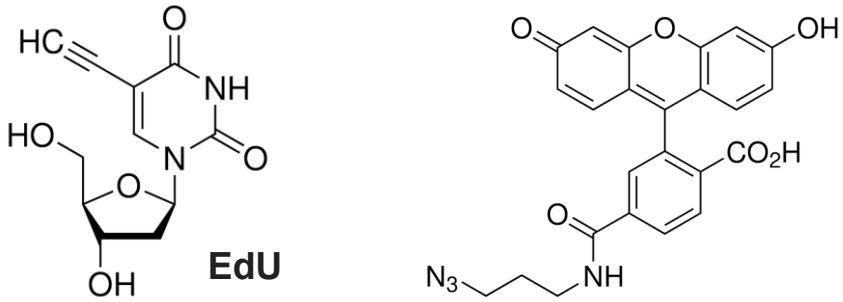
Click Chemistry

- Nobel Prize Chemistry 2022: "…for the development of click chemistry and biorthogonal chemistry"
  - labelling/modification through azides and alkynes
  - minimal invasive for biological systems!
  - simple, modular
  - e.g. copper(I)-catalyzed cycloaddition (CuAAC)
  - e.g. strain-promoted cycloaddition (SPAAC)

Source: © Nobel Prize Outreach/Niklas Elmehed

The 2022 chemistry laureates (left to right) Carolyn Bertozzi, Morten Meldal and Barry Sharpless





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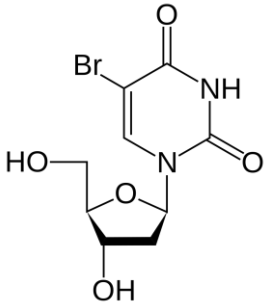
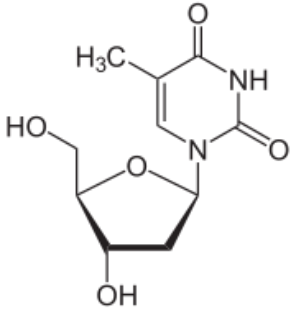
  - *in vivo* use possible



BrdU



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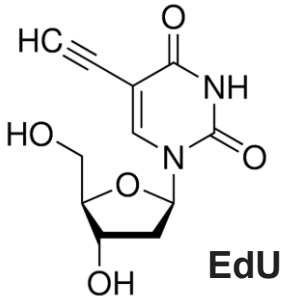

EdU

Q: In which phase of the cell cycle do these analogues act?

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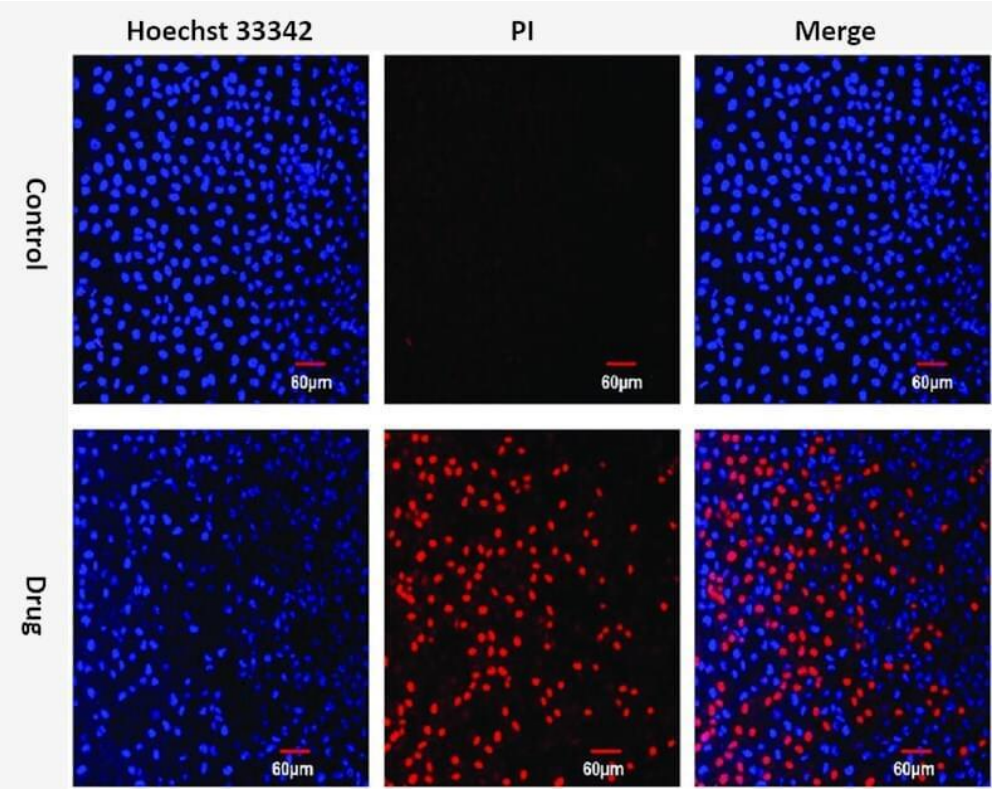
Labelling – Examples



Q: What does the drug do here?

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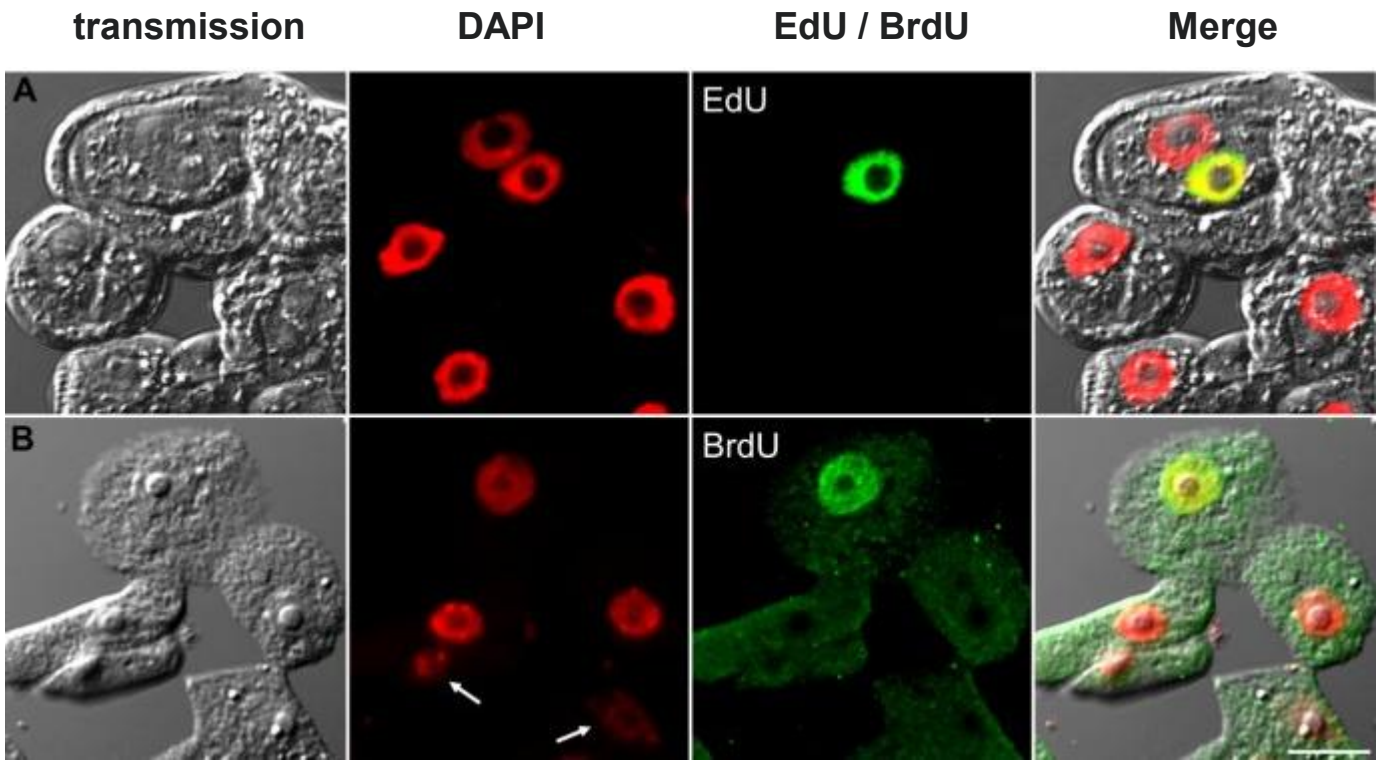
Labelling – Examples



Q: What can you say about the green cells?

How would these samples look in flow cytometry (2D plot)?

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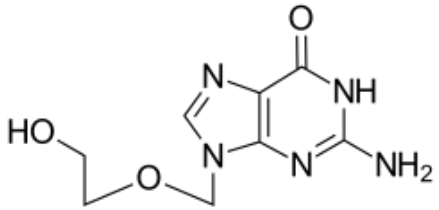
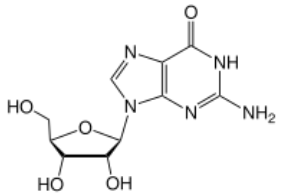
Nucleoside Analogues – Therapeutics

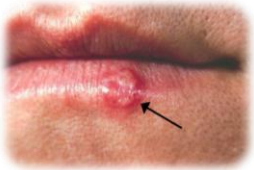
- Aciclovir (Zovirax)
  - antiviral medication
  - herpes simplex virus (amongst others)
  - inhibits viral DNA polymerase



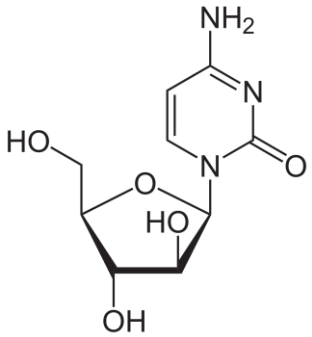
acyclovir



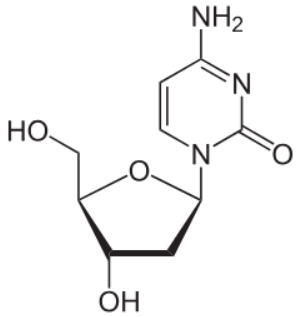
guanosine



- Cytarabine (cytosine arabinoside)
  - chemotherapeutic medication
  - different forms of leukemia
  - incoporates in DNA, DNA damage (in S phase)
  - inhibits DNA and RNA polymerases



cytosine arabinoside



deoxycytidine

Q: What could be a second mode of action for acyclovir?

What must these drugs not do to be of medical use?

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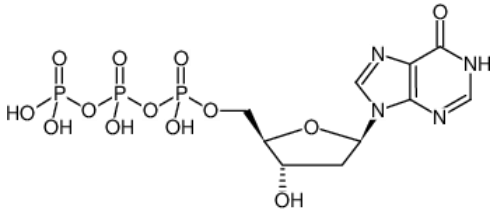
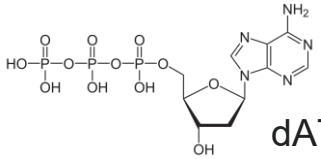
Nucleotide Analogues for Mutagenesis

- Deoxyinosine-triphosphate (dITP)
  - pairs with A, G or C
  - mostly A→G / G→A mutations

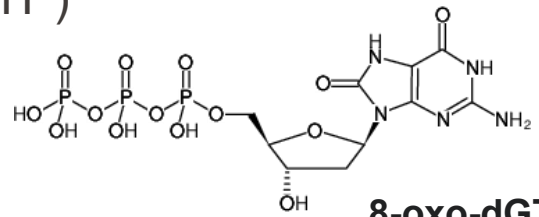


dITP

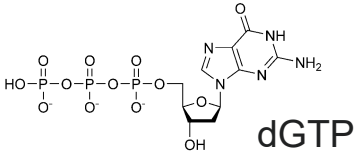


dATP

- 8-oxo-2'-deoxyguanosine (8-oxo-dGTP)
  - pairs with C and A
  - various mutations (mainly G→T/A)



8-oxo-dGTP



dGTP

- Used in error-prone PCR (epPCR)

→ will be discussed in “Engineering” part of the lecture

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RNA

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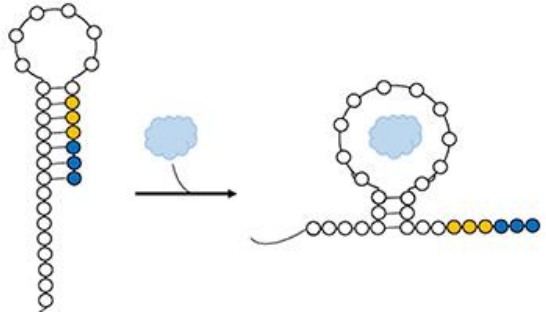
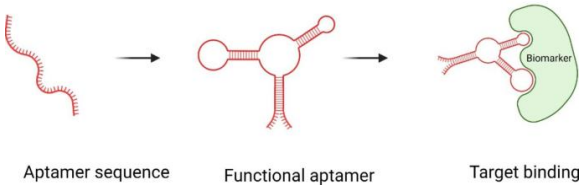
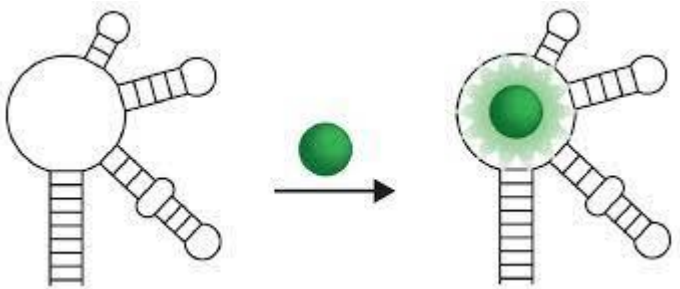
RNA Aptamers

- Short, single stranded RNA molecule
- Defined 3D structure
- Specific binding of molecules with high affinity
- *In vitro* selection for improved binders: SELEX method
- Can be used as versatile sensors/reporters
  - Spinach aptamer
  - catalytic aptamers
  - Quenching-/FRET-based beacons
  - riboswitches







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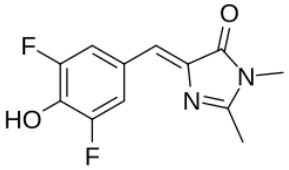
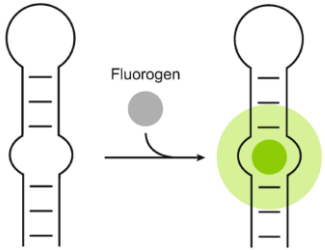
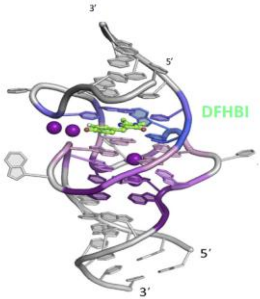
RNA Aptamers (examples)

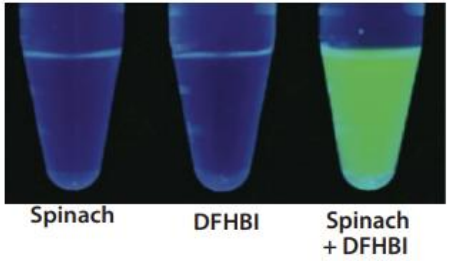


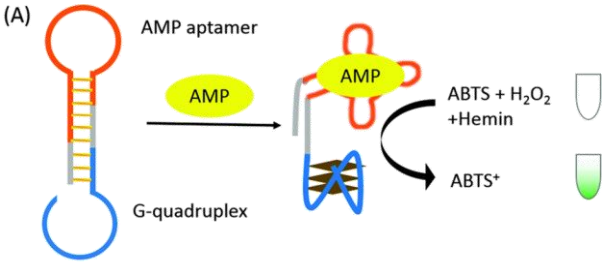
DFHBI
(3,5-Difluoro-4-hydroxy-benzylidene imidazolinone)

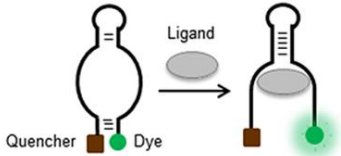


“spinach aptamer”

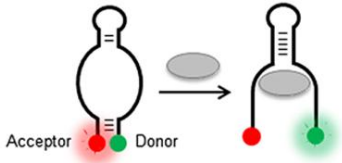








Quenching-based beacon



FRET-based beacon

Q: What could this such RNA aptamers be used for?

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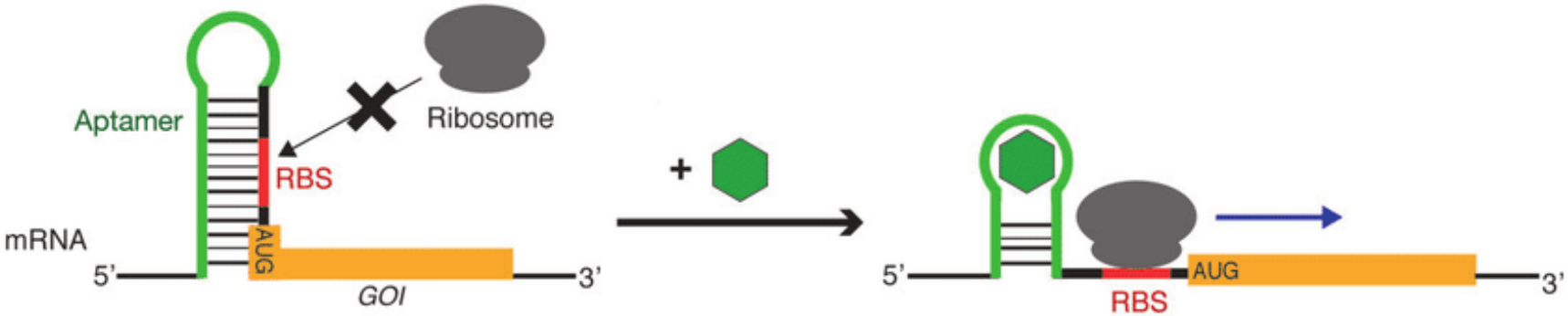
Riboswitches

- part of mRNA
- contain an aptamer
- “expression platform”: interaction with gene expression machinery
- Can act as ON-/OFF-switches via different mechanisms

(e.g. translation, transcription, mRNA degradation etc.)



Q: What could riboswitches be used for in nature and biotechnologically?
What are pros and cons compared to the aptamer examples before (e.g. Spinach)?

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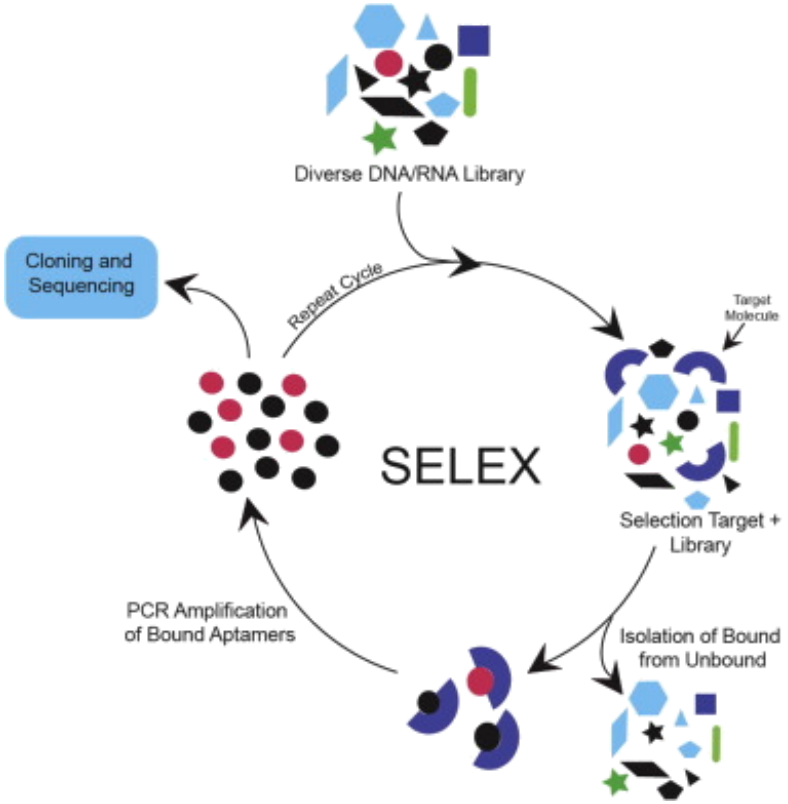
SELEX

- Systematic Evolution of Ligands by EXponential enrichment
- Process of *in vitro* selection for RNA (or DNA aptamers)
- Binders are enriched from large pools of random sequences
- Iterative enrichment, increasing stringency
- Routinely leads to binders with

(sub-)nanomolar affinity



Q: How is binding affinity commonly calculated here?