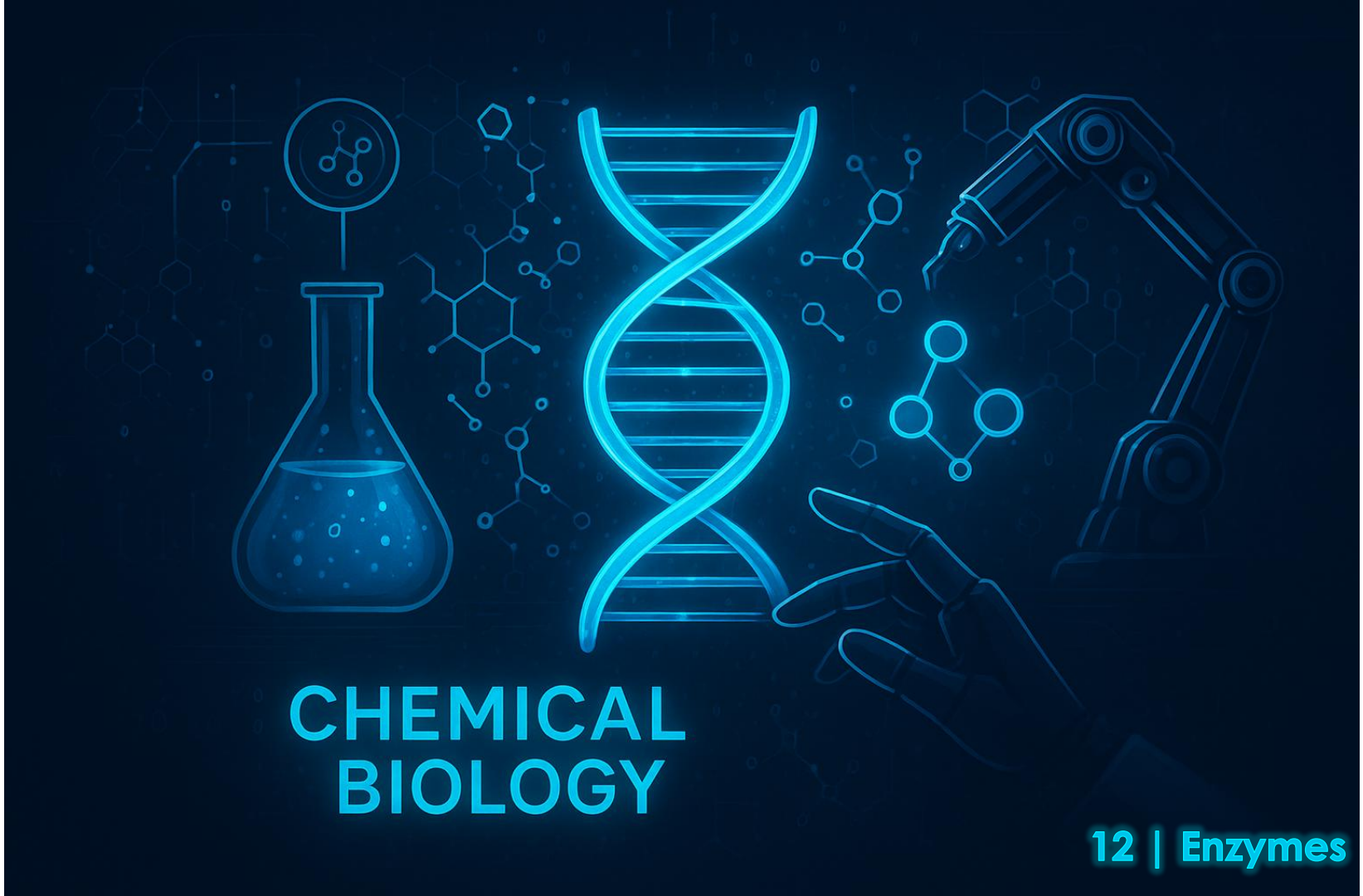

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General Course Information

- 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 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 | tbd
- Week 14 | LSAM Intro + Exam Preparation

[tentative schedule]

Protein – Enzymes
(Kinetics)

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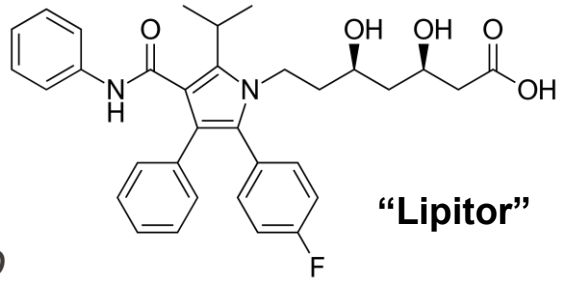
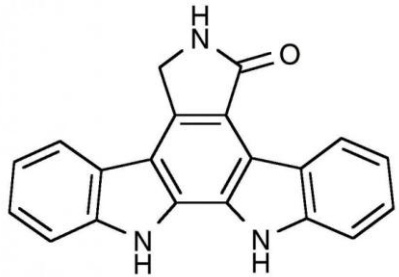
Competitive Inhibitor Drugs

- Structural similarity to substrates
- Rather straight-forward design
- Can be outcompeted by natural substrate *in vivo*

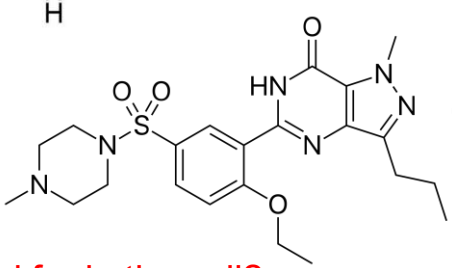


“Lipitor”

- Examples:
  - Atorvastatin (“Lipitor”)
  - Staurosporine aglycone
  - Sildenafil (“Viagra”)



Staurosporine aglycone



“Viagra”

Q: How can the effect of a competitive inhibitor be compensated for in the cell?

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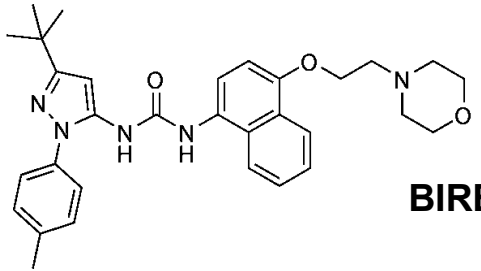
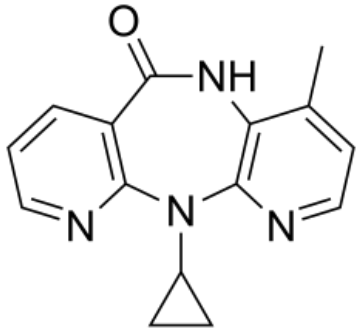
Uncompetitive Inhibitor Drugs

- Allosteric binding to target
- No structural resemblance to substrate
- Difficult to design, discovery by screening
- Not outcompeted by natural substrate



BIRB 796

- Examples:
  - BIRB 796
  - Nevirapine



Nevirapine

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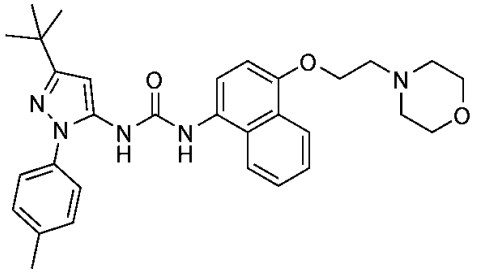
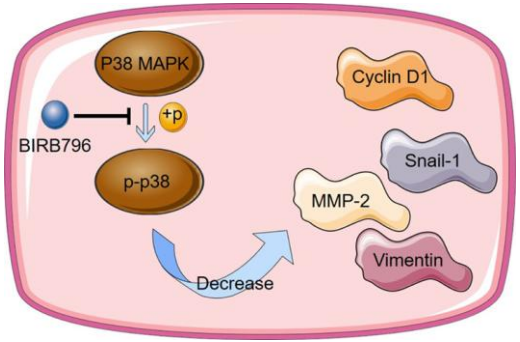
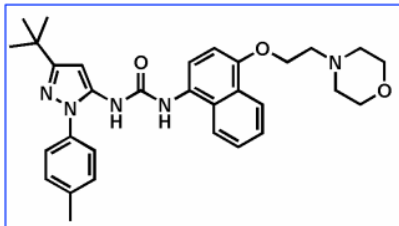
BIRB 796



- Inhibits p38 MAP (mitogen-activated protein) kinase
- p38 MAP kinase:
  - Stress response
  - Regulates cell differentiation, apoptosis, autophagy etc.
- BIRB 796 inhibits uncontrolled proliferation

→ anti-tumor (e.g. glioblastoma), anti-inflammatory etc.





BIRB 796

$k_{on} = 8.5 \times 10^4\ M^{-1}s^{-1}$

$k_{off} = 8.3 \times 10^{-6}\ s^{-1}$

$K_d = 0.1\ nM$

$$K_d = \frac{k_{off}}{k_{on}}$$

Pargellis *et al.*, *Nat Struct Mol Biol* (2002) **9**, 268

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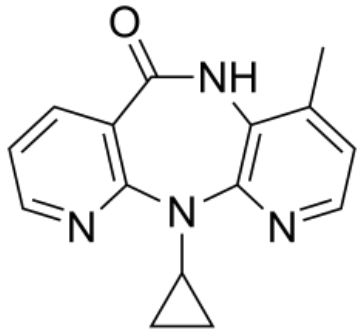
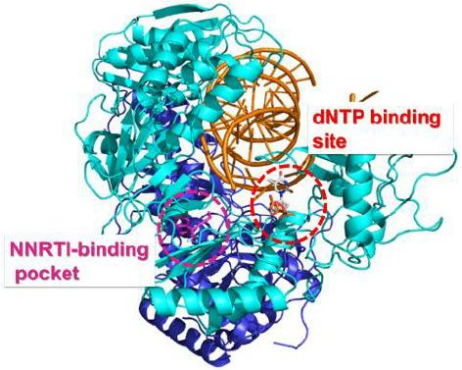
Nevirapine

- Non-nucleoside inhibitor of HIV reverse transcriptase (NNRTI)
- Binds to allosteric site (“NNRTI pocket”)
- HIV therapy
- WHO standard: antiretroviral (ART) regimen

→ combination with other drugs (e.g. AZT)



Nevirapine



Q: What does the reverse transcriptase do?

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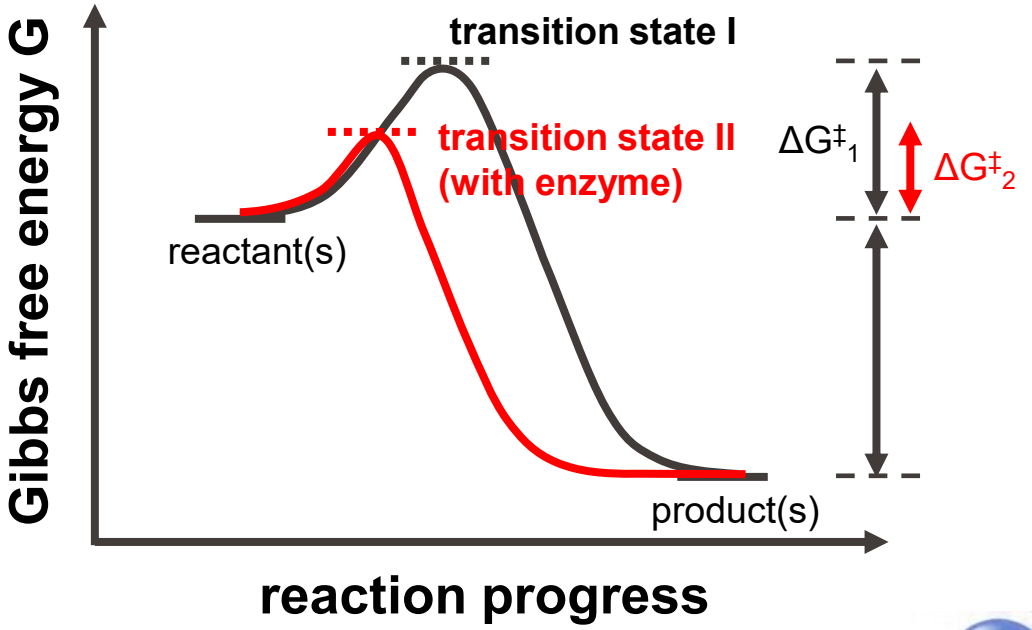
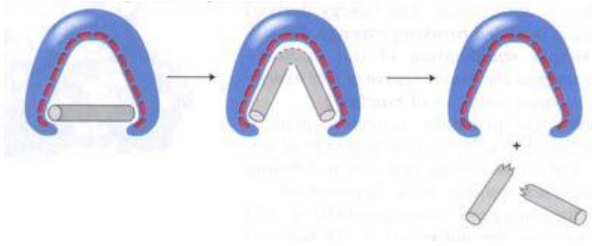
Transitions State



- Transitions state: intermediary state, short-lived, high-energy, instable
- Stabilize structurally by the enzyme (H-bonds, VdW, ionic interactions)



Recap

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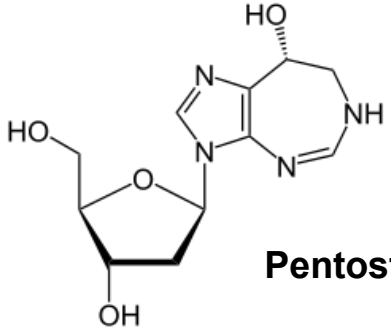
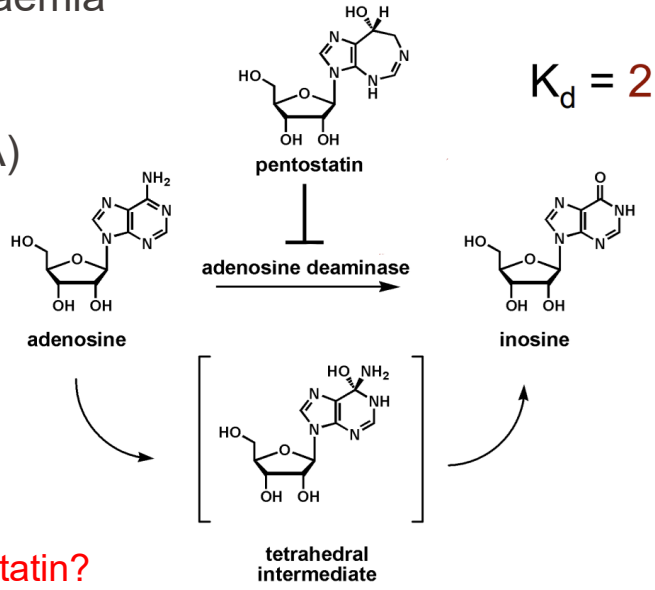
Transitions State Analog Inhitors

- mimick the structure of the transition state in the form of a stable compound that does not react
- Example: Pentostatin
  - Anticancer drug against different forms of leukaemia
  - Purin analog from *Streptomyces*
  - Inhibits adenosine deaminase (breakdown of A)
  - Various effects leading to apoptosis
  - Considered an “irreversible inhibitor”



Pentostatin



- Q: What could be the reason for the irreversible effect of pentostatin?

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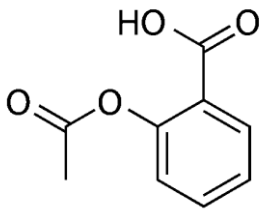
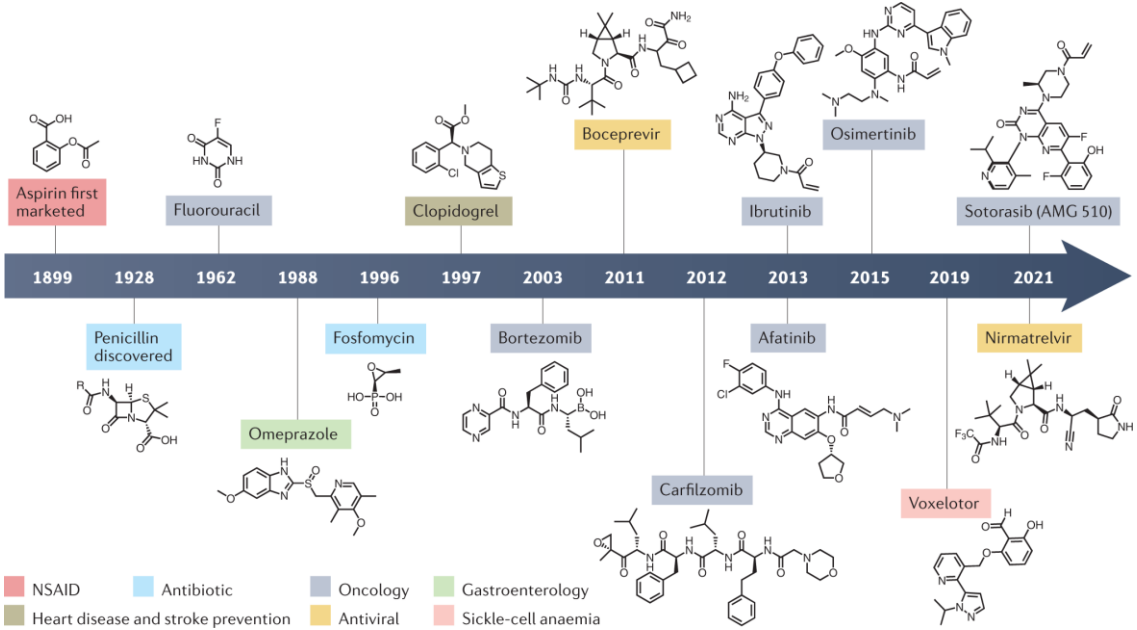
Covalent Drugs

- Covalent modification leads to inhibitory effects
- Often irreversible deactivation
- Examples:
  - Aspirin
  - Omeprazol
  - Penicillin
  - Etc.





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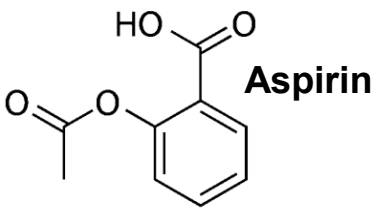
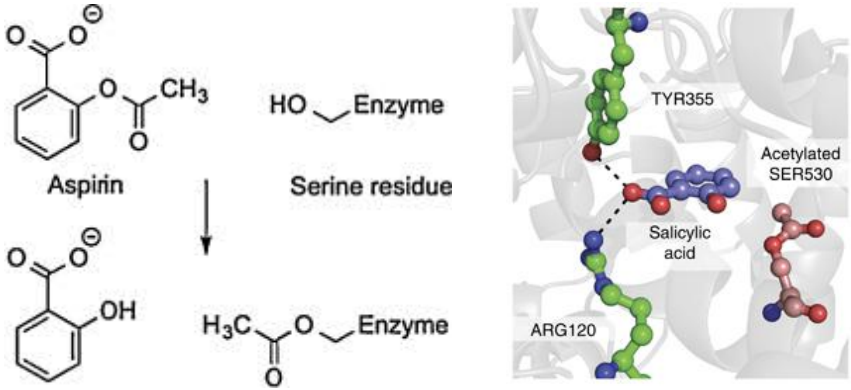
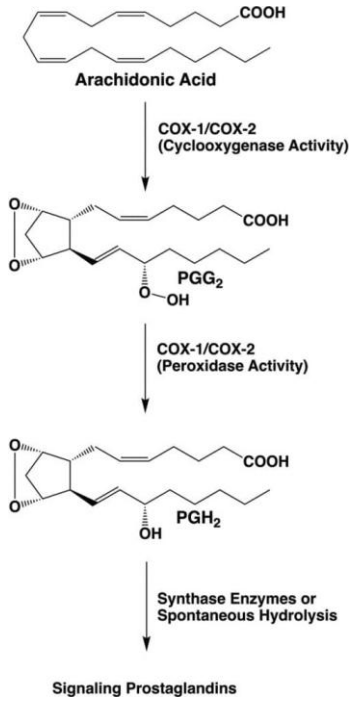
Aspirin



- Irreversible inactivation of cyclooxygenase (COX)
- COX is required for prostaglandin and thromboxane synthesis
- analgesic, antiinflammatory, anticoagulant etc.
- So-called “suicide inactivation”/”mechanism-based inhibition”: covalent acetylation of serine residue in substrate tunnel





- Q: Why are metrics like Ki or IC50 less useful for covalent drugs?

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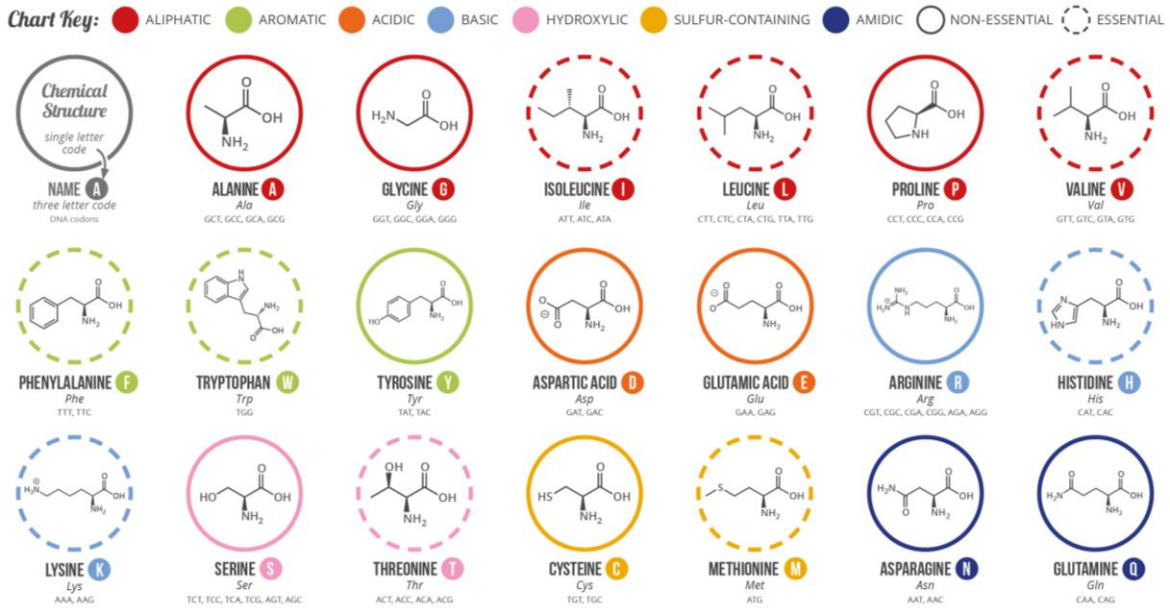
Nucleophilic Amino Acids as Drug Targets

- Targeted by covalent electrophilic drugs
- Reactive groups (drug): esters, strained rings, epoxides, nitriles, Michael acceptors
- Nucleophilicity: Cys >>Lys~His>Ser/Thr>Tyr



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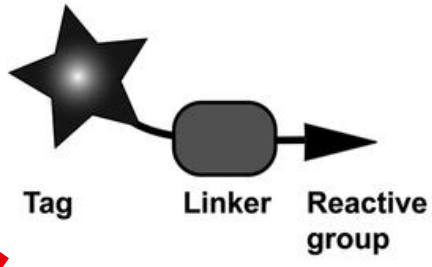
Activity-Based Protein Profiling (ABPP)

- 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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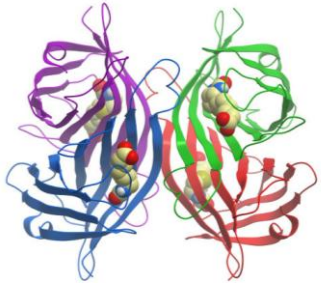
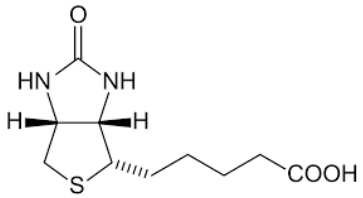
The Biotin-Streptavidin Technology

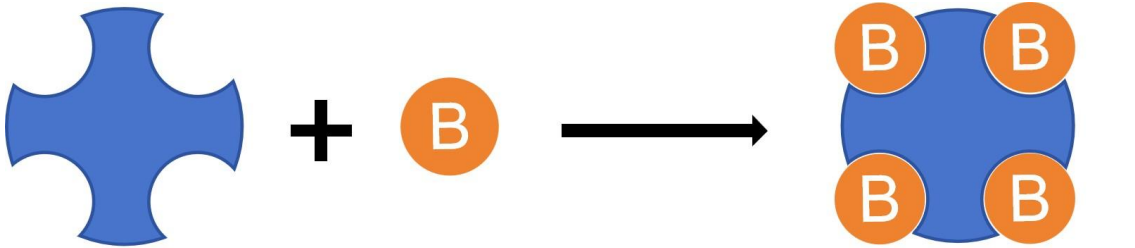
- Streptavidin: bacterial protein (4-mer) with high, non-covalent affinity to biotin
- Avidin = eukaryotic homologue
- $K_D \sim 10^{-15}$ M
- Extremely stable (T, solvents, pH etc.)
- Detection, labelling, purification, conjugation etc. etc.



streptavidin
(4-mer)



D-biotin
("vitamin H")



Streptavidin Biotin Streptavidin-biotin complex

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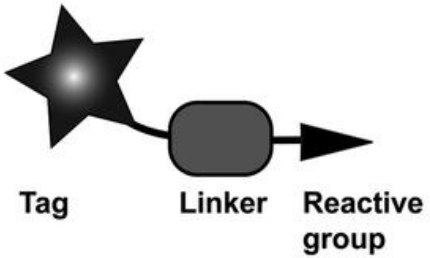
Activity-Based Protein Profiling (ABPP)

- 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

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

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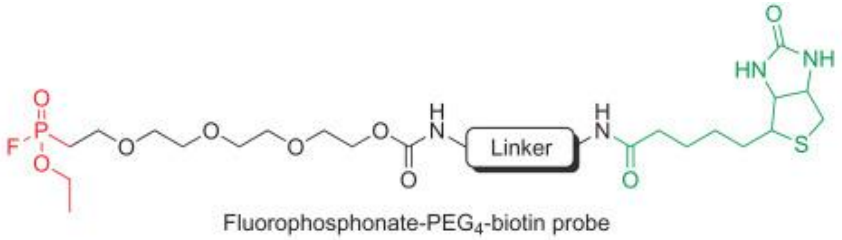
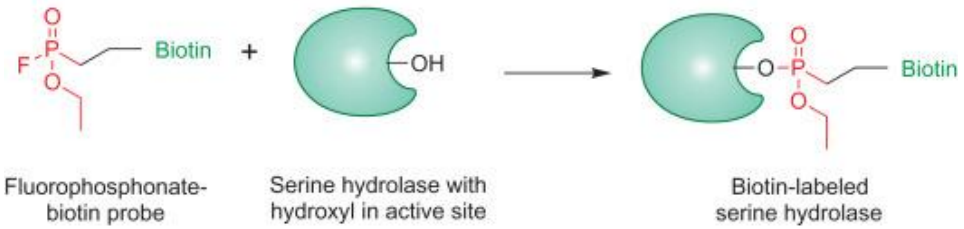
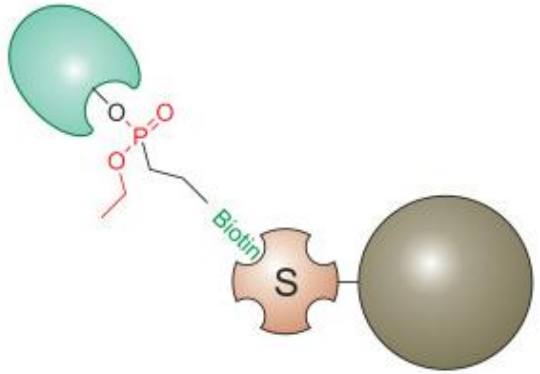
Fluorophosphonate ABPs







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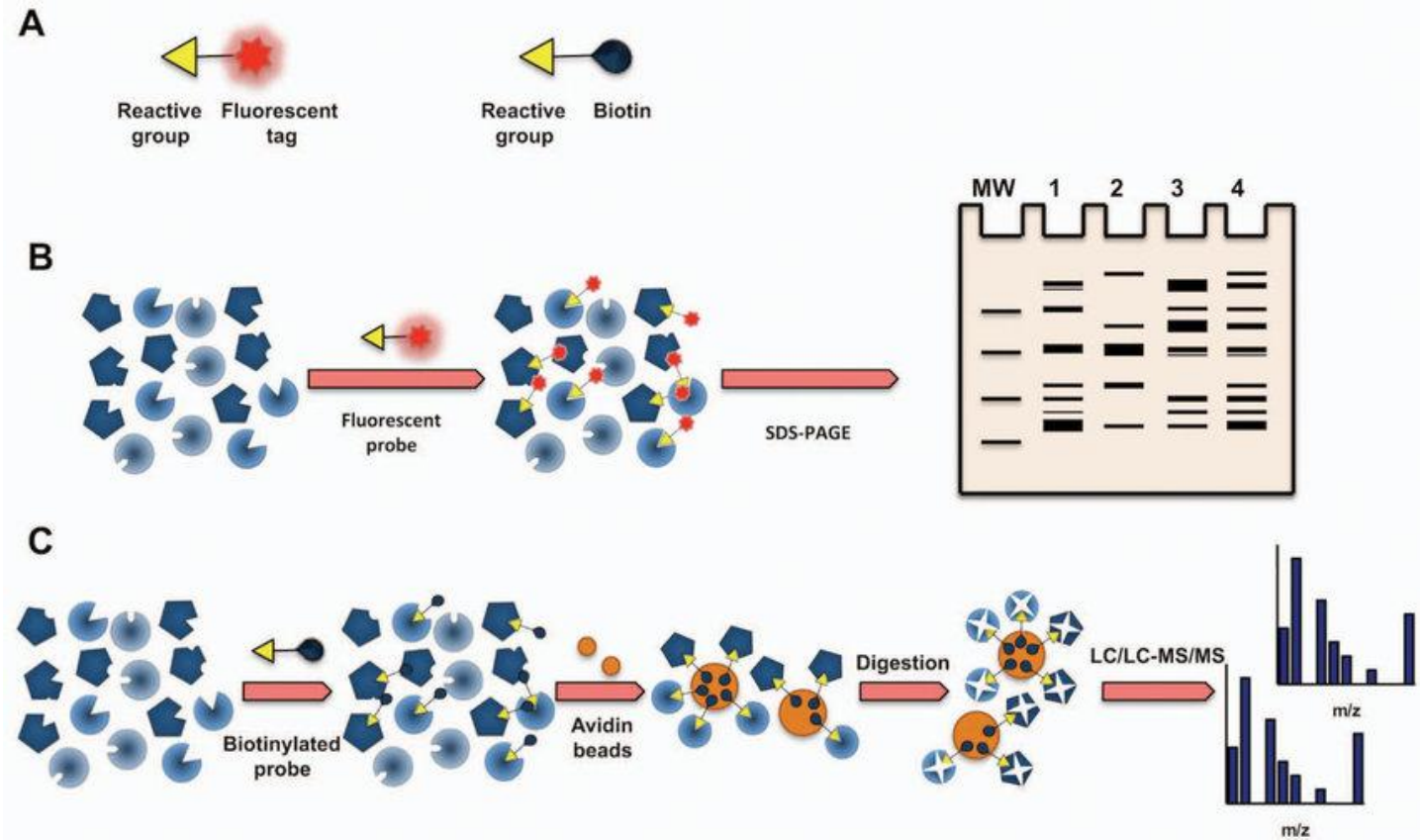
ABPP – Direct Target Capture



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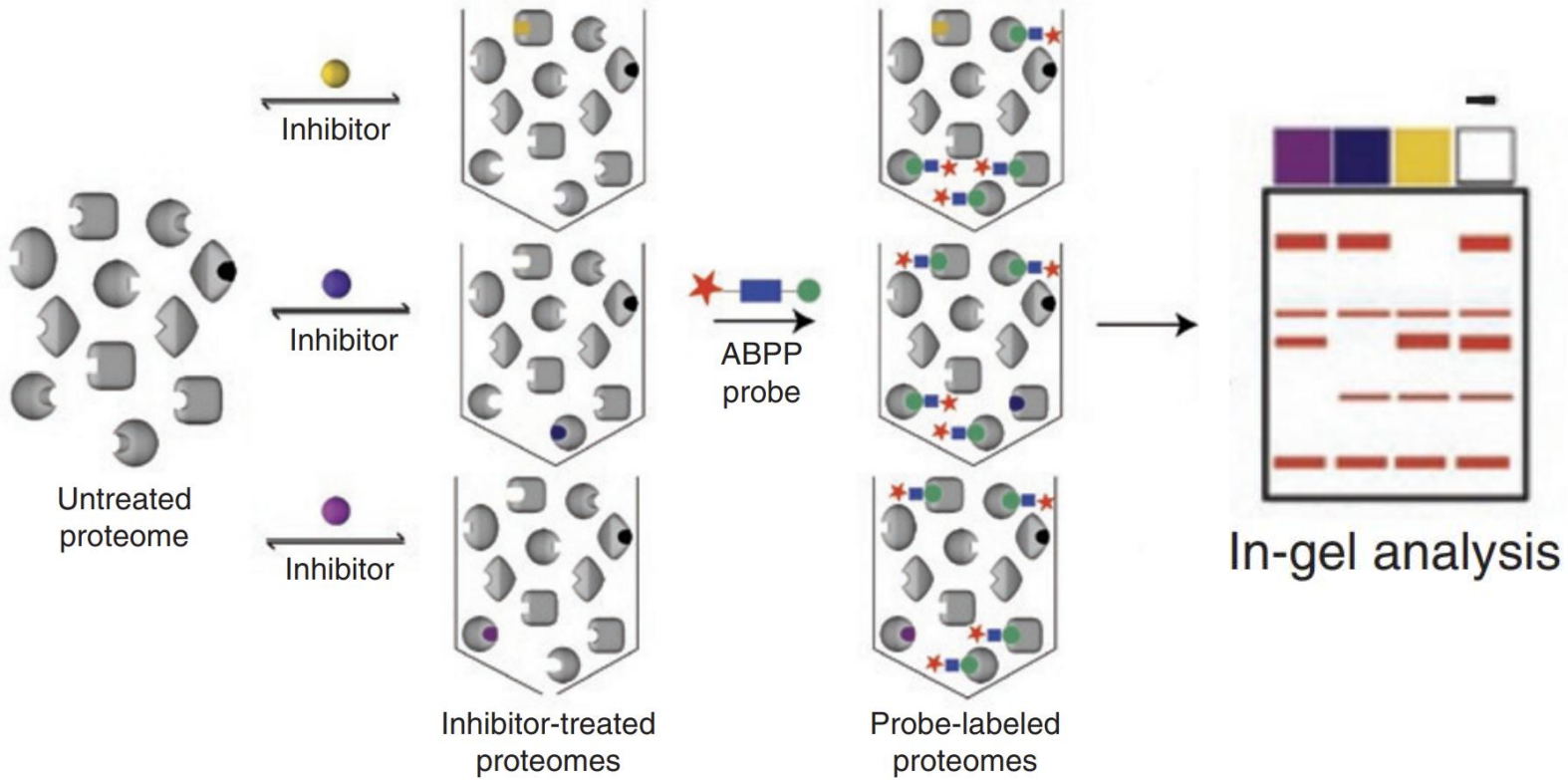
Competitive ABPP



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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