Module 5

Drug Development and Metabolism

5_1 Drug development

5_2 Drug metabolism

5_3 Rational design of drugs

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Case study: anti HIV drugs & rational drug design

Summary of the global HIV epidemic (2017)

	People living with HIV in 2017	People newly infected with HIV in 2017	HIV-related deaths 2017
Total	36.9 million [31.1 million – 43.9 million]	1.8 million [1.4 million – 2.4 million]	940 000 [670 000 – 1.3 million]
Adults	35.1 million [29.6 million – 41.7 million]	1.6 million [1.3 million – 2.1 million]	830 000 [590 000 – 1.2 million]
Women	18.2 million [15.6 million – 21.4 million]	–	–
Men	16.8 million [13.9 million – 20.4 million]	–	–
Children (<15 years)	1.8 million [1.3 million – 2.4 million]	180 000 [110 000 – 260 000]	110 000 [63 000 – 160 000]

Source: UNAIDS/WHO estimates

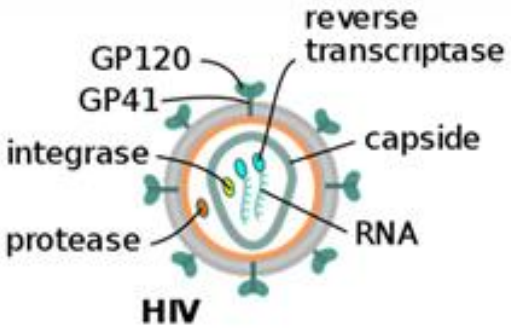
World Health Organization

Leading causes of death in USA (2015)

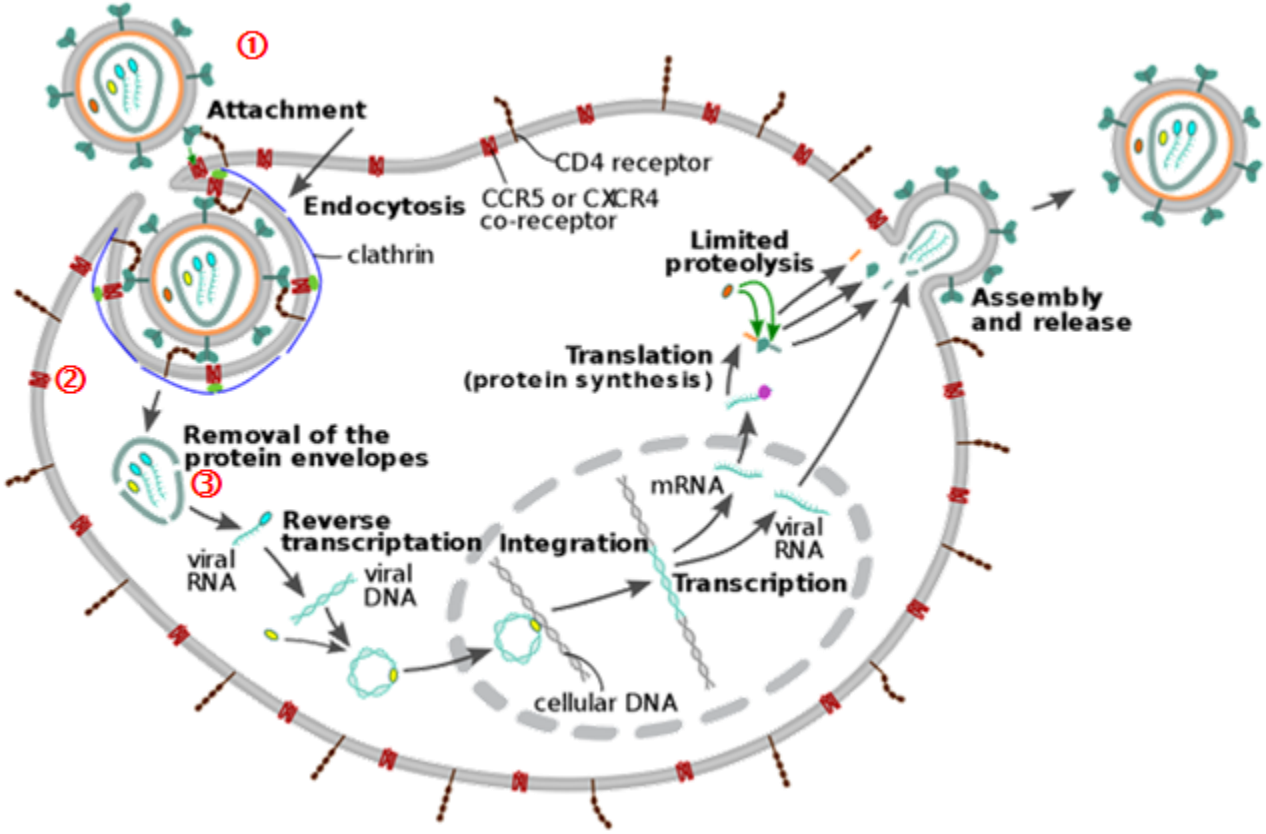
• Heart disease:	633,842
• Cancer:	595,930
• Respiratory diseases:	155,041
• Accidents	146,571
• Stroke	140,323
• Alzheimer's	110,561
• Diabetes:	79,535
• Influenza & pneumonia	57,062
• Nephritis	49,959
• Suicide	44,193
• AIDS:	6,465

- HIV infects CD4 T lymphocytes (T-helper cells) and eventually destroys them
- As the viral load increases and CD4 cell count decreases, the immune system is weakened (Aquired Immune Deficiency Syndrome)
- The patient becomes increasingly vulnerable to opportunistic infections and some forms of cancer
- HIV infection is chronic, without a known cure, but can be effectively controlled by Anti Retro Viral Treatment (ART). Without it the patient progresses to AIDS

HIV-1 REPLICATION CYCLE



HIV components

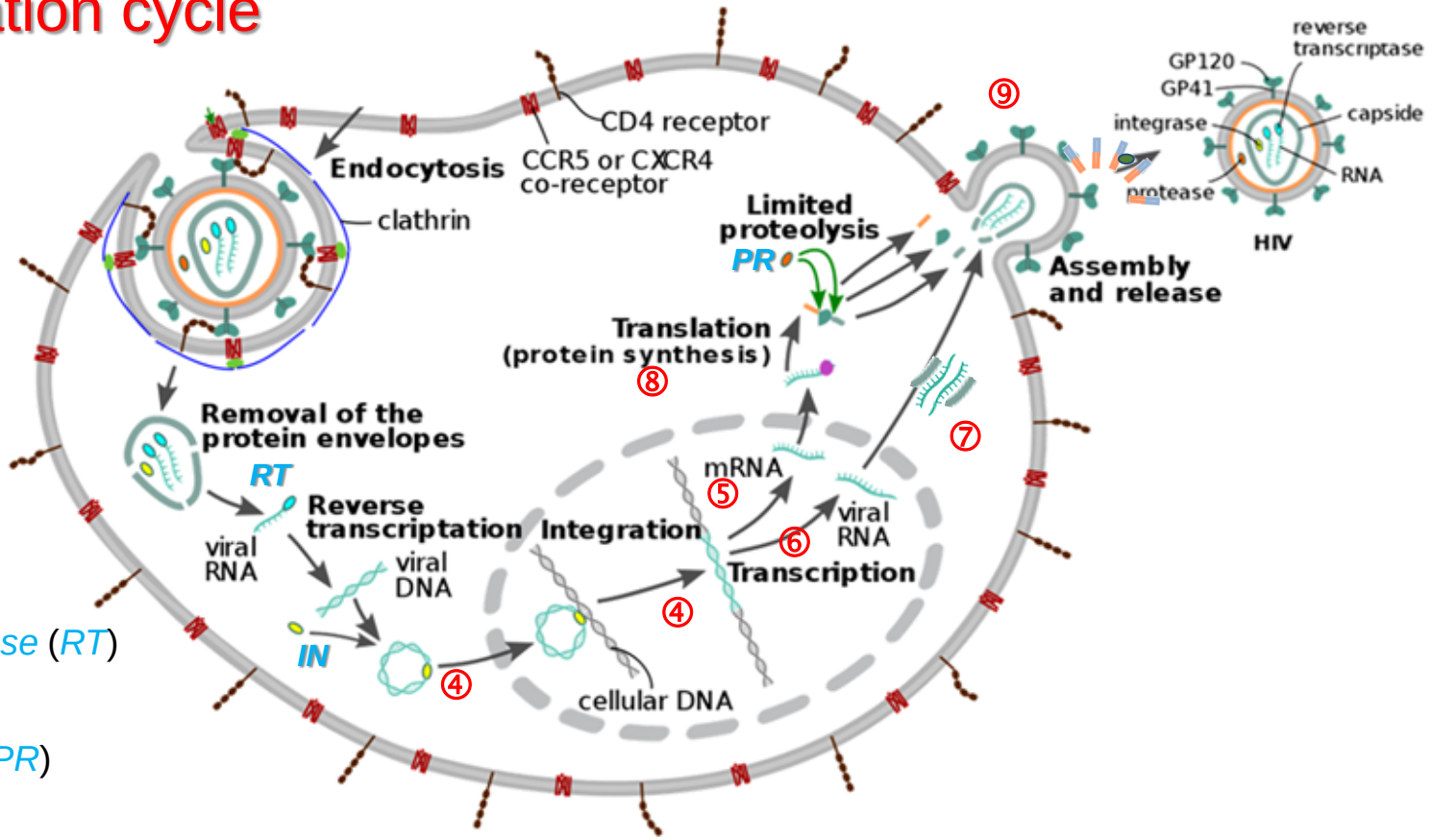


① HIV binds to the CD4 receptor on helper T-cells or macrophages with its surface gp20 and gp41 (spike) glycoproteins. The co-receptors CCR5 and/or CXCR4 play an essential role in binding.

② The viral particle fuses with the cell membrane and injects the capsid with 2 copies of the viral RNA as well as *reverse transciptase* (RT), *integrase* (IN) and other viral proteins (e.g. *protease* PR).

③ RT retrotranscribes a single stranded DNA copy of the RNA, using a cellular tRNA as primer, degrades the RNA and then makes the complementary cDNA strand. This is a very error-prone process.

HIV-1 replication cycle



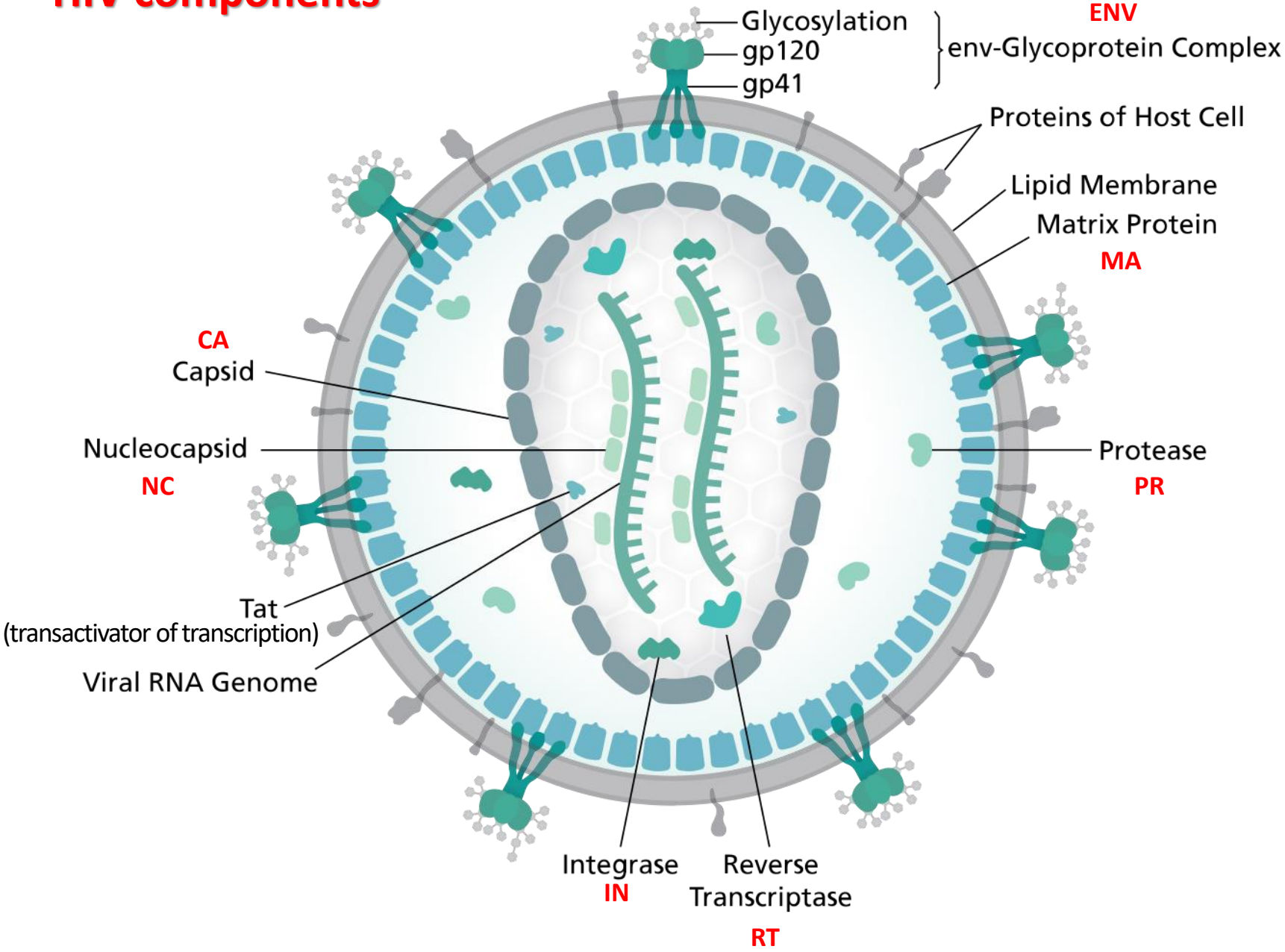
- *Reverse transcriptase* (*RT*)
- *Integrase* (*IN*)
- *Aspartic protease* (*PR*)

④ The integrase introduces sticky ends, carries the viral DNA through a nuclear pore to the nucleus and integrates it into the host chromosome, where it can remain latent until activated by cellular TFs.

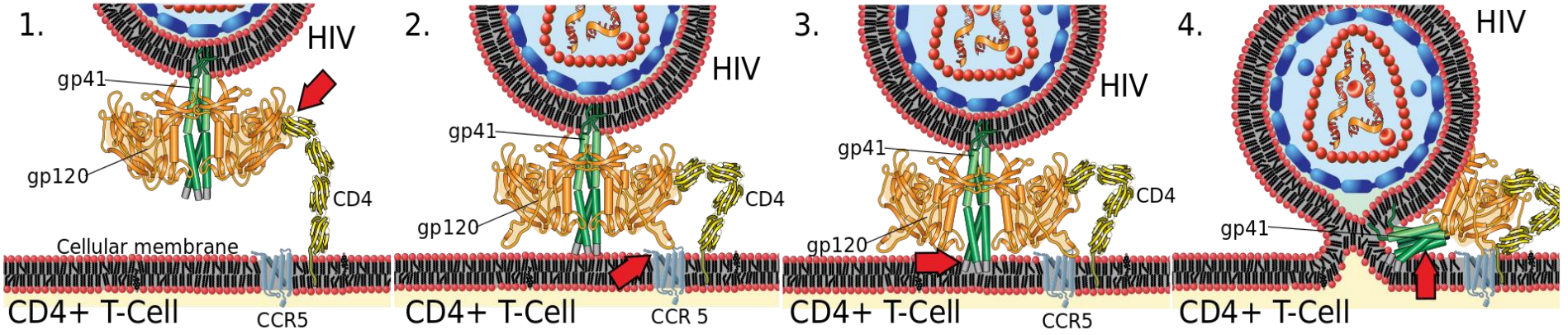
⑤ The provirus is transcribed to mRNA by cellular machinery. ⑥ In part the full length genomic RNA (gRNA) folds as a dimer and binds to the nucleocapsid protein (NC), in part it is processed and spliced to form several mRNAs that are ⑧ translated into polypeptides that contain structural and functional viral proteins [Matrix (MA), Capsid (CA), Envelope proteins (ENV, gp), nucleocapsid (NC), RT, IN & PR].

⑨ These polypeptides locate in the budding particle along with the gRNA, and the single proteins are released by PR, which autocatalyses its excision from one of the polypeptides.

HIV components



HIV-1 fusion process



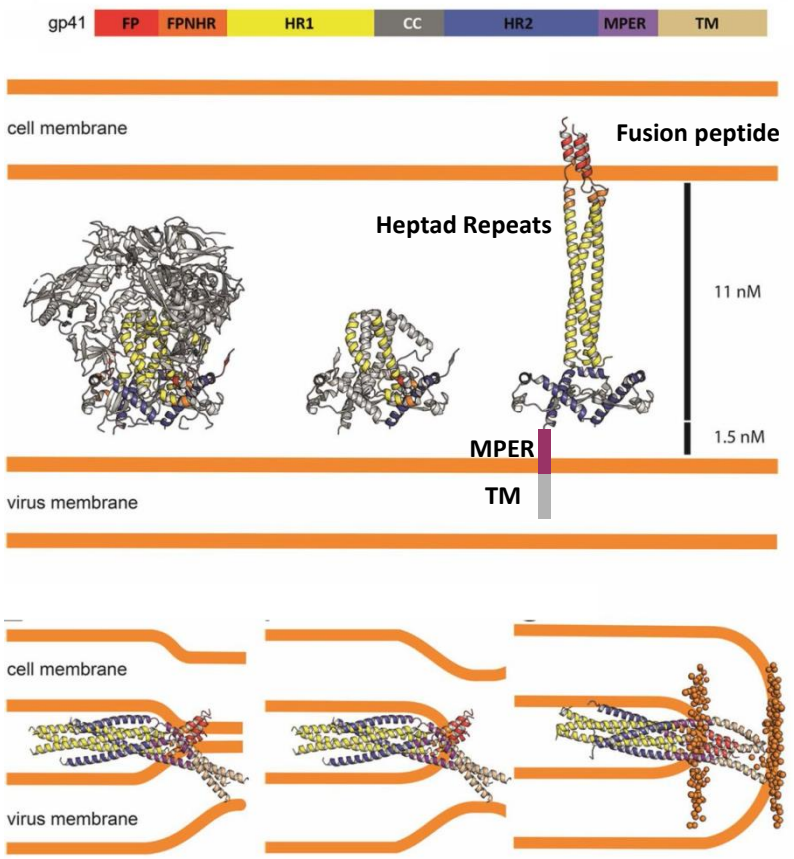
Mechanism of viral entry:

1. Initial interaction between gp120 and CD4.

2. Conformational change in gp120 allows for secondary interaction with CCR5.

3. The distal tips of gp41 are inserted into the cellular membrane.

4. gp41 undergoes significant conformational change; folding in half and forming coiled-coils. This process fuses viral and cellular membranes.



HIV AntiRetroViral drug classes

Entry inhibitors

① CCR5 antagonists

② Post-attachment inhibitors→CD4

③ Fusion inhibitors

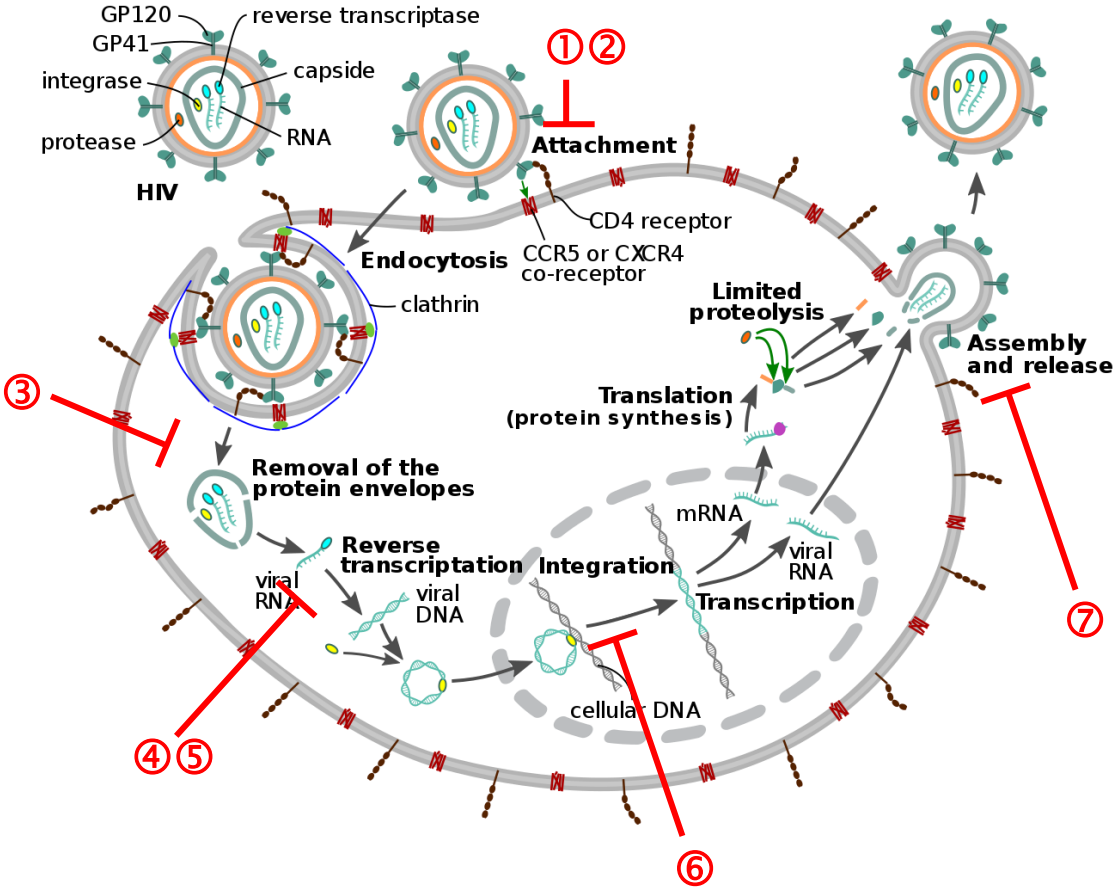
Enzyme inhibitors

④ Nucleoside RT inhibitors (NRTI)

⑤ Non-nucleoside RT inhibitors (NNRTI)

⑥ Integrase inhibitors (INI)*

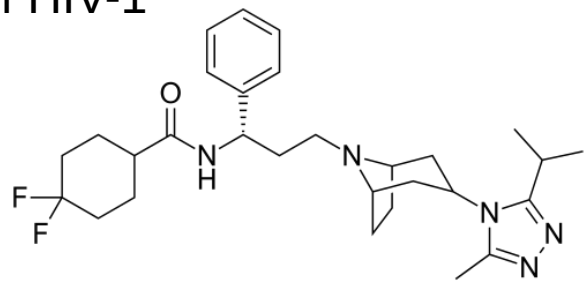
⑦ Protease inhibitors



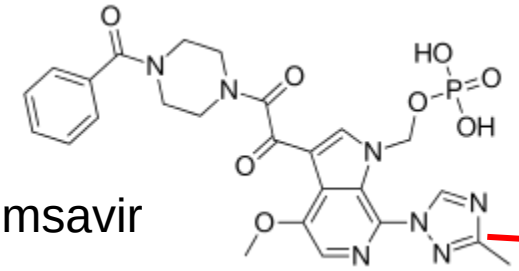
(*) aslo known as **Integrase Strand Transfer Inhibitors** (**INSTI**)

Antiretroviral (ARV) drugs

- Entry inhibitors interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets.

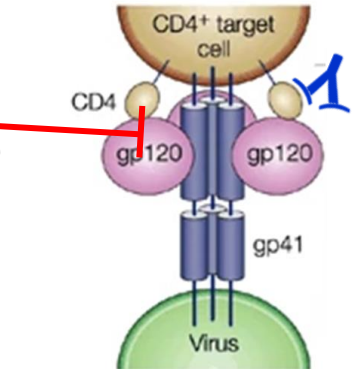


① Maraviroc (2007): a CCR5 antagonist that prevents binding of gp120 to the co-receptor

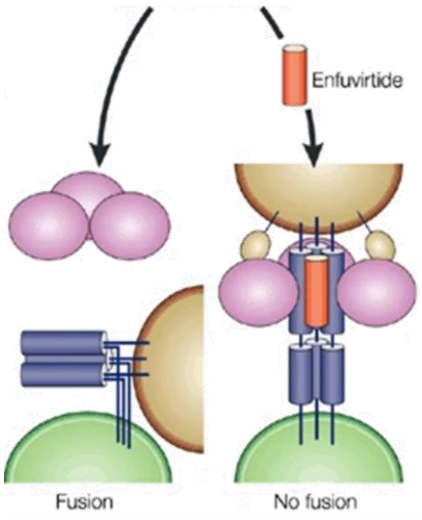


② Fostemsavir (2020): binds to gp120 and prevents binding to CD4.

Prodrug hydrolysed to active drug Temsavir



③ Ibalizumab (2018): Binds to CD4. Does not prevent binding of gp120 to CD4 but prevents binding of the gp120/CD4 complex to CCR5 or CXCR4



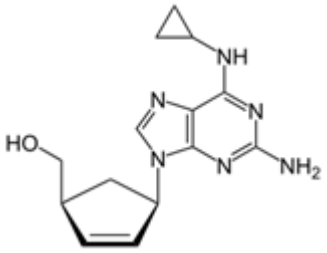
④ Enfuvirtide (Fuzeon) (2003): 36 residue peptide that binds gp41 and prevents the conformational change necessary for fusion.

It is produced by solid phase peptide synthesis and is administered by subcutaneous injection (disadvantage) but it has a low tendency to elicit resistance (advantage).

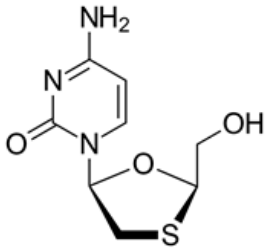
Antiretroviral (ARV) drugs

- Enzyme inhibitors block the activity of key enzymes in viral replication – *Reverse transcriptase* (RT), *Integrase* (IN) and the viral *Aspartic protease* (PR).

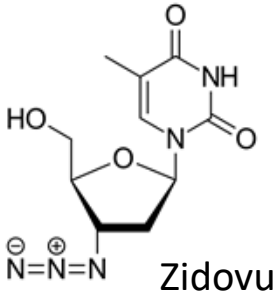
- Nucleoside/nucleotide *Reverse transcriptase* inhibitors (NRTI) are nucleoside/nucleotide analogues that inhibit reverse transcription by being incorporated into the newly synthesized cDNA strand (as nucleotides) and terminating polymerization (they lack the 3' phosphate); they are competitive substrate inhibitors. (Administered as pro-drugs)



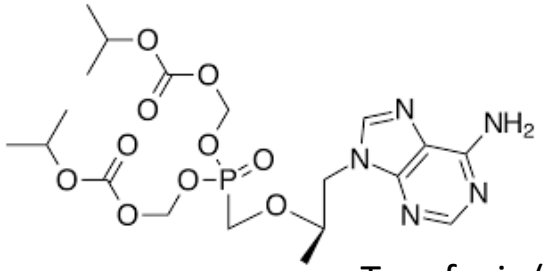
Abacavir (1998)



Lamivudine (1995)

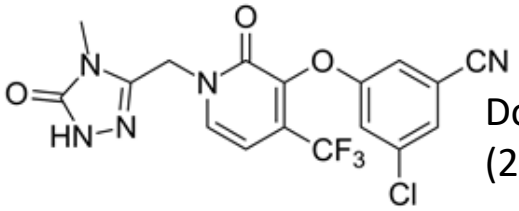


Zidovudine (1987)

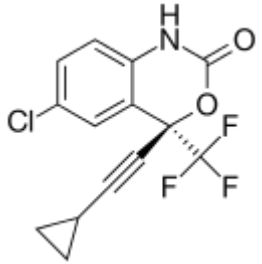


Tenofovir (2001)

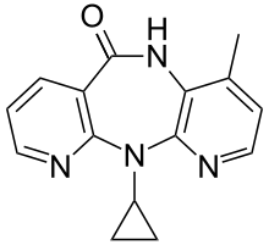
- Non-Nucleoside *Reverse transcriptase* inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of specific to HIV-1 RT; they are non-competitive inhibitors of reverse transcriptase that reduce its activity.



Doravirine (2018)

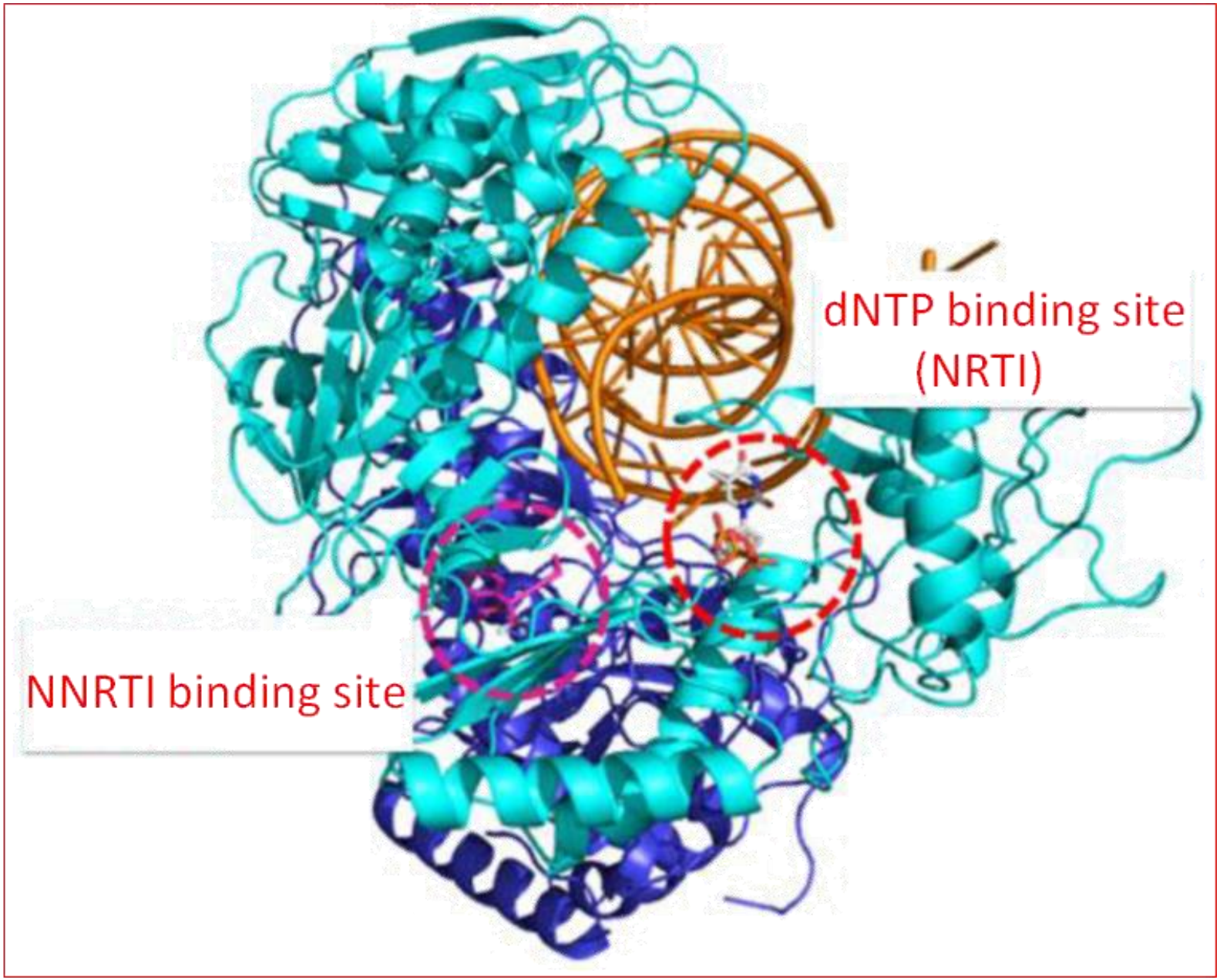


Efavirenz (1998)



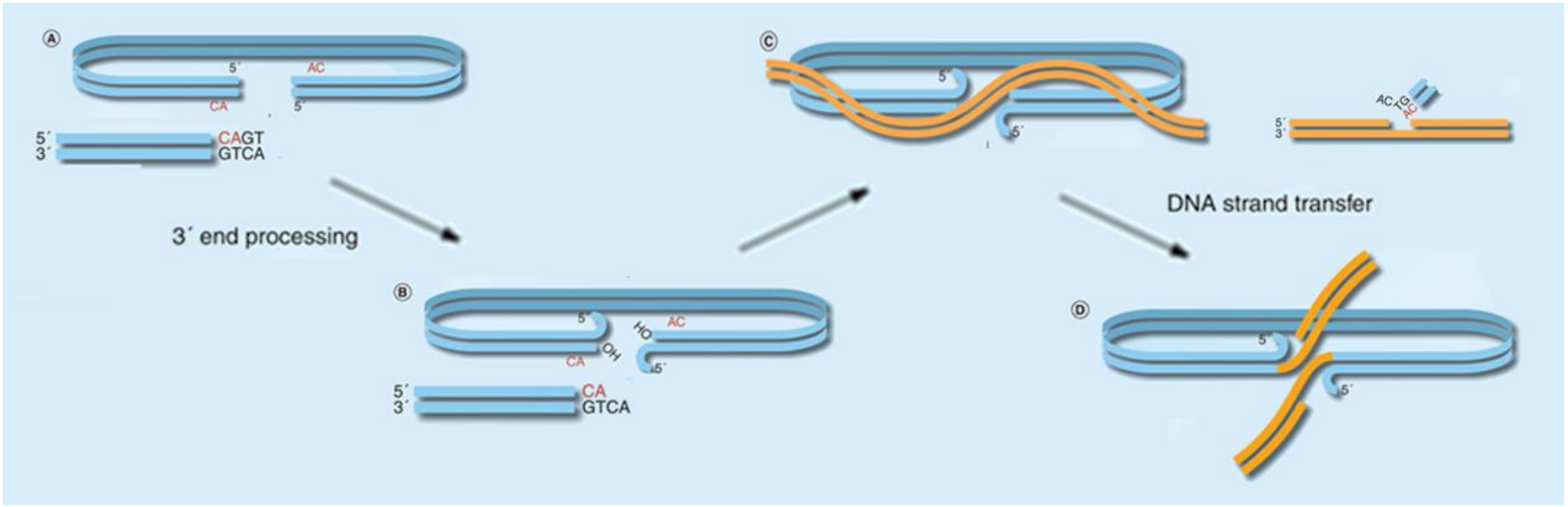
Nevirapine (1996)

NRTI & NNRTI

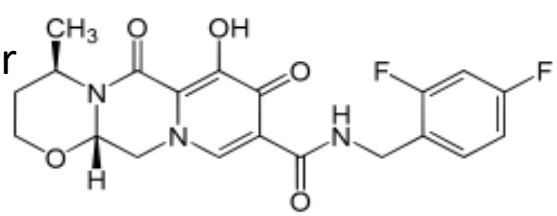


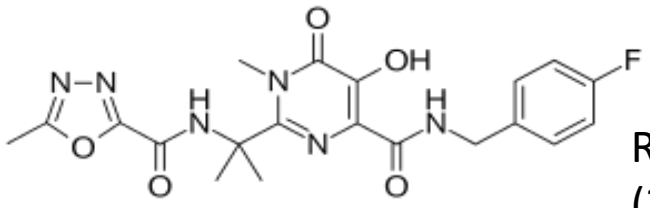
Antiretroviral (ARV) drugs

- INIs or INSTIs inhibit viral *Integrase*, which is responsible for 3' processing of viral cDNA and its integration into the infected cell's chromosomic DNA. Those in clinical use inhibit the strand transfer step - INSTI). Raltegravir was the first to received FDA approval in 2007.



Dolutegravir (2013)

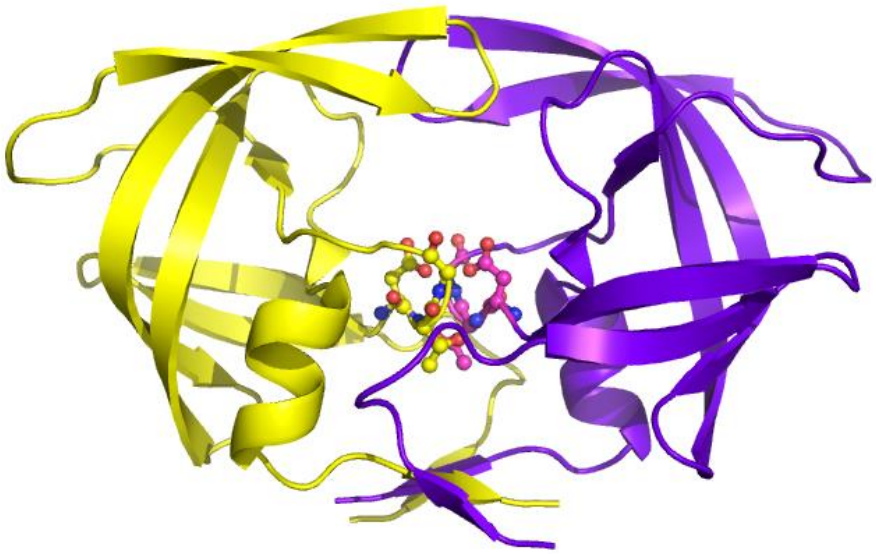


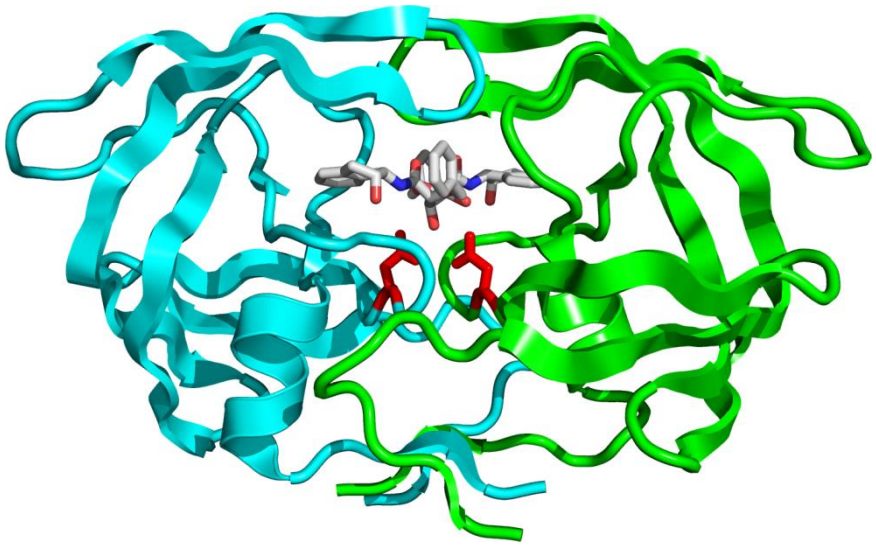


Raltegravir (2007)

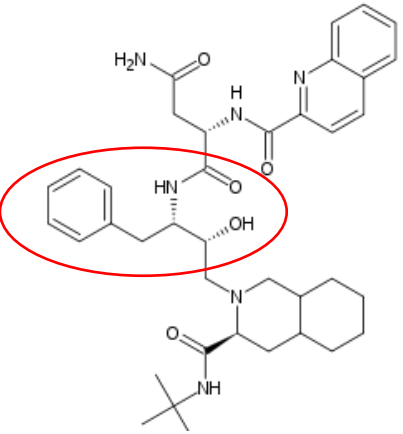
Protease inhibitors

- PIs block viral assembly by inhibiting viral *Aspartic protease*, the enzyme that cleaves HIV polyproteins to the proteins required for the final assembly of new virions.
- They were first developed as peptidomimetics, based on peptide substrates of the protease that:
  1) cannot be hydrolized
  2) have s small size and reduced peptidic nature (bioavailability & stability).
- They are extensively metabolized by CYP 3A4.



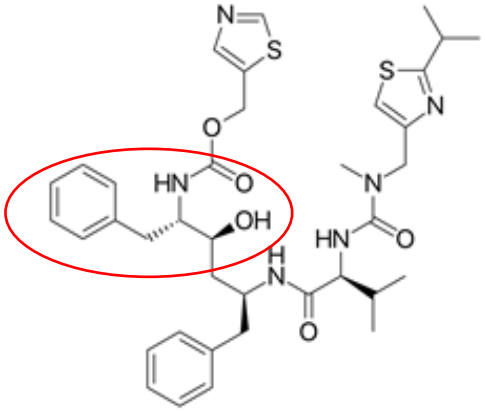


Protease inhibitors (PI)



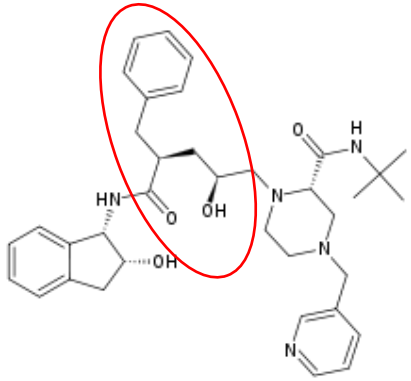
Saquinavir (1995)

EC_{50} ~ 40 nM



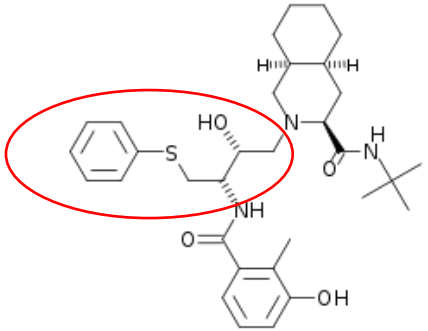
Ritonavir (1996)(*)

EC_{50} ~ 25 nM



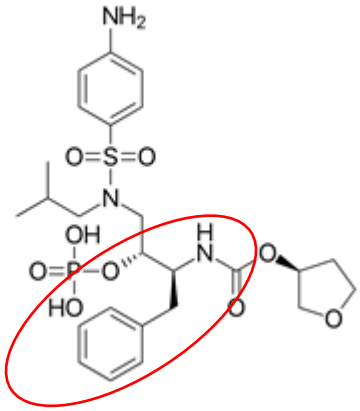
Indinavir (1996)

EC_{50} ~ 5 nM



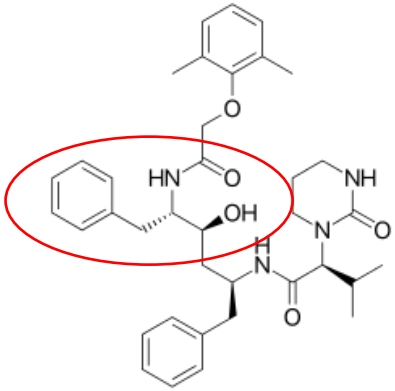
Nelfinavir (1997)

EC_{50} ~ 30-60nM



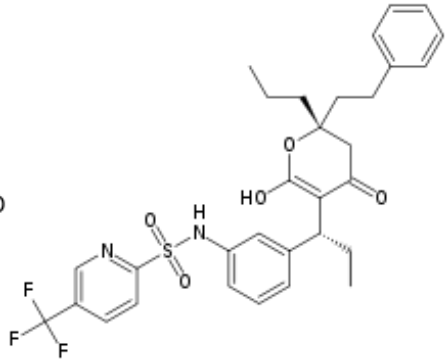
(Fos)Amprenavir (1999/2003)

EC_{50} ~ 10-80 nM



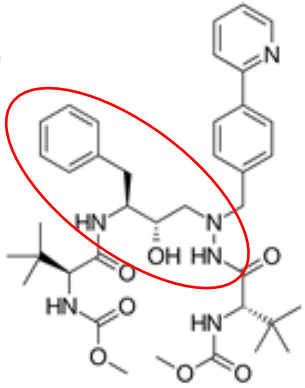
Lopinavir (2000)

EC_{50} ~ 20 nM



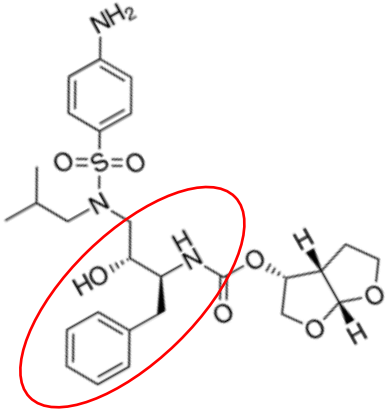
Tipranavir (2005)

EC_{50} ~ 30-70 nM



Atazanavir (2003)

EC_{50} ~ 5 nM

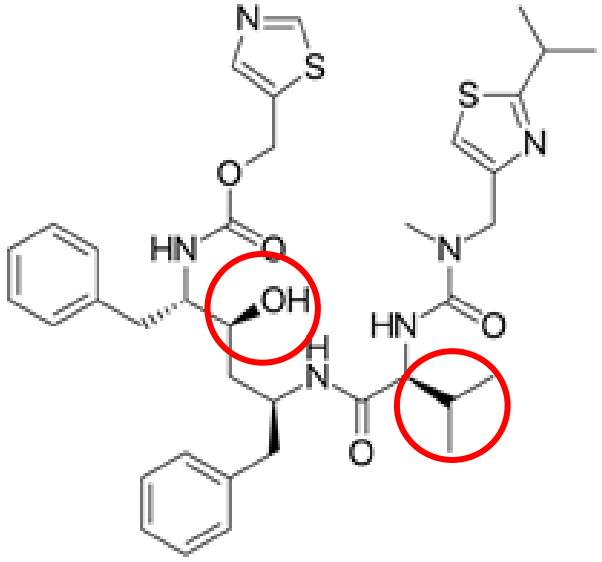


Duranavir (2006)

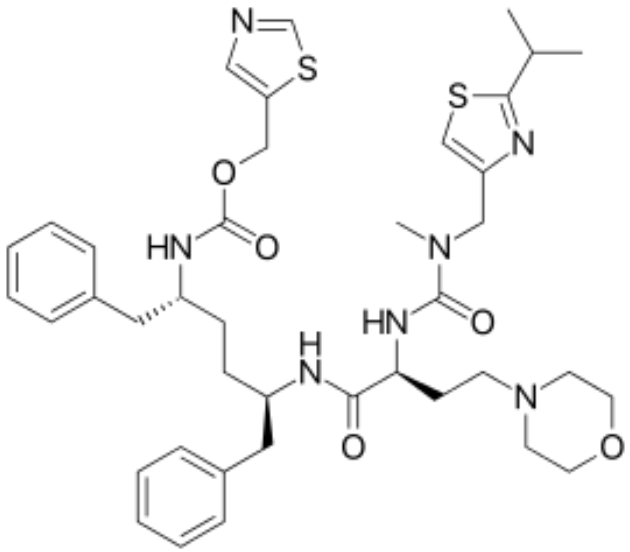
EC_{50} ~ 1-2 nM

Metabolic boosters

- **Ritonavir** is subject to resistance by HIV PR. However, it is a very potent inhibitor of CYP P450 3A4
- At sub-therapeutic doses it is used to boost the plasma concentration of second generation HIV PI.
- Cobicistat is an analogue of ritonavir where the backbone hydroxyl group is removed.
- These changes eliminate any anti-HIV activity but preserve CYP3A inhibitory effects
- It can increase plasma concentration of other co-administered anti-HIV drugs without the risk of causing resistant mutations in the HIV virus.



Ritonavir



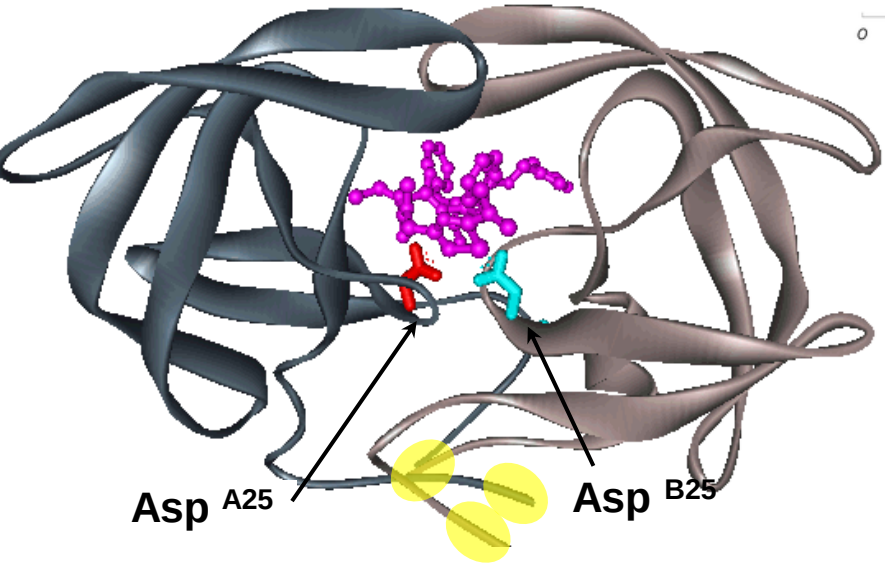
Cobicistat

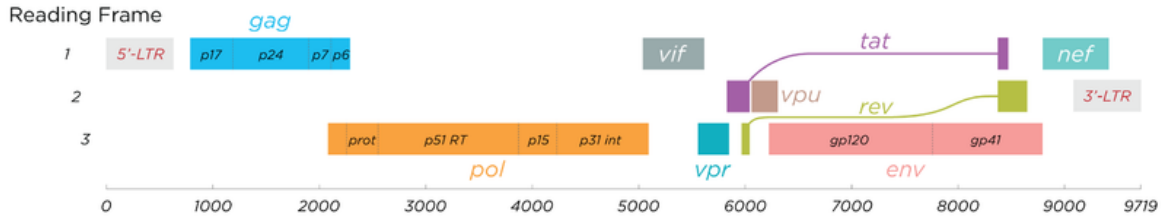
Aspartico proteasi virale

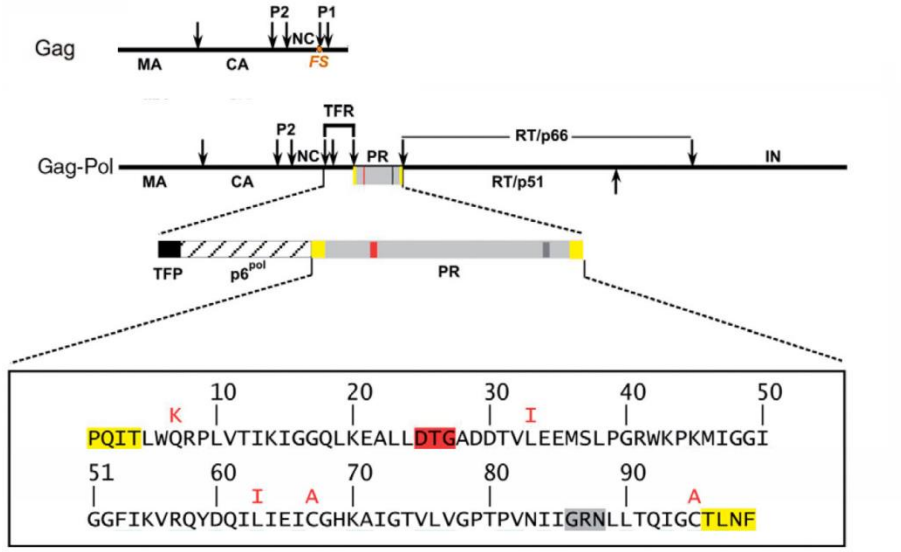
- L'endopeptidasi virale è necessaria per la maturazione di HIV

RNA virale —*Trascrittasi inversa*→ **DNA virale** —*Integrasi*→ **DNA GENOMICO** —*Enzimi cellulari*→ **POLIPEPTIDI virali** —*Aspartico proteasi*→ **PROTEINE virali**

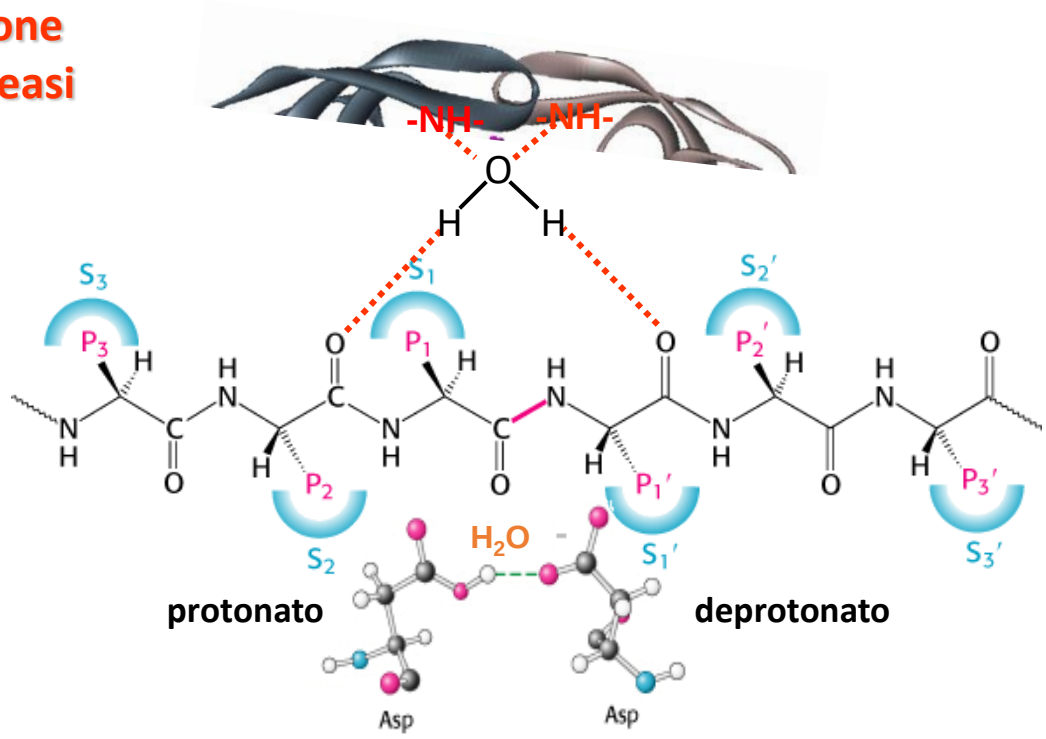
- È una proteina omodimerica. Ogni catena ha 99 residui.
- Taglia le singole proteine attive dalle poliproteine virali (incluso se stessa per autocatalisi).
- Ha una certa preferenza di taglio fra Phe e Pro.



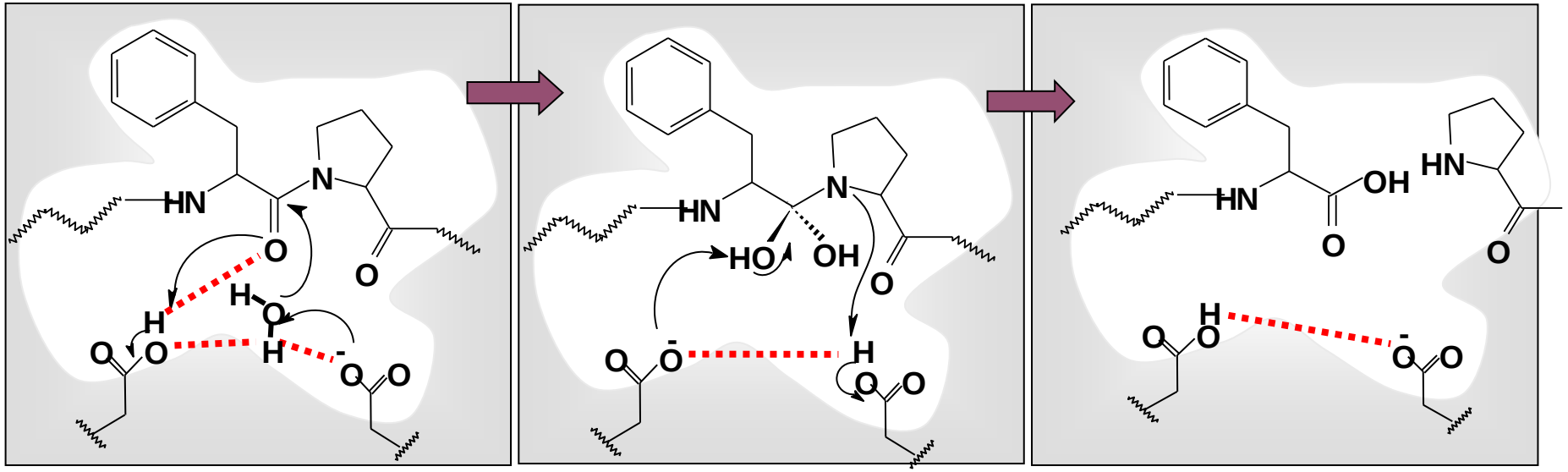




Meccanismo d'azione dell'aspartico proteasi di HIV



P_1 preferenzialmente Phe
P_1' preferenzialmente Pro
P_2' preferenzialmente Glu

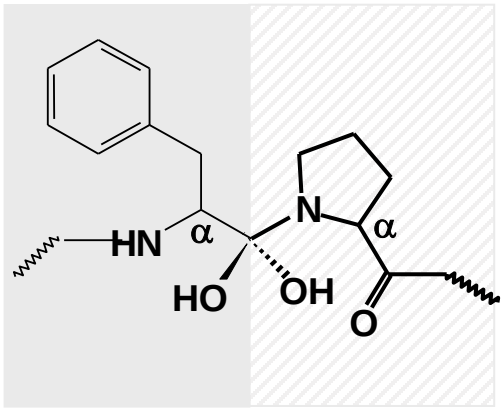


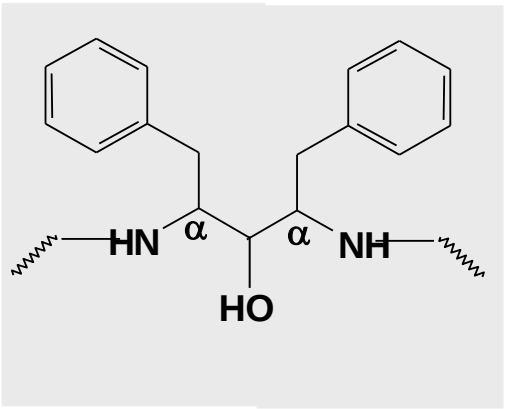
stato di transizione = gem diolo

- La progettazione di inibitori di proteasi si basa sulle caratteristiche

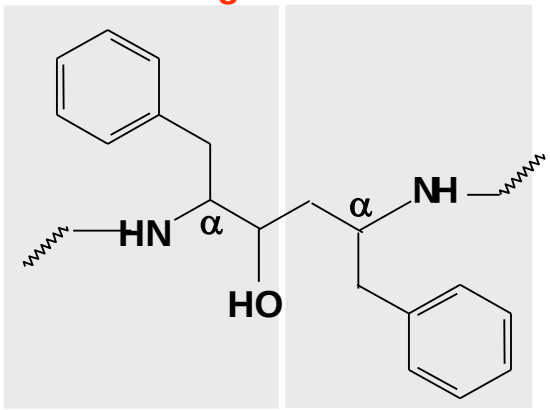
1) del sito di legame (P_3-P_2-P_1-P_1'-P_2'-P_3' e relativi S_n / S_n')
2) del meccanismo d'azione (Asp-COOH / Asp-COO^-)
3) dello stato di transizione (TS) (gemdiolo)

TS

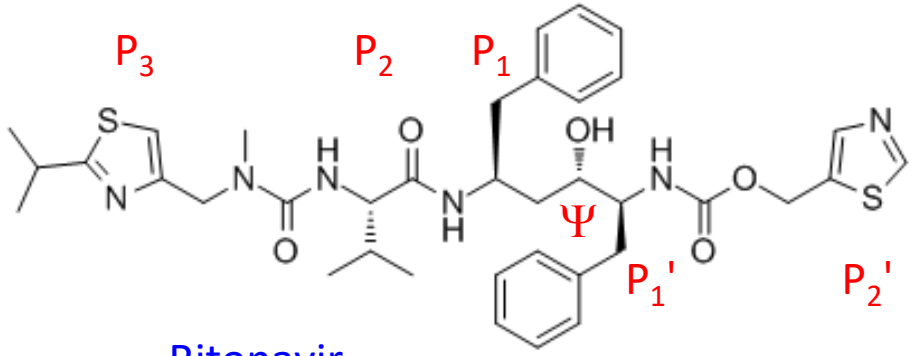




analogo del TS



Ψ = idrossietilene

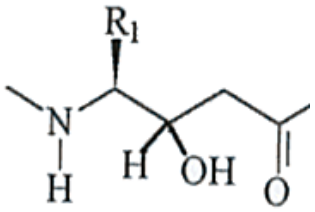


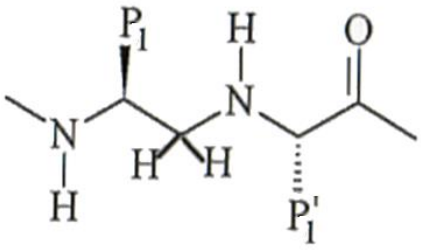
Ritonavir

Ψ = isostere non idrolizzabile del legame peptidico

Inibitori delle aspartico proteasi - Isosteri del legame peptidico (Ψ)

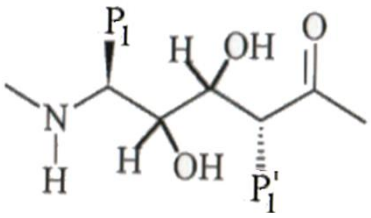
statina

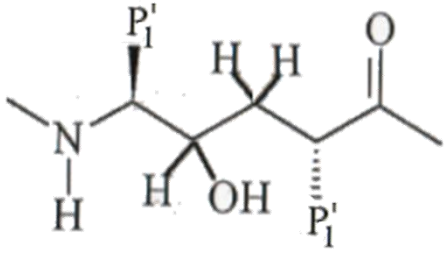




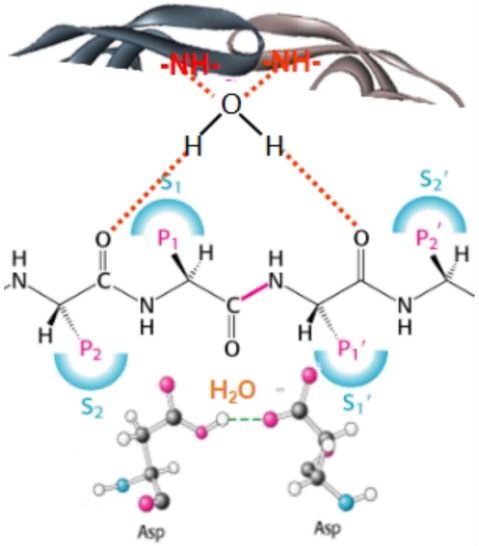
ammide ridotto

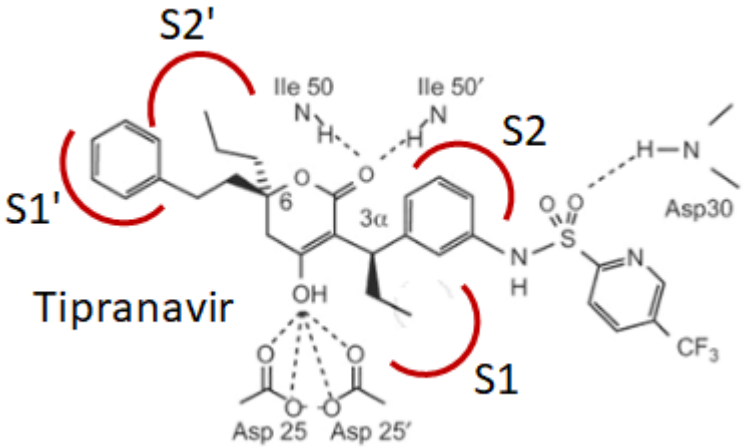
diidrossietilene



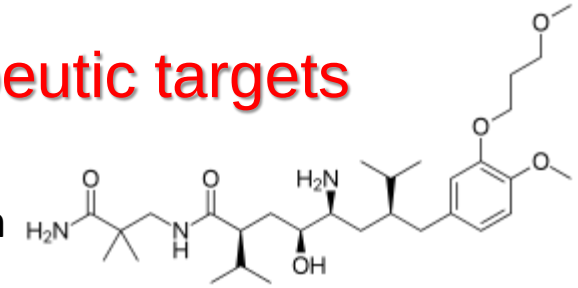


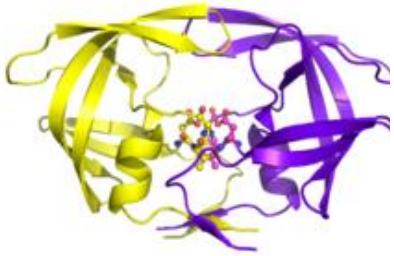
monoidrossietilene



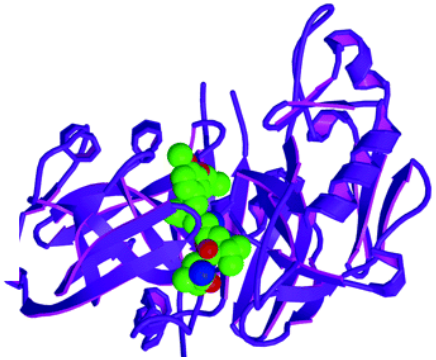


Other aspartic proteases as therapeutic targets

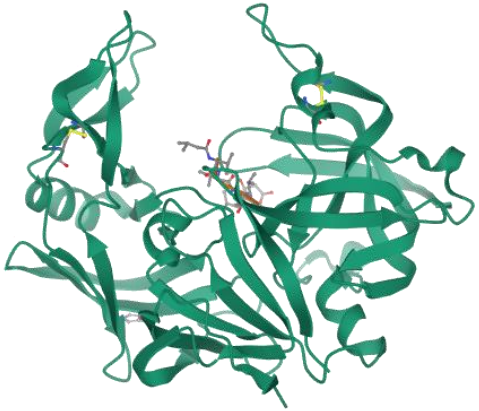
Renin	⇒ Hypertension	Aliskiren 
Cathepsin D	⇒ Alzheimer's disease	(development phase)
Plasmepsins	⇒ Malaria	(development phase)
Candidapepsins	⇒ *Candida* Infections	(development phase)



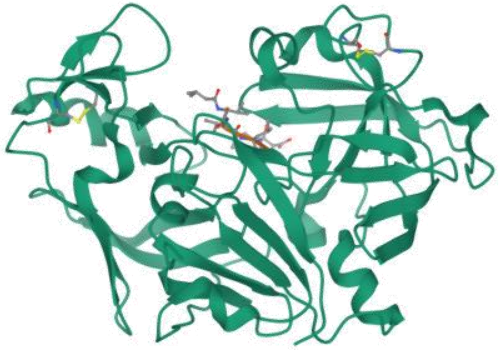
HIV-1 aspartic protease



human renin



candidapepsin (SAP)



plasmepsin

Highly Active Antiretroviral Therapy - HAART

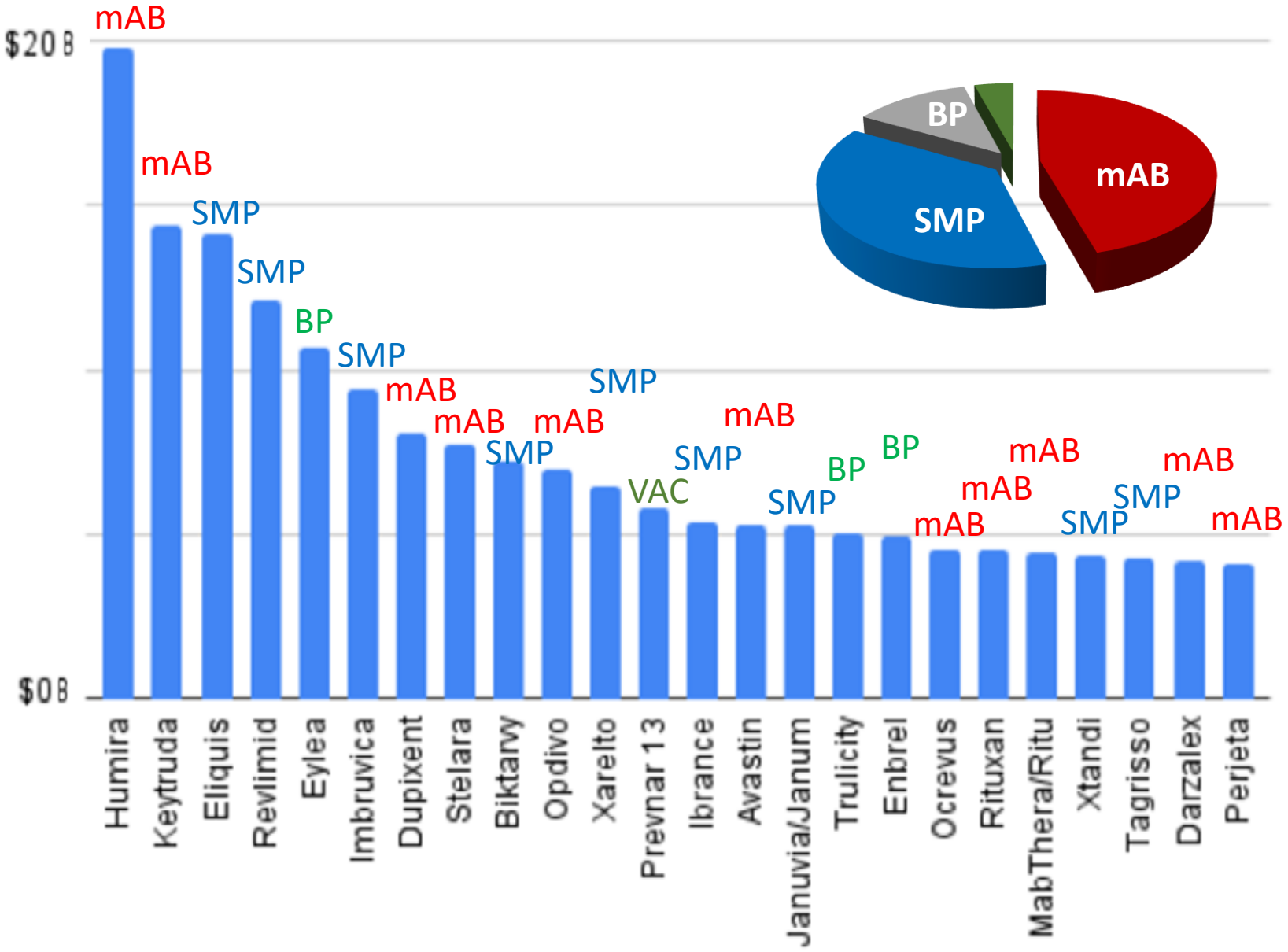
- Multiple antiretroviral drugs in single pill → Resistance ↓ Compliance ↑
- Fixed-dose combinations → Compliance ↑
- Preferably taken once a day → Compliance ↑
- With ritonavir → Metabolism ↓

HAART Regimens	
Two Nucleoside Reverse Transcriptase Inhibitors (NRTI) +	**Regimen Type**
(1) A Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)	NNRTI
(2) Ritonavir plus one or more Protease Inhibitor (PI)	Boosted PI
(3) A Protease Inhibitor (PI)	Unboosted PI
(4) An Integrase Inhibitor (INI)	INI
(5) Three or more NRTI containing abacavir and not including an INI, PI, or NNRTI	3NRTI
(6) Three or more antiretroviral medications from at least two different categories (NRTI, PI, NNRTI, INI) not meeting any other criteria	Other

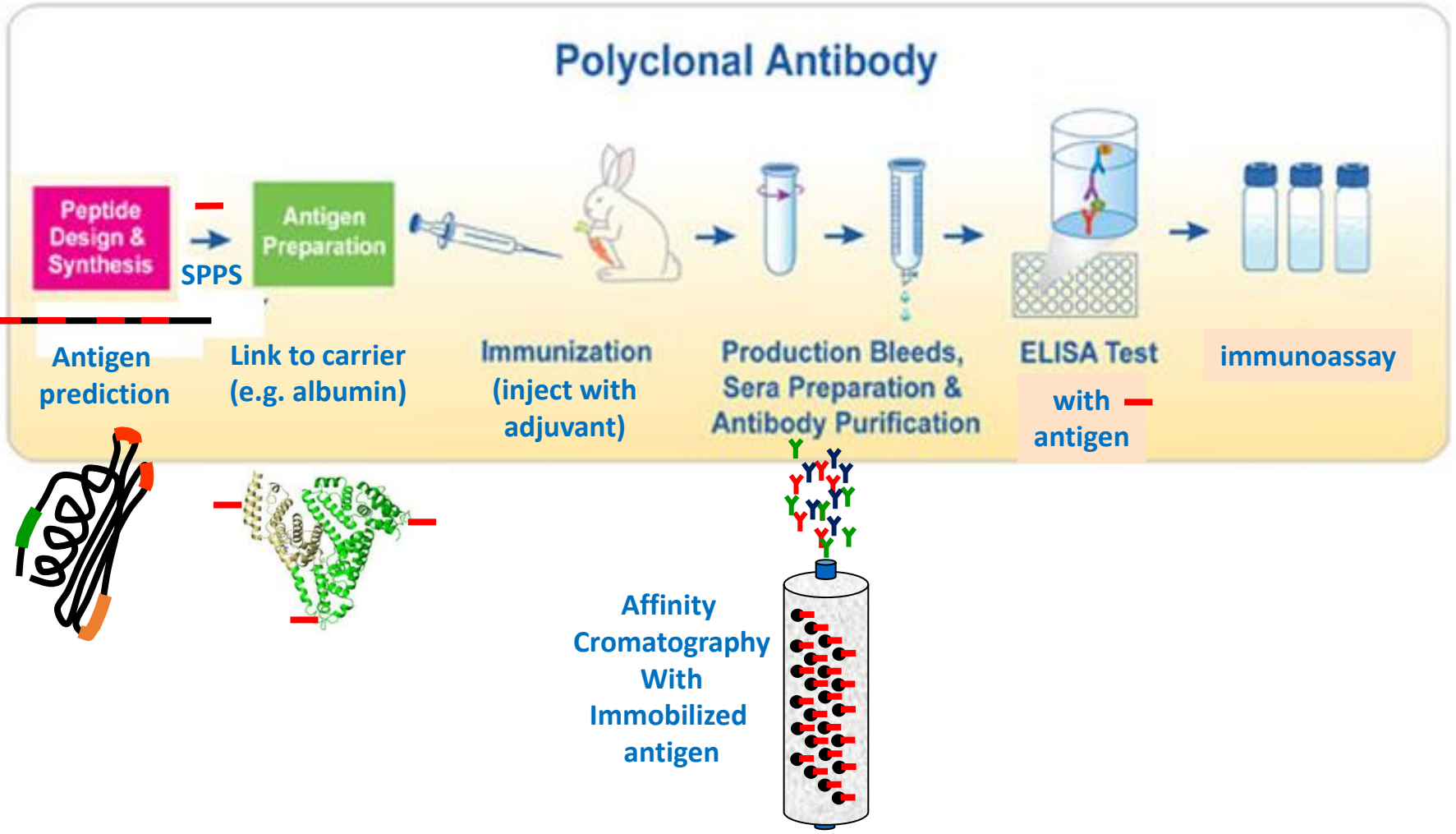
Biopharmaceuticals

- Biopharmaceuticals are medical drugs produced by biotechnology, by means other than direct extraction from a native (non-engineered) biological source.
- They are often called biologics, but this is also used to indicate vaccines, blood, blood components, cells, allergens, genes and tissues for therapeutic uses.
- They include proteins and nucleic acids (DNA, RNA or antisense oligonucleotides).
- They can be used for therapeutic or *in vivo* diagnostic purposes.
- The first such substance approved for therapeutic use was recombinant "human" insulin (rHI) under the trade name Humulin (1982).
- Biopharmaceuticals are very expensive due to their complex manufacturing process and represent a growing share of the global market (~240 billion $ in 2018 - 33% mAb).
- The most common biologics are **monoclonal antibodies** (mAb, ~ 80 approved).
- Another type are **recombinant proteins** (hormones, cytokynes or enzymes)
- A third type are **fusion proteins** that combine the binding domain of a receptor with part of an immunoglobulin (soluble form of receptor that competes for a ligand)
- **Nucleic acid biopharmaceuticals** include DNA/RNA vaccines, antisense oligonucleotides (ASO), siRNA and constructs for gene therapies (adeno-associated).

24 of 2020's best-selling pharmaceuticals

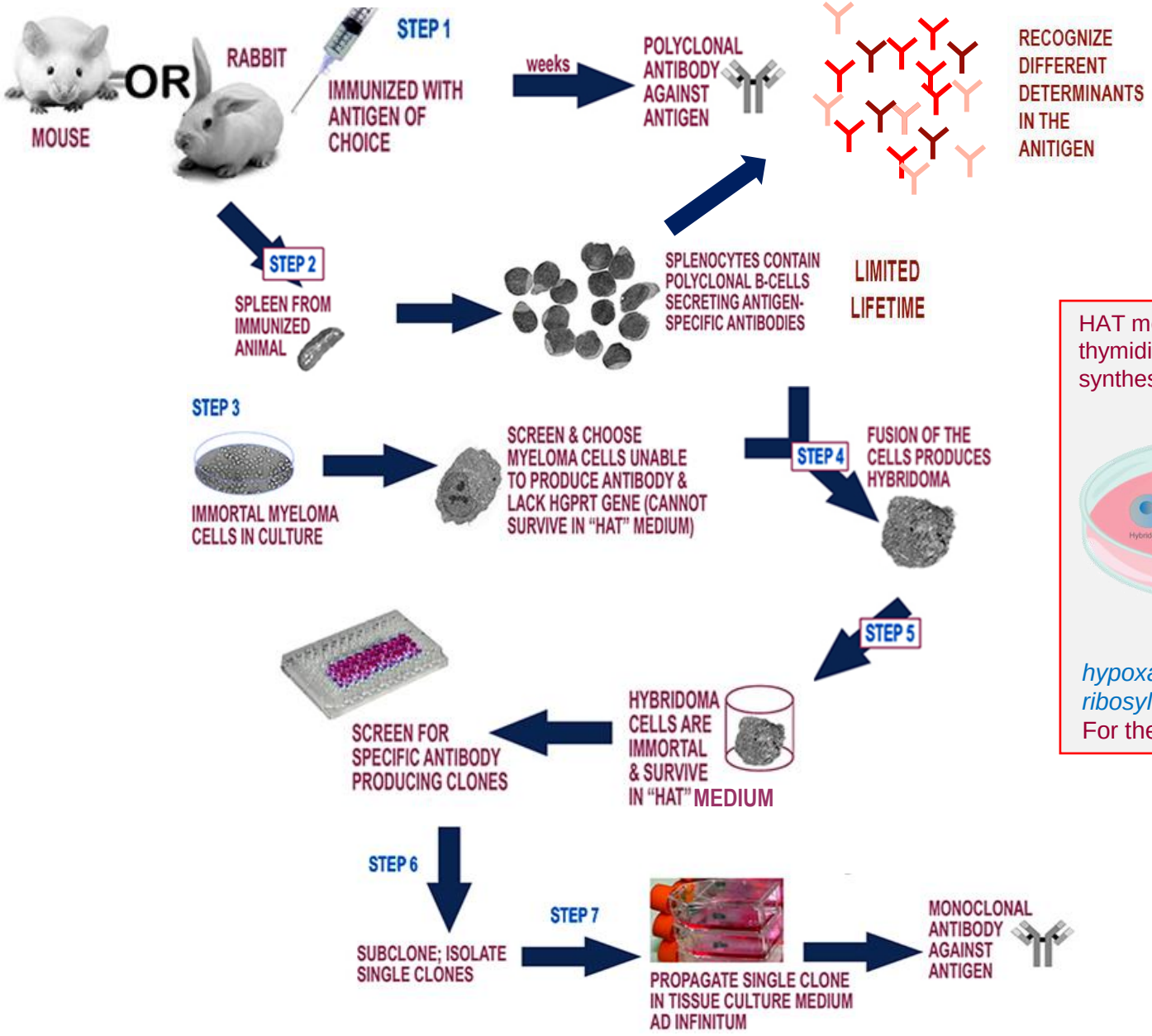


Produzione di anticorpi

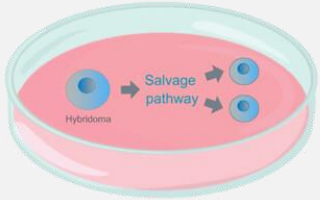


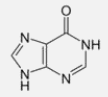
Gli anticorpi **policlonali** non derivano da una unica linea di linfociti B, ma da alcune diverse linee che riconoscono diverse parti dell'antigene. Sono comunque molto utili a scopo diagnostico.

Produzione di anticorpi monoclonali – metodo ibridoma

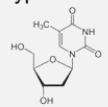


HAT medium = hypoxanthine-aminopterin-thymidine medium (cells lack *de novo* synthesis pathway)





Hypoxanthine

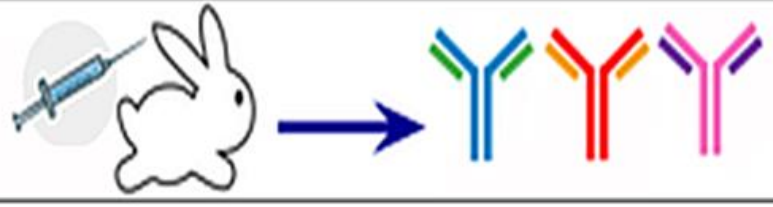


Thymidine

hypoxanthine-guanine phospho ribosyltransferase (*HGPRT*) is essential For the salvage pathway

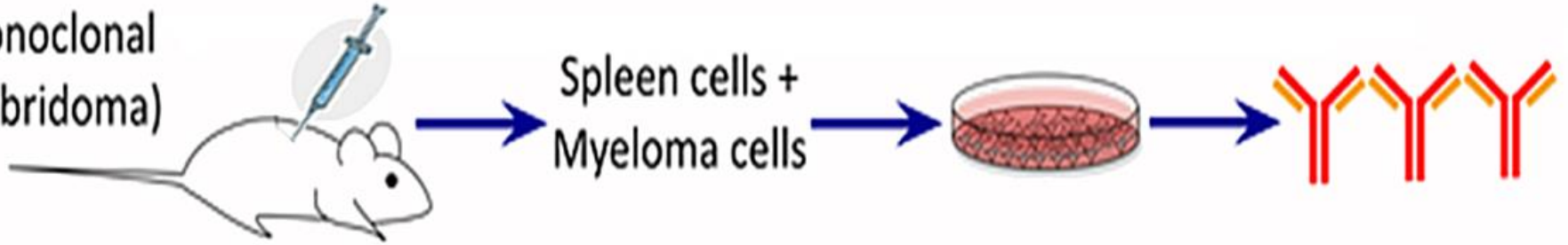
Antibody types – mAB, rAB and minibodies

Polyclonal

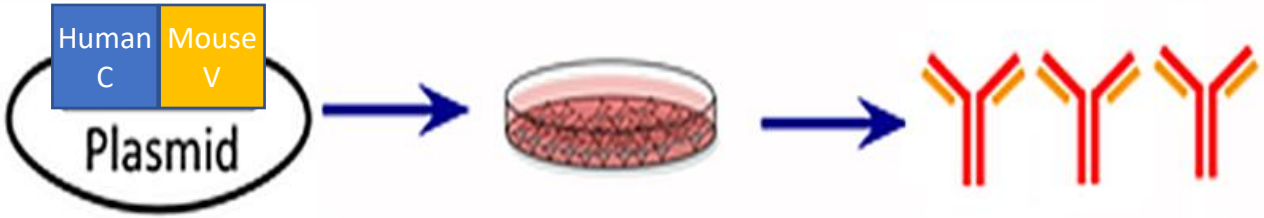


- Insufficient batch-to-batch reproducibility for use as drugs or diagnostic agents
- Ok for lab analyses (e.g. ELISA / N BLOT)

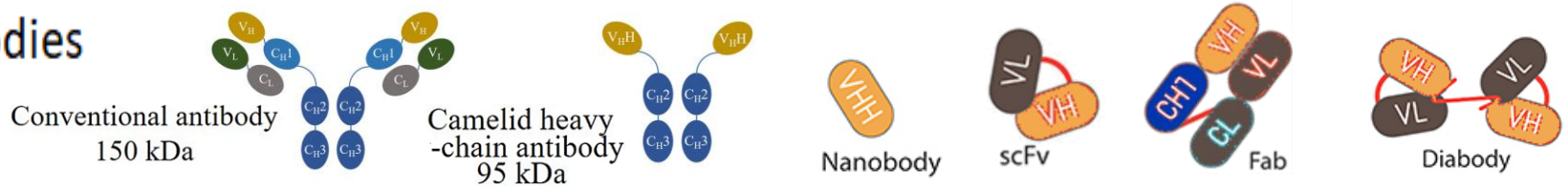
Monoclonal (hybridoma)



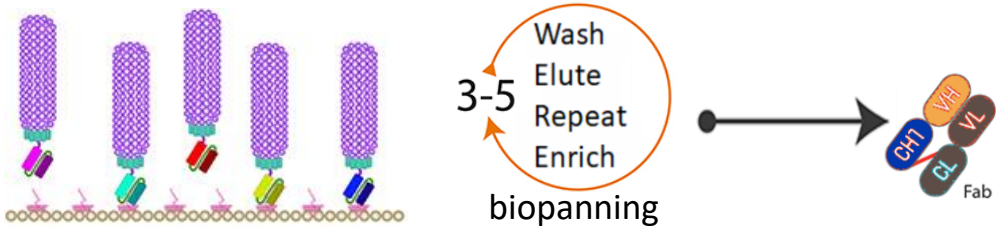
Recombinant monoclonal (rAb)



Minibodies



Antibody phage display library



- possible altered conformation when attached to solid surfaces
- cell-based biopanning can be utilized for membrane proteins

Antibody production

1. Attainment in the laboratory

- Hybridoma, phage display etc. used to obtain mAb
- **Humanization of mAb**.

2. Industrial production

- **Genetically modified cells** used to produce the humanized antibody
- **Chinese hamster ovary cells** are the most commonly used.[3]
- These must be meet certain **pH, temperature and oxygen conditions, etc., and require the necessary nutrients (culture medium)**.
- Each company prepares the most appropriate for the antibody they want to produce.
- Cells are grown in **bioreactors**. The most efficient have **cells in suspension in a liquid culture medium**.
- Bioreactors can be multiple or single use (disposable)
- Disposable bioreactors are more flexibile and eliminate any possibility of cross-contamination.
- Antibodies are then **purified and concentrated**.

Anticorpi (o Fab) terapeutici (…mab → monoclonal ab)

Example FDA approved therapeutic monoclonal antibodies[1]

Antibody	Brand name	Company	Approval date	Type	Target	Indication (Targeted disease)
Abciximab	ReoPro	Eli Lilly	1994	chimeric	inhibition of glycoprotein IIb/IIIa	Cardiovascular disease
Adalimumab	Humira	Abbot	2002	human	inhibition of TNF-α signaling	Several auto-immune disorders
Alemtuzumab	Campath	Genzyme	2001	humanized	CD52	Chronic lymphocytic leukemia
Basiliximab	Simulect	Novartis	1998	chimeric	IL-2Rα receptor (CD25)	Transplant rejection
Belimumab	Benlysta	GlaxoSmithKline	2011	human	inihibition of B- cell activating factor	Systemic lupus erythematosus
Bevacizumab	Avastin Ig	Genentech/Roche	2004	humanized	Vascular endothelial growth factor (VEGF)	Colorectal cancer, Age related macular degeneration (off-label)
Brentuximab vedotin	Adcetris		2011	Chimeric	CD30	Anaplastic large cell lymphoma (ALCL) and Hodgkin lymphoma
Canakinumab	Ilaris	Novartis	2009	Human	IL-1β	Cryopyrin-associated periodic syndrome (CAPS)
Cetuximab	Erbitux	Bristol-Myers Squibb/Eli Lilly/Merck KGaA	2004	chimeric	epidermal growth factor receptor	Colorectal cancer, Head and neck cancer
Certolizumab pegol[19]	Cimzia	UCB (company)	2008	humanized	inhibition of TNF-α signaling	Crohn's disease
Daclizumab	Zenapax	Genentech/Roche	1997	humanized	IL-2Rα receptor (CD25)	Transplant rejection
Denosumab	Prolia , Xgeva	Amgen	2010	Human	RANK Ligand inhibitor	Postmenopausal osteoporosis , Solid tumor`s bony metasteses
Eculizumab	Soliris	Alexion Pharmaceuticals	2007	humanized	Complement system protein C5	Paroxysmal nocturnal hemoglobinuria
Efalizumab	Raptiva	Genentech/Merck Serono	2002	humanized	CD11a	Psoriasis
Gemtuzumab	Mylotarg	Wyeth	2000	humanized	CD33	Acute myelogenous leukemia (with calicheamicin)
Golimumab	Simponi	Johnson & Johnson/Merck & Co, Inc.	2009	Human	TNF-alpha inihibitor	Rheumatoid arthritis, Psoriatic arthritis, and Ankylosing spondylitis
Ibritumomab tiuxetan	Zevalin	Spectrum Pharmaceuticals, Inc.	2002	murine	CD20	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Infliximab	Remicade	Janssen Biotech, Inc./Merck & Co	1998	chimeric	inhibition of TNF-α signaling	Several autoimmune disorders
Ipilimumab (MDX-101)	Yervoy		2011	Human	blocks CTLA-4	Melanoma
Muromonab-CD3	Orthoclone OKT3	Janssen-Cilag	1986	murine	T cell CD3 Receptor	Transplant rejection
Natalizumab	Tysabri	Biogen Idec/Élan	2006	humanized	alpha-4 (α4) integrin,	Multiple sclerosis and Crohn's disease
Ofatumumab	Arzerra		2009	Human	CD20	Chronic lymphocytic leukemia
Omalizumab	Xolair	Genentech/Novartis	2004	humanized	immunoglobulin E (IgE)	mainly allergy-related asthma
Palivizumab	Synagis	MedImmune	1998	humanized	an epitope of the RSV F protein	Respiratory Syncytial Virus
Panitumumab	Vectibix	Amgen	2006	human	epidermal growth factor receptor	Colorectal cancer
Ranibizumab	Lucentis F_{ab}	Genentech/Novartis	2006	humanized	Vascular endothelial growth factor A (VEGF-A)	Macular degeneration
Rituximab	Rituxan, Mabthera	Biogen Idec/Genentech	1997	chimeric	CD20	Non-Hodgkin lymphoma
Tocilizumab (or Atlizumab)	Actemra and RoActemra		2010	Humanised	Anti- IL-6R	Rheumatoid arthritis
Tositumomab	Bexxar	GlaxoSmithKline	2003	murine	CD20	Non-Hodgkin lymphoma
Trastuzumab	Herceptin	Genentech	1998	humanized	ErbB2	Breast cancer

Humira (Adalimumab)

- Humira è un anticorpo monoclonale umano ottenuto con la tecnologia phage display

- Si lega e inibisce l'attività del tumor necrosis factor-alpha (TNFα). Quando TNFα si lega al suo recettore provoca una risposta infiammatoria.

- Approvato per artrite reumatoide, artrite psoriatica, spondilite anchilosante, malattia di Crohn, colite ulcerativa, psoriasi cronica da severa a moderata e artrite idiopatica giovanile. Dose: 40 mg ogni due settimane.
- Effetti collaterali: poiché l'αTNF, che fa parte del sistema immunitario, possono essere riattivate le infezioni latenti come la tubercolosi, e il sistema immunitario potrebbe non essere in grado di combattere nuove infezioni.
- Costa circa più di $ 3.000 al mese ed è riconosciuto come il farmaco con le maggiori vendite dal 2014
- Ma già nel 2014 L'Indiana Cadila Healthcare ha lanciato il primo biosimilare adalimumab ad un quinto del costo negli Stati Uniti.