How do researchers design tomorrow's drugs?

Examples: pain and cancer

www. drug-design-workshop.ch

Marie-Claude.Blatter@sib.swiss
Antoine.Daina@sib.swiss
Vincent.Zoete@sib.swiss

FNSNF
FONDS NATIONAL SUISSE
DE LA RECHERCHE SCIENTIFIQUE

Chimiscope
UNIGE

SIB
Swiss Institute of Bioinformatics

All the material is available online

http://education.expasy.org/cours/Outreach/Chimiscope/

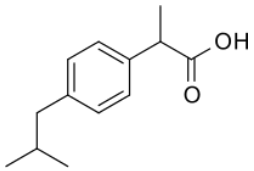
http://www2.grifil.com/album.html

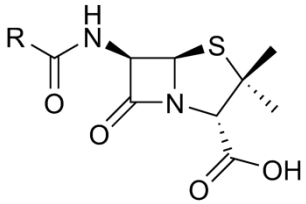
- ✓ What is a drug?
- ✓ How does it work?
- ✓ How to select the best drug candidates with the help of bioinformatics?
- ✓ Why do a genetic test before a treatment?

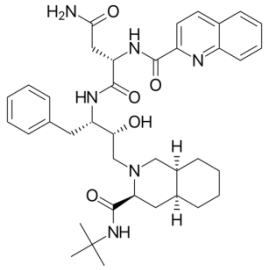
http://www2.grifil.com/album.html

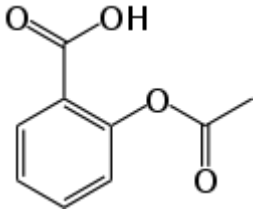
A drug is a small organic molecule (100 atomes)

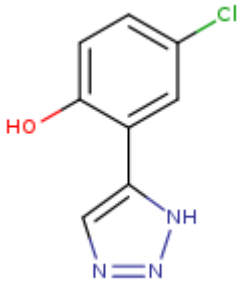
- ✓ 10^{60} molecules (theory)
- ✓ 35 million molecules
- ✓ 2,000 ‘drugs’





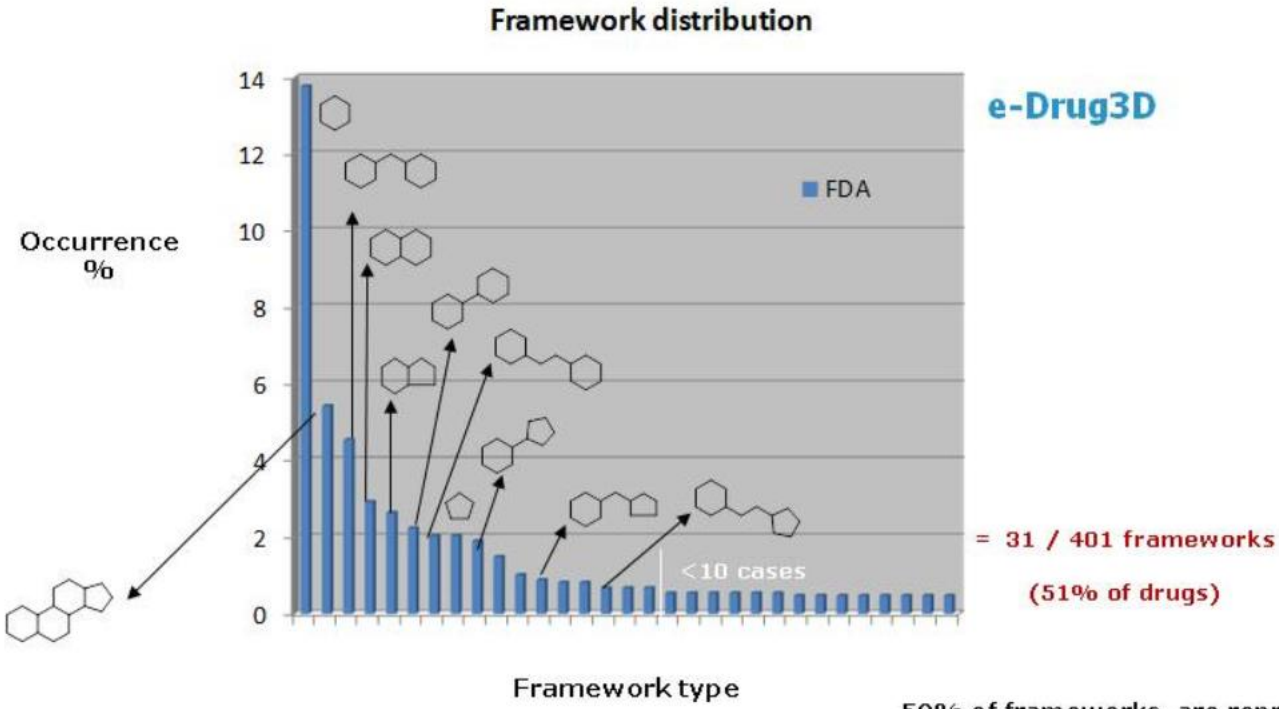




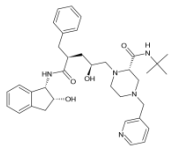


Aspirin (500 mg):
1.65 x 10^{21} molecules

• 51% of drugs are represented by only 31 "frameworks"

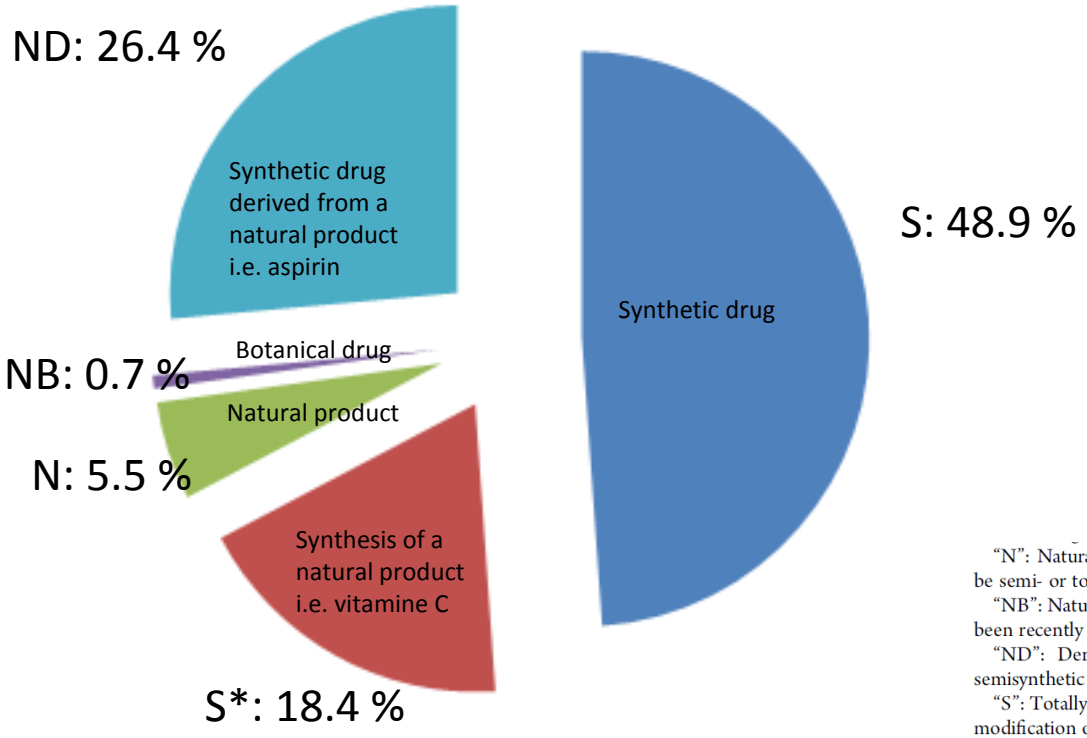


59% of frameworks are represented by 1 drug (eg: Indinavir)



http://chemoinfo.ipmc.cnrs.fr/MOLDB/statistics.html

Where do the drug molecules come from ?



"N": Natural product, unmodified in structure, though might be semi- or totally synthetic

"NB": Natural product "botanical drug" (in general these have been recently approved)

"ND": Derived from a natural product and is usually a semisynthetic modification

"S": Totally synthetic drug, often found by random screening/ modification of an existing agent

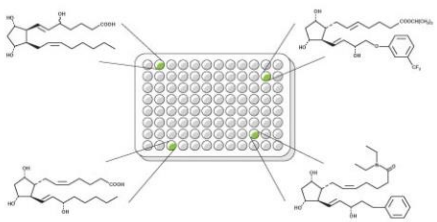
"S*": Made by total synthesis, but the pharmacophore is/was from a natural product

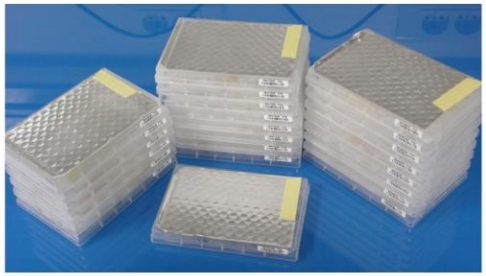
categorie	%	number
S	48.88	592
ND	26.43	320
S*	18.41	223
N	5.53	67
NB	0.74	9

http://pubs.acs.org/doi/pdf/10.1021/acs.jnatprod.5b01055 (2016)

Molecular screening – experimental HTS

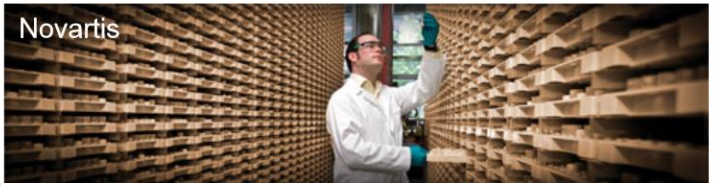
Format of the experimental collections:





University collection…

… pharmaceutical company collection.



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- ✓ 35 million molecules
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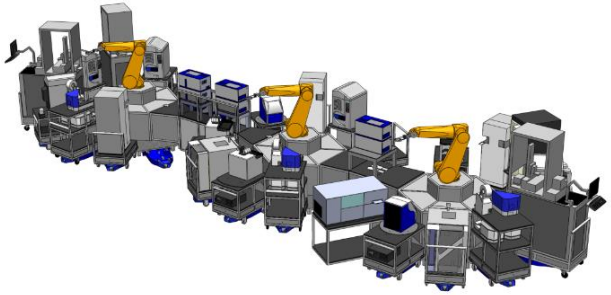
The industrial version :



Typical capacity: more than **100,000 compounds per day** in high-density 1536-well formats.

Molecular screening: an alternative

With the help of computers - **Computer-Aided Drug Design (CADD)** - it is possible to select molecules:

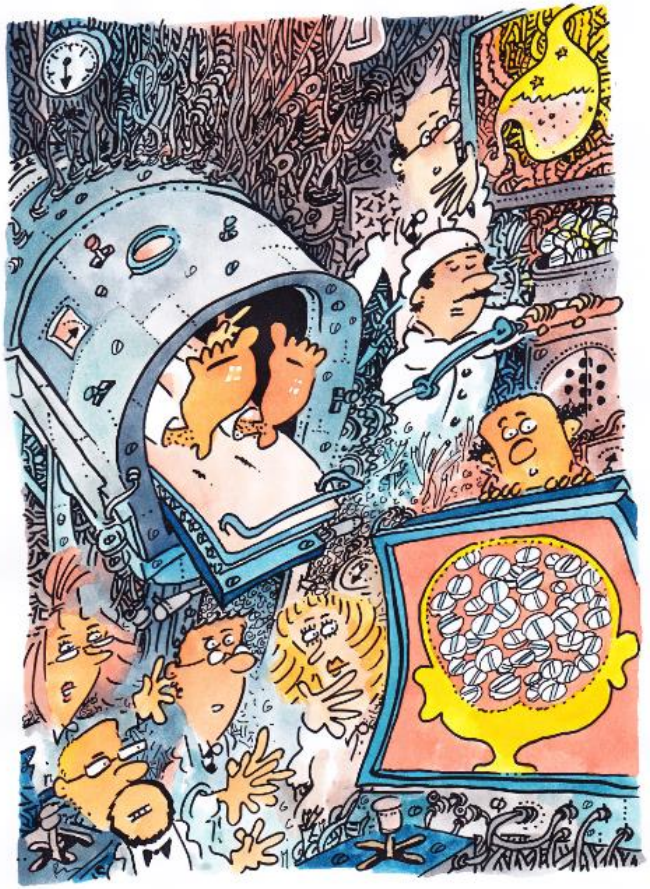
- ✓ with the highest affinity for their target
- ✓ which are as specific as possible for their target
- ✓ which have the potential of becoming a drug (i.e. to be taken orally, for the patient's comfort and compliance)

… evaluate **the fate** of the molecule in the body: absorption, distribution, metabolism and excretion

… evaluate the **solubility** of the molecule

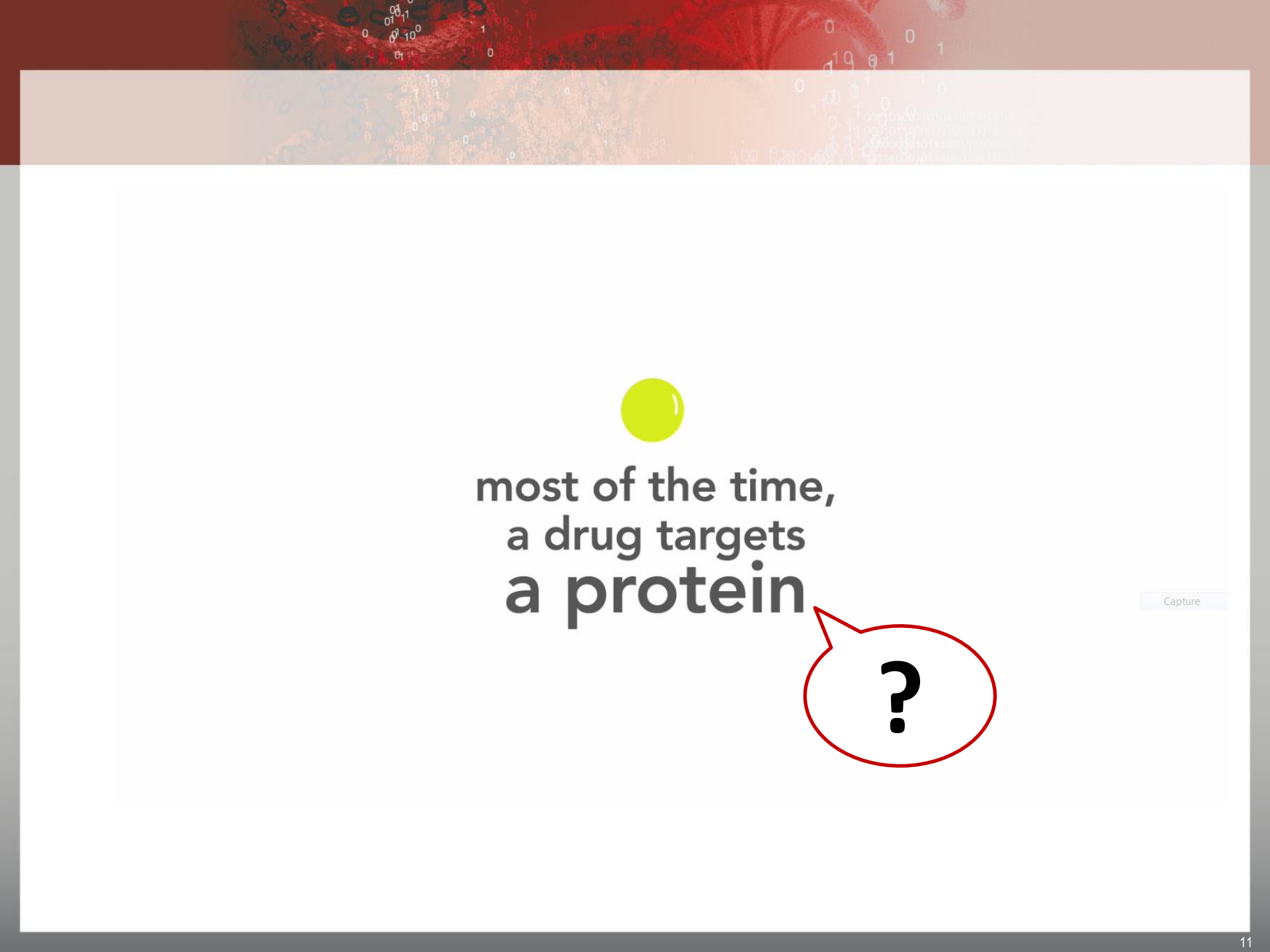
Computer-Aided Drug Design (CADD)

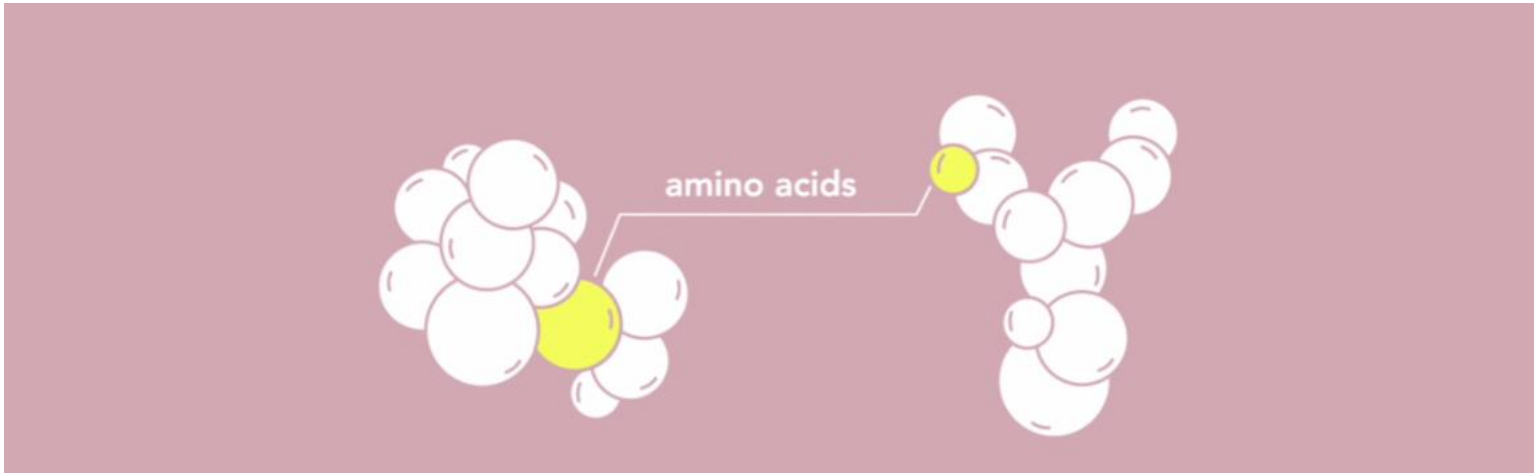
… evaluate the **toxicity** of the molecule



… can the molecule **reach the brain**?

Computer-Aided Drug Design (CADD)

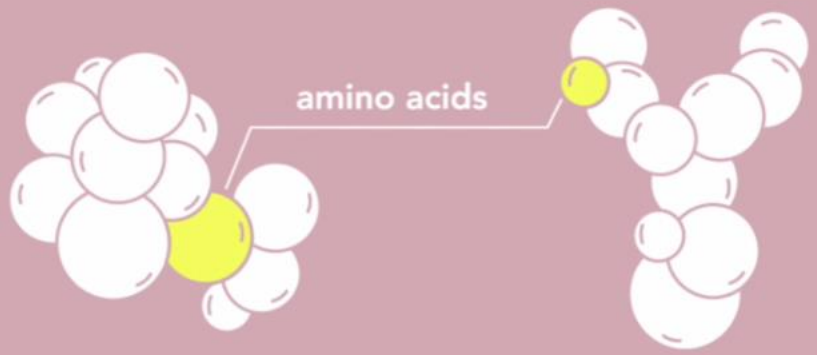

most of the time,
a drug targets
a protein
?



A protein may be compared to a necklace of beads
(one bead = one amino acid),
folded over onto itself.

There are 20 different amino acids.

This protein controls cell division

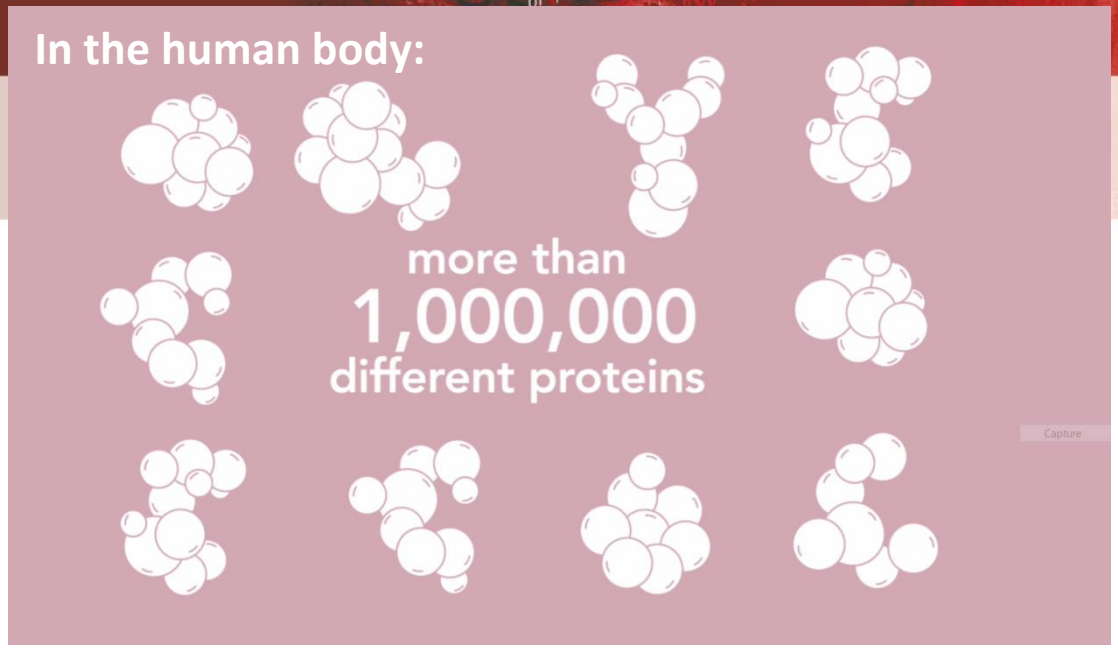


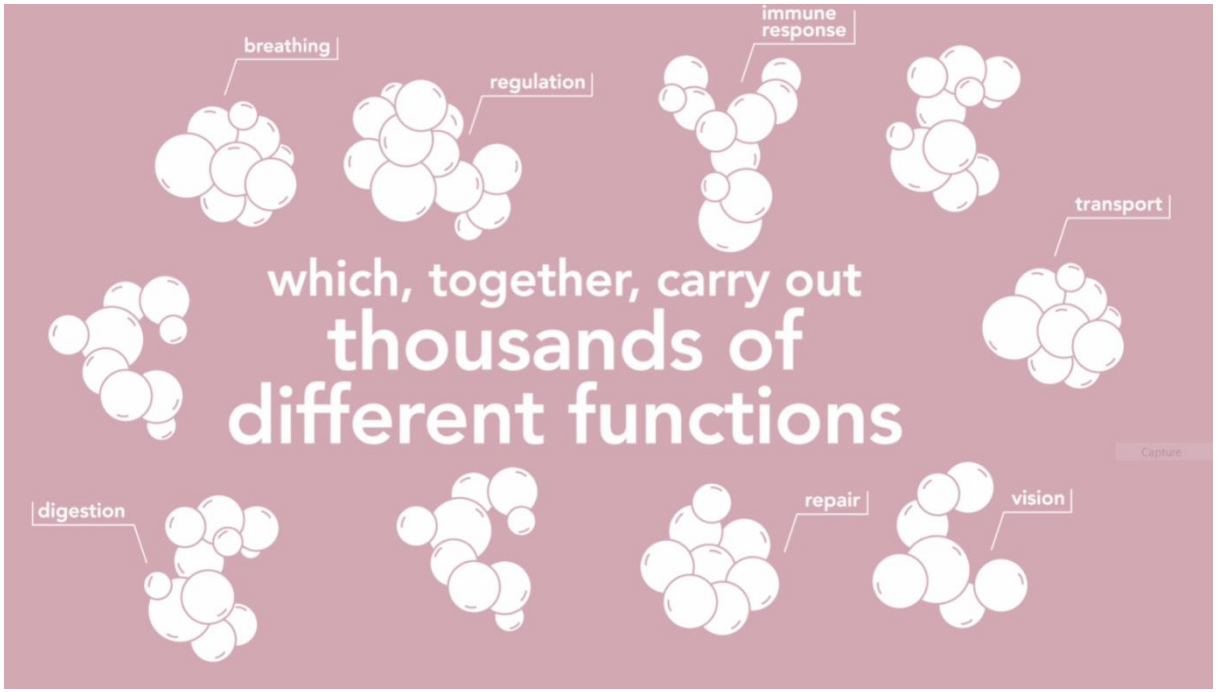
This protein is an antibody

a protein's **function** depends on its **composition**

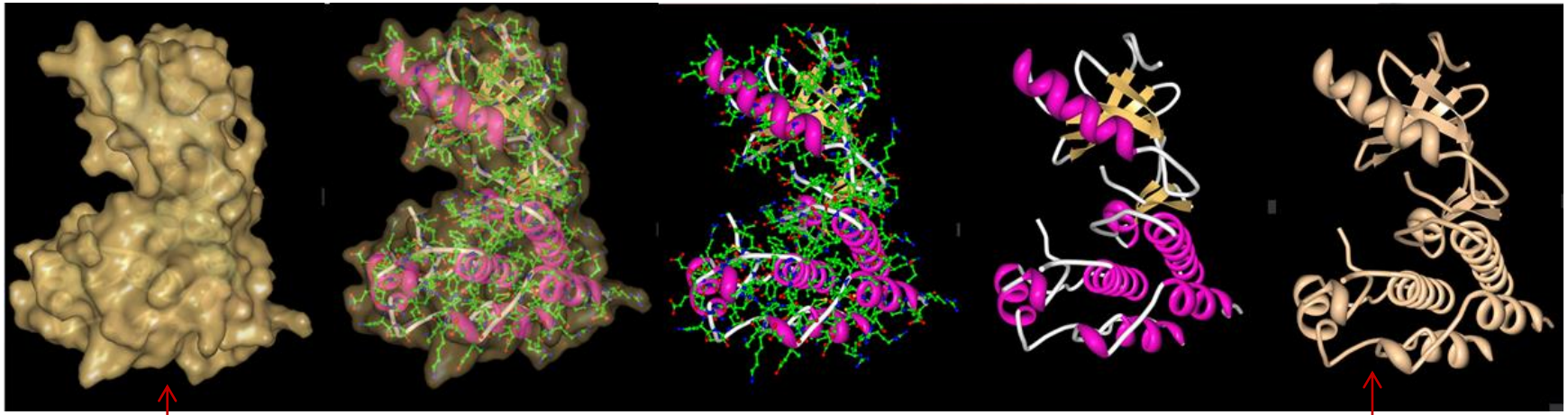
(3D structure)

In the human body:





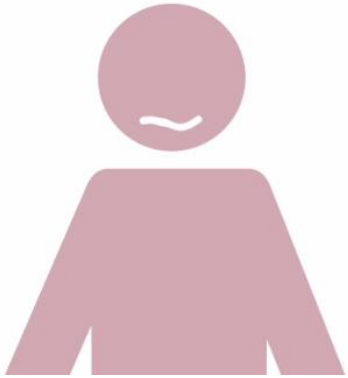
Different representations of the 3D structure of a protein
(a necklace of amino acids)



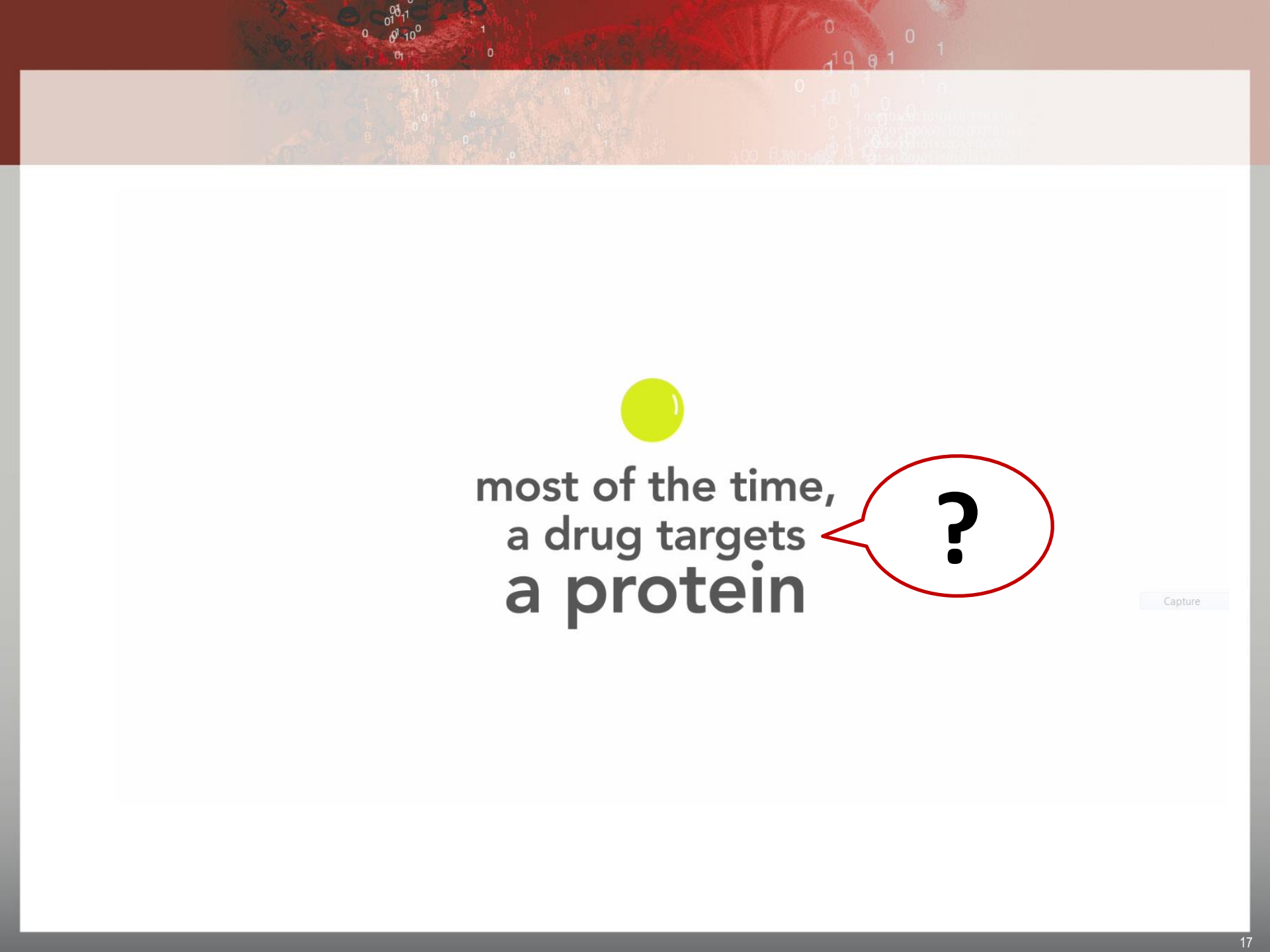
the necklace‘s surface

the thread on which the beads (amino acids) are

someone
falls ill...



- Protein's active site is **altered** (mutated)
- there is **too little** of a protein
- there is **too much** of a protein


most of the time,
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Antibiotics & Antivirals

1 2

Antibiotics and antiviral drugs are specific poisons. They need to kill pathogenic organisms like bacteria and viruses without poisoning the patient at the same time. Often, these drugs attack proteins that are only found in the targeted bacterium or virus and which are crucial for their survival or multiplication. For instance, **penicillin** attacks the enzyme that builds bacterial cell walls, and HIV protease inhibitors like **saquinavir** attack an enzyme that is needed for HIV maturation.

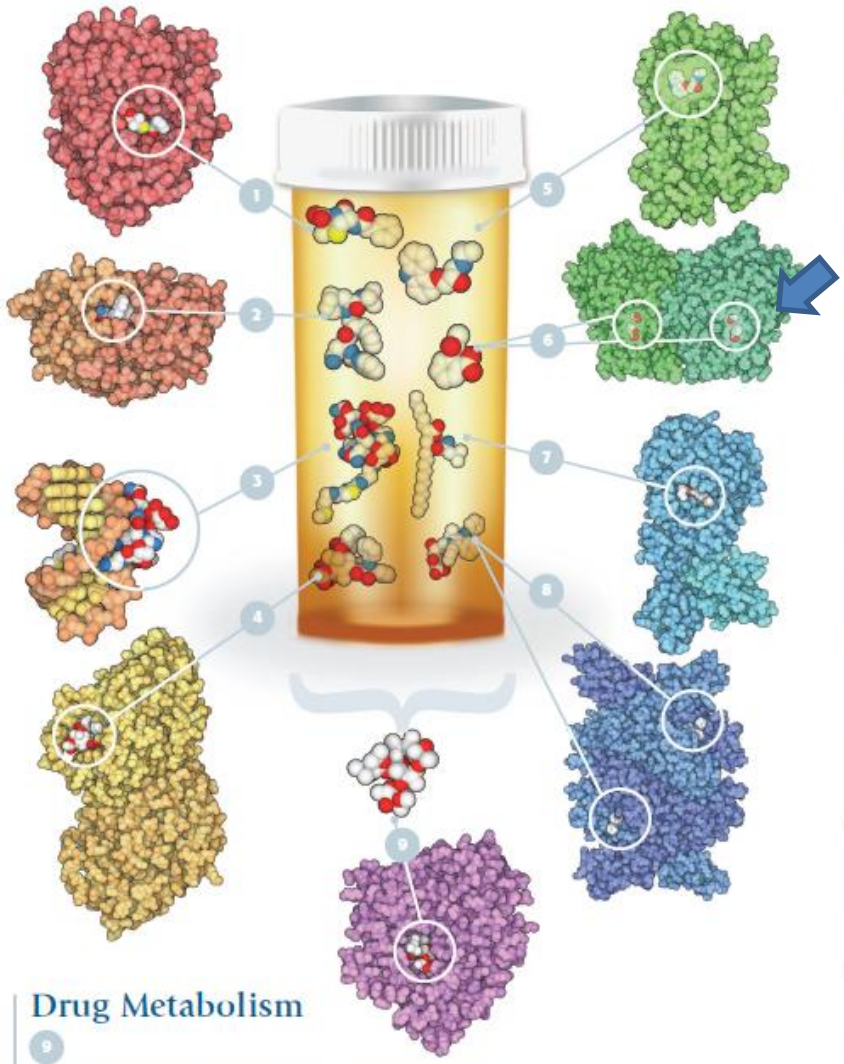
1. D-alanyl-D-alanine carboxypeptidase with penicillin (1pwc)
2. HIV protease with saquinavir (1hxb)

Anticancer Chemotherapy

3 4

Cancer cells grow and multiply without control. Since these cells are still similar to normal cells, it is difficult to kill them selectively with drugs that can't distinguish between the two. Many drugs currently used for cancer chemotherapy attack all growing cells, including cancer cells and normal cells. This causes the severe side effects of cancer chemotherapy, because the drugs attack rapidly-growing cells in hair follicles and the stomach. Two examples are shown here. **Bleomycin** attacks DNA in actively growing cells, often cleaving the DNA chain and killing the cell. Paclitaxel (**Taxol**) binds to tubulin, preventing the action of microtubules during cell division.

3. DNA with bleomycin (1mxk)
4. Tubulin with taxol (1jff)



Drug Metabolism

9

You have probably noticed that when you take drugs, the effects gradually wear off in a few hours. Enzymes like cytochrome P450 continually search for drugs and destroy them. This is important because it protects us from poisonous molecules in our diet and in the environment, but it means that we have to take multiple doses of drugs when being treated for a disease.

9. Cytochrome P450 3A4 with erythromycin (2j0d)

Drugs of Signaling Proteins

5 6

Many drugs are designed to keep bodily processes at normal healthy levels. Much of the body's regulation is done through elaborate communications between cells, so some of the most widely prescribed drugs function by blocking the signaling proteins that allow cells to communicate. G protein-coupled receptors, which transmit signals across cell membranes, are targets for many drugs. For instance, the drug loratadine (**Claritin**) is used to treat allergies because it blocks the histamine receptor; losartan (**Cozaar**) is used to treat high blood pressure because it blocks the angiotensin II receptor; and **carazolol** is one of a large class of beta-blockers that bind to the adrenergic receptor, making it useful for treating heart disease. Signals can also be stopped by blocking the enzymes that create a signaling molecule. **Aspirin** blocks pain at the source by inhibiting the enzyme cyclooxygenase, which makes pain-signaling prostaglandin molecules.

5. Adrenergic receptor with carazolol (2rh1)
6. Prostaglandin H2 synthase with aspirin (1pth). The drug breaks into two pieces when it binds to the enzyme, and the smaller piece (an acetyl group) is attached to the enzyme with a covalent bond. The closeup shows the drug in one piece.

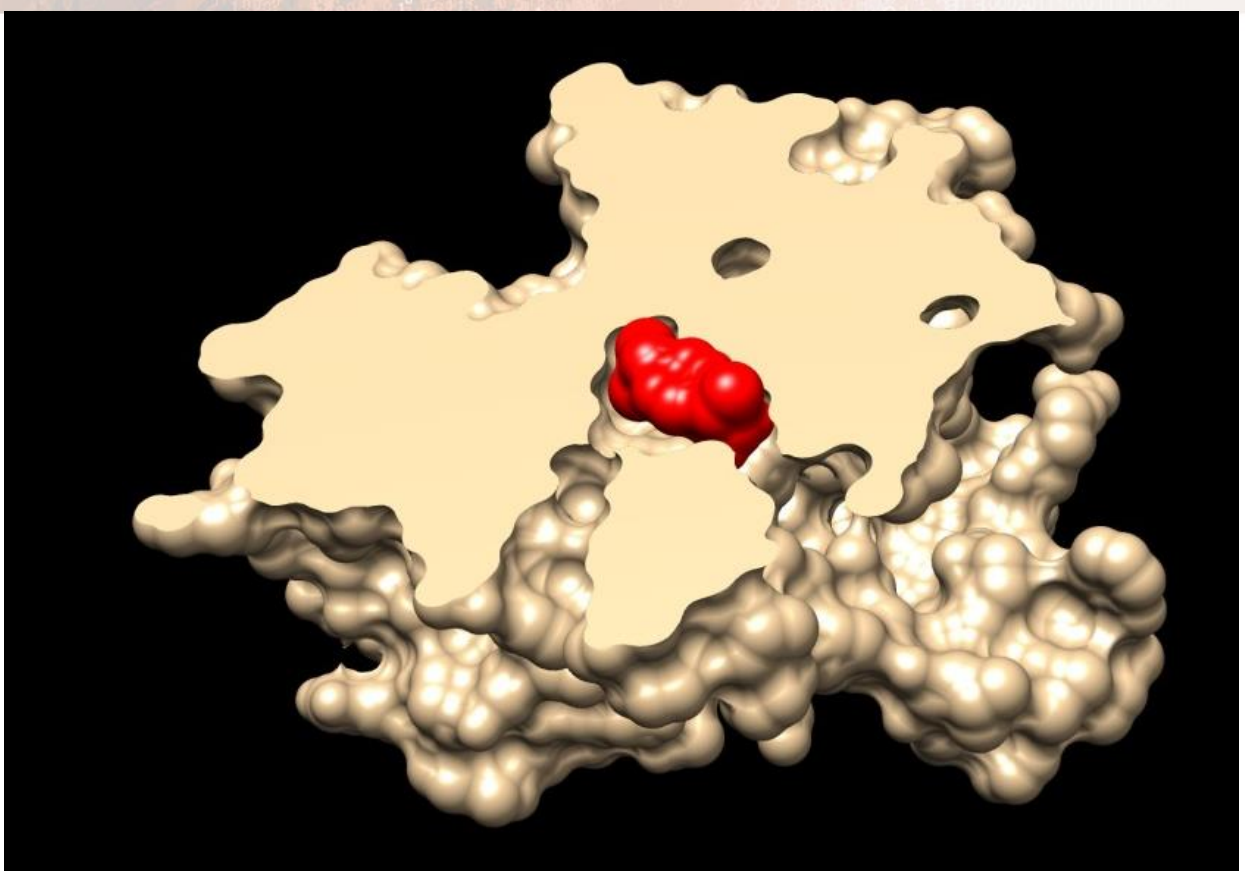
Lifestyle Drugs

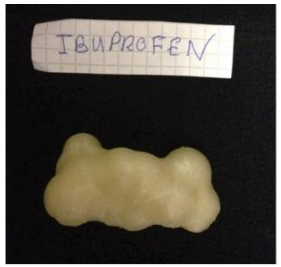
7 8

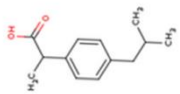
Pharmaceutical scientists have developed a number of drugs that help people modify their own health and bodily function. The drug orlistat (**Xenical** or **alli**) blocks the action of pancreatic lipase, and thereby reduces the amount of fat that is absorbed from food. Atorvastatin (**Lipitor**) and simvastatin (**Zocor**) lower cholesterol by blocking the action of HMG-CoA reductase, an enzyme involved in the synthesis of cholesterol. These drugs can be used, along with changes in diet and exercise, to help lose weight, regulate cholesterol levels, and control heart disease.

7. Pancreatic lipase with an alkyl phosphonate inhibitor (1lpb). The drug orlistat shown on the right is similar to the inhibitor found in the crystal structure.
8. HMG-CoA reductase with atorvastatin (1hwk)

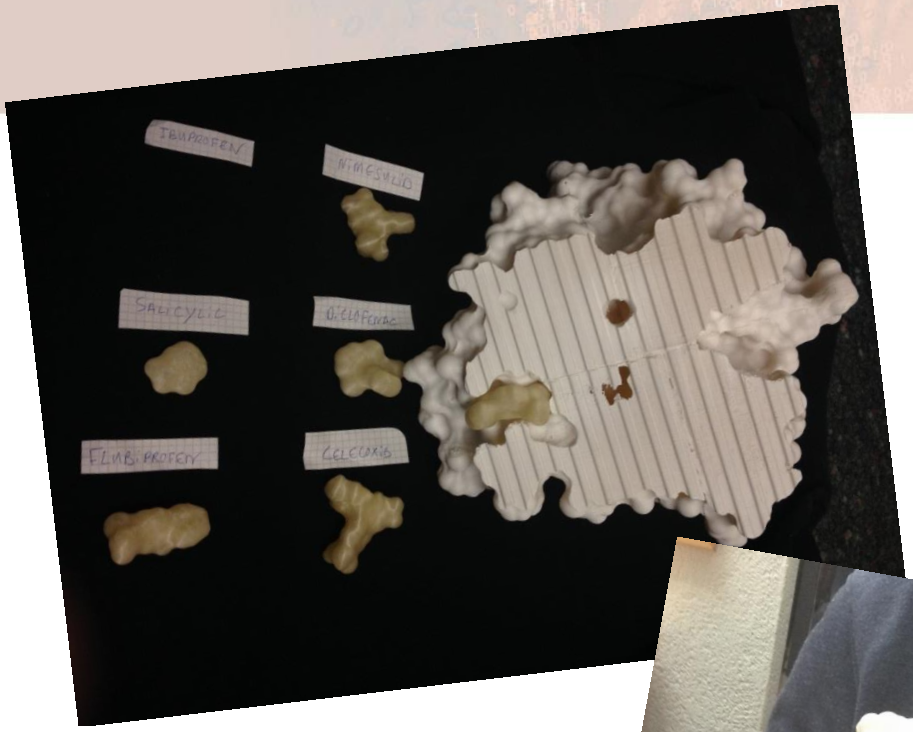
http://cdn.rcsb.org/pdb101/learn/resources/how-do-drugs-work-poster-high-res.pdf

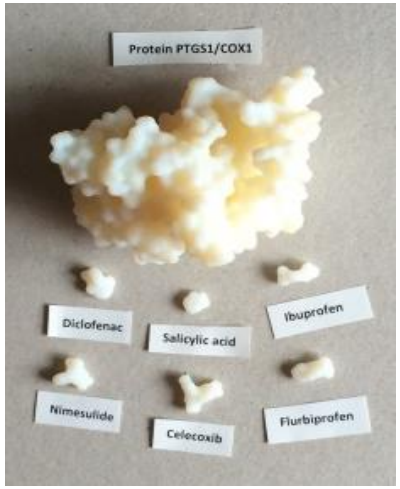






ibuprofen and COX1
(pain treatment)


IBUPROFEN
NIMESULID
SALICYLIC
DICLOFENAC
FLURBIPROFEN
CELECOXIB


Protein PTGS1/COX1
Diclofenac
Salicylic acid
Ibuprofen
Nimesulide
Celecoxib
Flurbiprofen

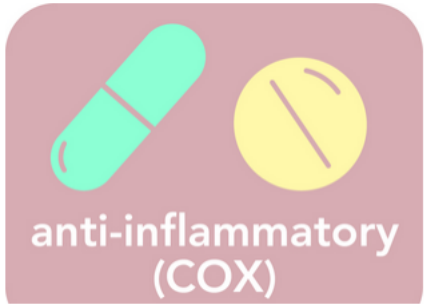
Drug Design workshop

www.drug-design-workshop.ch

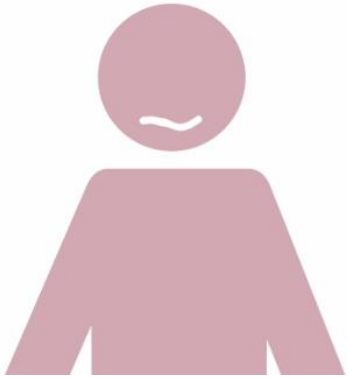
www.drug-design-workshop.ch

COX workshop

Have a try to design a drug...



someone
falls ill...



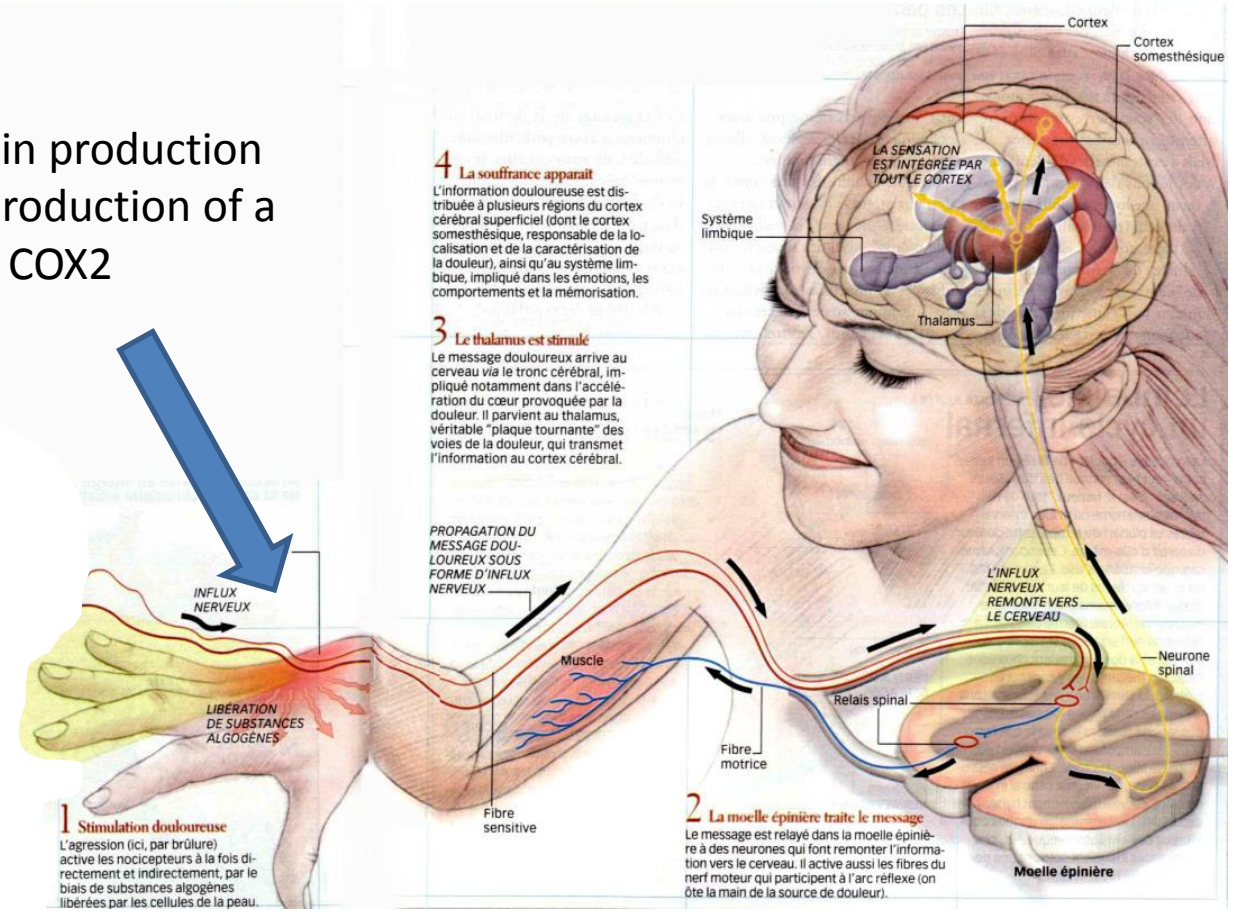
- ❑ Protein's active site is **altered** (mutated)
- ❑ there is **too little** of a protein
- ➡ ❑ there is **too much** of a protein

anti-inflammatory drugs

More often than not, pain is the result of excessive prostaglandin production due to the local high production of a protein called COX2*

* Also called **Prostaglandin G/H synthase 2 (PTGS2)**

excessive prostaglandin production due to the local high production of a protein called COX2

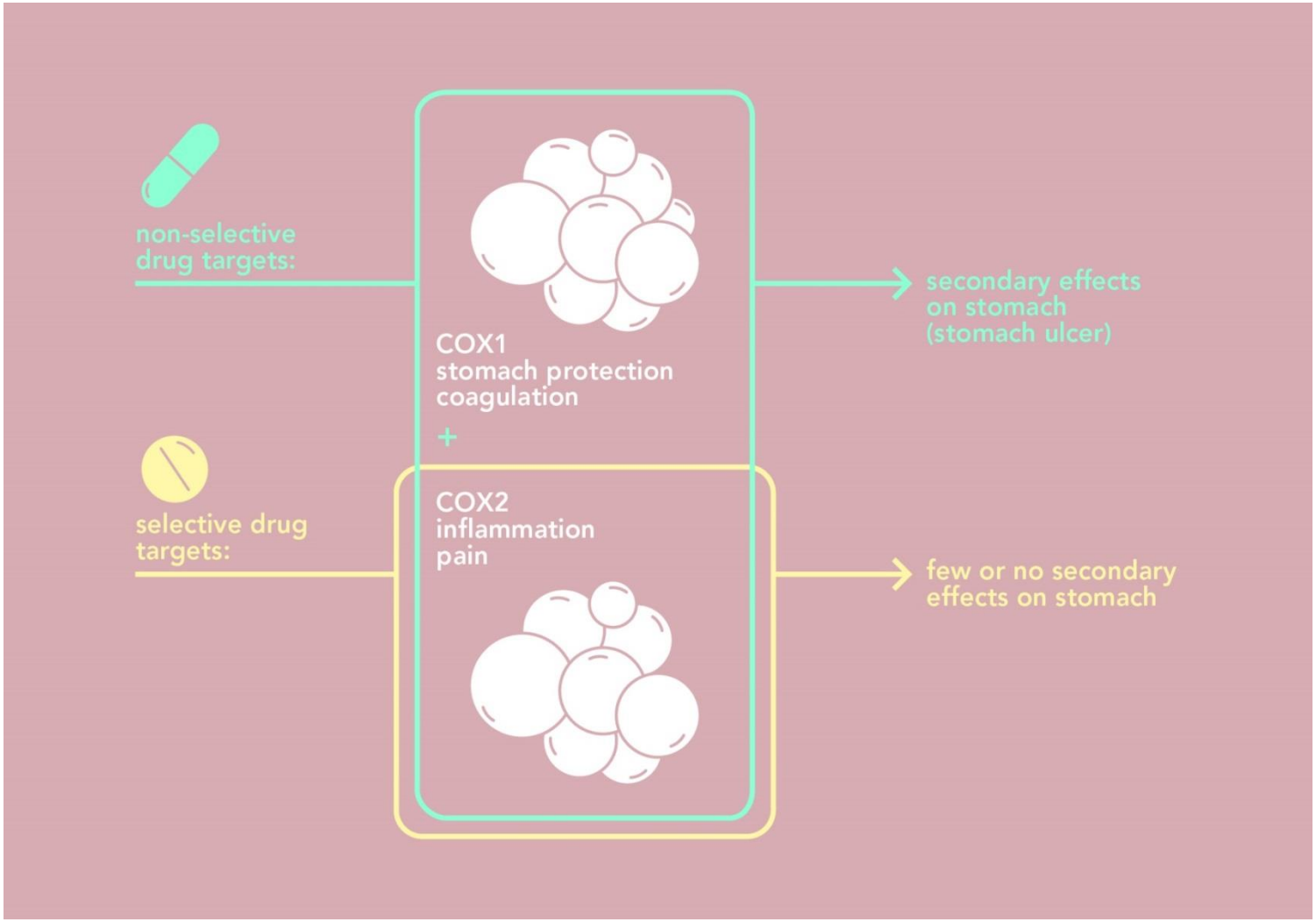


The challenge COX1 and COX 2

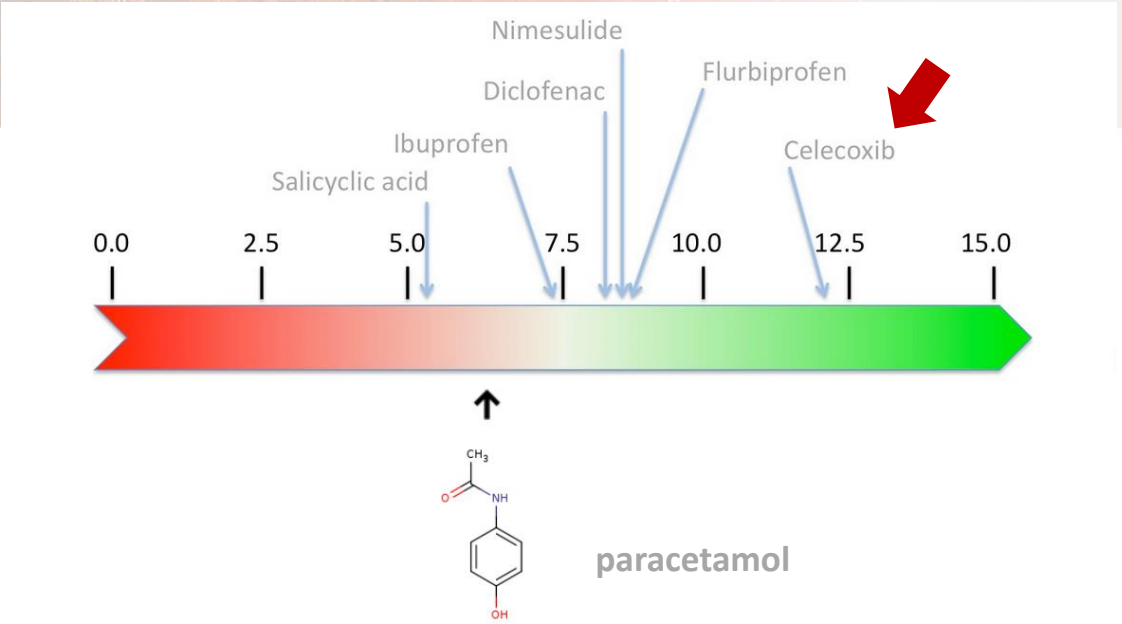
COX1 is expressed constitutively and produces prostaglandins to fine-tune **physiological processes**

COX2 is inducible and typically produces prostaglandins that mediate responses to **physiological stresses** such as infection and inflammation

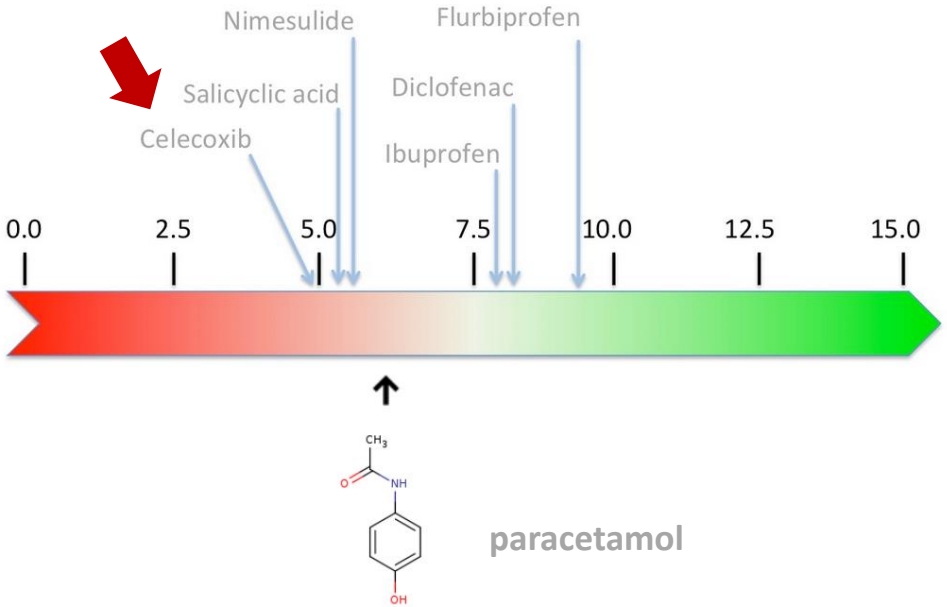
The challenge COX1 and COX 2



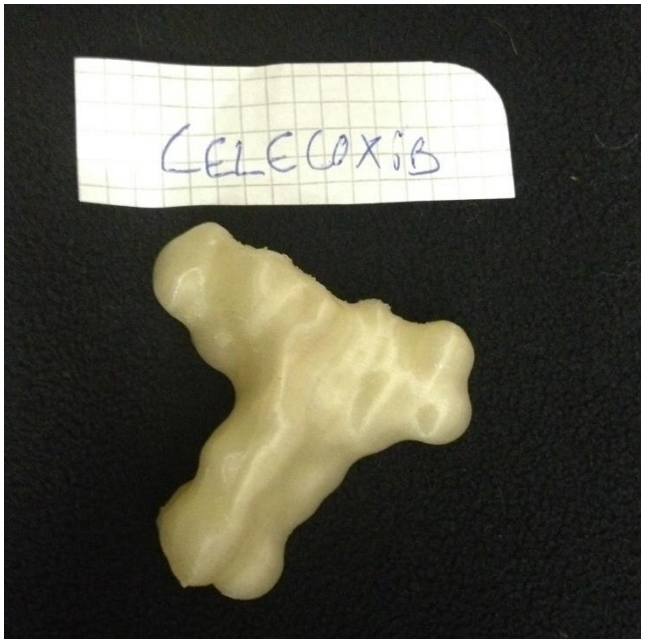
Affinity for COX2

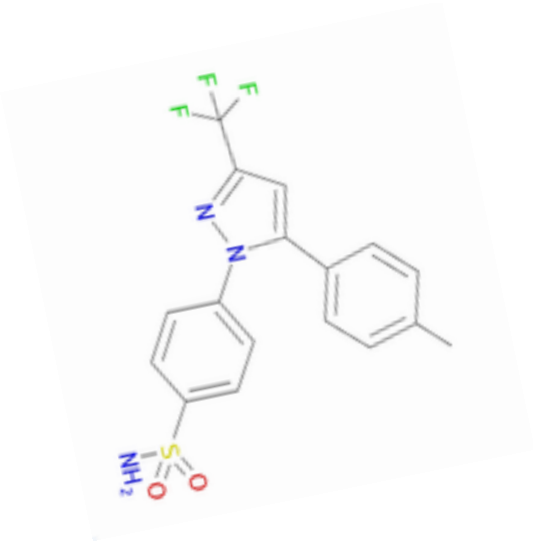


Affinity for COX1



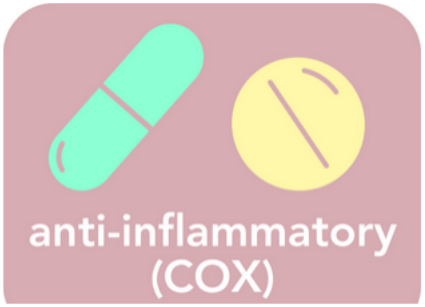
Find a molecule with a higher affinity for COX2 compared to COX1 (i.e. Celecoxib)…



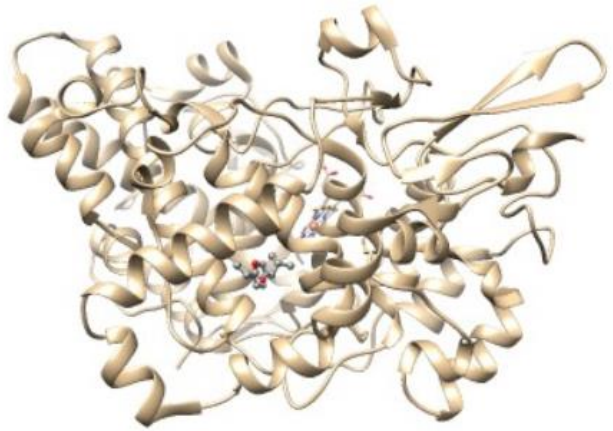


Celecoxib is a selective inhibitor of COX2 and belongs to the « coxibs » family, which was put on the market in 2000. It is used to treat rheumatic diseases (arthrosis and rheumatoid polyarthritis). It does not inhibit COX1 and has no side effects on the stomach. As a precaution, celecoxib is not a first-choice treatment because other coxibs family molecules seem to be toxic for the heart.

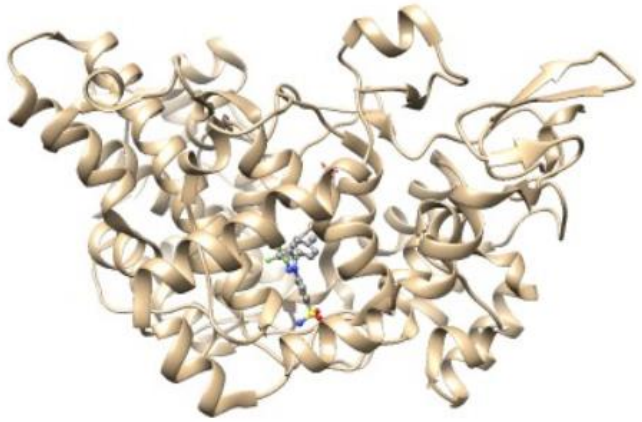
Have a try to design a drug...



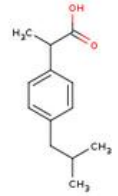
COX1



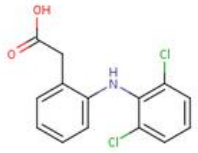
COX2



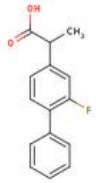
Ibuprofen



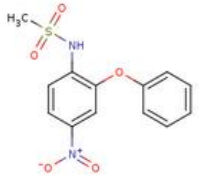
Diclofenac



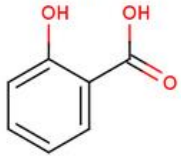
Flurbiprofen



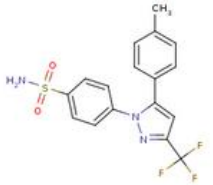
Nimesulide



Salicylic acid



Celecoxib

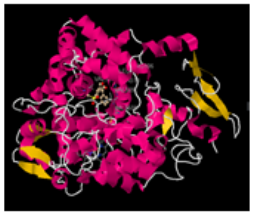


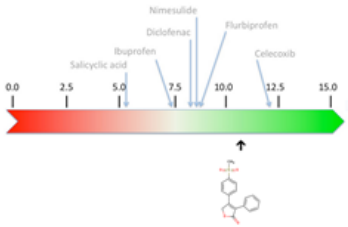
Dessinez votre propre molécule

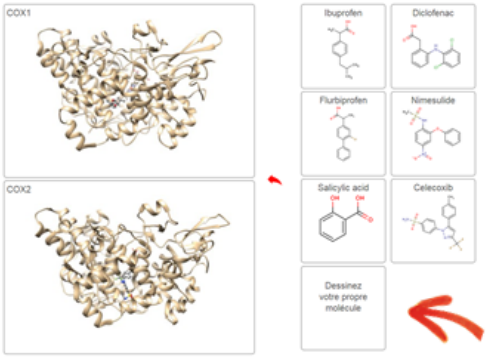
www.drug-design-workshop.ch/cox.php

an iterative approach - 4 steps

2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)





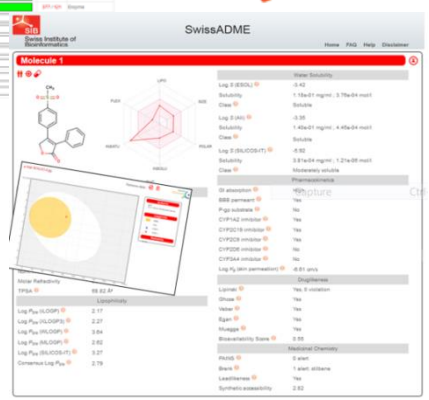


a molecular docking simulation (AutoDock Vina) predicts the binding mode and compares molecule-protein affinity with existing drugs (score)

1. Draw a new molecule

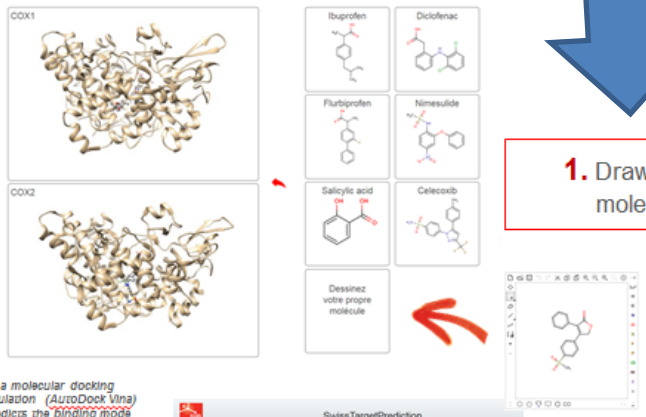
iterative approach

3. predict possible targets (SwissTargetPrediction)

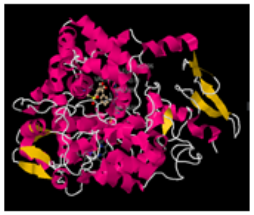


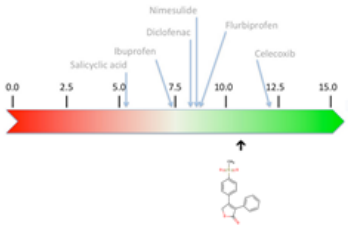
4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

1. Draw a new molecule



2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)

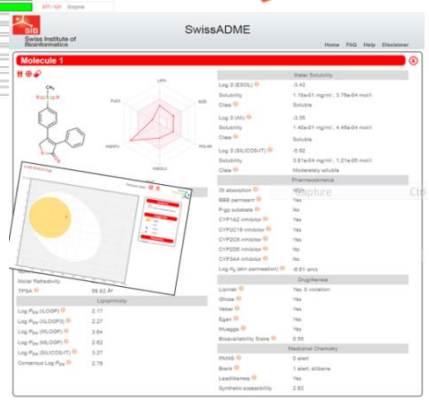




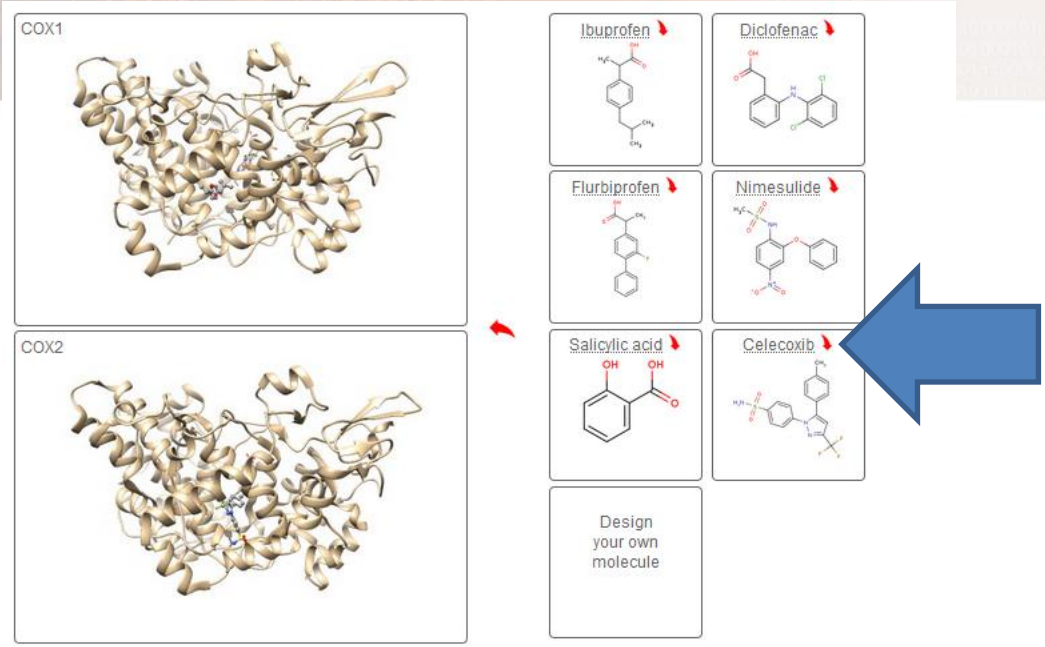
a molecular docking simulation (AutoDock Vina) predicts the binding mode and compares molecule-protein affinity with existing drugs (score)

Iterative approach

3. predict possible targets (SwissTargetPrediction)

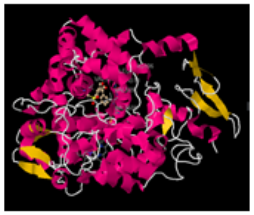


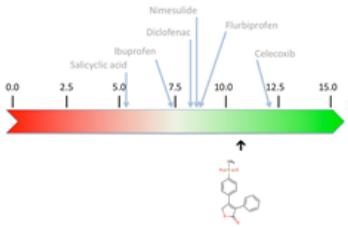
4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

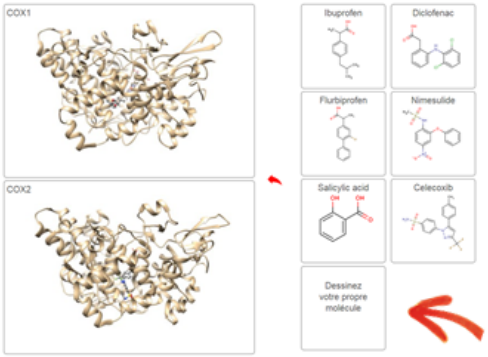


draw a new molecule
or
'improve' an existing molecule

2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)





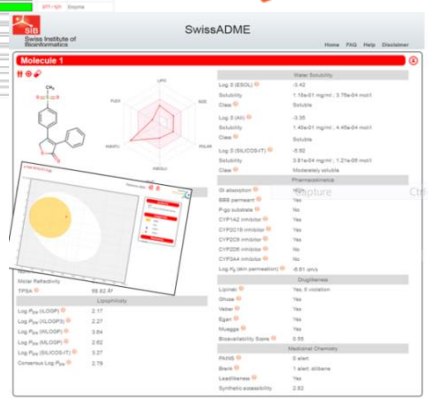


a molecular docking simulation (AutoDock Vina) predicts the binding mode and compares molecule-protein affinity with existing drugs (score)

1. Draw a new molecule

Iterative approach

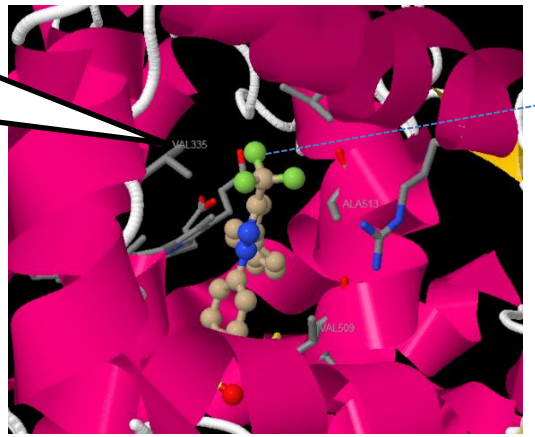
3. predict possible targets (SwissTargetPrediction)

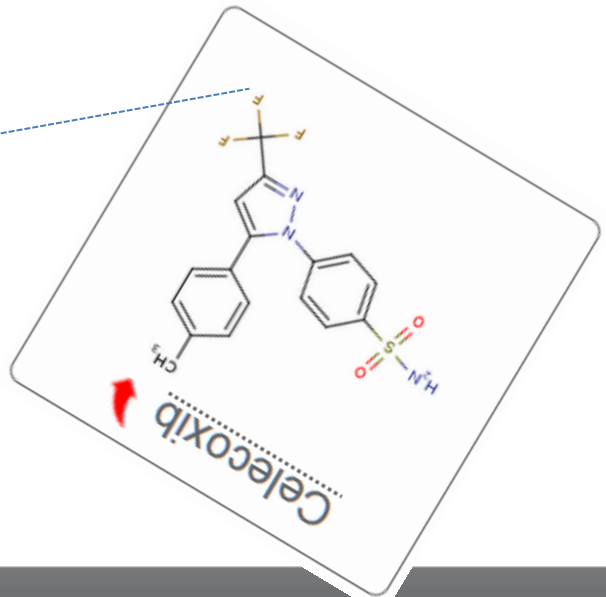


4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

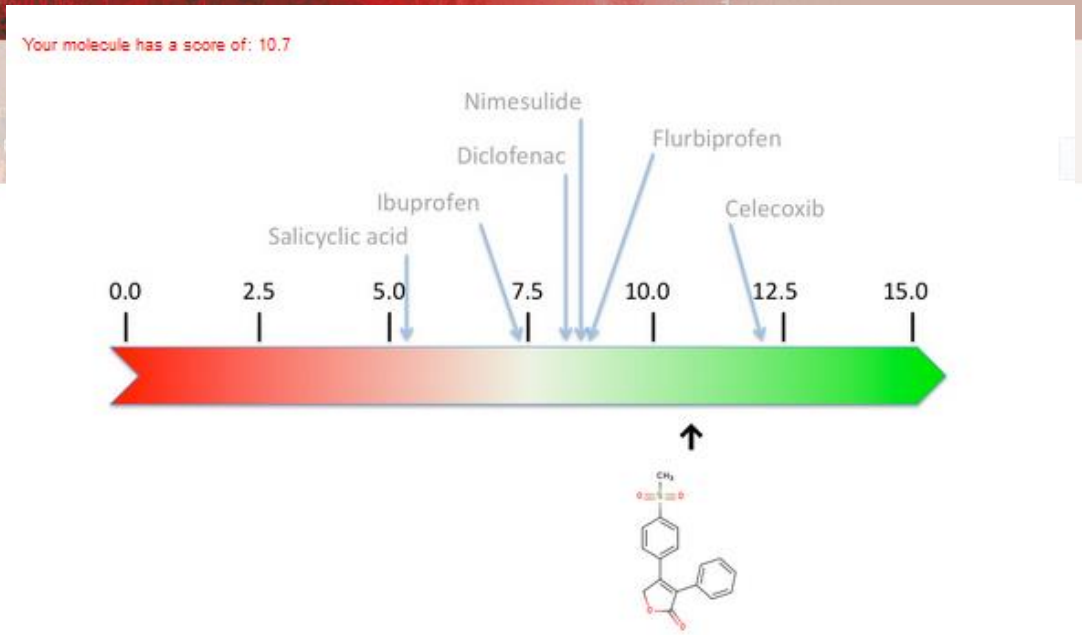
- ✓ **Docking computation is launched on a remote computer** thanks to the Internet
- ✓ Tens of thousands of different geometries and positions of the ligand in the protein are evaluated!
- ✓ The most probable position is shown in 3D (the amino acids interacting with the molecule are named) and an affinity score is provided

Amino acid at position 335 is a Valine, which interacts with Celecoxib

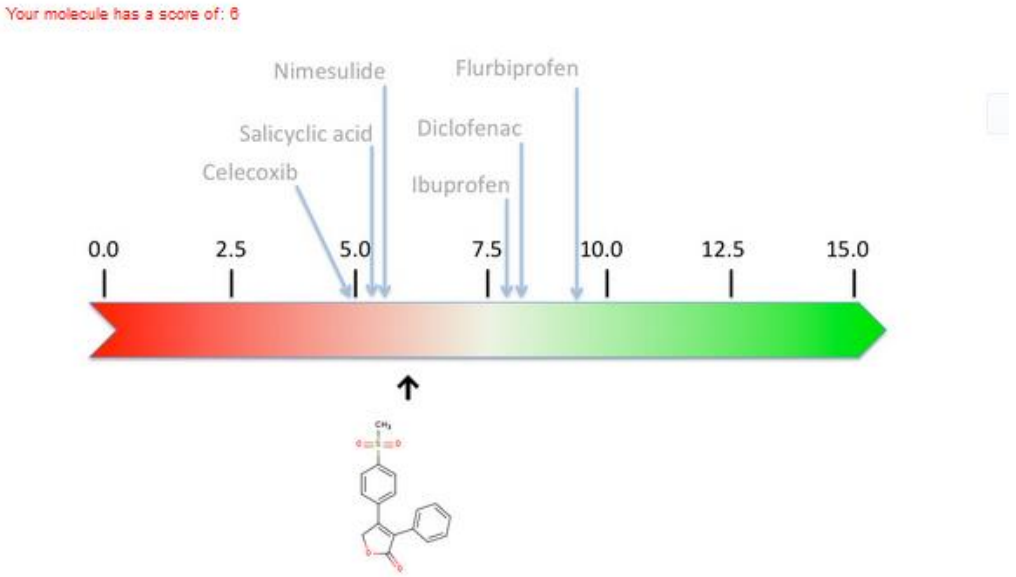




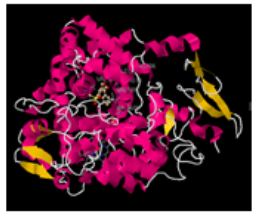
Docking and scores - COX2

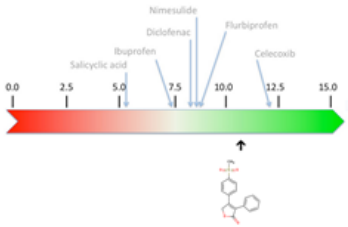


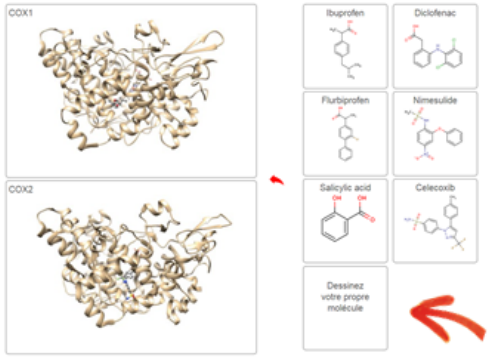
Docking and scores – COX1



2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)





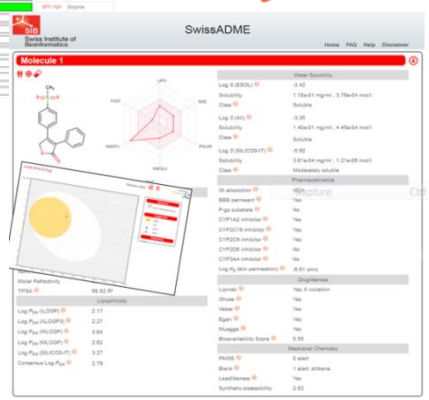


a molecular docking simulation (AutoDock Vina) predicts the binding mode and compares molecule-protein affinity with existing drugs (score)

1. Draw a new molecule

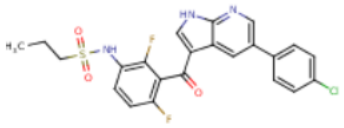
Iterative approach

3. predict possible targets (SwissTargetPrediction)



4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

Query Molecule

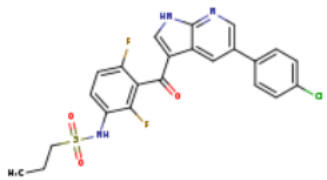
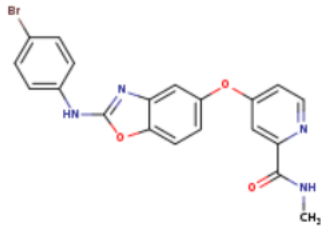
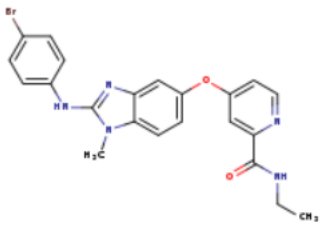
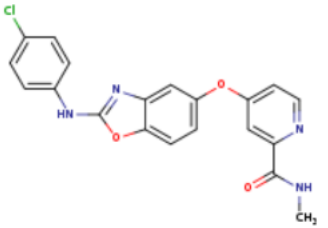


SwissTarget Prediction compares your molecules (at the 2D and 3D structure levels) with 280,000 other molecules known to be active on 2,000 proteins.

'similar molecules are prone to exhibiting similar biological activities'

Retrieve data:

Ligands of RAF proto-oncogene serine/threonine-protein kinase (RAF1) with 3D-based similarity ;

CHEMBL1229517	CHEMBL1778393	CHEMBL470359	CHEMBL1162958
Similarity: 0.992	Similarity: 0.814	Similarity: 0.792	Similarity: 0.788
			

See later: how to compare molecules (molecular fingerprint)

Aspirin

SIB Swiss Institute of Bioinformatics

SwissTargetPrediction

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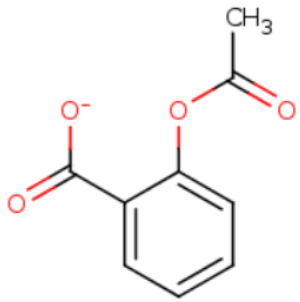
List of predicted targets

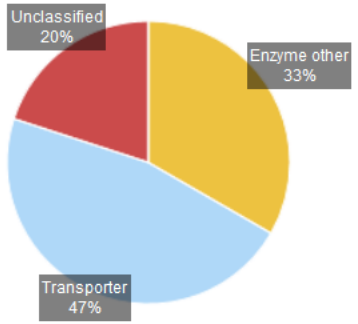
These targets have been predicted using the method described in:

Gfeller D., Michielin O. & Zoete V. Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Query Molecule



General Target Classes



Retrieve data:

Target	Common name	Uniprot ID	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Prostaglandin G/H synthase 1	PTGS1	P23219	CHEMBL221		1 / 4	Enzyme
Prostaglandin G/H synthase 2	PTGS2	P35354	CHEMBL230		1 / 4	Enzyme
Sodium-dependent noradrenaline transporter	SLC6A2	P23975	CHEMBL222		2 / 3	Transporter
Sodium-dependent serotonin transporter	SLC6A4	P31645	CHEMBL228		2 / 2	Transporter
Sodium- and chloride-dependent glycine transporter 1 *(by homology)*	SLC6A9	P48067	CHEMBL2337		2 / 4	Transporter
Sodium-dependent dopamine transporter	SLC6A3	Q01959	CHEMBL238		2 / 3	Transporter

Swiss Institute of Bioinformatics

SwissTargetPrediction

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List of predicted targets

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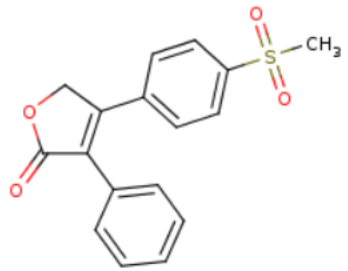
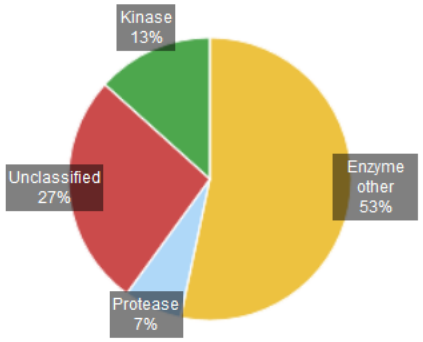
Query Molecule



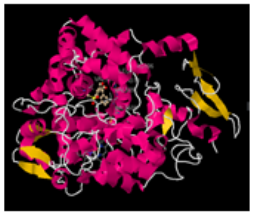
General Target Classes

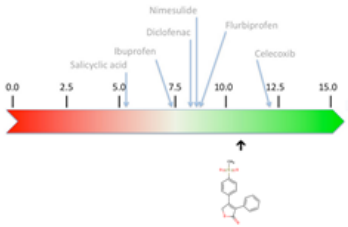


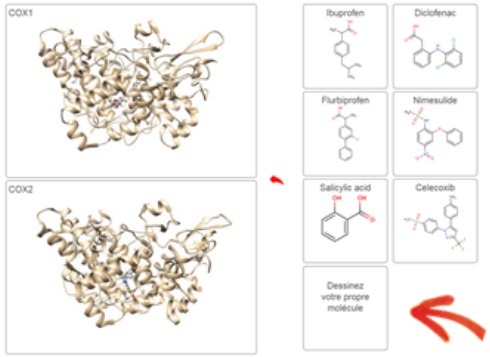
Retrieve data:

Target	Common name	Uniprot ID	ChEMBL ID	Probability*	# sim. cmpds (3D / 2D)	Target Class
Prostaglandin G/H synthase 1	PTGS1	P23219	CHEMBL221		377 / 121	Enzyme
Lipid-phosphate phosphatase	EPHX2	P34913	CHEMBL2409		95 / 1	Serine Protease
Prostaglandin G/H synthase 2	PTGS2	P35354	CHEMBL230		377 / 121	Enzyme
Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978		263 / 3	Enzyme
Muscleblind-like protein 1	MBNL1	Q9NR56	CHEMBL1293317		907 / 3	Unclassified
Muscleblind-like protein 2 *(by homology)*	MBNL2	Q5VZF2			907 / 3	Unclassified
Muscleblind-like protein 3 *(by homology)*	MBNL3	Q9NUK0			907 / 3	Unclassified

2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)





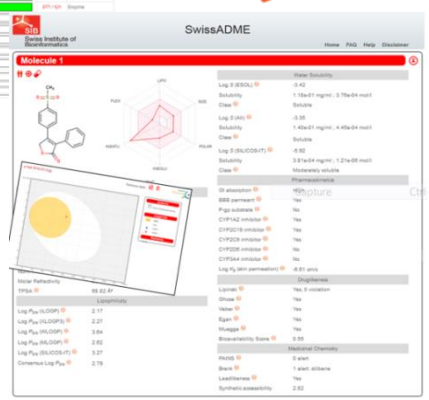


a molecular docking simulation (AutoDock Vina) predicts the binding mode and compares molecule-protein affinity with existing drugs (score)

1. Draw a new molecule

Iterative approach

3. predict possible targets (SwissTargetPrediction)



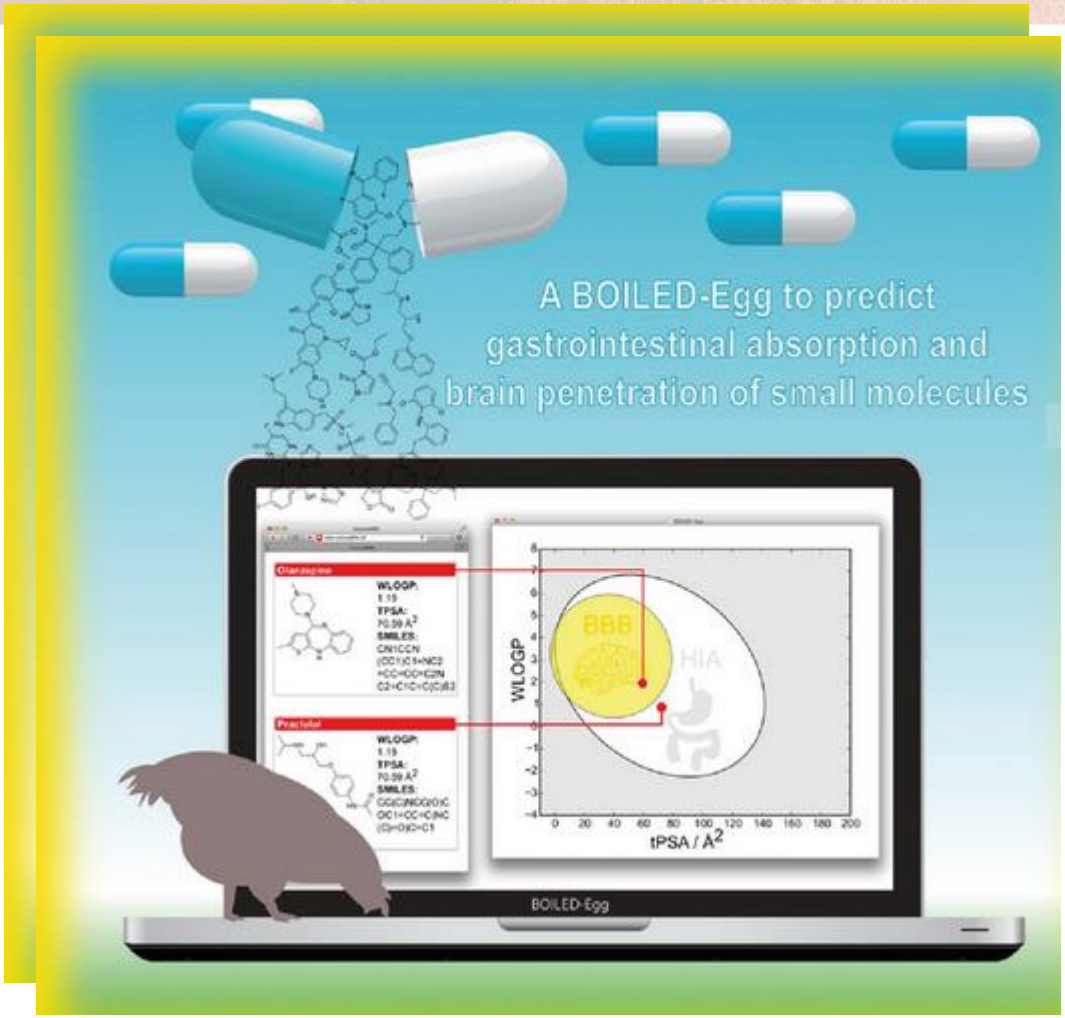
4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

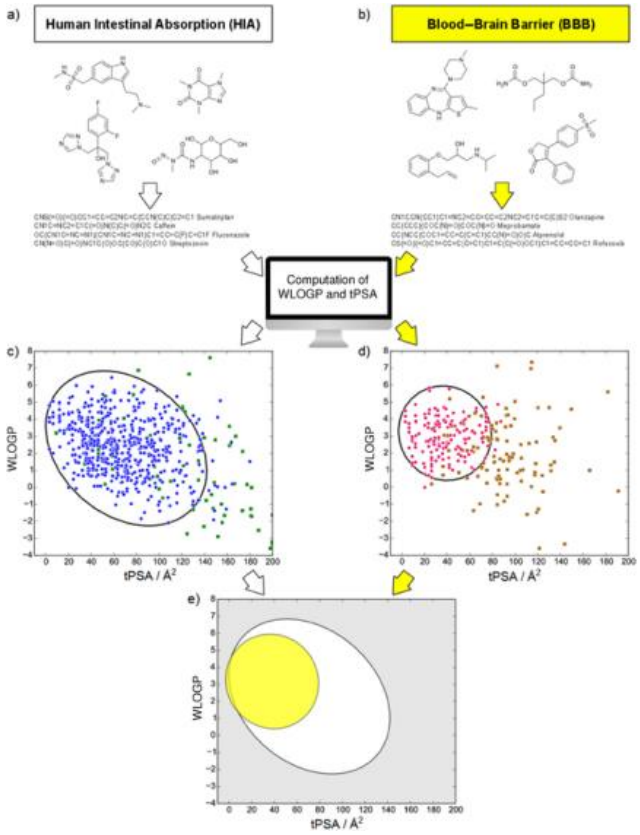
www.SwissADME.ch

Predict important properties of the molecule
in order to evaluate its potential of
becoming a drug to be taken orally

Absorption, Distribution, Metabolism, Excretion

www.SwissADME.ch

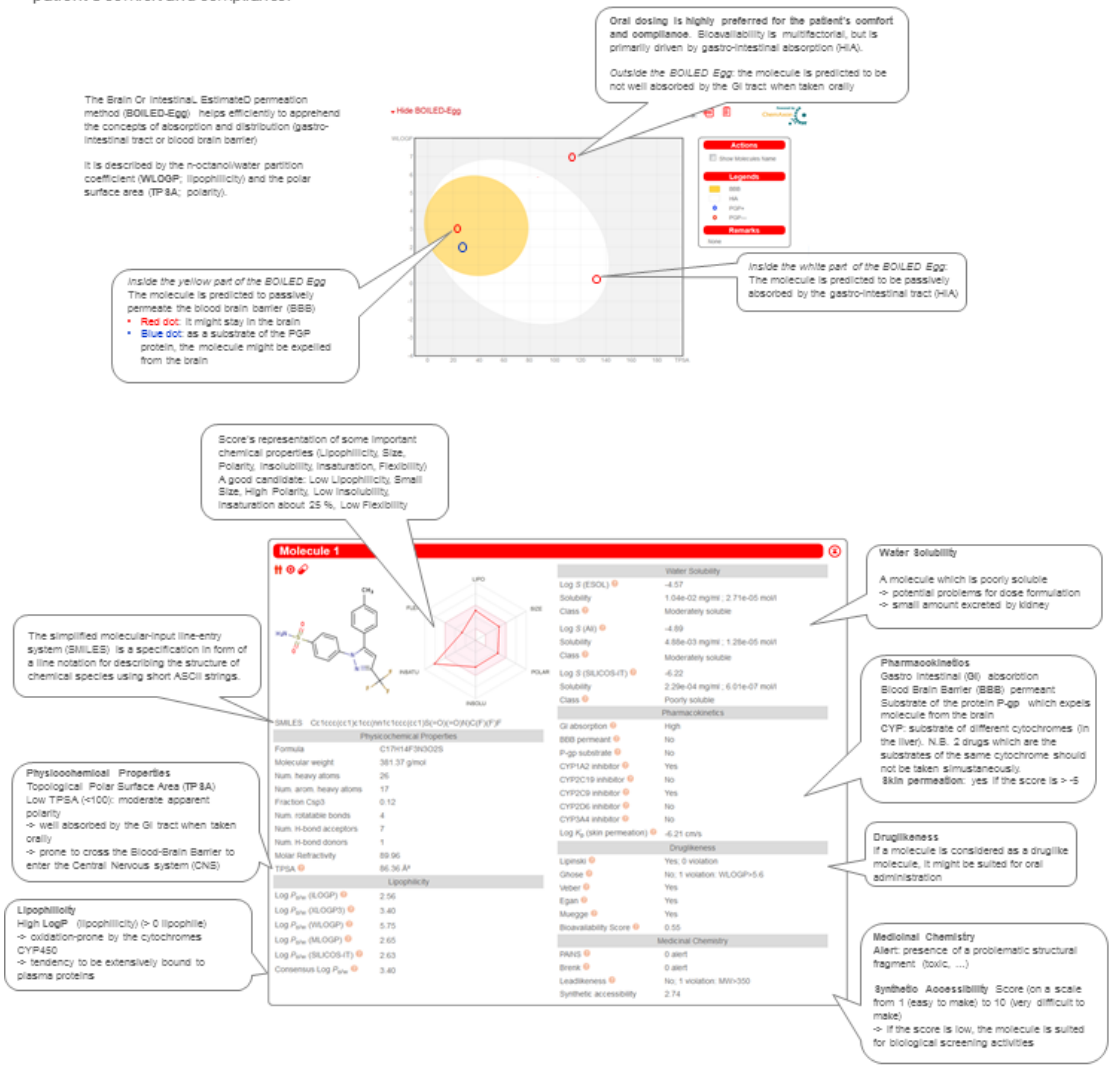




PMID:27218427

evaluate the potential of a molecule of becoming a drug
absorption, distribution, metabolism and excretion (SwissADME)

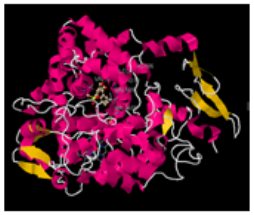
For designing, discovering or developing a therapeutically relevant molecule, potency and selectivity to the target protein is only one side of the problem. Indeed **absorption, distribution, metabolism, and excretion (ADME)** must be optimized for the molecule to reach its tissue/target in sufficient amount and to **reduce off-target and unwanted effects**. Aim: design a drug which can be taken orally, for the patient's comfort and compliance.

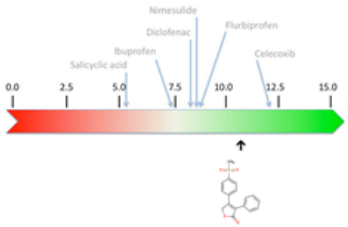


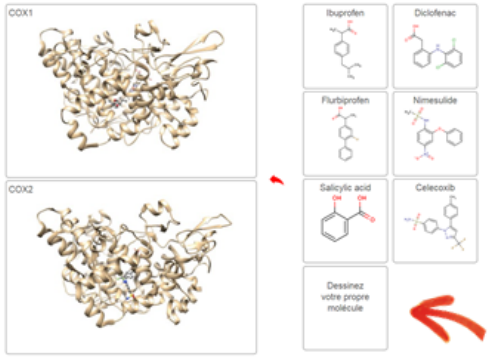
http://education.expasy.org/cours/Outreach/SCS2016/SwissADME_Output_explanation.pdf

Iterative process…..you may have to go back to the beginning

2. visualize its binding mode and estimate its affinity for the target protein (compare the score with existing drugs)







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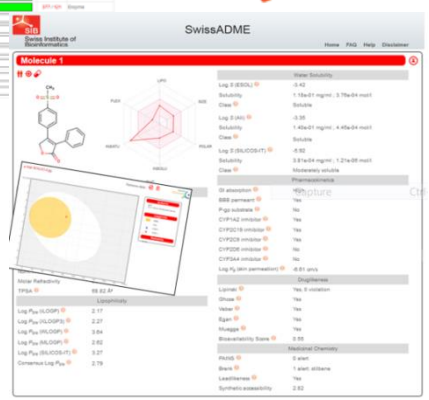
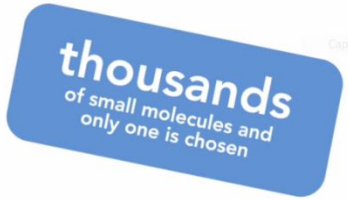
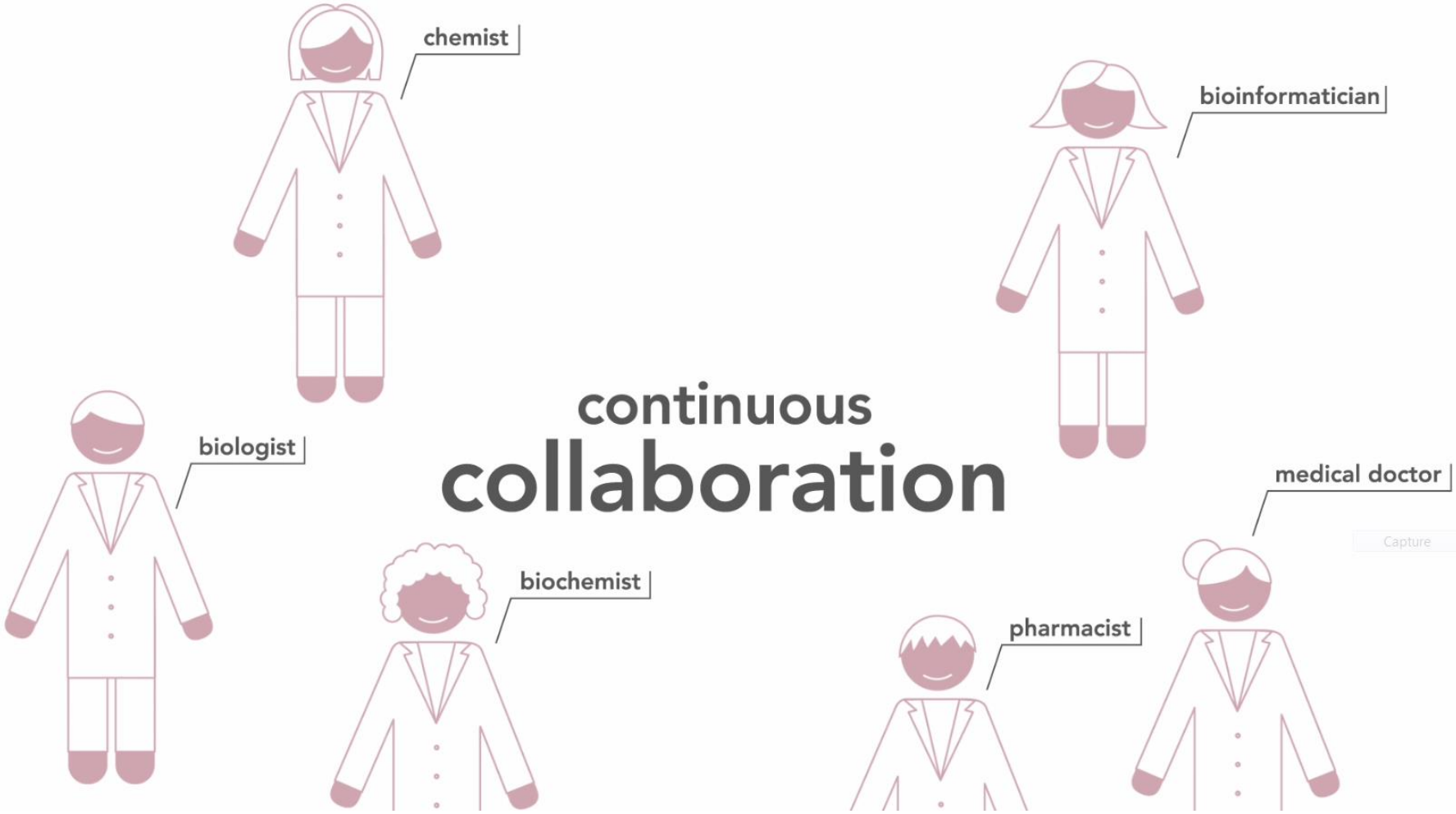


4. predict important properties of the molecule in order to evaluate its potential of becoming a drug (SwissADME)

- ✓ 10^{60} molecules (potential)
- ✓ 35 millions of existing molecules (http://zinc.docking.org/)
- ✓ 2,000 molecules are drugs approved by the FDA
- ✓ 280,000 molecules are known to be active on 2,000 target proteins (SwissTarget)

- ✓ More than 10 years to find a new drug….including at least 2 years of '*in silico*' analysis…



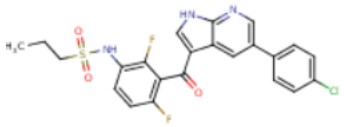

chemist
bioinformatician
biologist
continuous
collaboration
medical doctor
biochemist
pharmacist

BONUS

How to search for molecule similarity?

Molecular Fingerprint

Query Molecule

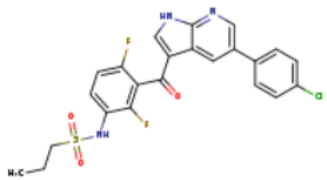
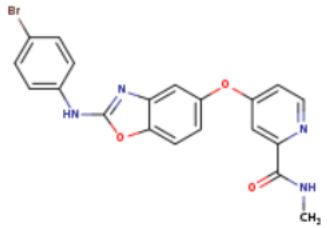
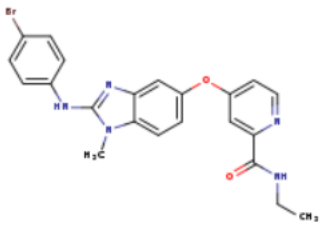
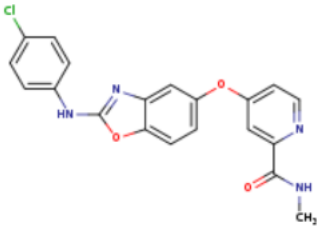


SwissTarget Prediction compares your molecules (at the 2D and 3D structure levels) with 280,000 molecules known to be active on 2,000 proteins.

'similar molecules are prone to exhibiting similar biological activities'

Retrieve data:

Ligands of RAF proto-oncogene serine/threonine-protein kinase (RAF1) with 3D-based similarity ;

CHEMBL1229517	CHEMBL1778393	CHEMBL470359	CHEMBL1162958
Similarity: 0.992	Similarity: 0.814	Similarity: 0.792	Similarity: 0.788
			

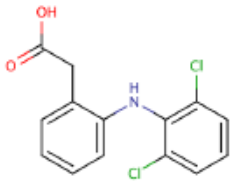
See later: how to compare molecules (molecular fingerprint)

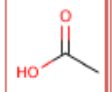
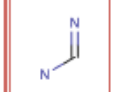
How to compare molecules -> Molecular fingerprints

Molecular fingerprints is a technique that compares molecules by describing a chemical structure as a vector of 0 and 1 (a bit string)

(i) establish a short list of molecular features (e.g. chemical functions or combination of bound atoms);
(ii) for each molecule, either put a 1 in the correct position of a vector if a given feature is found in the molecule, or a 0 .
(iii) Create the fingerprint vectors for several molecules, and then calculate the Tanimoto coefficients
(iv) Comparison of Tanimoto coefficient values and known biological activities will then illustrate the assumption that **similar molecules are prone to having similar biological activities**

Diclofenac

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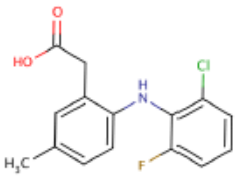
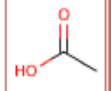
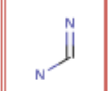
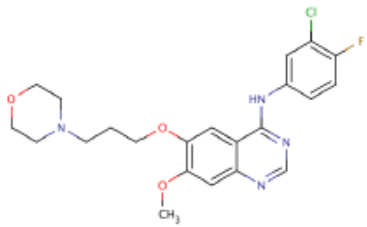
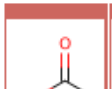
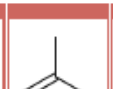
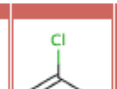
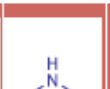
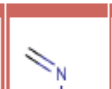
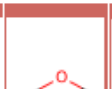
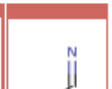
Lumiracoxib



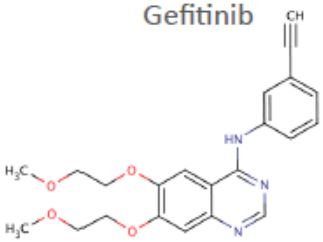
							

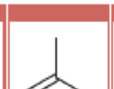
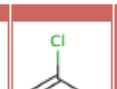
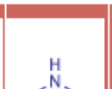
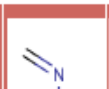
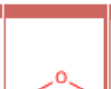
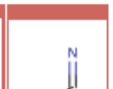
Erlotinib



Gefitinib



Calcul de similarité
Coefficient de Tanimoto

$$T = \frac{M_{11}}{M_{11} + M_{10} + M_{01}}$$

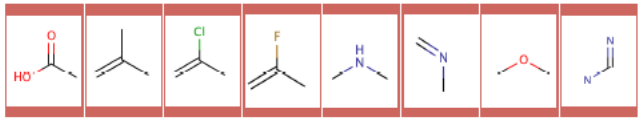
M_{11}: nombre de cases où l'on trouve 1 à la fois chez A et chez B
M_{10}: nombre de cases où l'on trouve 1 chez A et 0 chez B
M_{01}: nombre de cases où l'on trouve 0 chez A et 1 chez B

$0 \leq T \leq 1$

T = 0 : molécules totalement différentes
T = 1 : molécules identiques

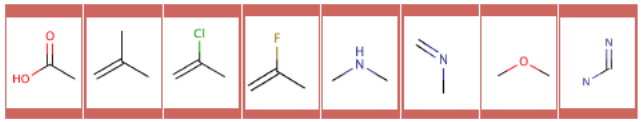
Example:

A



1	1	1	0	1	0	0	0

B



1	1	0	1	1	0	0	1

$M_{11} = 3$
$M_{10} = 1$
$M_{01} = 2$

$$T = \frac{3}{3+1+2} = 0.5$$

Calcul de similarité
Coefficient de Tanimoto

$$T = \frac{M_{11}}{M_{11} + M_{10} + M_{01}}$$

M_{11}: nombre de cases où l'on trouve 1 à la fois chez A et chez B
M_{10}: nombre de cases où l'on trouve 1 chez A et 0 chez B
M_{01}: nombre de cases où l'on trouve 0 chez A et 1 chez B

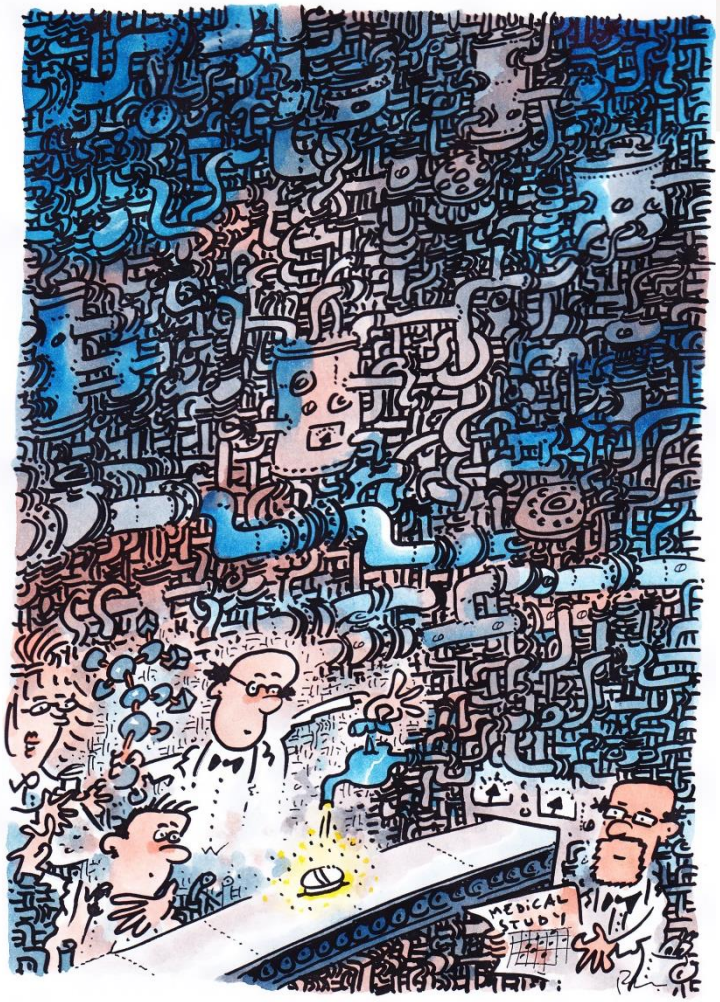
$0 \leq T \leq 1$

T = 0 : molécules totalement différentes
T = 1 : molécules identiques

	Diclofenac	Lumiracoxib	Erlotinib	Gefinitib
Diclofenac				
Lumiracoxib				
Erlotinib				
Gefinitib				

Quelles sont les paires de molécules les plus similaires?

Many thanks !



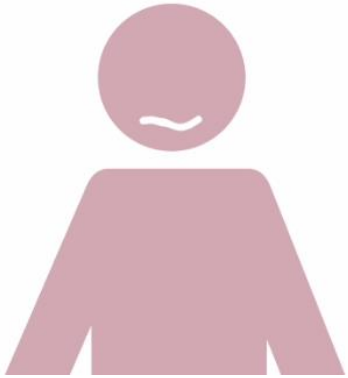
FNSNF
FONDS NATIONAL SUISSE
DE LA RECHERCHE SCIENTIFIQUE

SIB
Swiss Institute of
Bioinformatics

BRAF workshop

Have a try to design a drug...

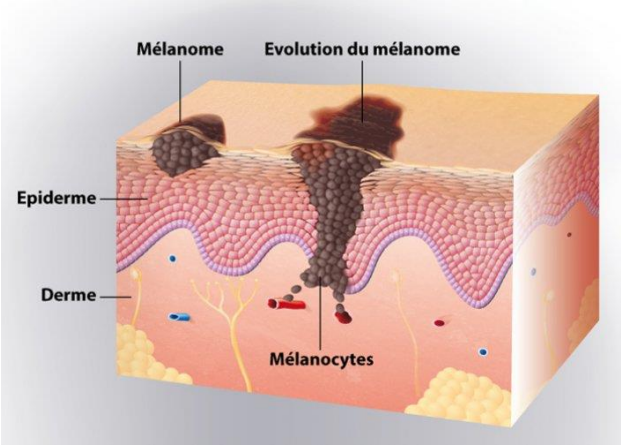
someone
falls ill...



- ❑ Protein's active site is **altered** (mutated)
- ❑ there is **too little** of a protein
- ❑ there is **too much** of a protein

- Melanoma is a skin cancer: it is the result of the abnormal proliferation of cells called ' melanocytes '.

- The tumor cells all derive from a cell ' initiator ' which has acquired certain characteristics enabling it to divide indefinitely (loss of cell division control).



http://ramsaygds.fr/nos-soins-nos-soins/m%C3%A9lanome

Identify the protein involved in cancer (melanoma)

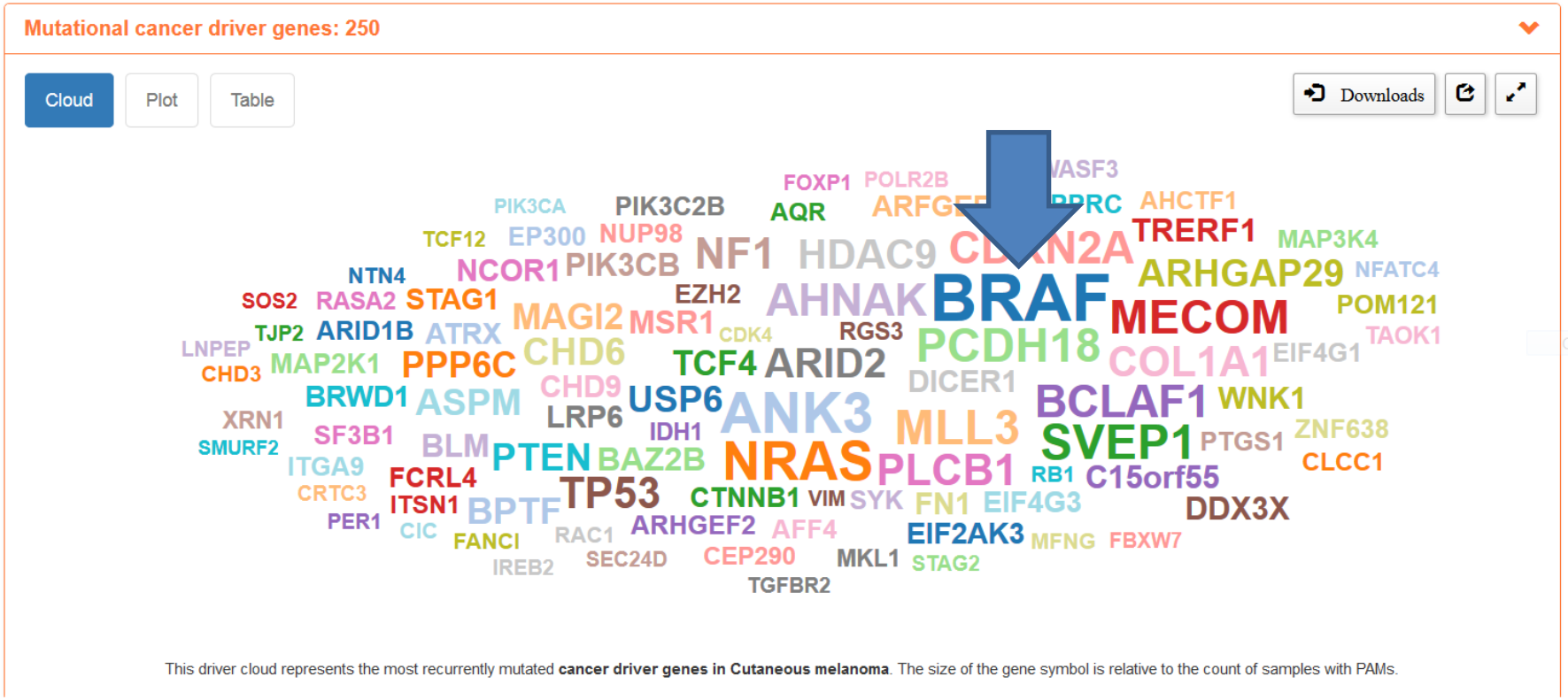
=

Identify the protein to be targeted

Cutaneous melanoma

250 mutational cancer drivers have been detected in 2 *Cutaneous melanoma* (CM) projects. The most mutated drivers are: *BRAF*, *NRAS*, *TP53*, *CDKN2A*, *PTEN*, etc.

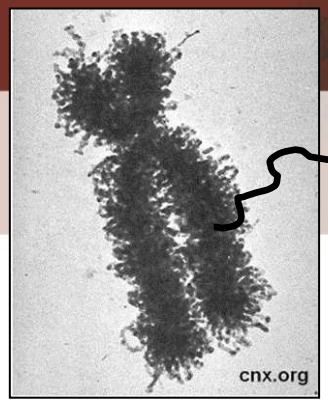
Genome wide association studies (GWAS): associations between single-nucleotide polymorphisms (SNPs) and traits like major human diseases



https://www.intogen.org/search?cancer=CM

Approximately 40-60% of melanomas contain a mutation in the BRAF gene, which leads to the constitutive activation of downstream signaling in the MAP kinase pathway.

In 80-90% of these cases, the activating mutation consists in the substitution of glutamic acid for valine, at amino acid 600 (V600E).



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GCATGGTGGCAGGCTCCTGTCATCCTGGCTACTAGGGAGGCTGAGGCAGGAGAATCACTTGAAC[illegible]GGA
GGCGGAGGTTGTAGTGAGCCAAGACTGTGCCACTGCACTCCAGCCTGGGTGACAGAGTGAGAC[illegible]
CAAAAAATATATATATATAAAATTCATATAATTTATACTTTATATATAATGTATATAATTTAT[illegible]
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BRAF gene
A genetic variation
T -> A
found in ~60 % of the melanoma cells

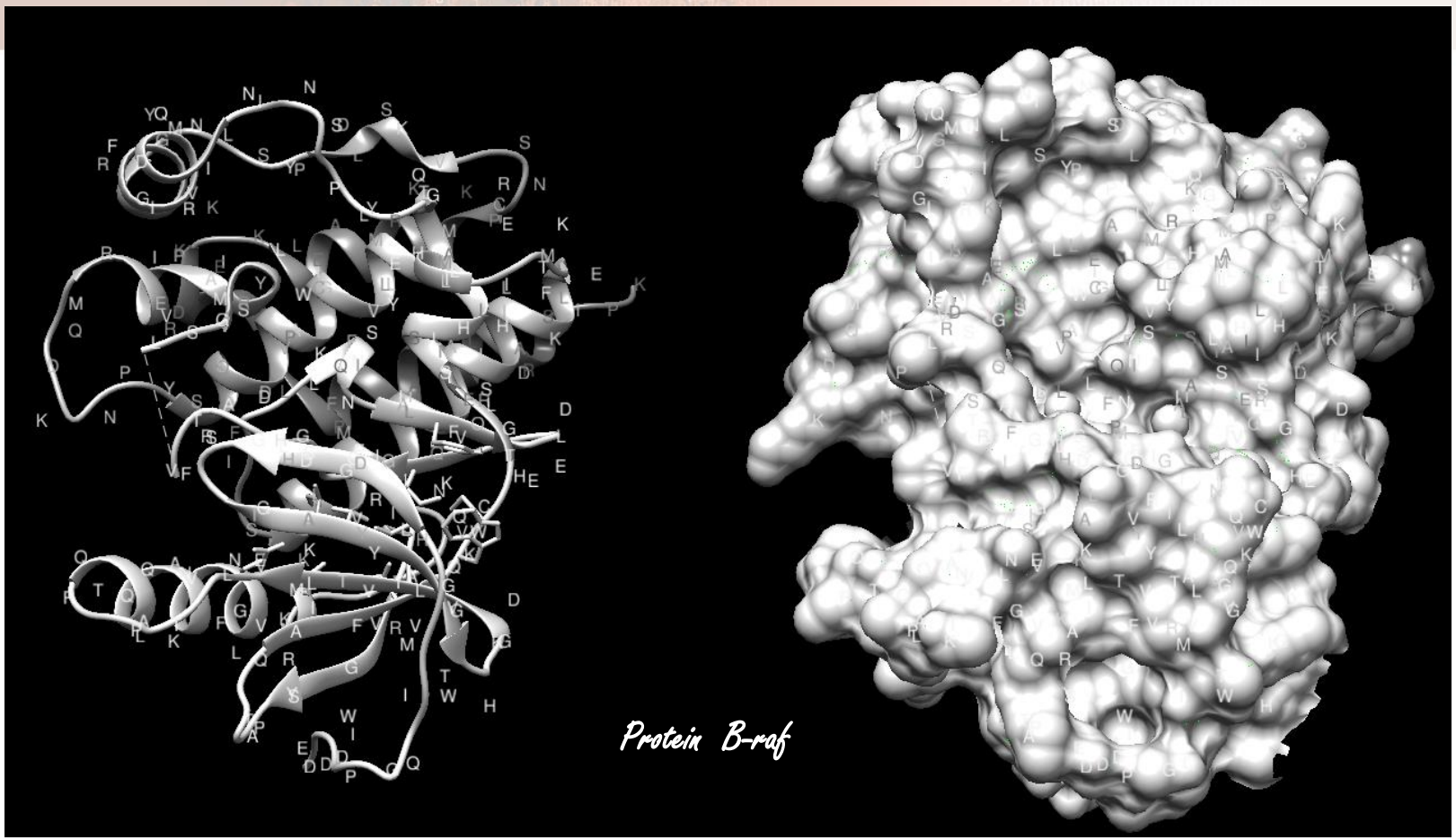
http://www.ncbi.nlm.nih.gov/nuccore/568815591?report=fasta

chromosome 7 DNA sequence

(GenBank database; 159’345’973 bp)

Bioinformatics

SIB
Swiss Institute of Bioinformatics

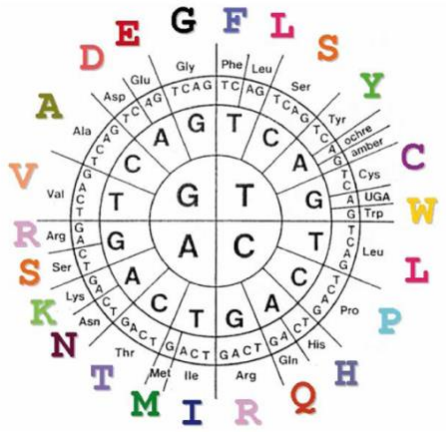


The protein produced by the BRAF gene is called B-raf

The B-raf protein is involved in cell division control

DNA
BRAF gene

…ggt gat ttt ggt cta gct aca gtg aaa tct cga tgg…

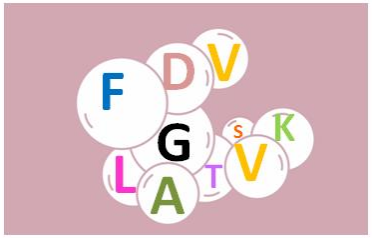


Genetic code

B-Raf protein

… G D F G L A T V K S R W …

One amino acid
There are 20 different amino acids

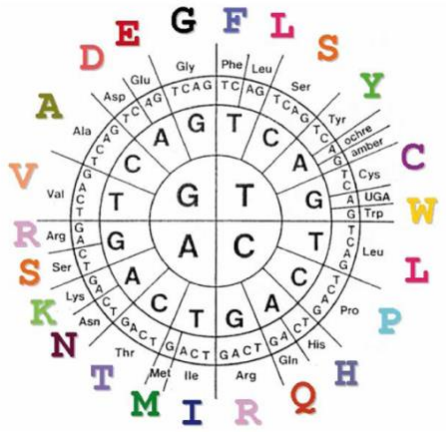


DNA
BRAF gene

Mutation

somatic
(in 40-60 % of the melanoma cells)

…ggt gat ttt ggt cta gct aca gag aaa tct cga tgg…

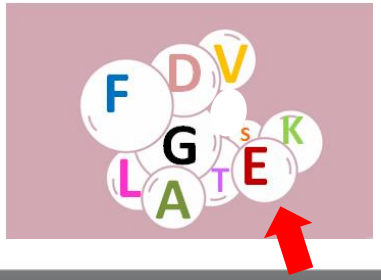


Genetic code

B-Raf protein

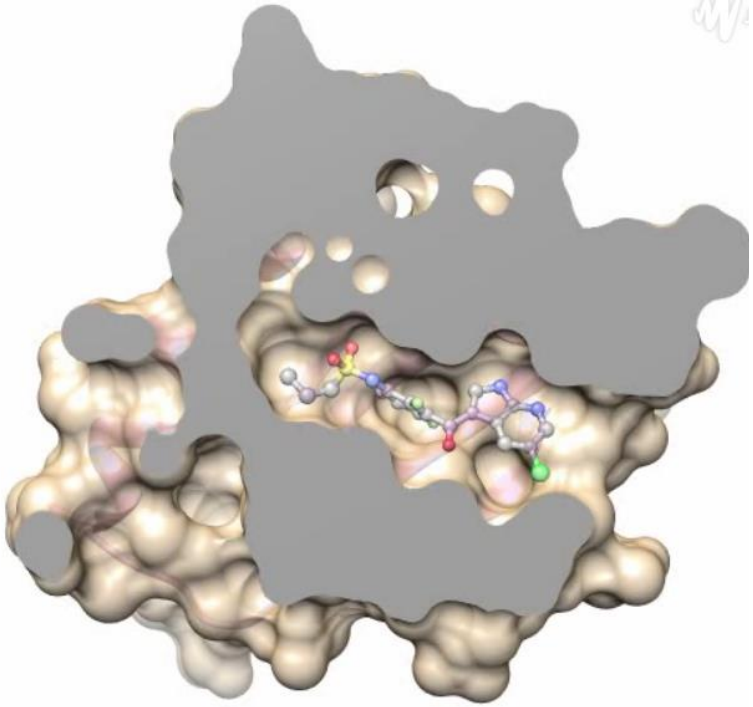
… G D F G L A T E K S R W …

Mutation

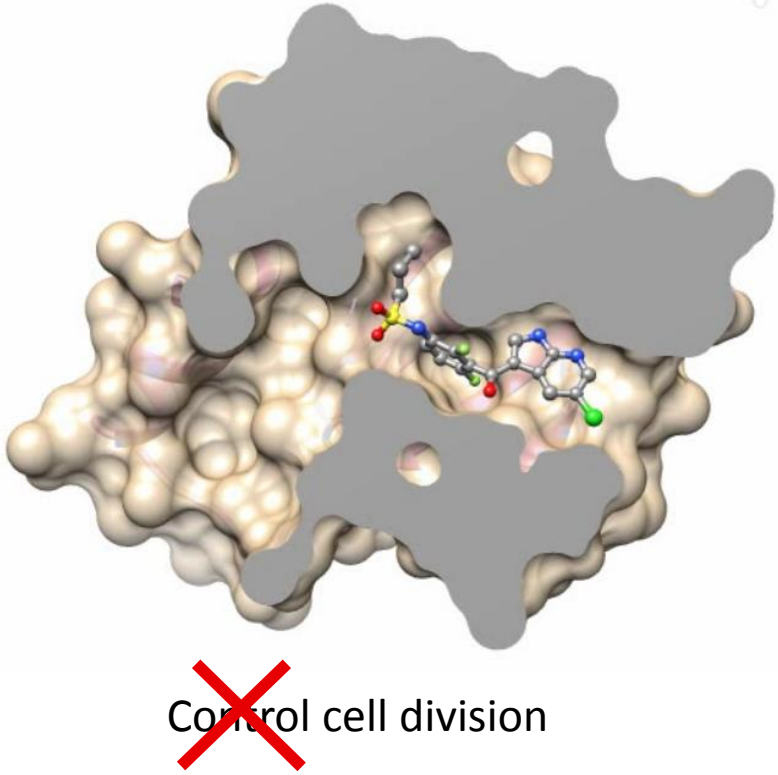


The mutation in the BRAF gene (V600E) leads to a change in the shape of B-Raf protein

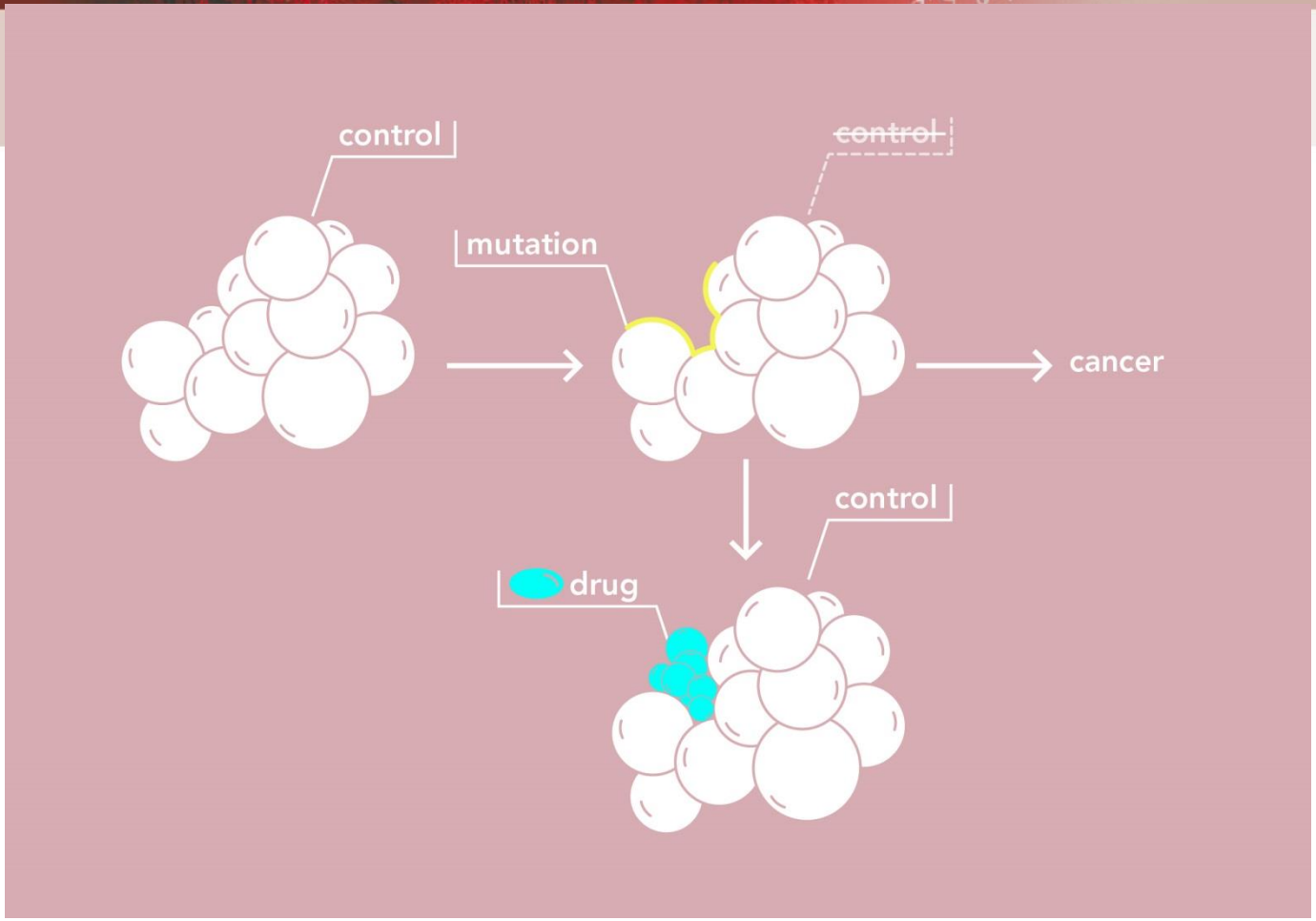
Video: transition BRAF inactive -> BRAF active (V600E)



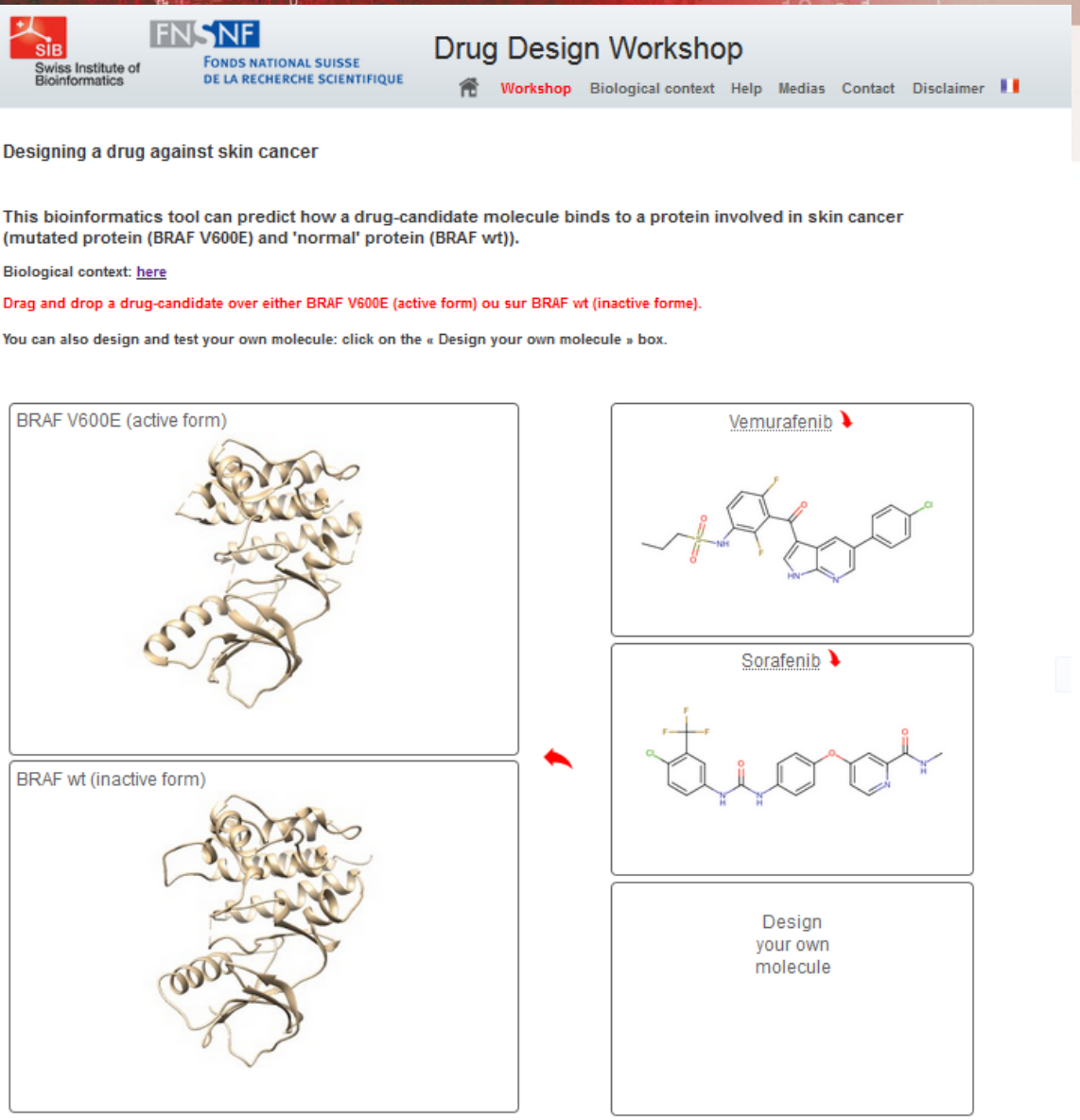
Control cell division



Control cell division



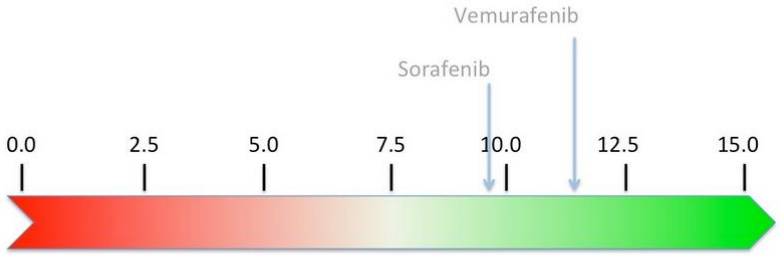
Design a drug that targets the mutated B-Raf protein, and which restores its 'control' function.

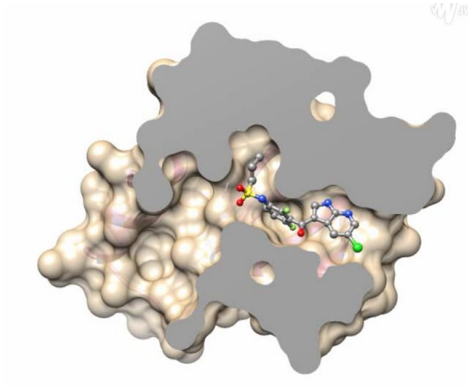


www.drug-design-workshop.ch/braf.php

Vemurafenib is the most suitable drug for the treatment of melanoma with the BRAF V600E mutation: its docking score is higher compared to that of Sorafenib

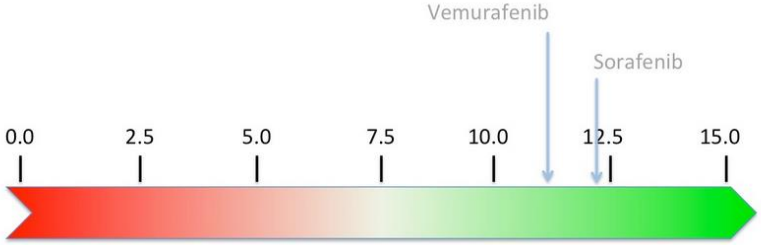
BRAF V600E

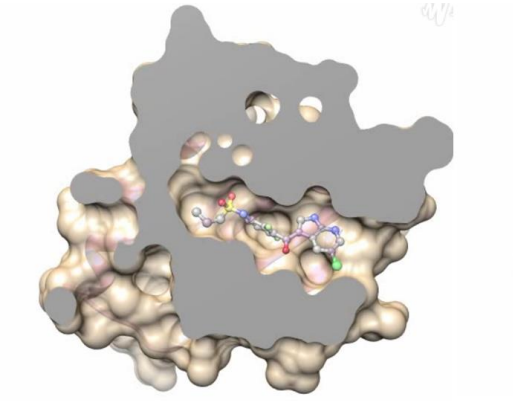




BRAF V600E + Vemurafenib

BRAF wild type





BRAF + Vemurafenib

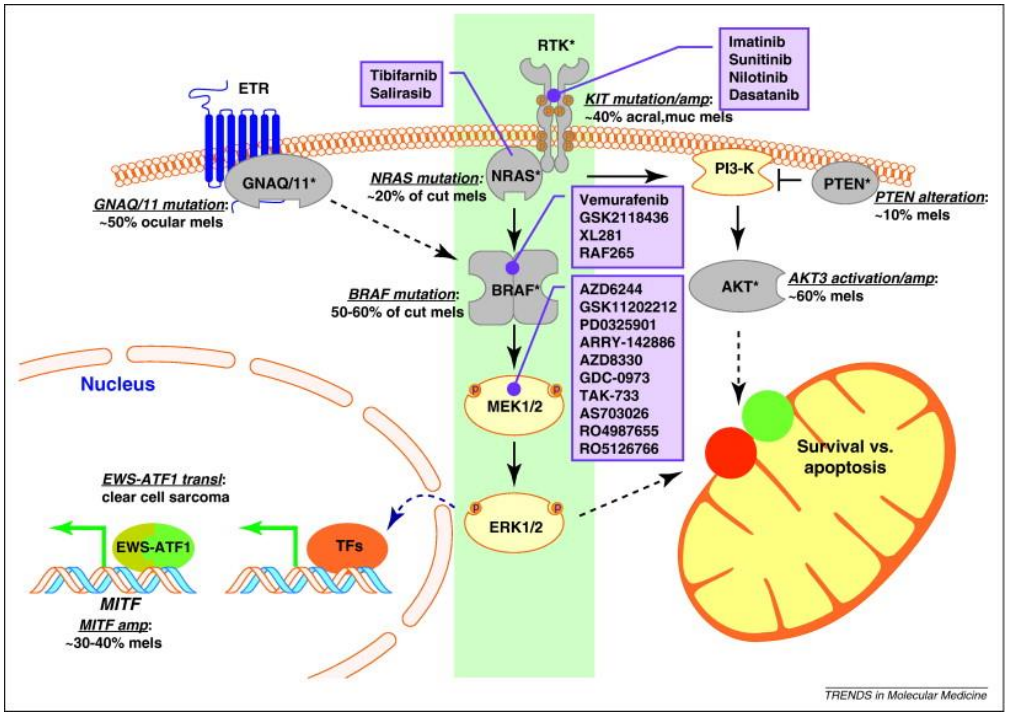
Find a molecule with a higher affinity for BRAF V600E compared to BRAF wild type…

- Inhibitors that are specific for the V600E B-raf mutant have been recently introduced for treating late-stage melanoma
- Vemurafenib (Zelboraf®) is an example of a specific inhibitor of V600E B-Raf
- Vemurafenib has been shown to dangerously favor tumor growth when the melanoma cells do not carry the B-Raf V600E mutation
- Doctor prescriptions are only allowed after having sequenced the BRAF gene of the patient cancer cells, using the FDA-approved cobas® 4800 BRAF V600 Mutation Test, to ascertain sequence alteration.

Summary of RAS pathway inhibitors

Drug	Targets	Stage of clinical development
Imatinib (Gleevec, STI571)	KIT, ABL, PDGFR, NQO2 [84, V-ATPase [85	Approved for CML and GIST [86
Sunitinib (SU11248)	KIT, PDGFR, VEGFR [87	Approved for RCC and GIST [87
Nilotinib	KIT, ABL, LCK, NQO2, DDR1 [84	Approved for CML [88
Dasatanib (BMS-354825)	KIT, ABL, SRC [89, DDR1, BTK, TEC [84	Approved for CML [90
Tipifarnib (R115777)	RAS and other proteins that require farnesyl transferase [91	Phase II/III [91
Salirasib (FTS)	RAS, mTOR [92	Phase II [43
Sorafenib (BAY 43-9006)	BRAF	Approved for RCC and HCC Failed at phase II for advanced melanoma [52
PLX4720	BRAF, CRAF, VEGF, PDGF, FLT3, KIT [46	Precursor of PLX4032
Vemurafenib (PLX4032)	$BRAF^{V600E}$, $BRAF^{WT}$, BRK [56	Phase III [61
GSK2118436	$BRAF^{V600E}$, CRAF, $BRAF^{WT}$, ARAF, ACK1, SRMS and MAP4K5 [57, 58]	Phase I/II [68
PD0325901	MEK	Phase II [75
AZD6244 (ARRY-142886)	MEK	Phase II [93
Anthrax lethal toxin	MEK	
GSK1120212 (JTP-74057)	MEK	Phase III

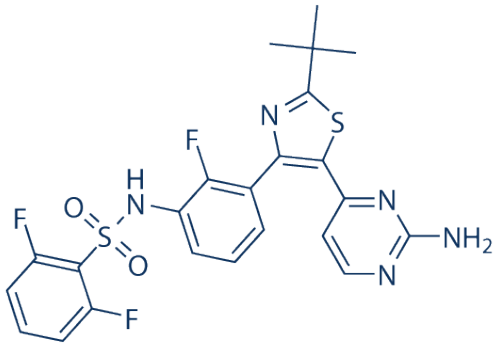
Pathway involved in the control of cell division



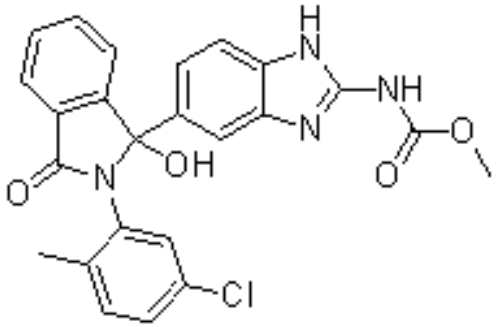
DOI: http://dx.doi.org/10.1016/j.molmed.2011.08.001

Molecules targeting BRAF

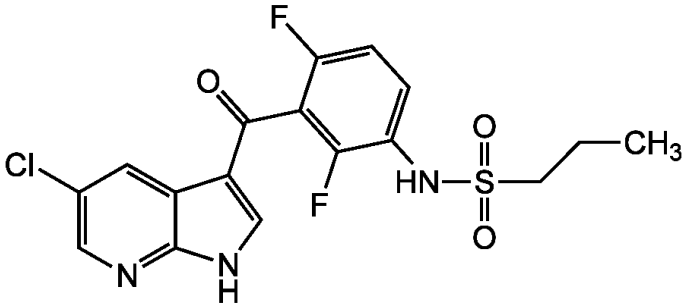
- Dabrafenib (GSK2118436)



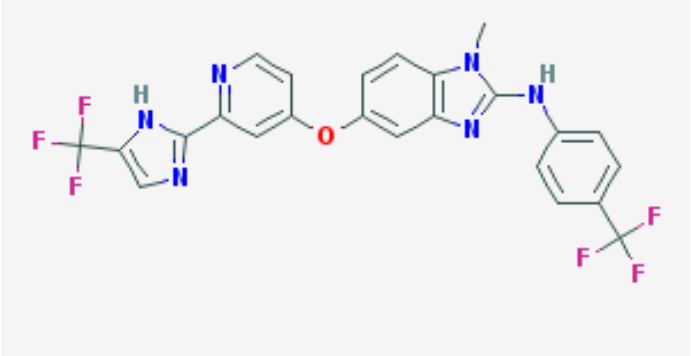
XL281



- PLX4720



RAF265



LE TEMPS

Septembre 2015

Médecine personnalisée, la révolution

> Santé Un programme a été lancé en juin aux Etats-Unis pour faire progresser les thérapies ciblées sur le profil génétique

> Les grandes pharmaceutiques, en particulier Roche et Novartis, sont à la pointe

Willy Boder

Début juillet a commencé, dans 2400 hôpitaux américains, une vaste opération de dépistage de patients atteints d'une forme ou d'une autre de cancer.

Contrairement aux études cliniques traditionnelles, l'objectif n'est pas de recruter des malades souffrant d'un même type de cancer, du sein, des poumons, ou de la prostate par exemple, pour tester l'efficacité d'un seul nouveau médicament. Il s'agit, au contraire, de rassembler les patients selon le profil génétique de leur tumeur, toutes catégories confondues.

Les médecins administreront une vingtaine de médicaments, dont certains proviennent de groupes pharmaceutiques suisses, qui sont déjà sur le marché ou encore en développement. Une patiente souffrant d'un cancer du sein avancé recevra par exemple une thérapie normalement destinée à un patient souffrant d'un cancer du poumon.

Cette nouvelle approche des programmes d'essais cliniques via le profil génétique des tumeurs et des patients, désignée par le terme «essais en corbeille» est suivie d'un œil bienveillant par la Food and Drug Administration (FDA), chargée du contrôle des médicaments aux Etats-Unis. Cette vaste étude s'intègre dans la tendance scientifique générale suivie actuellement par tous les grands groupes pharmaceutiques, à savoir rendre chaque médicament plus efficace en le modulant selon le profil génétique de la maladie dont souffre exactement tel ou tel patient.

La méthode d'essais cliniques en «corbeille», menée aux Etats-Unis sous le contrôle de l'Institut national du cancer (NCI), vise à élargir et à affiner ce qu'on appelle la médecine personnalisée, ou la médecine de précision, selon le terme utilisé par Barack Obama. Le président des Etats-Unis est persuadé de pouvoir faire progresser rapidement la recherche scientifique de cette manière et tente d'obtenir un financement par le Congrès de ces nouvelles formes de thérapie.

Joe Jimenez, patron de Novartis, deuxième entreprise au monde, derrière Roche, dans la mise à disposition de médicaments contre le cancer, estime qu'un quart des médicaments sont actuellement gaspillés de différentes manières. L'une des principales causes de ce gaspillage, qui coûte très cher aux systèmes de santé, provient du manque de connaissances scientifiques précises sur le profil génétique de telle ou telle maladie à mettre en corrélation avec l'ADN du patient. Le médecin est dès lors contraint de tester plusieurs médicaments sur un patient avant de trouver celui qui est le plus efficace.

Cette approche empirique se produit pour de très nombreuses maladies, de l'hypertension aux maladies infectieuses, en passant par des affections très graves. Pour les maladies où le pronostic vital est engagé, comme certaines formes de cancer, ce tâtonnement, synonyme de perte de temps, peut conduire à la mort.

Selon les spécialistes, la médecine de précision a aussi pour avantage de réduire les coûts de la santé. Les médicaments de ce type associés à un diagnostic (biomarqueur ou test spécifique d'ADN accompagnant le traitement) sont, certes, nettement plus chers que les produits thérapeutiques traditionnels, mais ils évitent le tâtonnement médical et la facturation aux caisses maladie de médicaments inefficaces.

> Rassembler les patients selon le profil génétique de leur tumeur, toutes catégories confondues

Le programme du NCI, qui touche 2400 hôpitaux et 1000 patients sélectionnés au sein d'un groupe de 3000 malades du cancer, est doté d'un budget de 30 à 40 millions de dollars. Le critère de succès de la thérapie sera principalement basé sur une réduction rapide de la tumeur d'au moins un tiers.

GETTY IMAGES

La médecine personnalisée combine profil génétique et médicaments thérapeutiques de manière ciblée. ARCHIVES

Depuis le premier séquençage d'un génome humain, en 2003, l'analyse des maladies, en particulier le cancer, repose de manière de plus en plus précise sur la découverte de mutations génétiques et l'activation ou la désactivation de protéines, au sein même ou à la surface des cellules. Ces mécanismes provoquent la prolifération des cellules cancéreuses dans l'organe touché, puis sous forme de métastases.

Les cancers ne sont désormais plus considérés comme différentes formes d'une même maladie, mais comme une multitude de maladies ayant des caractéristiques et un profil génétique propre. Le cancer évolue différemment selon chaque patient, ce qui entraîne, si le mécanisme génétique qui dysfonctionne peut être ciblé et corrigé, la prescription d'un médicament spécifique et une approche thérapeutique personnalisée.

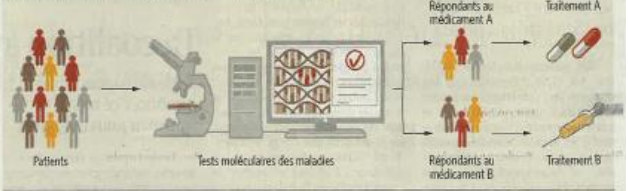
«Grâce la médecine personnalisée nous obtenons immédiatement des taux de réponse inédits aux traitements. Il y a vraiment un changement de paradigme dans ce domaine scientifique», s'enthousiasme Dietmar Berger, responsable du développement de Roche en oncologie. Il cite le cas du médicament Alectinib, contre le cancer du poumon au stade avancé, en phase d'homologation. Les métastases dans le cerveau se réduisent rapidement avec un taux de réponse jusqu'à 70% d'une durée jusqu'à onze mois.

Roche place aussi beaucoup d'espoir dans Atezolizumab, un médicament, associé à la présence de la protéine PD-L1, qui fait appel à la stimulation du système immunitaire pour détruire les cellules cancéreuses. Ce mécanisme, identique chez certains patients spécialement diagnostiqués, peut être appliqué dans la lutte contre les cancers du poumon, de la prostate, du sein et du rein. «70% de nos médicaments en phase clinique II et III en oncologie ont désormais un test diagnostic spécifique associé», constate Dietmar Berger.

«On assiste réellement à une révolution dans le traitement de ces maladies, confirme Severin Schwan, patron de Roche. Auparavant, un cancer métastasé menait le plus souvent à la mort. Aujourd'hui, dans de nombreux cas, grâce à la médecine personnalisée, on peut prolonger la vie durant des années, et peut-être guérir de ce type de cancer.»

Comment ça marche

Le profil génétique du patient et de sa tumeur détermine le traitement à suivre

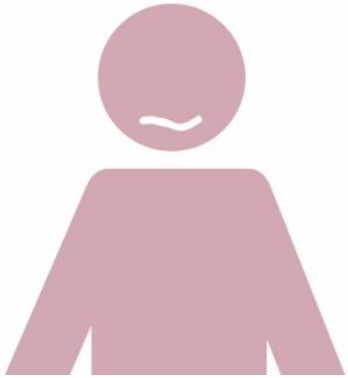


La majorité des thérapies contre le cancer à l'étude sont associées à un ou plusieurs tests génétiques développés en parallèle avec l'élaboration finale de la substance active contenue dans le médicament. Le taux d'efficacité de la thérapie, ainsi adaptée au profil précis du patient et de sa tumeur, peut dépasser 70% et augmente fortement ses chances de survie à la suite d'un cancer métastasé. SOURCE: PFIZER

IDO1 workshop

Have a try to design a drug...

someone
falls ill...



- ❑ Protein's active site is **altered** (mutated)
- ❑ there is **too little** of a protein
- ➡ ❑ there is **too much** of a protein

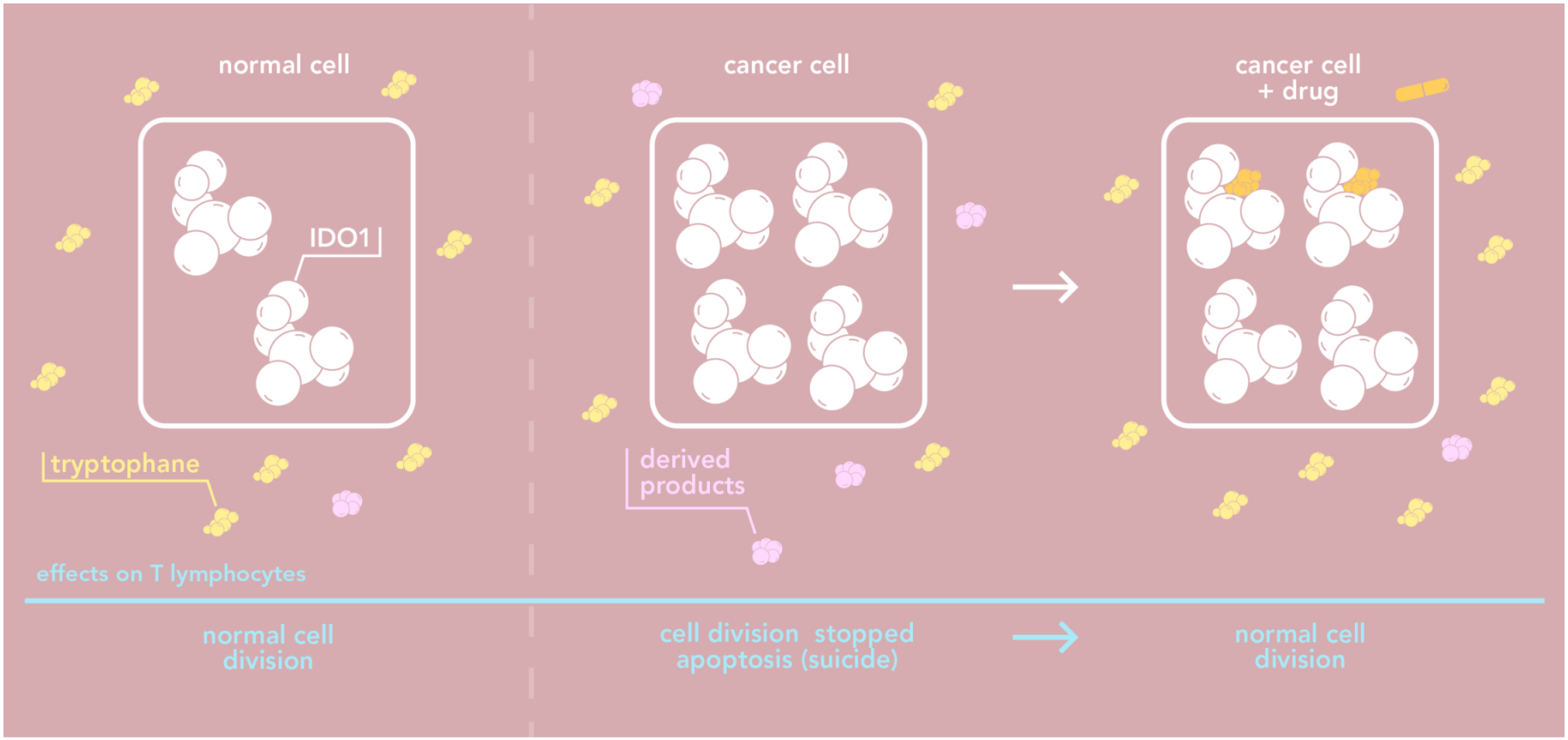
The immune system plays a key role in fighting off cancer by tracking down and eliminating cancer cells.

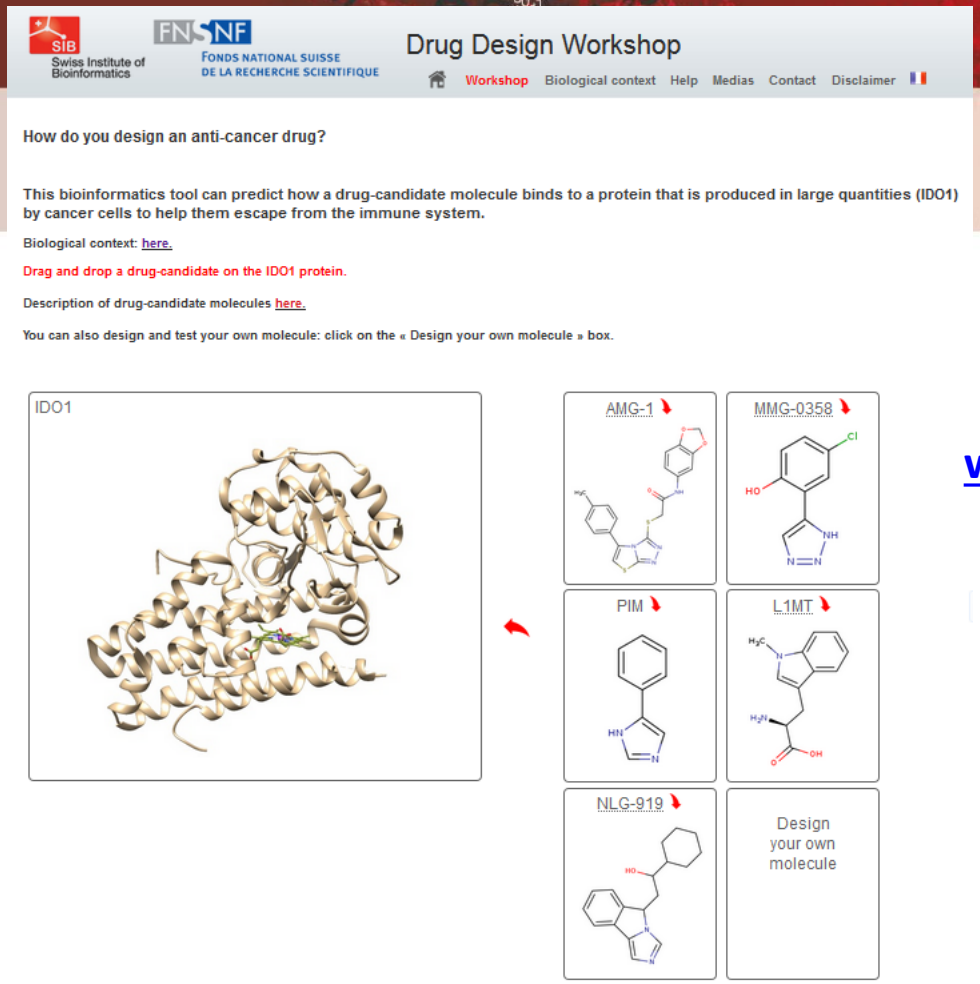
Cancer cells, however, also have ways of evading the immune system:

- Many cancer cells produce a protein known as indoleamine 2,3-dioxygenase 2 (IDO1) in large quantities.

- IDO1 degrades tryptophane, which is an essential amino acid and has to be present in sufficient quantities for cells to divide normally. A high concentration of IDO1in the environment of a cancer cell decreases the amount of tryptophane, which in turn stops immune cells, or T lymphocytes, from proliferating. Furthermore, certain side products which result from tryptophane degradation are toxic for T lymphocytes.

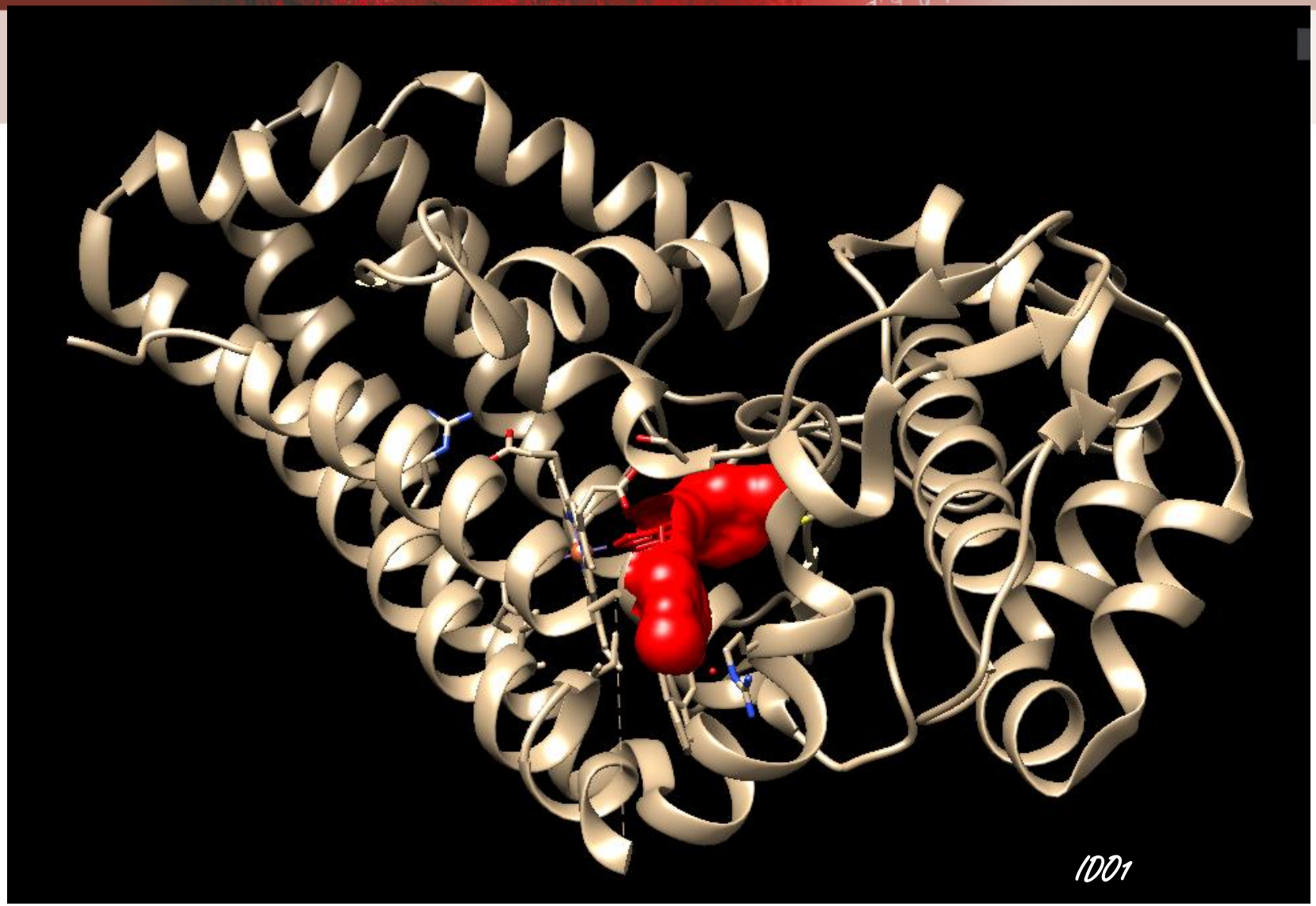
- IDO1 is a promising therapeutic target in anti-cancer treatment: inhibiting IDO1 increases the effectiveness of immunotherapies as well as other treatments by restoring the immune response.


normal cell
IDO1
tryptophane
cancer cell
derived products
cancer cell + drug
effects on T lymphocytes
normal cell division
cell division stopped apoptosis (suicide)
normal cell division



www.drug-design-workshop.ch/ido1.php

AMG-1, an imidazothiazole developed by Dainippon Sunimoto Pharma.
MMG-0358, a triazole designed by the SIB Swiss Institute of Bioinformatics. MMG-0358 has a strong affinity for IDO1 and is active *in vivo*.
PIM, an imidazole. PIM is one of the first inhibitors whose 3D structure complexed with IDO1 has been characterized. PIM has a weak affinity for IDO1 and is not very specific.
L1MT, or L-1-methyltryptophane, an inert analog of tryptophane that inhibits IDO1. L1MT is undergoing clinical tests for the treatment of breast and prostate cancer.
NLG-919, an imidazole developed by NewLink Genetics. NLG-919 is under clinical evaluation to treat solid tumors.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Additional links

- www.drug-design-workshop.ch (EN, FR)
  http://www.atelier-drug-design.ch/CADD-FormationContinue.pdf (EN)
  http://education.expasy.org/bioinformatique/DrugDesign_complement.html (FR)

- www.chromosomewalk.ch (DE, EN, FR): discover the world of DNA, genes, proteins, common genetic variations and bioinformatics tools which are used to visualize genomic data.

- Atelier de Bioinformatique (FR): http://education.expasy.org/bioinformatique/ a selection of additional bioinformatics activities such as 'Phylogeny and Biodiversity' and 'BLAST discovery'

- Protein Spotlight (EN): small articles about a specific protein - or family of proteins – written on an informal tone.

- www.sib.swiss/bioinformatics-for-all/workshops: online bioinformatics-related workshops for different levels of understanding