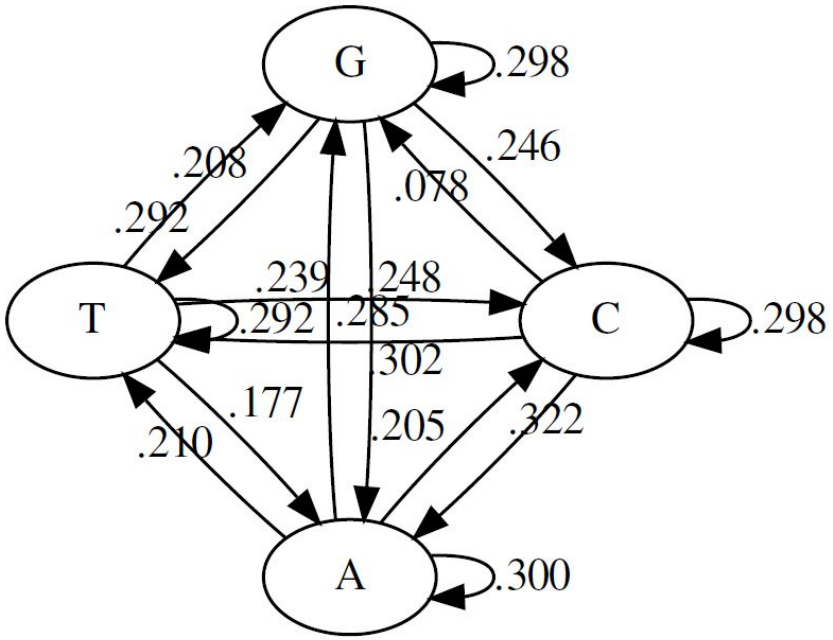
Given the Markov chain model, what is the ratio between p(ACGTGGT|M) and p(ACCTGGT|M)?

Answers:

- 0.18 (6 votes)
- 0.246 (G→C, 1 vote)
- 0.262 (1 vote)
- 0.078 (C→G, 1 vote)
- 18.18 (1 vote)

Which one is correct, and why?



Exercises of Levenshtein distance and BLAST

	H	E	A	G	A	W	G	H	E	E
P	-2	-1	-1	-2	-1	-4	-2	-2	-1	-1
A	-2	-1	5	0	5	-3	0	-2	-1	-1
W	-3	-3	-3	-3	-3	15	-3	-3	-3	-3
H	10	0	-2	-2	-2	-3	-3	10	0	0
E	0	6	-1	-3	-1	-3	0	0	6	6
A	2	-1	5	0	5	-3	0	2	-1	-1
E	0	6	1	3	1	-3	-3	)	6	6

-2 -2

Adapted from *Biological Sequence Analysis* (R. Durbin, S. Eddy, A. Krogh, G. Mitchison), Figure 2.3. We assume that a gap cost per unaligned residue of *d*=*-8*. Try to use the information to perform global alignment between the two amino-acid sequences:

1. HEAGAWGHEE
2. PAWHEAE

		H	E	A	G	A	W	G	H	E	E
	0	-8	-16	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	-17	-25	-33	-42	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	-20	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	-5	-13	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-13	-7	-3	-11	-19
E	-40	-34	-8	-16	-16	-9	-12	-15	-7	3	-5
A	-48	-42	-16	-3	-11	-11	-12	-12	-15	-5	2
E	-56	-50	-24	-11	-6	-12	-14	-15	-12	-9	1

```
HEAGAWGHE-E
--P-AW-HEAE
```

What does Fomivirsen target?

It is possible to search for local sequence matches in large databases of nucleotides, for instance using the BLAST algorithm. An implementation is freely available at National Institute of Health (NIH, US): https://blast.ncbi.nlm.nih.gov/Blast.cgi. Try to search for the RNA/protein targeted by fomivirsen, given its sequence 5'-GCG TTT GCT CTT CTT CTT GTT GCG-3'.

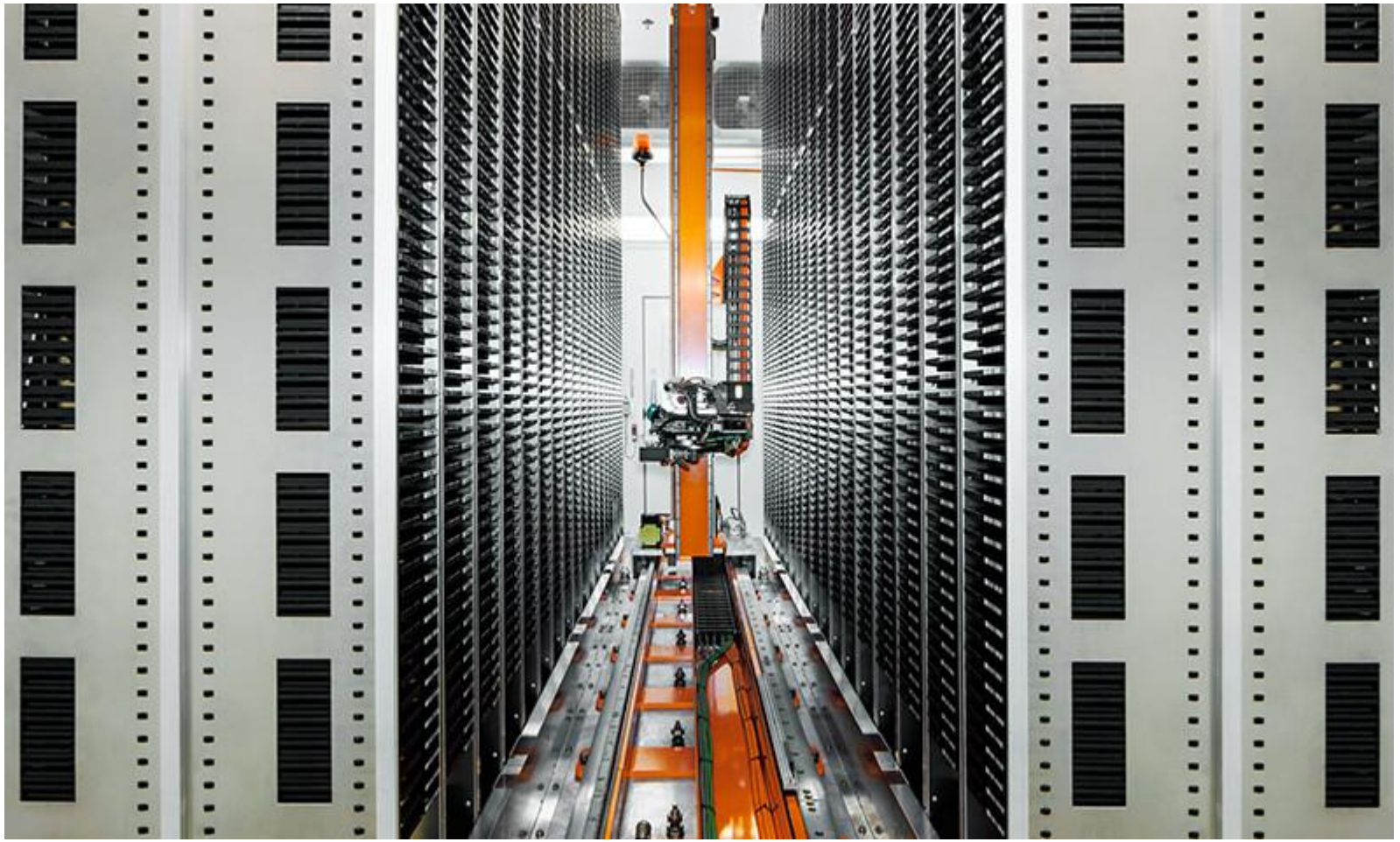
I thank Jessica Falkowski who pointed out my mistake!

Human betaherpesvirus 5 strain SYD-SCT1, complete genome	42.1	42.1	100%	0.14	100.00%	MT044485.1
Human betaherpesvirus 5 strain HAN-SOT4, complete genome	42.1	42.1	100%	0.14	100.00%	MT044484.1
Human betaherpesvirus 5 strain HAN-SOT3, partial genome	42.1	42.1	100%	0.14	100.00%	MT044483.1
Human betaherpesvirus 5 strain GLA-SOT3, complete genome	42.1	42.1	100%	0.14	100.00%	MT044482.1
Human betaherpesvirus 5 strain GLA-SOT2, complete genome	42.1	42.1	100%	0.14	100.00%	MT044481.1
Human betaherpesvirus 5 strain SYD-SCT2, complete genome	42.1	42.1	100%	0.14	100.00%	MT044480.1
Human betaherpesvirus 5 strain HAN-SOT5, complete genome	42.1	42.1	100%	0.14	100.00%	MT044479.1
Human betaherpesvirus 5 strain HAN-SOT1, complete genome	42.1	42.1	100%	0.14	100.00%	MT044478.1
Human betaherpesvirus 5 strain GLA-SOT4, complete genome	42.1	42.1	100%	0.14	100.00%	MT044477.1

Additional questions

- I wondered when you use which approach the probabilistic or the deterministic one, when should we use which model or is there a difference in their performance. How do you chose the model, which one to prefer? Depend on the purpose. Deterministic methods are easy to use; probabilistic models are more powerful.
- Any recommendations for what direction of master thesis/PhD thesis to choose that's more related to the field of work in pharmaceutical/biotech companies? In either experimental or data analysis aspect? Many companies offer positions of both types (and some even combining the two). For instance see careers.roche.com for positions openned by Roche, or LinkedIn. I also post my groups openings on my personal blog *David Discovers Drug Discovery* at *jdzhang.me*.

AMIDD Lecture 5: Proteins and Ligands



The chemical library at Novartis headquarters in Basel currently contains roughly 3 million molecules. We aim to expand that number radically within the next few years.

Jay Bradner, President of NIBR, in an interview in 2017

Dr. Jitao David Zhang, Computational Biologist

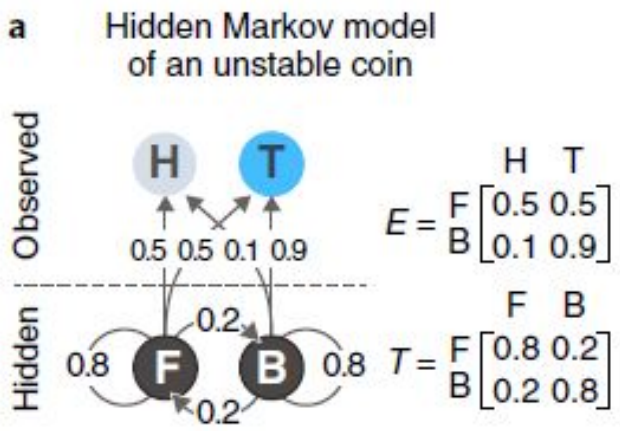
[1] Pharmaceutical Sciences, Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche

[2] Department of Mathematics and Informatics, University of Basel

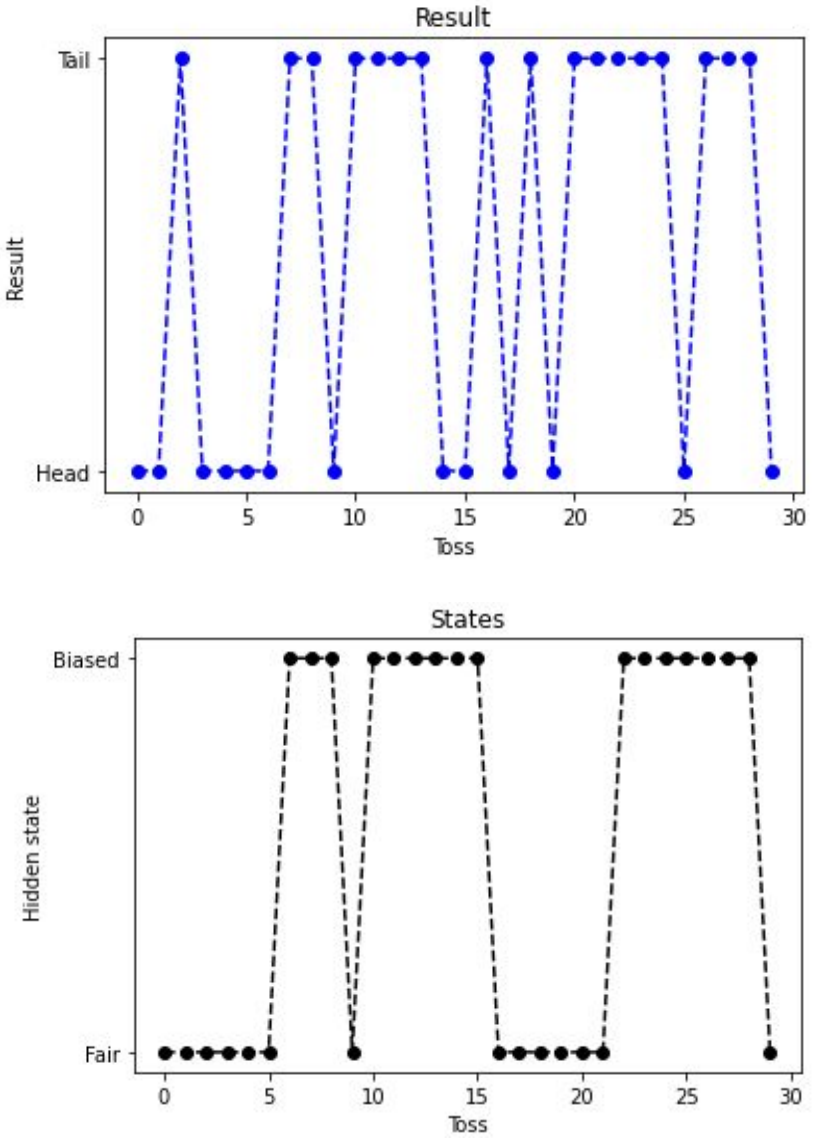
Today's goals

- Protein biology and structure determination
- Representation and molecular descriptors of small molecules
- Workflow of ligand- and structure-based drug discovery programs

Recap of Hidden Markov Chains



Grewal, Jasleen K., Martin Krzywinski, and Naomi Altman. 2019. "Markov Models — Hidden Markov Models." Nature Methods 16 (9): 795–96.




```
Transmission Matrix Generated:
[[0.8 0.2]
 [0.2 0.8]]

Transmission Matrix Recovered:
[[0.774 0.226]
 [0.104 0.896]]


Emission Matrix Generated:
[[0.5 0.5]
 [0.1 0.9]]

Emission Matrix Recovered:
[[0.539 0.461]
 [0.152 0.848]]
```

Inferred model parameters with 1000 observations and the Viterbi algorithm, see source code

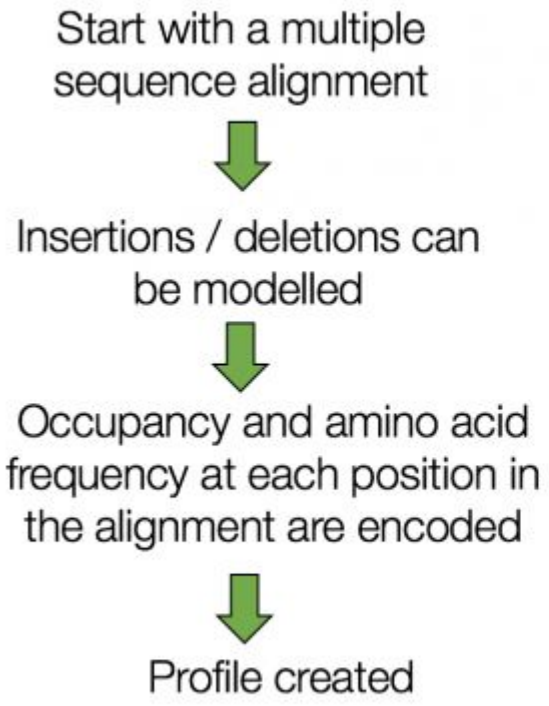
Profile Hidden Markov models capture evolutionary changes in homologs

M: match states. In the match state, the probability distribution is the frequency of the amino acids in that position.

I: insert states, which model highly variable regions in the alignment

D: delete states, which allows gaps and deletion.

Profile HMMs belongs to ***generative models***.



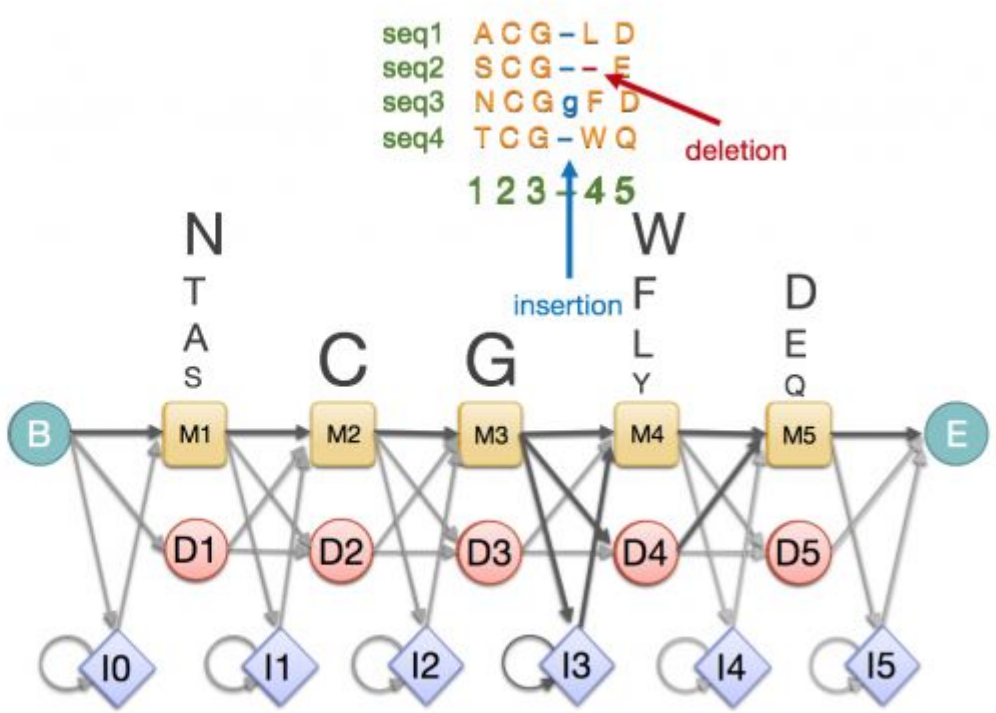
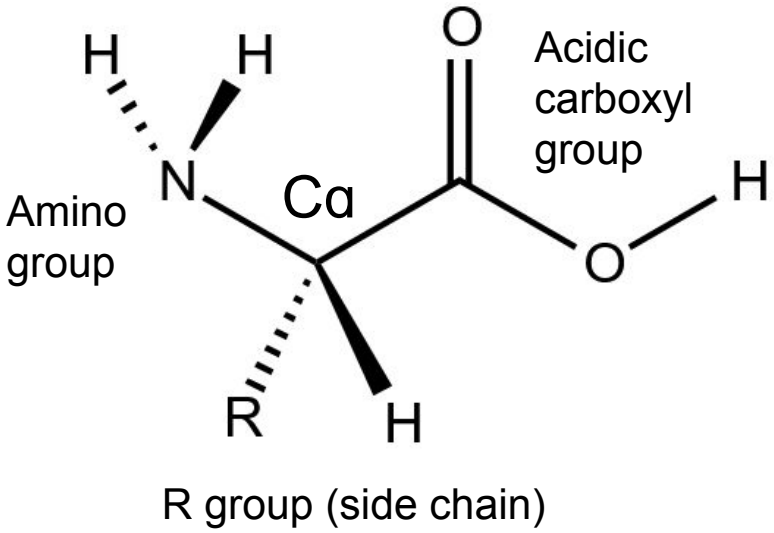
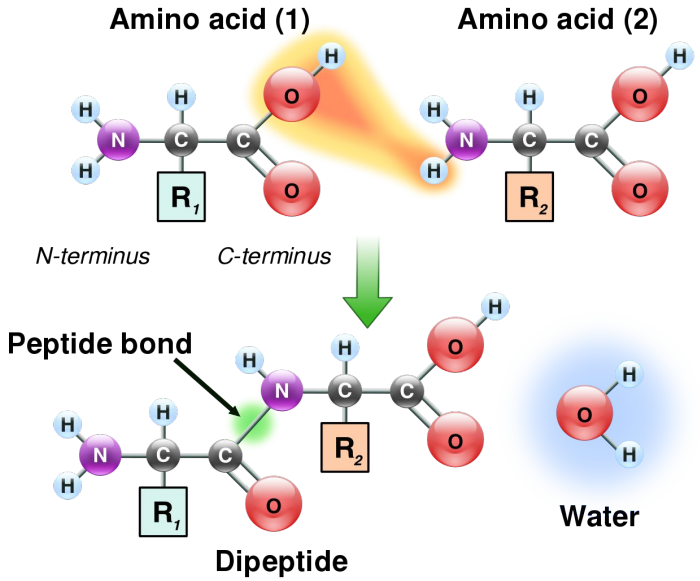


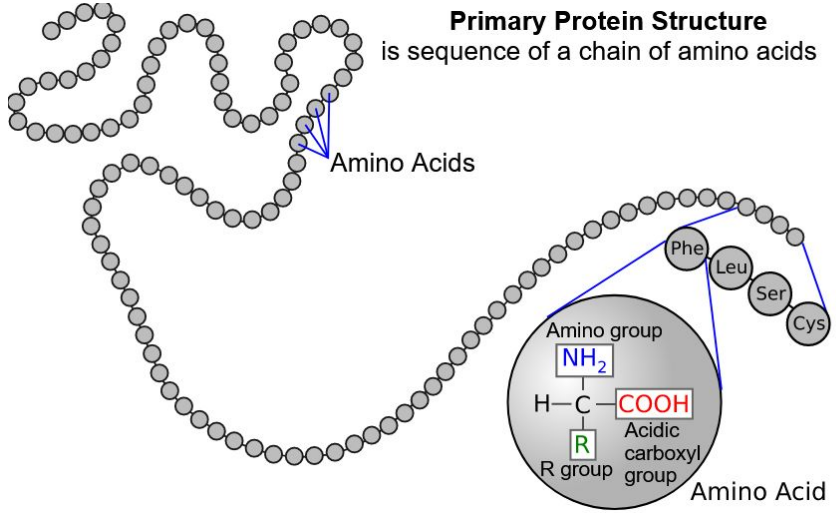
Figure from Pfam, a database of protein domains

Amino acids form polypeptide chains

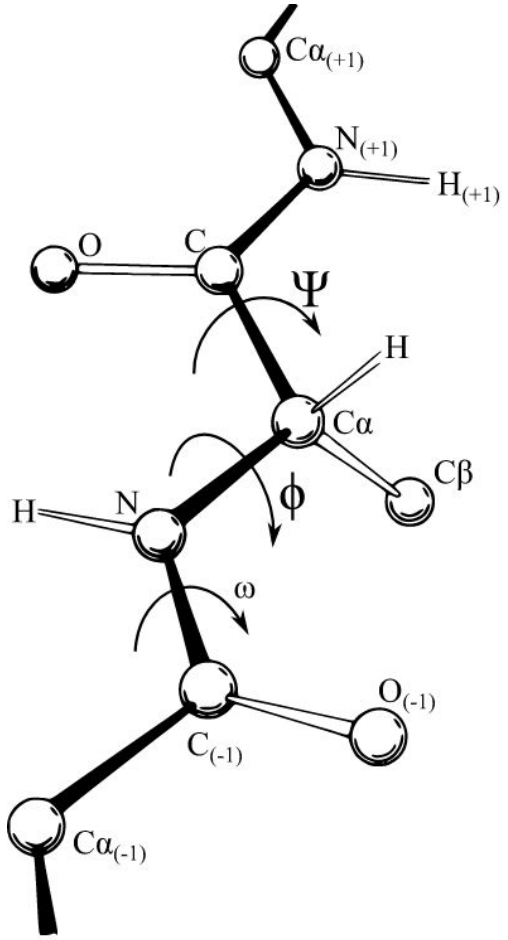
Proteins in human consist of chains of 21 amino acids. Each amino acid has a hydrogen (Glycine) or a side chain (R group) attached to the alpha carbon, which are connected to both an amino group and a carboxyl group. The amino group and the carboxyl group of two adjacent amino acids form a peptide bond.

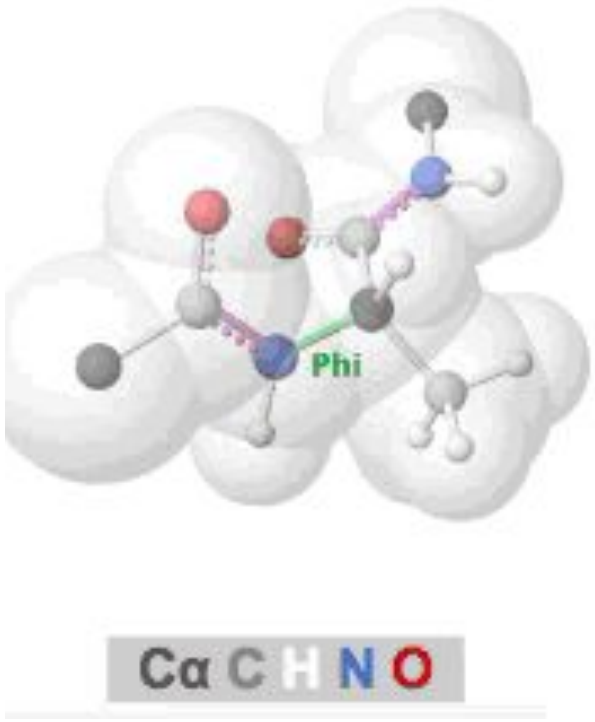


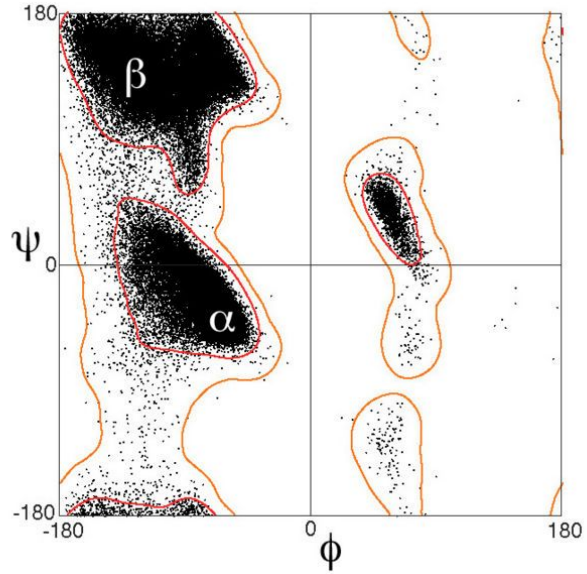


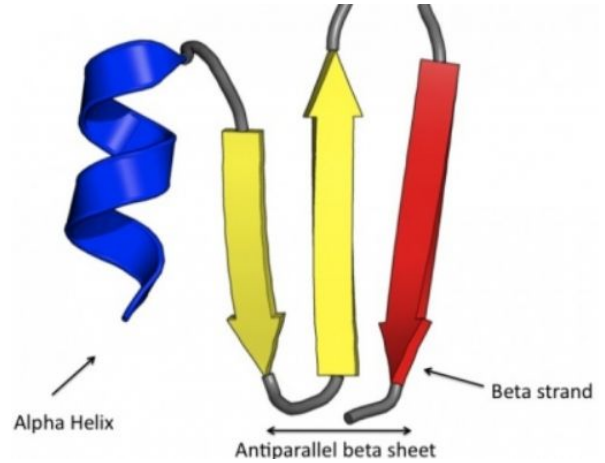


The Ramachandran Principle: Alpha helices, beta strands, and turns are the most likely conformations of a polypeptide chain









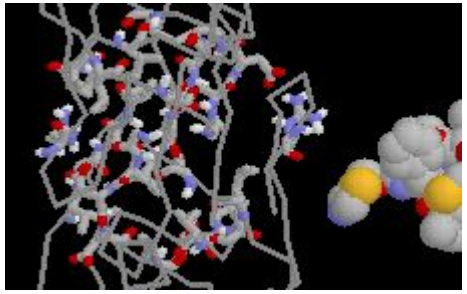
The Ramachandran Principle, explained by Eric Martz on YouTube, discovered via Proteopedia

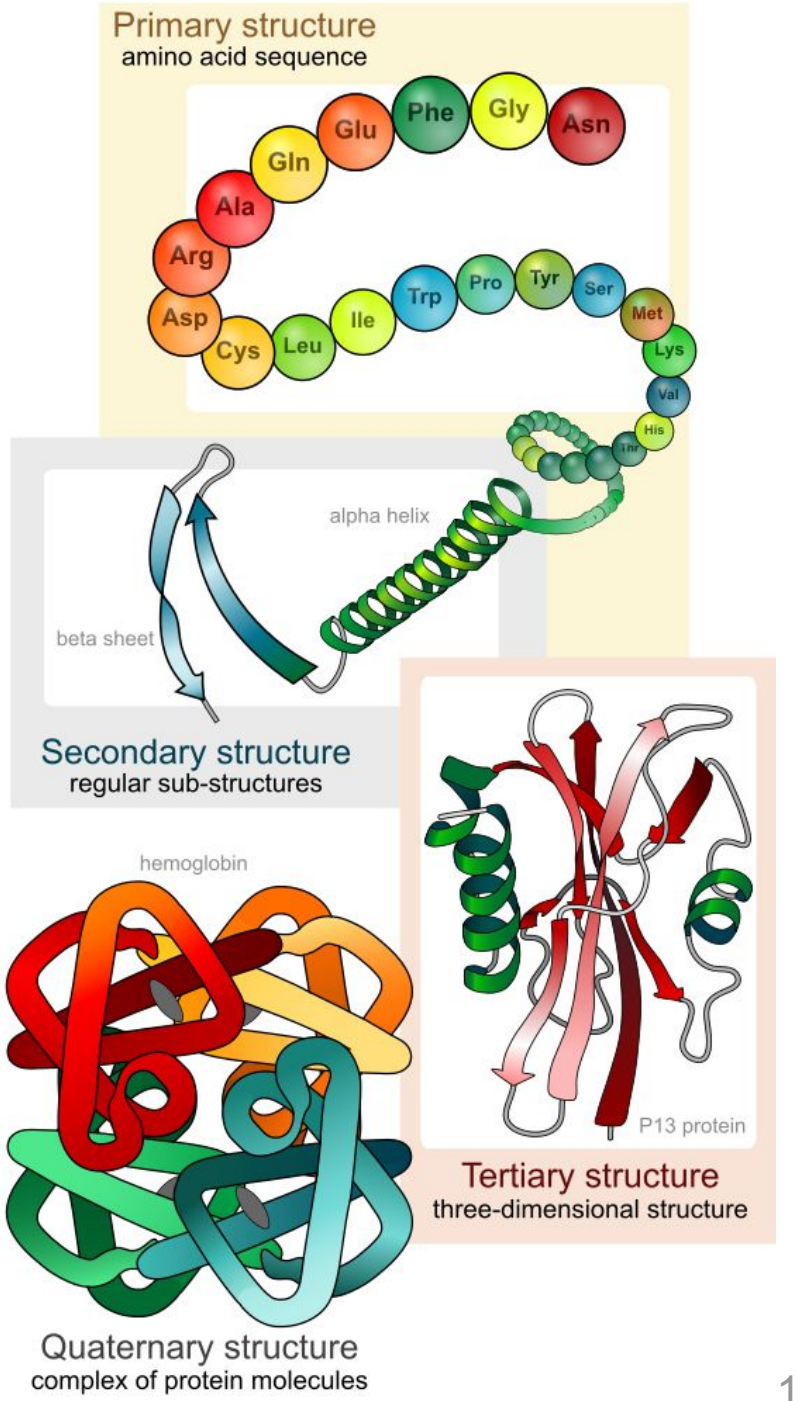
Protein structure is hierarchical

Protein structure is hierarchical:

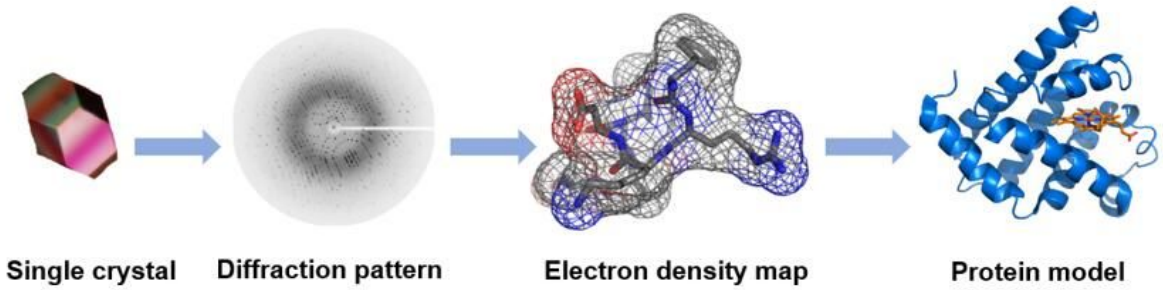
- Primary amino-acid sequences form secondary structures (alpha helices, beta sheets, and turns)
- Secondary structures form 3D structures of proteins (tertiary structures)
- Proteins interact with each other and form complexes (quaternary structure).

Many drugs induce changes in tertiary structure.

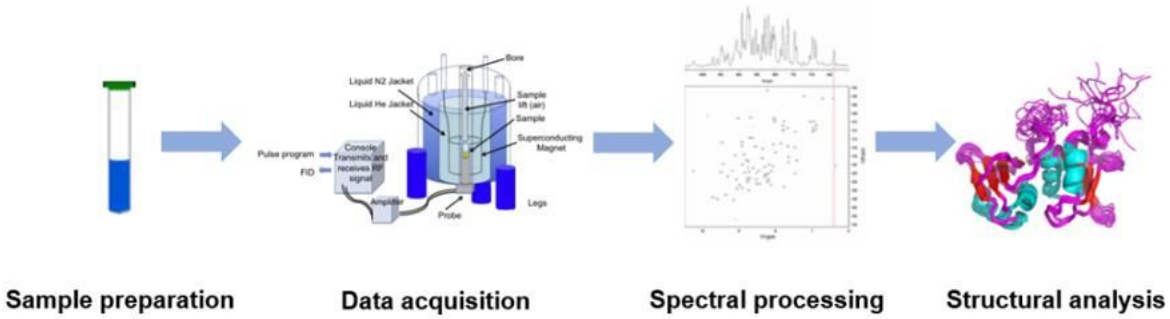




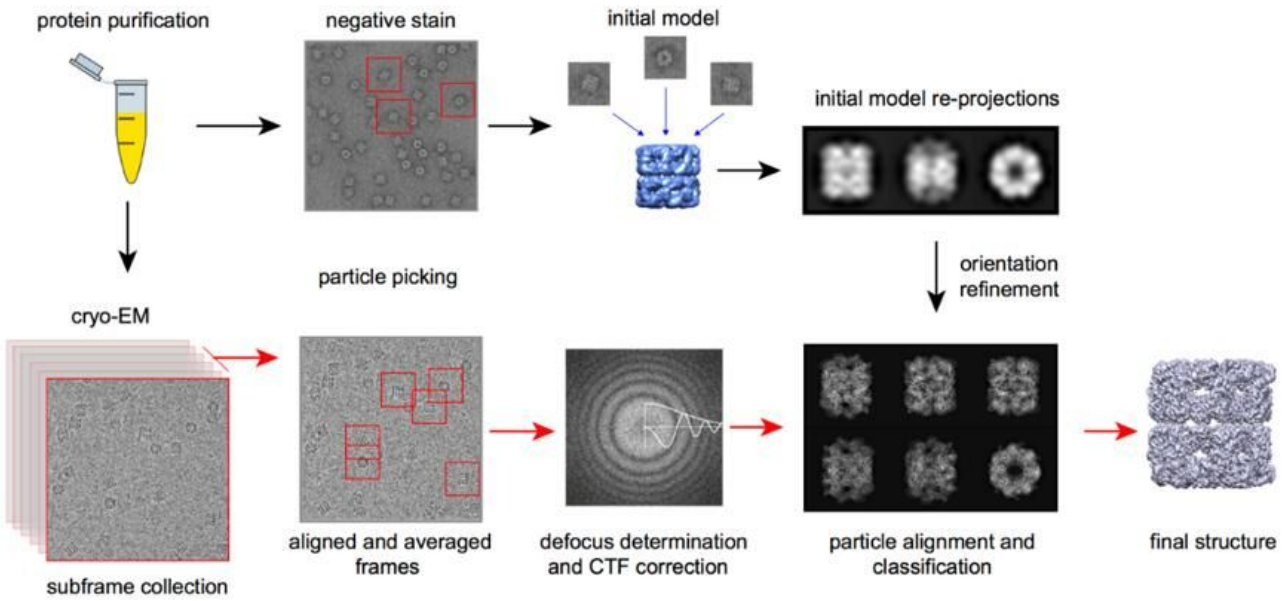
Three major experimental approaches to determining protein structures



X-ray crystallography



Nuclear Magnetic Resonance (NMR)



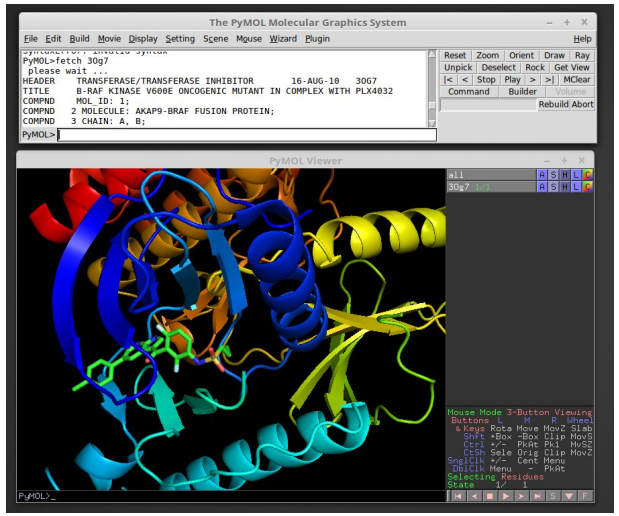
Cryo-electron microscopy (CryoEM)

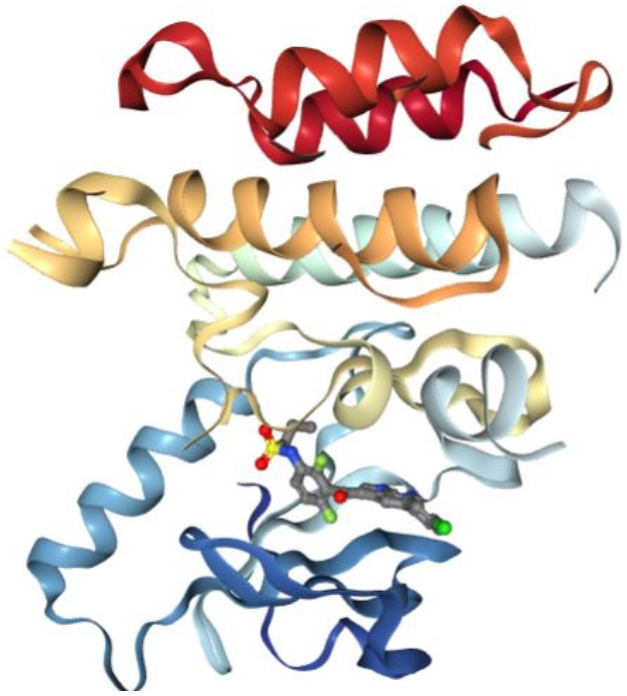
Figure sources:
https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm

Three major experimental approaches to determining protein structures

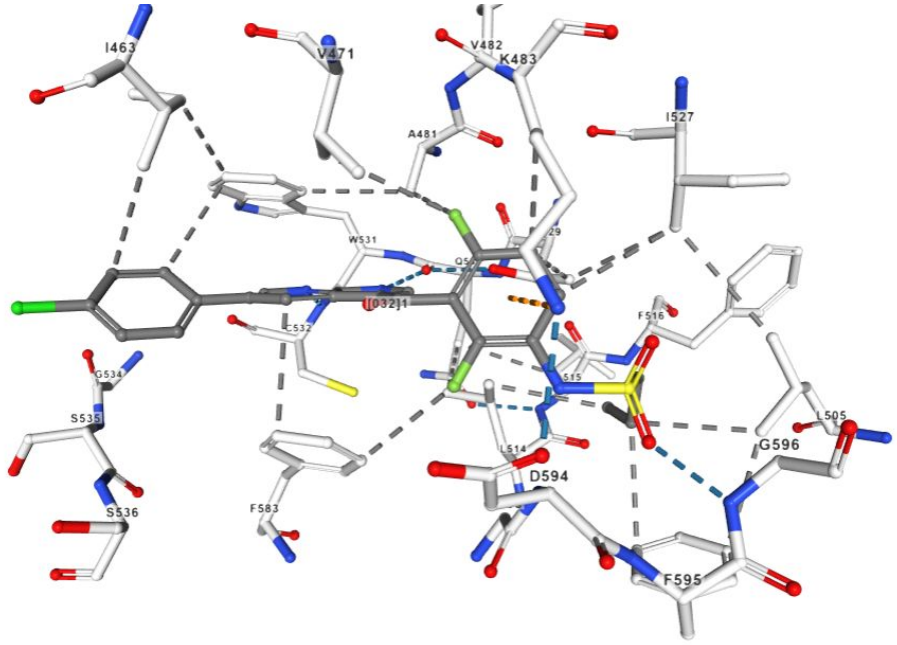
Method	Underlying physical properties	Main mathematical technique used	Advantages	Limitations
X-ray crystallography	The crystalline structure of a molecule causes a beam of incident X-rays to diffract into many specific directions.	Fourier series and Fourier transform	• Established • Broad molecular weight range • High resolution	• Crystallization • Static model
Nuclear Magnetic Resonance (NMR)	Nuclei with odd number of protons and/or neutrons in a strong constant magnetic field, when perturbed by a weak oscillating magnetic field, produce an electromagnetic signal with a frequency characteristic of the magnetic field at the nucleus.	Distance geometry (the study of matrices of distances between pairs of atoms) of and discrete differential geometry of curves	• 3D structure in solution • Dynamic study possible	• High sample purity needed • Molecular weight limit (~<40-50 kDa) • Sample preparation and computational simulation
Cryo-electron microscopy	An electron microscope using a beam of accelerated electrons (instead of protons) as a source of illumination. Samples are cooled to cryogenic temperatures and embedded in an environment of vitreous water (amorphous ice).	An inverse problem of reconstruction - the estimation of randomly rotated molecule structure from a projection with noise; Fourier transform; iterative refinement	• Easy sample preparation • Ntive-state structure • Small sample size	• Costly EM equipment • Challenging for small proteins

In silico presentation of protein structures: PDB





Structural view



Ligand view

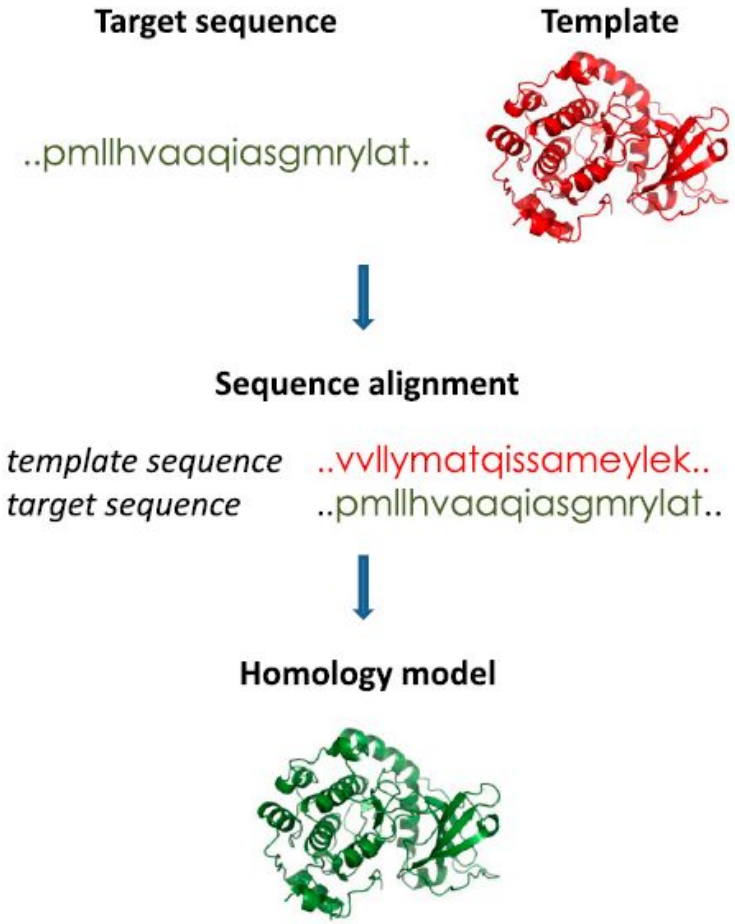
Balls and sticks: protein V600E and ligand (PLX4032)

Blue dashes: hydrogen bonds (<3.5 Angstrom)

Gray dashes: hydrophobic interactions (<4 Angstrom)

Working with PDB files with ***PyMol*** from the command-line

If no structure is available, homology model building and *in silico* prediction may help



Sliwoski, Gregory, Sandeepkumar Kothiwale, Jens Meiler, und Edward W. Lowe. „Computational Methods in Drug Discovery“. *Pharmacological Reviews* 66, Nr. 1 (1. Januar 2014): 334–95. https://doi.org/10.1124/pr.112.007336.

W296–W303 Nucleic Acids Research, 2018, Vol. 46, Web Server issue *Published online 21 May 2018*
doi: 10.1093/nar/gky427

SWISS-MODEL: homology modelling of protein structures and complexes

Andrew Waterhouse[1,2,†], Martino Bertoni[1,2,†], Stefan Bienert[1,2,†], Gabriel Studer[1,2,†], Gerardo Tauriello[1,2,†], Rafal Gumienny[1,2], Florian T. Heer[1,2], Tjaart A. P. de Beer[1,2], Christine Rempfer[1,2], Lorenza Bordoli[1,2], Rosalba Lepore[1,2] and Torsten Schwede[1,2,*]

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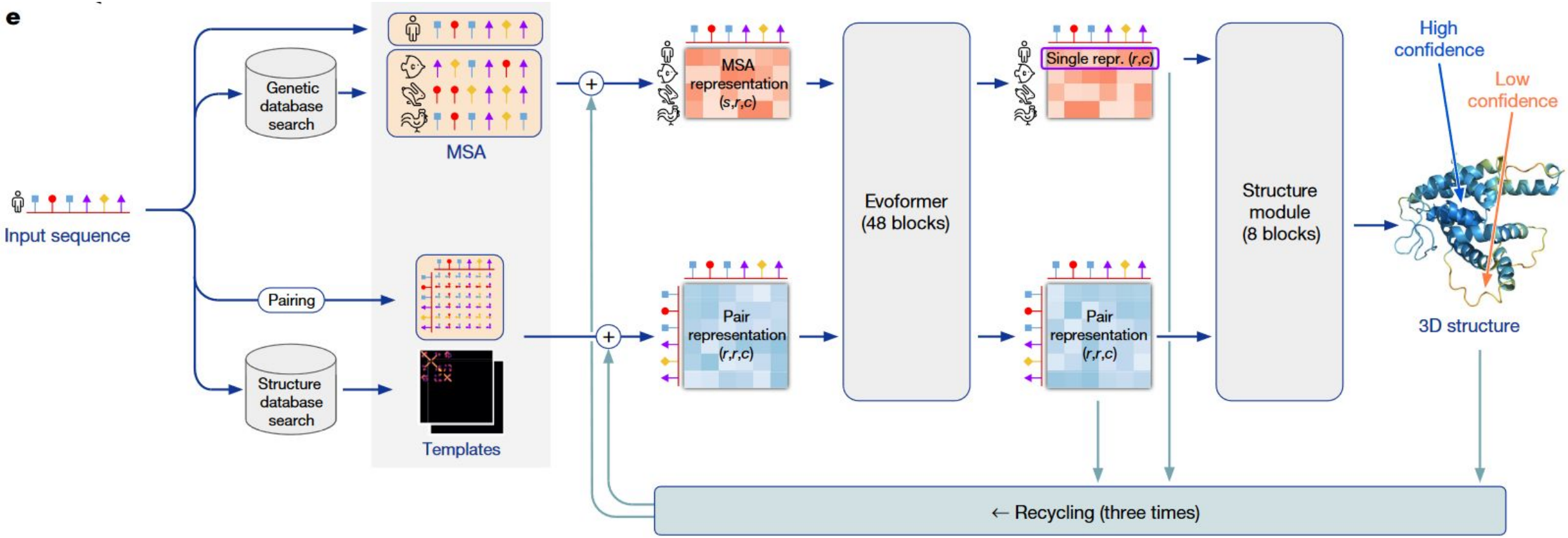
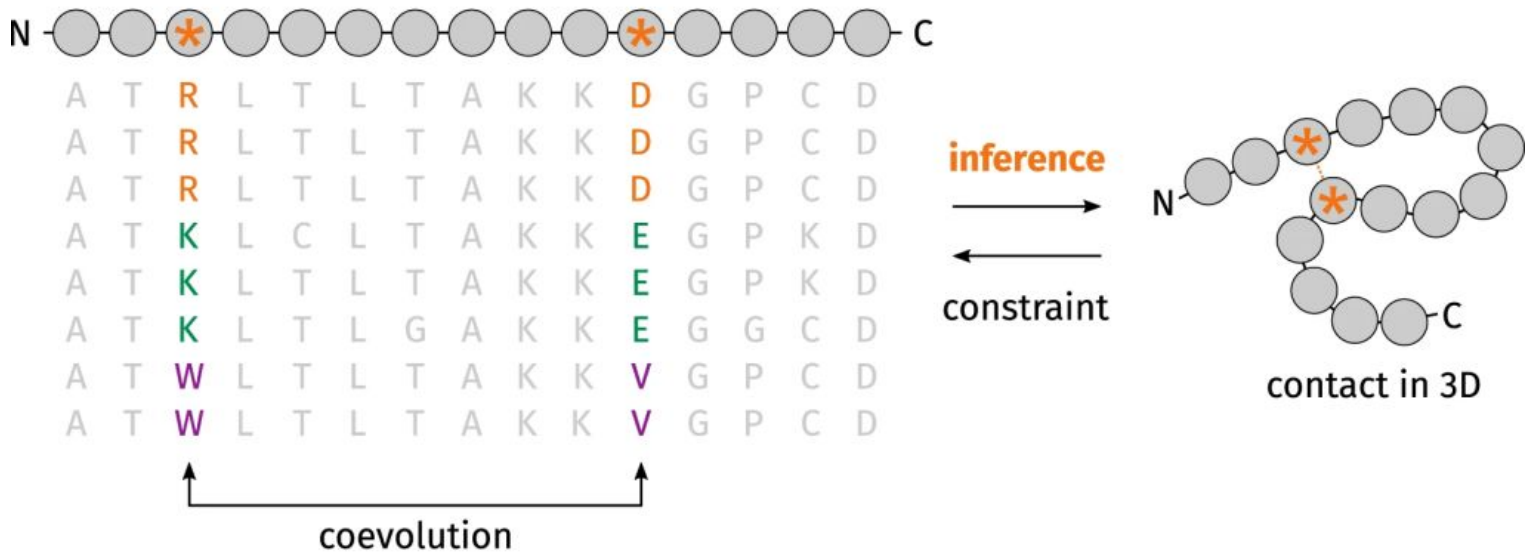
- **Levinthal's paradox:** *It would take a protein the present age of the universe to explore all possible configurations and find the minimum energy configuration. Yet proteins fold in microseconds*.
- **CASP: Critical Assessment of Techniques for Protein Structure Prediction**
- A thought-provoking blog from Mohammed AlQuraishi: AlphaFold @ CASP13: “What just happened?”, with an informal but good overview of history of protein structure prediction, and his indictment (criminal accusations) of both academia and pharma.
- By 2021 AlphaFold2 and RoseTTAfold reach experiment-level accuracy in some predictions of protein static structure

AlphaFold2 uses co-evolution of residues, determined structures, and neural networks to achieve the high performance



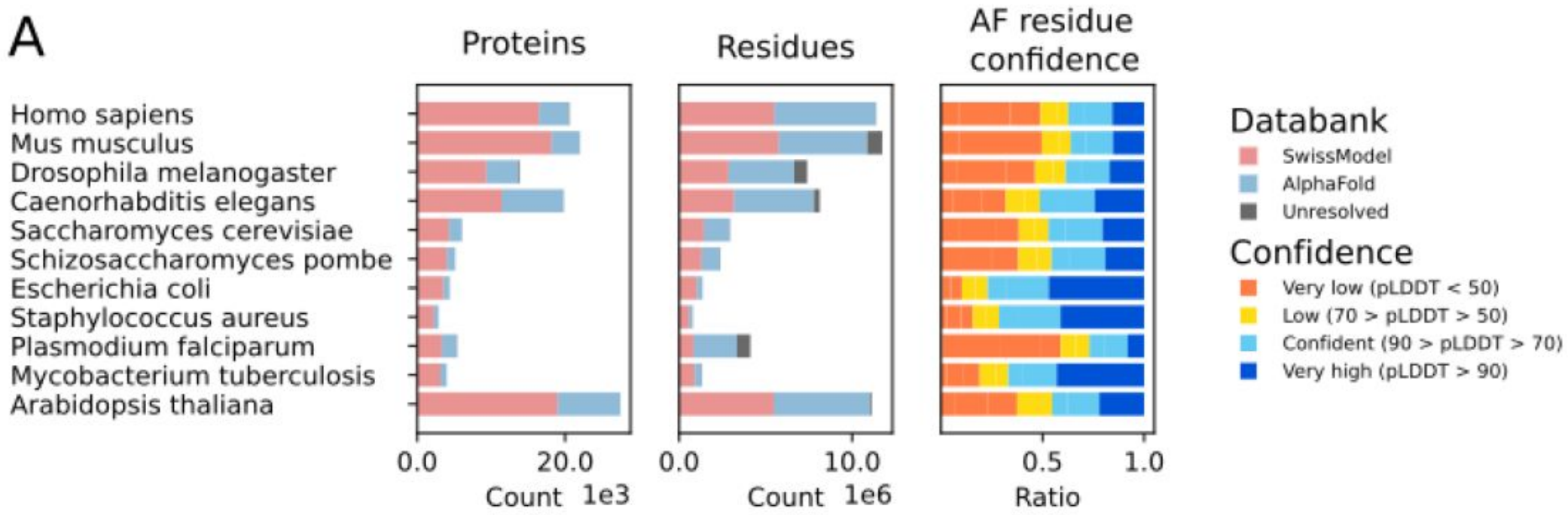
- Jumpe et al. “Highly Accurate Protein Structure Prediction with AlphaFold.” Nature 596, no. 7873 (August 2021): 583–89. https://doi.org/10.1038/s41586-021-03819-2.
- A blog post that explains how AlphaFold2 works: blogpig.com

The key idea (beyond using 2D and 3D structure mapping): learning from evolutionary constraints



Marks, Debora S., Lucy J. Colwell, Robert Sheridan, Thomas A. Hopf, Andrea Pagnani, Riccardo Zecchina, and Chris Sander. “Protein 3D Structure Computed from Evolutionary Sequence Variation.” PLOS ONE 6, no. 12 (December 7, 2011): e28766. https://doi.org/10.1371/journal.pone.0028766.

AlphaFold2 & RoseTTAfold extend our understanding of protein biology, while their impact on drug discovery remains to be seen



Akdel, Mehmet, Douglas EV Pires, Eduard Porta-Pardo, Jurgen Janes, Arthur O. Zalevsky, Balint Meszaros, Patrick Bryant, et al. “A Structural Biology Community Assessment of AlphaFold 2 Applications,” September 26, 2021. https://doi.org/10.1101/2021.09.26.461876.

Offline activities: promises and challenges of leveraging predicted protein structures in drug discovery

Your answers about promising applications of protein structure prediction tools, summarized:

- Target finding
- **Design drugs that interact with proteins**
- **Complement experimental approaches to solve protein structure**
- **Virtual screening**
- **Predicting 3D structure changes induced by mutations**

Your answers about limitations, summarized:

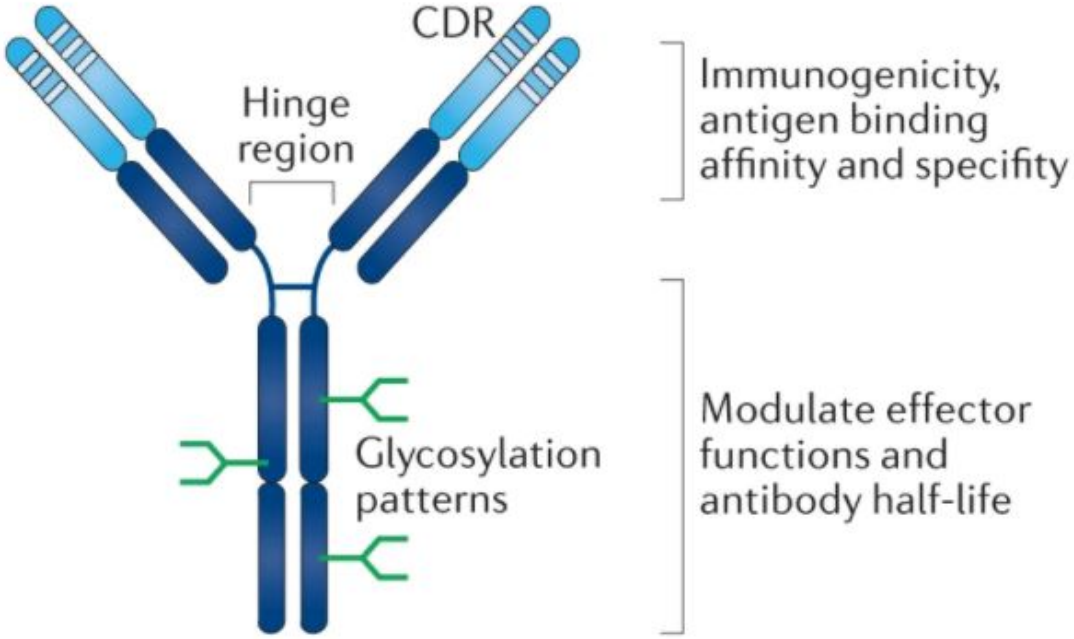
- **Low accuracy of sites in proteins where the drug molecule binds to, either active or allosteric, because they tend to break the folder rules**
- Training based on public data only
- **Difficulty in predicting flexible and rare conformations of proteins**
- **Difficulty in predicting off-target effects**

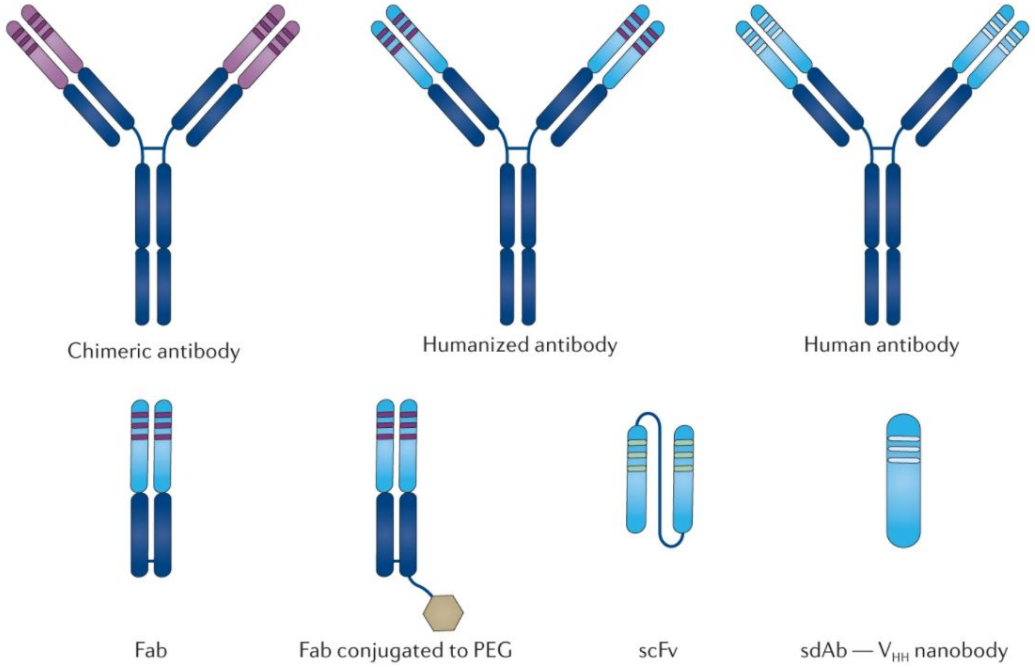
Your question: difference between AlphaFold, AlphaFold2, and RoseTTAFold.

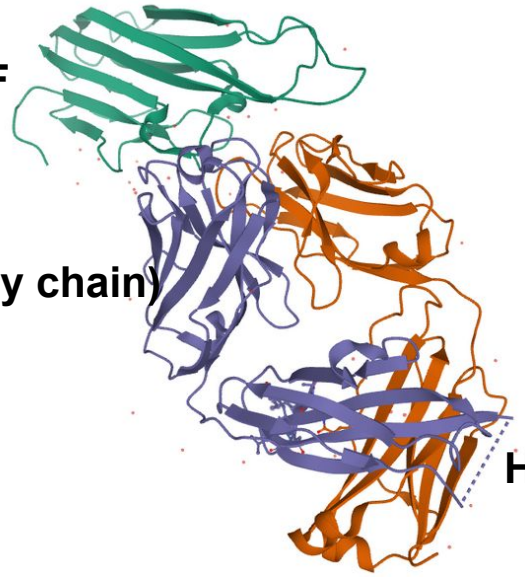
Brief introduction to AlphaFold (2) and RoseTTAFold

- AlphaFold (available in 2018, relevant research since ~2010s)
  - Key assumption: **a distance map,** created by following the observation that co-evoluting amino acids have close physical interactions.
  - Key algorithm: graph neural networks that predict distances between distances, as well as φ (Psi, dihedral angle of the N-Cα bond) and ψ (Phi, C-Cα bond) angles for each amino acid. Trained with amino-acid and structural data of 29,000 proteins, with neural network and gradient descent.
- AlphaFold2 (available in 2020)
  - Improving drawback of AlphaFold1, which overwrites interactions between nearby residues over residues further apart.
  - Major changes
    - Transformers that refine a vector representation of each relationship between two amino acids in the protein. Attention mechanism is used to learn from data.
    - A single differentiable end-to-end model instead of modular models
    - Local physicals is applied only at the final refinement step.
- RoseTTAFold (Science 2021): a three-track network integrating 1D (sequence), 2D (distance), and 3D (coordinate) level information. Possible to model protein-protien complexes. Code and server available.

Antibodies are also proteins







PDB 3WD5, Crystal structure of *TNF-alpha* in complex with *Adalimumab* (*Humira*) Fab fragment, PubMed: 23943614

Attwood, Misty M., Jörgen Jonsson, Mathias Rask-Andersen, and Helgi B. Schiöth. 2020. “Soluble Ligands as Drug Targets.” Nature Reviews Drug Discovery 19 (10): 695–710. https://doi.org/10.1038/s41573-020-0078-4.

What properties must a drug satisfy?

- **Potency**
- **Selectivity**
- Physico-chemical properties
- Administration, Distribution, Metabolism, Excretion (ADME)
- Safety
- Formulation
- Stability
- ...

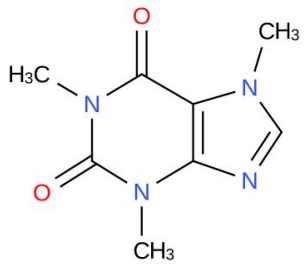
ChEMBL as information source of small molecules

Nomenclature

caffeine
1,3,7-trimethylxanthine
methyltheobromine

Bioactivity

Affinity to human proteins and drug targets



Chemical data

Formula: $C_8H_{10}N_4O_2$
Charge: 0
Mass: 194.19

Database Xrefs

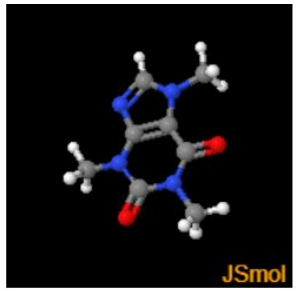
PubChem: CID2519
BindingDB: 1849

Chemical Informatics

***InChI**=1/C8H10N4O2/c1-10-4-9-6-5(10)7(13)12(3)8(14)11(6)2/h4H,1-3H3*

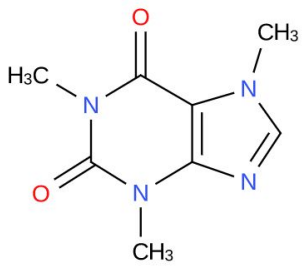
***SMILES**: CN1C(=O)N(C)c2ncn(C)c2C1=O*

Visualisation



A subset of available information from EBI ChEBI/ChEMBL, inspired by EBI's roadshow *Small Molecules in Bioinformatics*

Representation of small molecules



Molfile: </> View Raw | Download | Editor | Copy

Canonical SMILES: CN1C(=O)N(C)c2ncn(C)c2C1=O

Standard InChI: InChI=1S/C8H10N4O2/c1-10-4-9-6-5(10)7(13)12(3)8(14)11(6)2/h4H,1-3H3

Standard InChI Key: RYYVLZVUVIJVGH-UHFFFAOYSA-N

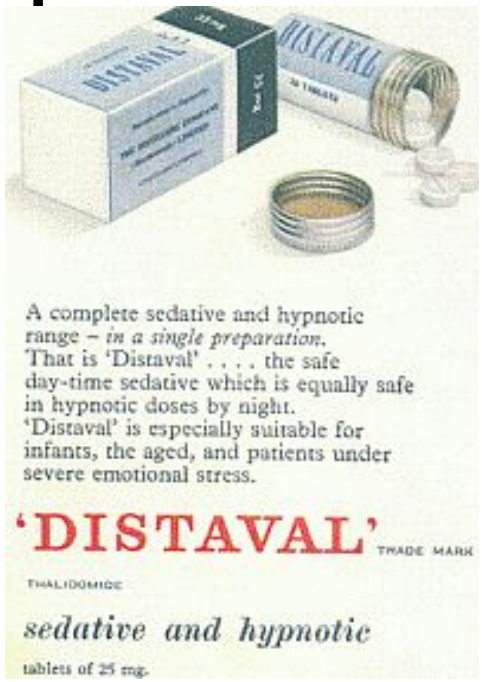
```
CHEMBL113

SciTegic12231509382D

14 15 0 0 0 0 999 V2000
-1.1875 -9.6542 0.0000 C 0 0
-1.1875 -8.9625 0.0000 C 0 0
-1.8125 -10.0292 0.0000 N 0 0
-2.4167 -8.9625 0.0000 N 0 0
-2.4167 -9.6542 0.0000 C 0 0
-1.8125 -8.6000 0.0000 C 0 0
-0.5000 -9.8917 0.0000 N 0 0
-0.5000 -8.7625 0.0000 N 0 0
-0.1125 -9.3042 0.0000 C 0 0
-3.0250 -10.0375 0.0000 O 0 0
-1.8125 -7.8917 0.0000 O 0 0
-1.8125 -10.7417 0.0000 C 0 0
-3.0250 -8.6000 0.0000 C 0 0
-0.2917 -8.0750 0.0000 C 0 0
2 1 2 0
3 1 1 0
4 5 1 0
5 3 1 0
6 2 1 0
7 1 1 0
8 2 1 0
9 7 2 0
10 5 2 0
11 6 2 0
12 3 1 0
13 4 1 0
```

- Simplified Molecular-Input Line-Entry System (SMILES)
- IUPAC International Chemical Identifier (InChI)
- InChiKey: a 27-character, hash version of InChI
- Molfile: a type of chemical table files

The tragedy of thalidomide and the importance of representation

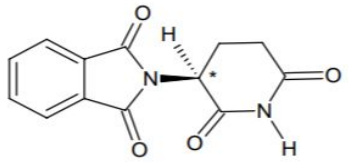


(1957)

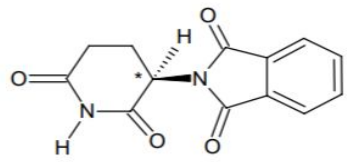
Frances Oldham Kelsey received the President's Award for Distinguished Federal Civilian Service from President John F. Kennedy, 1962

Canonic SMILES of thalidomide

C1CC(=O)NC(=O)C1N2C(=O)C3=CC=CC=C3C2=O



(-)(*S*)-thalidomide



(+)(*R*)-thalidomide

Isomeric SMILES of (-)(S)-thalidomide

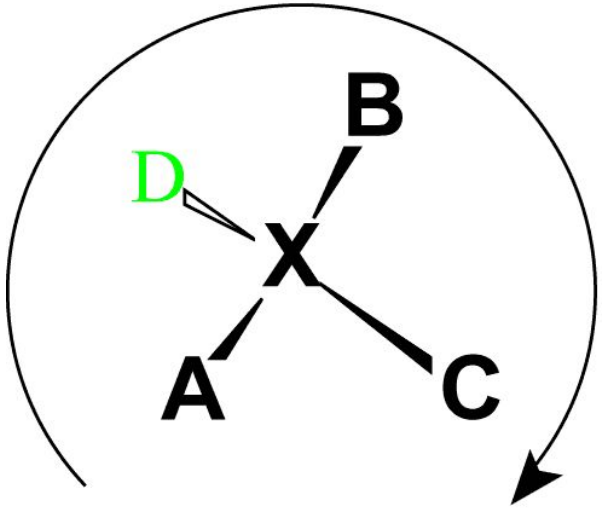
C1CC(=O)NC(=O)[C@H]1N2C(=O)C3=CC=CC=C3C2=O

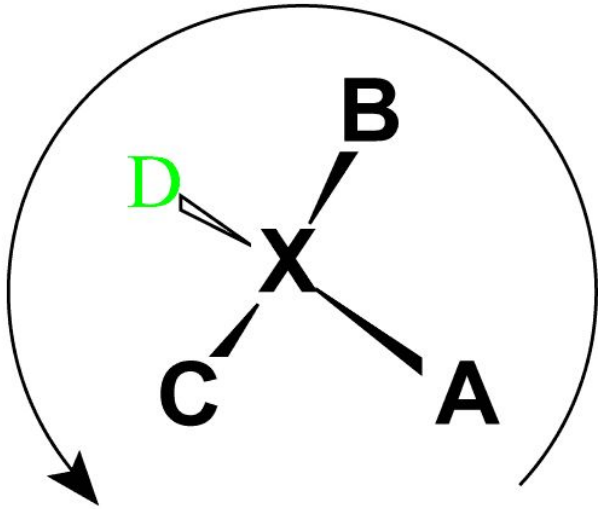
Isomeric SMILES of (+)(R)-thalidomide

C1CC(=O)NC(=O)[C@@H]1N2C(=O)C3=CC=CC=C3C2=O

I thank Manuela Jacklin for her help preparing this slide.

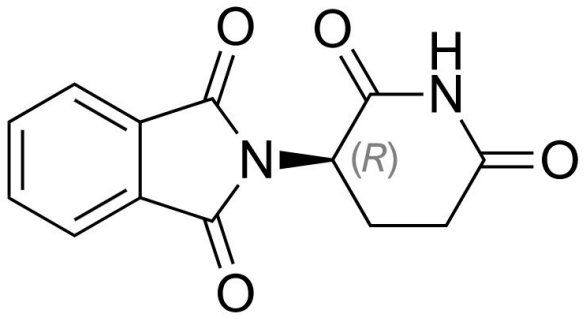
Absolute configuration of atoms within a chiral molecule





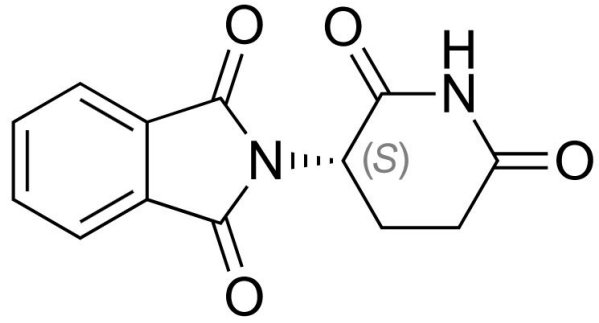
R

S



Sedative
Non-teratogenic

Embryo-toxic
Teratogenic



Conclusions

- **A successful drug must possess many properties**, among others potency, selectivity, physico-chemical/ADME properties, and safety profiles. These need to be considered in the screening process.
- **Drug screening means to identify drug candidates (small molecules, antibodies, oligonucleotides, *etc.*) to modulate target function.** We need to understand the target (mostly proteins), the ligand (small molecules, antibodies, oligonucleotides), and the interaction between them (binding mode, affinity, consequence of modulation, *etc.*).
- **Protein structures can be determined experimentally (X-ray, NMR, CyroEM) or by *in silico* prediction (homology modelling, AlphaFold2/RoseTTAfold).**
- **Small molecules can be presented by symbols and by molecular descriptors.**

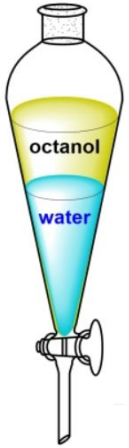
Offline activities

- **Protein structure and protein-ligand interaction**
  - Watch the YouTube video about the Ramachandran Principal by Prof. Eric Martz, or read the notes (including slides) on Proteopedia, and finish a Practice Quiz.
- **Required reading:**
  - Selected pages of *Evaluation of the Biological Activity of Compounds: Techniques and Mechanism of Action Studies* by Dougall and Unitt and answer questions (see the next slide). Please submit your results to the Google Form.
- **Optional reading** based on your interests**:**
  - [Mathematics and structural biology] Mathematical techniques used in biophysics by J. R. Quine.

Backup slides

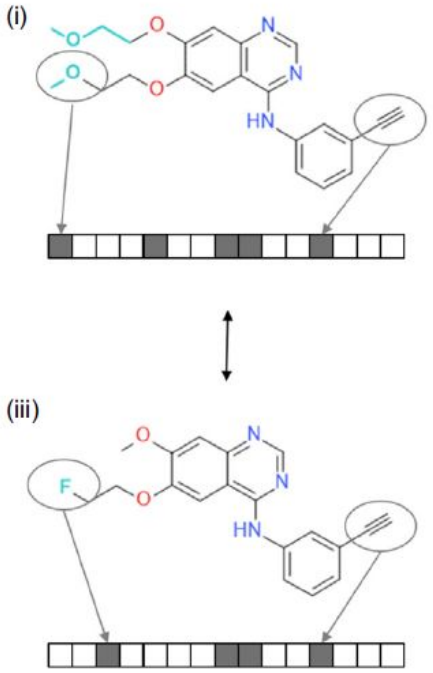
Molecular descriptors: numeric values that describe chemical molecules.

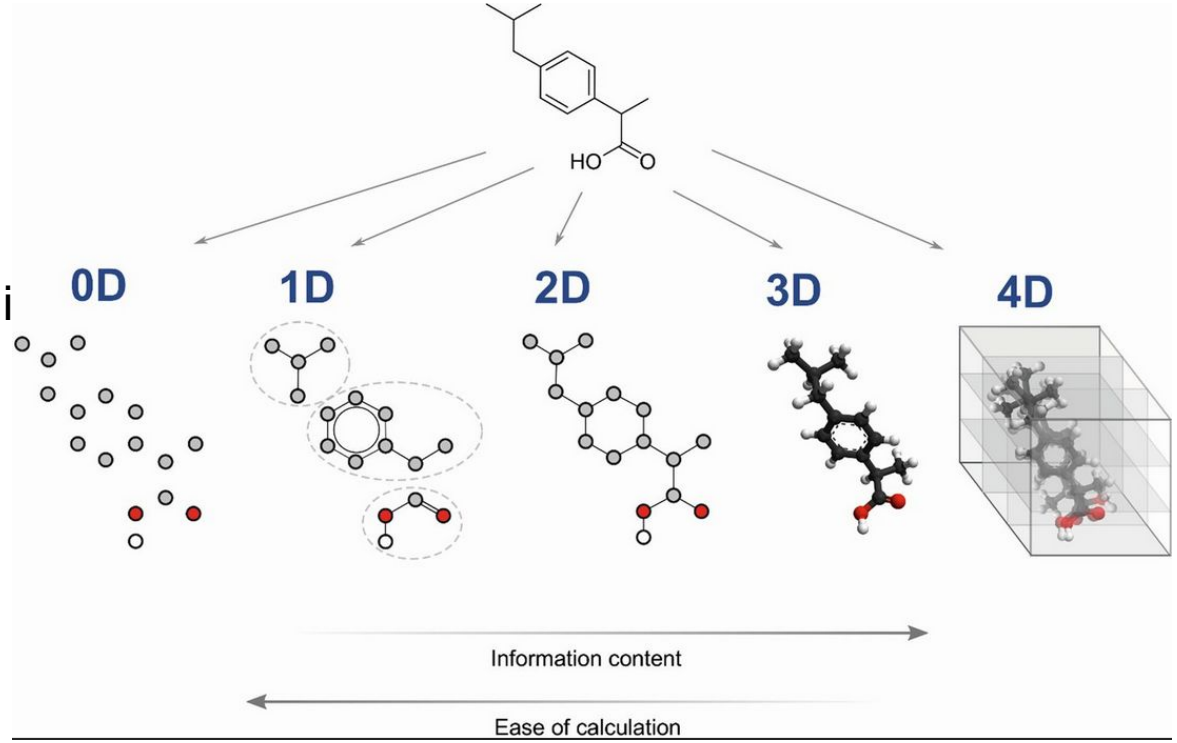
In contrast to symbolic representations, molecular descriptors enable **quantification of molecular properties**. It allows mathematical operations and statistical analysis that associate biophysical/biochemi properties with molecule structures.



$$\log P_{\mathrm{oct/wat}} = \log\left(\frac{[\mathrm{solute}]^{\text{un-ionized}}_{\text{octanol}}}{[\mathrm{solute}]^{\text{un-ionized}}_{\text{water}}}\right)$$

logP is an experimental molecular descriptor. Calculated version (cLogP) exists as well.





- Atom count
- Molecular weight
- Sum of atomic properties

- Fragment counts, *e.g.* # of -OH
- Fingerprints

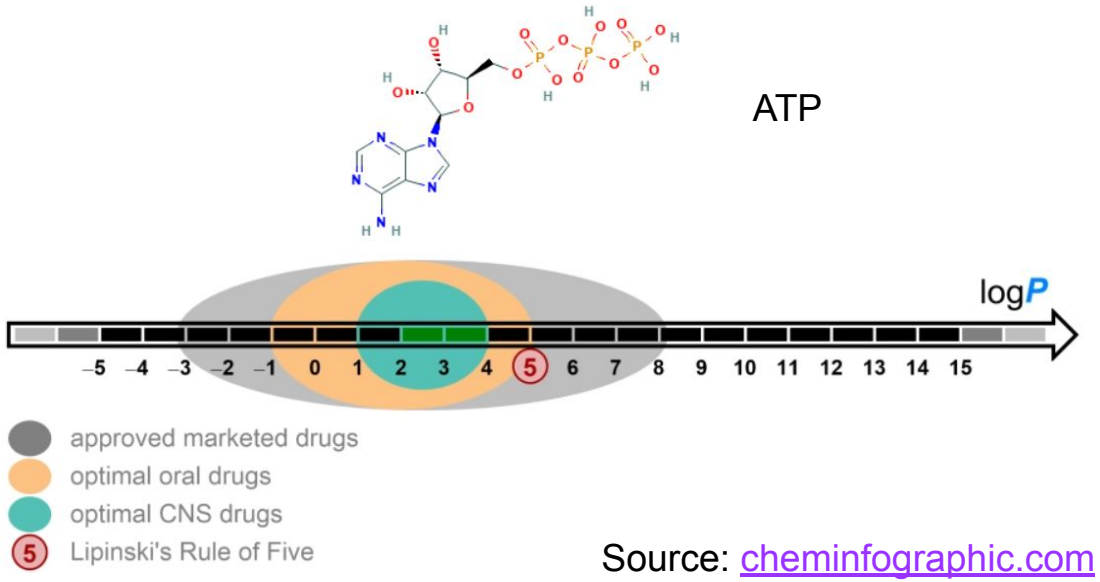
- Topological descriptors, *e.g.* the Wiener Index, sum of lengths of the shortest paths between all non-H atoms

- Geometrical
- Atomic coordinates
- Energy grid

- Combination of atomic coordinates and sampling of possible conformations

Lipinski's Rule of Five of small-molecule drugs

- **HBD<=5:** No more than **5 hydrogen-bond donors,** *e.g.* the total number of nitrogen–hydrogen and oxygen–hydrogen bonds.
- **HBA<=10**: No more than **10 hydrogen-bond acceptors**, *e.g.* all nitrogen or oxygen atoms
- **MW<500**: A **molecular weight** less than **500 Daltons, or** 500 g/mol. Reference: ATP has a molecular mass of ~507.
- **logP<=5**: An **octanol-water partition coefficient (log P)** that does not exceed **5.** (10-based)



Source: cheminfographic.com

Table 1. New FDA Approvals (2014 to Present)a of Oral bRo5 Drugs

drug	year approved	therapeutic area	MW	cLogP	HBD	N+O
velpatasvir	2016	HCV	883.02	2.5	4	16
venetoclax	2016	oncology	868.44	10.4	3	14
elbasvir	2016	HCV	882.0	2.6	4	16
grazoprevir	2016	HCV	766.90	−2.0	3	15
cobimetinib	2015	oncology	531.31	5.2	3	5
daclatasvir	2015	HCV	738.88	1.3	4	14
edoxaban	2015	cardiovascular	548.06	−0.9	3	11
ombitasvir	2014	HCV	894.13	1.3	4	15
paritaprevir	2014	HCV	765.89	1.1	3	14
netupitant	2014	nausea from chemotherapy	578.59	6.8	0	5
ledipasvir	2014	HCV	889.00	0.9	4	14
ceritinib	2014	oncology	558.14	6.5	3	8

DeGoey, *et al.*. 2018. "Beyond the Rule of 5: Lessons Learned from AbbVie's Drugs and Compound Collection." Journal of Medicinal Chemistry 61 (7): 2636–51.

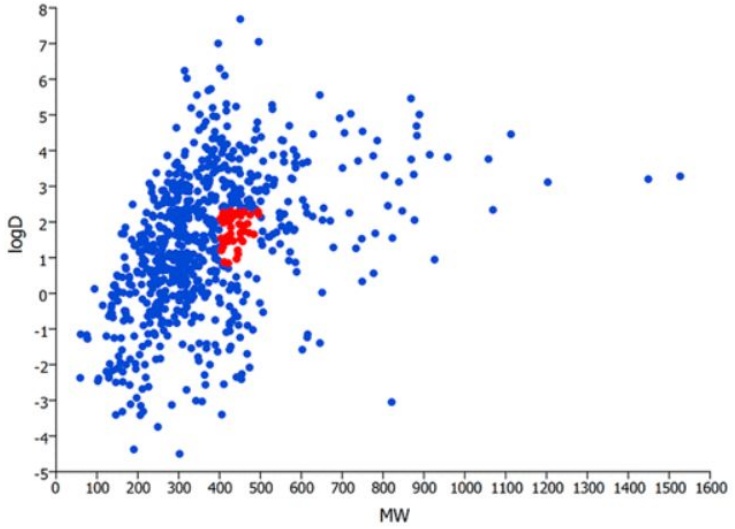
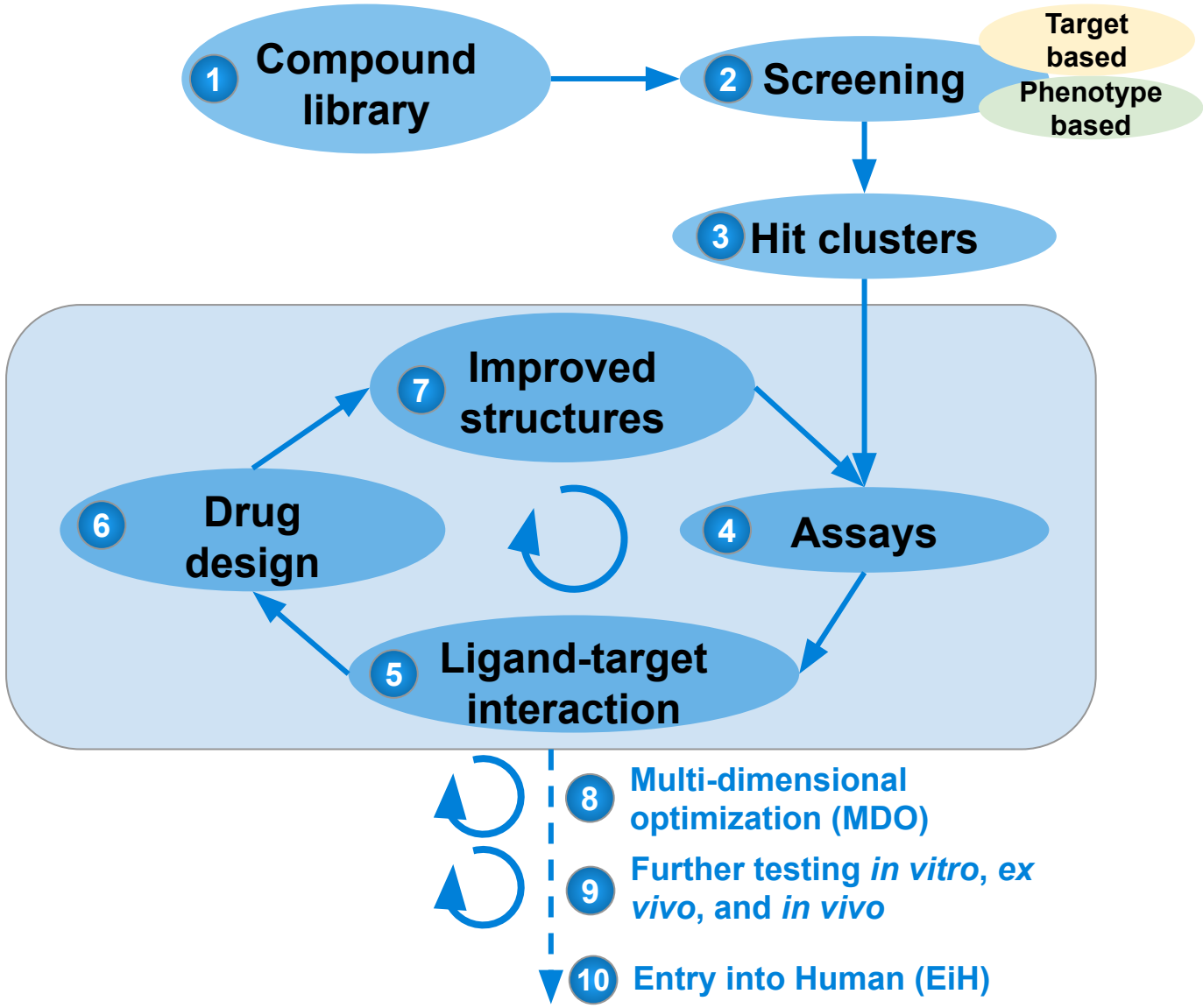


Figure 7: Plot of MW vs cLogD of FDA approved oral drugs. Red points: 'high probability area' supposed by (questionable) data analysis. Shultz, Michael D. 2019. "Two Decades under the Influence of the Rule of Five and the Changing Properties of Approved Oral Drugs." Journal of Medicinal Chemistry 62 (4): 1701–14.

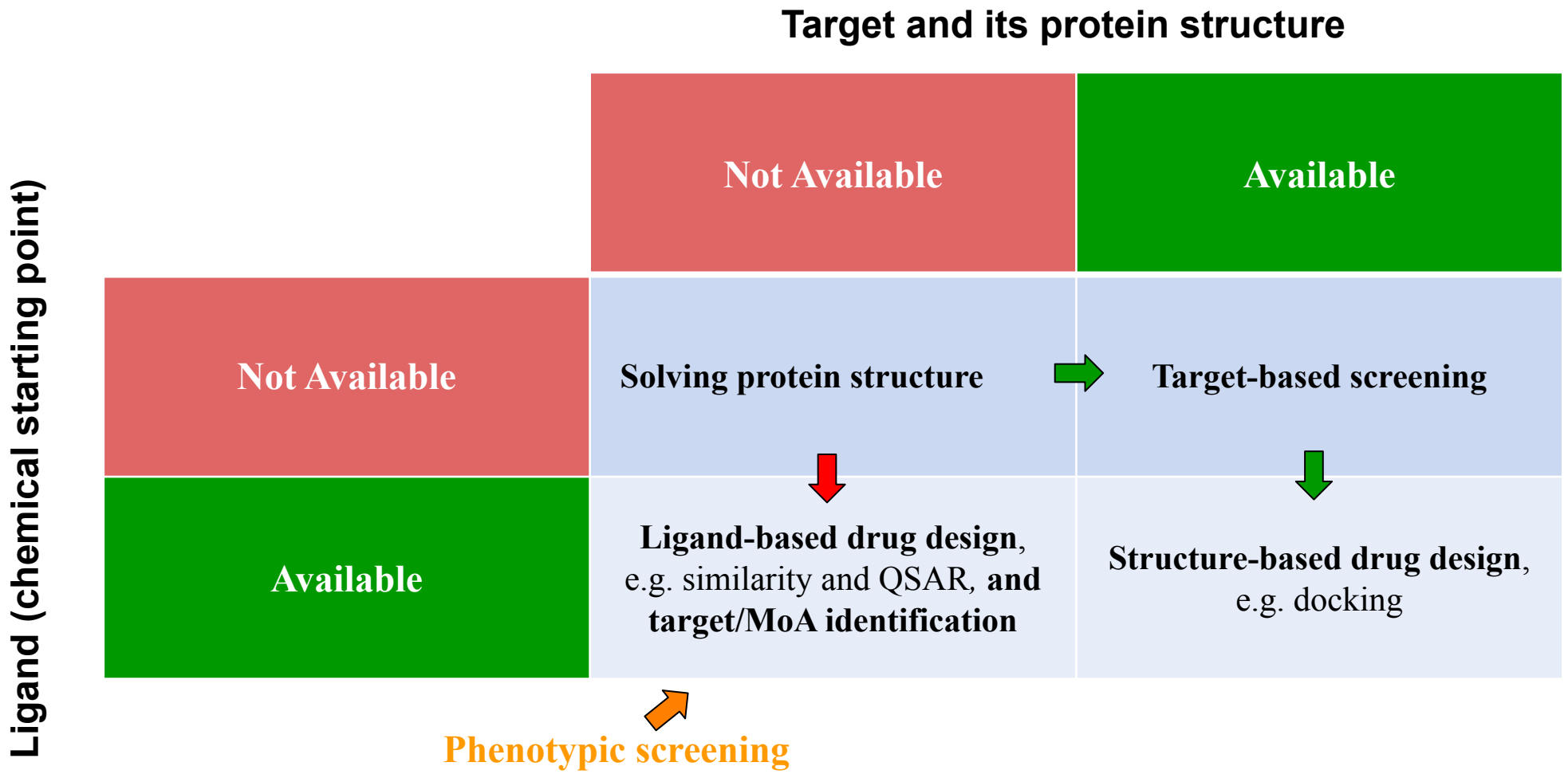
Workflow in a typical drug-discovery program

1. Compound library construction;
2. Screening compounds with ***bioassays***, or ***assays***, which determine potency of a chemical by its effect on biological entities: proteins, cells, *etc*;
3. Hit identification and clustering;
4. More assays, complementary to the assays used in the screening, maybe of lower throughput but more biologically relevant;
5. Analysis of ligand-target interactions, for instance by getting the co-structure of both protein (primary target, and off-targets if necessary) and the hit;
6. *Drug design,* namely to modify the structure of the drug candidate;
7. Analog synthesis and testing (back to step 4);
8. Multidimensional Optimization (MDO), with the goal to optimize potency, selectivity, safety, bioavailability, *etc;*
9. Further *in vitro*, *ex vivo*, and *in vivo* testing, and preclinical development;
10. Entry into human (Phase 0 or phase 1 clinical trial).



A schematic presentation of structure-based drug discovery

Ligand-based and structure-based drug design



QSAR= quantitative structure activity relationship; MoA= mechanism of action, or mode of action