Computational biology

Co-evolution to predict protein structures

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Topic: protein structure prediction

Topic of this series of lecture

- Introductory lectures about protein structure prediction
- Project: I picked up one protein I found in marine viruses with unidentified function, will you determine what function this protein is likely to carry out?

Evaluation:

- Project-based (3-5 pages report)
- Explanations of the methods used, with documented references will be the most important criteria for the evaluation.

Today's outline: from gene sequence to protein structure

- Reminder about the central dogma
  - Genomes, genes, proteins
- Protein structure prediction methods
- Focus on *de novo* from covariation
  - Sequence evolution and selective pressure
  - Multiple sequence alignment
  - Residue co-variation

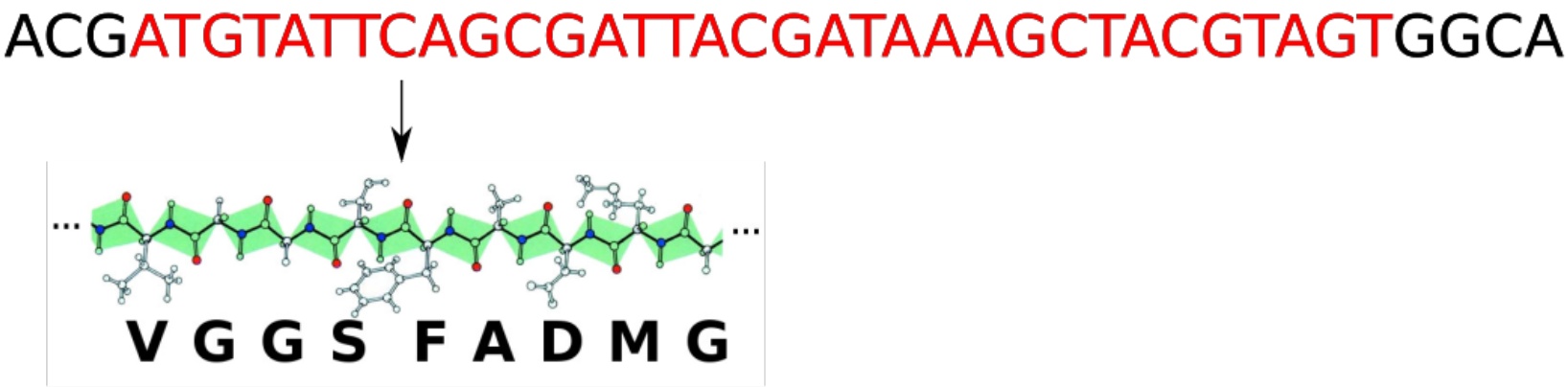
Some background

From genome to function, the very big picture

ACGATGTATTCAGCGATTACGATAAAGCTACGTAGTGGCA

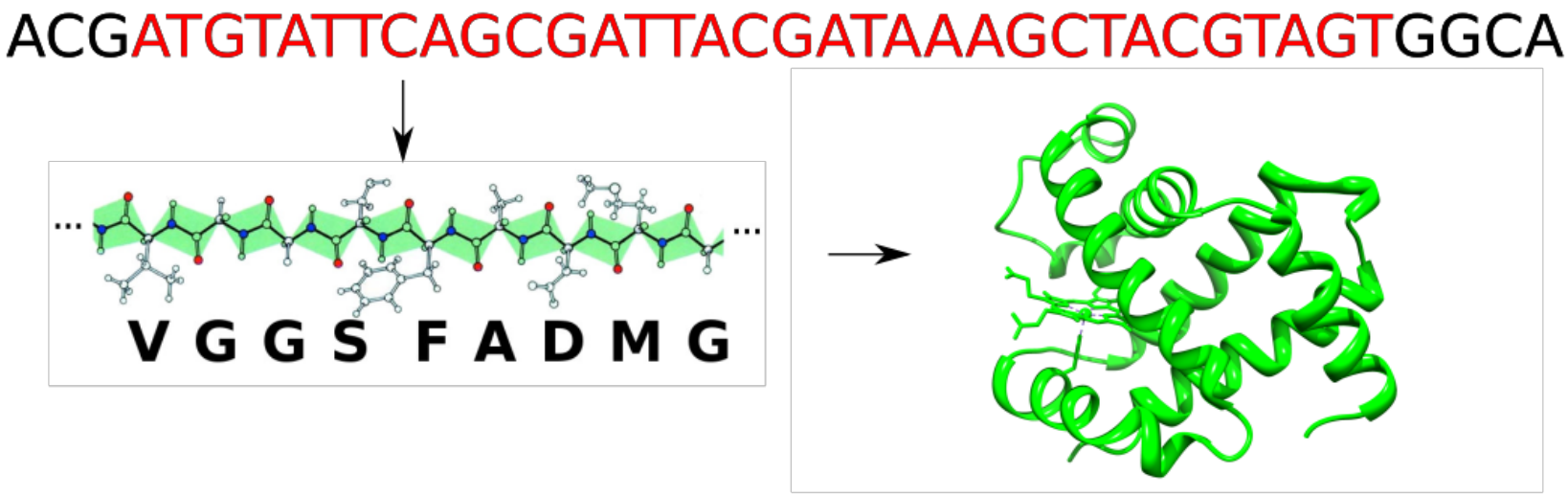
On a genome (∼5Mbp), specific motifs define begining and end of a gene

From genome to function, the very big picture



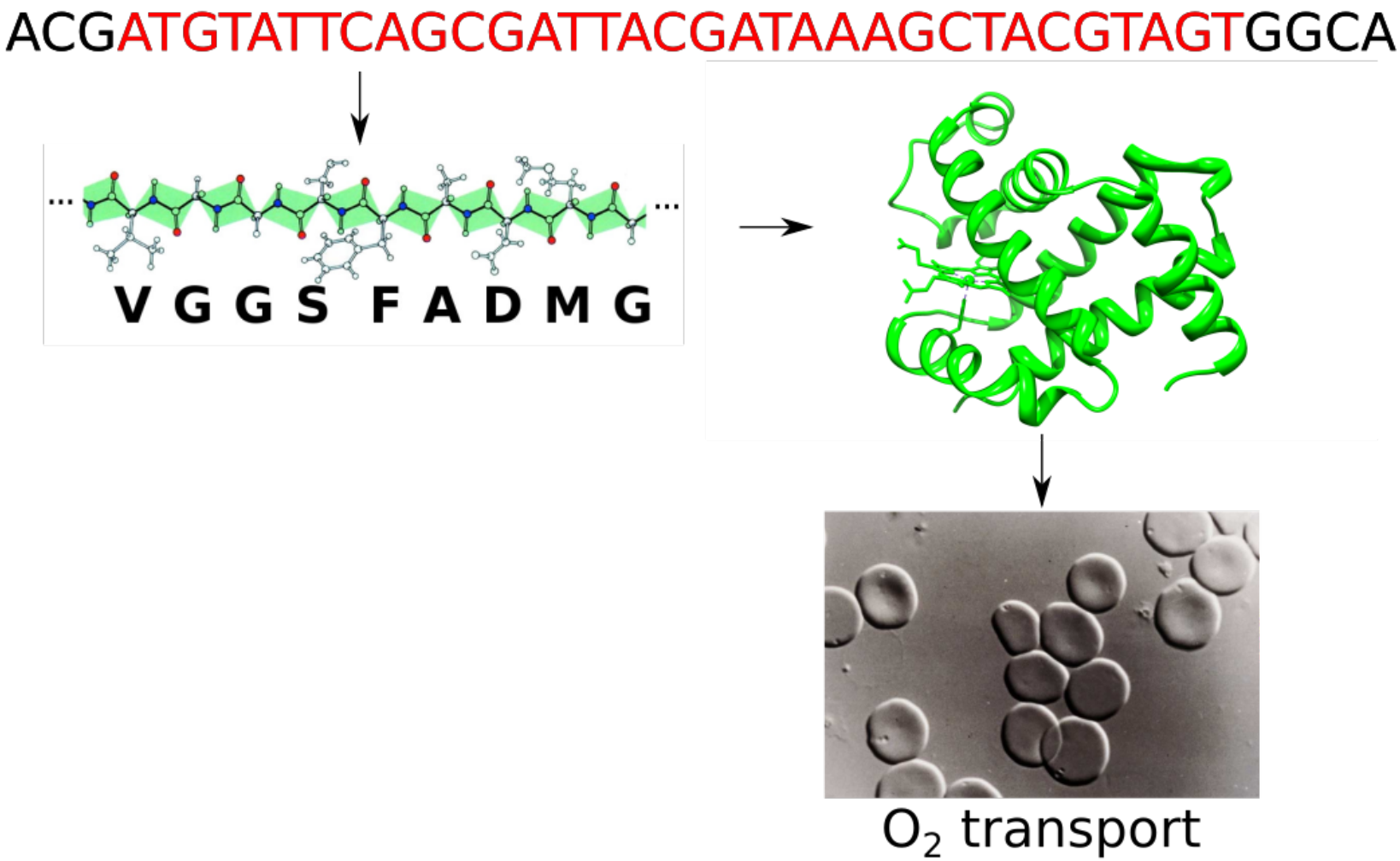
Transcription + *translation*, to form a chain of amino acids (∼300-3000AA)

From genome to function, the very big picture



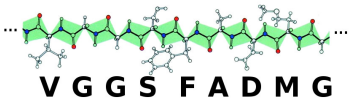
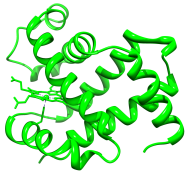
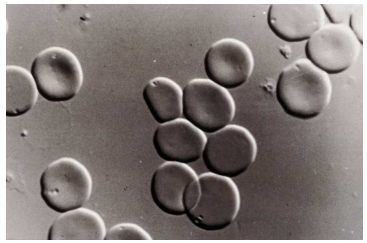
Protein folding under pysico-chemical interactions, diameter $\sim$ few nanometers

From genome to function, the very big picture



Protein endowed with a function (biochemical reactions, transport, etc.)

Data at every steps

Nucleic seq.	Amino acid seq.	Protein	Function
..ATTGTCGAAC..			

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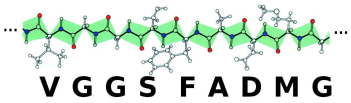
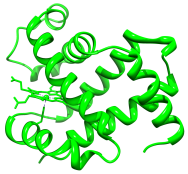
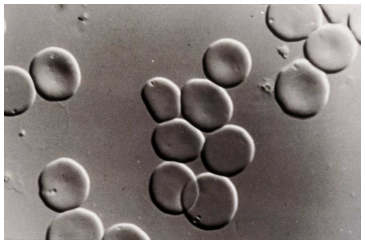
..ATTGTCGAAC..			
NCBI	UniProt	RCSB PDB PROTEIN DATA BANK	
`ncbi.nlm.nih.gov`	`uniprot.org`	`rcsb.org`	`ebi.ac.uk/interpro`

How to predict gene function?

Some gene functions have been previously identified by biologists.

When having an unknown sequence, how can you guess its function?

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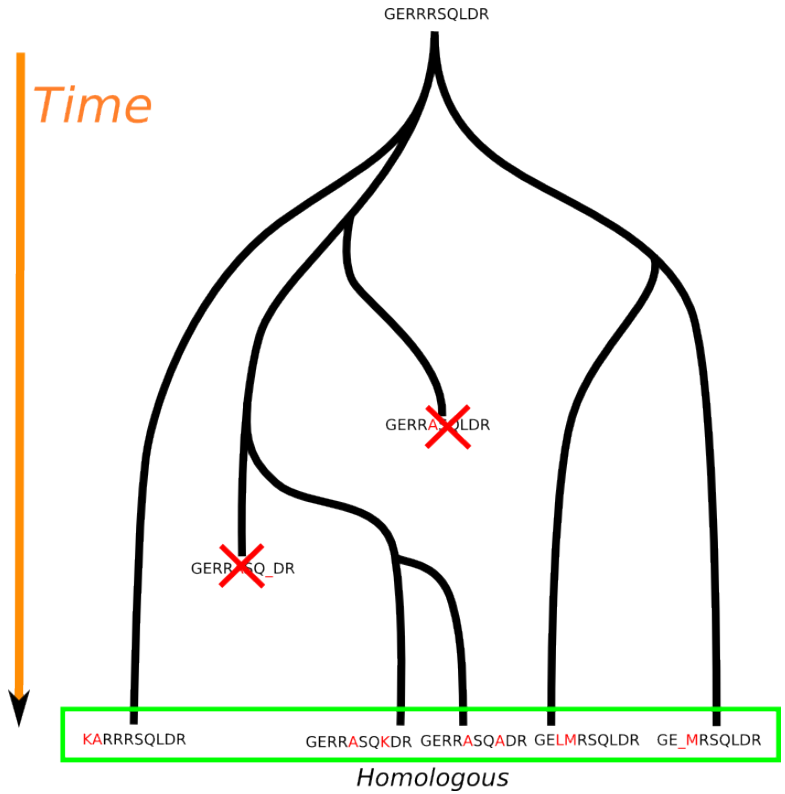

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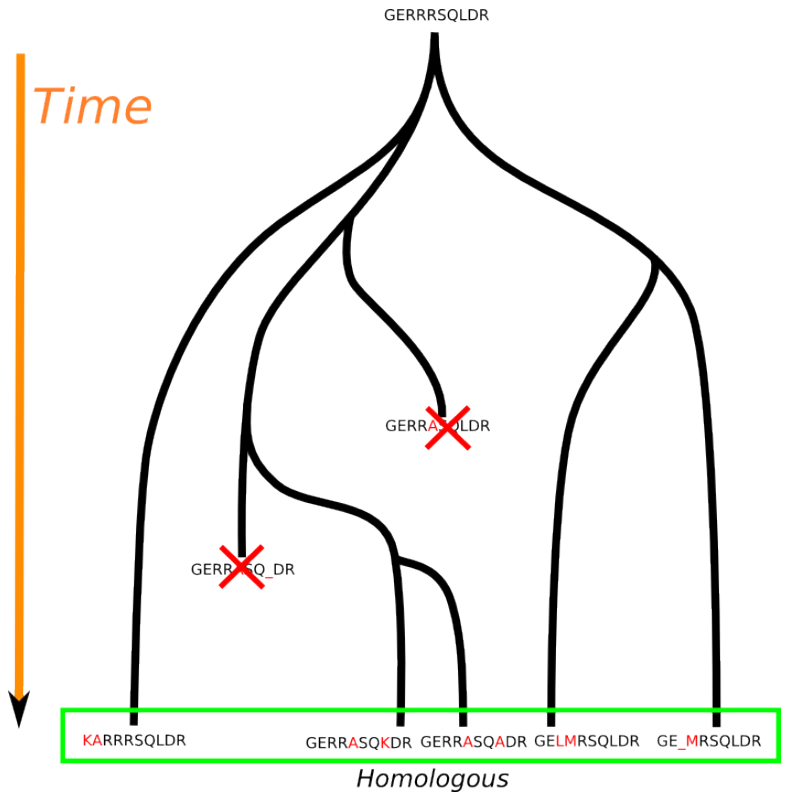

By comparing to millions of existing sequences and hope that **homologuous** genes are already known.

Get insights from the structure

What if no homologous sequences or if they have no functional annotation?

Get insights from the structure

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Look at the structure!

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The bad news is...

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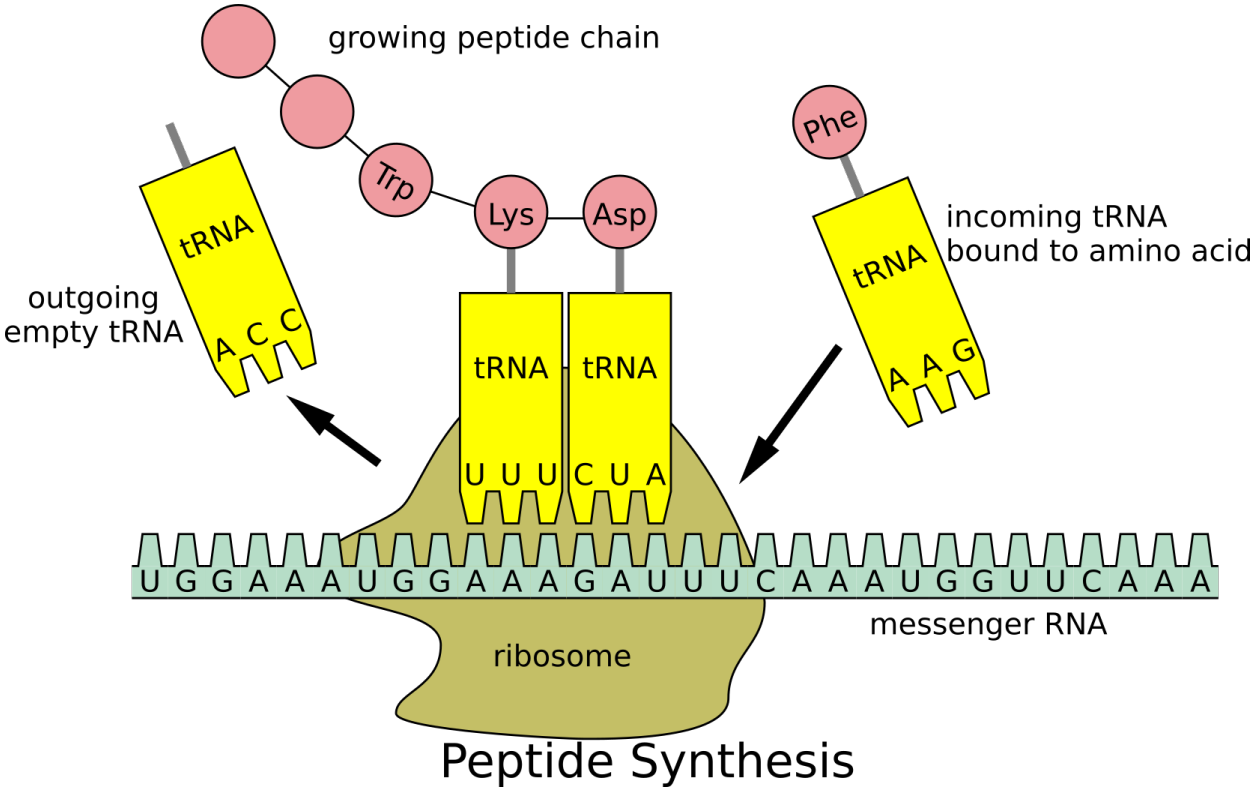
... so have to predict the structure

Zoom: genes to proteins



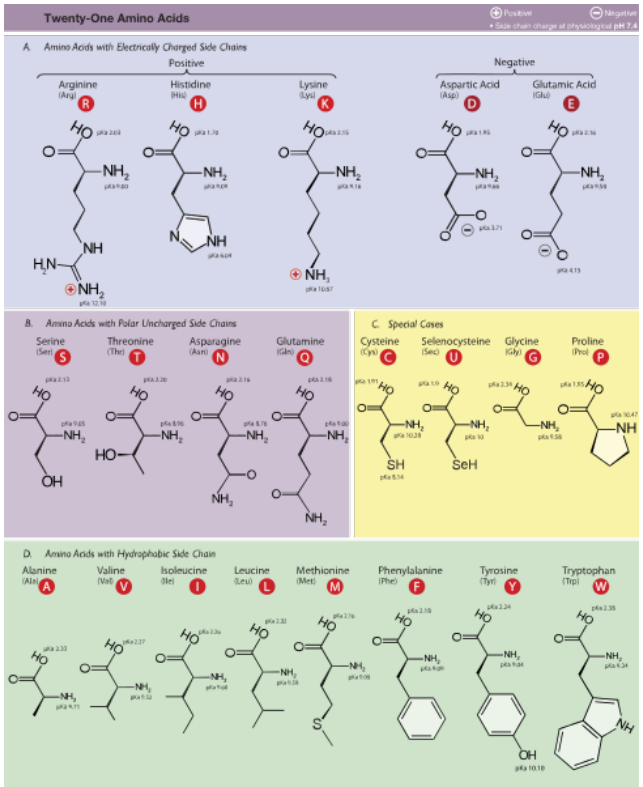
RNA codon table

1st position	2nd position U	2nd position C	2nd position A	2nd position G	3rd position
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	stop	stop	A
	Leu	Ser	stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

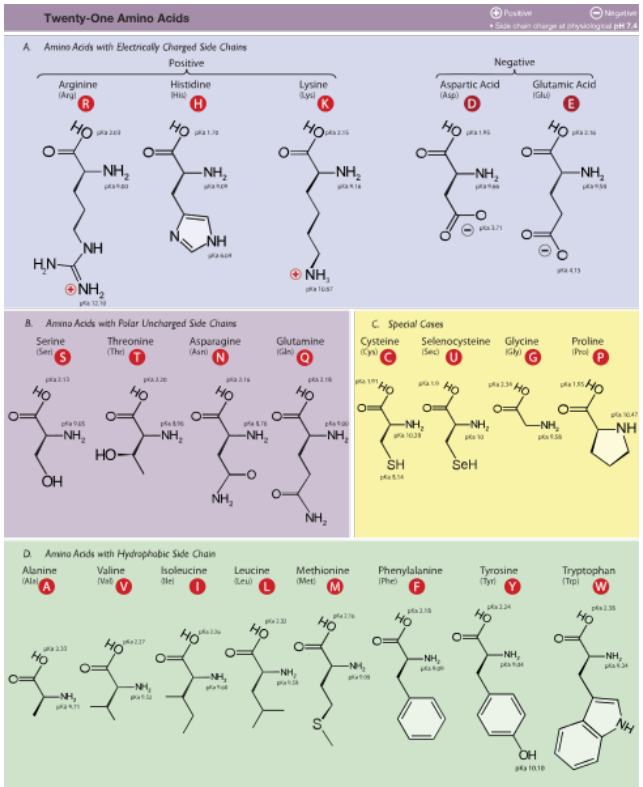
Amino Acids

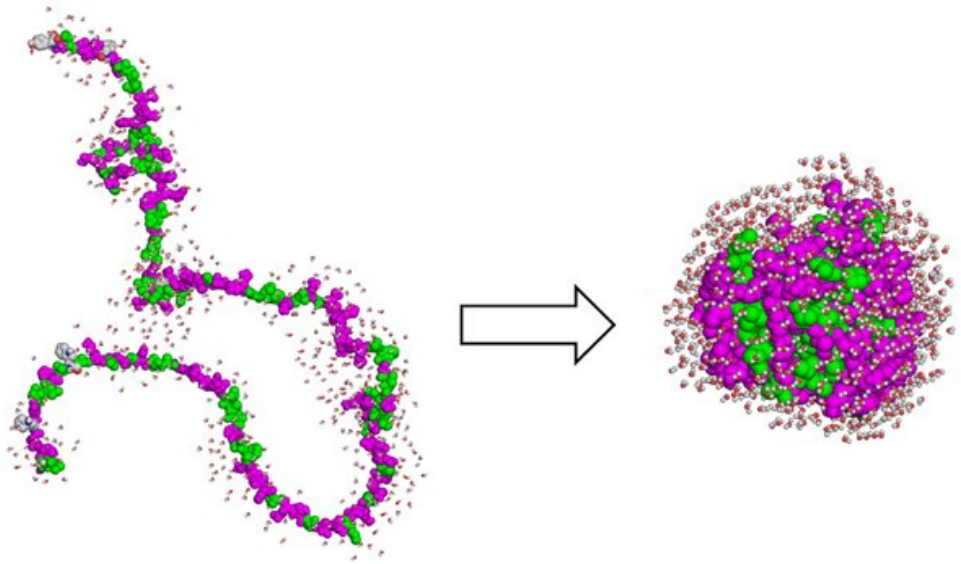
Ala: Alanine
Arg: Arginine
Asn: Asparagine
Asp:Aspartic acid
Cys:Cysteine
Gln: Glutamine
Glu: Glutamic acid
Gly: Glycine
His: Histidine
Ile: Isoleucine
Leu: Leucine
Lys: Lysine
Met: Methionine
Phe: Phenylalanine
Pro: Proline
Ser: Serine
Thr: Threonine
Trp: Tryptophane
Tyr: Tyrosisne
Val: Valine

Primary to tertiary protein structure



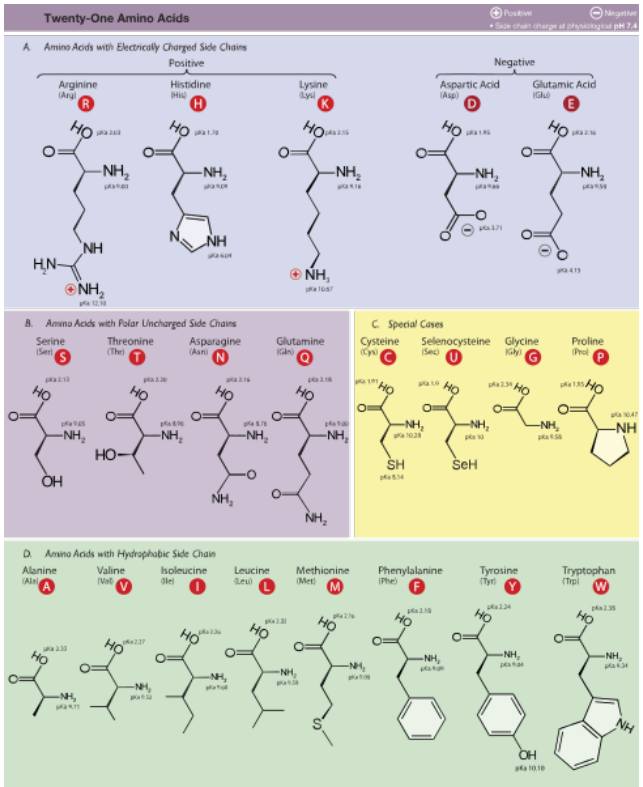
Primary to tertiary protein structure

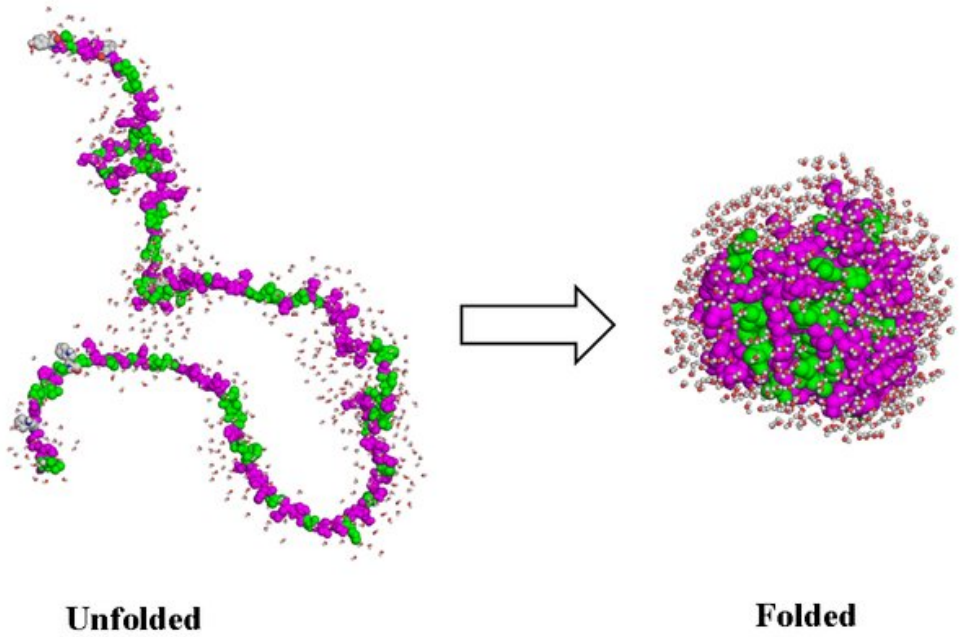




Unfolded **Folded**

Primary to tertiary protein structure

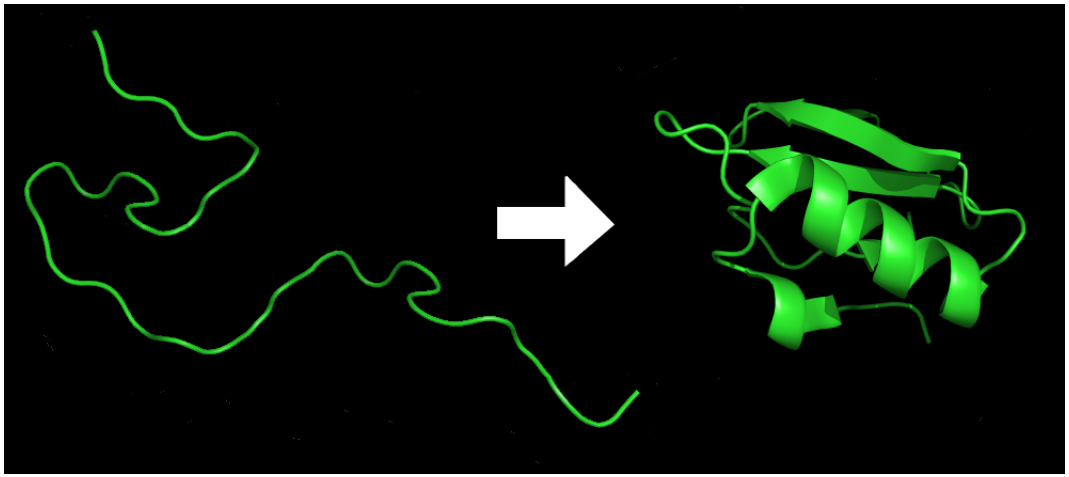




The amino acid sequence is called the **primary** structure of the protein and the final structure is called the **tertiary** protein structure.

Protein folding

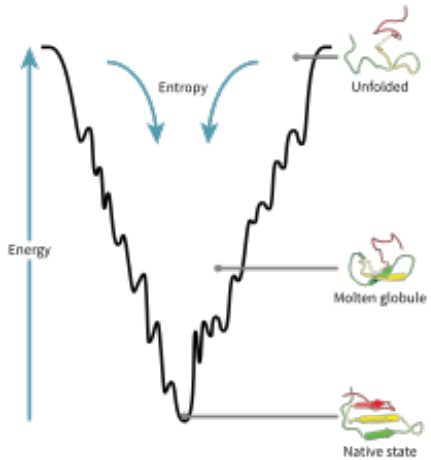
Protein folds to their stable structure in milliseconds [Karplus, 97] under interactions between their amino acids, as well with the environment (mostly water).



Given the large number of possible conformations, the energy landscape cannot be flat (*aka* Levinthal's paradox), and it hints to be a problem computationally tractable.

Not a single perfect model

Several models have been proposed for the folding mechanism, like the funnel energy landscape:



[Wikipedia]

No model gives full satisfaction on all aspect of folding (folding times, physically realistic, computationally tractable, etc.)

Predicting the structure

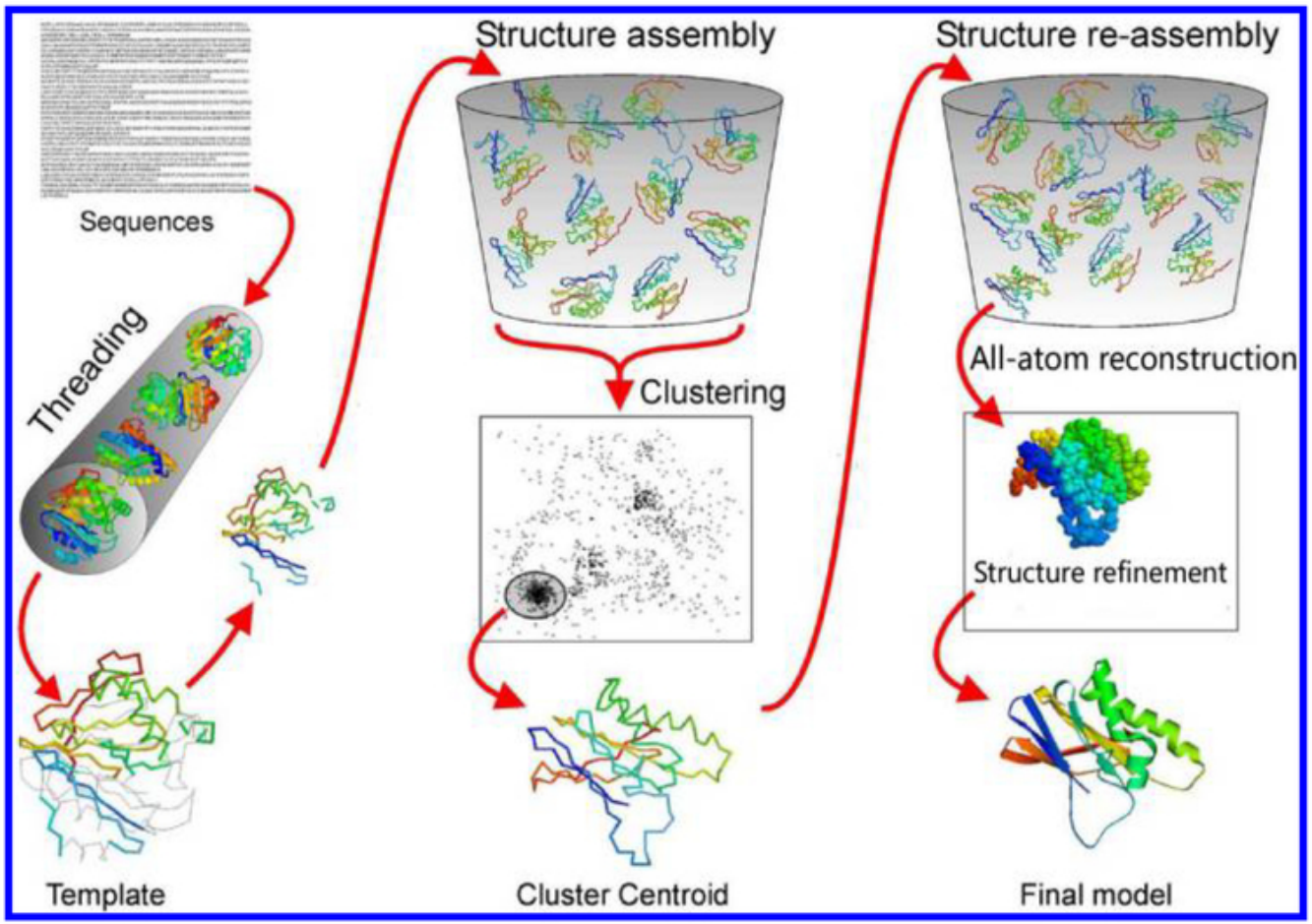
Several types of approaches:

- Molecular dynamics (much costly!): simulate force field between amino acids
- Fragment assembly: Rosetta [Baker Lab 2004]
- Template-based modelling: use known structures with similar sequences [Zhang Lab 2010]
- Coevolution based: [Weigt et al 09], [Jones et al. 2012]
- Hybrid+machine learning methods: AlphaFold2 [Jumper et al. 2021]

The last mentioned method has been a *revolution* for science in 2021. It combines **threading** and **co-evolution** based methods in a **modern AI framework** with attention mechanisms.

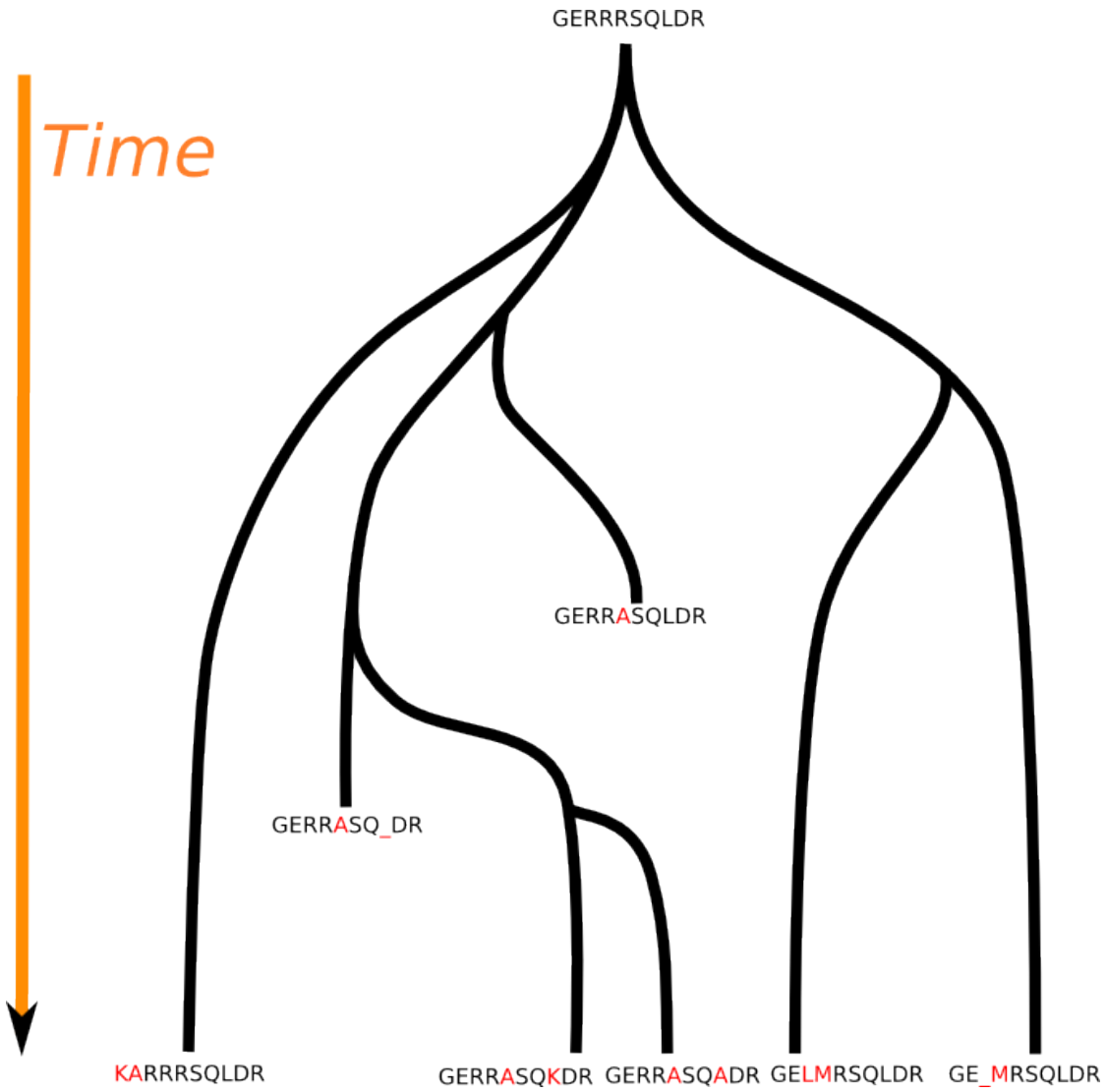
Threading

I-TASSER [Roy et al. 10]:



Covariation for structure prediction

Sequence evolution



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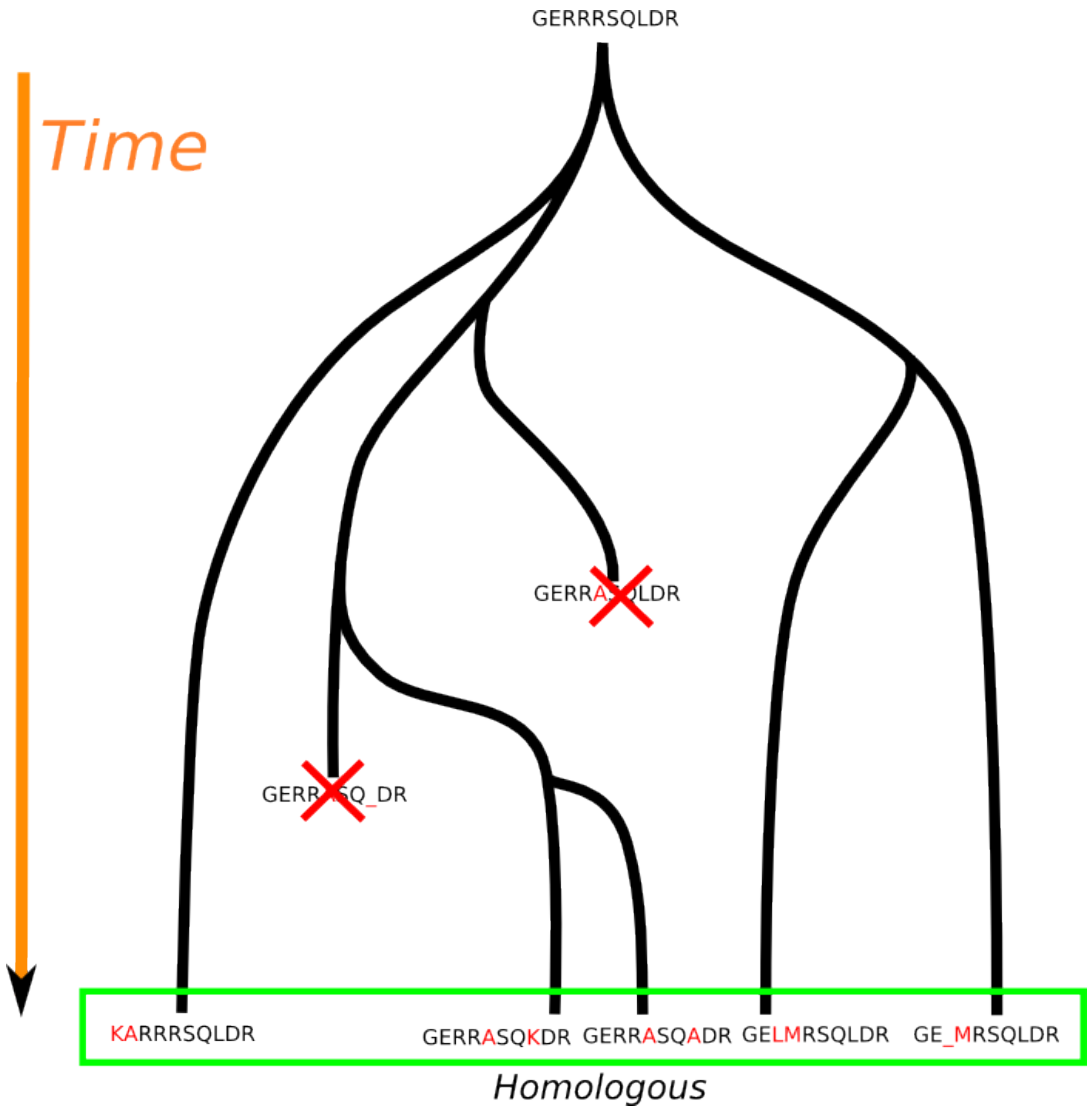

Sequence conservation

Aligning the sequences (MSA, multiple sequence alignment):

Tools	Database
ClustalW [Larkin et al. 07]	Pfam `ebi.ac.uk/interpro/entry/pfam`

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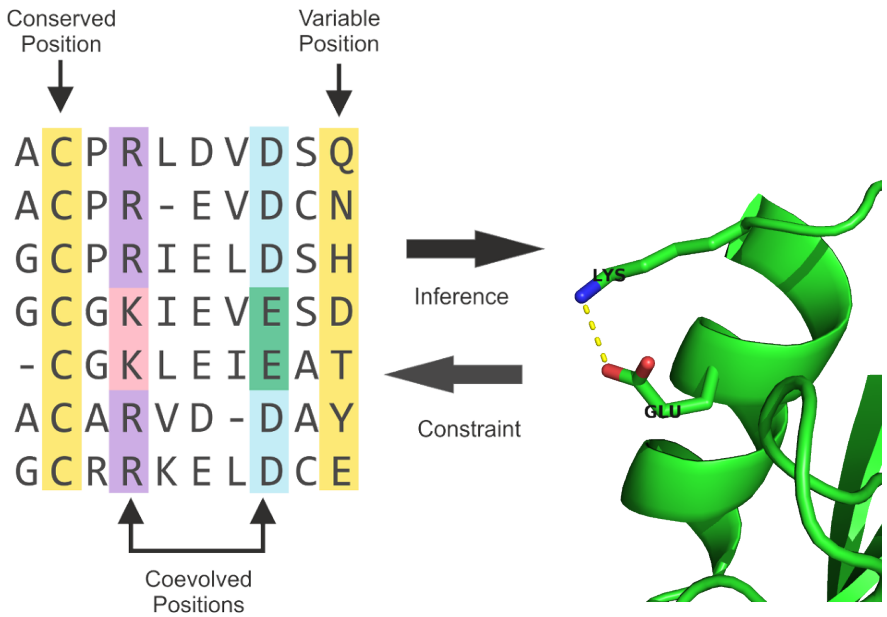
Why some positions are conserved, some other aren't?

Structure is determined by amino acid interactions

Preserving the function: coevolution of residues

As protein function is vital, **evolution selects mutations preserving structures**.
Leading to **compensatory** mutations:



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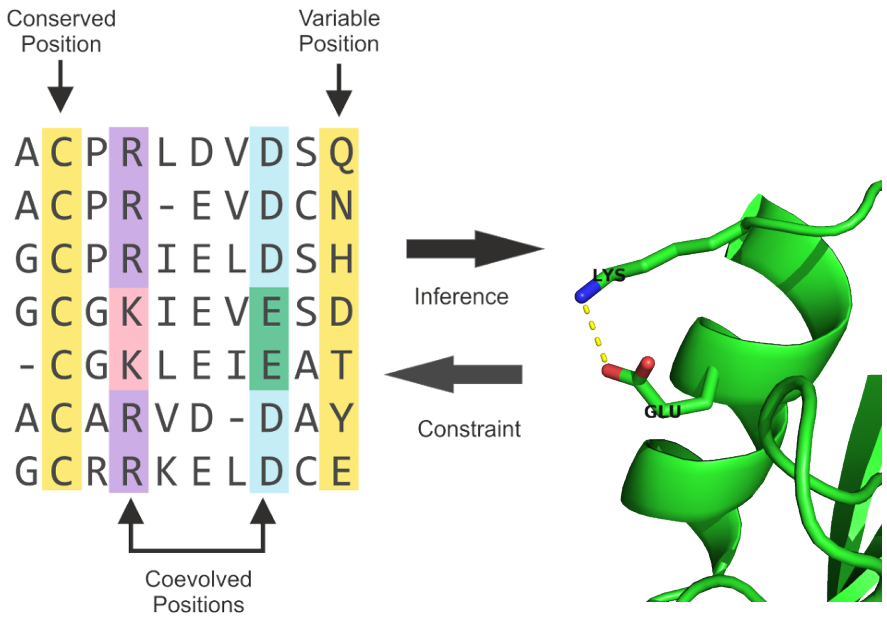

To predict a structure:

- Build or get multiple amino acid sequence alignments
- Infer what are the position in contact using machine learning

Conservation vs. co-evolution

How to measure co-variation?

Conservation vs. co-evolution

How to measure co-variation? A standard approach is to measure it through Mutual Information:

$$MI(i,j) = \sum_{a,b} p(x_i = a, x_j = b) \log \frac{p(x_i=a, x_j=b)}{p(x_i=a)\, p(x_j=b)}$$

Where

- x_i is the amino acid at position i
- $p(x_i = a)$ is estimated in the MSA by $\frac{\#\text{sequences having "a" at position } i}{N}$
- N the number of sequences in the MSA
- $p(x_i = a, x_j = b)$ is estimated in the MSA by $\frac{\#\text{sequence having "a" at } i \text{ and "b" at } j}{N}$

In practice you need $N > 1,000$ to have reasonable estimation of $p(x_i = a, x_j = b)$.

Issue: indirect dependencies

The later approaches suffer from indirect dependencies.
Proposed solutions:

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Proposed solutions:

- Direct Coupling Analysis: infer J, h by maximizing the likelihood $P(x|J,h) = \frac{1}{Z} \exp\left(\sum_{i=1}^{N-1} \sum_{j=i+1}^{N} J_{ij}(x_i, x_j) + \sum_{i=1}^{N} h_i(x_i)\right)$
- Sparse Inverse Covariance matrix: the precision matrix ($\Lambda = \Sigma^{-1}$) represents the partial correlations ($\rho_{x_i x_j|\text{other positions}} = -\frac{\Lambda_{ij}}{\sqrt{\Lambda_{ii}\Lambda_{jj}}}$), infer it with a Lasso regularization.

Toward machine learning

That was the state-of-the-art until ≈ 2018.

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Critics for the previous approaches:

- The link: covariation $\rightarrow$ contact in 3D may be suboptimal
- There are a lot of parameters to infer (at least 20x20 amino acids x length of the sequence^2) $\rightarrow$ need for a lot of sequences in the MSA

Machine learning models to the rescue to cope with these 2 issues.

CASP competition

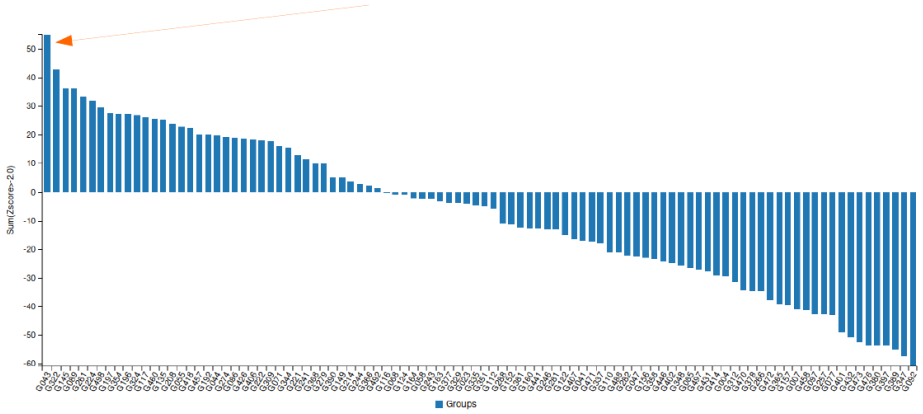
Blind competition. Simple principle:
- a sequence is given
- have to predict the structure.

Prior to 2018 it used to be (pseudo) physical models that where best performing.

CASP13 (2018)

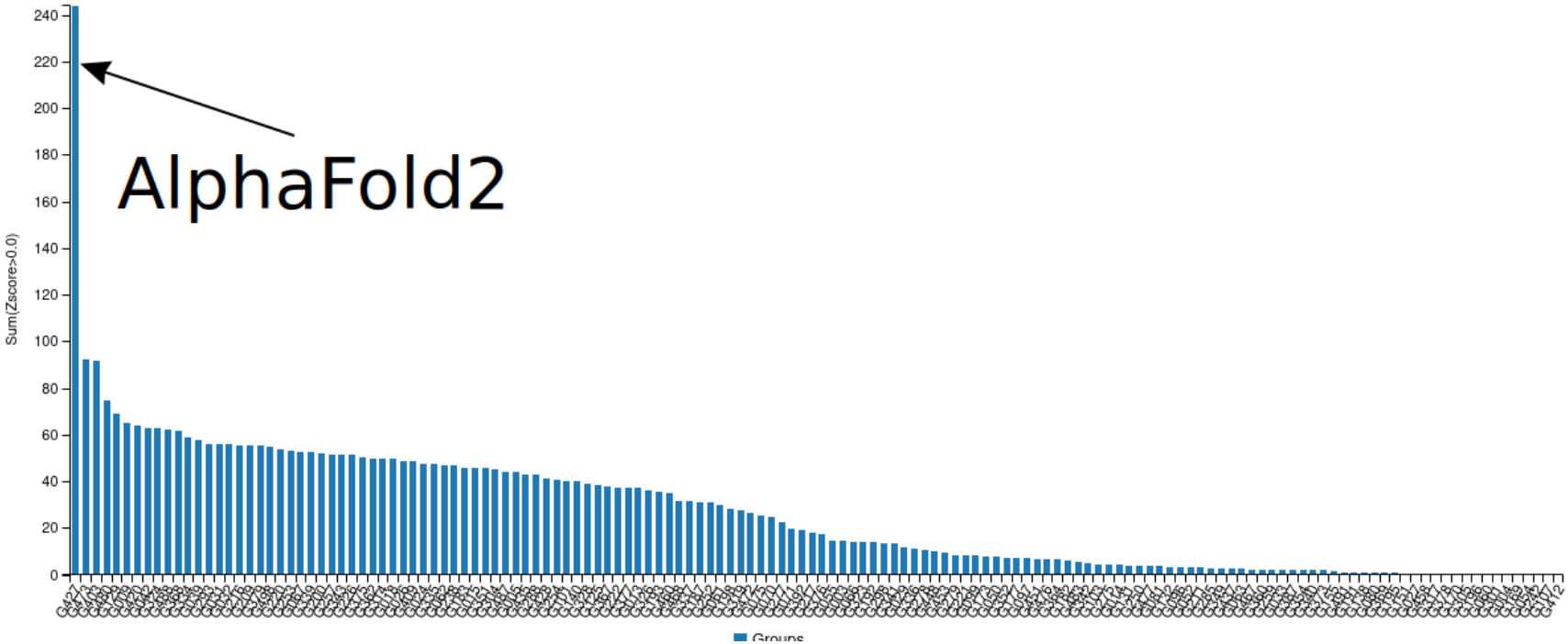
AI wins the challenge for the first time.

Google's DeepMind



CASP14 (2020)

"The big leap forward"

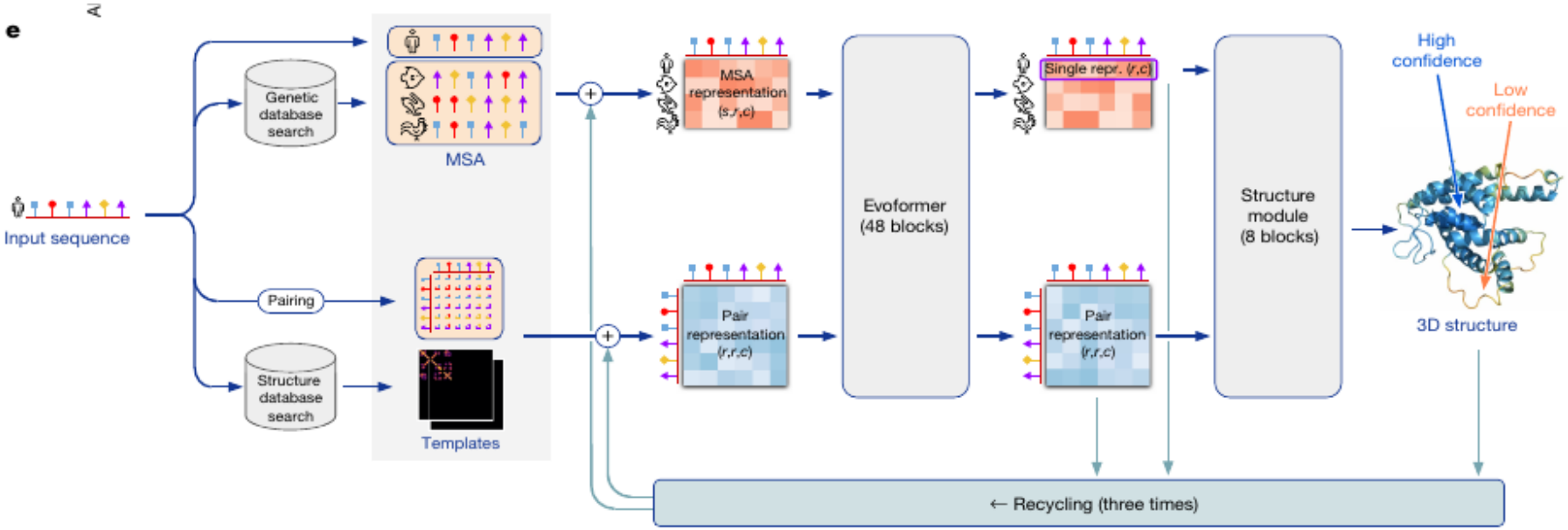


Alphafold2: attention-based learning on protein sequence alignments

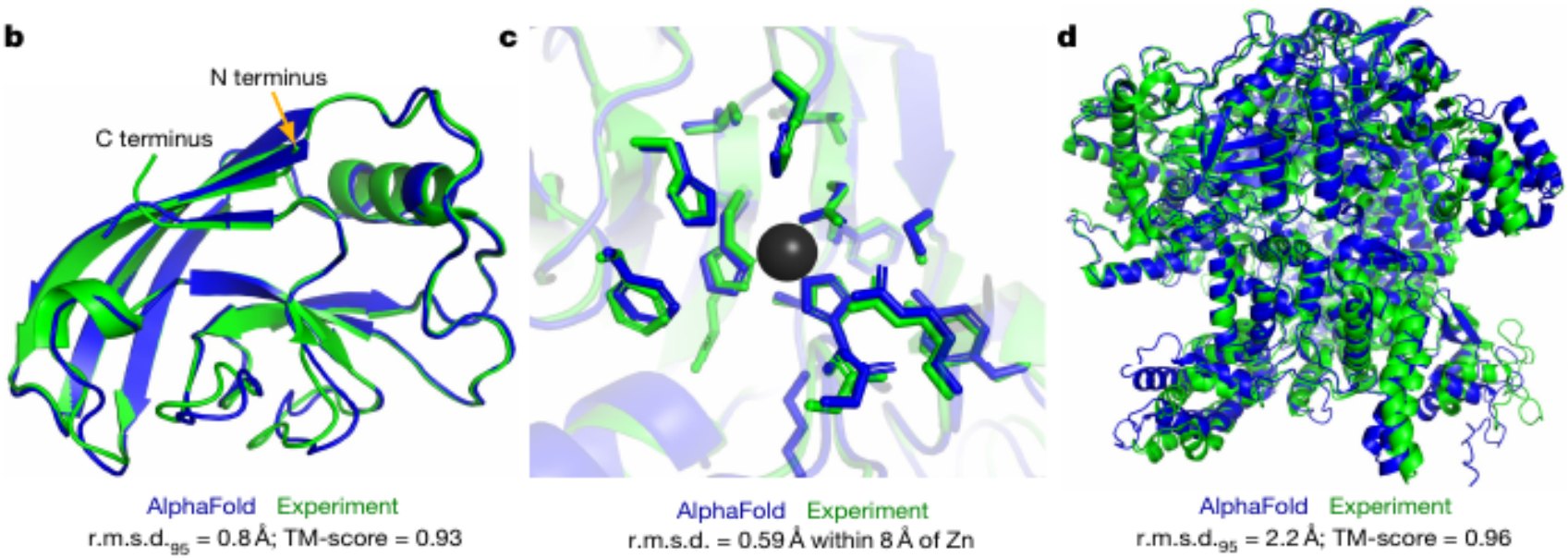
[Casp14.]

[Article in Nature]

AlphaFold2 architecture



AlphaFold2 results



Information for your project

Find out the function of a gene extracted from a viral metagenome

See the git of the project here: `https://gitlab.ensimag.fr/galiezc/predict-protein-x-function`.
Short syllabus and protein sequence is provided.

Let's find the function of protein X!

Sequence comparison

Sequence alignement: algorithm and p-value

Find the best alignment between your query sequence S_Q and a reference sequence S_R:

```
MEAIGNA.GSAI
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Algorithm (sketch):

- given a 20×20 matrix of scores between amino-acids, set gap penalties
- find the alignment maximizing the total score.

Can be solved by **dynamic programming** in $\mathcal{O}(L^2)$ (see *Smith-Waterman algorithm*).

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Under a given p-value threshold we estimate the function to be similar.

Big data: need for heuristic

Even with optimized versions of Smith-Waterman, it is still too heavy to compare sequences to all know sequences.
Tools have developed heuristics to filter down the possible target sequences:

- Blast (the historical tool)
- Diamond
- MMseqs2
- ...

Heuristics are mostly based on similar k-mers, and efficiently filtering through hash tables.

More on mutual information

Over-prediction at entropic position

When applying the rule

$$MI(i,j) > \tau \Rightarrow \text{contact between } i \text{ and } j$$

some positions predict too many contacts, often position with high entropy. Several corrections can be applied[1].

In your project

You can try using the simple correction:

$$MI'(i,j) = MI(i,j) - \frac{1}{N}\sum_k (MI(k,j) + MI(i,k))$$

and fix a τ to predict a contact as soon as:

$$MI'(i,j) > \tau$$

[1] See `https://doi.org/10.1093/bioinformatics/bti671`