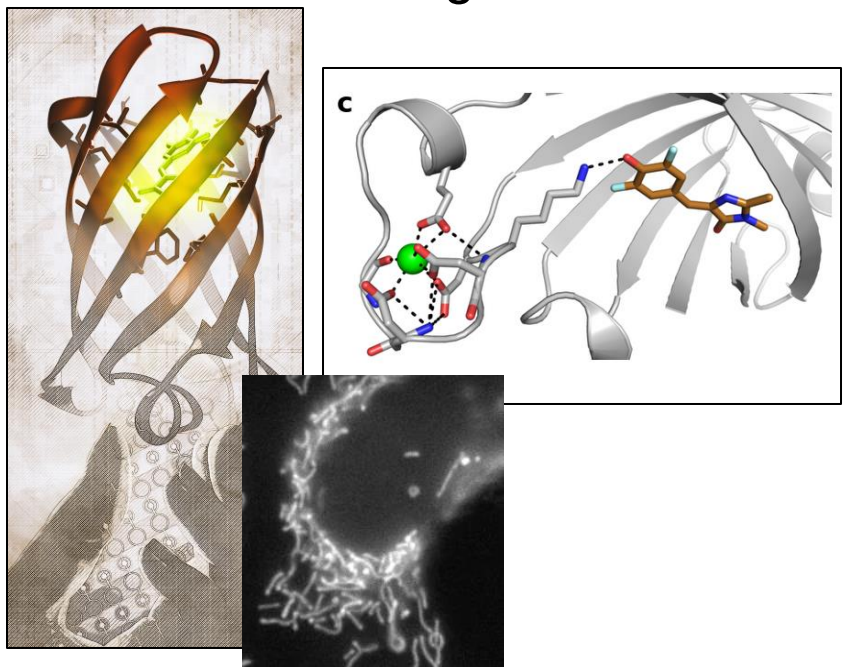
Protein design
Part 1: structure prediction

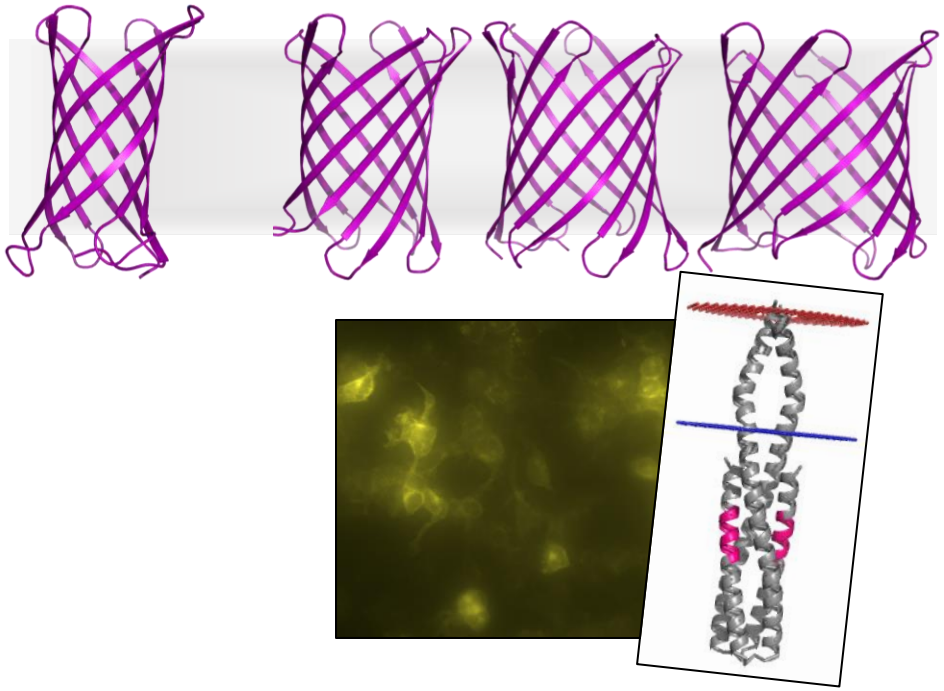
Anastassia Vorobieva

Small-molecule binder/sensor design



Enzyme design

Membrane protein design



2009 — PhD UCLouvain, BE — Directed evolution

2014 — Postdoc UW, Seattle — David Baker

2019 — Postdoc EPFL

2021 — Group leader, VIB

2022 — Assistant professor, VUB

📝 Workshop Schedule

TIME	WEDNESDAY 28 MAY	THURSDAY 29 MAY	FRIDAY 20 MAY
09:00 – 10:30	Protein modelling and structure prediction: Intro to key concepts, from physics-based modelling to AI	De novo protein design: introduction, minimal sequence design, structure-based design principles	De novo design with AI models: RFDiffusion, ProteinMPNN, and ColabFold
10:30 – 11:00	Break and questions	Break and questions	Break and questions
11:00 – 12:00	Introduction to protein design: predicting the effect of mutations on protein stability	Structure-based de novo design: How to generate new structures? The chicken-and-egg problem	Practical session: De novo design of a SARS-CoV-2 RBD binder using RFDiffusion and ProteinMPNN
12:00 – 13:30	Lunch	Lunch	Lunch
13:30 – 15:00	Practical session: AlphaFold hands-on	Practical session: Parametric design of alpha-helical bundles	Practical session: Data analysis and group slide preparation
15:15 – 17:45	Practical session: In silico mutational scanning and ΔΔG calculations	Practical session: Sequence design for parametric bundles with PyRosetta	Practical session: Group presentations and results discussion

Structure prediction - outline

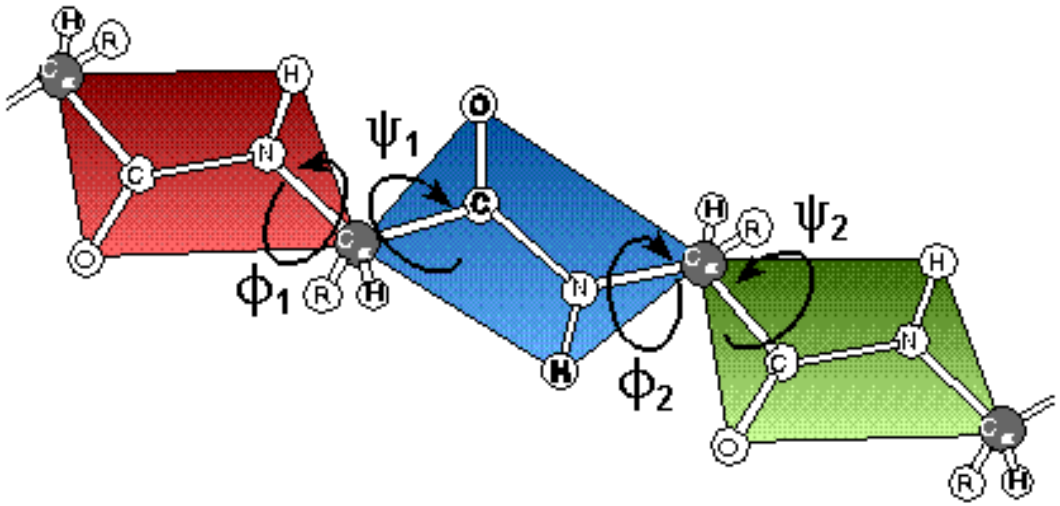
1. Introduction to protein folding and structure:
    - Backbone torsion and secondary structure
    - Levinthal's paradox
    - The thermodynamic hypothesis of protein folding
    - Structure prediction/design *vs* molecular dynamics simulations

2. Predicting the structure of proteins from their sequence
    - Physics-based models:
        - Scoring
        - Sampling
        - Building a prediction pipeline
    - Co-evolution and contacts prediction
    - AI models: AlphaFold 1 and 2 architectures
    - AI models: protein language models

Introduction - protein folding and structure

Accelerated *in vitro* evolution, inspired by natural selection.

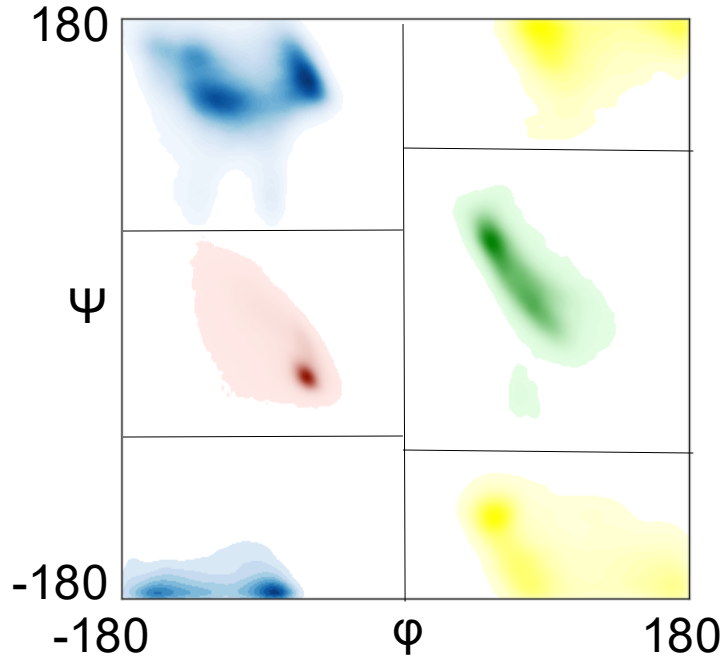
Polypeptide folding: torsion around several degrees of freedom



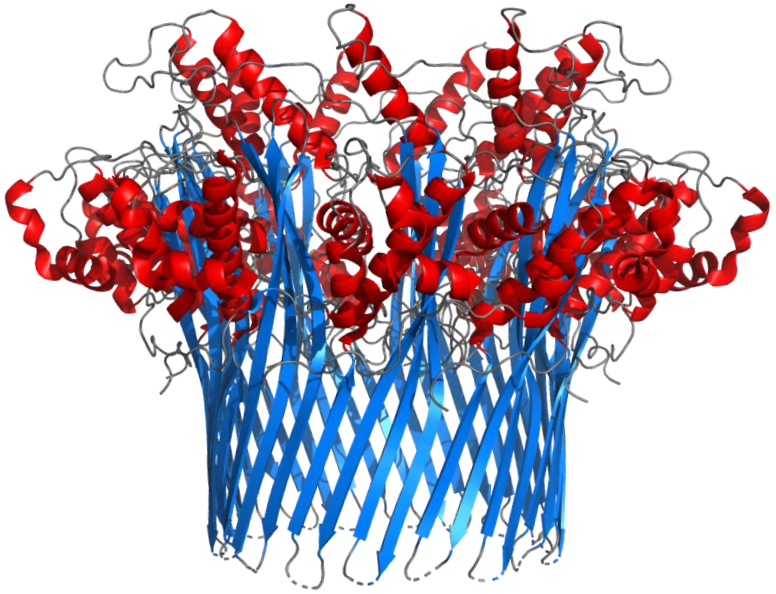
Φ – rotation around the N-C_α bond

Ψ – rotation around the C_α-C bond

Ω – peptide bond

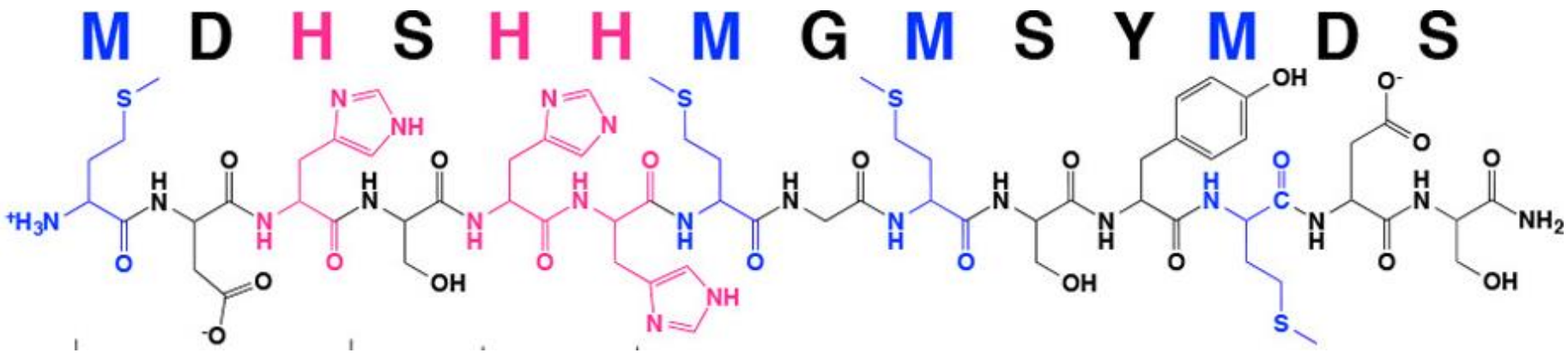


Secondary structure



Tertiary structure

The torsion in the backbone is stabilized by the side chains



20 letters

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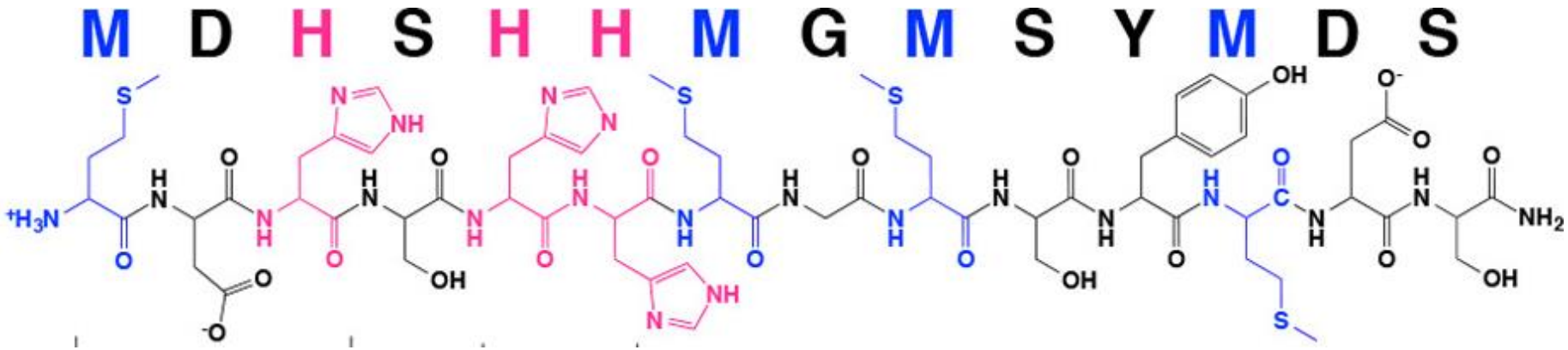
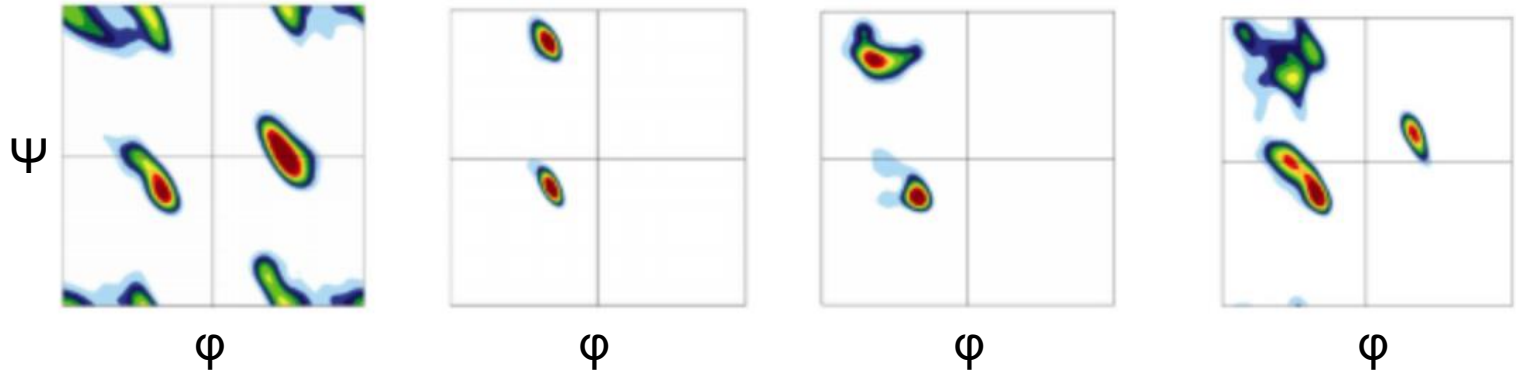


20 letters



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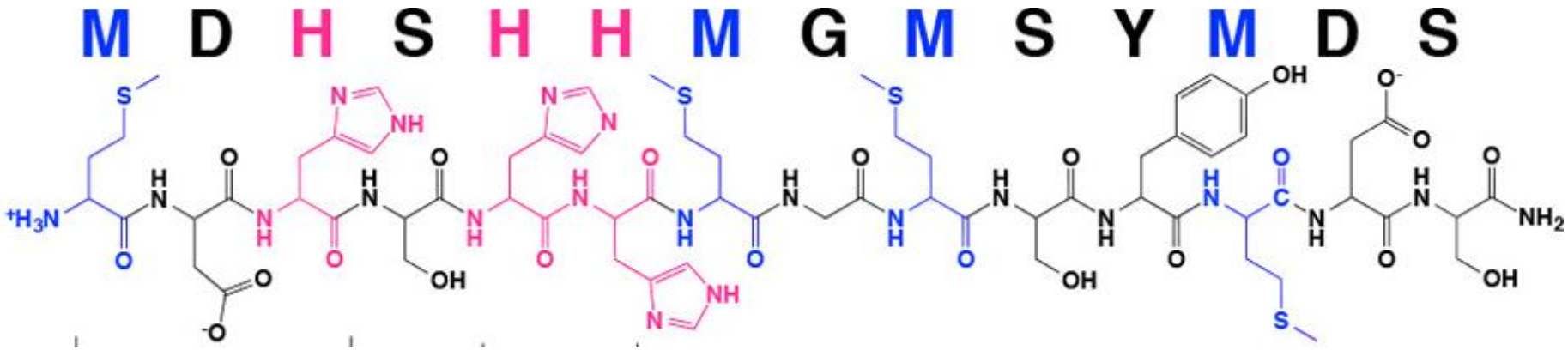
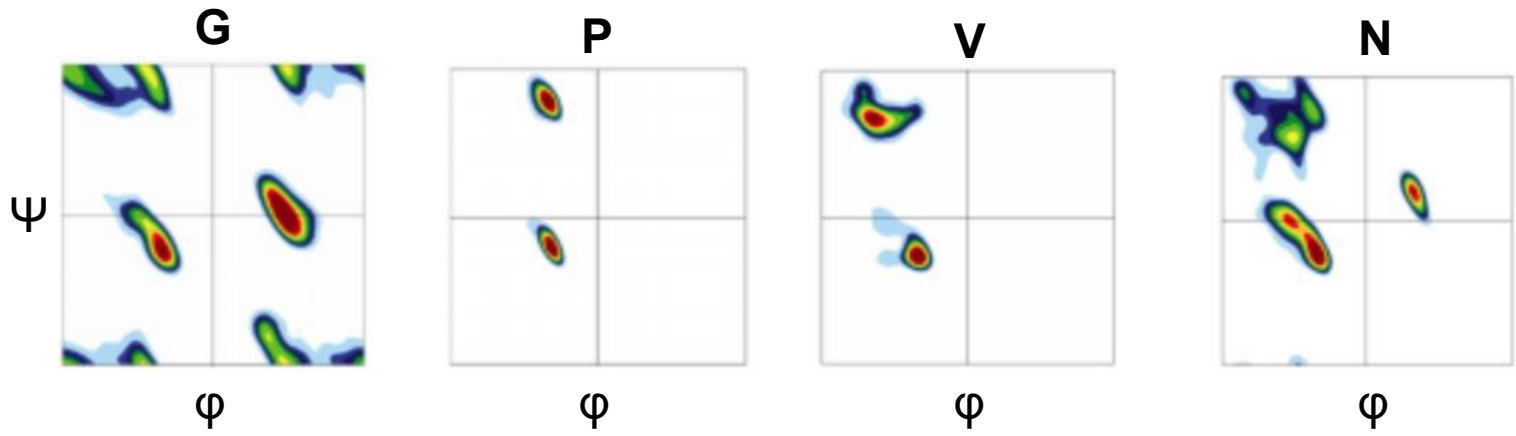


20 letters



The folded state of a protein is likely the energy minima for its sequence (C. Anfinsen)

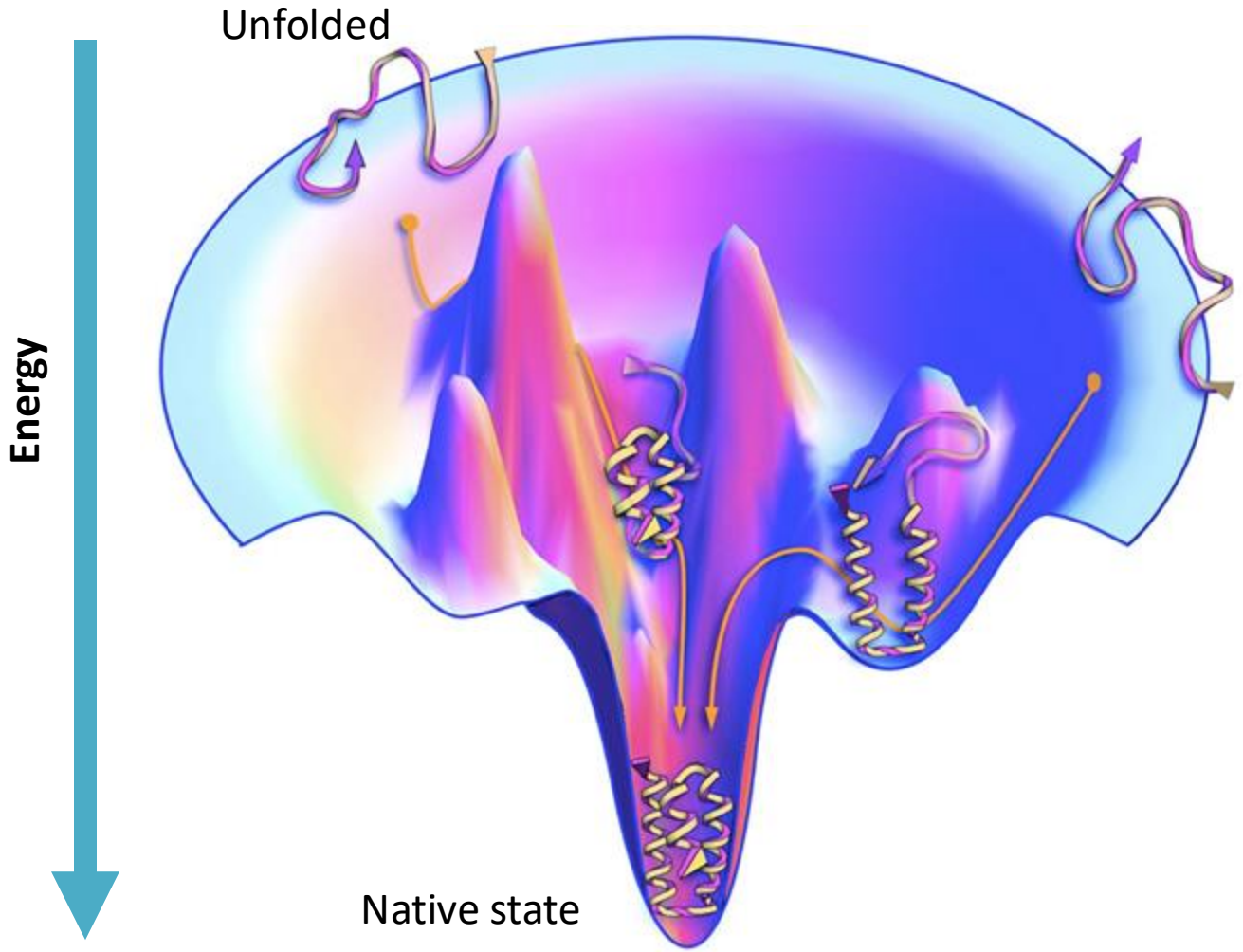


Image from: "The protein-folding problem, 50 years on." *science* 338, no. 6110 (2012): 1042-1046.

The native state of a protein is key to its function

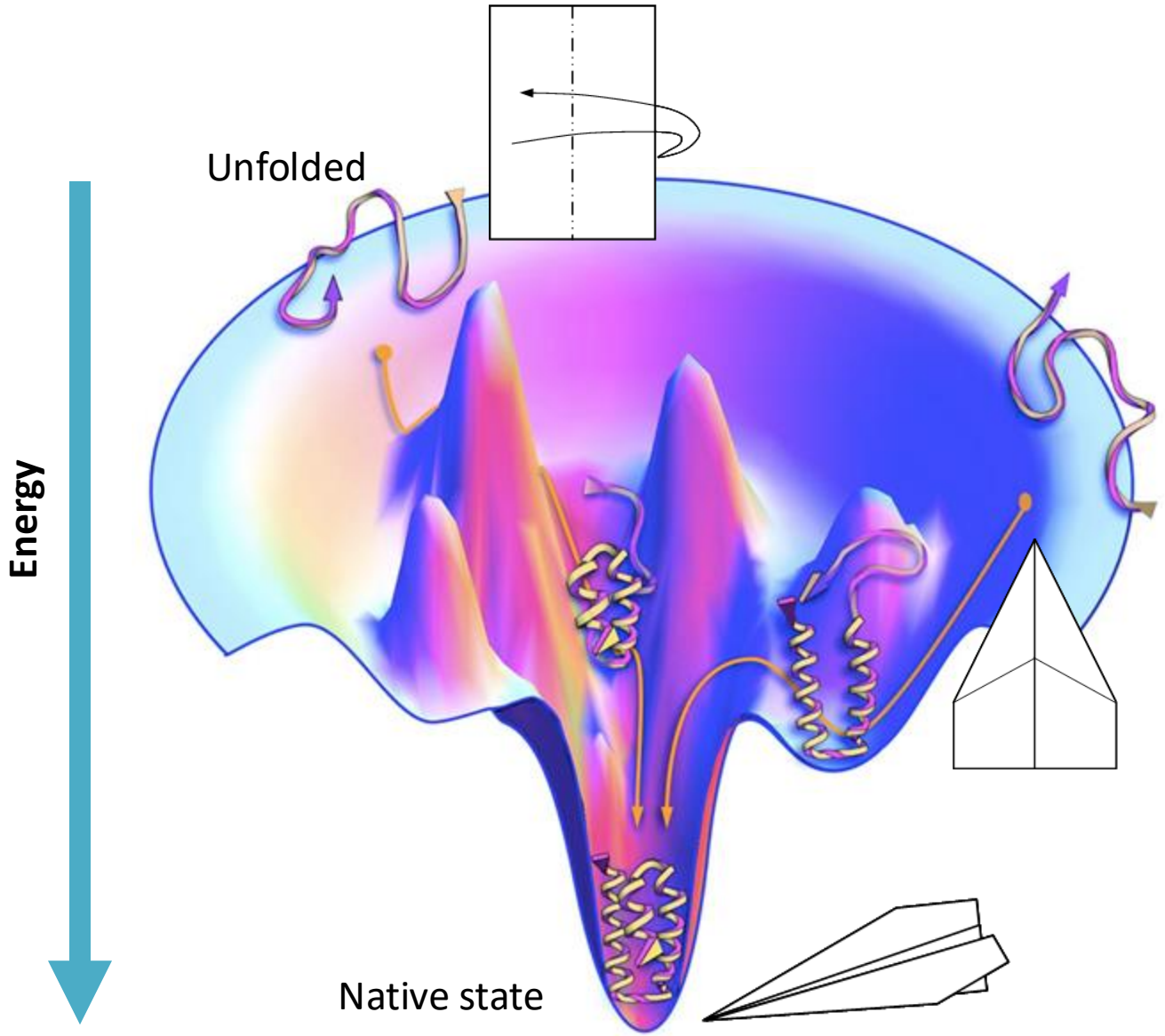


Image from: "The protein-folding problem, 50 years on." *science* 338, no. 6110 (2012): 1042-1046.

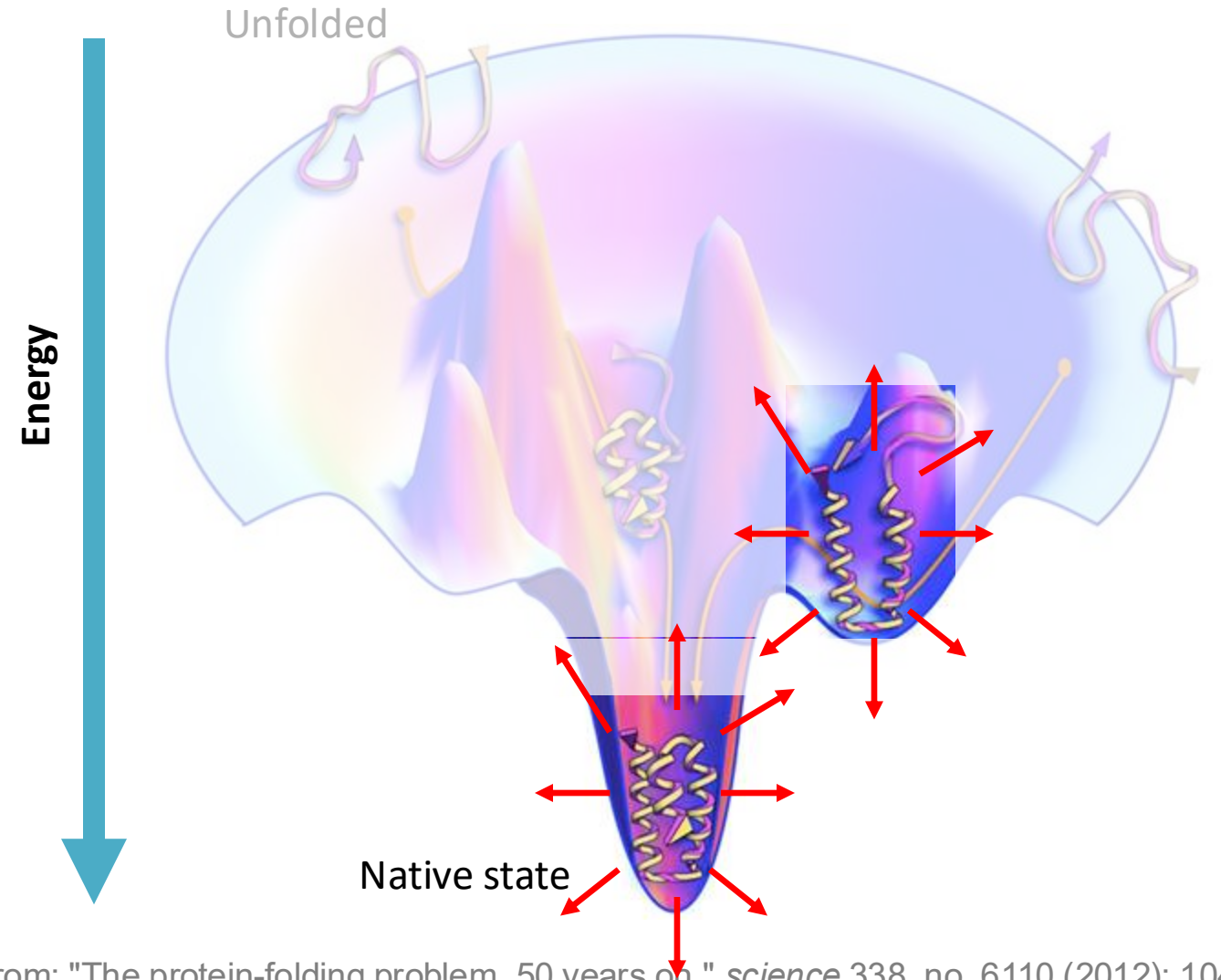
Levinthal's paradox

An unfolded protein has an astonishing number of possible conformations.

Yet, most proteins fold on a µs-ms timescale.

➔ Protein folding is driven by a few stable interaction formed early-on (folding pathway).

Molecular Dynamics simulations are used to study the dynamics and the stability of an existing state



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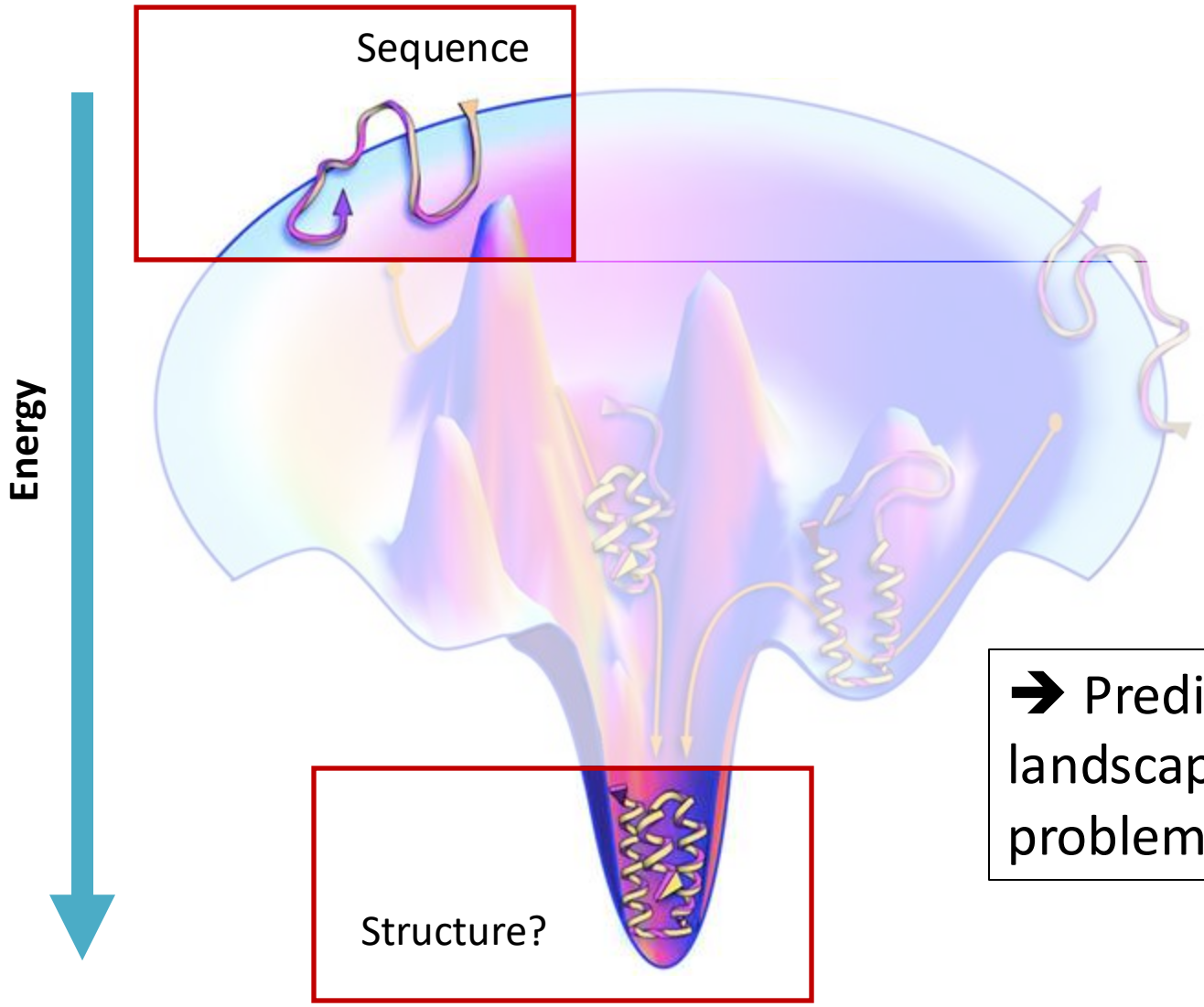
Structure prediction(*ab initio*) and design



Structure prediction

Design

➔ Predicting the entire energy landscape is an un-tracktable problem

Image from: "The protein-folding problem, 50 years on." *science* 338, no. 6110 (2012): 1042-1046.

Modelling and predicting protein structures

From physics-based modelling to deep learning

“Classic” physics-based models

- Both MD simulations and protein structure prediction/design
- Explicit modeling of possible conformations
- **Sampling** conformations from the energy landscape
  Proteins can fold into an astronomically large number of conformations, and sampling methods aim to explore these efficiently.
- **Scoring** the sampled conformations with an energy function or force field
  Estimate the free energy of a state or a conformation as accurately as possible.

Scoring – energy functions

- Mathematical models used to evaluate the stability or quality of a protein model
  ➔ Models ranking and comparison tools

- Trade-off between speed (e.g. energy functions for design) and accuracy (e.g. MD simulations force fields)

Scoring – energy functions

- Mathematical models used to evaluate the stability or quality of a protein model
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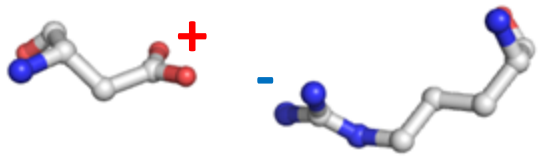
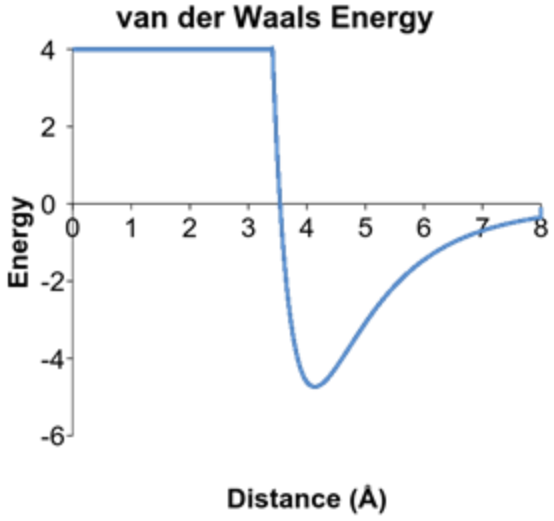
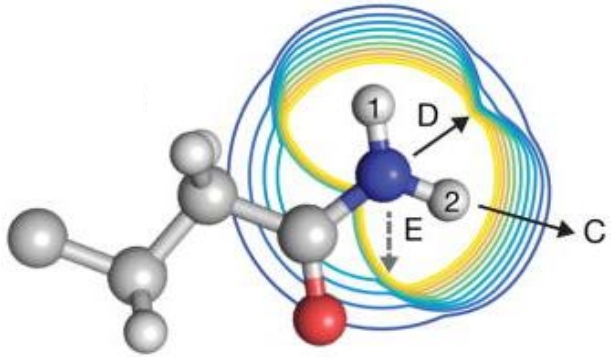
- Trade-off between speed (e.g. energy functions for design) and accuracy (e.g. MD simulations force fields)

- Physics-based parameters

Electrostatic interactions







Lazaridis-Karplus solvation

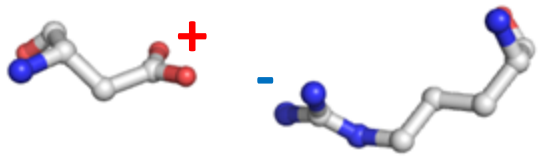
Scoring – energy functions

- Mathematical models used to evaluate the stability or quality of a protein model
  ➔ <u>Models ranking and comparison tools</u>

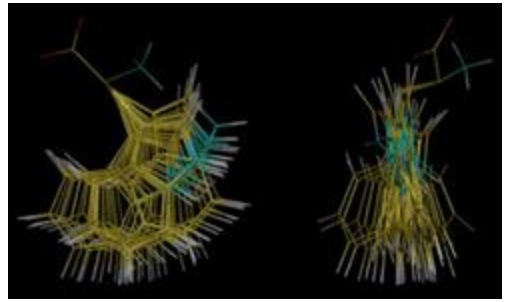
- Trade-off between speed (e.g. energy functions for design) and accuracy (e.g. MD simulations force fields)

- Physics-based parameters
- Statistical potentials (from PDB)

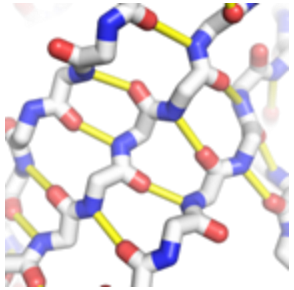
Electrostatic interactions

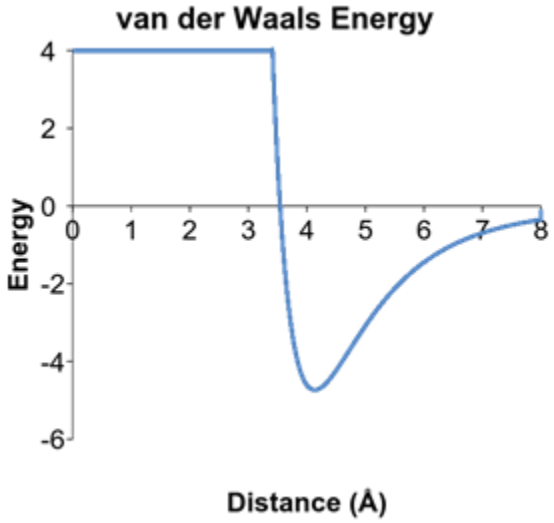


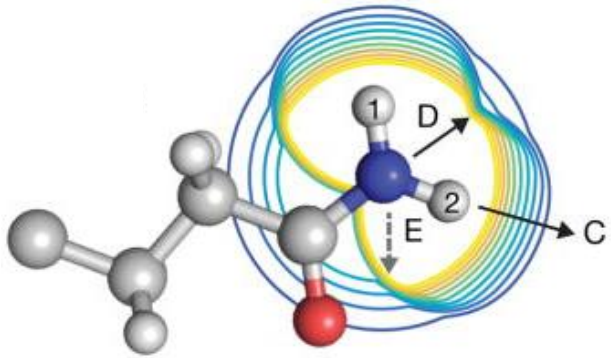
Sidechain conformations



Hydrogen bond

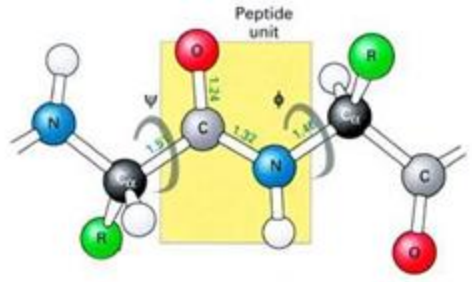






Lazaridis-Karplus solvation

Backbone conformations



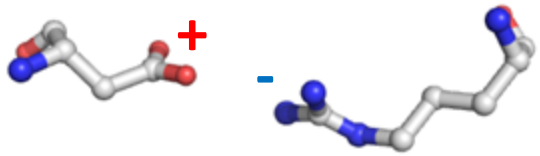
Scoring – energy functions

- Mathematical models used to evaluate the stability or quality of a protein model
  ➔ <u>Models ranking and comparison tools</u>

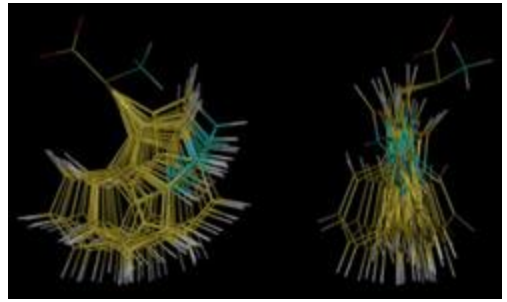
- Trade-off between speed (e.g. energy functions for design) and accuracy (e.g. MD simulations force fields)

- Physics-based parameters
- Statistical potentials (from PDB)
- Hybrid potentials: **e.g. Rosetta**

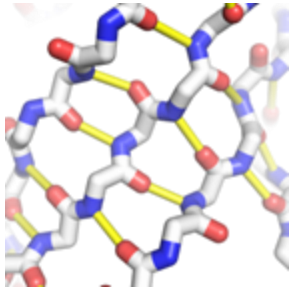
Electrostatic interactions

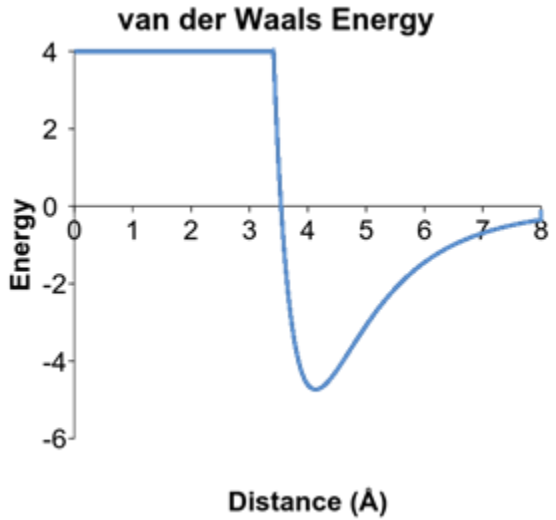


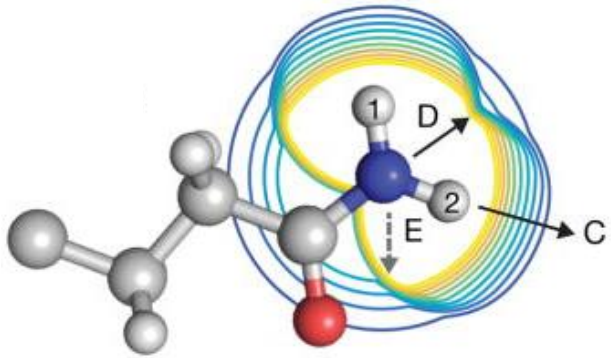
Sidechain conformations



Hydrogen bond







Lazaridis-Karplus solvation

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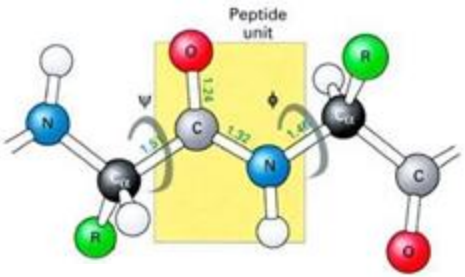


weight

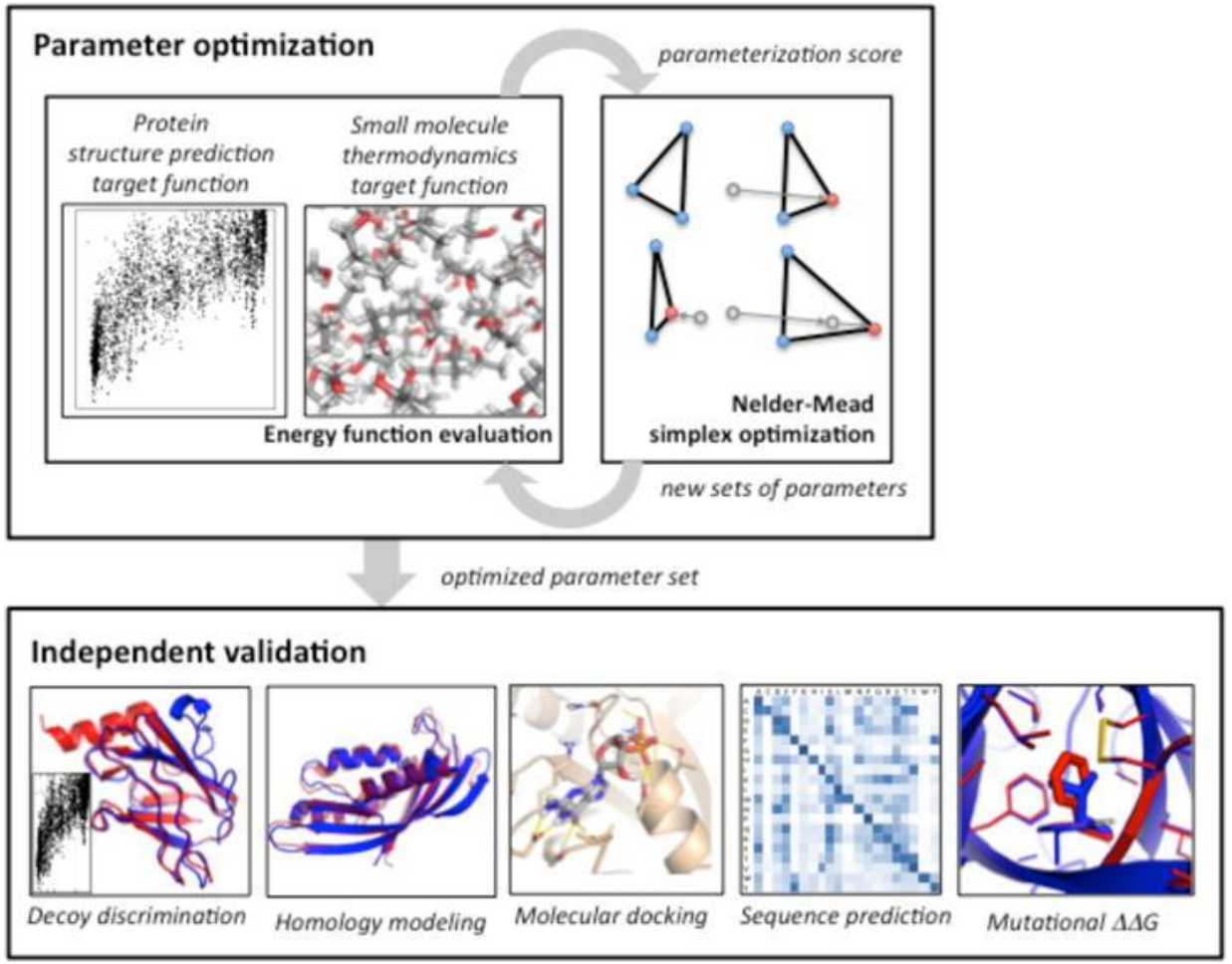
$$\Delta E_{\text{total}} = \sum_i w_i E_i(\Theta_i, \text{aa}_i)$$

https://doi.org/10.1021/acs.jctc.7b00125

Training an energy function

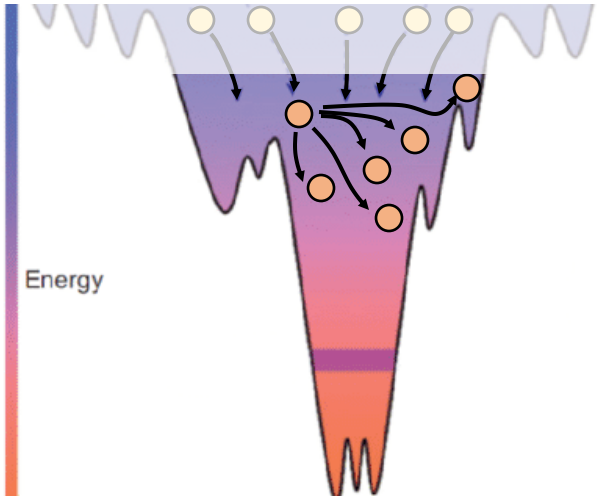
- Adjusting the weights of the parameters in the energy function to fit experimental data

- Example: training the Rosetta energy function

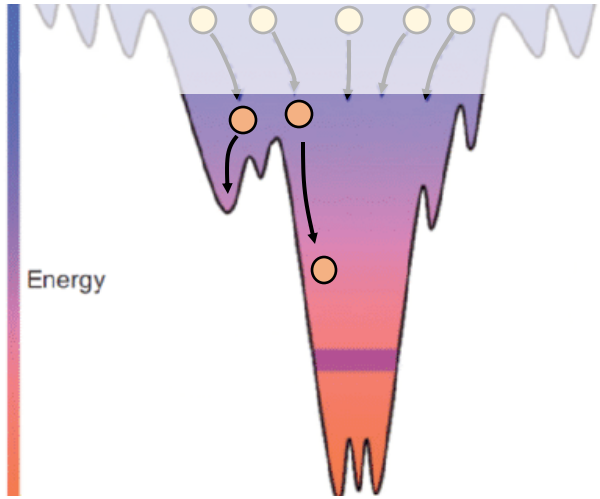


https://doi.org/10.1021/acs.jctc.6b00819

Sampling *vs* minimization



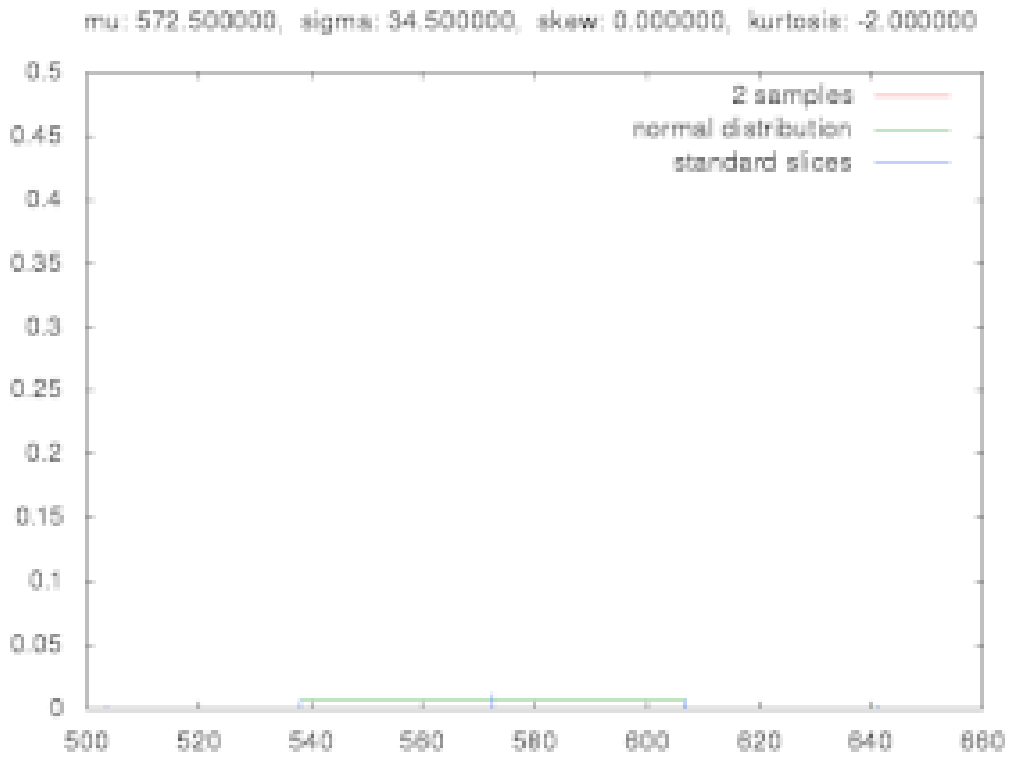
Sampling: random exploration



minimization: rolling down the energy gradient

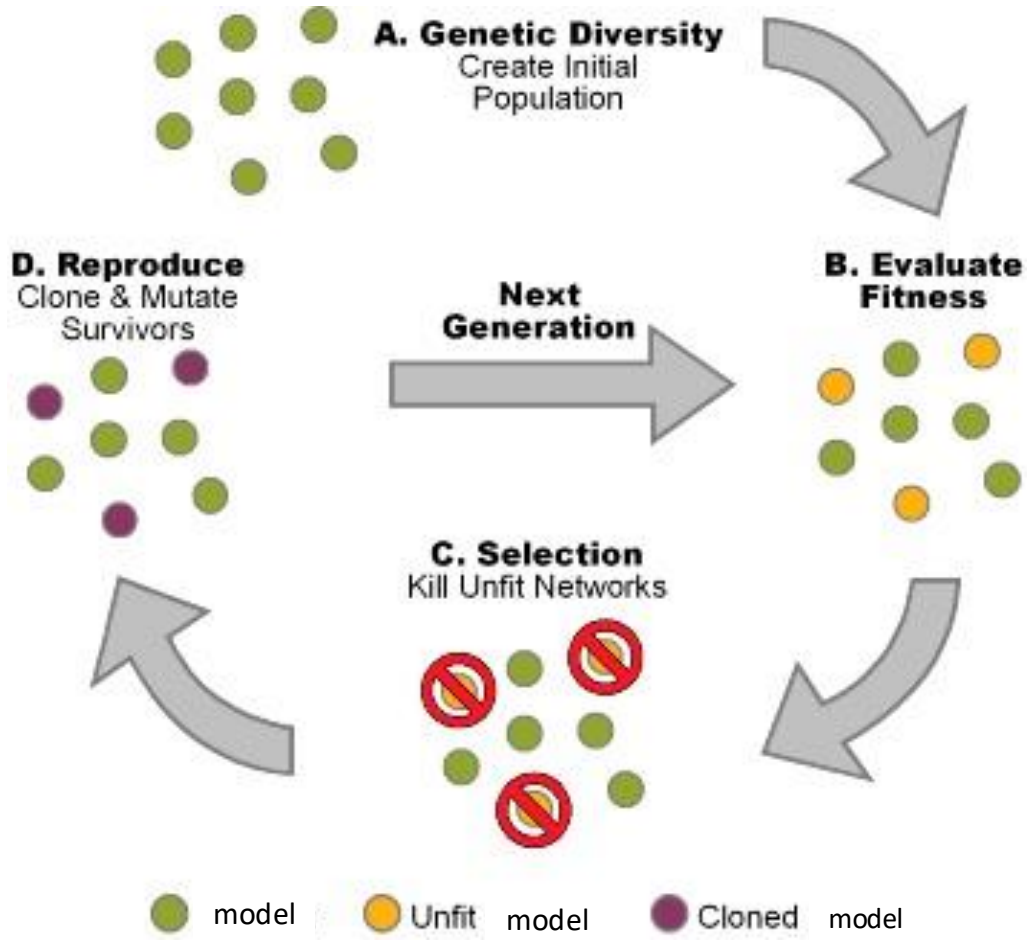
Sampling methods

- Approaches and algorithms to efficiently navigating the conformational landscape
- Monte Carlo algorithm to simulate random sampling of a normal distribution

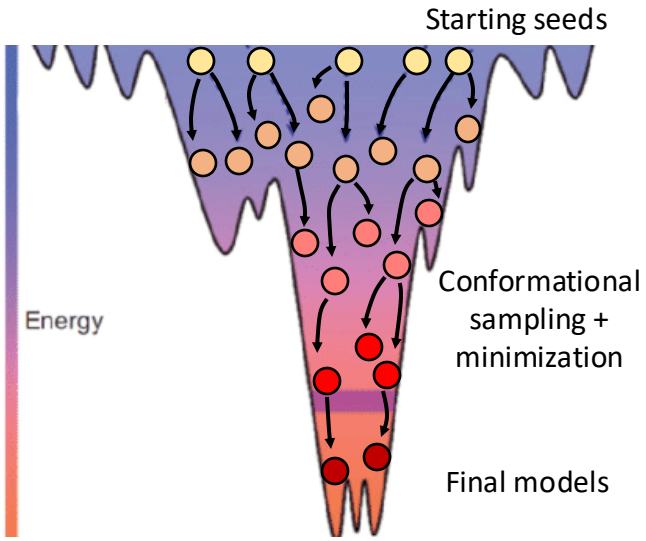


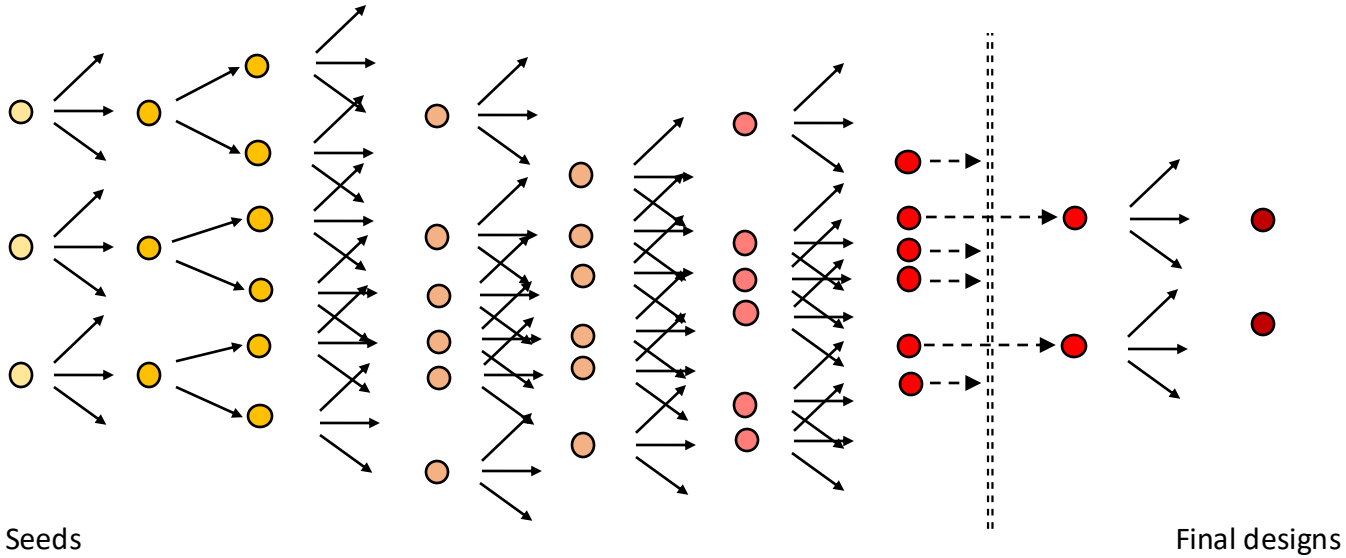
Sampling methods

- Approaches and algorithms to efficiently navigating the conformational landscape
- Monte Carlo algorithm to simulate random sampling of a normal distribution
- Genetic algorithm

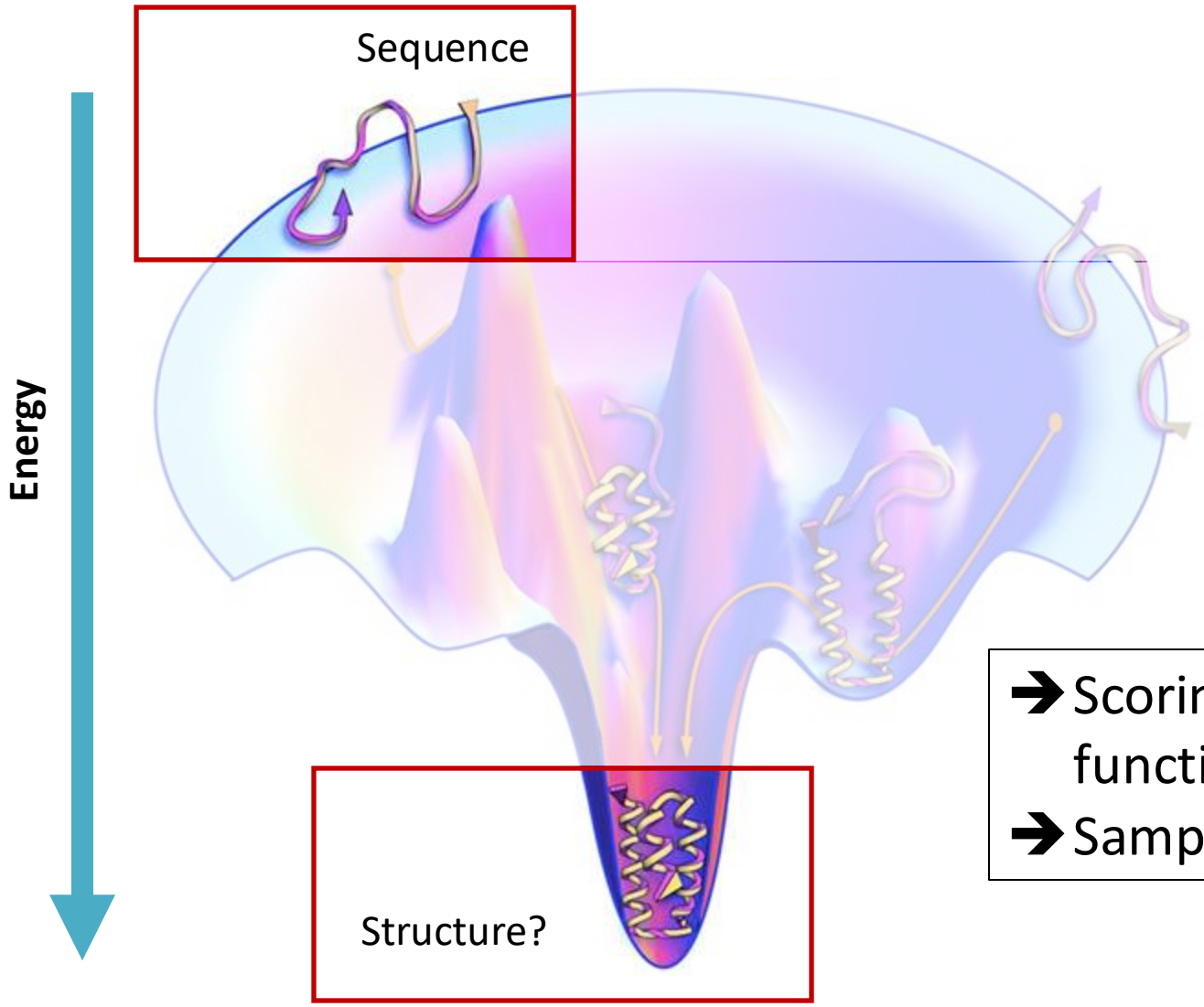


Example modelling trajectory





Predicting the structure of a protein based on its sequence (*ab initio*)



➔ Scoring: Rosetta energy function

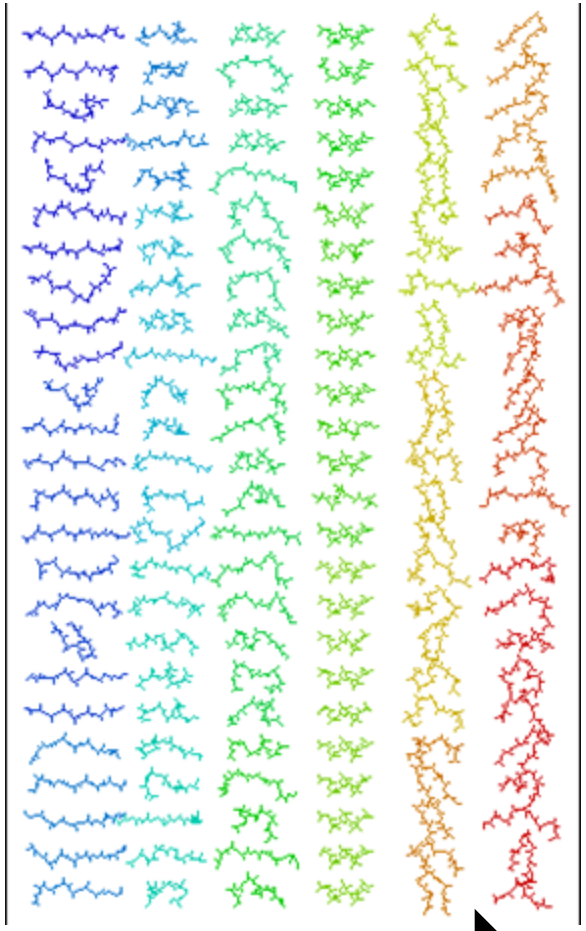
➔ Sampling structures ?

Image from: "The protein-folding problem, 50 years on." *science* 338, no. 6110 (2012): 1042-1046.

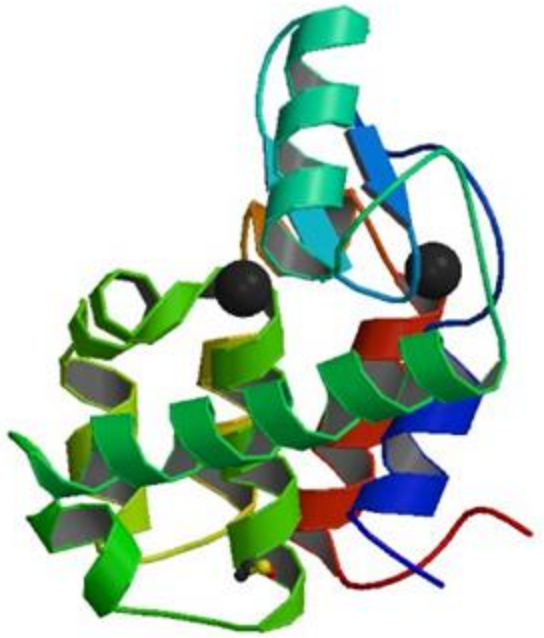
Classic structure prediction: **Monte Carlo sampling** of existent conformations

MNIFEMLRIDEGLRLKI
YKDTEGYYTIGIGHLLT
KSPSLNASKSELDKAIG
RNTNGVITKDEAEKLFN
QDVDAAVRGILRNAKLK
PVYDSLDAVRRAALINM
VFQMGETGVAGFTNSLR
MLQQKRWDEAAVNLAKS
RWYNQTPNRAKRVITTF
RTGTWDAYKNL

Primary Sequence

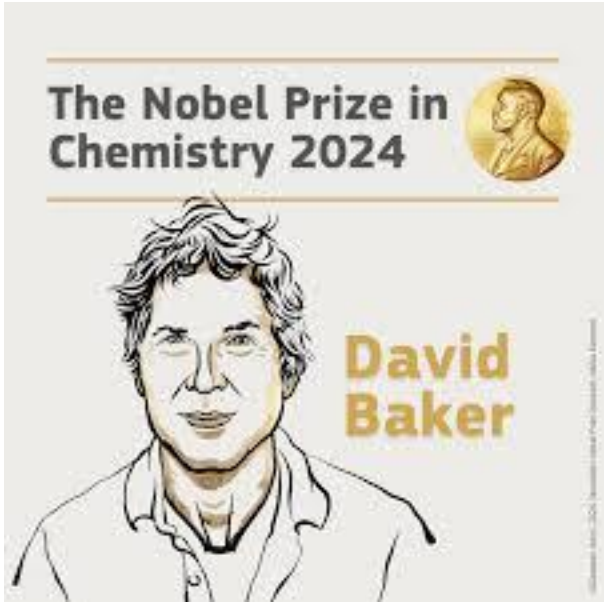


***Ab initio* folding**



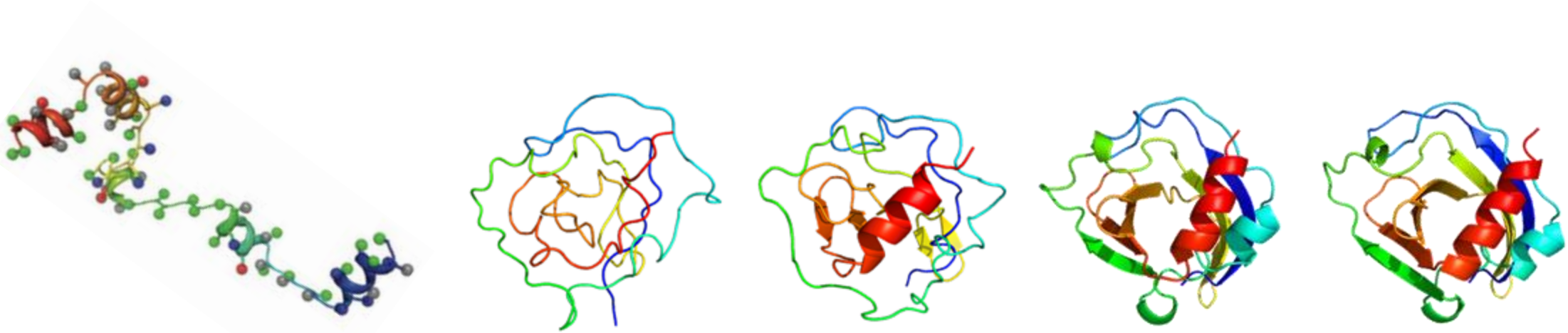
Tertiary Structure

Rosetta structure prediction (state-of-the-art years ago)



This is not a folding pathway simulation, but one successful trajectory of random exploration of the conformational space.

Ab initio Relax



Sequence

uniform: 9 (25) → **Stage1** → **score0**: vdw

uniform: 9 (25) → **Stage2** → **score1**: vdw, pair, env, hs_pair, ss_pair, sheet

uniform: 9 (25) → **Stage 3a** → **score2**: vdw, cenpack, pair, env, cbeta, hs_pair, ss_pair, sheet

uniform: 9 (25) → **Stage 3b** → **score5**: vdw, cenpack, pair, env, cbeta, hs_pair, ss_pair, sheet

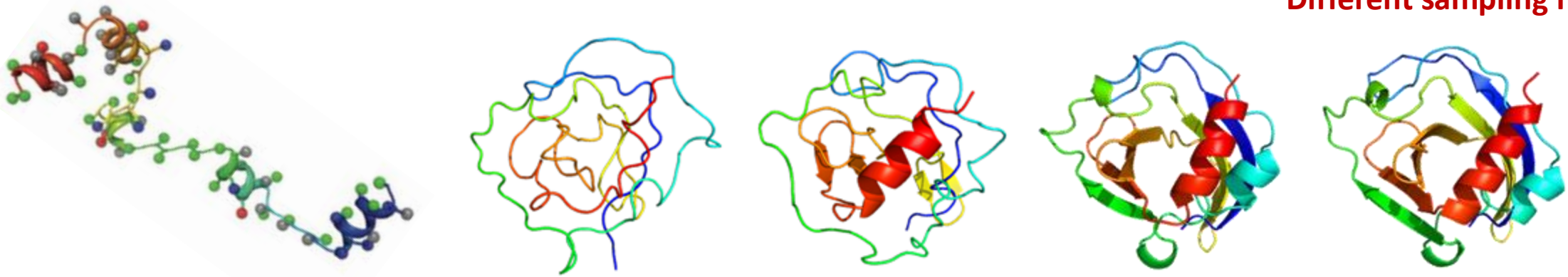
uniform: 3 (200)
smooth: 3 (200) → **Stage4** → **score3**: vdw, cenpack, pair, env, cbeta, **rg**, hs_pair, ss_pair, rsigma, sheet

pack
minimize → **Relax** → **ref2015**

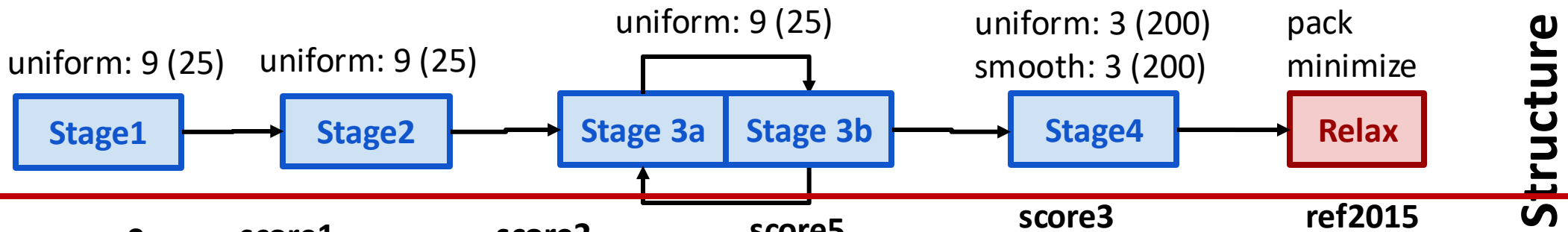
Structure

Ab initio Relax

Different sampling methods



Sequence



Structure

score0
vdw

score1
vdw, pair
env, hs_pair
ss_pair, sheet

score2
vdw, cenpack
pair, env,
cbeta, hs_pair,
ss_pair, sheet

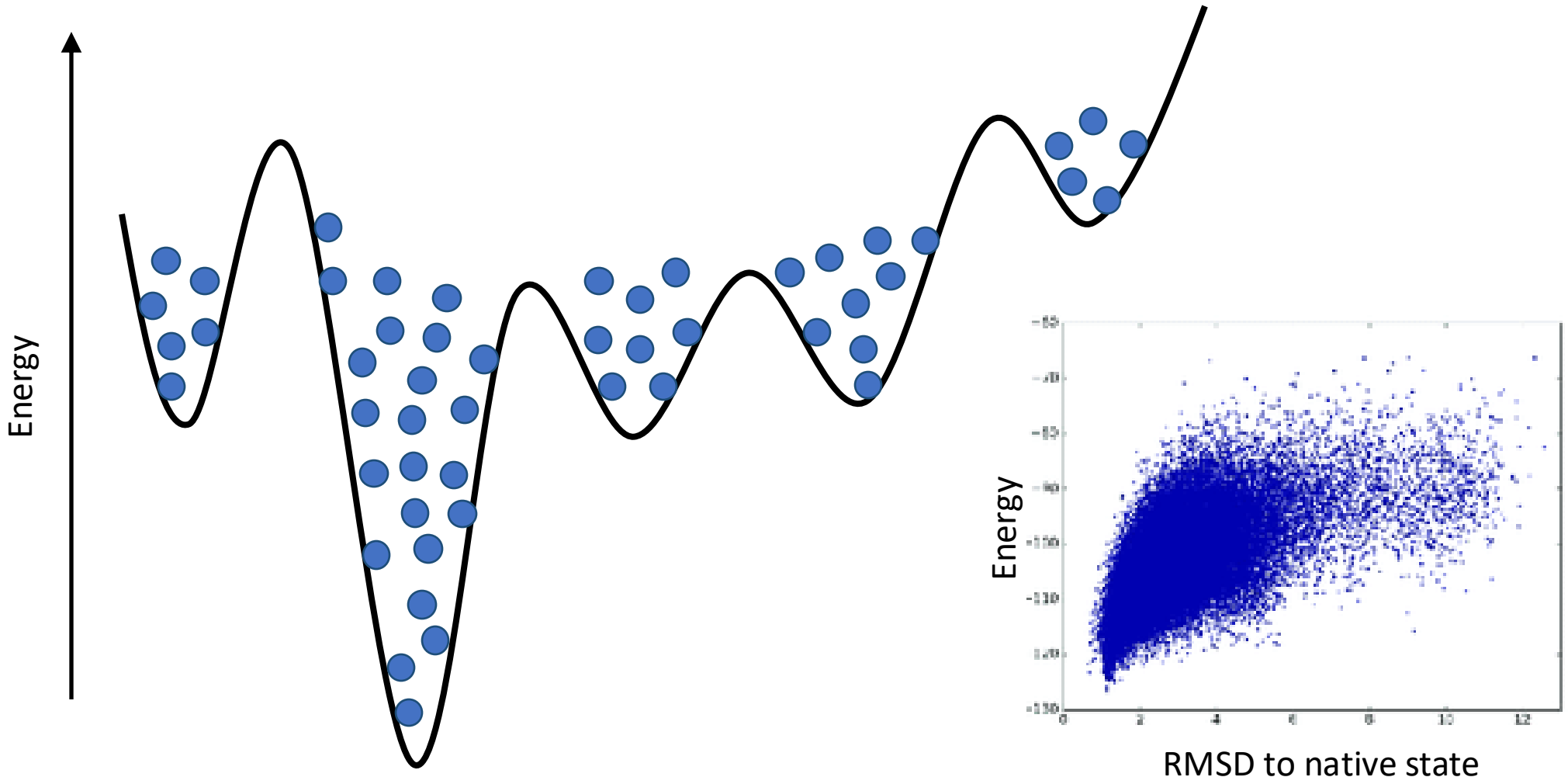
score5
vdw, cenpack
pair, env, cbeta,
hs_pair, ss_pair,
sheet

score3
vdw, cenpack
pair, env, cbeta,
rg, hs_pair, ss_pair
rsigma, sheet

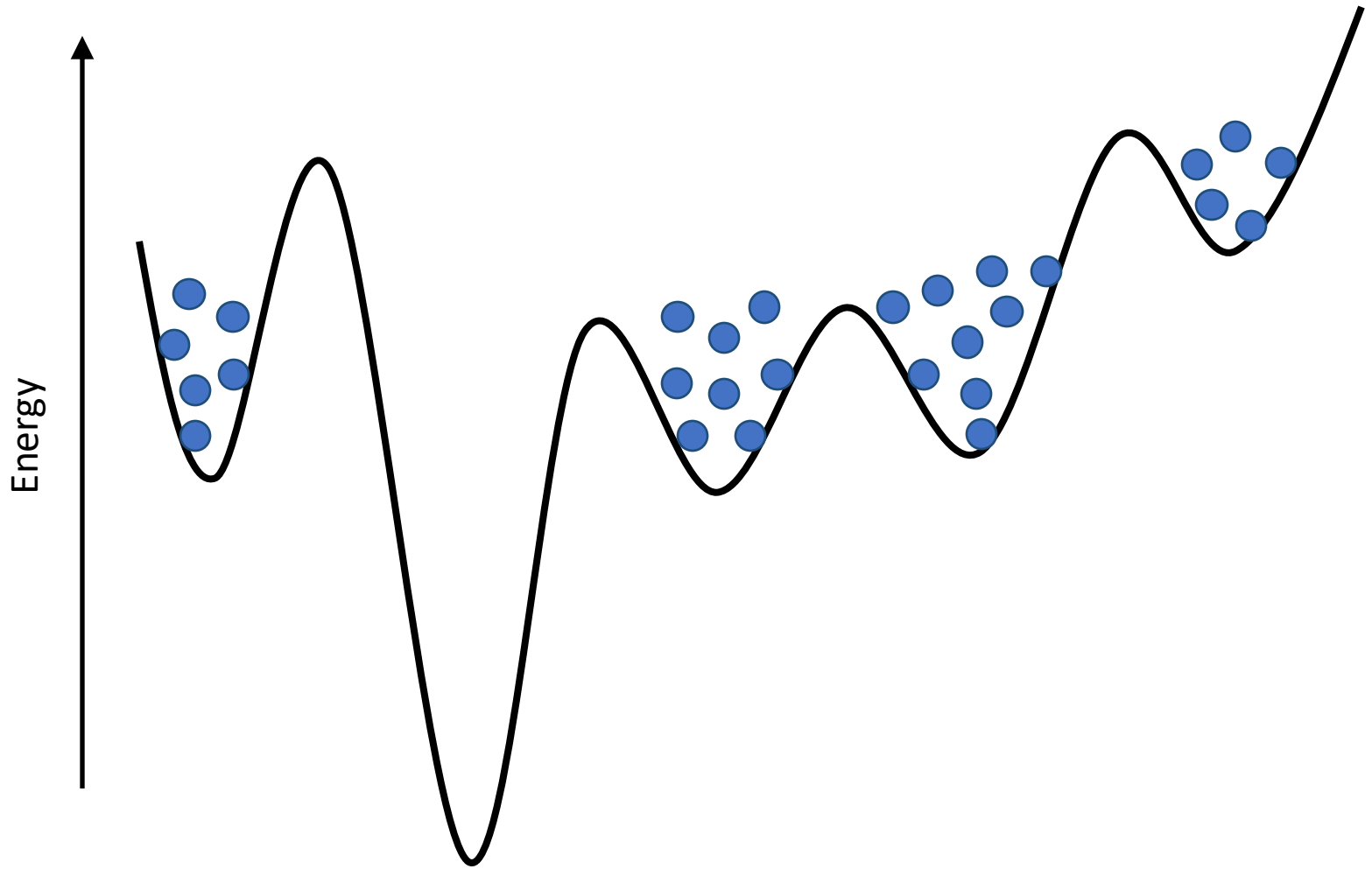
ref2015

Custom energy function

Lowest energy structures converge to same solution



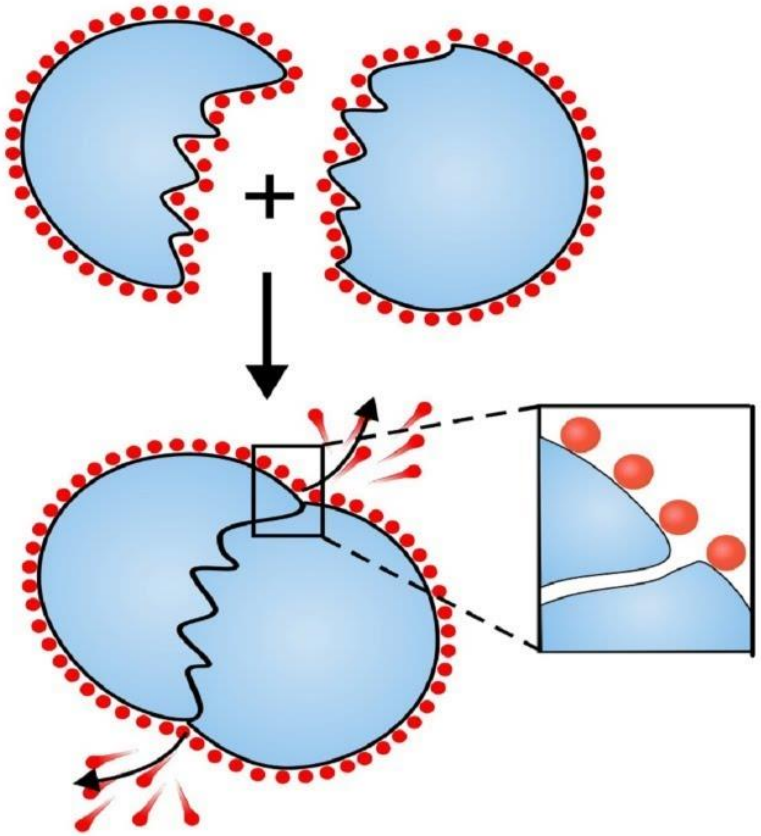
BUT… for many proteins **no** convergence



Why would you see no convergence?

What is the major driving force of protein folding?

The hydrophobic effect



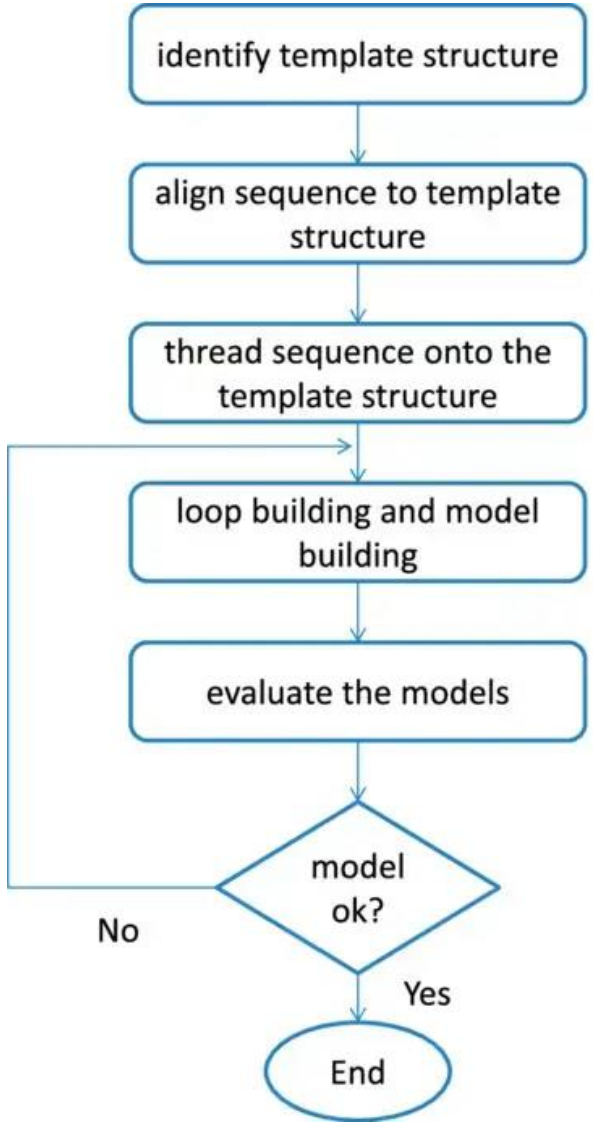
- Entropic effect

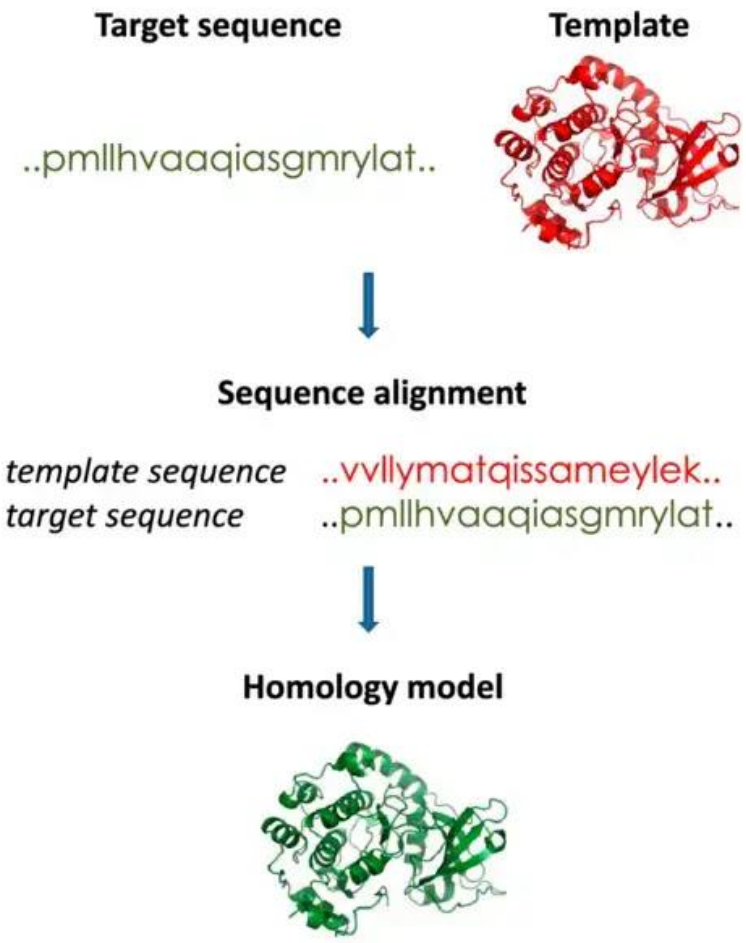
- Water-mediated (water molecules are not explicitely represented to save computing)

- long-range interactions in the protein

➔ The hydrophobic effect is missing from the energy function

Solution? Use a homologous protein with a known structure as a template

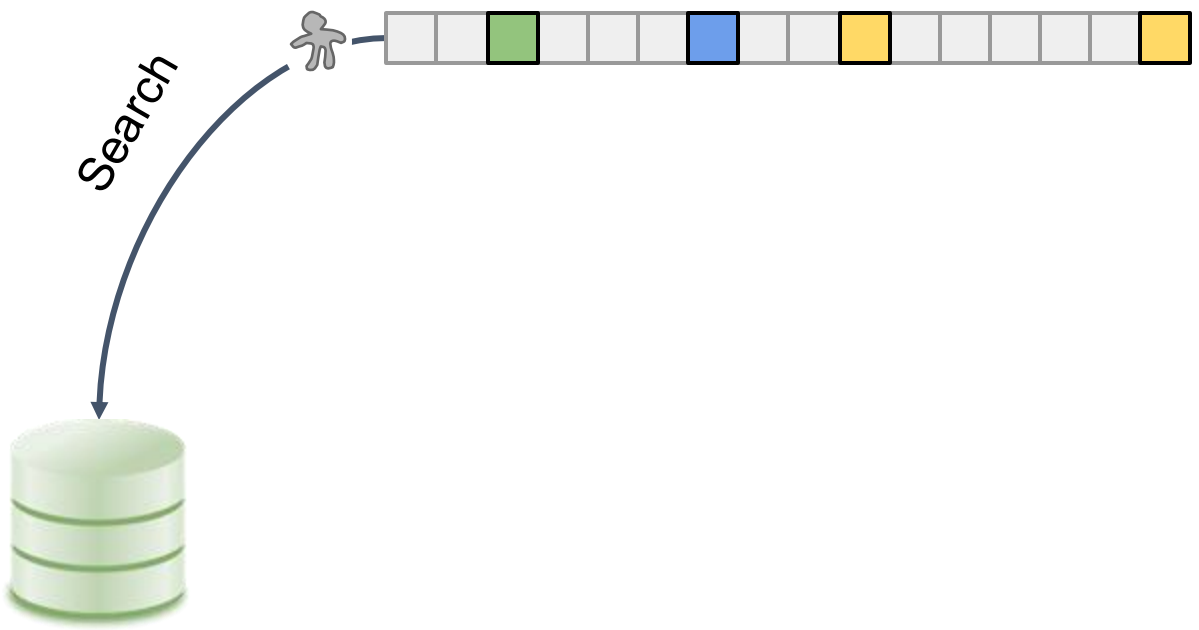




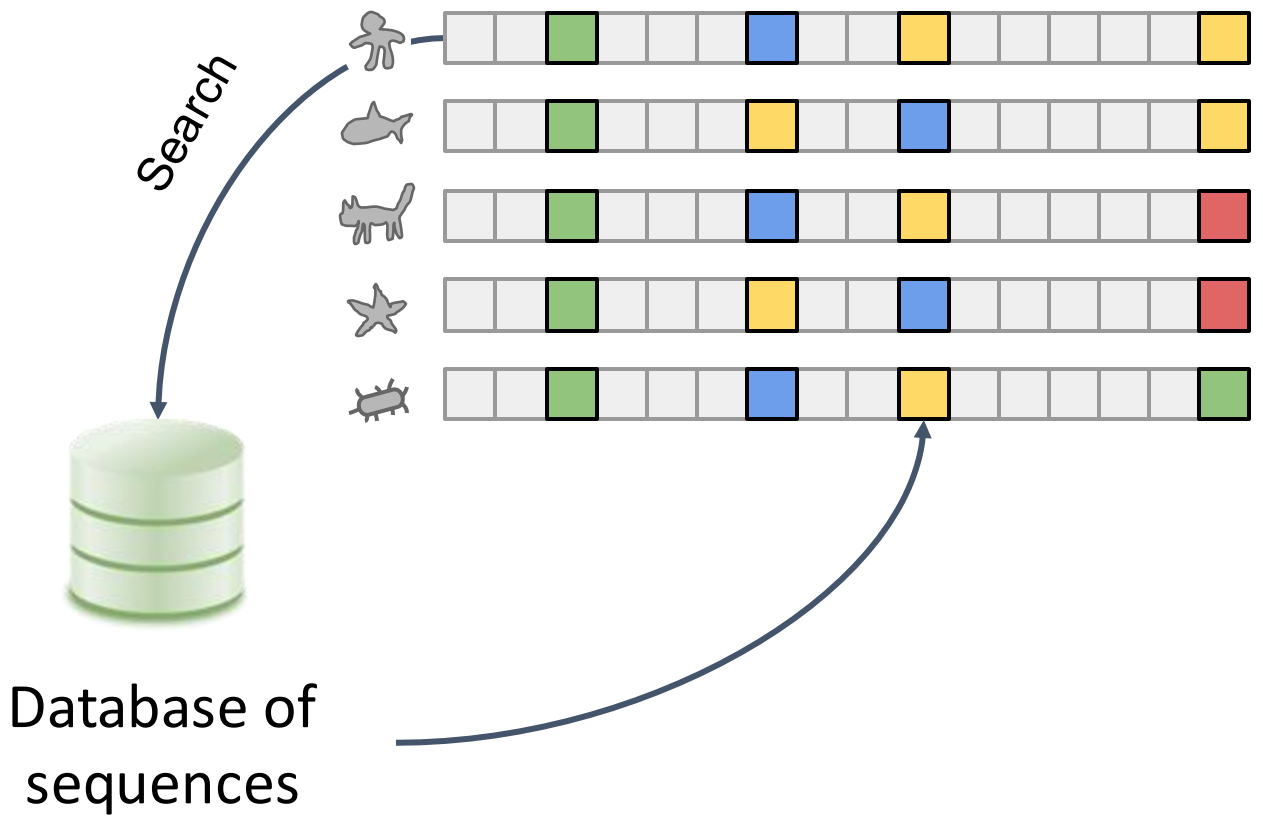
What happens if there is no homolog with a known structure?

We can **CHEAT** by using evolutionary information!

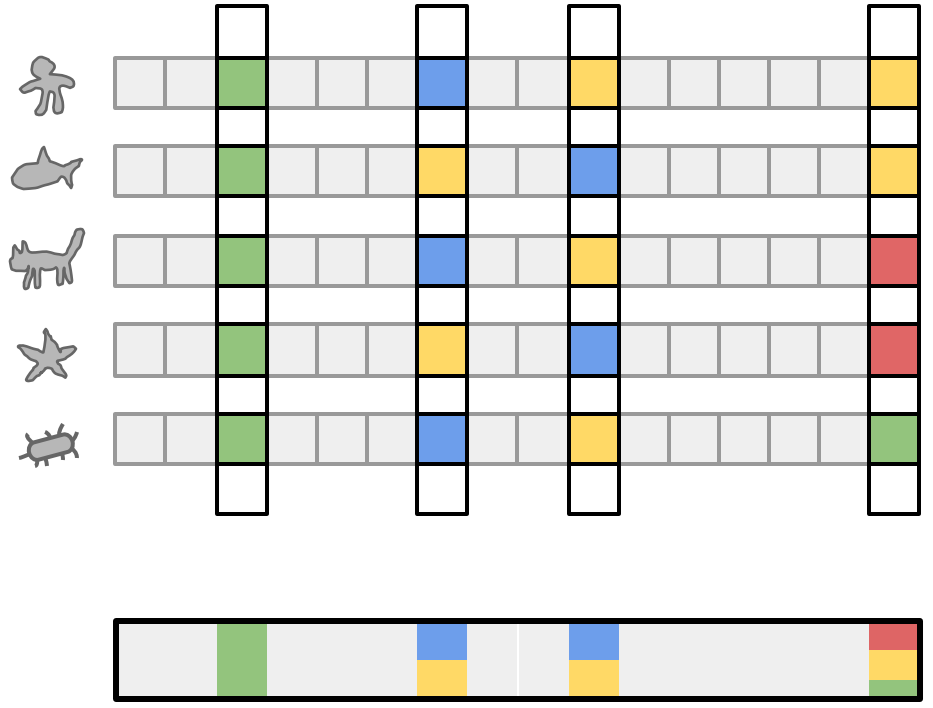
Search against a database of sequences



Generate a multiple sequence alignment

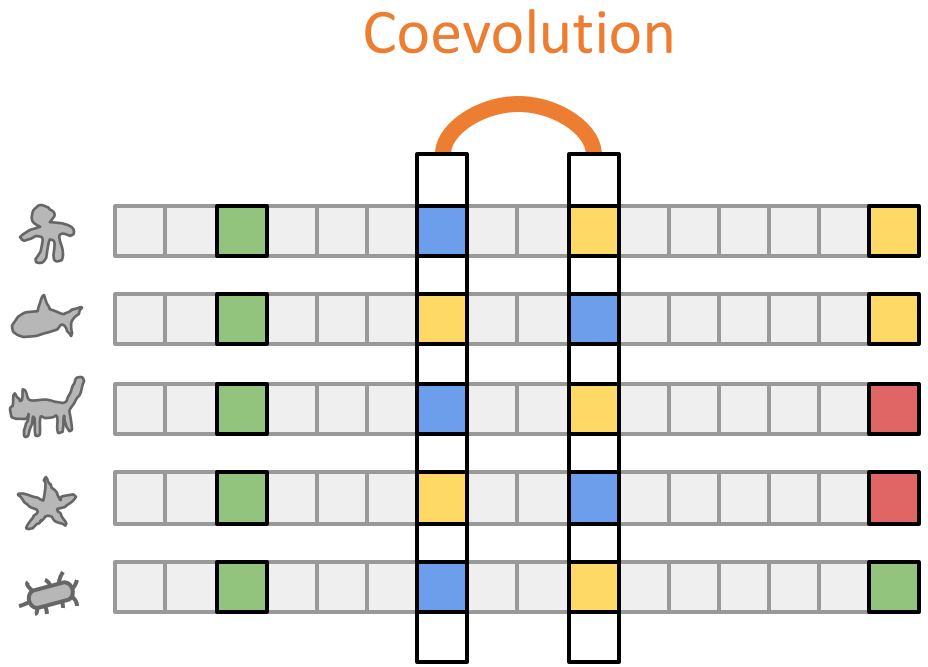


Analyze the MSA for conservation

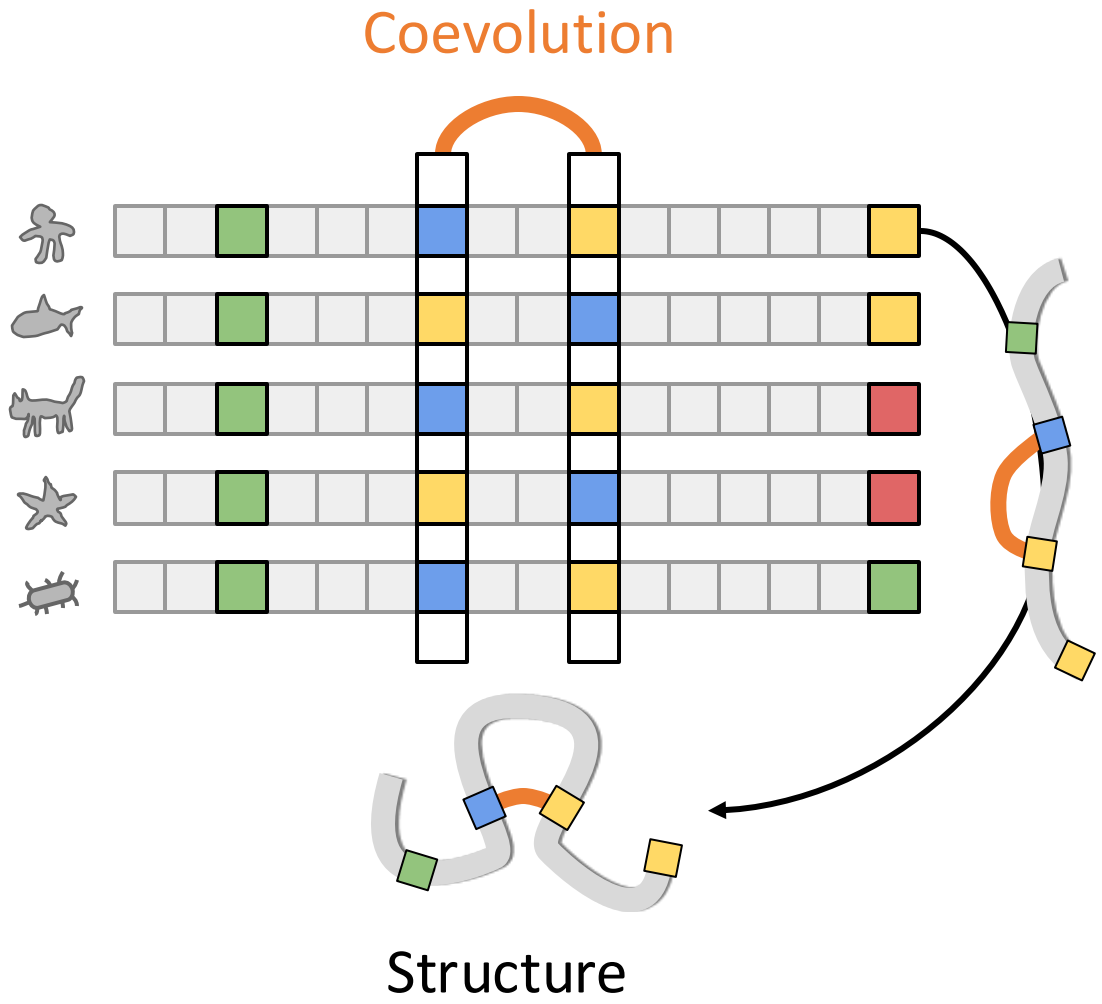


PSSM = Position-specific-scoring matrix

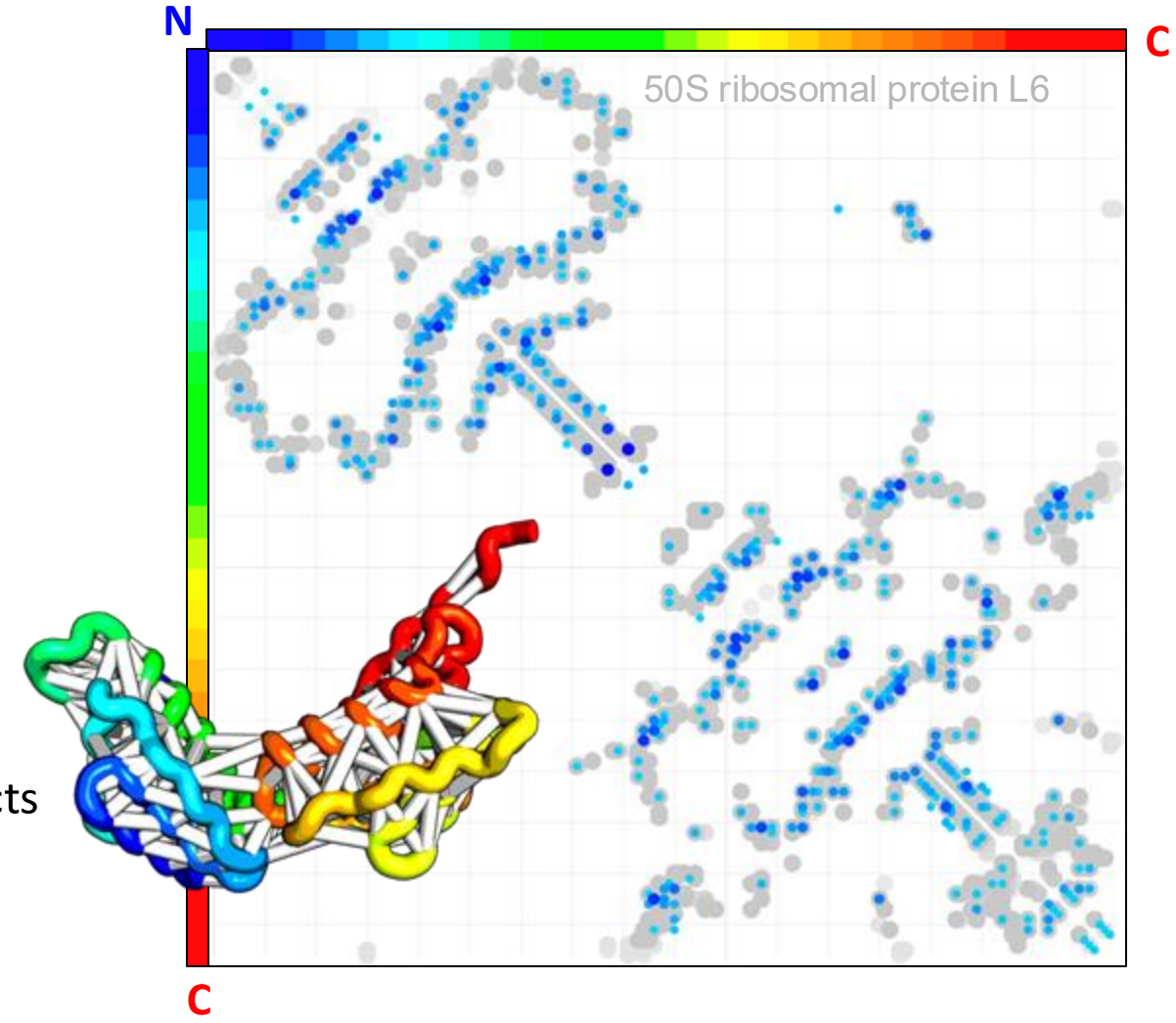
Analyze the MSA for coevolution



Use the as restraints in folding simulations!



The Contact map is correlated to physical contacts

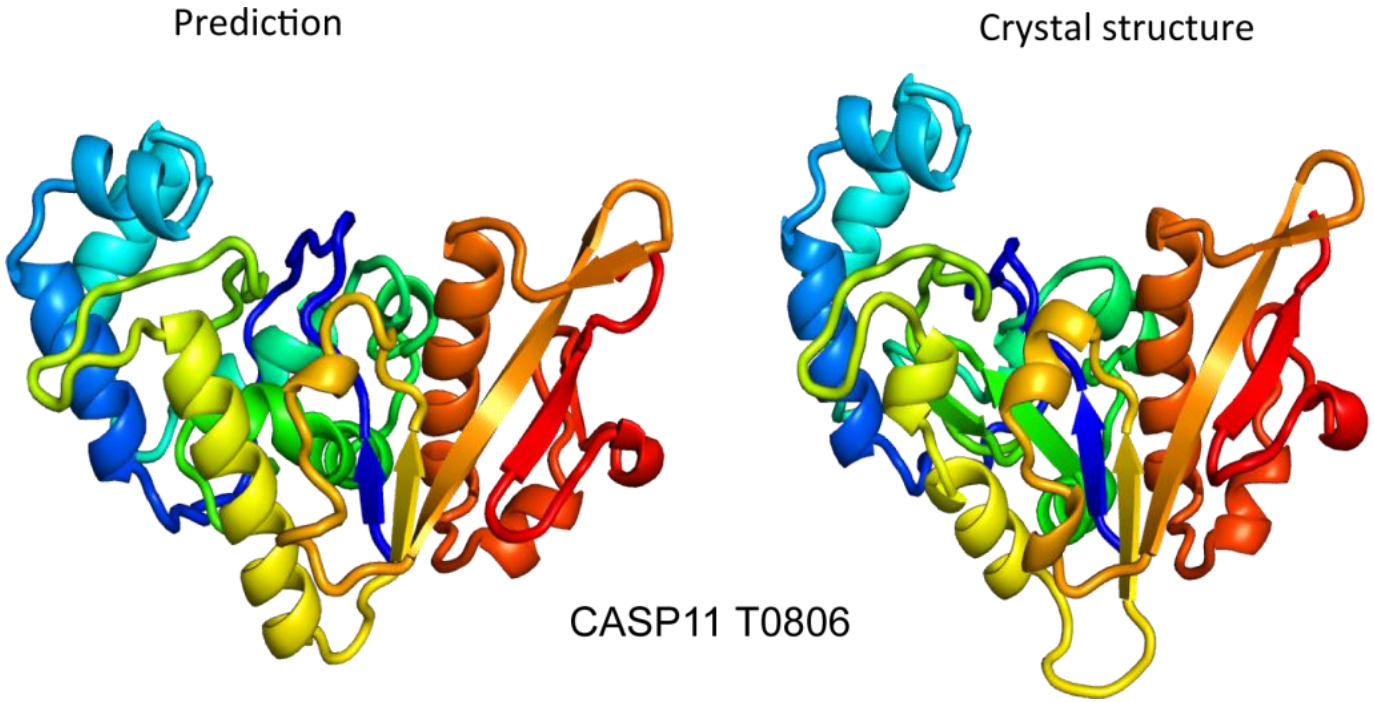


XRAY Contacts

Predicted Contacts

CASP-11 (2014)

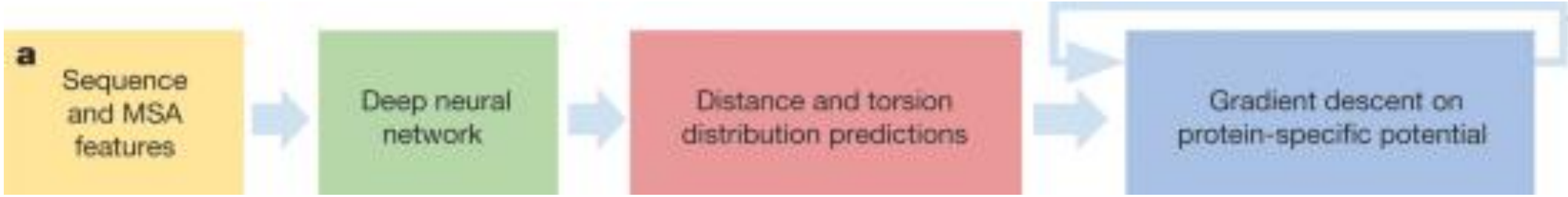
Sergey Ovchinnikov, David Baker

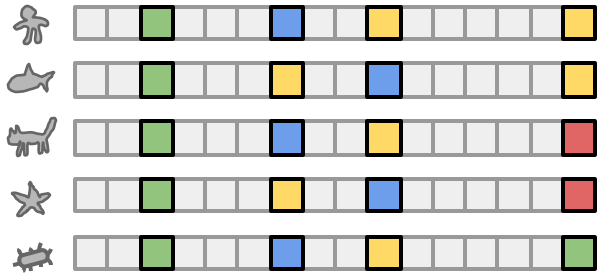


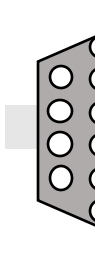
https://doi.org/10.7554/eLife.02030

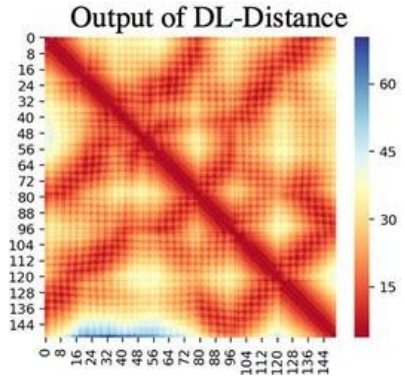
*S*tructure prediction:
Introducing AI

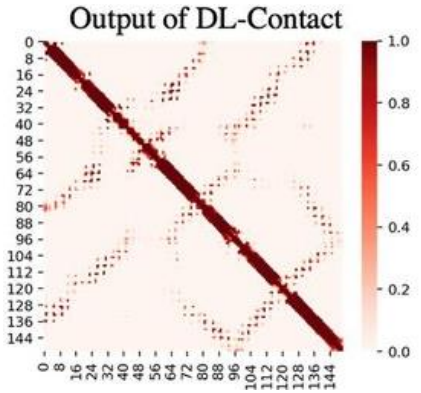
AlphaFold1: coevolution contacts from MSA



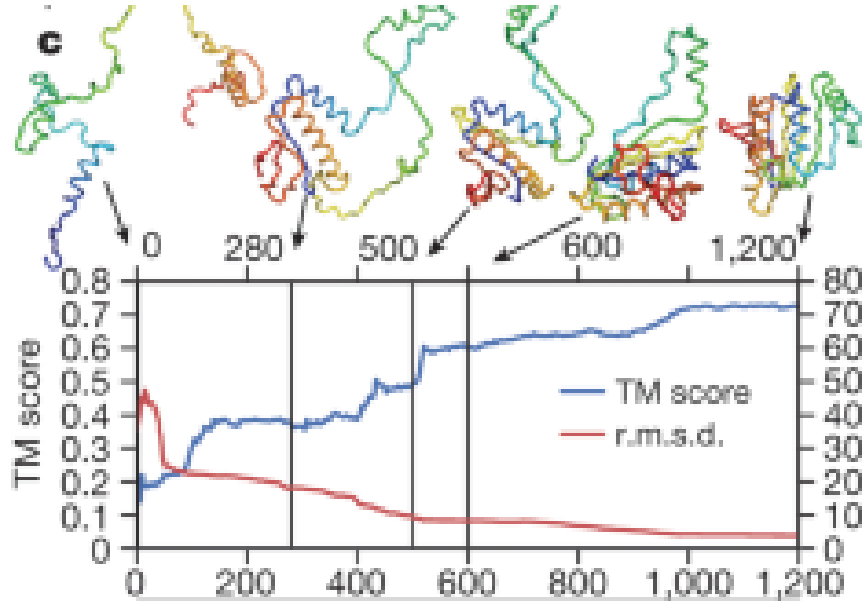








No physical parameters !!



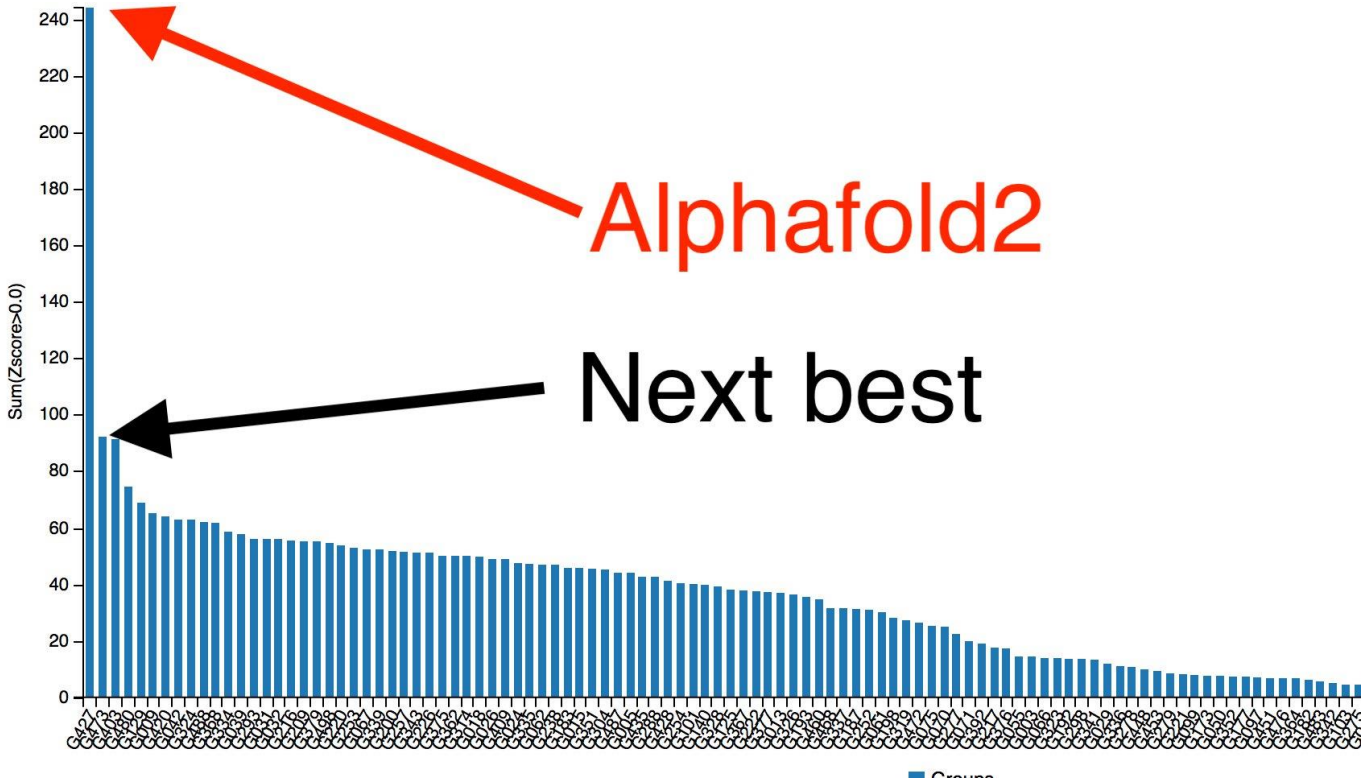
Minimize the restraints

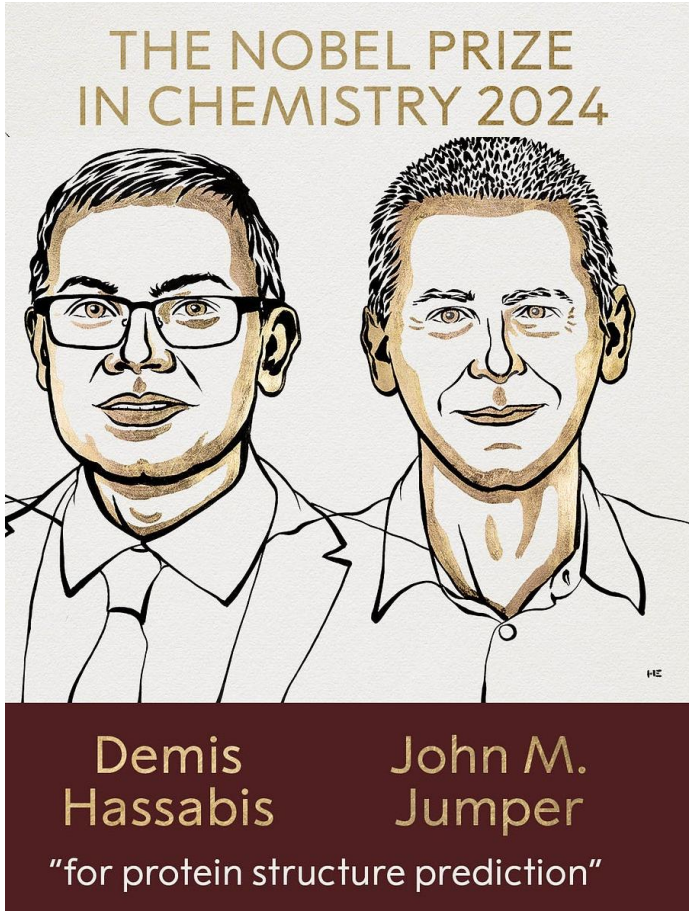
Contacts prediction module

Structure module

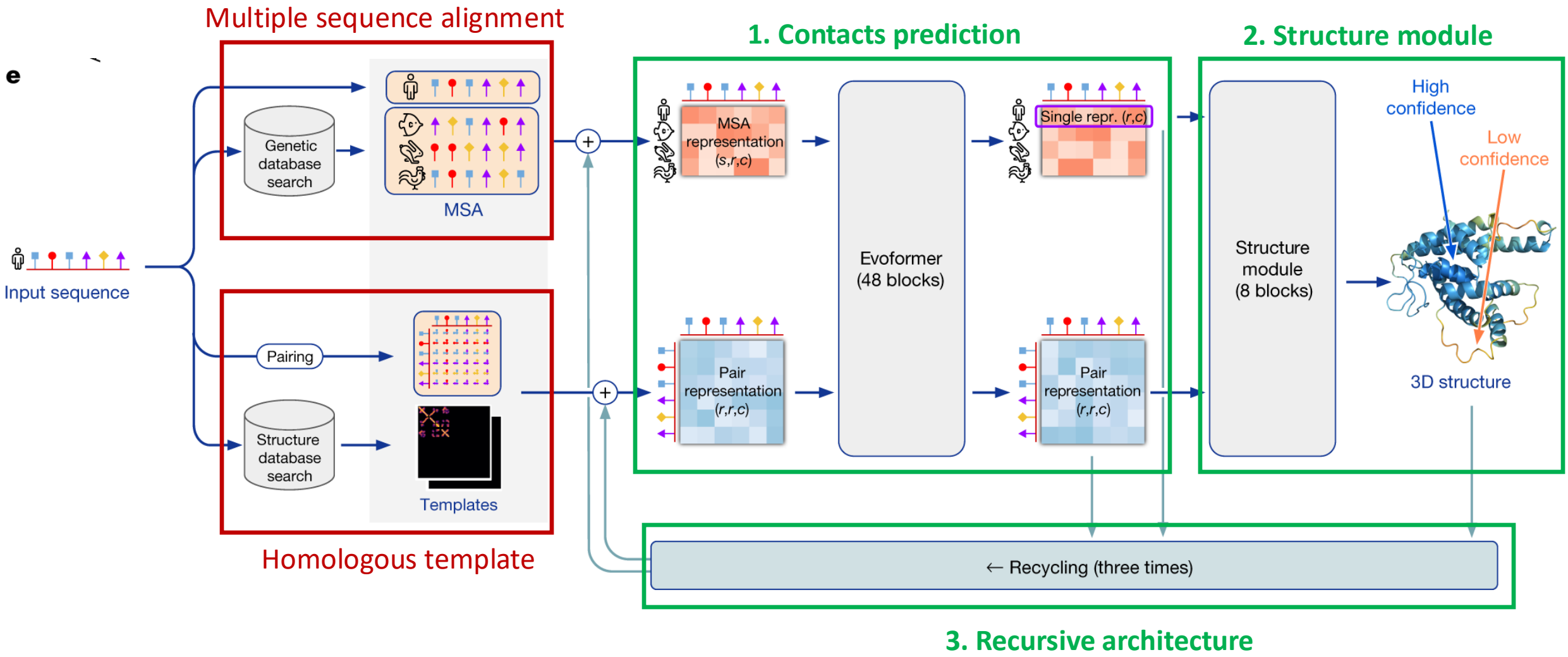
https://doi.org/10.1038/s41586-019-1923-7

AlphaFold2 on CASP-13 competition (2020)





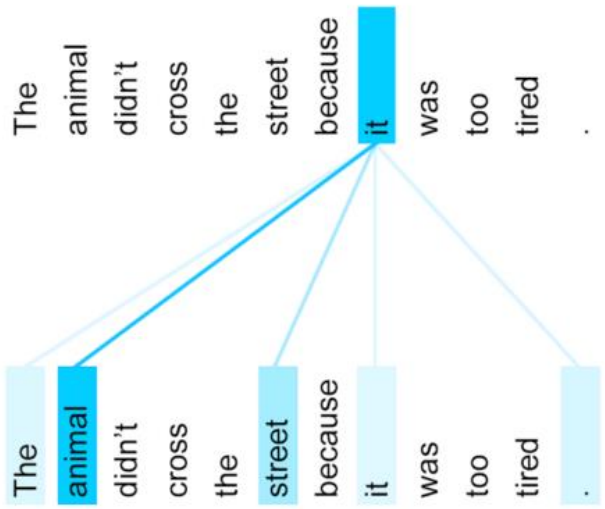
AlphaFold 2 - Architecture

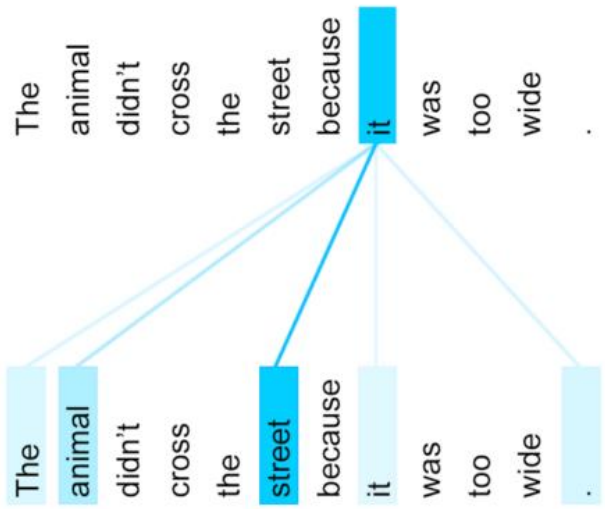


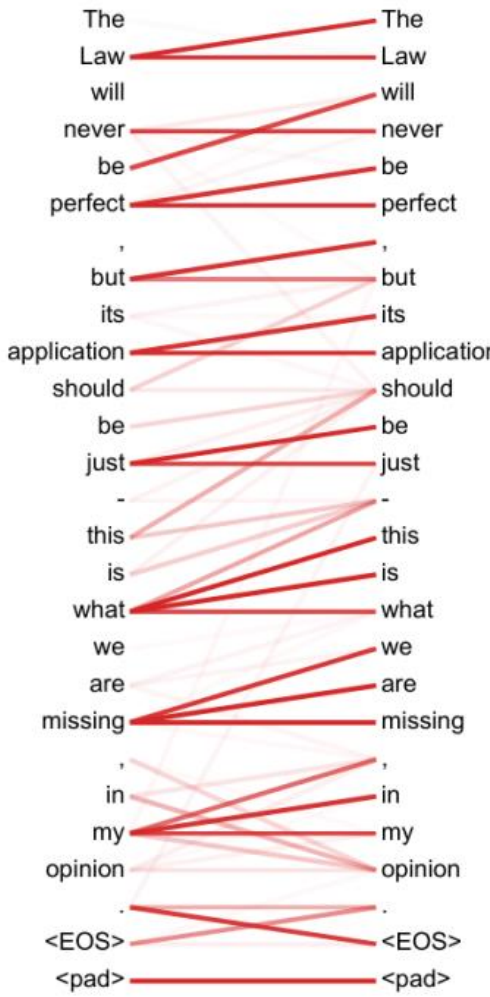
https://doi.org/10.1038/s41586-021-03819-2

1. Contacts prediction module

A **TRANSFORMER** with an **ATTENTION** mechanism



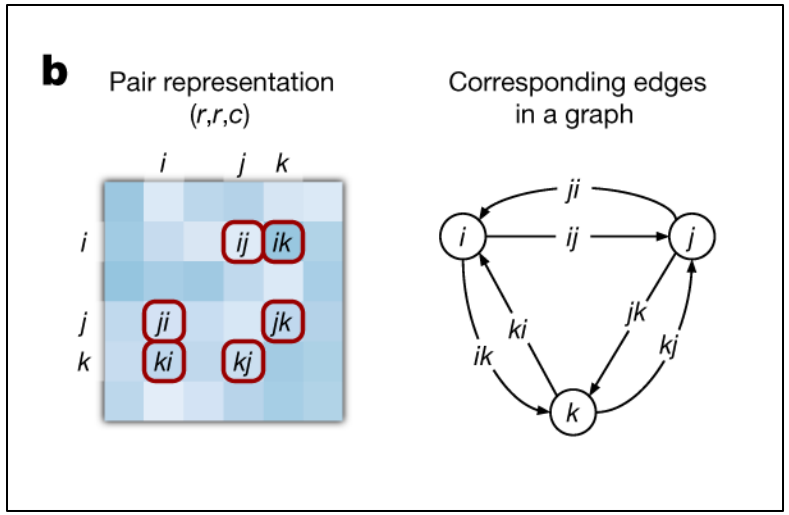


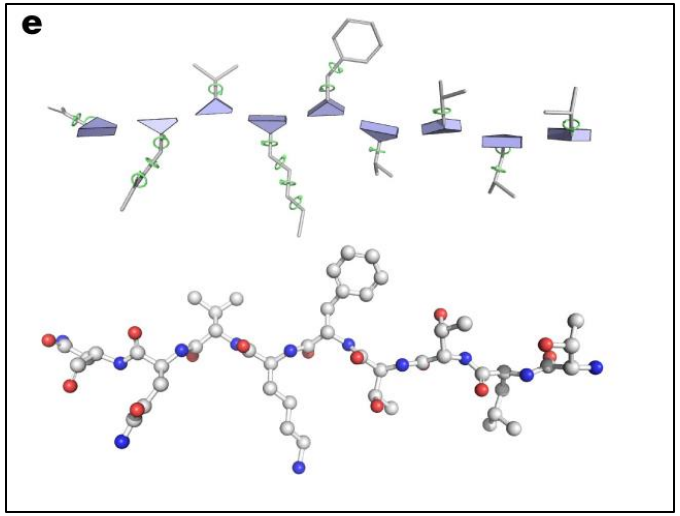


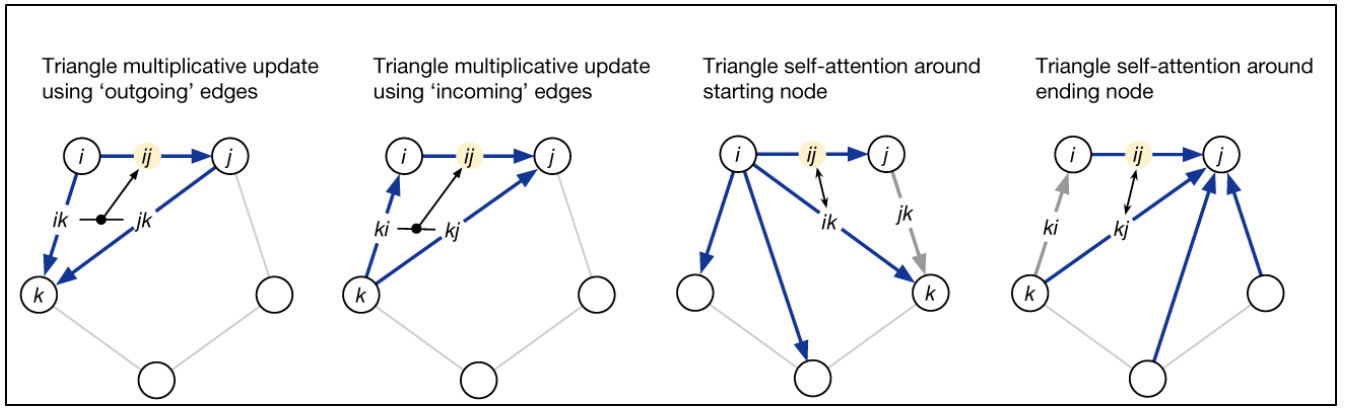
The **TRANSFORMER** that predicts contacts from evolution relationships ➔ **EVOFORMER**

2. Structure prediction module

All residues move independently in the space to satisfy the predicted contacts

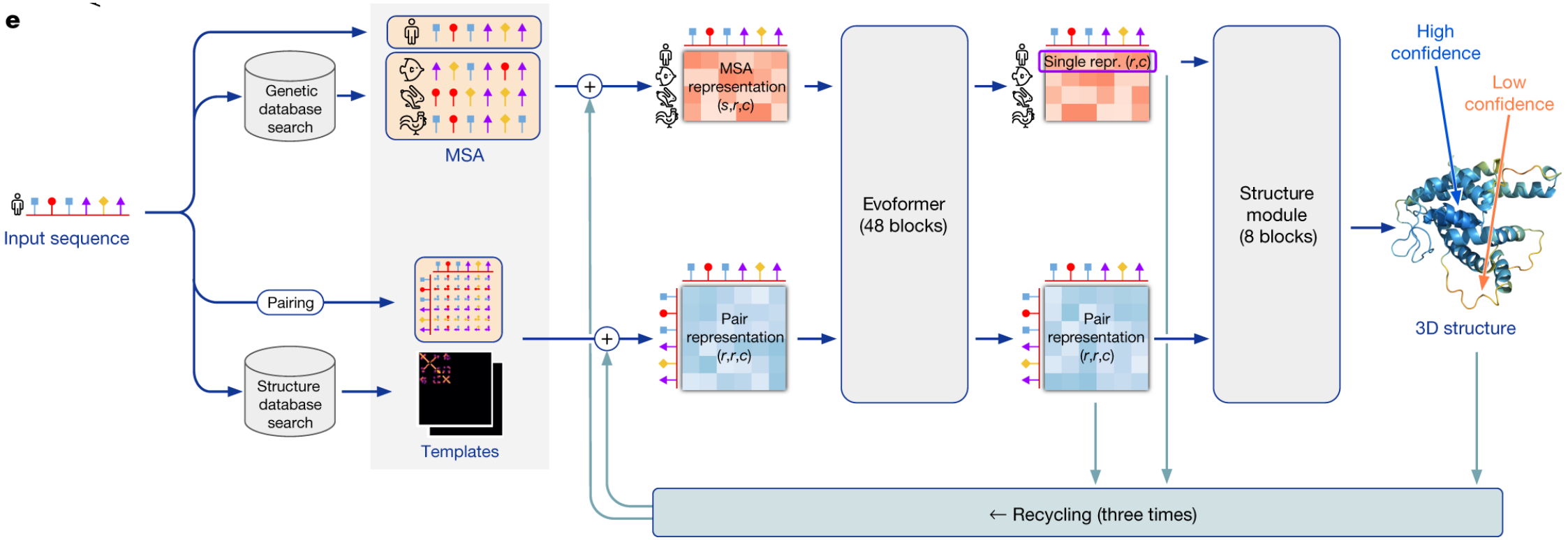






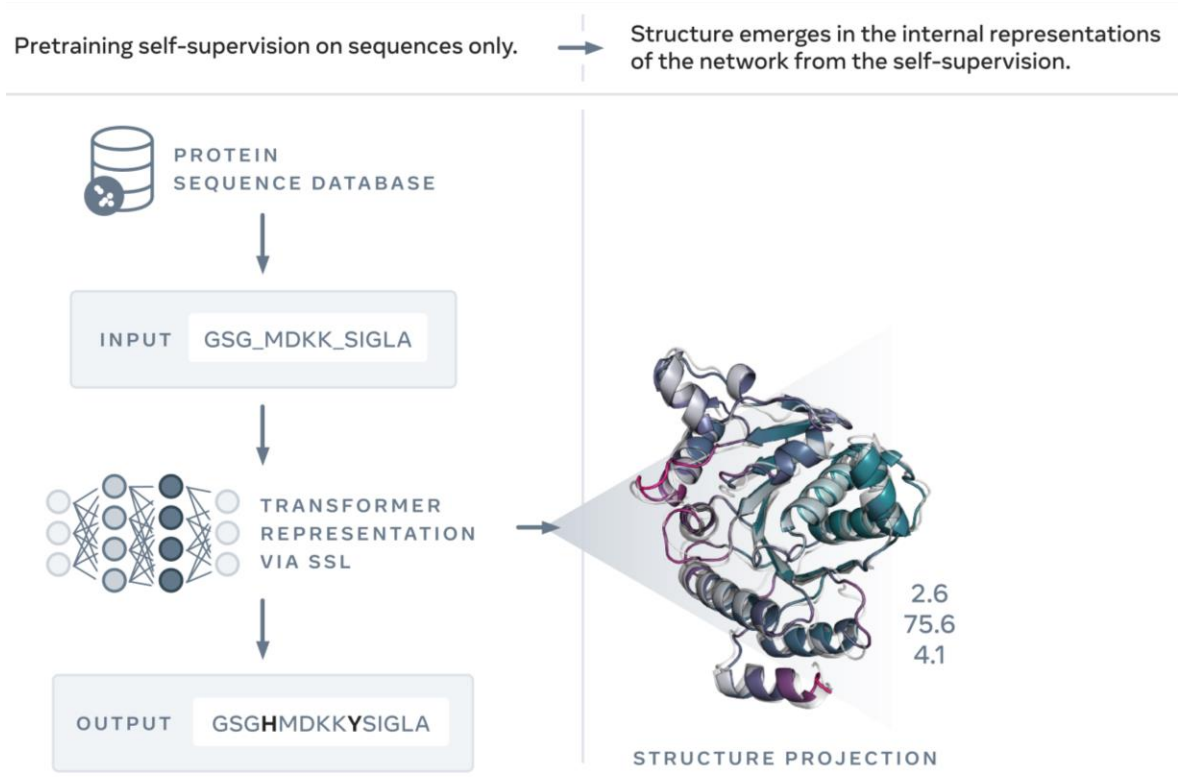
3. Recursive architecture to refine the solution

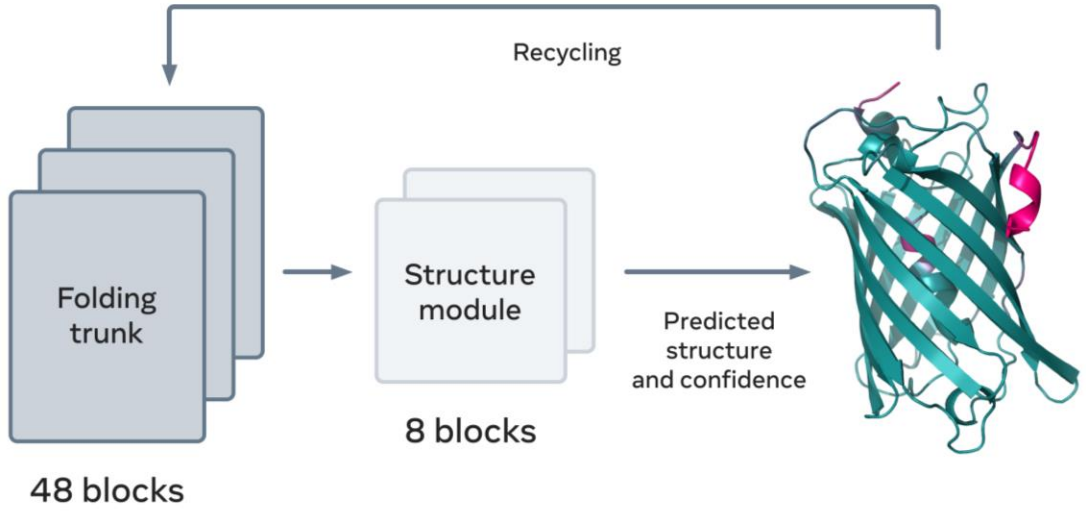
A multiple sequence alignment is a **HYPOTHESIS**



RECYCLING to refine the predicted structure based on local confidence assignments

Other AI models: Structure prediction with a protein language model (e.g. ESMFold from Meta AI)





Less accrate than AlphaFold2 but predicts structure from a single sequence (rather than from MSA)

https://doi.org/10.1126/science.ade2574