# Ab Initio Protein Structure Prediction: AlphaFold

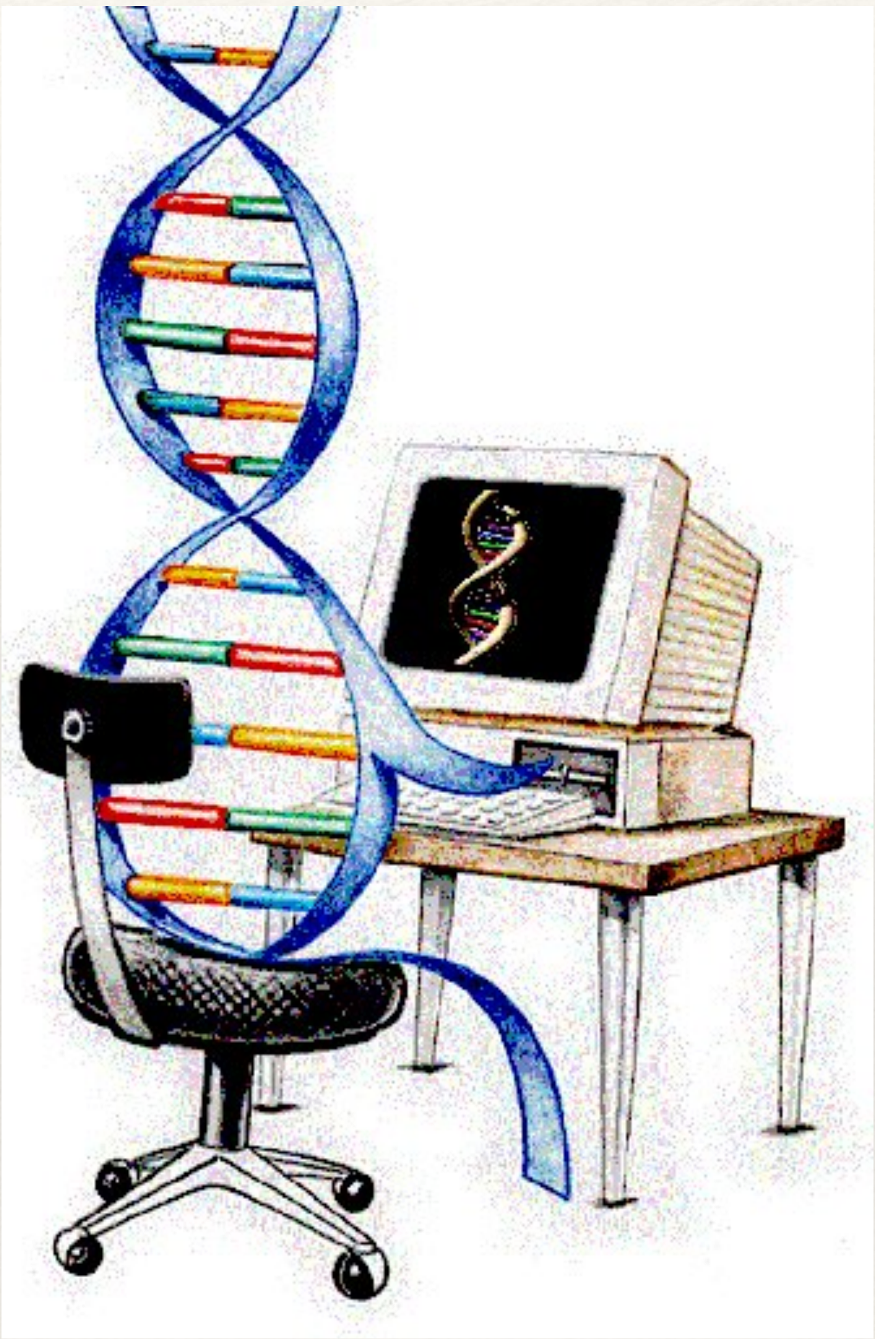
# Ab initio Protein Structure Prediction

Ab initio prediction before AlphaFold

Ab initio prediction: Predicting Contacts

AlphaFold 1

AlphaFold 2



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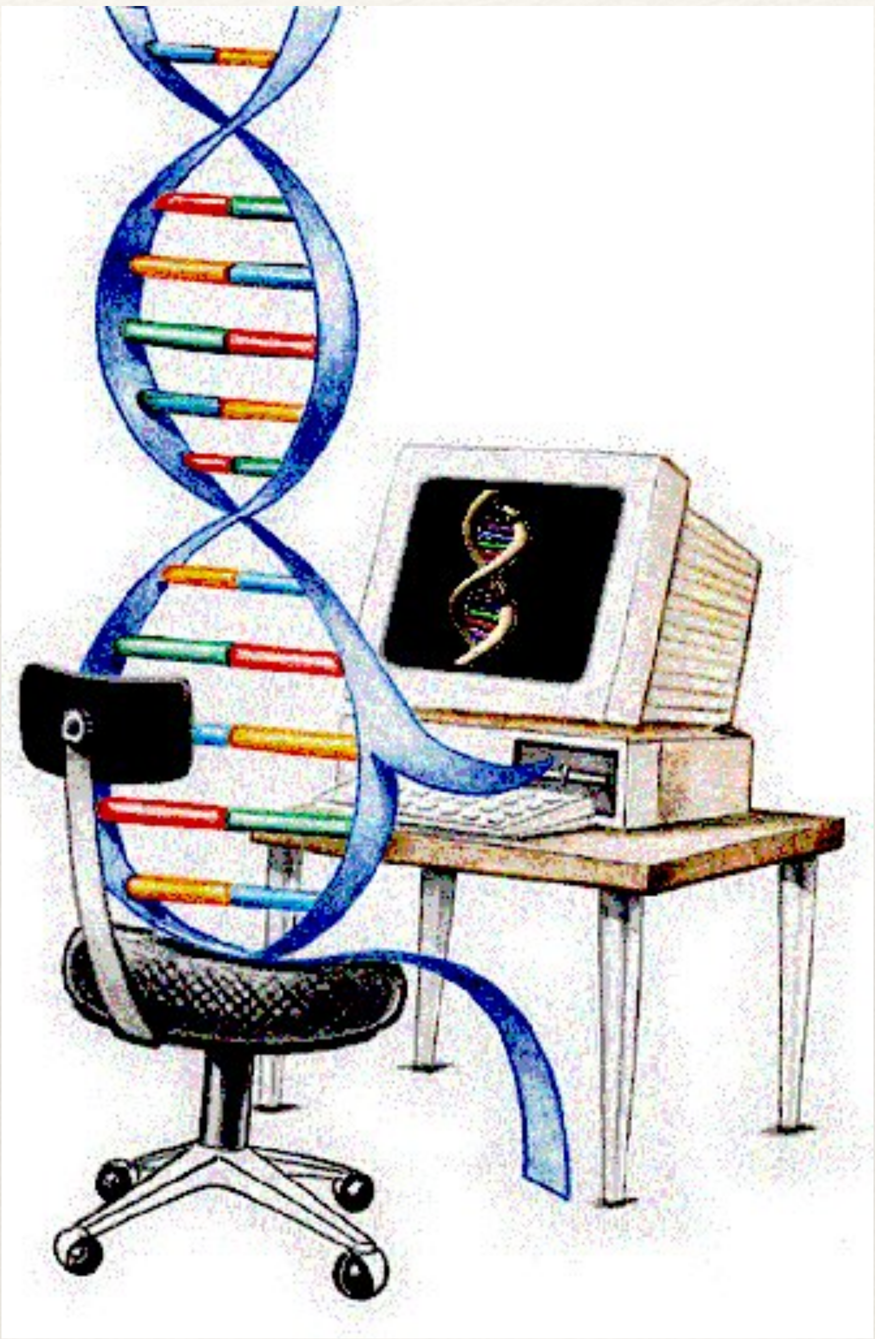
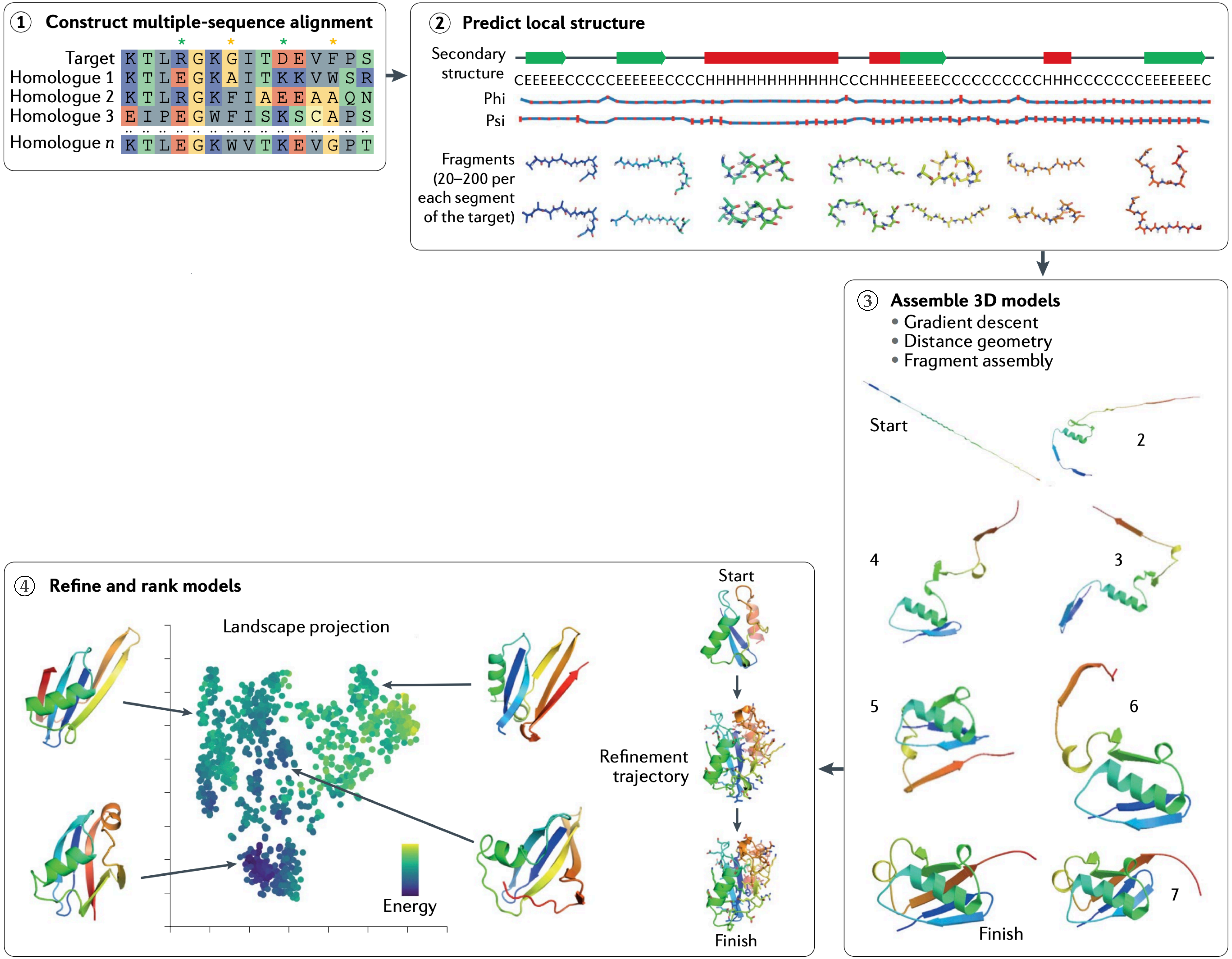
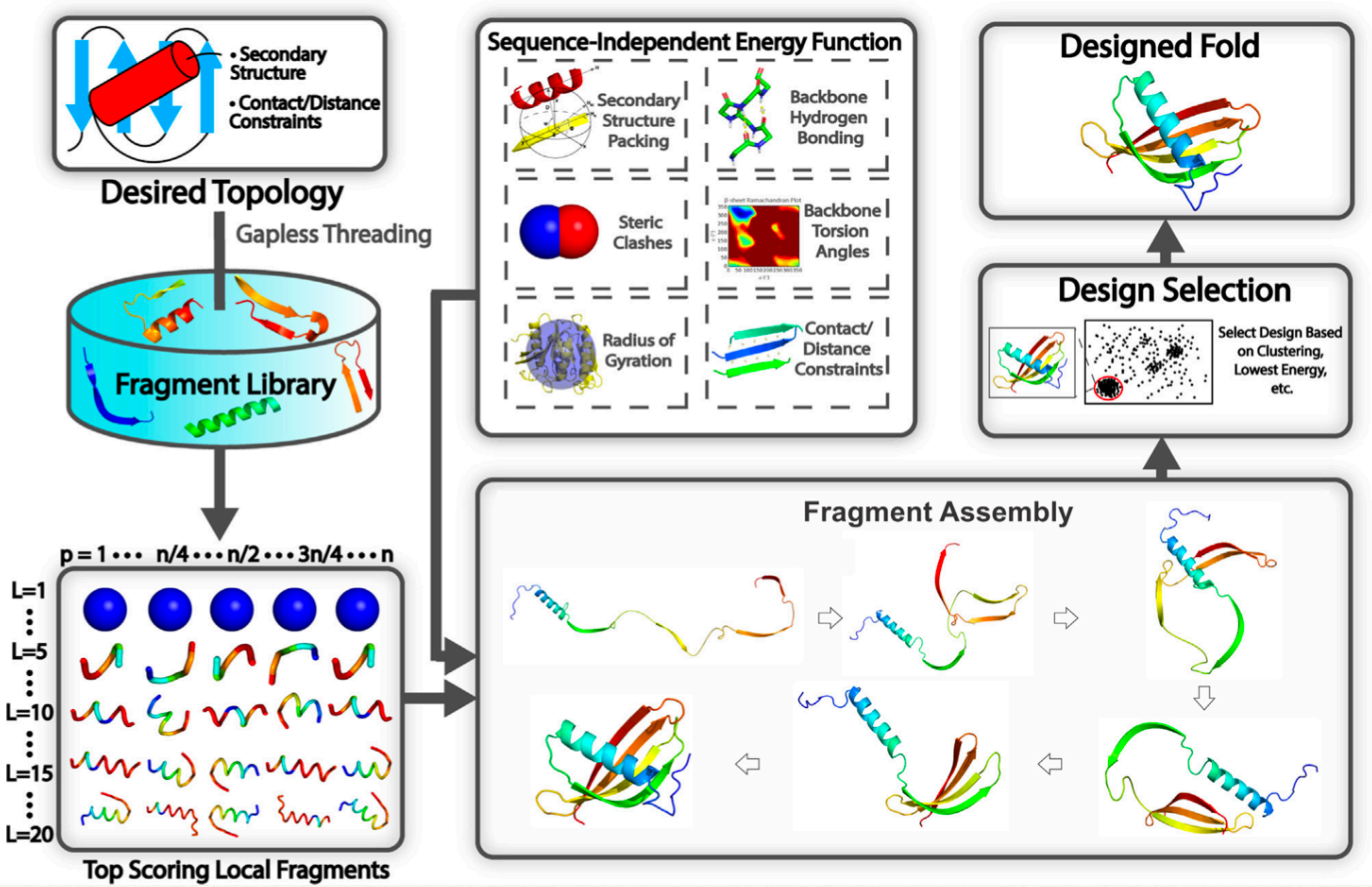


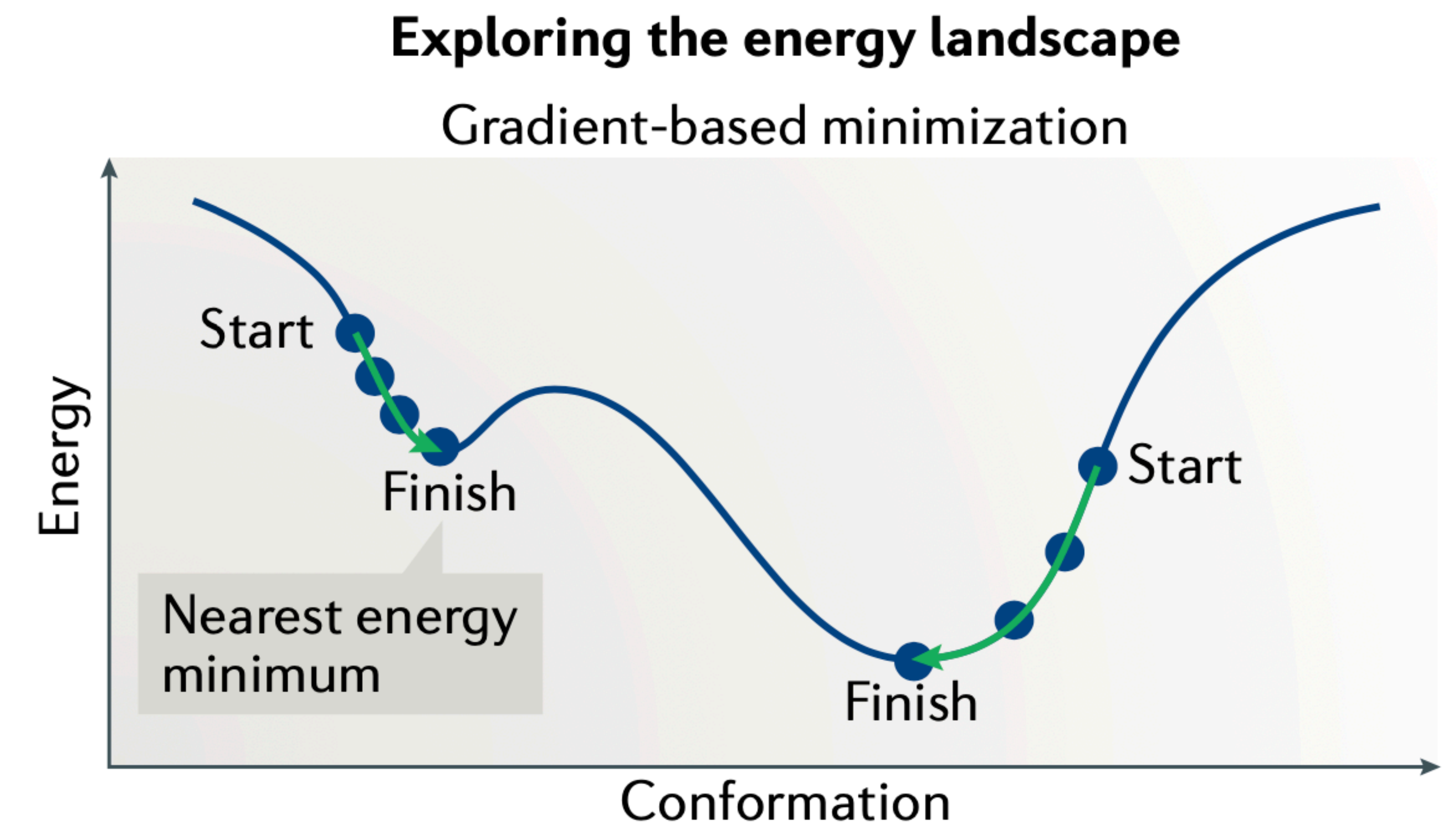


| | | | | * | | | * | | | * | | | * | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Target | K | T | L | R | G | K | G | I | T | D | E | V | F | P | S |
| Homologue 1 | K | T | L | E | G | K | A | I | T | K | K | V | W | S | R |
| Homologue 2 | K | T | L | R | G | K | F | I | A | E | E | A | A | Q | N |
| Homologue 3 | E | I | P | E | G | W | F | I | S | K | S | C | A | P | S |
| | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Homologue *n* | K | T | L | E | G | K | W | V | T | K | E | V | G | P | T |

Secondary structure CEEEEECCCCCEEEEEECCCCHHHHHHHHHHHHHCCCHHHEEEEECCCCCCCCCCHHHCCCCCCCEEEEEEEC

# Fragment based methods


• Secondary Structure
• Contact/Distance Constraints
Desired Topology
Gapless Threading
Fragment Library
p = 1 • • • n/4 • • • n/2 • • • 3n/4 • • • n
L=1
L=5
L=10
L=15
L=20
Top Scoring Local Fragments
Sequence-Independent Energy Function
Secondary Structure Packing
Backbone Hydrogen Bonding
Steric Clashes
Backbone Torsion Angles
Radius of Gyration
Contact/ Distance Constraints
Fragment Assembly
Designed Fold
Design Selection
Select Design Based on Clustering, Lowest Energy, etc.



Exploring the energy landscape

Gradient-based minimization

Metropolis Monte Carlo
Energy
Conformation
P = 0.03
ΔE = 2.1
P = 0.58
P = 1
P = 1
P = 0.07
Start
ΔE = 0.33
Global minimum
P = 1
ΔE = 1.6
Finish
Monte Carlo moves
Fragment replacement
Rotamer substitution

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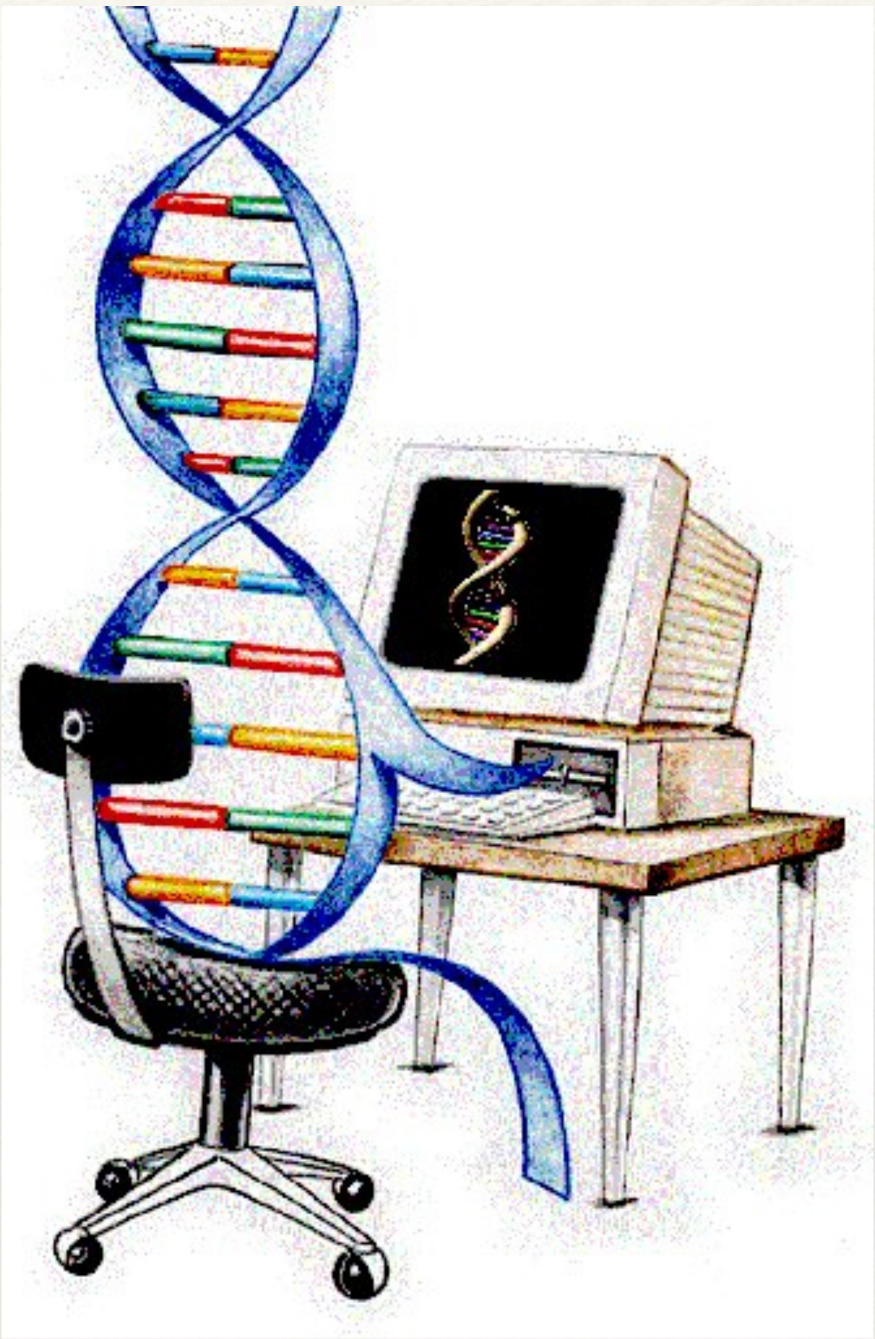
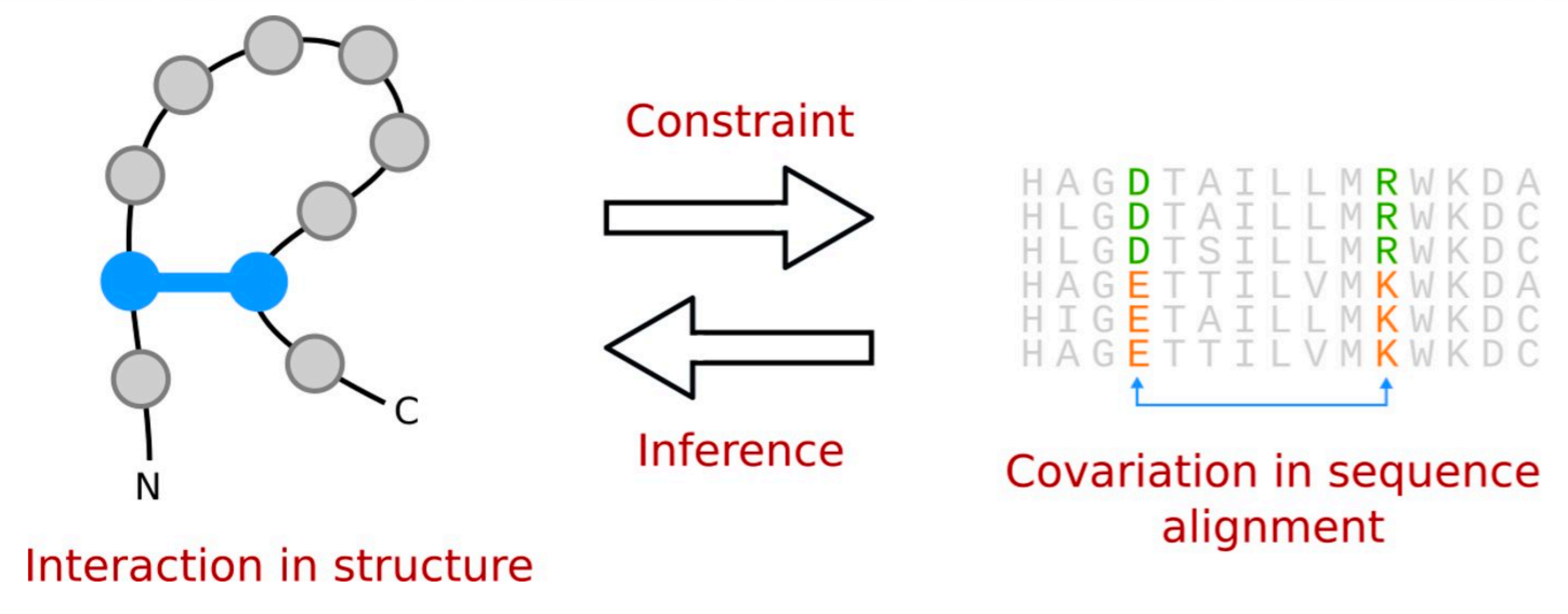


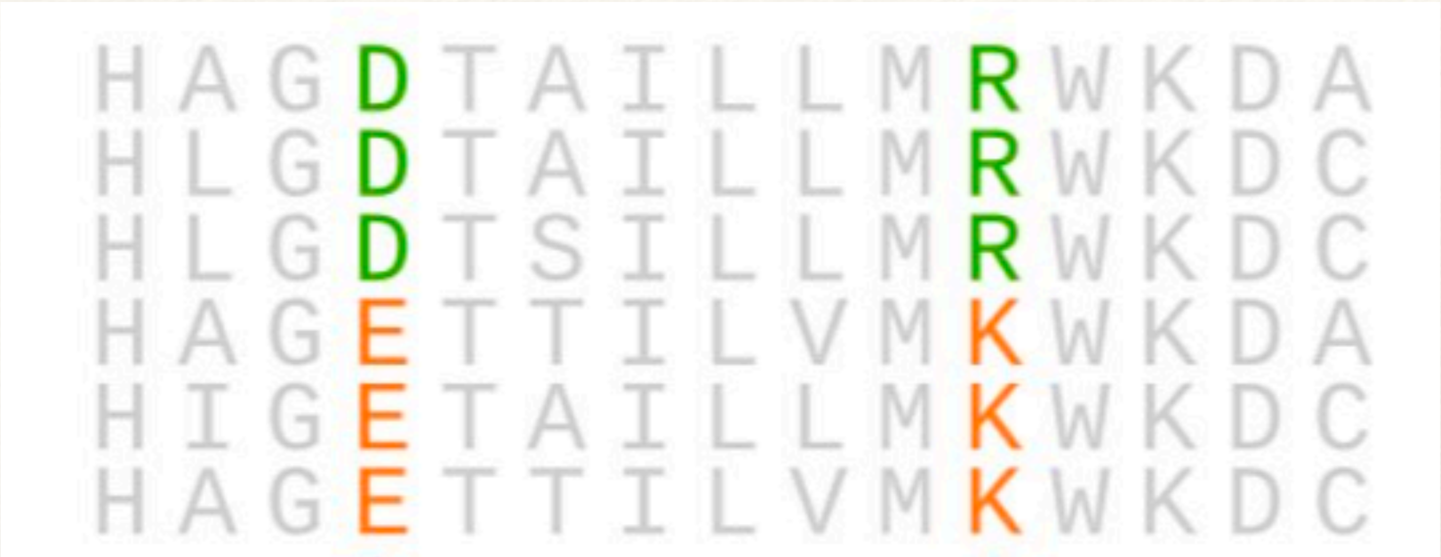
# Predicting residue contacts



```
HAGDTAILLMRWKDA
HLGDTAILLMRWKDC
HLGDTSILLMRWKDC
HAGETTILVMKWKDA
HIGETAILLMKWKDC
HAGETTILVMKWKDC
```

# Predicting residue contacts

*1. Given a multiple sequence alignment (MSA):*



$X_1$ HAGDTAILLMRWKDA
HLGDTAILLMRWKDC
HLGDTSILLMRWKDC
HAGETTILVMKWKDA
HIGETAILLMKWKDC
$X_N$ HAGETTILVMKWKDC

*2. Compute "mean" sequence and covariance matrix:*

$$\overline{\mathrm{X}} = \frac{1}{N}\sum_{n=1}^{N} \mathrm{X}_n$$

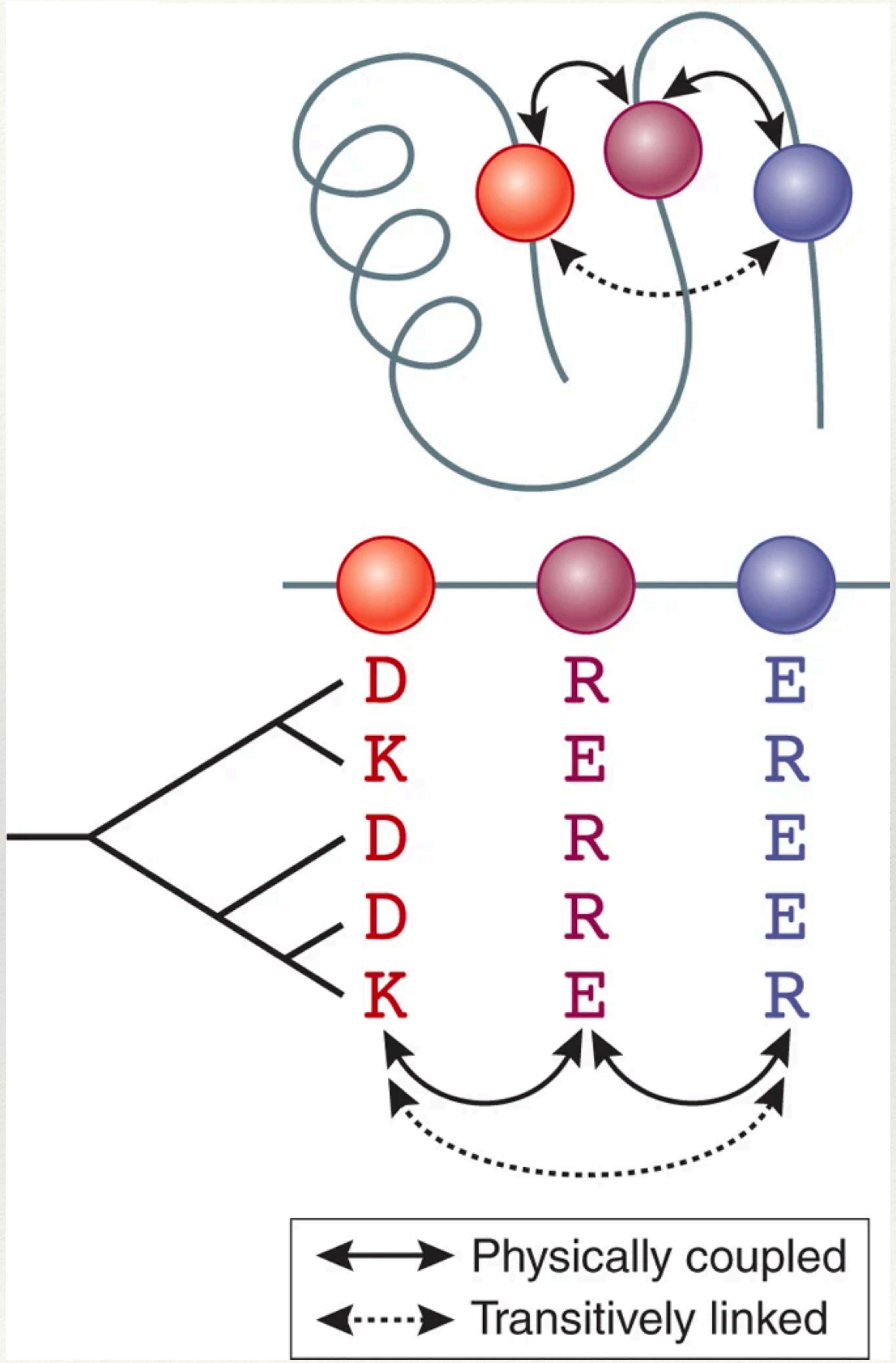
$$\overline{C} = C(MSA, \overline{\mathrm{X}}) = \frac{1}{N}\sum_{n=1}^{N} (\mathrm{X}_n - \overline{\mathrm{X}})^T(\mathrm{X}_n - \overline{\mathrm{X}})$$

*3. Compute contact J(i,j)*

$$J(i,j) = C(i,j)?$$

# Predicting residue contacts

*No! We need to pay attention to indirect effects:*



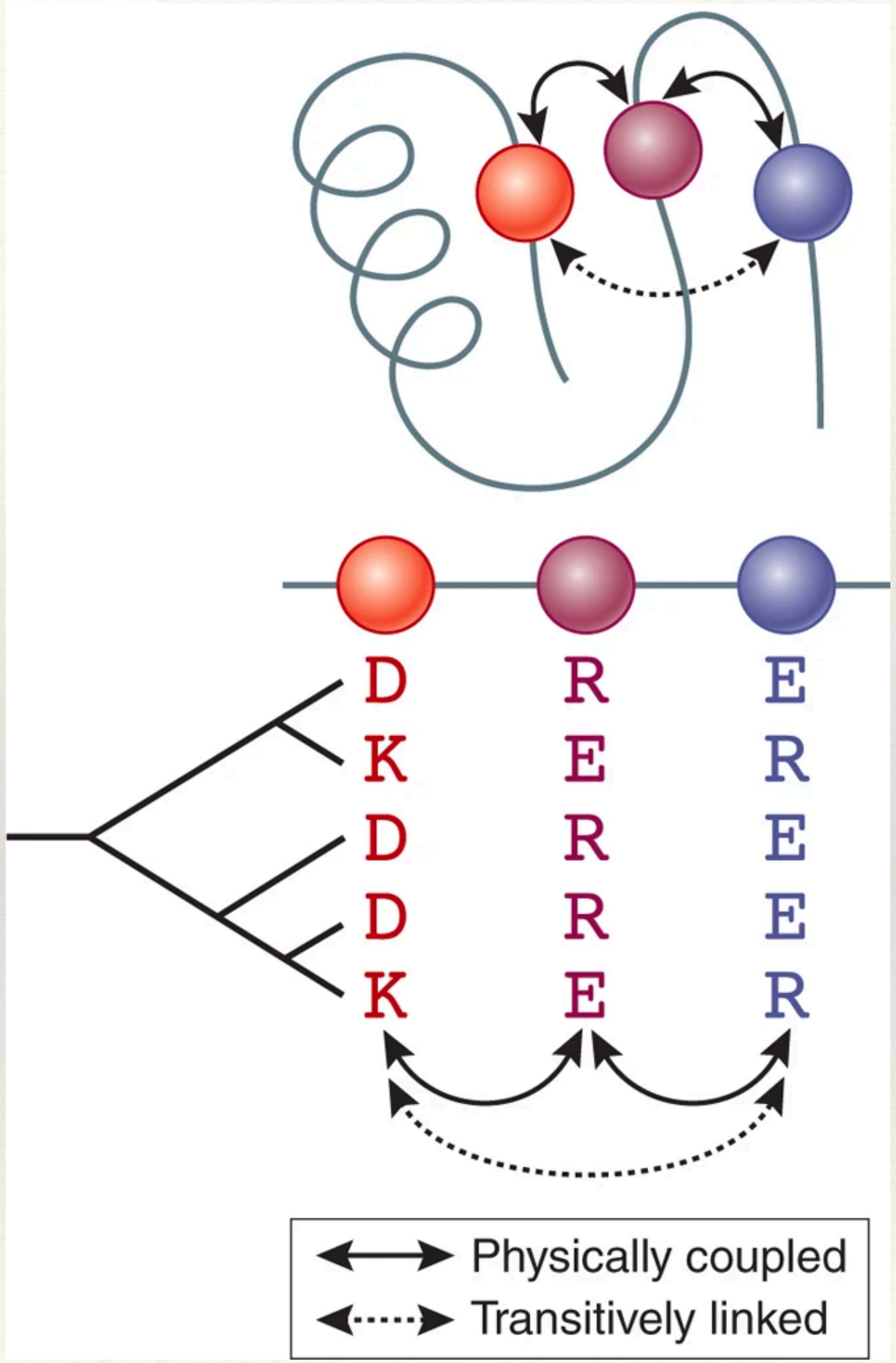
# Predicting residue contacts

***No! We need to pay attention to indirect effects:***

*Gaussian model:*

Each sequence $X_i$ in the MSA is drawn from a multivariate Gaussian distribution characterized by a mean vector $\boldsymbol{\mu}$ and a covariance matrix $\Sigma$, with the probability:

$$P(\mathrm{X}_n \,|\, \boldsymbol{\mu}, \Sigma) = (2\pi)^{-\frac{Ls}{2}} \,|\,\Sigma\,|^{-\frac{1}{2}} \exp\left[-\frac{1}{2}(\mathrm{X}_n - \boldsymbol{\mu})^T \Sigma^{-1} (\mathrm{X}_n - \boldsymbol{\mu})\right]$$



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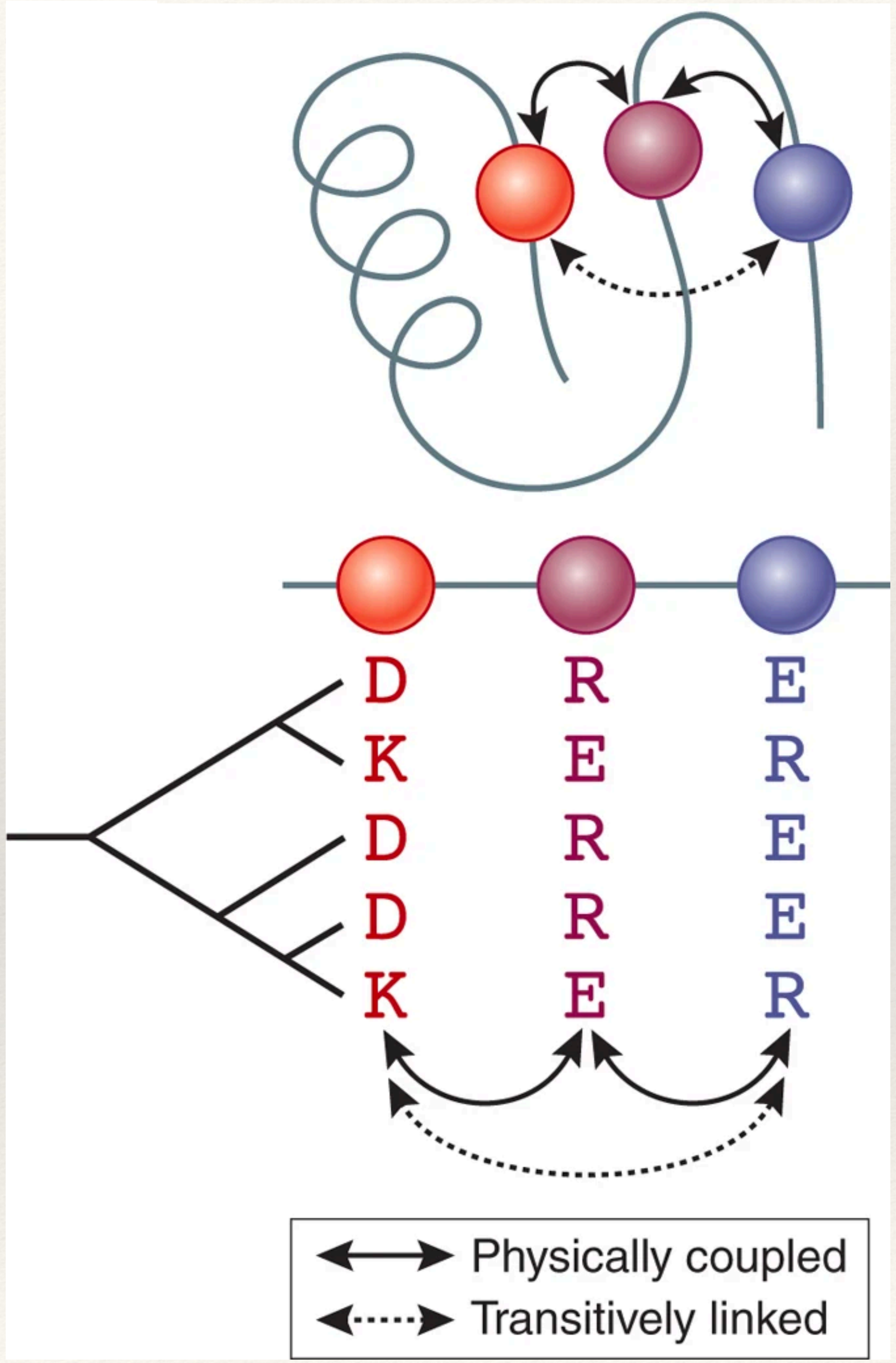
Assuming that the $N$ sequences in the MSA are statistical independent, the probability, or likelihood of the data under this model is given by

$$P(MSA \,|\, \boldsymbol{\mu}, \Sigma) = \prod_{n=1}^{N} P(\mathrm{X}_n \,|\, \boldsymbol{\mu}, \Sigma)$$



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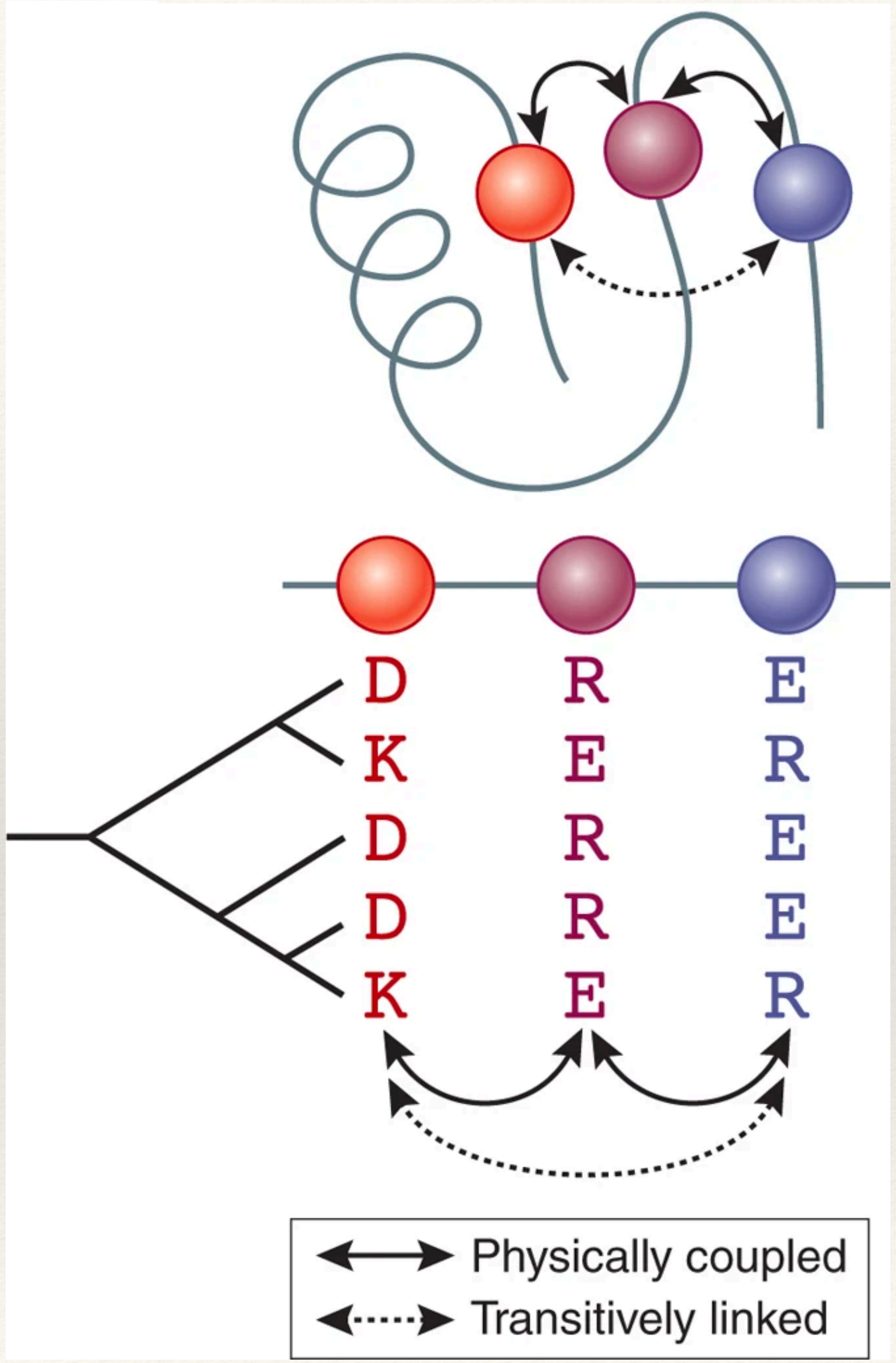
$$P(MSA \,|\, \boldsymbol{\mu}, \Sigma) = \prod_{n=1}^{N} P(\mathrm{X}_n \,|\, \boldsymbol{\mu}, \Sigma)$$

Using the maximum likelihood estimator for this probability

$$\boldsymbol{\mu} = \overline{\mathrm{X}}$$

$$\Sigma = \overline{C} = C(MSA, \overline{\mathrm{X}})$$



# Predicting residue contacts

***No! We need to pay attention to indirect effects:***

*Gaussian model:*

$$P(\mathrm{X}_n|\boldsymbol{\mu},\Sigma) = (2\pi)^{-\frac{Ls}{2}}|\Sigma|^{-\frac{1}{2}}\exp\left[-\frac{1}{2}(\mathrm{X}_n-\boldsymbol{\mu})^T\Sigma^{-1}(\mathrm{X}_n-\boldsymbol{\mu})\right]$$

$$\boldsymbol{\mu} = \overline{\mathrm{X}} \qquad \Sigma = \overline{C} = C(MSA,\overline{\mathrm{X}})$$

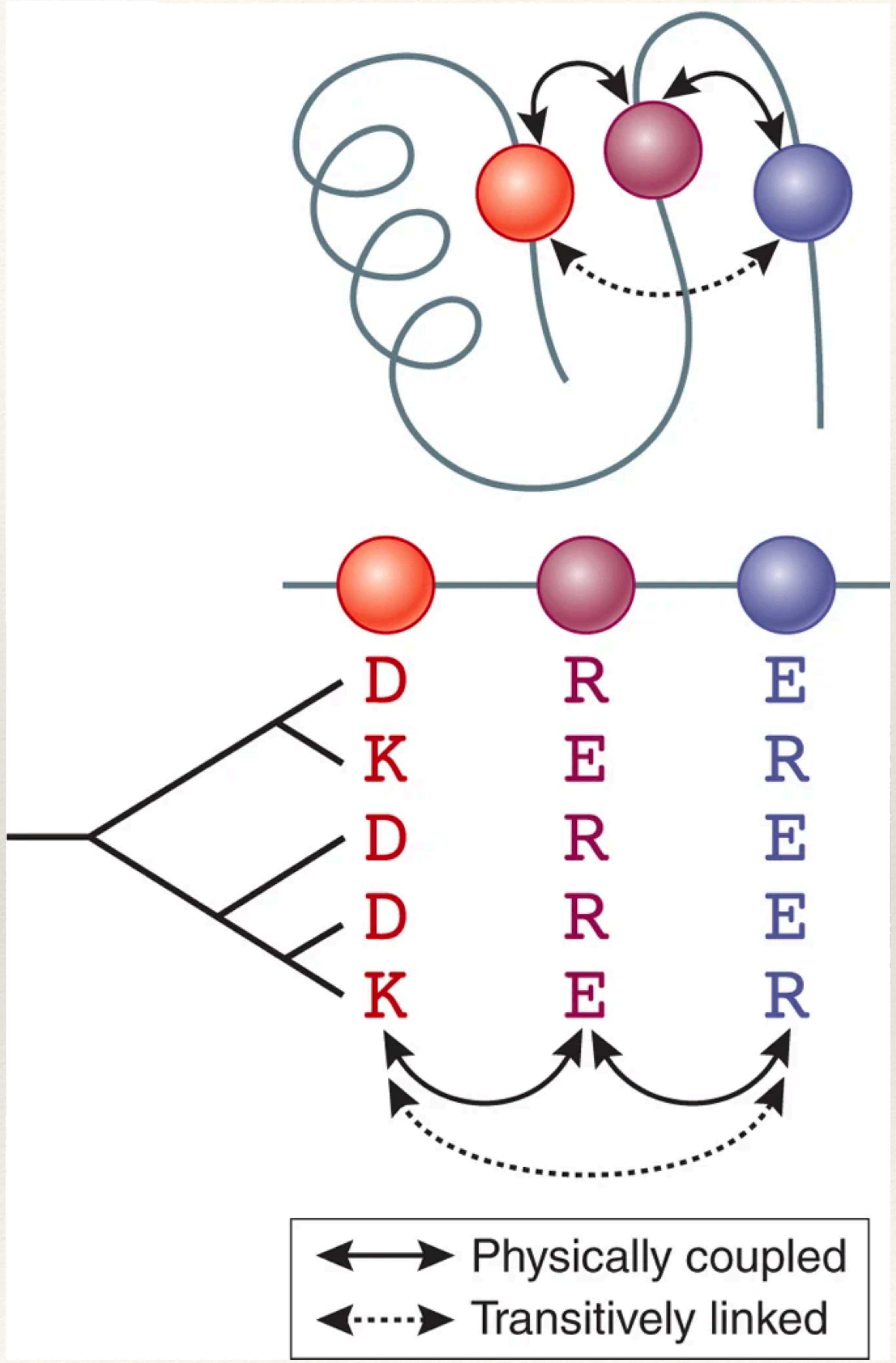
Note that:

$$(\mathrm{X}_n-\boldsymbol{\mu})^T\Sigma^{-1}(\mathrm{X}_n-\boldsymbol{\mu}) = \sum_{k=1}^{N}\sum_{l=1}^{N}(X_k-\mu_k)(\Sigma^{-1})(k,l)(X_l-\mu_l)$$

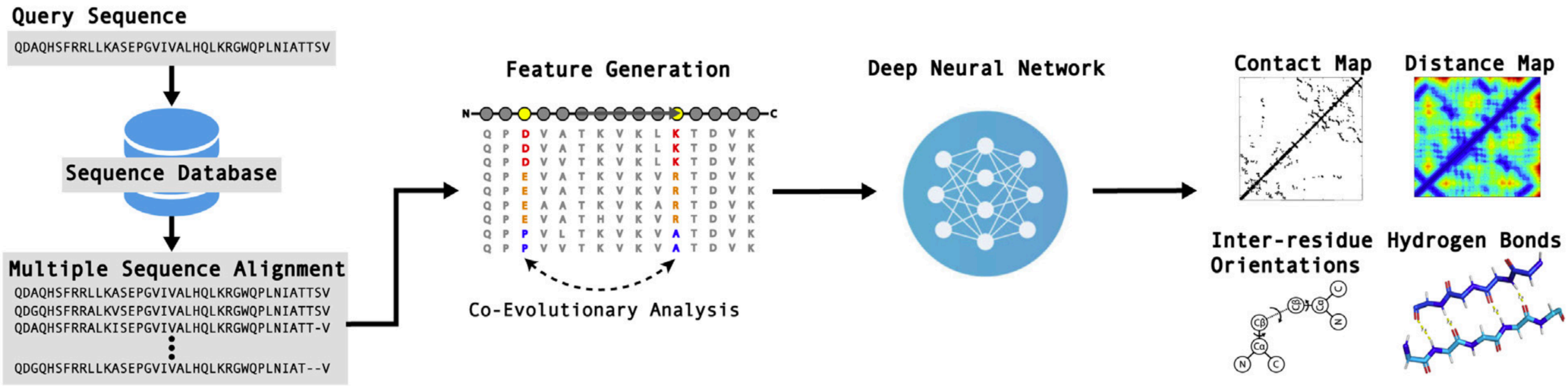
This shows that $(\Sigma^{-1})(k,l)$ serves as a coupling between positions $k$ and $l$ in the MSA.

Therefore:

$$J = \Sigma = (C(MSA,\overline{X}))^{-1}$$



# Predicting residue contacts



Query Sequence

QDAQHSFRRLLKASEPGVIVALHQLKRGWQPLNIATTSV

Sequence Database

Multiple Sequence Alignment

```
QDAQHSFRRLLKASEPGVIVALHQLKRGWQPLNIATTSV
QDGQHSFRRALKVSEPGVIVALHQLKRGWQPLNIATTSV
QDAQHSFRRALKISEPGVIVALHQLKRGWQPLNIATT-V
⋮
QDGQHSFRRLLKASEPGVIVALHQLKRGWQPLNIAT--V
```

Feature Generation

```
N-○-○-○-○-○-○-○-○-○-○-○-○-○-○-○-C
  Q P D V A T K V K L K T D V K
  Q P D V A T K V K L K T D V K
  Q P D V V T K V K L K T D V K
  Q P E V A T K V K V R T D V K
  Q P E V A T K V K V R T D V K
  Q P E A A T K V K A R T D V K
  Q P E V A T H V K V R T D V K
  Q P P V L T K V K V A T D V K
  Q P P V V T K V K V A T D V K
```

Co-Evolutionary Analysis

Deep Neural Network

Contact Map

Distance Map

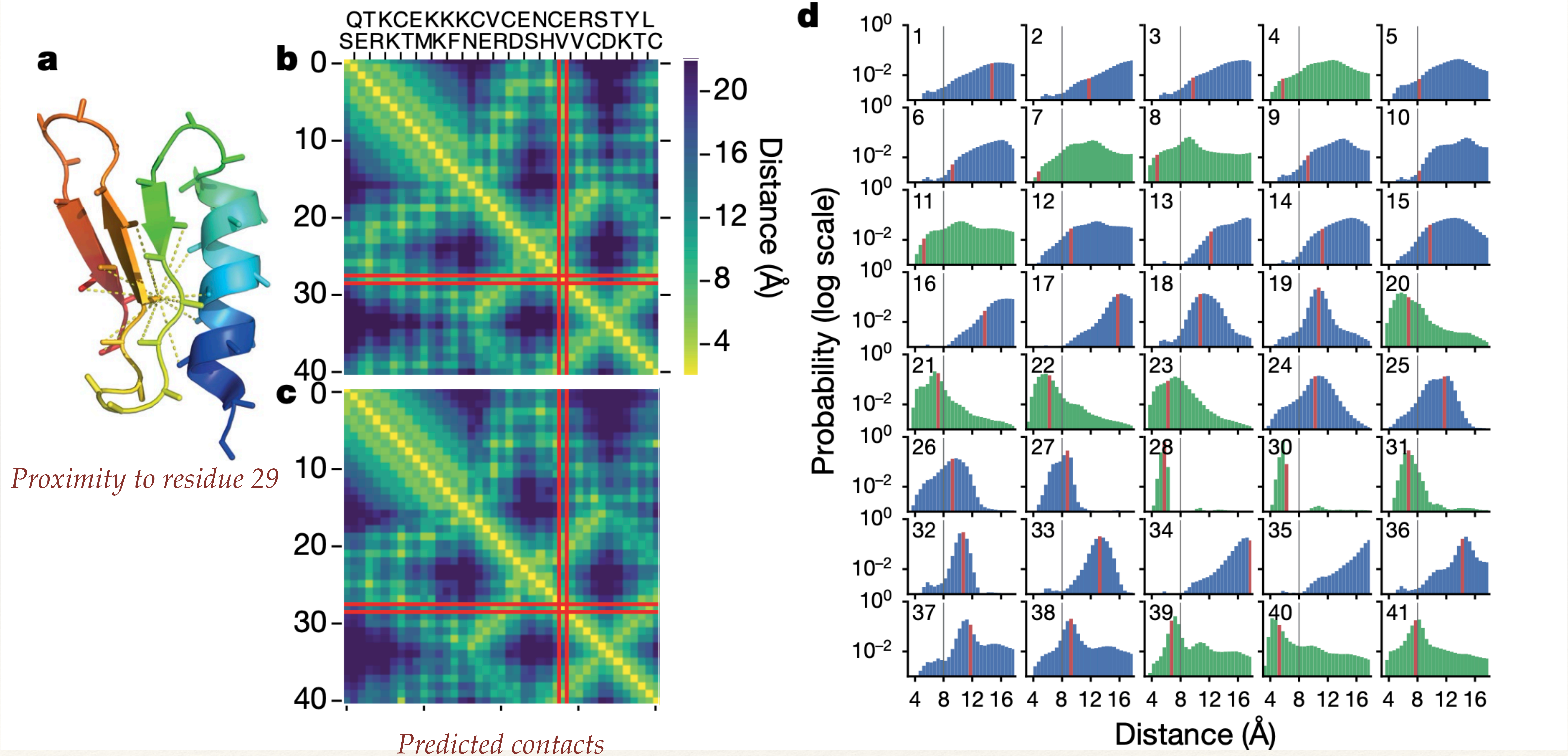
Inter-residue Orientations

Hydrogen Bonds

# Predicting residue contacts: How well does it work?

*Actual contacts*

*Predicted distances to residue 29*



*Proximity to residue 29*

*Predicted contacts*

***CASP target T0995***

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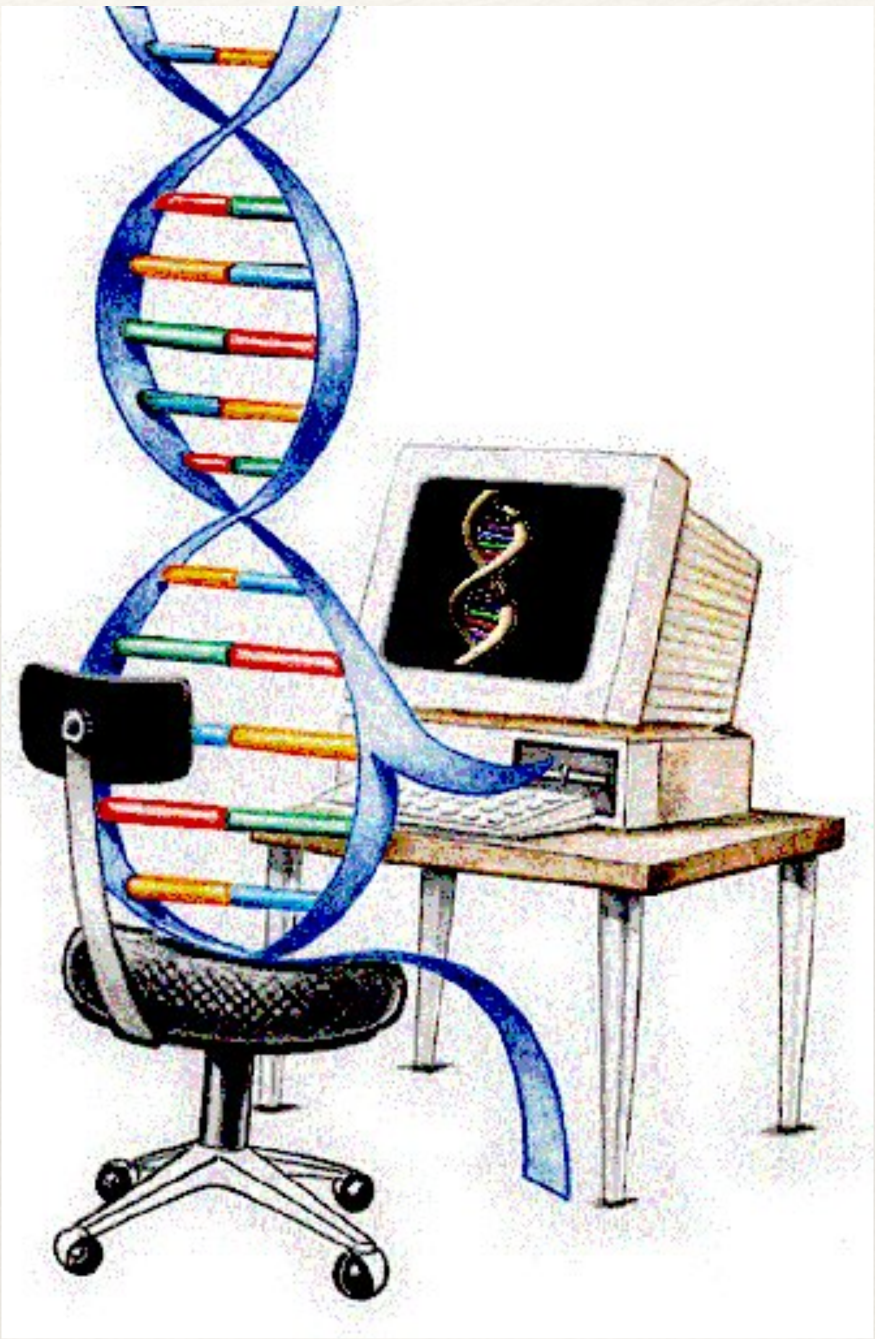
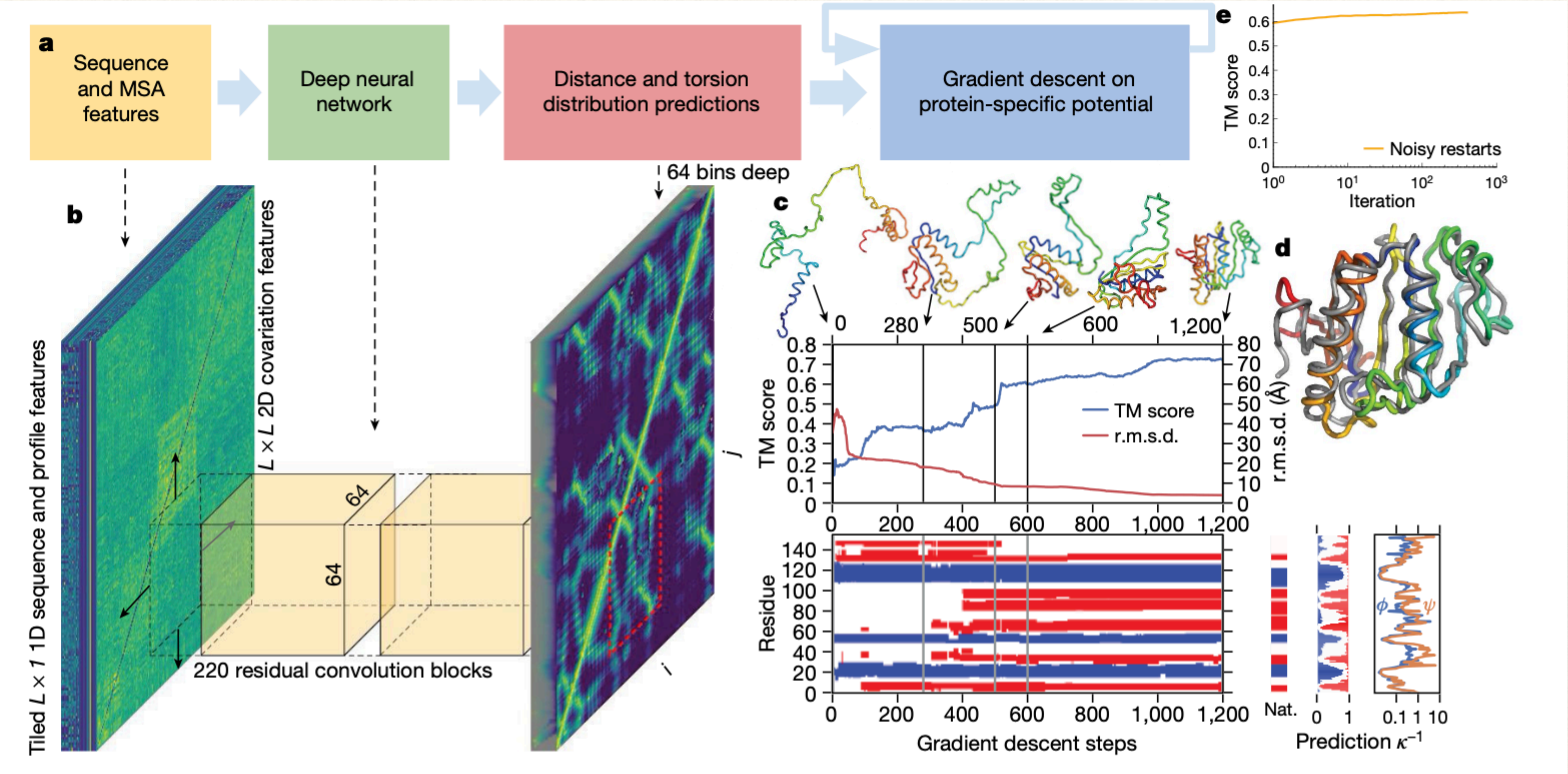
Ab initio prediction: Predicting Contacts

AlphaFold 1

AlphaFold 2



# AlphaFold 1



AlphaFold1

# AlphaFold 1

*Reminder:*

***To compare two sets of points (atoms) $A=\{a_1, a_2, \ldots a_N\}$ and $B=\{b_1, b_2, \ldots, b_N\}$:***

**-Define a 1-to-1 correspondence between A and B**

for example, $a_i$ corresponds to $b_i$, for all i in [1,N]

**-Compute RMS as:**

$$\boxed{RMS(A,B) = \sqrt{\frac{1}{N}\sum_{i=1}^{N} d(a_i,b_i)^2}}$$

**Compute TM score:**

$$\boxed{TM(A,B) = \frac{1}{N}\sum_{i=1}^{N} \frac{1}{1+\left(\frac{d(a_i,b_i)}{d_0(N)}\right)^2}}$$

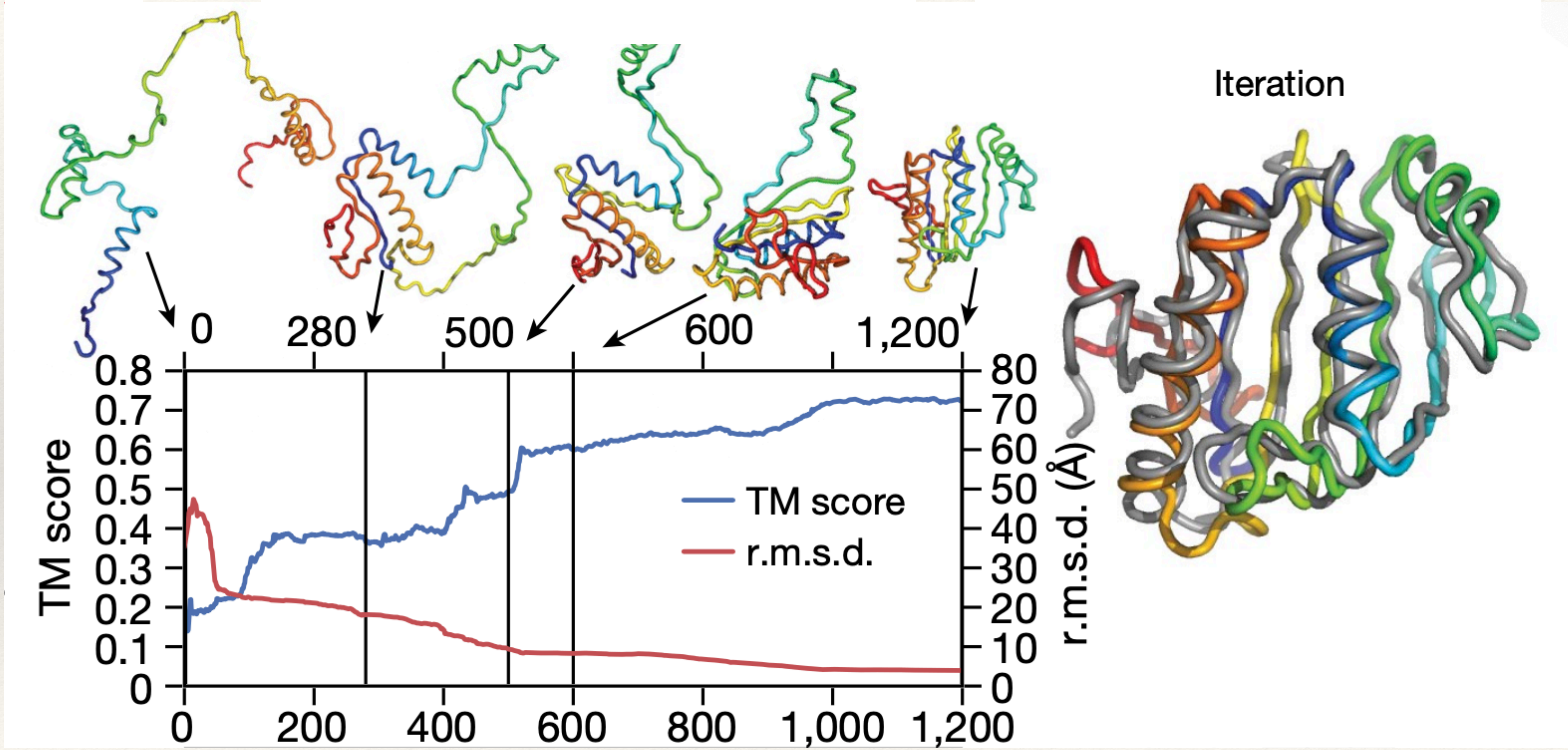
with $d_0(N) = 1.24\sqrt[3]{N-15} - 1.8$

$d(a_i,b_i)$ is the Euclidian distance between $a_i$ and $b_i$ after optimal alignment of B onto A

RMS: the lower, the better

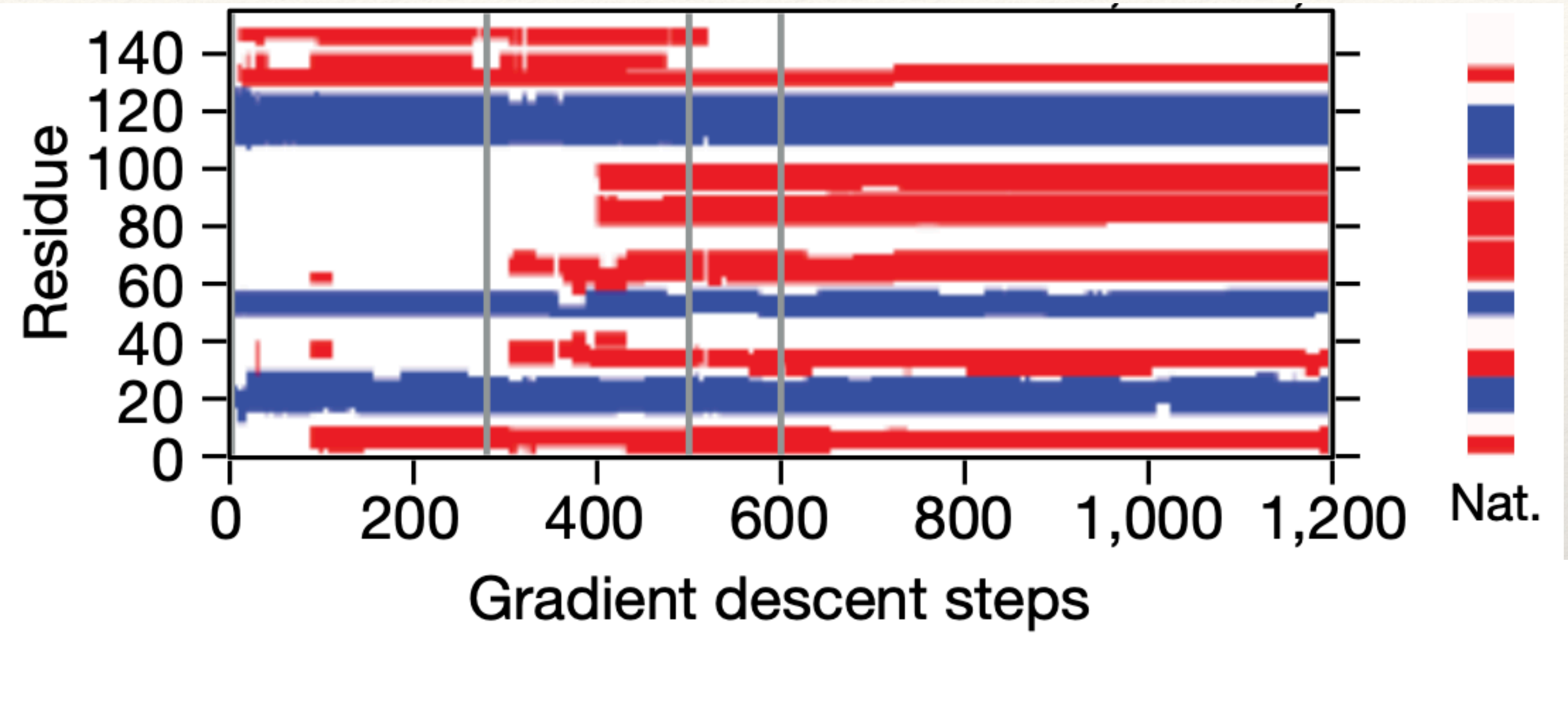
TM: between [0,1]; the higher the better

# AlphaFold 1



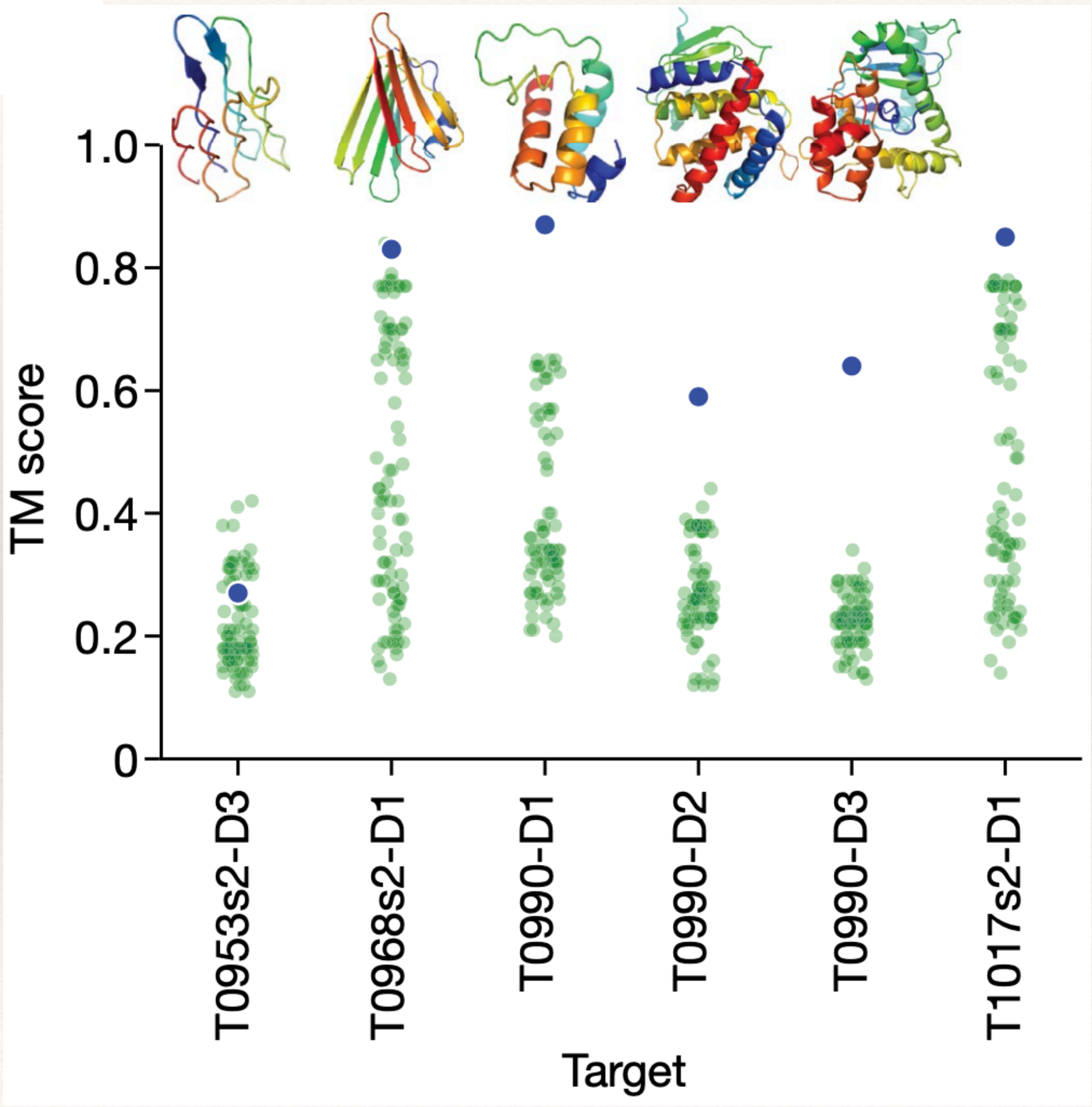
Iteration
0
280
500
600
1,200
TM score
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
r.m.s.d. (Å)
80
70
60
50
40
30
20
10
0
0
200
400
600
800
1,000
1,200
TM score
r.m.s.d.

# AlphaFold 1



*Helix in blue, strand in red*

# AlphaFold 1: Success



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Ab initio prediction before AlphaFold

Ab initio prediction: Predicting Contacts

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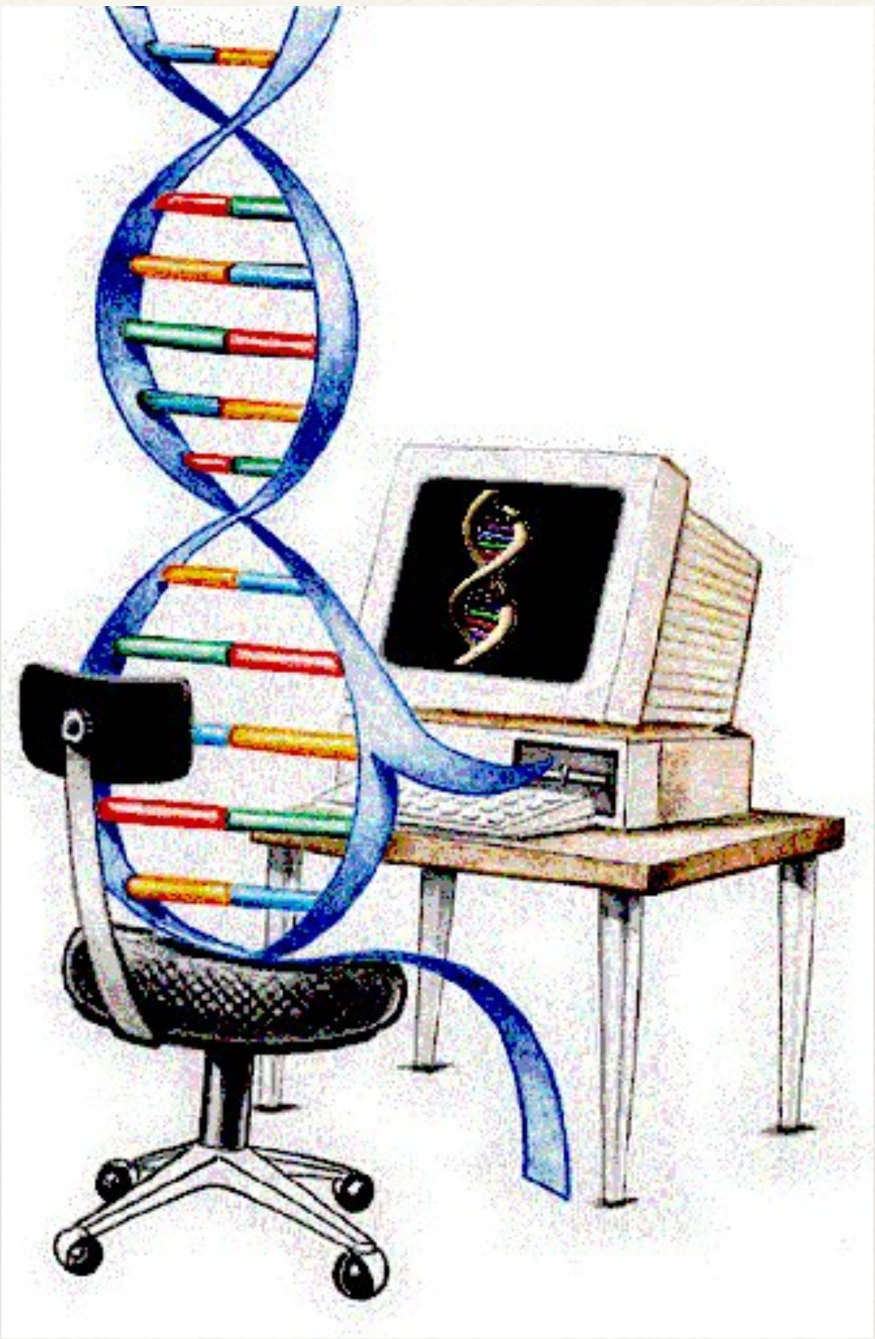
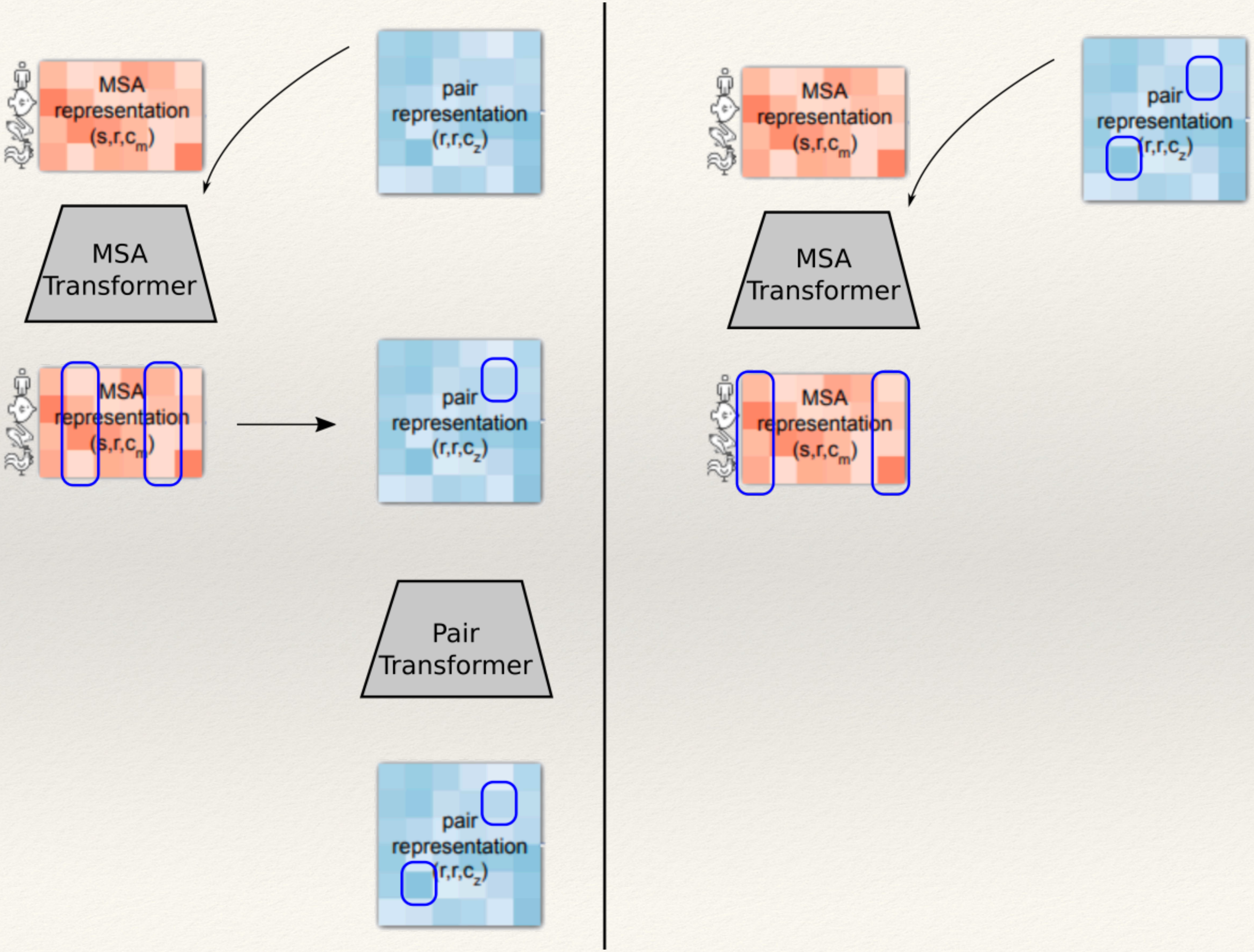
AlphaFold 2



# AlphaFold 2

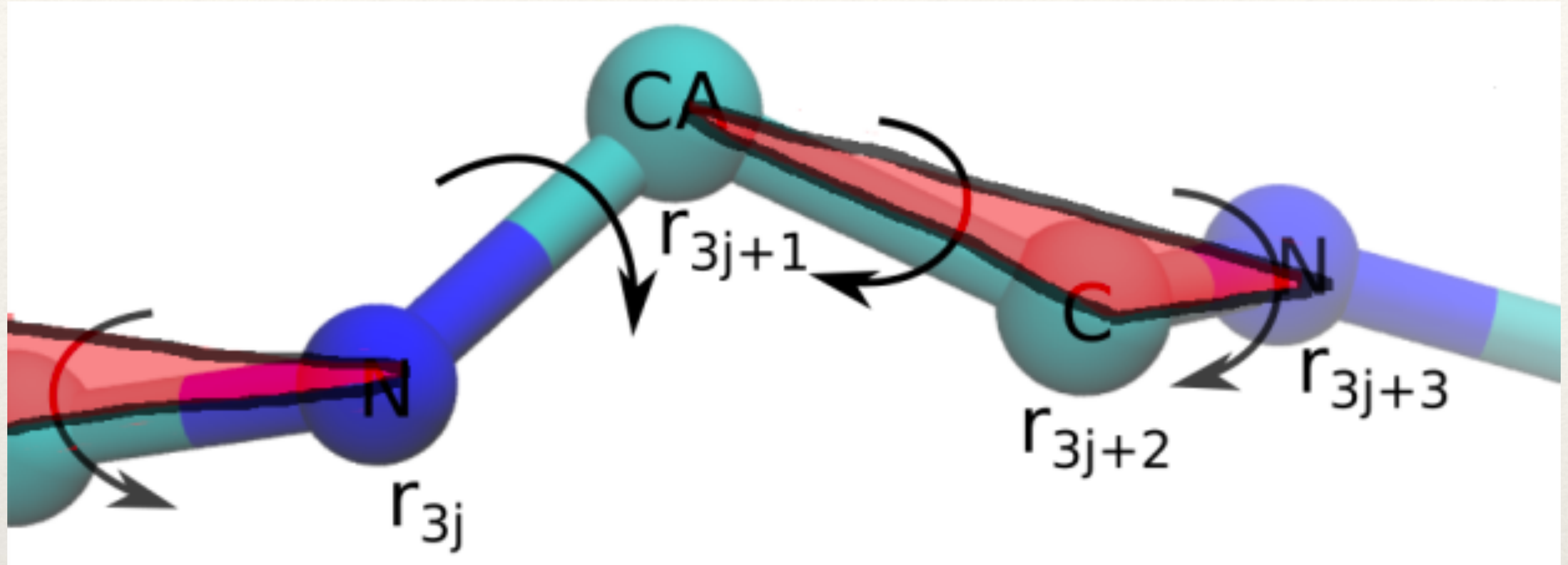
High confidence
Low confidence
Genetic database search
MSA
Input sequence
Pairing
Structure database search
Templates
MSA representation (s,r,c)
Pair representation (r,r,c)
Evoformer (48 blocks)
Single repr. (r,c)
Pair representation (r,r,c)
Structure module (8 blocks)
3D structure
← Recycling (three times)

# AlphaFold 2: some intuition


MSA representation (s,r,c$_m$)
pair representation (r,r,c$_z$)
MSA Transformer
MSA representation (s,r,c$_m$)
pair representation (r,r,c$_z$)
Pair Transformer
pair representation (r,r,c$_z$)
MSA representation (s,r,c$_m$)
pair representation (r,r,c$_z$)
MSA Transformer
MSA representation (s,r,c$_m$)

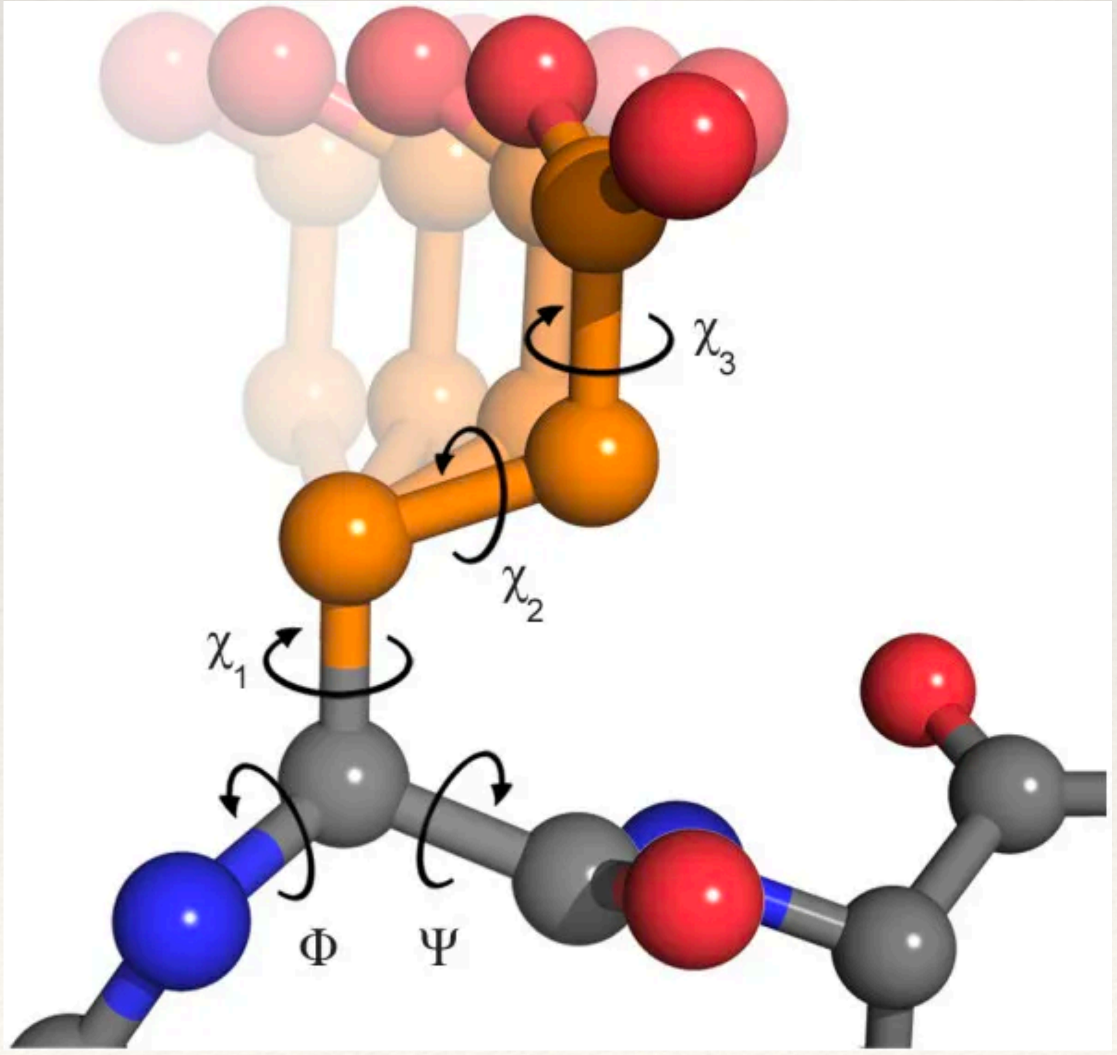
# AlphaFold 2: the structure module

*Predicting backbone:*
*the residues form a gas soup of triangles whose relative positions are characterized by affine transformation*

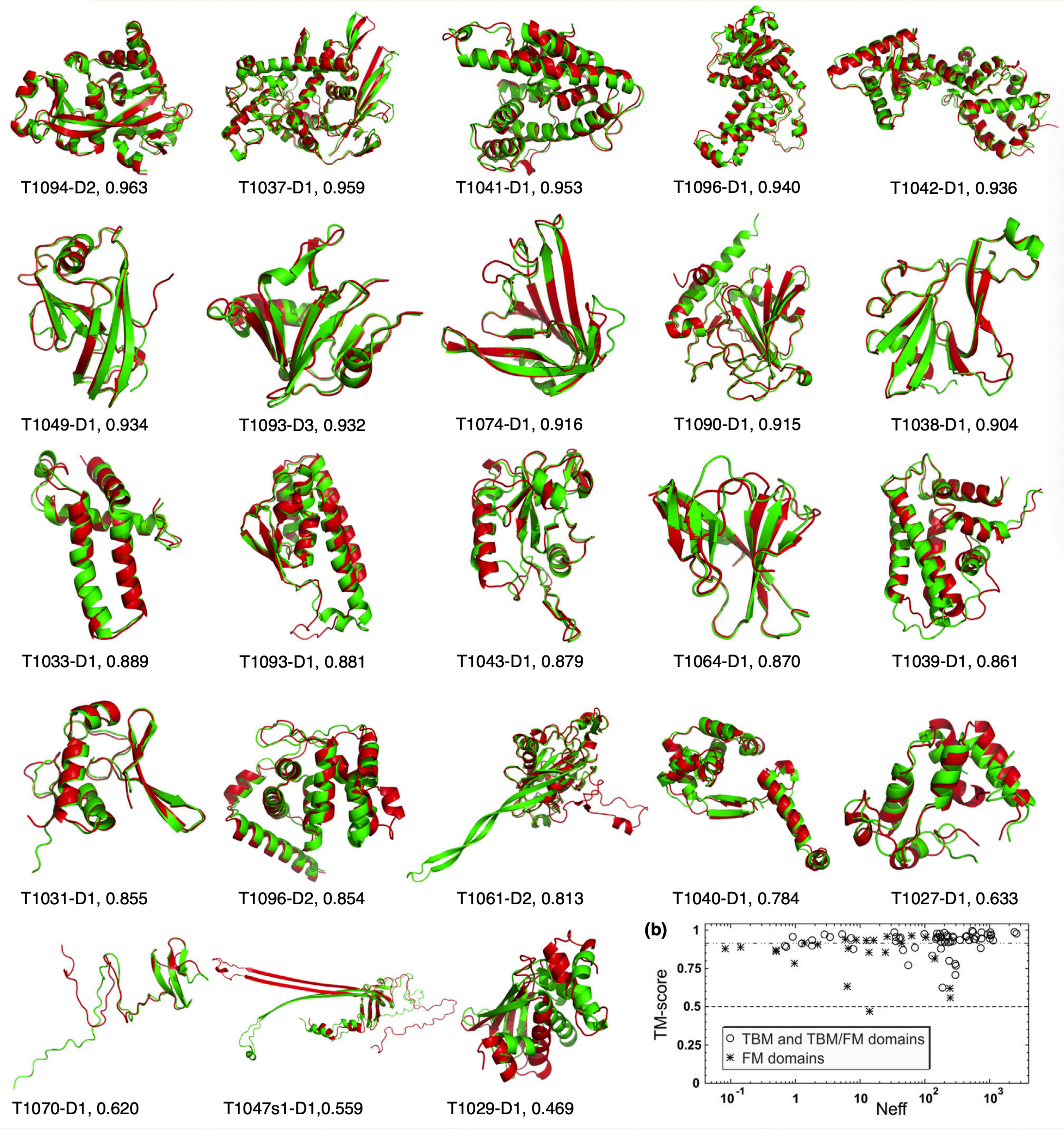


$$\mathbf{M} = \begin{pmatrix} a_{11} & a_{12} & a_{13} & a_{14} \\ a_{21} & a_{22} & a_{23} & a_{24} \\ a_{31} & a_{32} & a_{33} & a_{34} \\ 0 & 0 & 0 & 1 \end{pmatrix}$$

*Predicting side chains:*



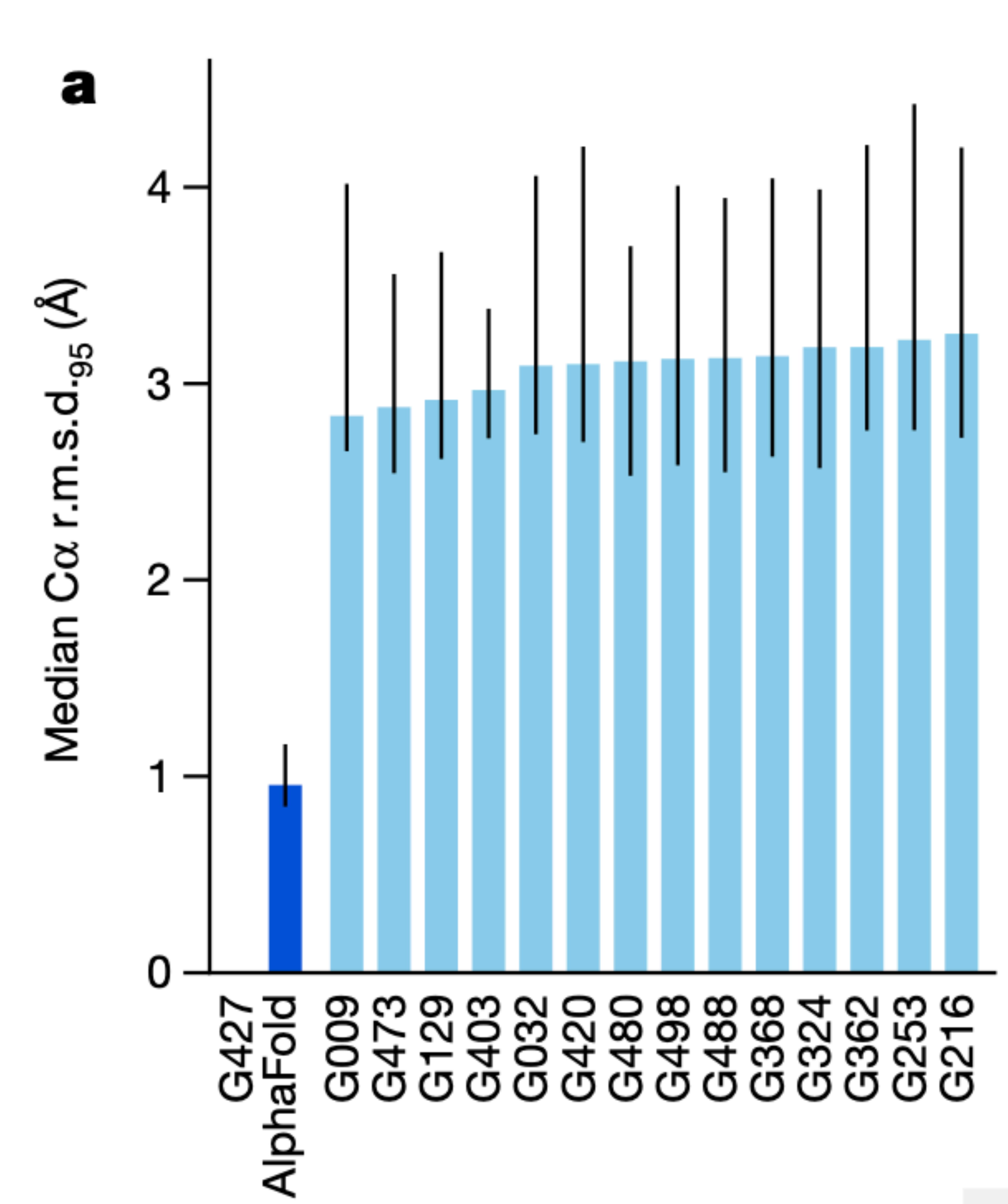
# *Successes at CASP14*

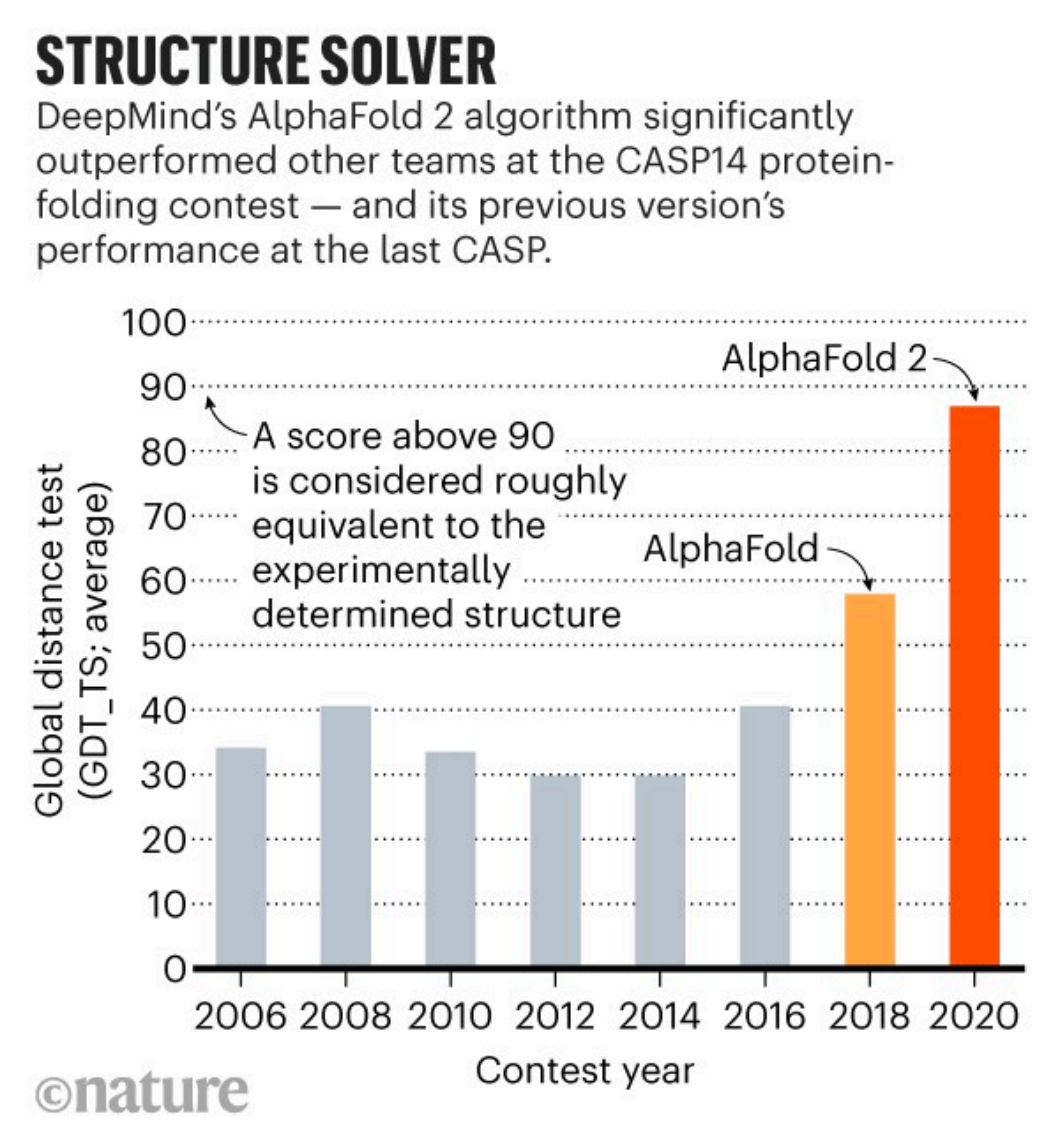


*TBM: template-based modeling*

*FM: free modeling*

## *Successes at CASP14*





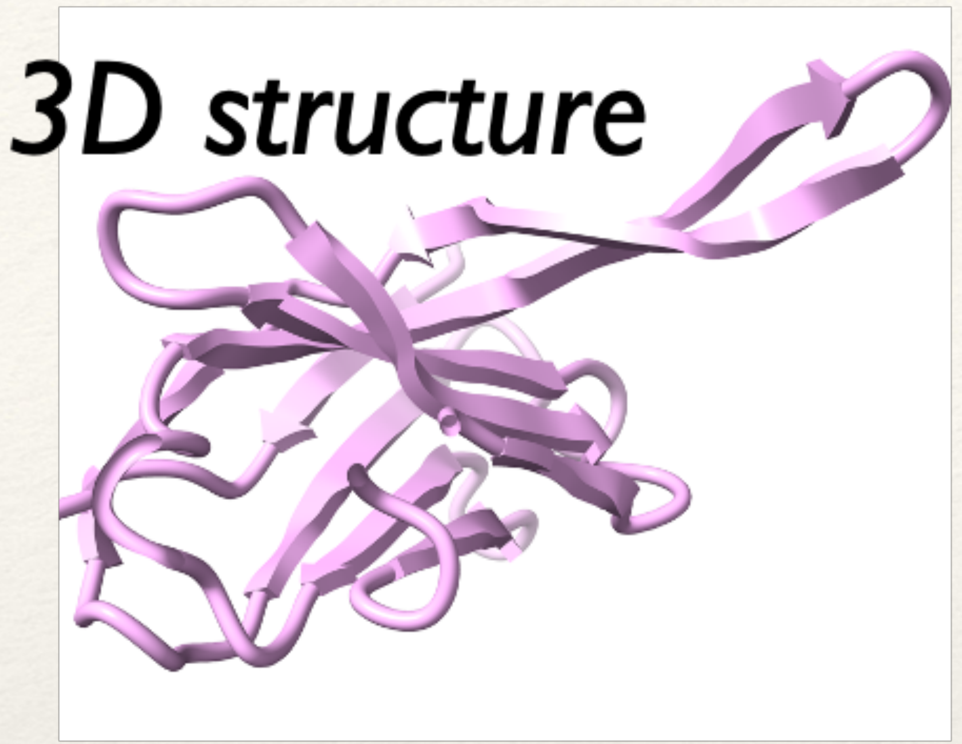
**STRUCTURE SOLVER**

DeepMind's AlphaFold 2 algorithm significantly outperformed other teams at the CASP14 protein-folding contest — and its previous version's performance at the last CASP.

©nature

**Training**

- *Sequence*
- *Multiple sequence alignment*
- *3D structure*



→ **21 million parameters**

**Prediction**

- *Sequence*
- *Multiple sequence alignment*

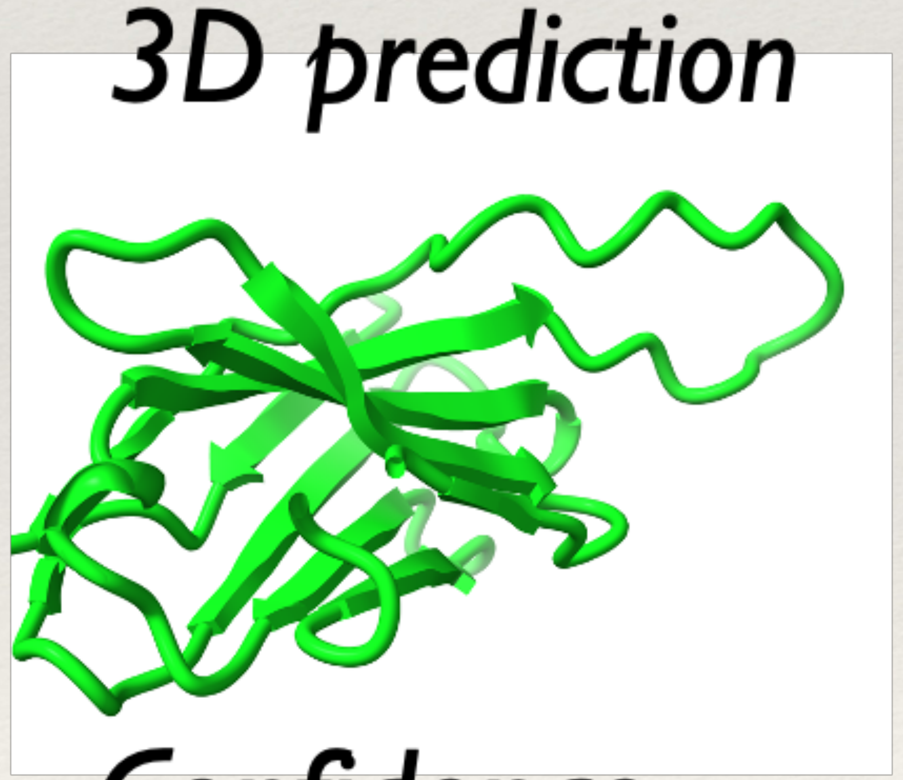
```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M........Q........
..................K.........Y....L.......A.........
..............A.............................V......
....................A..............................
............................L....V....E............
.........A...................................Q.....
```

**21 million parameters**

→

*Focus attention on important relationships*

3D prediction



Confidence estimates

*Credit: Tom Terwilliger, Los Alamos NL*

## Multiple sequence alignment

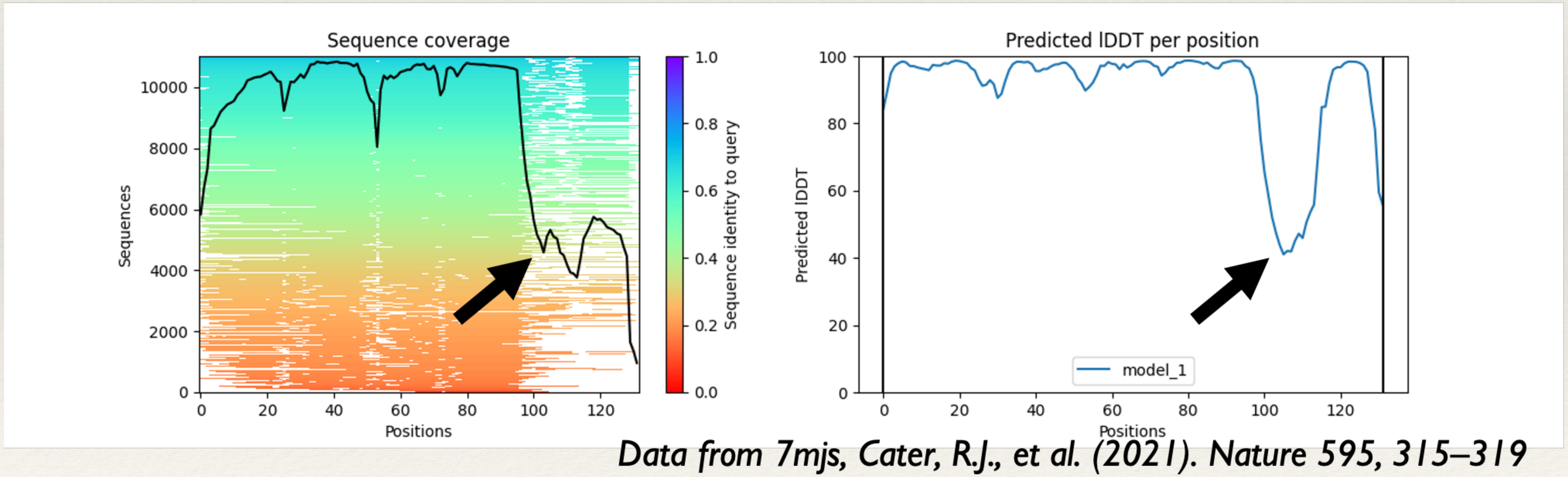
```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M........Q........
..................K.........Y....L.......A.........
..............A.............................V......
....................A..............................
............................L....V....E............
.........A...................................Q.....
```

*Residues that* ***co-vary*** *are probably close in 3D structure*

*All sequences in alignment should be compatible with the right structure*

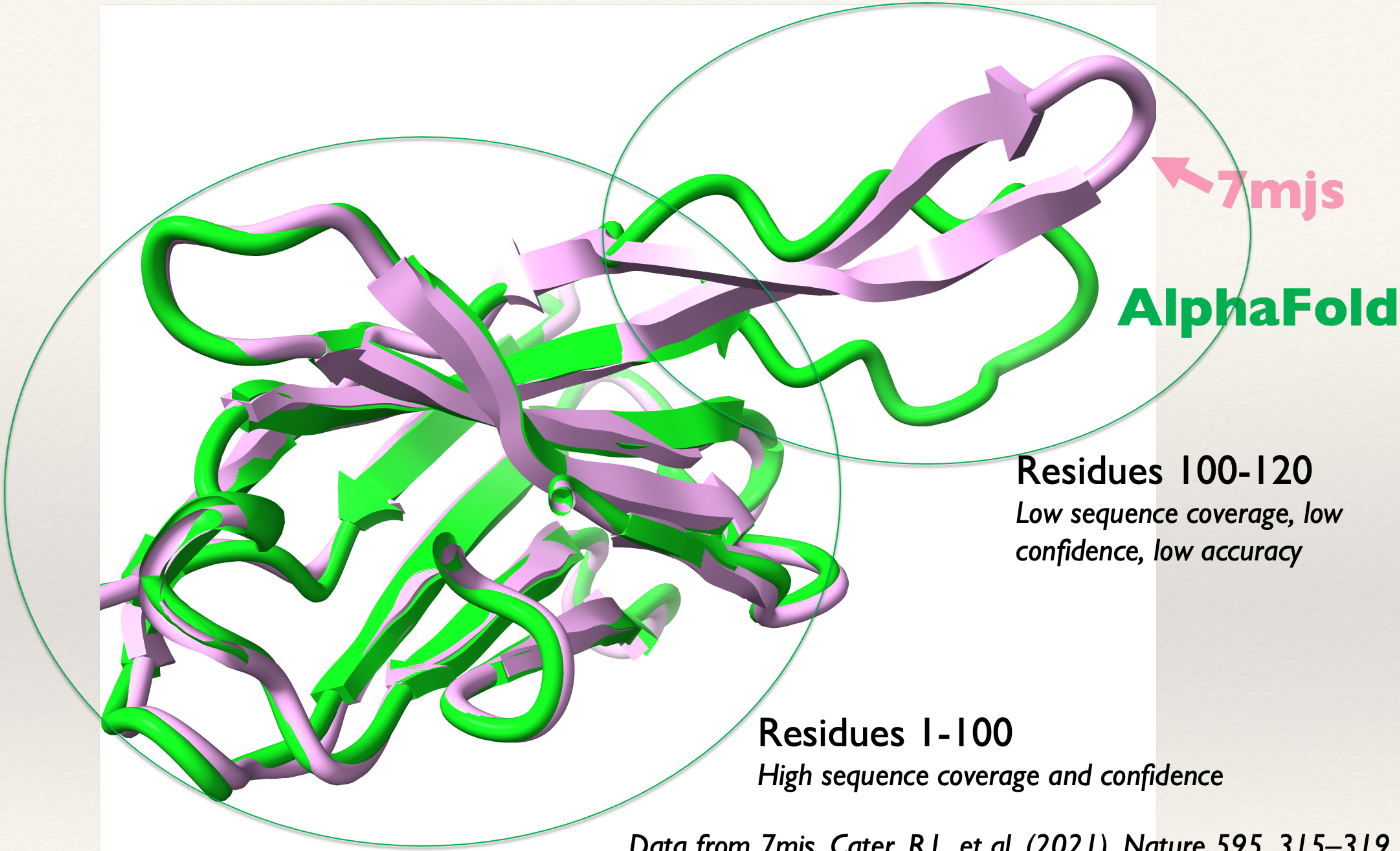
## Sequence coverage

## Confidence



*Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319*

*Credit: Tom Terwilliger, Los Alamos NL*



**Residues 100-120**

*Low sequence coverage, low confidence, low accuracy*

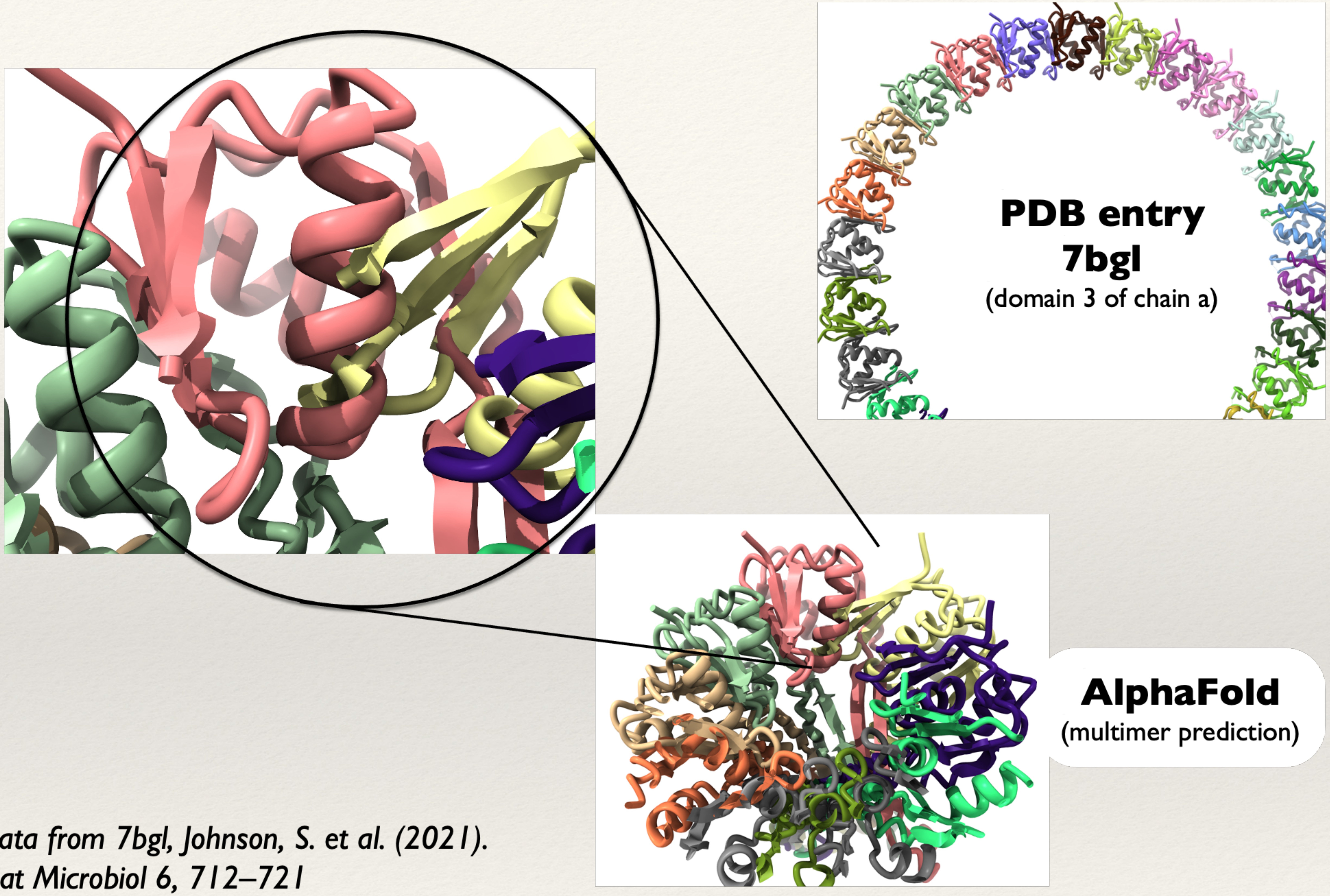
**Residues 1-100**

*High sequence coverage and confidence*

*Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319*

*Credit: Tom Terwilliger, Los Alamos NL*

# Multimeric proteins



**PDB entry 7bgl**

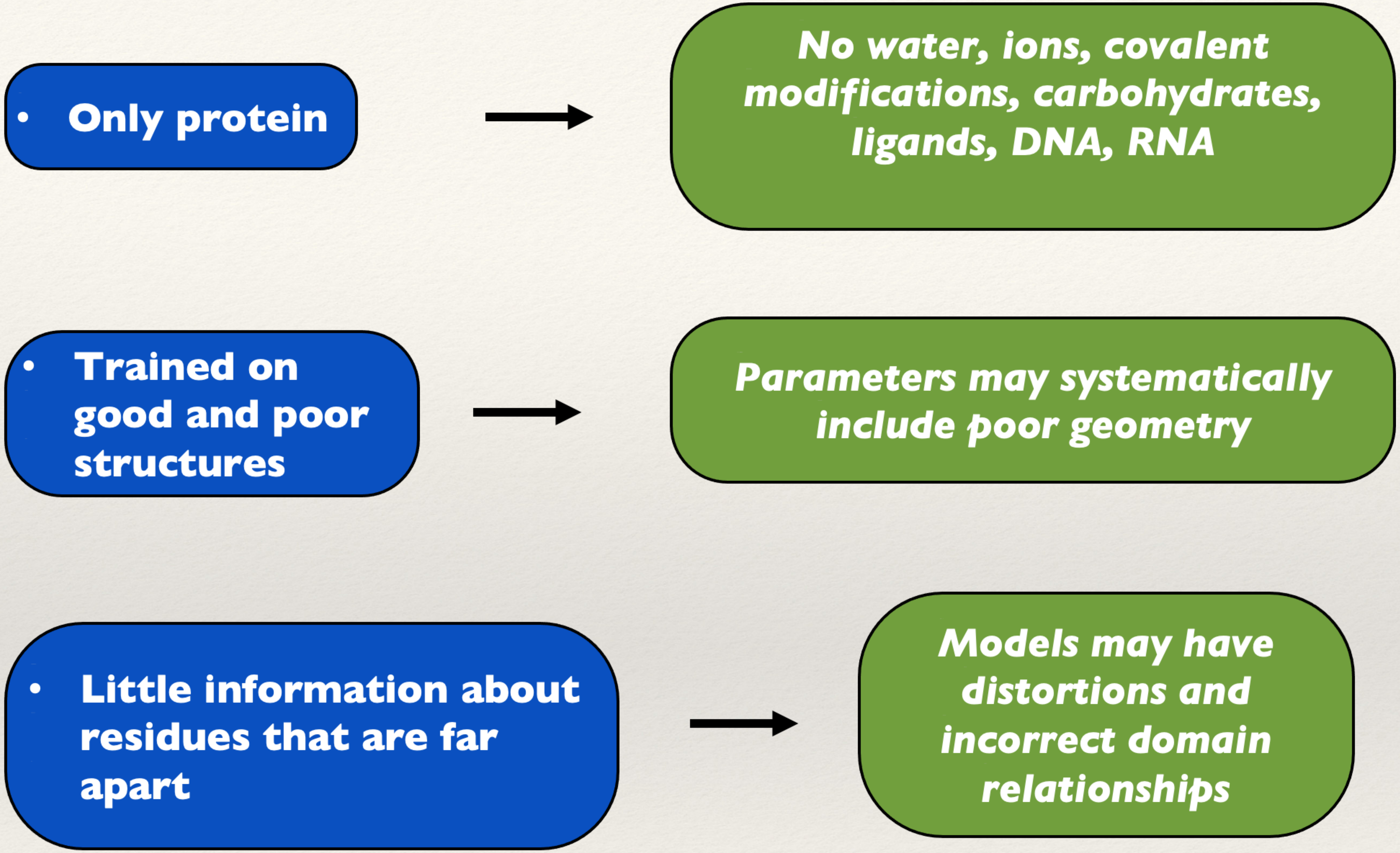
(domain 3 of chain a)

**AlphaFold**

(multimer prediction)

*Data from 7bgl, Johnson, S. et al. (2021). Nat Microbiol 6, 712–721*

*Credit: Tom Terwilliger, Los Alamos NL*



- Only protein → *No water, ions, covalent modifications, carbohydrates, ligands, DNA, RNA*
- Trained on good and poor structures → *Parameters may systematically include poor geometry*
- Little information about residues that are far apart → *Models may have distortions and incorrect domain relationships*

*Credit: Tom Terwilliger, Los Alamos NL*