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Protein 3D Structure Identification by AlphaFold: a Physics-Based *Prediction* or *Recognition* Using Huge Databases?

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Two main problems of protein folding

«Protein folding problem» №1:

HOW a protein can fold spontaneously so fast?

⬇

Solved: "Folding funnel" with phase separation

(Finkelstein, Badretdinov, 1997-98; Garbuzynskiy et al., 2013)

«Protein folding problem» №2:

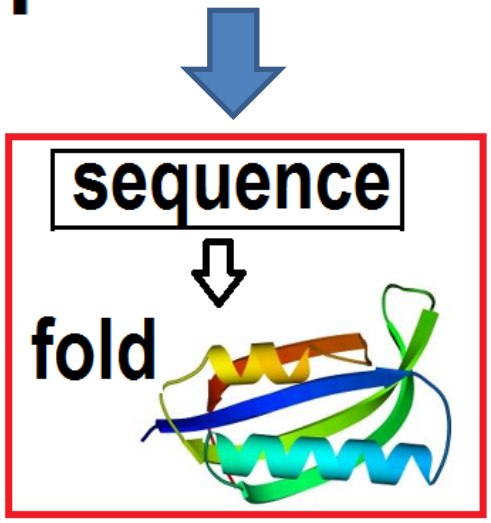
Predict 3-dimensional structure of a protein from its amino acid sequence

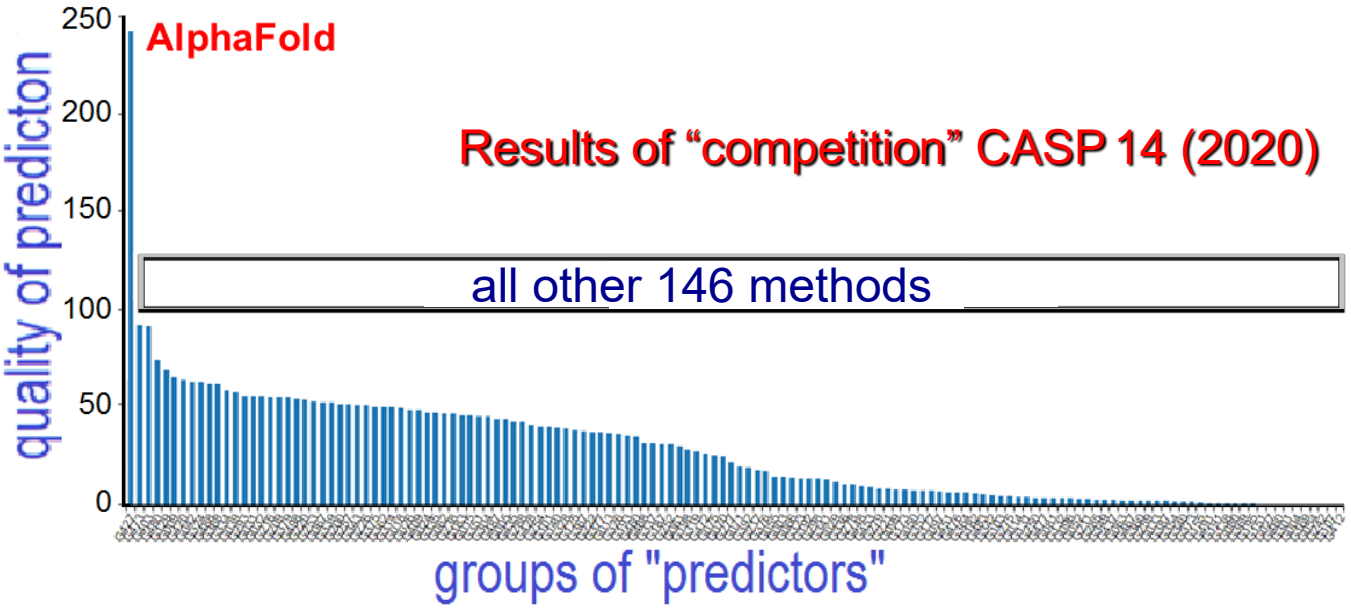
⬇

AlphaFold

(Senior *et al.*; Jumper *et al.*)

prediction





AlphaFold – great success

A. Senior, J. Jumper, et al., 2018-21

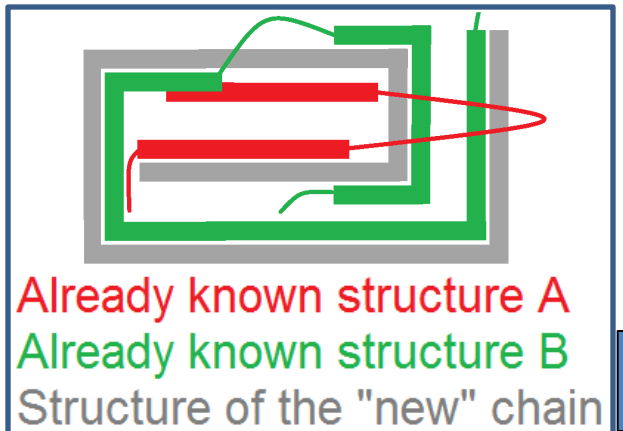
1) What is the main reason for this success?

2) What does **AlphaFold** do:

– does it *predict* protein structure from its a.a. sequence & *physics of protein chain folding?*

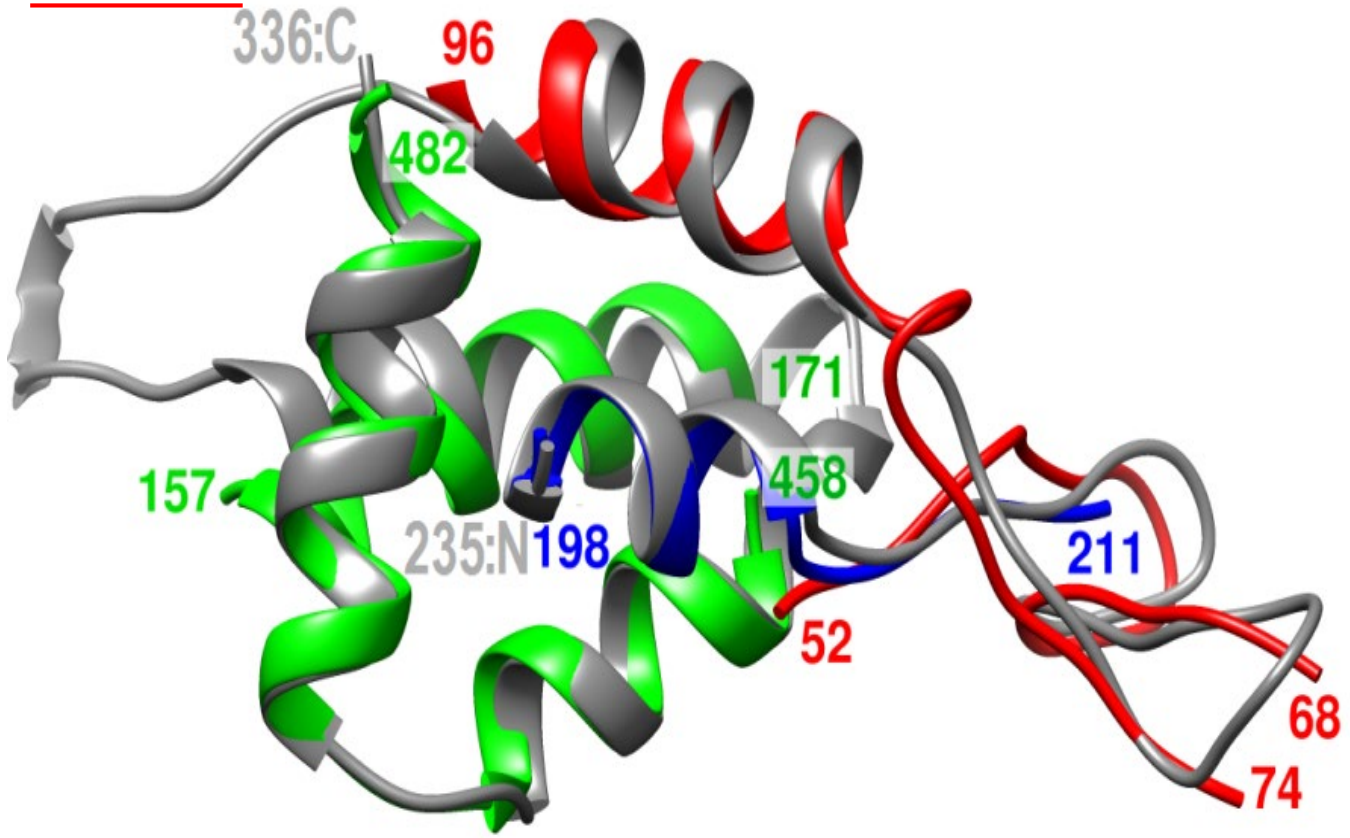
or

– *recognize* this structure by the *similarity* of large pieces of its a.a. sequence with **"joined" large pieces of sequences** that *already* are in PDB?



“Novel fold”: When it is impossible to superimpose *any* of already known 3D structures onto this “**novel** fold”

greatest success

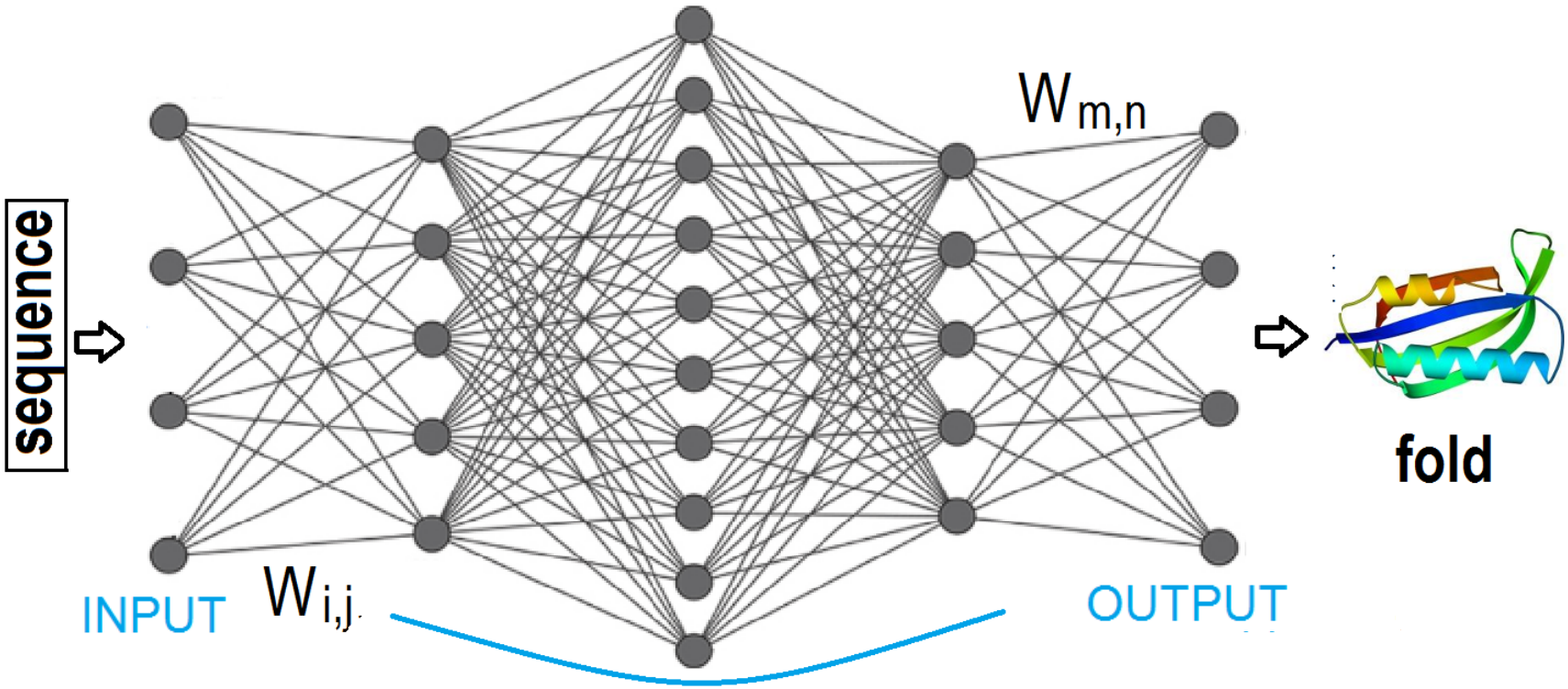


One of many dozens of examples of superposition of pieces of already known 3D structures onto a “**novel** fold”

“Novel fold” (6VR4, chain A - target T1035 from CASP 14 (2020)) as a combination of fragments of 3 already known structures available to AlphaFold during the training:

1GB3, chain A; 5A29, chain A; 5W40, chain B.

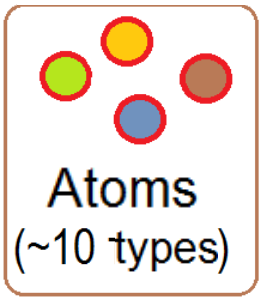
AlphaFold: neural network

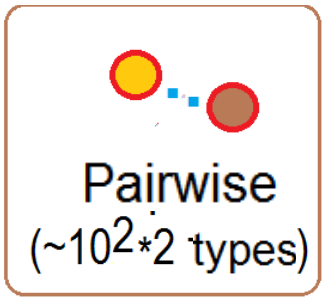


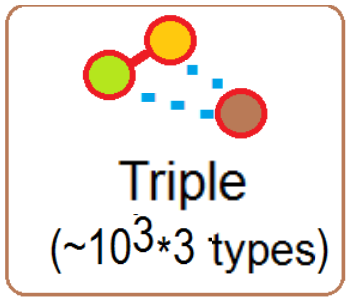
Hidden layers (many dozens)

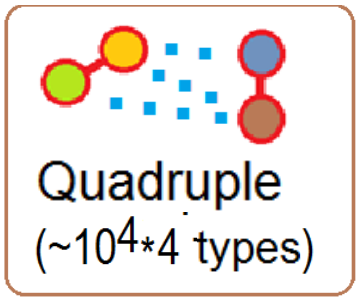
$W_{i,j}$, $W_{m,n}$ - "weights" (adjustable parameters): In Alphafold: **~21,000,000**

But physics of atomic interactions in proteins only needs **~43,200**







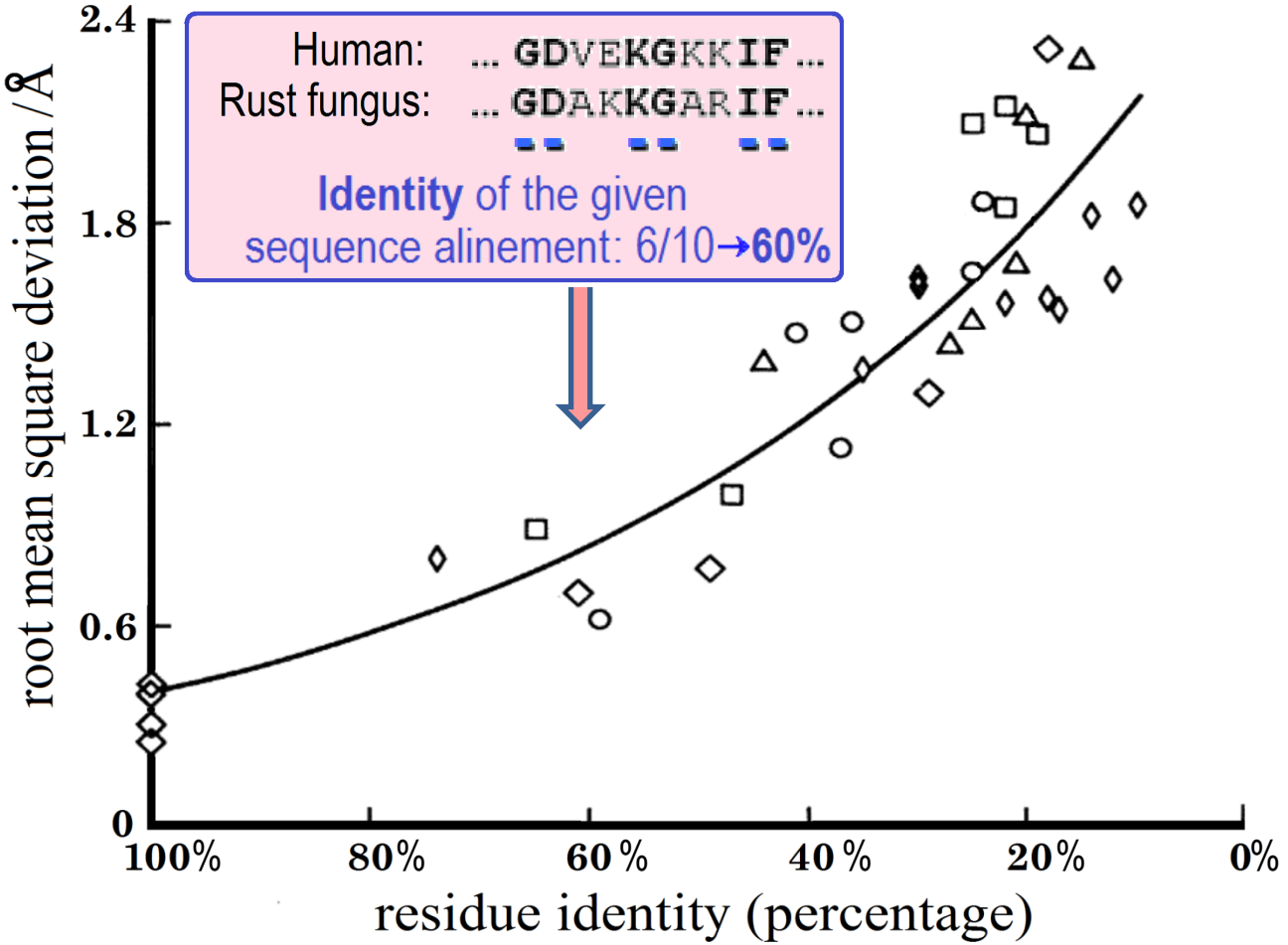


So, ~20,955,000 parameters are "trained" not in physics, but ?

KNOWN:

SIMILAR SEQUENCES ➔ *VERY* SIMILAR STRUCTURES

but only – with identity ≳15-20%



Lesk, A.M., Chothia, C. *Phil. Trans. R. Soc. Lond. A* **317**, 345–356 (1986)

WHAT IDENTITY WITH A "NEW" SEQUENCE IS EXPECTED - HAVING MODERN HUGE DATABASES?

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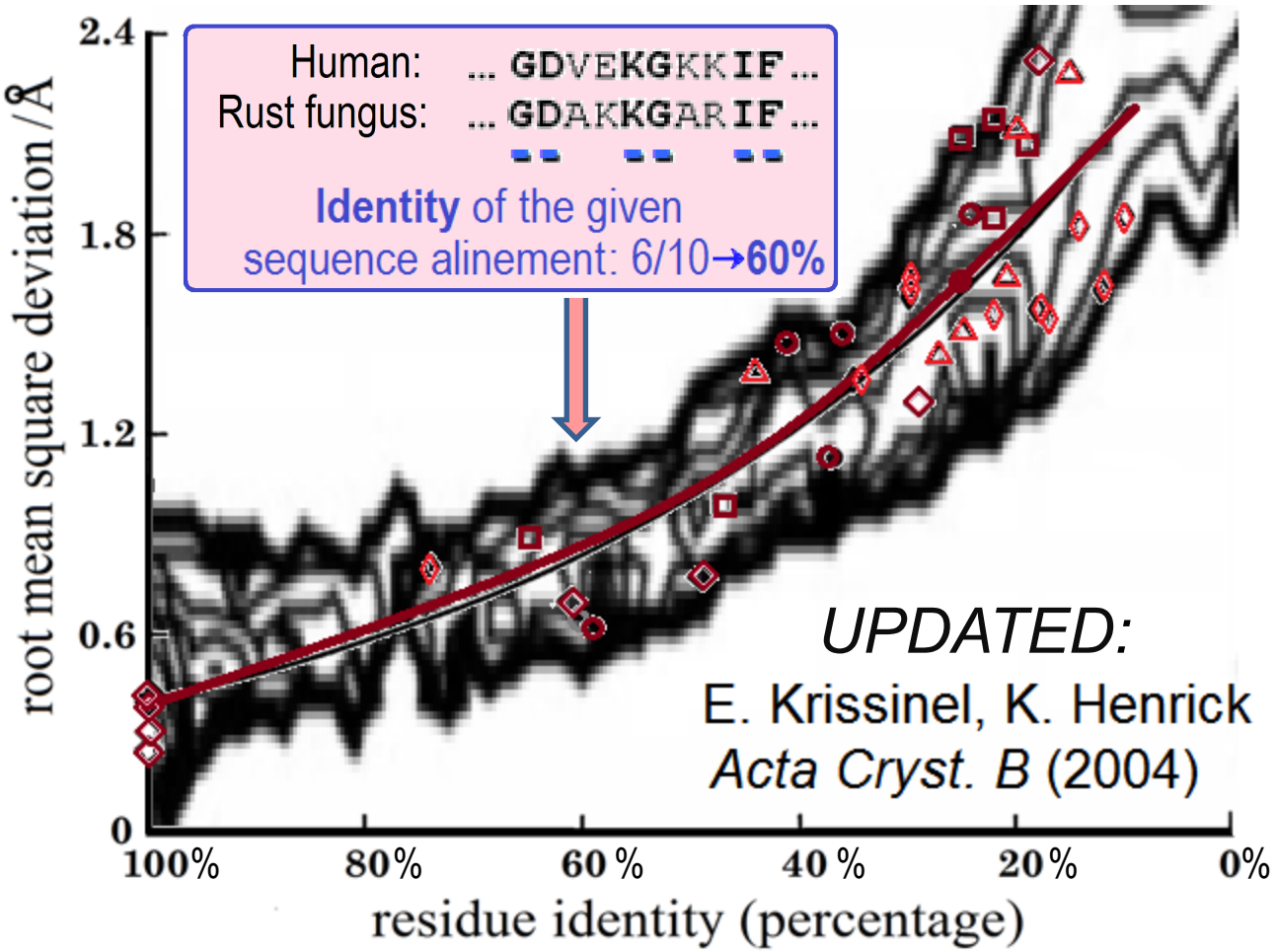

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The probability that two "random" sequences of n a.a. residues, each of which occurres with a probability p (in proteins, ~1/20), coincide in m positions, follows from

5%

$$P_{m,pn} = \frac{(pn)^m}{m!} e^{-pn}$$

Poisson distribution

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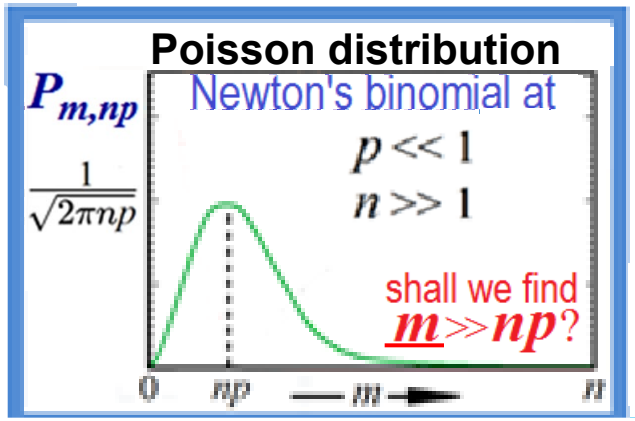
pn – average (*pairwise* comparison)



ACDEFGHI**K**LMNPQRSTVWY

KPYDSTFQ**K**HILAMNPQRST ➡ Expected for **1** pairwise comparison: $n \times 5\%$

Could we expect $n \times 20\%$ for **1** out of **1000000** pairwise comparisons?

ACDEFGHI**K**LMNPQRSTVWY	AC**D**EFGHIKLMNPQRSTVWY	ACDE**F**GHIK**L**MNPQRSTVWY
KPYDSTFQ**K**HILAMNPQRST	KP**D**DSTFQSHILAMNPQRST	KPYD**F**TFQH**L**ILAMNPQRST
1	2	1000000

and having shifts, insertions, deletions – when comparing sequences?

ACD**E**---FGHI**K**LMNPQRST**VW**Y

MNPD**A**TF**E**PYDSTFQ**K**HILA--MN**VW**RSTDSTF

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$$P_{m,pn} = \frac{(pn)^m}{m!} e^{-pn}$$

pn – average (*pairwise* comparison)

Poisson distribution

because $m! \approx (m/e)^m$ (Stirling's eq.), then $P_{m,pn} \approx \left(\frac{ep}{m/n}\right)^m e^{-pn}$

When **1** sequence is compared not with **1**, but with N others, then $P_{m,pn} \cdot N = 1$ gives the maximally expected number of matches (M) with the "most similar" of them. Thus, the expected residue identity M/n follows from the equation

$$\left(\frac{M/n}{pe}\right)\ln\left(\frac{M/n}{pe}\right) + \frac{1}{e} = \left(\frac{1}{npe}\right)\ln(N)$$

expected M/n

MODERN DATABASES

no chain shifts, insertions, deletions

n - any, N=1: $M/n = p = 5\%$

n=100 (domain), N~150000 (PDB): M/n = 20%

n=100 (domain), N~190000000 (UniProt): M/n = 24%

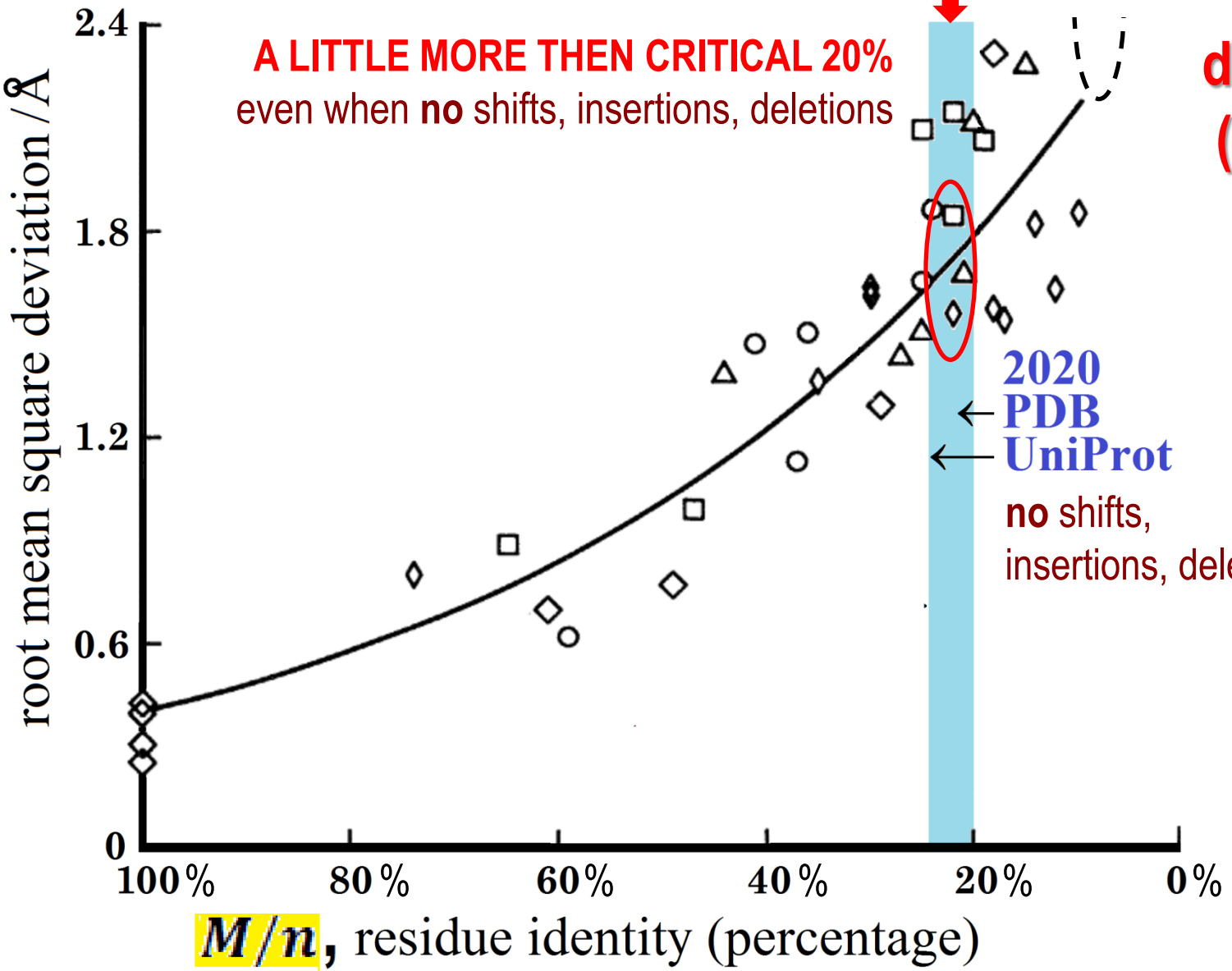
with chain shifts, insertions, deletions

n=100 (domain), N~150000 (PDB)$*10^6$: M/n = 25%

n=100 (domain), N~190000000 (UniProt)$*10^6$: M/n = 32%

8”

EXPECT TO FIND – HAVING MODERN DATABASES



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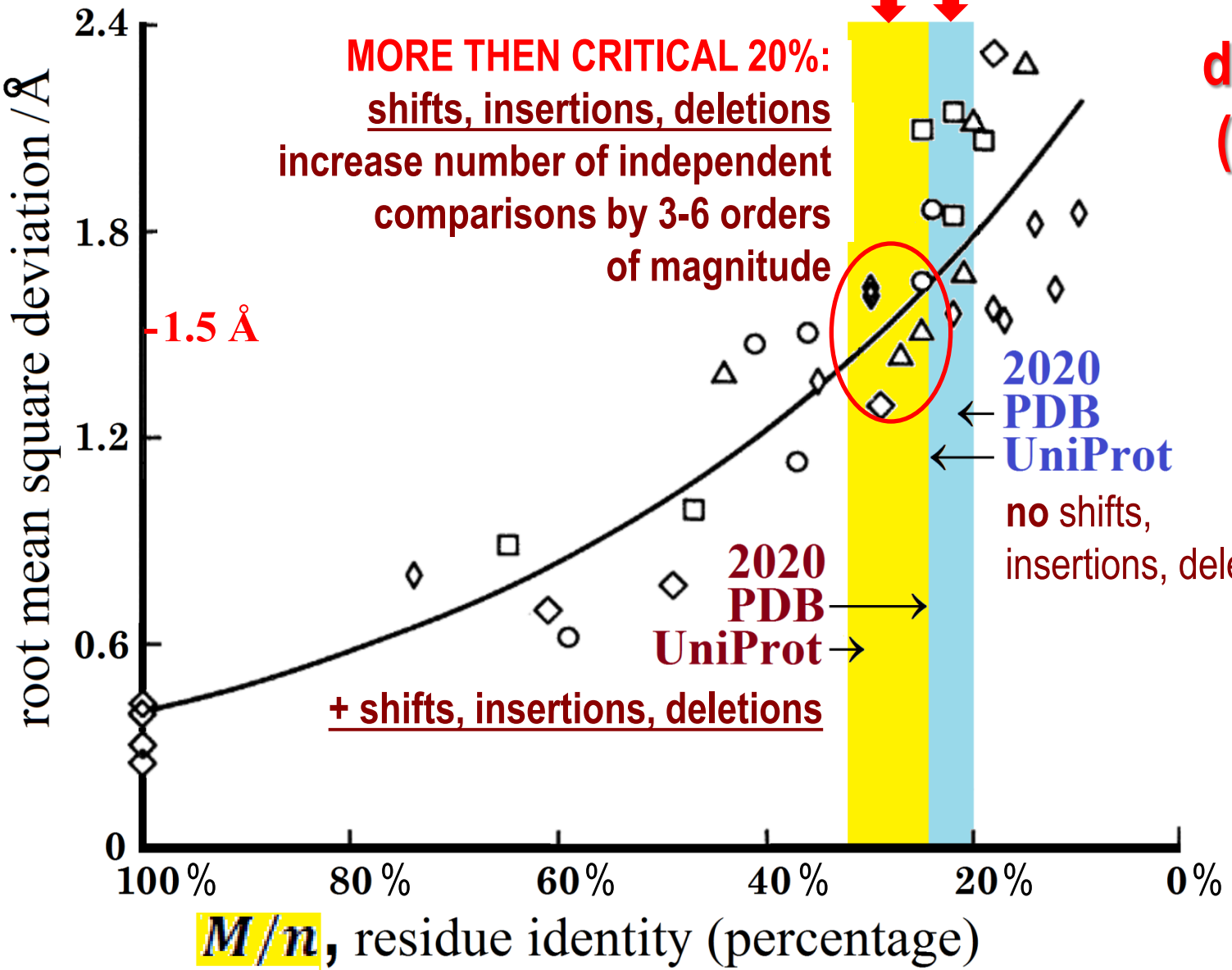

WITH MODERN DATABASES, ALPHAFOLD CAN RECOGNIZE PROTEIN STRUCTURE

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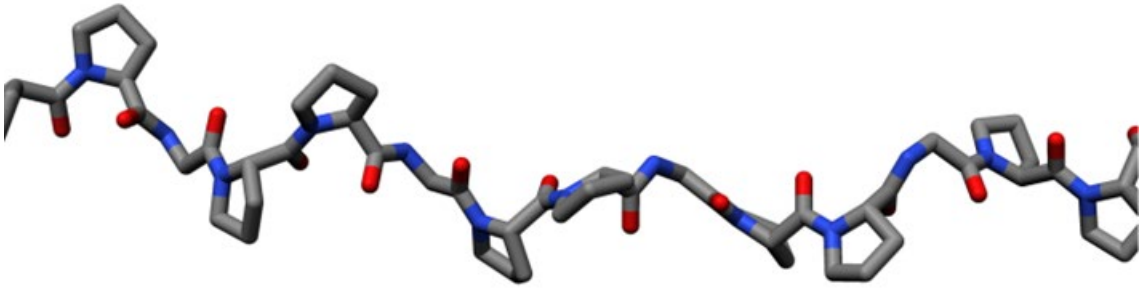
NOTE:

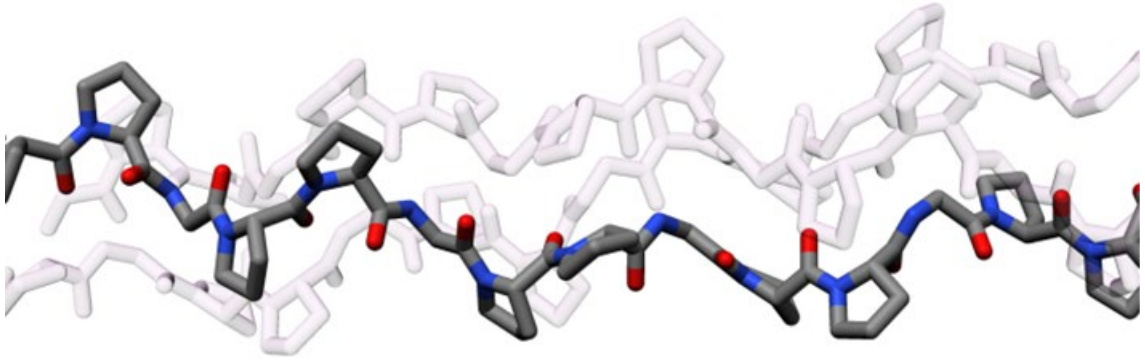
Bioinformatics is much more important than *physics* for AlphaFold predictions:



AlphaFold -predicted piece of $(Gly\text{-}Pro\text{-}Pro)_{13}$

- is a *contradicting to physics* prediction of a *non-compact* structure of separate collagen-like $(Gly\text{-}Pro\text{-}Pro)_{13}$ chain, which **lacks interactions** that can **support** it.

In collagen, such a chain is fixed by ***surrounding*** chains:



Collagen **PDB**: 4CTD

- but these have been *not* introduced to AlphaFold, asked to predict a structure of the **separate** $(Gly\text{-}Pro\text{-}Pro)_{13}$ chain!

Knowing similar complexes, AlphaFold makes correct *bioinformatic recognition, though contradicting to physics* of this separate chain.

A LITTLE PHILOSOPHY

1) "Predict fold" $\neq$ **"Predict folding"** (*folding rate*)

result — process ↓

AlphaFold

*(Garbuzynskiy et al,. PNAS, **110**:147–150, 2013;*
*Ivankov, Finkelstein, Biomolecules **10**:E250, 2020)*

2) Does *AlphaFold* know protein physics?

- *it knows only the **frequency of occurrence** in **PDB** of elements of protein structures, which is **related to their stability***
  (Finkelstein et al., Proteins, 23: 142-150, 1995)
- ***AlphaFold** relies on bioinformatics, and (yet) knows **nothing** about the process of protein **folding***

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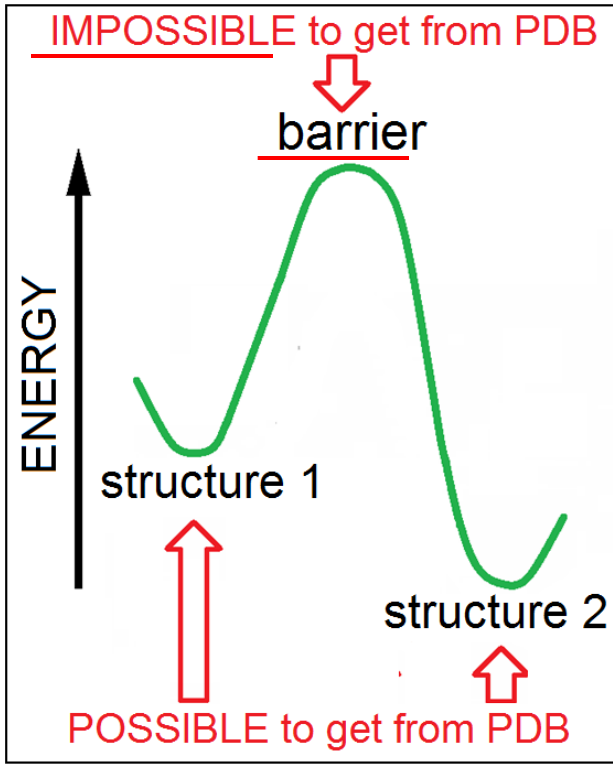

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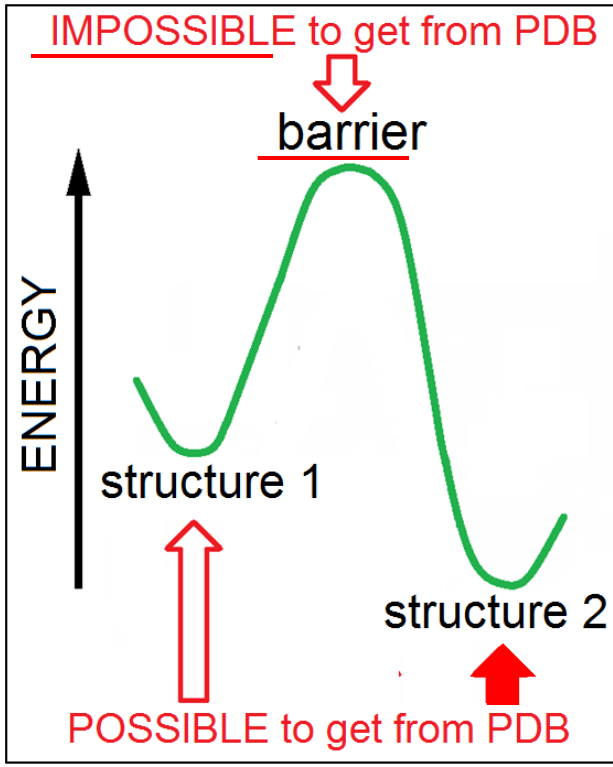


3) Does a **good prediction** mean a **correct understanding**?

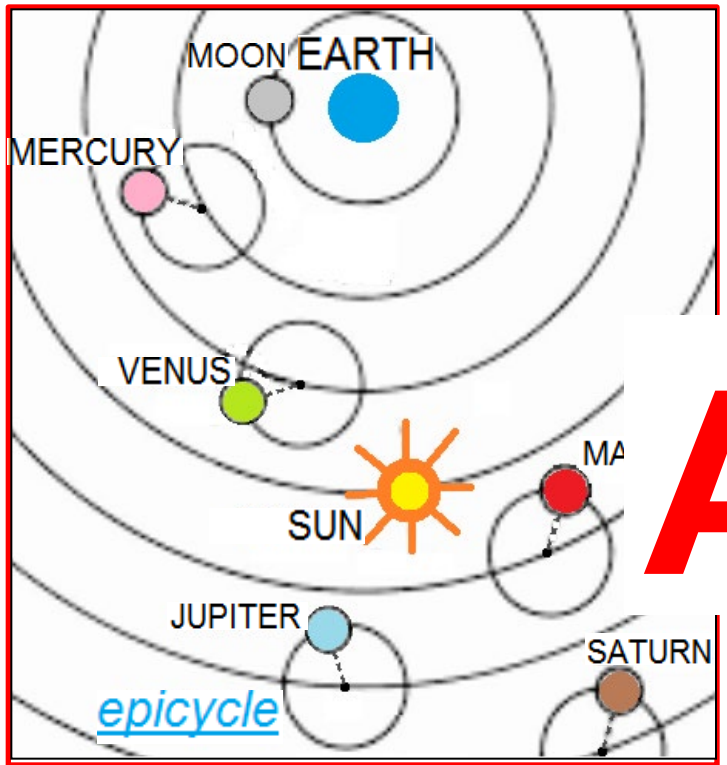
- ***NO***. ↓ *An example from the history of astronomy*

GOOD PREDICTION ⇏ CORRECT UNDERSTANDING

Priests of Egypt and Babylon:

GOOD PREDICTIONS
of eclipses of the Sun and Moon
(based on huge archives spanning 2500 years!),

BUT: *fundamentally* WRONG UNDERSTANDING
(The Earth is flat!)



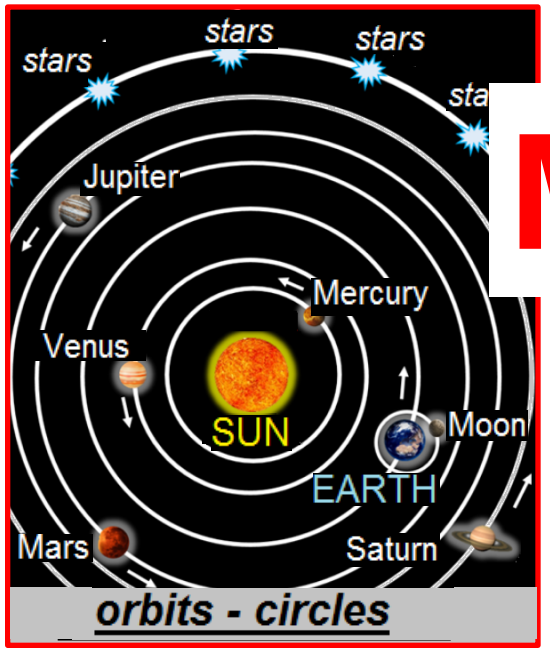
PTOLEMAEUS
(*using huge archives*):

GOOD PREDICTION

AlphaFold

***WRONG* UNDERSTANDING of the *PROCESS*!**

GOOD PREDICTION ⇎ CORRECT UNDERSTANDING

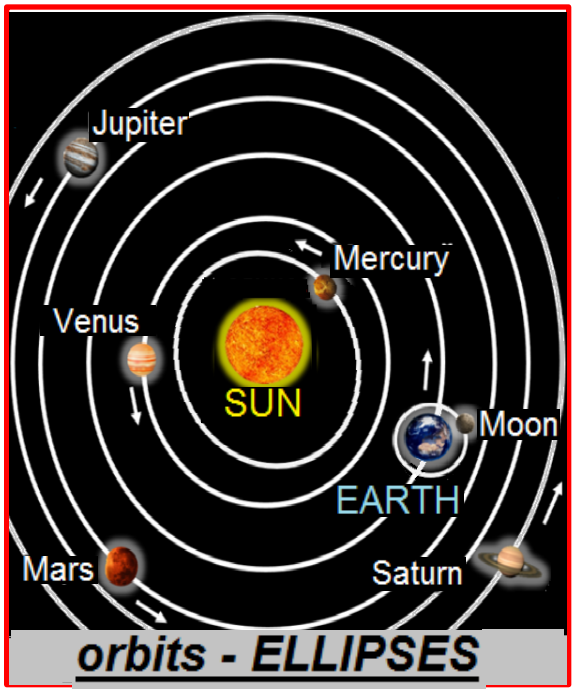


Copernicus:

Mol. dynamics

***(BUT – SMALL ERROR:** in parameters*),

IMPERFECT (worse than by Ptolemaeus)
PREDICTION OF PLANETARY MOVEMENTS

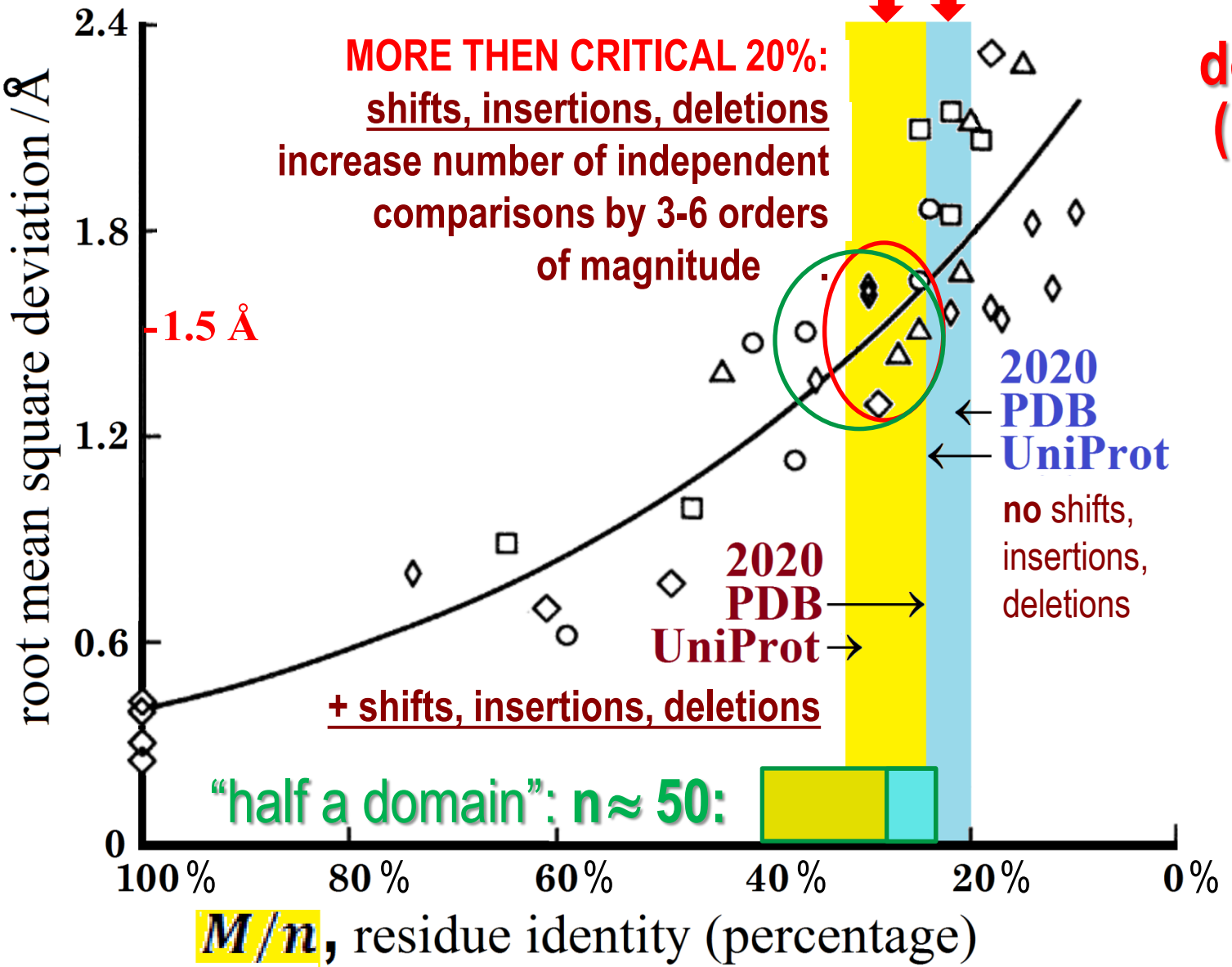


Kepler, Newton:

***CORRECT* UNDERSTANDING**
(exact equations of celestial mechanics!),

PERFECT PREDICTION OF MOVEMENTS OF PLANETS ,
COMETS, ROCKETS AND EVERYTHING ELSE

EXPECT TO FIND – HAVING MODERN DATABASES



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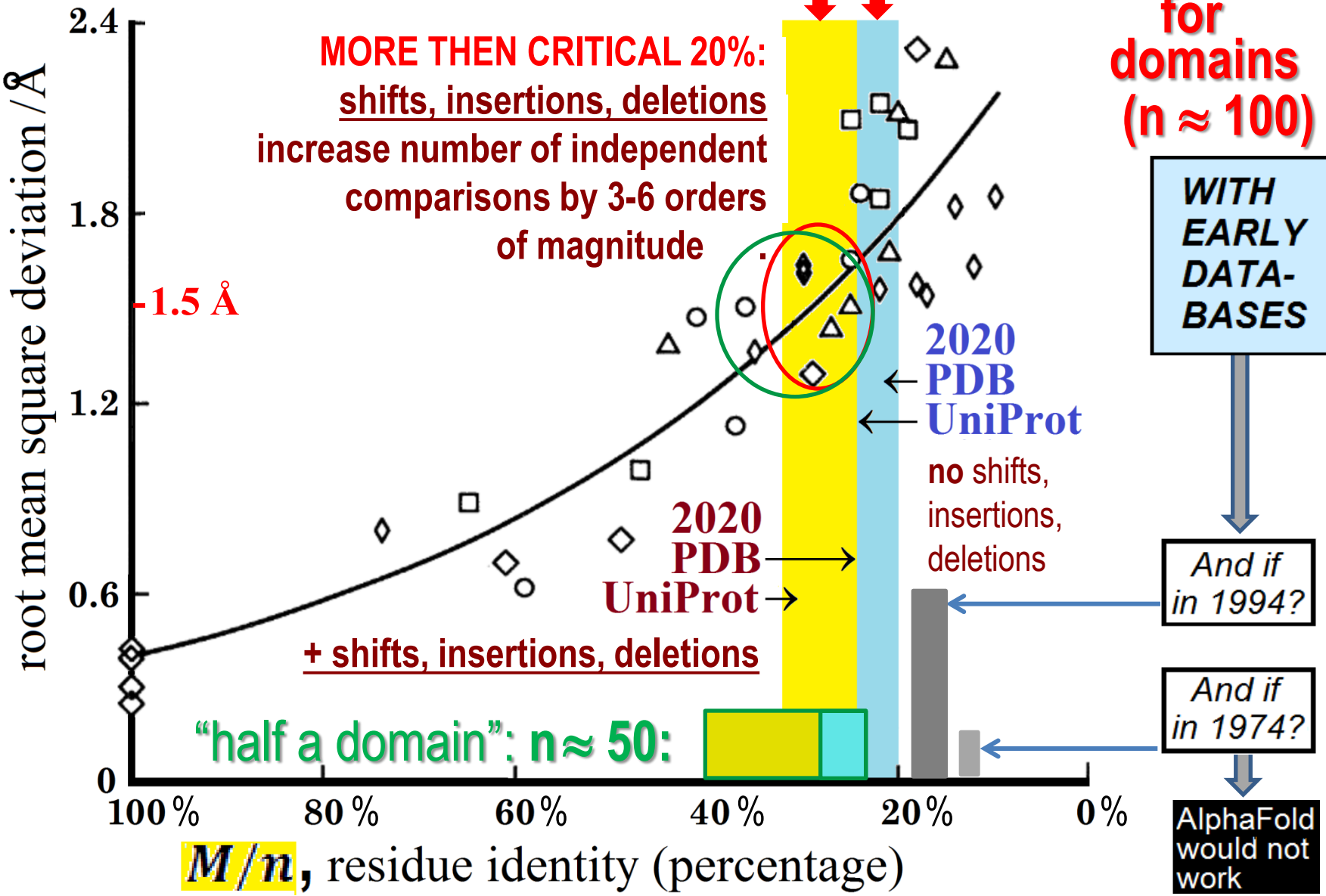

WITH MODERN DATABASES, ALPHAFOLD CAN RECOGNIZE PROTEIN STRUCTURE

The basis of AlphaFold's great success is a skillful usage of huge protein databases collected during 60 years and clearly presenting evolutionary conservation of stable features of 3D protein structures.
Now AlphaFold gives a possibility to predict, or rather recognize stable protein structures from their a.a. sequences without considering the process of protein folding that creates these structures.

We emphasize that the this study *does not* diminish the merit and utility of AlphaFolds; it only explains the basis of their success.

On the basis of AlphaFold:

RoseTTAFold:
Anishchenko I., … , Baker D. - De novo protein **design** by deep network hallucination. *Nature* **600**, 547–552 (2021). https://doi.org/10.1038/s41586-021-04184-w.

AF-multimer:
Gao, M., …, Skolnick J. - AF2Complex predicts direct physical **interactions in multimeric proteins** with deep learning. *Nat Commun* **13**, 1744 (2022). https://doi.org/10.1038/s41467-022-29394-2

OpenFold:
Ahdritz G., …, AlQuraishi M. - OpenFold: retraining AlphaFold2 yields new **insights into its learning** mechanisms and capacity for generalization. *Nat Methods* **21**, 1514–1524 (2024). https://doi.org/10.1038/s41592-024-02272-z

AlphaFold 3:
Abramson J., …, Jumper J.M. - Accurate structure prediction of **biomolecular interactions** with AlphaFold 3. *Nature* **630**, 493–500 (2024). https://doi.org/10.1038/s41586-024-07487-w

etc.

Thanks for your attention!

Protein 3D Structure Identification by AlphaFold: a Physics-Based *Prediction* or *Recognition* Based on Huge Databases?

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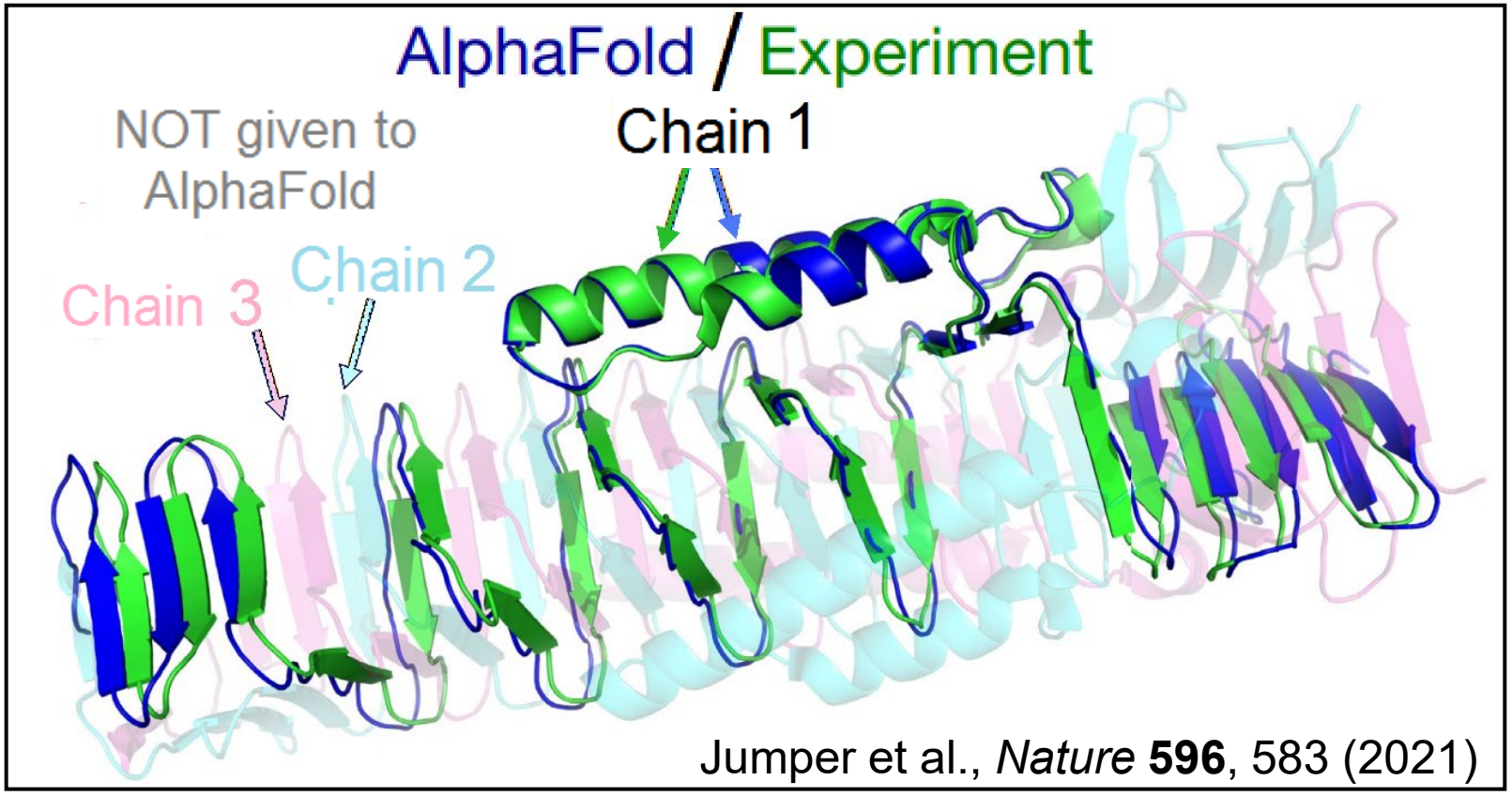
AlphaFold is NOT driven by physics:

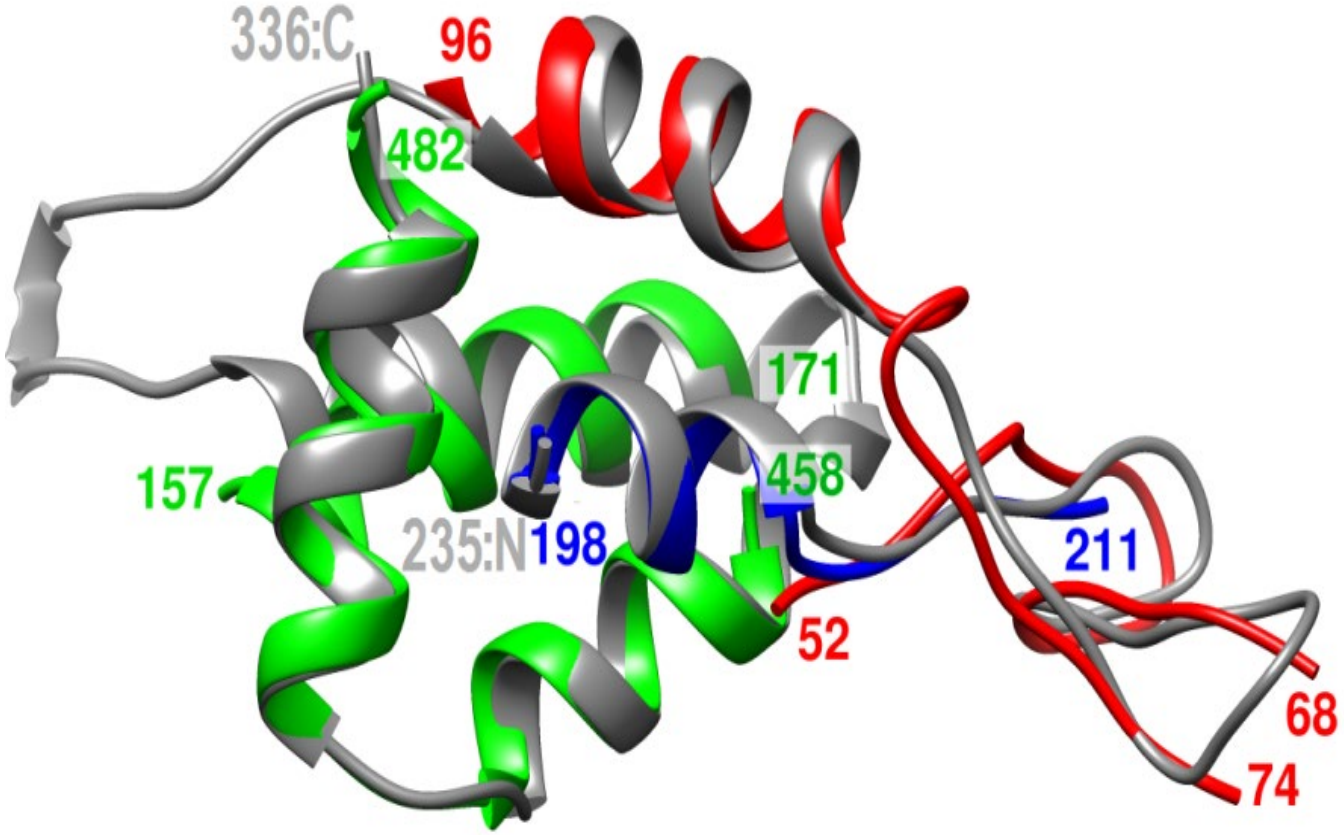
AlphaFold, which is given a.a. sequence of only one of the three intertwined protein chains, recognizes its spatial structure



— which, due to its complete non-compactness,

— **cannot** be stable on its own!

One of many dozens of examples of superposition of pieces of already known 3D structures on a novel fold



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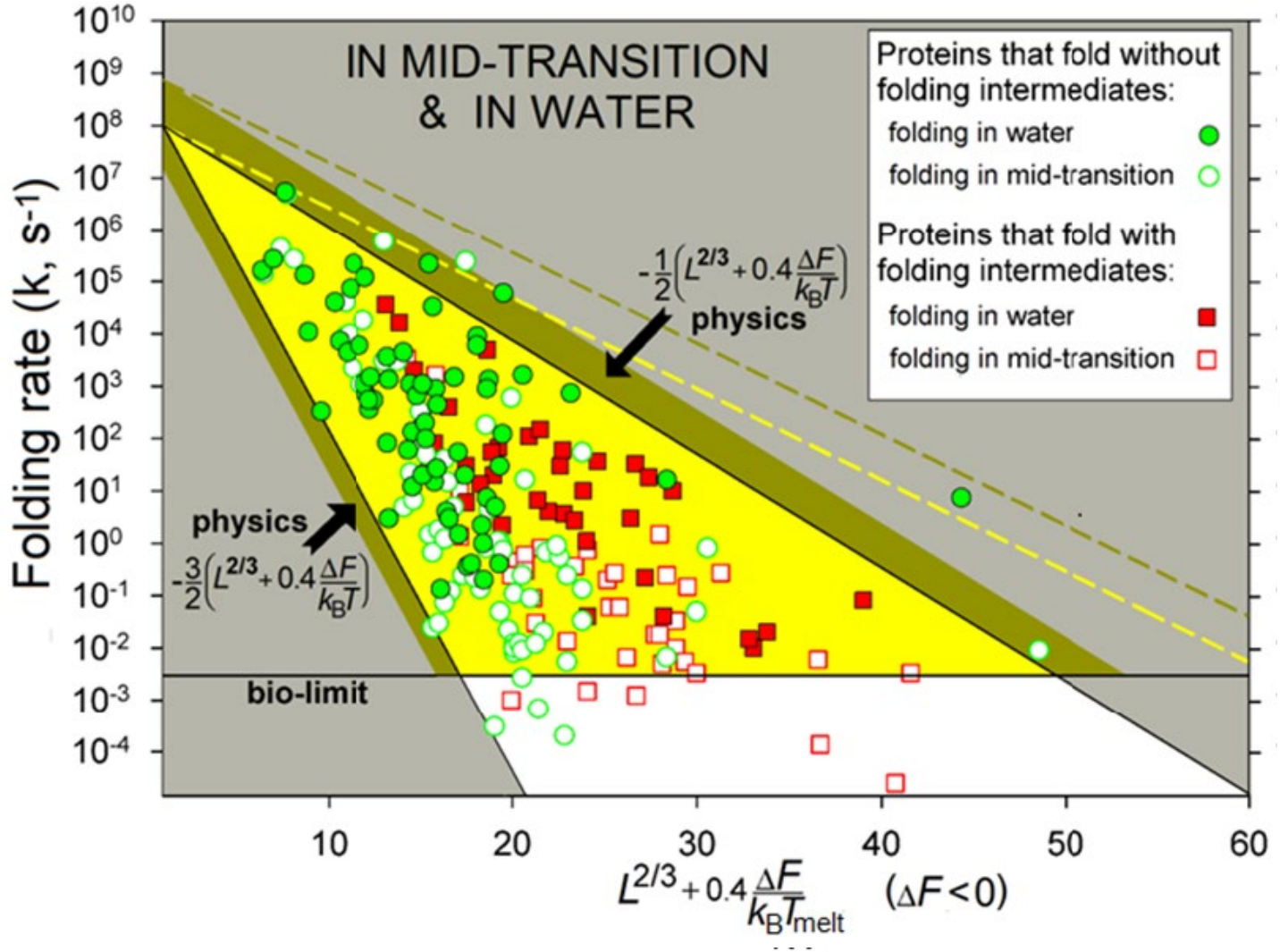
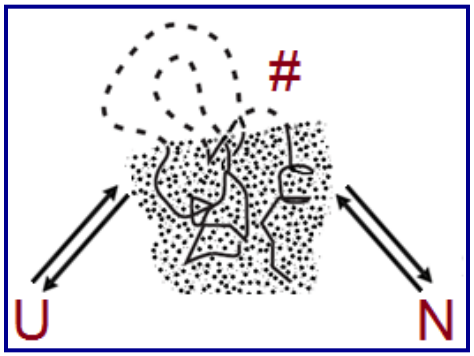
1GB3, chain A; 5A29, chain A; 5W40, chain B.

“Predict folding” (*folding rate*!) ≠ “Predict fold”

$$k_f \simeq \exp\left\{-\left(\frac{1}{2}\div\frac{3}{2}\right)\left[L^{2/3}+0.4\left[\frac{\Delta F}{k_B T}\right]\right]\right\}\frac{0.1}{\text{ns}}$$

Solution of the “Levinthal’s paradox





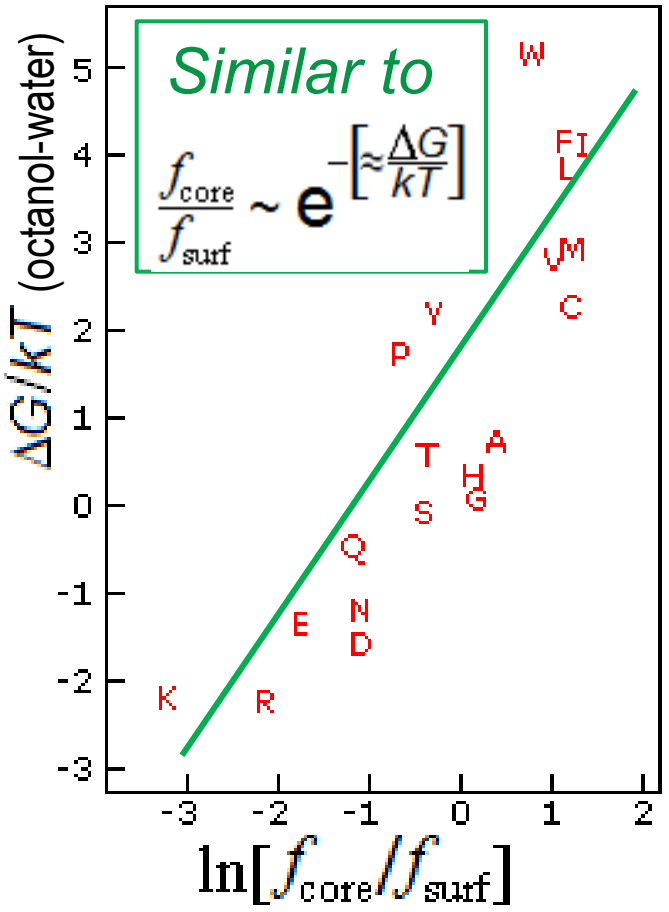
Phase transition

Finkelstein, Badretdinov, *1997,1998*; Garbuzynskiy, Ivankov, Bogatyreva, Finkelstein, *PNAS*, 2013

The occurrence of elements of protein structures is associated with their stability (Finkelstein et al., Proteins, 23: 142-150, 1995)

Small details of protein structures

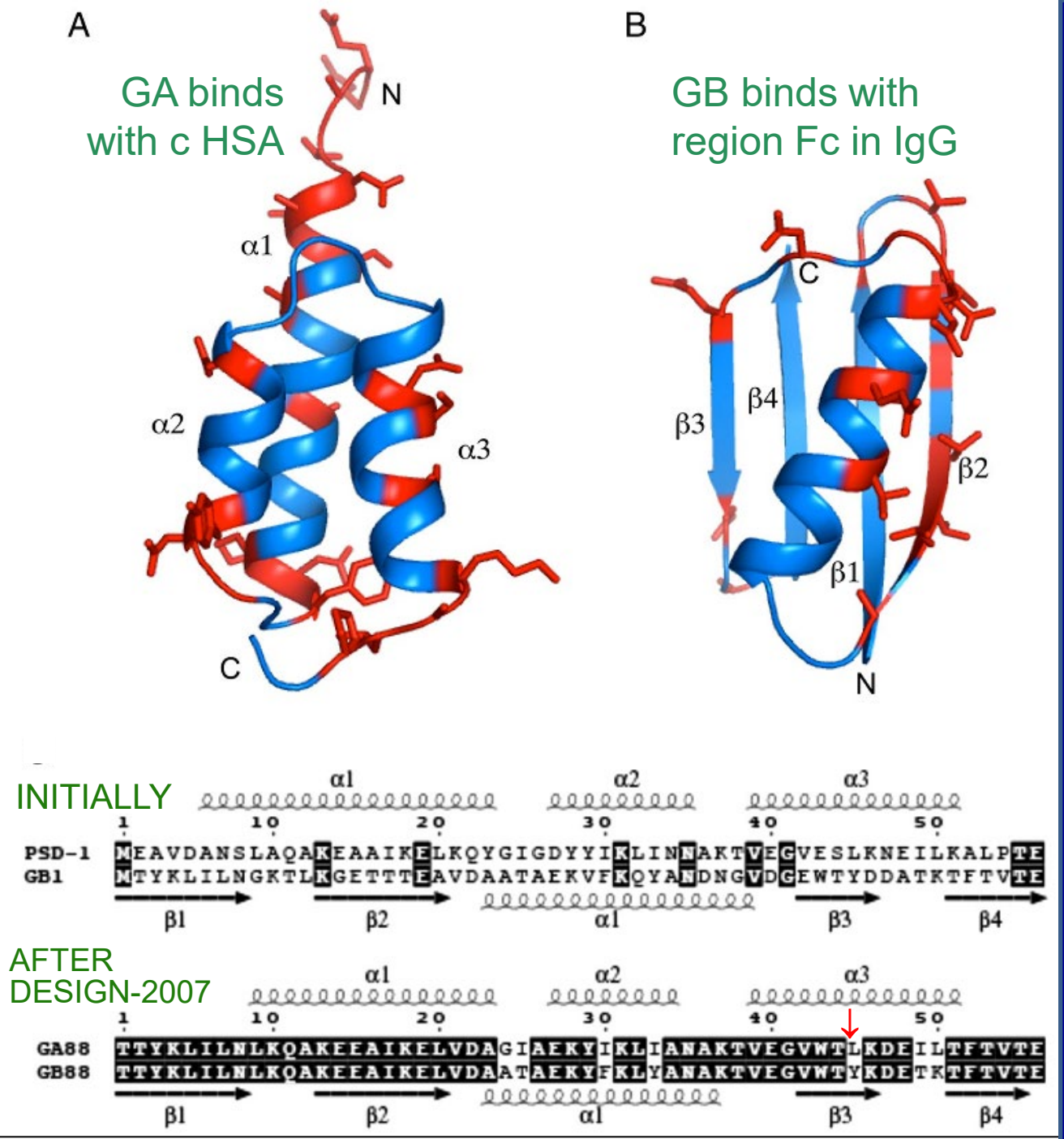
Example:



Miller, Janin, Chothia, 1984

Similar to Boltzmann-Gibbs statistics (F.M. Pohl, 1971)

The reason is a *selection* of *stable* structures



Needed:

Old chain fold and old activity – with a completely new a.a. sequence

P.A.Alexander, Y.He, Y.Chen, J.Orban, P.N.Bryan

PNAS, 2007, 104, 11963-8

The design and characterization of two proteins with 88% sequence identity but different structure and function

Y.He, Y.Chen, P.Alexander, P.N.Bryan, J.Orban

PNAS, 2008, 105, 14412-7

NMR structures of two designed proteins with high sequence identity but different fold and function

2012 (*Structure*, 20, 283-91):

Difference: **ONE** a.a. residue!

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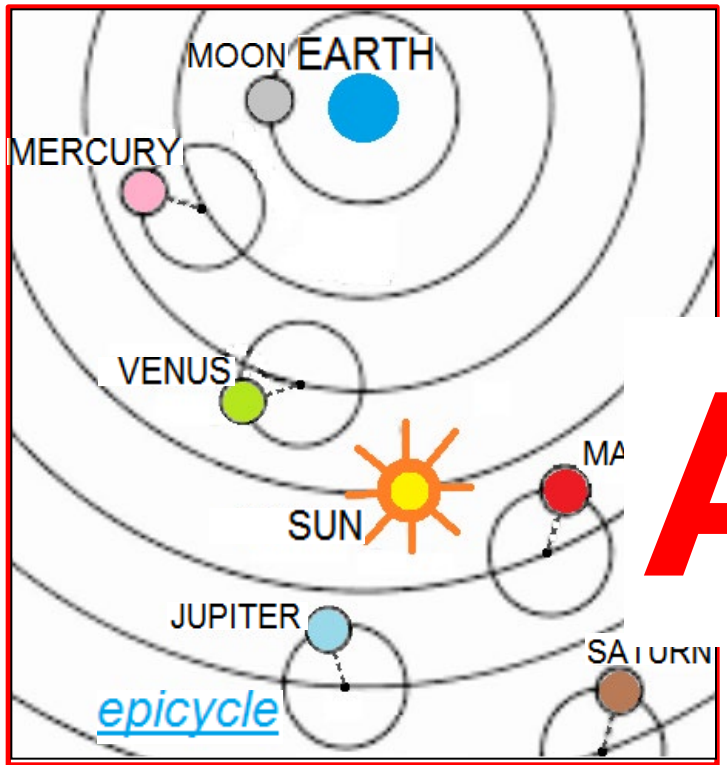
BUT: *fundamentally* WRONG UNDERSTANDING
(The Earth is flat!)



PTOLEMAEUS
(*using huge archives*):

GOOD PREDICTION

AlphaFold

***WRONG* UNDERSTANDING of the PROCESS!**