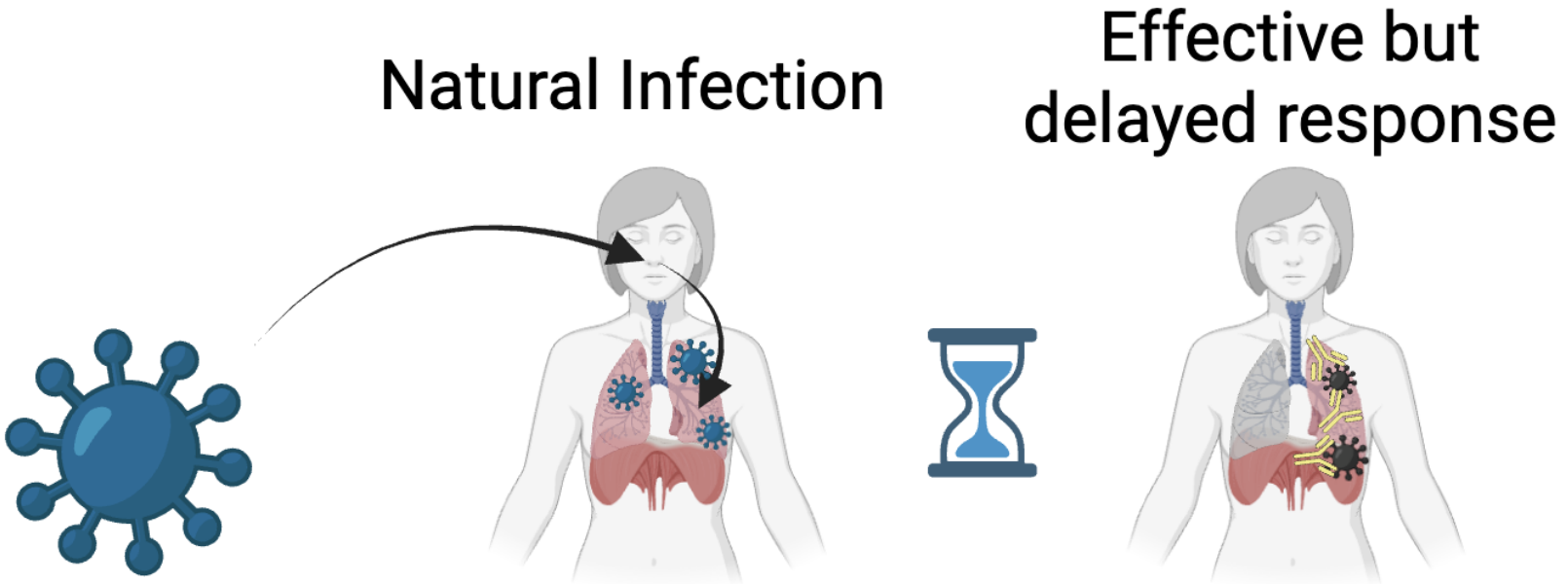
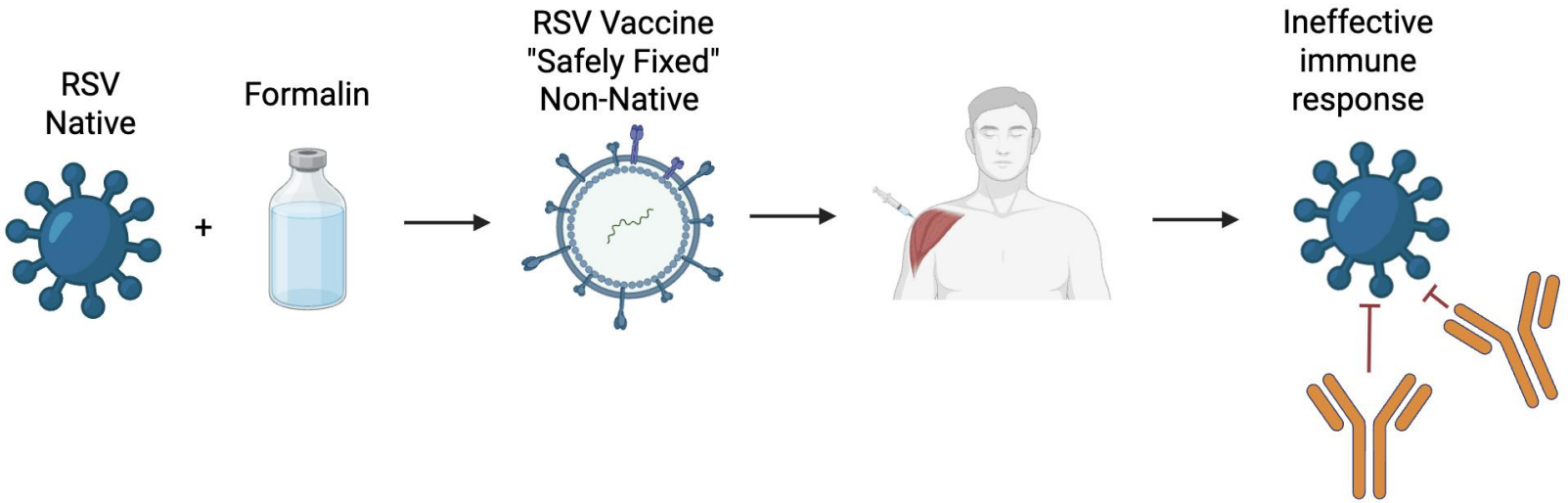
ML-Based Structure Prediction with RosettaFold-3

Immunogen Design Workshop
April 2026

Natural immunogens are typically effective, but unsafe

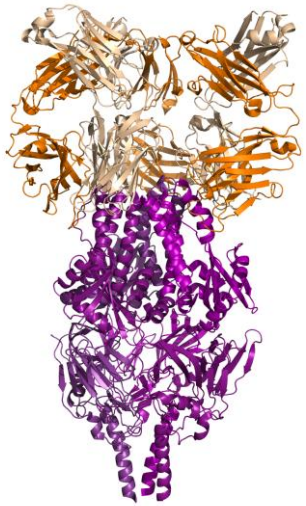


Naïve immunogen design can alter the shape of the immunogen



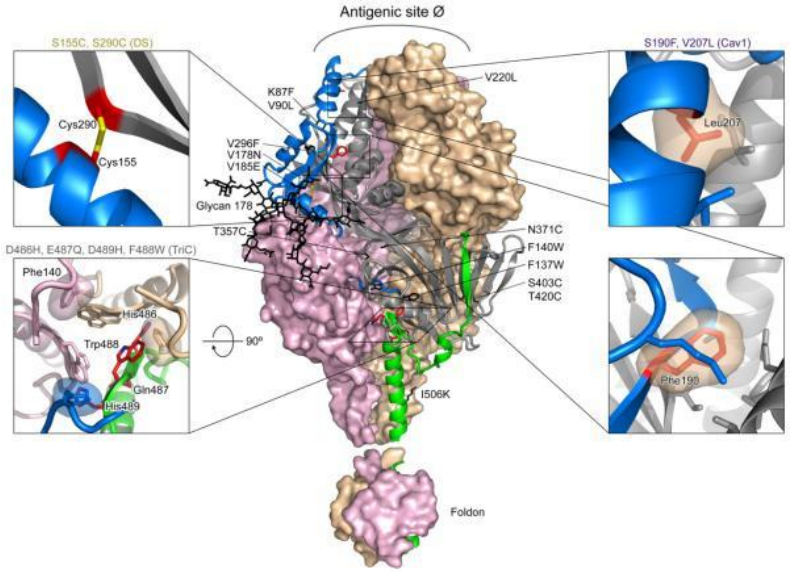
Antibody-Antigen structures can be essential for immunogen design

Step 1:
Build model of immunogen in desired conformation



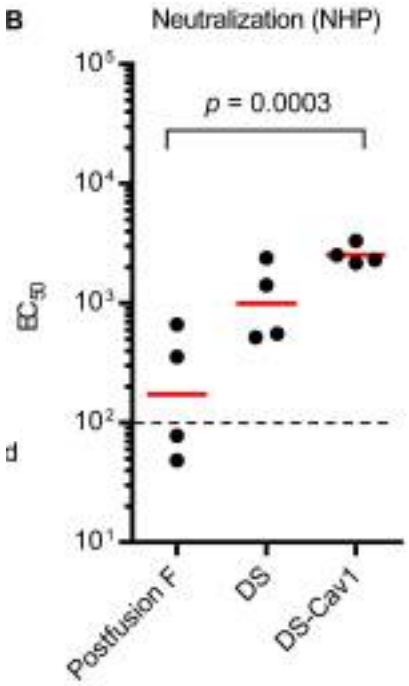
RSV (unstabilized)
Antibody Heavy chain
Light chain

Step 2:
Stabilize immunogen in desired conformation



RSV DS-Cav 1 (stabilized)

Step 3:
Evaluate immune response



PDB ID 4JHW (D25); Jason S. McLellan *et al.*, *Science* 2013

Predicting these models *de novo* is one of the hardest folding challenges

Reasons why antibody–antigen modeling is so challenging:

1. Size
2. Stoichiometry
3. Non-protein atoms
4. Transmembrane regions
5. Multiple conformations
6. Limited evolutionary information on many pathogens
7. Lack of co-evolutionary information

RosettaFold-3 is often the best folding model for immunogen design

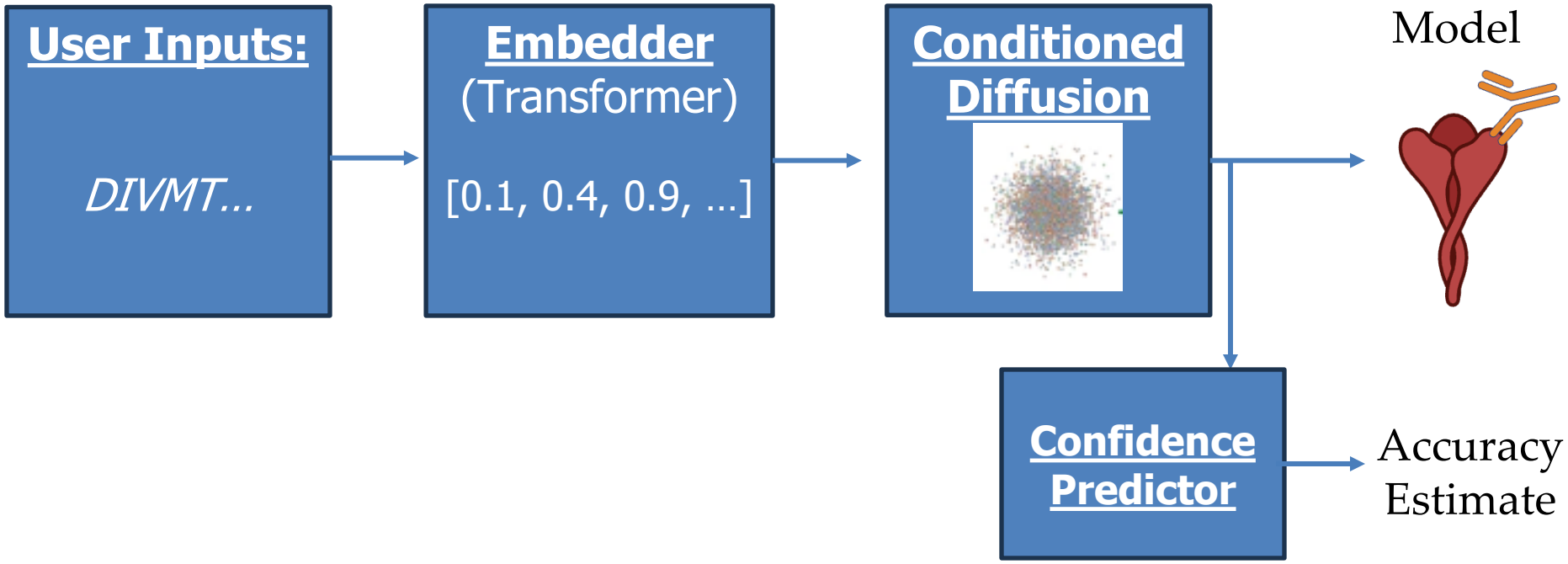
Cases to use RF3:

1. There is potential for intellectual property
2. Part of the structure is known
3. There are non-protein atoms

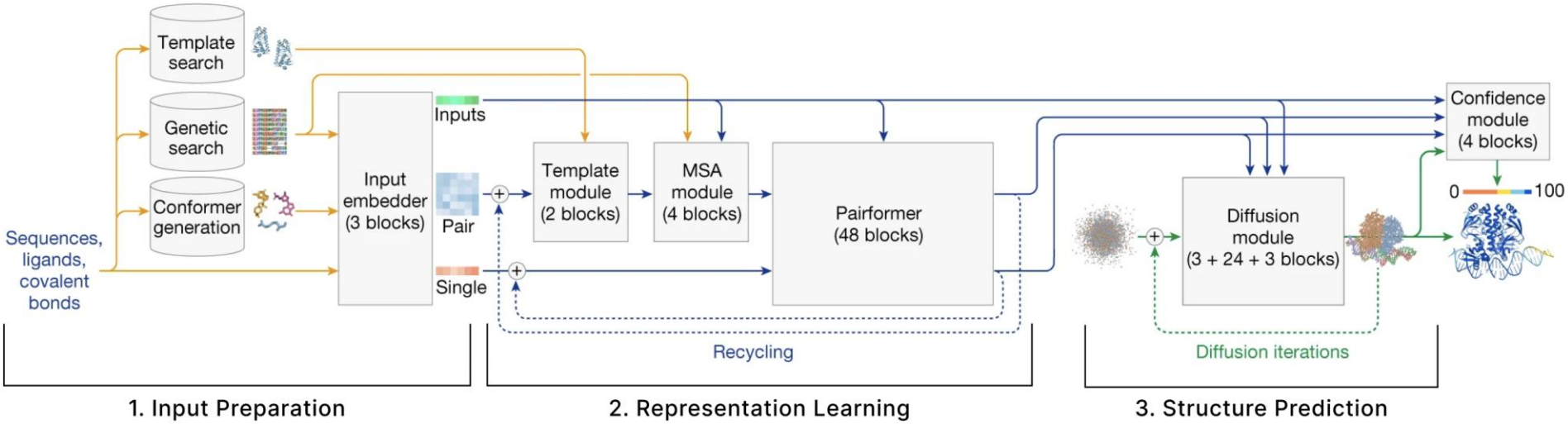
Cases to use other folding models:

1. There is very high (80%+) sequence identity to existing structures – try both homology modeling and templated RF3
2. There is little existing structural data and your MSA is small (for example certain viruses) – try both RF3 and AF2
3. No IP issues and you want an online server – **use AF3**

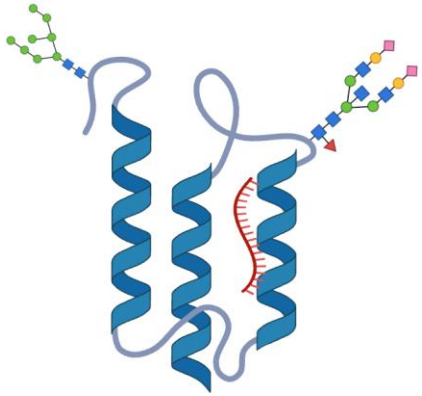
General overview of RF3



AlphaFold-3 model in detail (RF3 is nearly identical)



User inputs

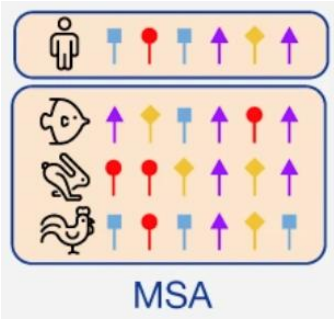


Model components:

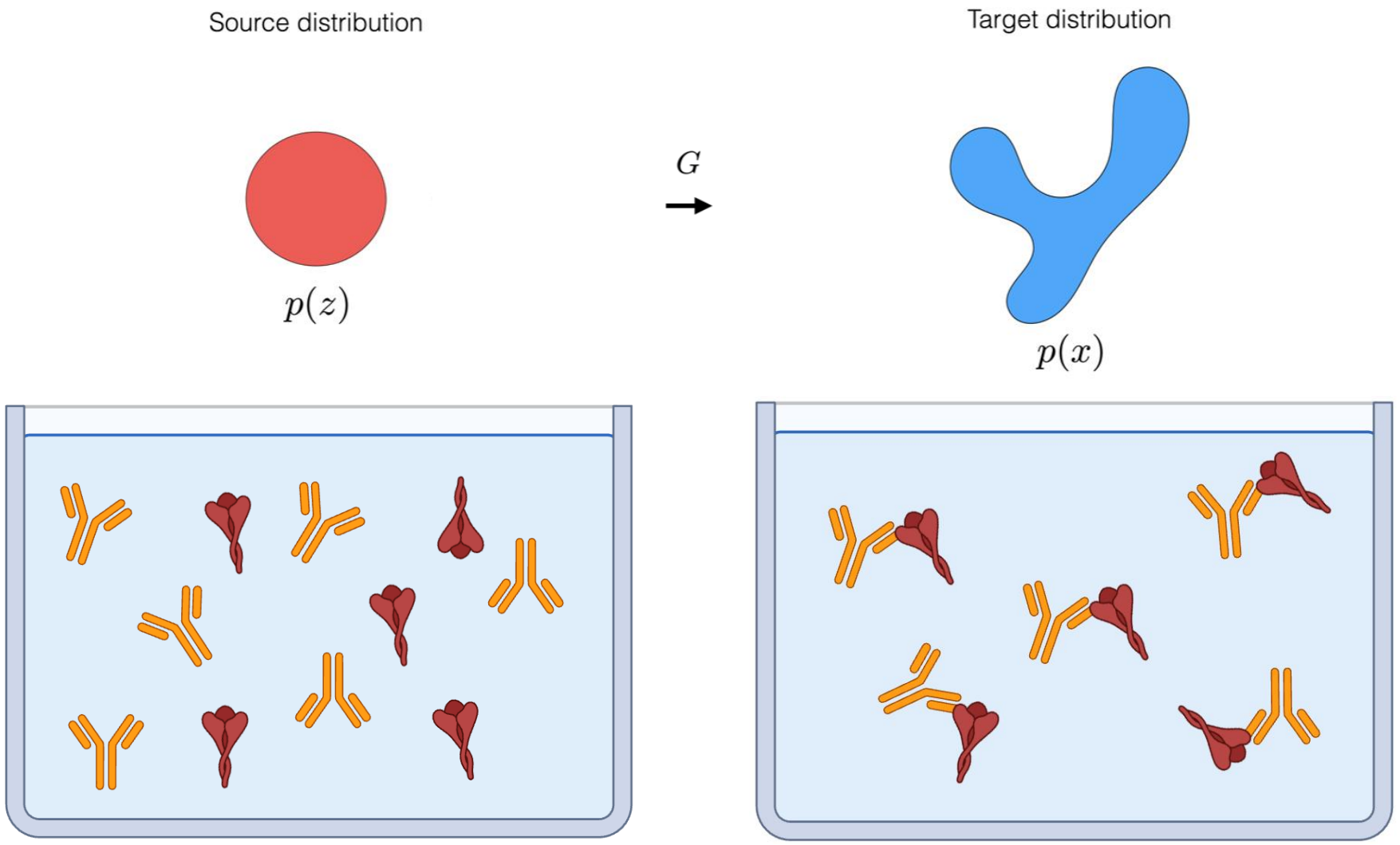
1. Amino acid sequence (for proteins)
2. Other biomolecules (ligands, RNA, DNA, etc.)
3. Covalent modifications (glycosylation, any element, etc.)

Extra Knowledge (Optional, but very helpful):

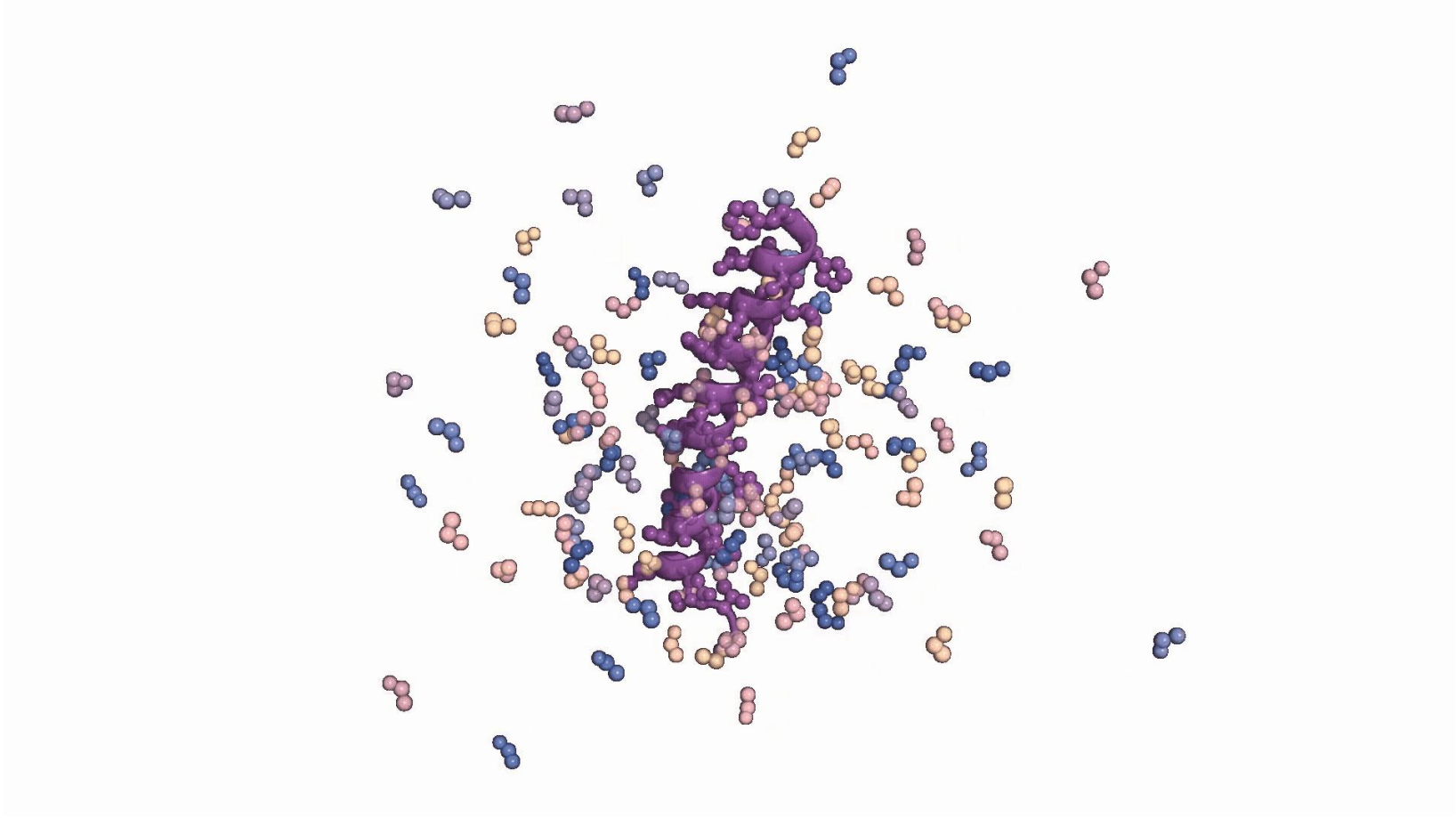
1. Multiple sequence alignment
2. Structural templates



RF3's goal is to map your inputs from a "starting" to a "target" distribution

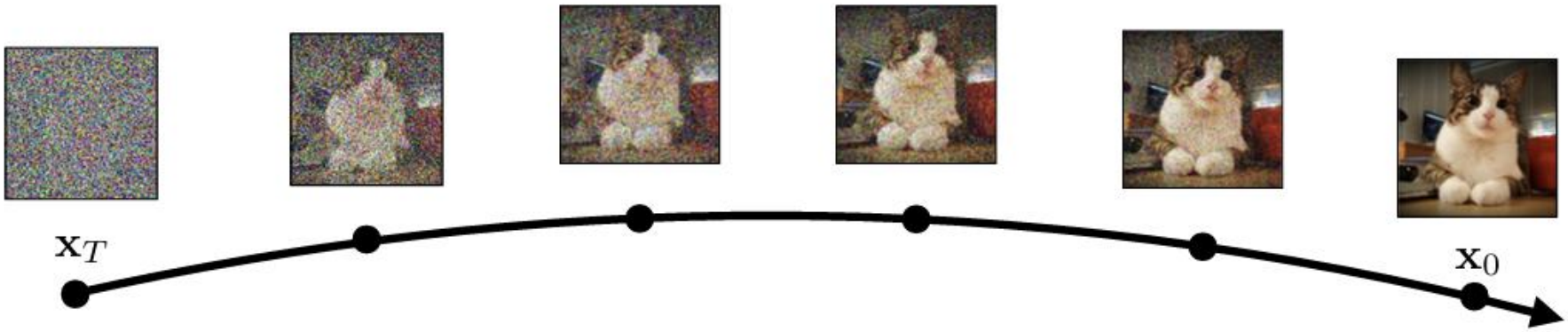


Folding of a protein with a templated section in a diffusion model

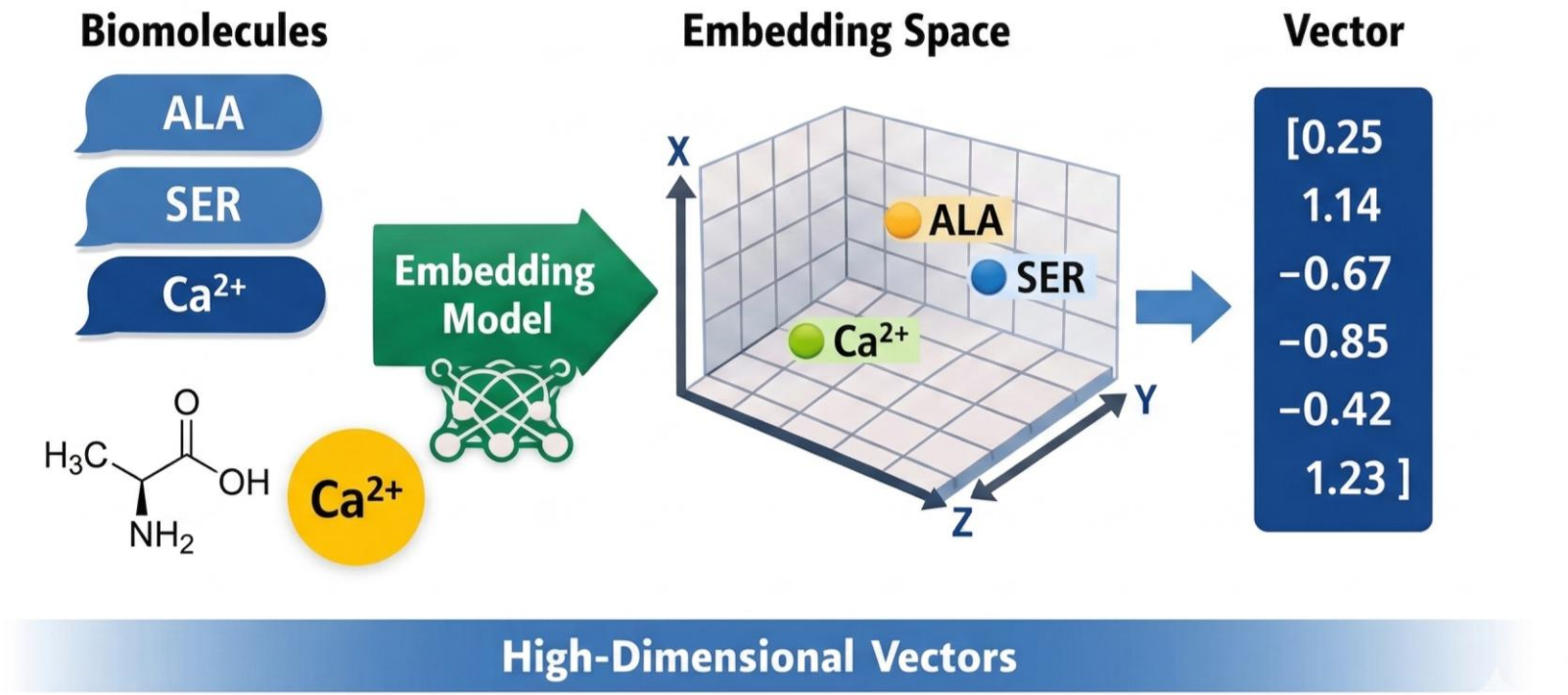


https://www.bakerlab.org/2023/07/11/diffusion-model-for-protein-design/

Diffusion models gradually denoise over a number of steps

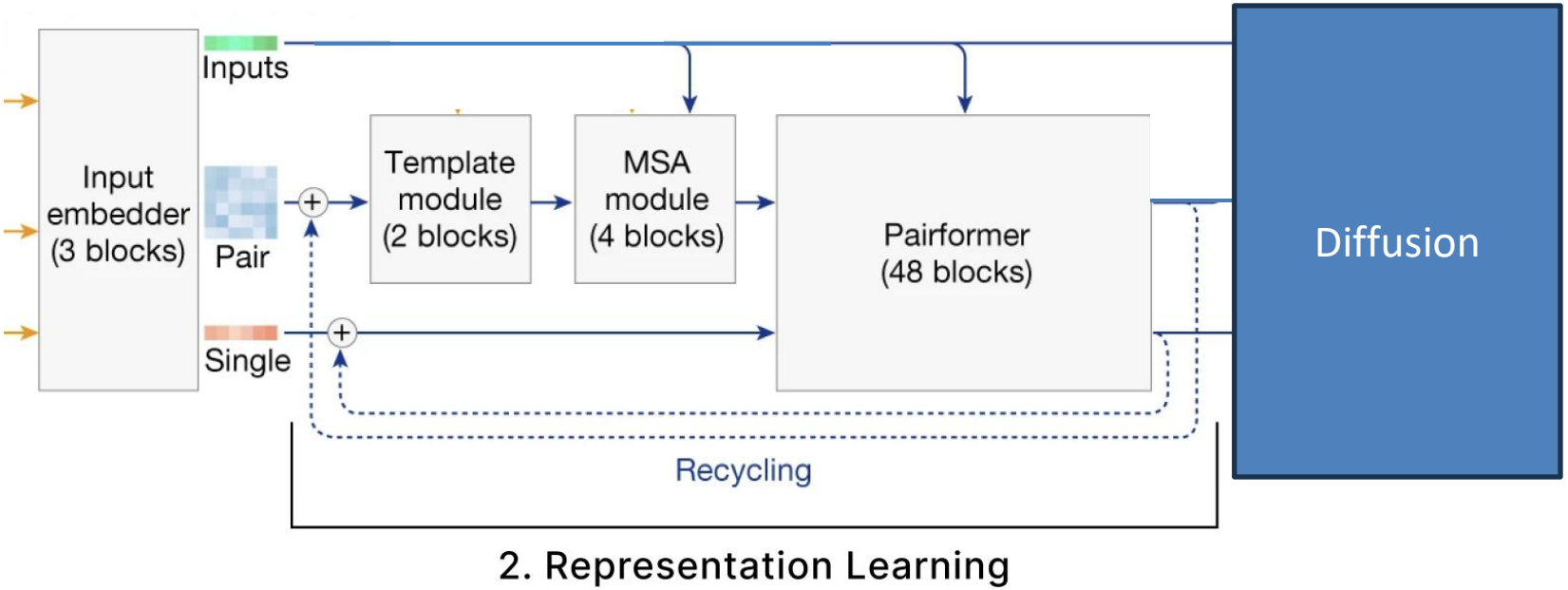


First RF3 **tokenizes and embeds** each of your inputs



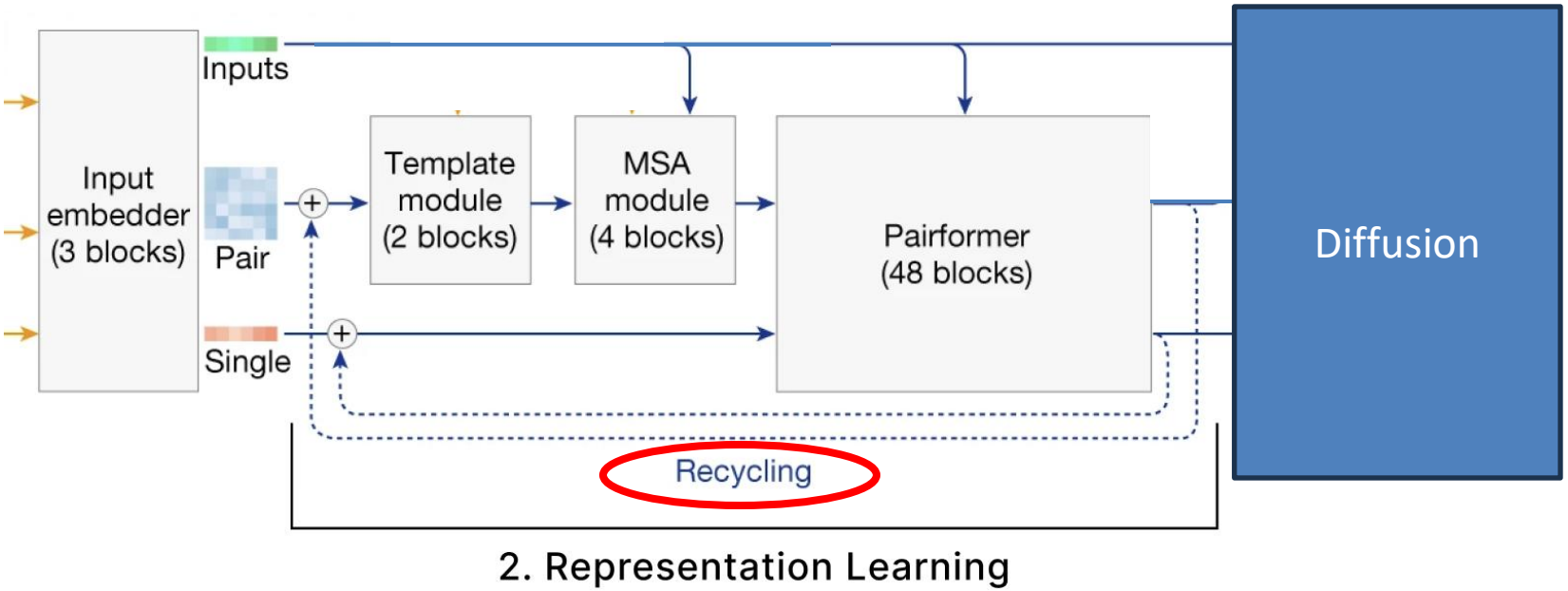
https://database.guide/what-is-an-embedding/ edited by Gemini

Eventually (1) **inputs**,
(2) **pairwise embeddings** and (3) **per-token embeddings** condition the diffusion



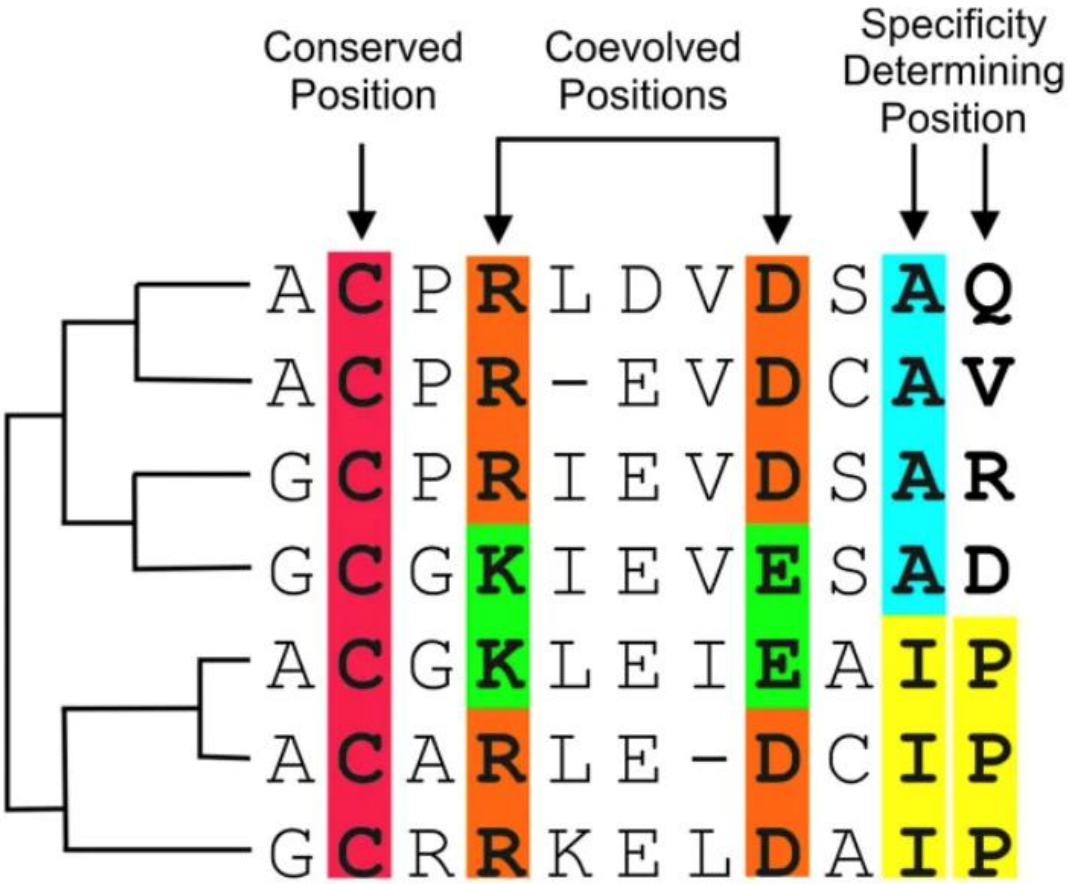
RF3 re-uses the same embedding weights R times

- This is the most time-intensive stage



2. Representation Learning

The MSA module attends to coevolution when updating pairwise embeddings

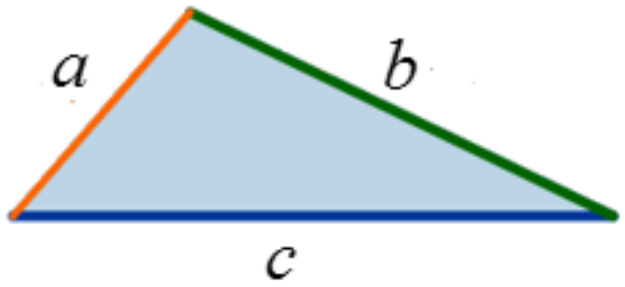


Tepp E., et al. BMC Bioinformatics, 2012

Triangle updates & attention enforce geometric consistency in pairwise embeddings

Triangle Inequality Theorem

The sum of the lengths of any two sides of a triangle is greater than the length of the third side



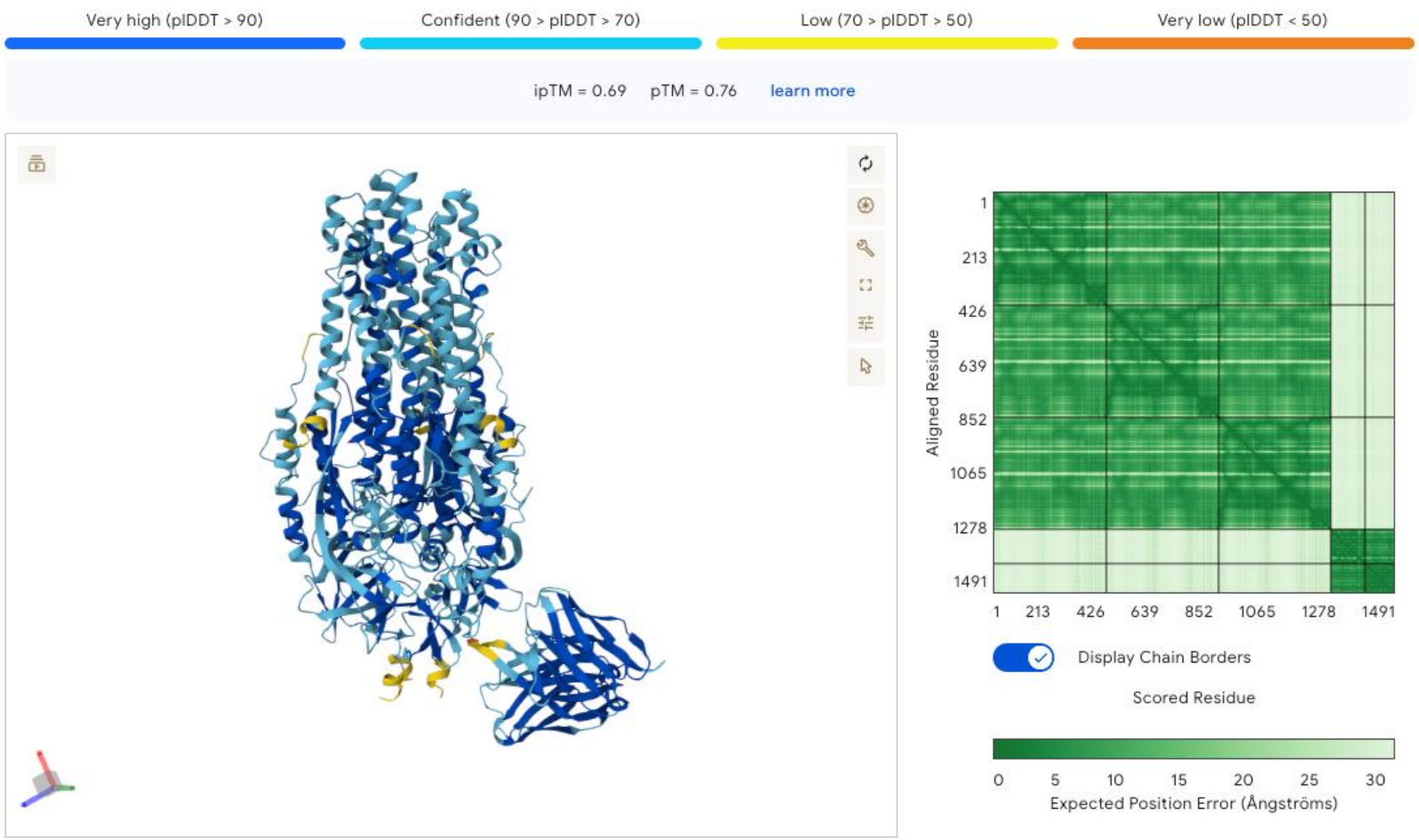
$$a + b > c$$

$$a + c > b$$

$$b + c > a$$

https://www.onlinemathlearning.com/triangle-inequality.html

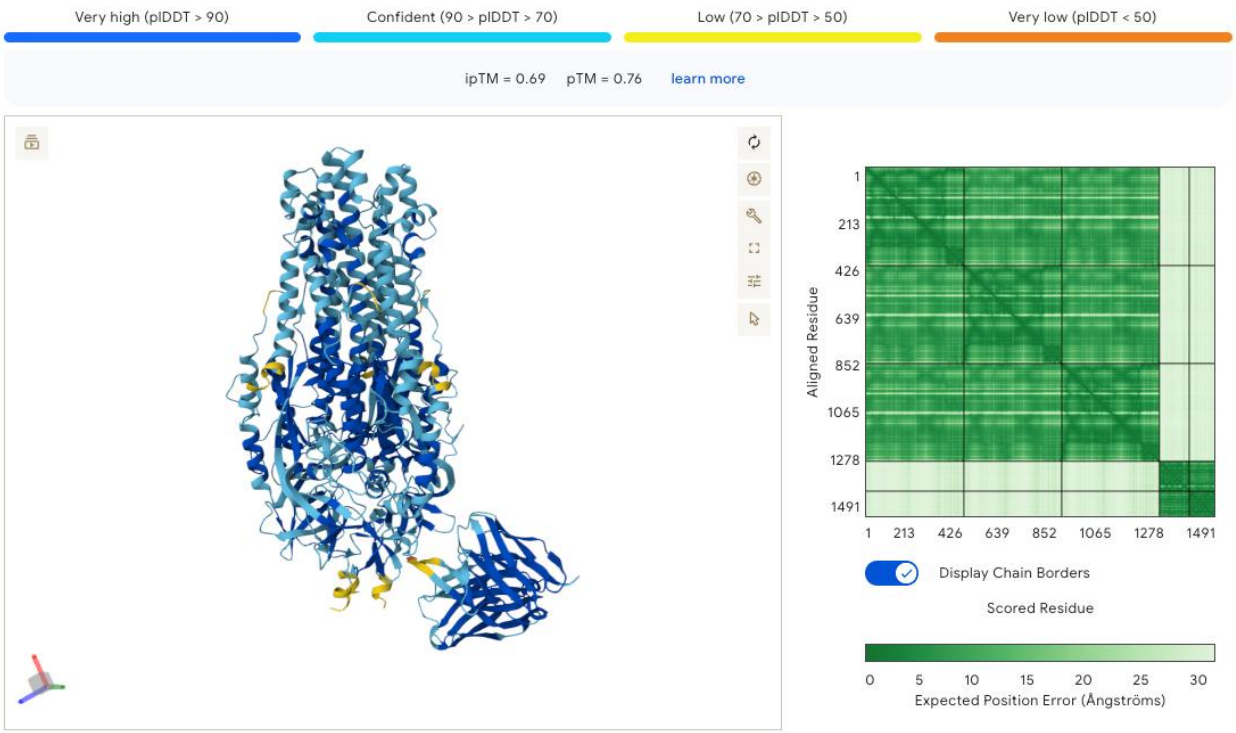
Confidence metrics: view of an Alphafold-3 server prediction



https://alphafoldserver.com/

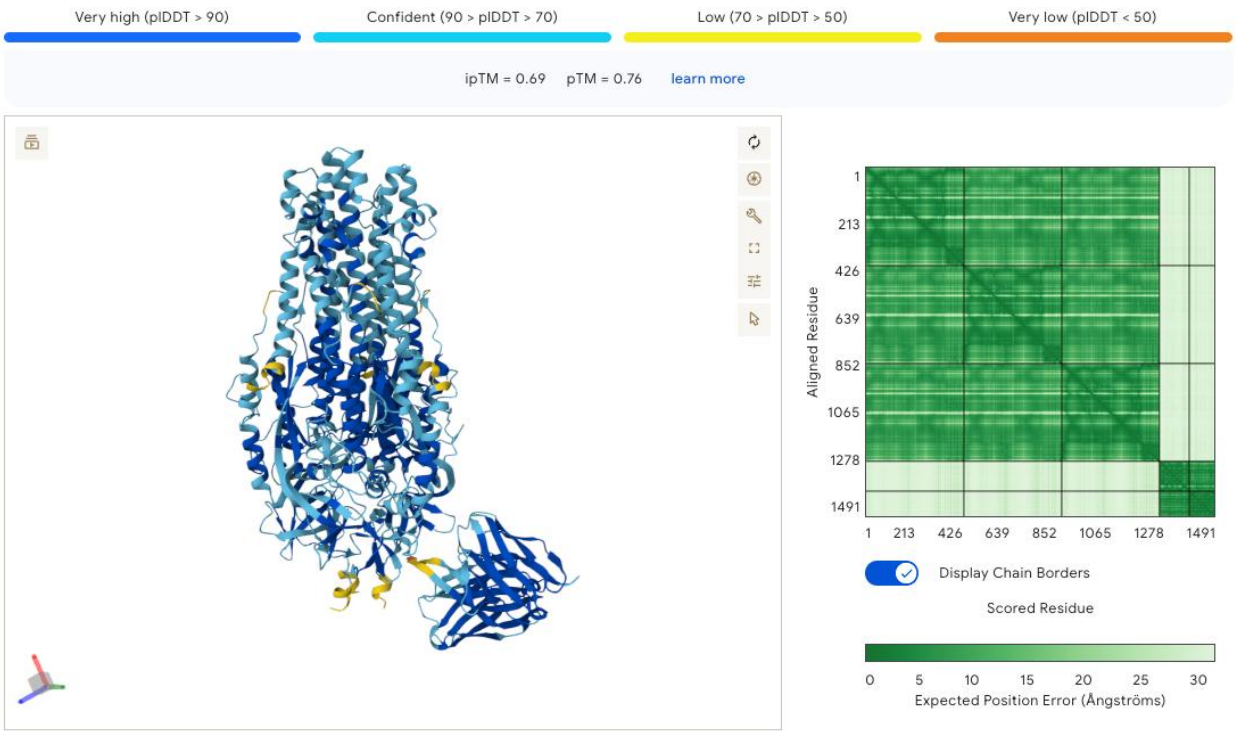
Confidence metric **pLDDT**: Per-residue confidence score (0-1)

- pLDDT = predicted Local Distance Difference Test
- $pLDDT_{ca,\ avg}$ >0.7: good enough prediction of viral protein
- $pLDDT_{ca,\ avg}$ >0.8: quality filter on designed immunogen



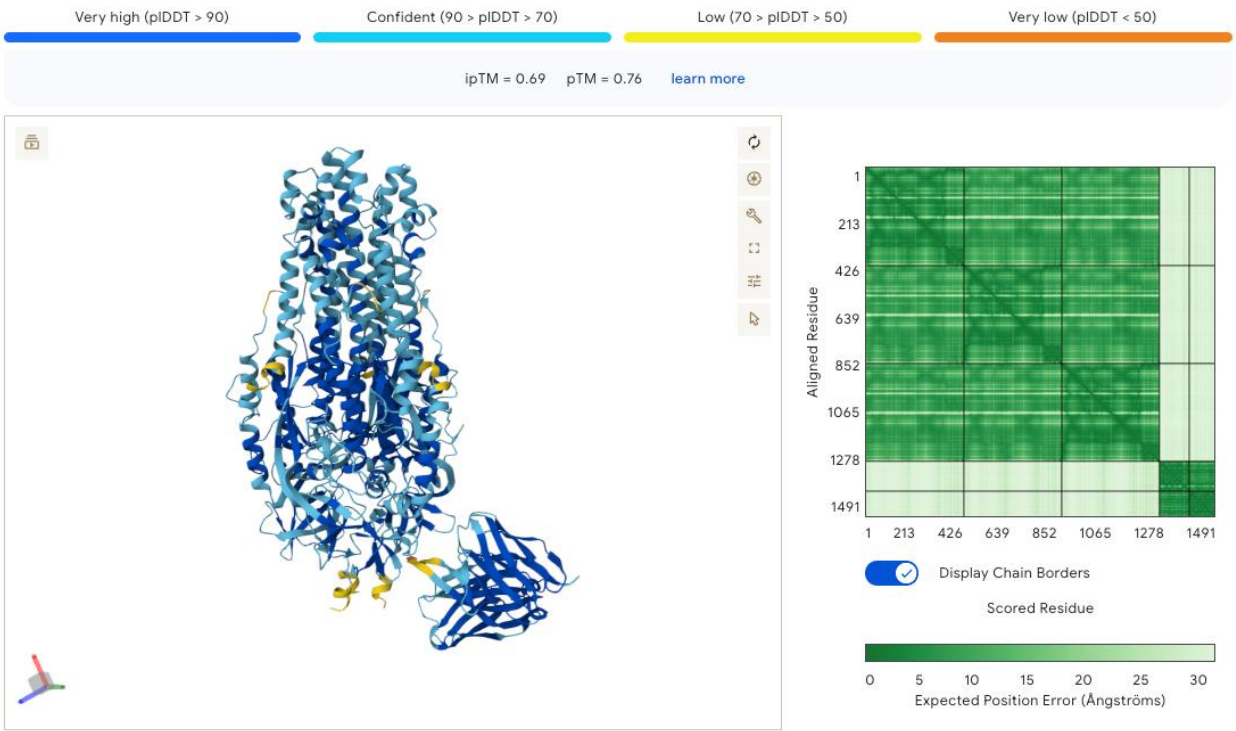
Confidence metric **pTM**: Global fold quality (0-1).

- pTM = predicted Template Modeling Score
- >0.8 suggests confident overall topology



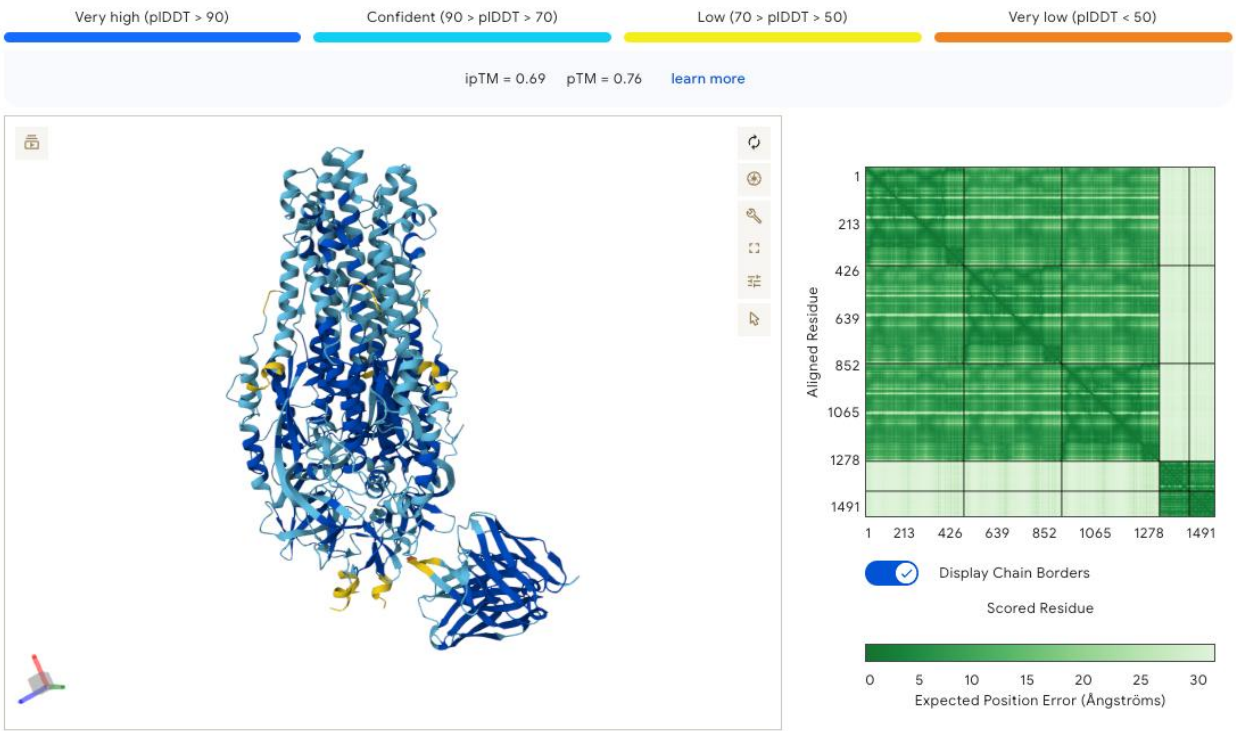
Confidence metric ipTM: Inter-chain quality (0-1).

- ipTM = interface pTM
- >0.8 suggests confident chain-chain placement



Confidence metric **pAE**: Pairwise residue-residue error (0-30+)

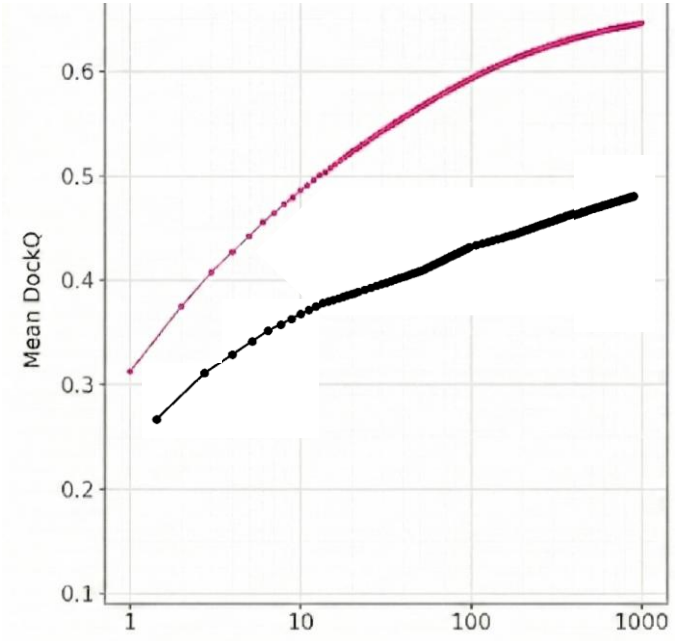
- pAE = predicted Aligned Error
- <5 high
- 5-12 moderate
- > 12 low



Additional Key Concepts

1. Using multiple seeds can significantly improve antibody-antigen modeling results

AF3 Antibody-Antigen Modeling Accuracy*



Best Model

Model's top choice

Image edited by AI to simplify components, source = Openfold3

Additional Key Concepts

1. Sampling with more seeds produces diversity
2. MSA depth matters, but less than in AF2-like models
3. Distillation enabled significant RF3 improvement

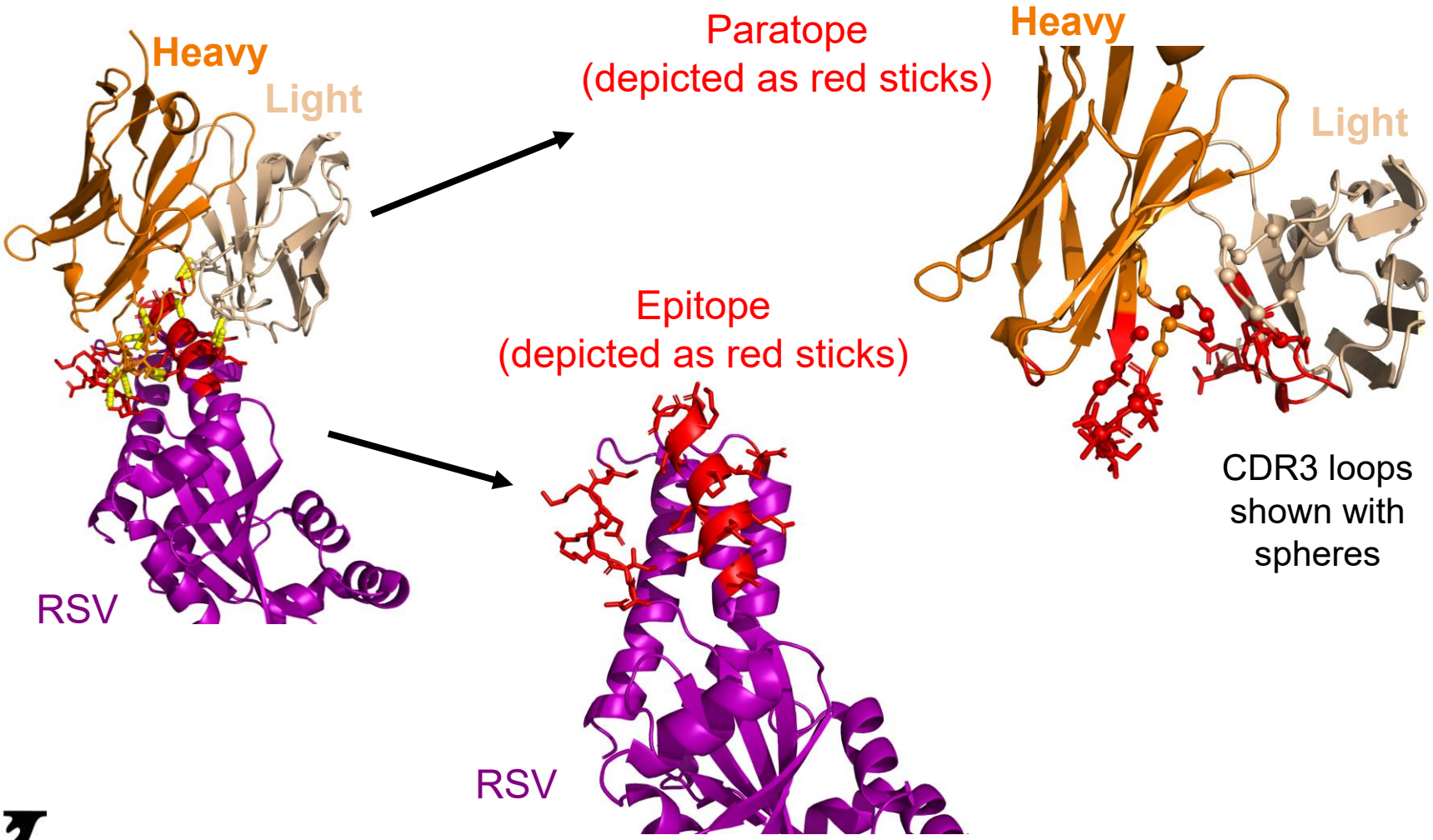
Summary

- External benchmarks place RF3 as the best open-source model for antibody-antigen complex prediction
- RF3's strict templating is a crucial capability separate from other models.
- Confidence metrics should always be checked, use pLDDT for local, pTM for global, and ipTM or pAE between chains
- Antibody-Antigen modeling is challenging. I recommend using higher computational resources (more recycles, steps, and seeds as well as MSAs and templates).

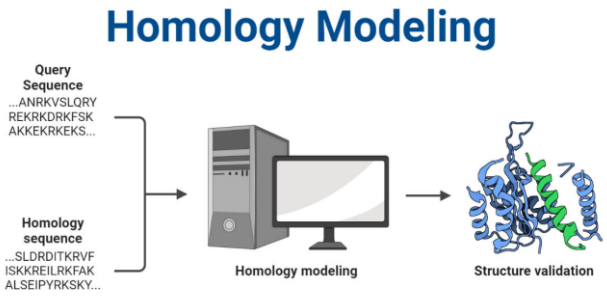
Now it’s time for the tutorial!

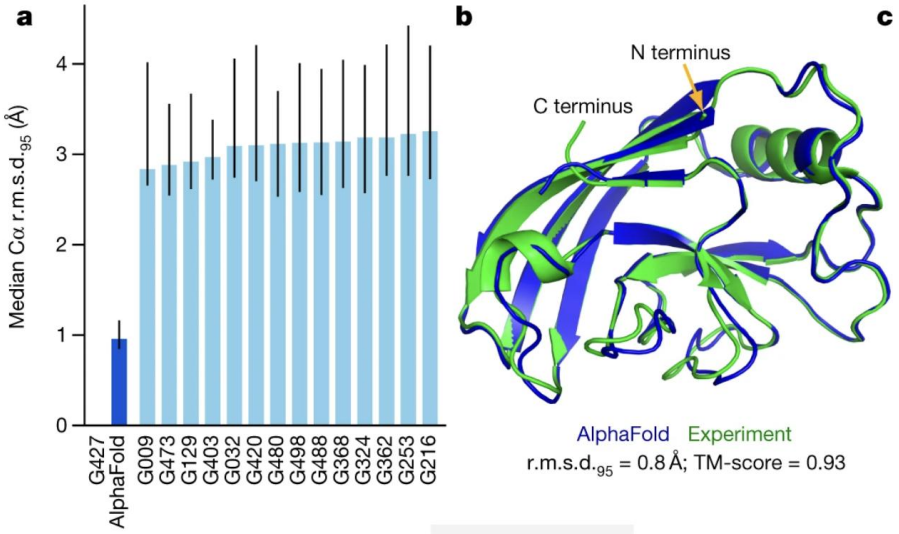
Extra Slides

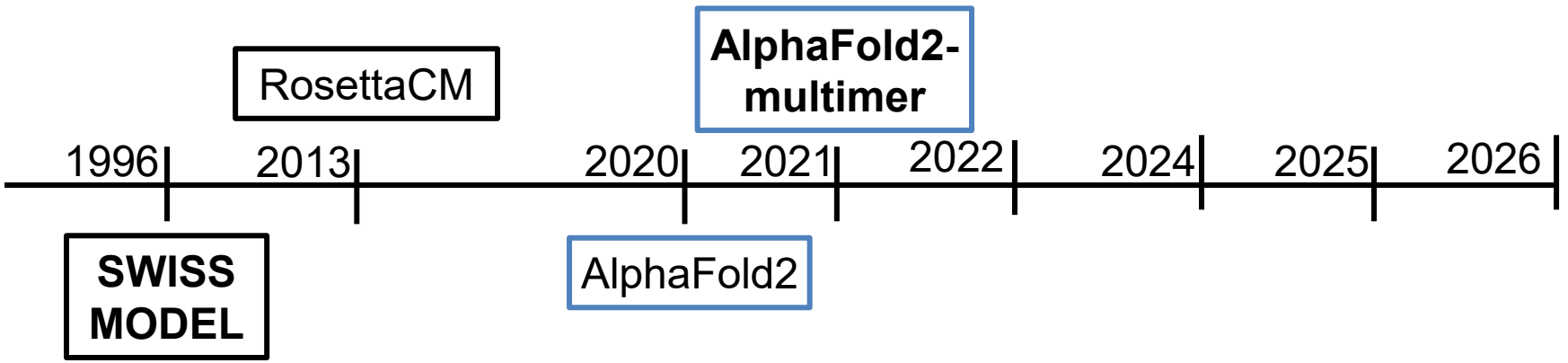
For many efforts the key components are the epitope and paratope



Model building approaches for immunogen design

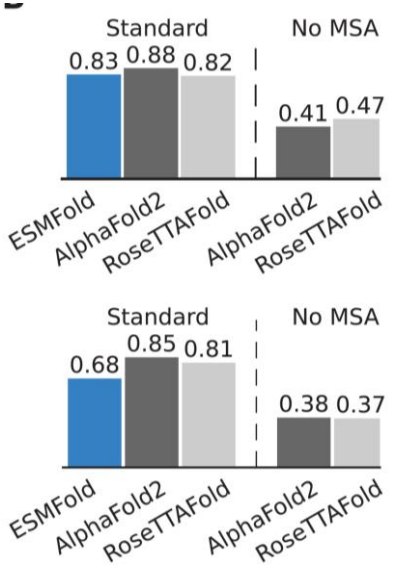


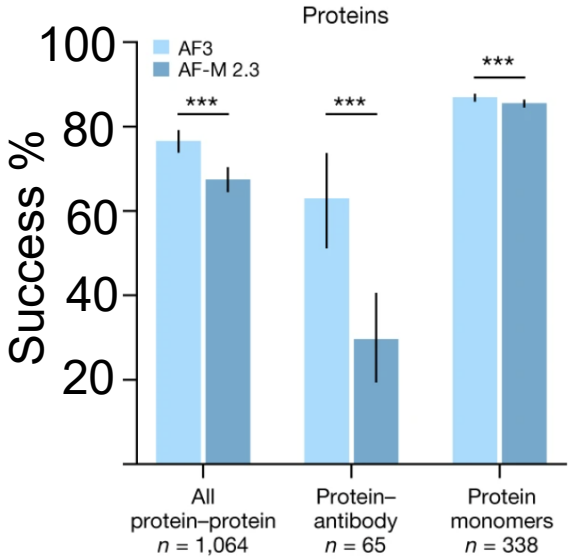


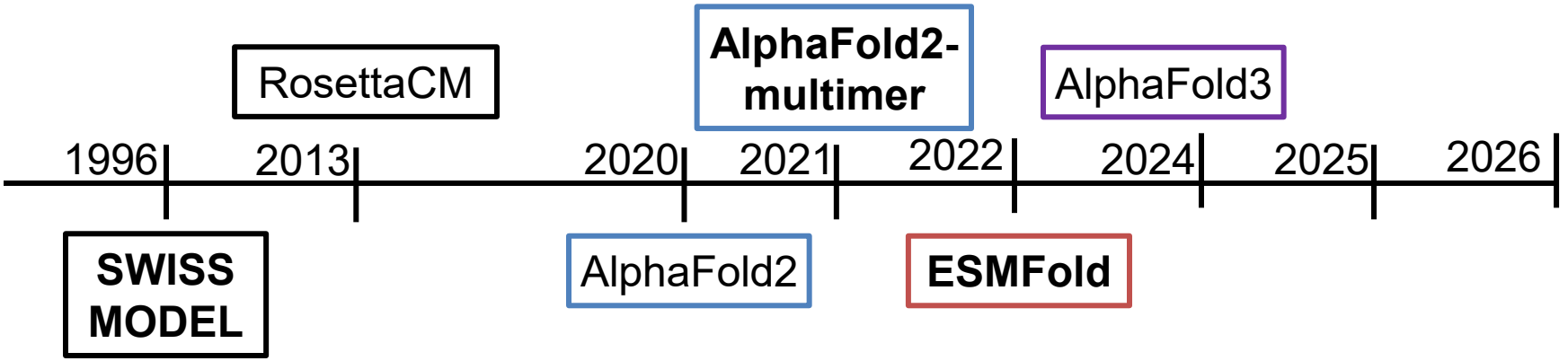


Jumper, J *et al*. *Nature* (2021)

Model building approaches for immunogen design



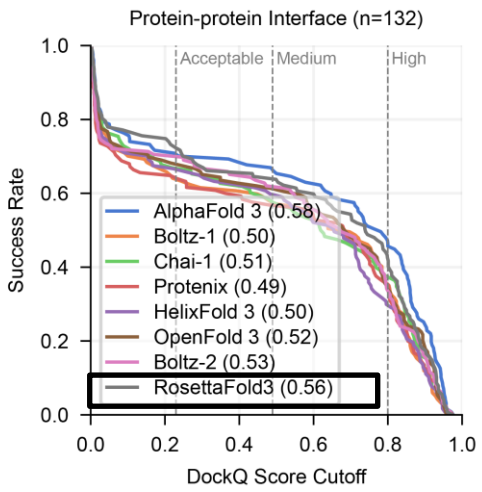


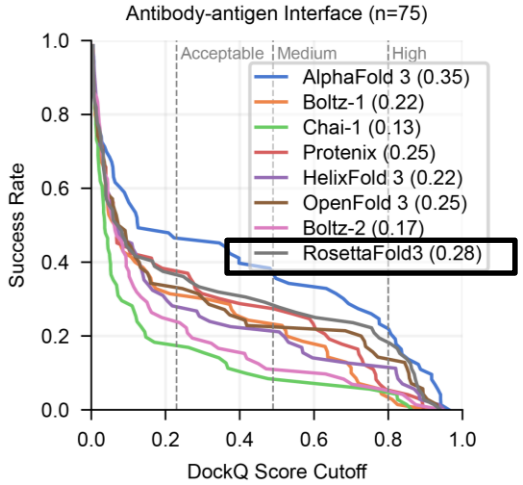


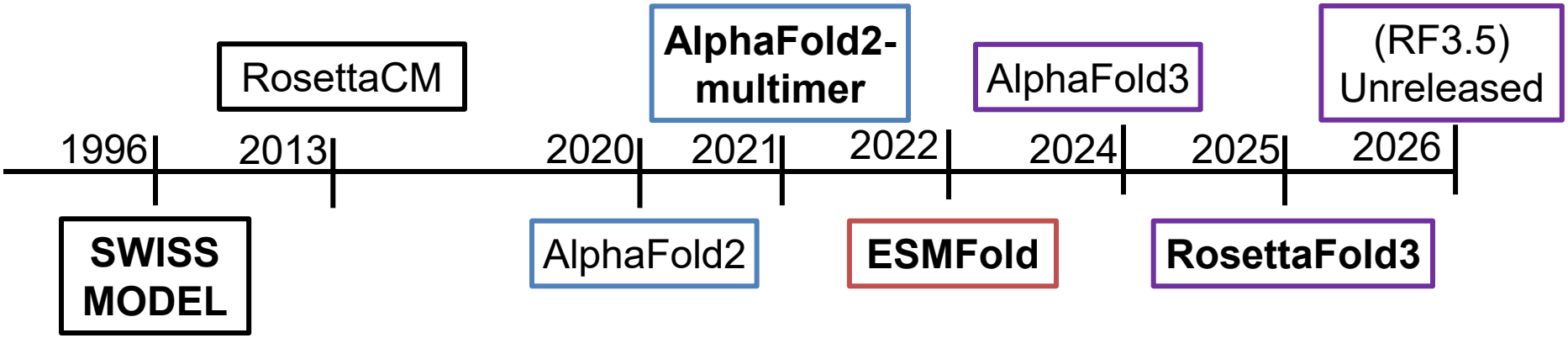
Zeming Lin *et al. Science* (2023), Abramson, J., *et al. Nature* (2024).

Model building approaches for immunogen design

External Benchmark: FoldBench







The embedding steps rely on attention