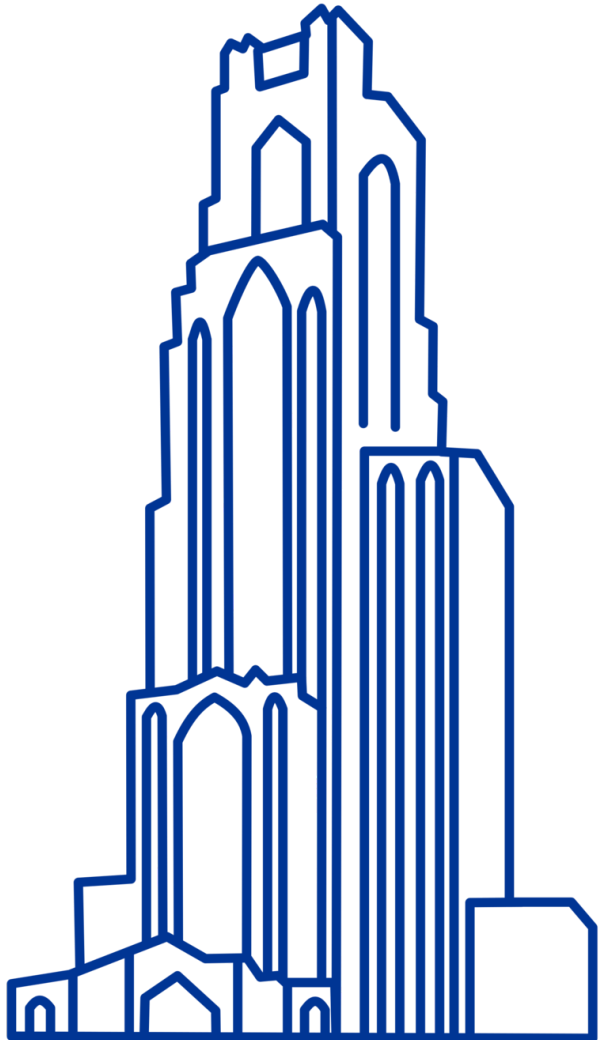


Computational Biology
(BIOSC 1540)

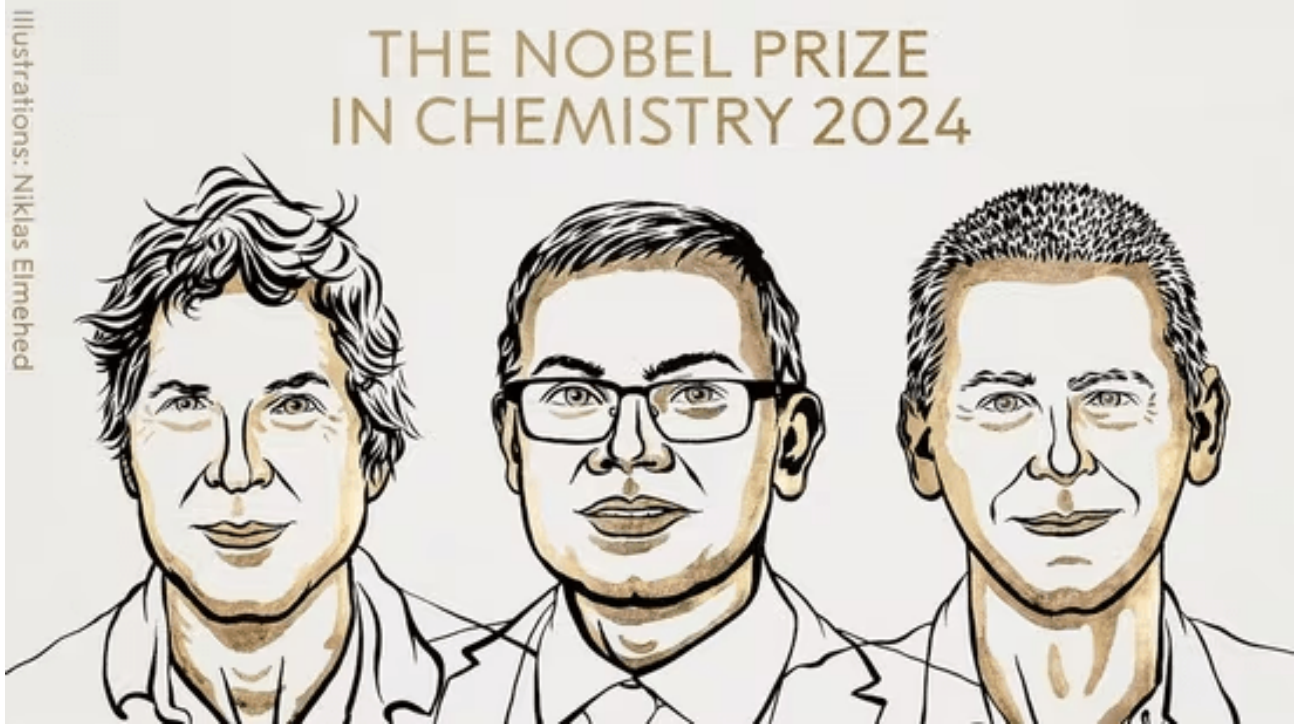
Lecture 12:
Protein structure prediction

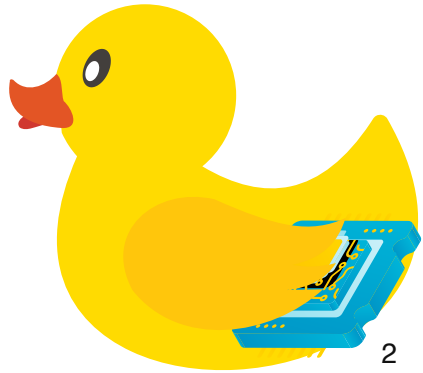
Oct 10, 2024

University of Pittsburgh

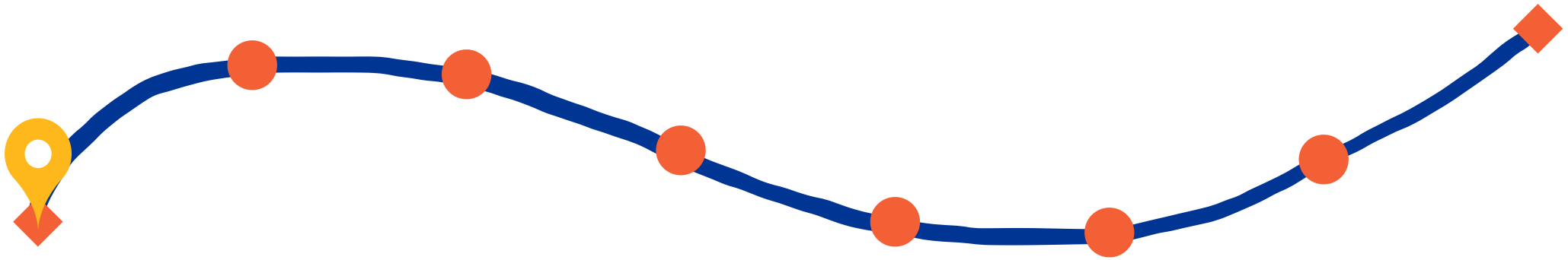
Announcements

- No class on Tuesday (10/15)
- No office hours (mine or UTA) next week - will resume on 10/22
- Will have Programming+ recitations
- A05 will be posted tomorrow
- David Baker, John Jumper, and Demis Hassabis won the Nobel Prize in Chemistry for "computational protein design" and "protein structure prediction"





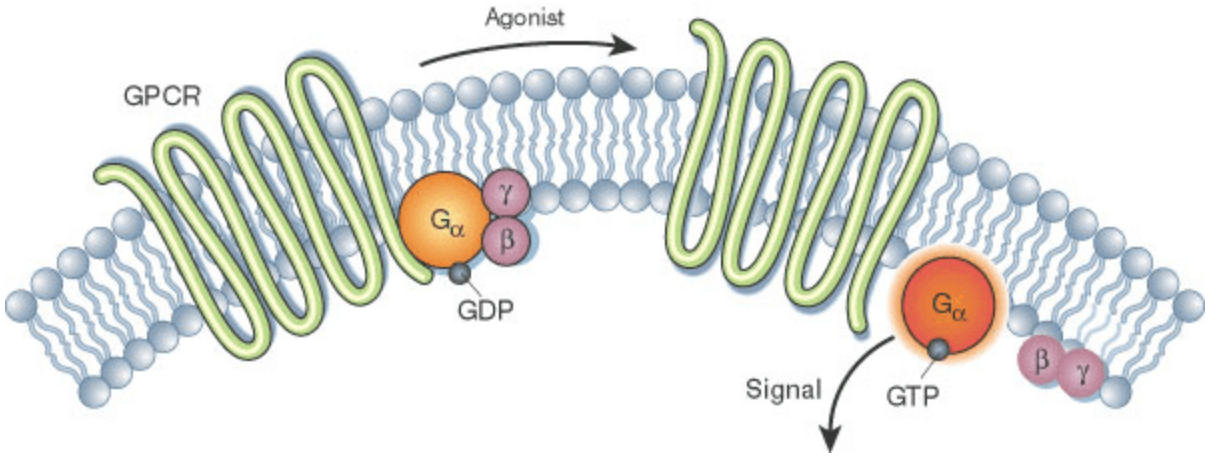
After today, you should be able to

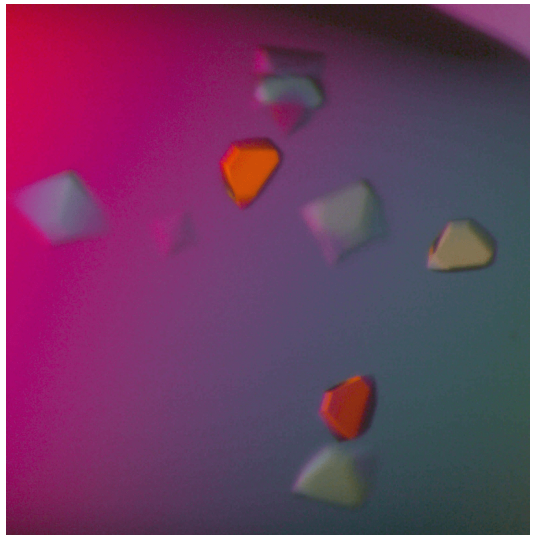


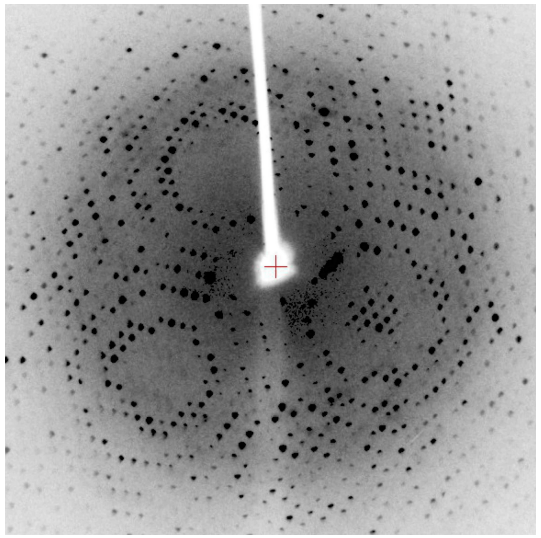
Why are we learning about protein structure prediction?

Why predict protein structure?

Protein structure dictates interactions, signaling, and biochemical roles







Experimental methods (X-ray, Cryo-EM) provide high-resolution structures but are resource-intensive and time-consuming

Structural insights can accelerate … everything?

- **Drug Discovery:** Designing small-molecule inhibitors or antibodies that target specific protein conformations.
- **Biotechnology:** Engineering proteins for industrial or therapeutic applications.
- **Disease Research:** Mutations causing structural defects linked to diseases like Alzheimer’s and cystic fibrosis.

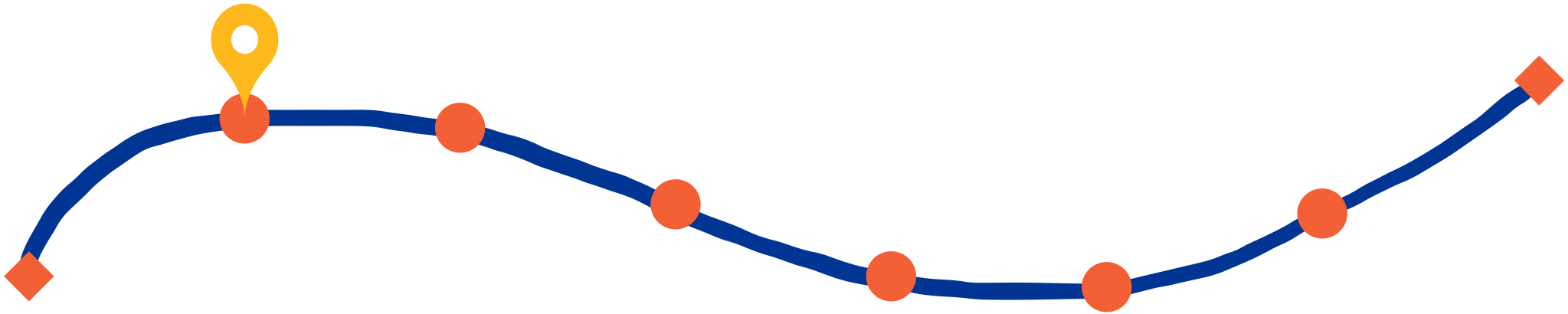
Prediction is critical for the future of biology

Advances in predictive accuracy are opening new frontiers in biology

Integrating predictive models with experimental data is the way forward

Structure prediction complements genomics and transcriptomics to create a holistic understanding of biological function

After today, you should be able to



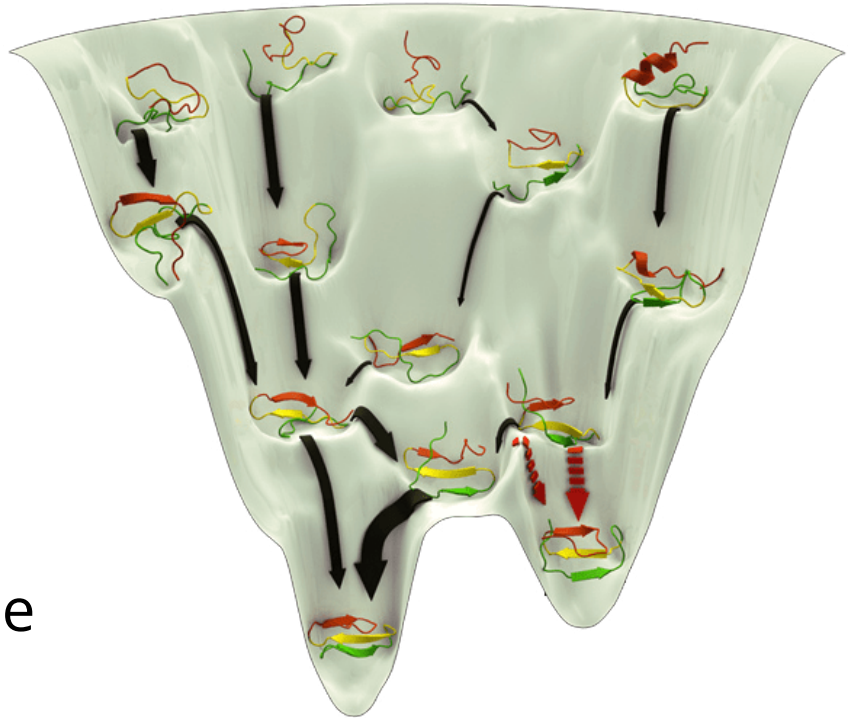
Identify what makes structure prediction challenging

What makes structure prediction hard: **Conformational space**

Proteins can adopt a large number of possible conformations

Levinthal's Paradox: A protein can't sample all conformations in a biologically reasonable time, yet it folds quickly

Example: A protein with 100 amino acids, each capable of adopting about 3 torsion angles, results in ~3^{100} possible conformations



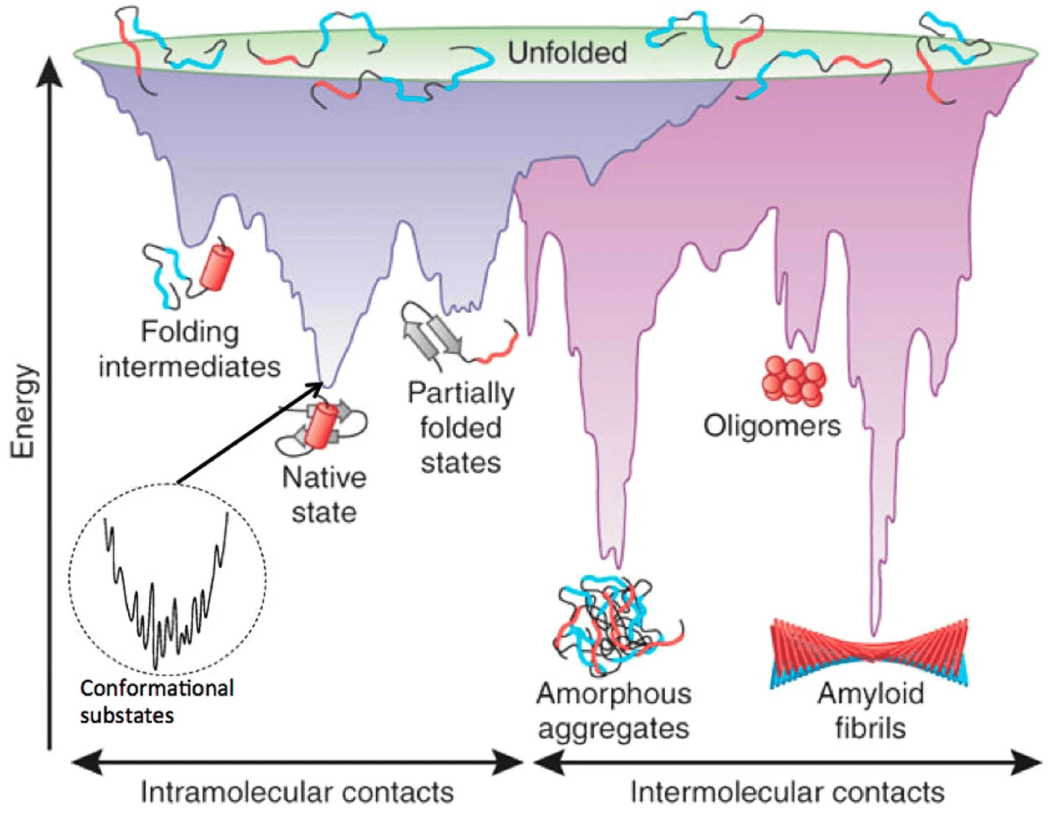
What makes structure prediction hard: **Complex energy landscape**

A **potential energy surface** (PES) is a represents the energy of a system as a function of the positions of its atoms

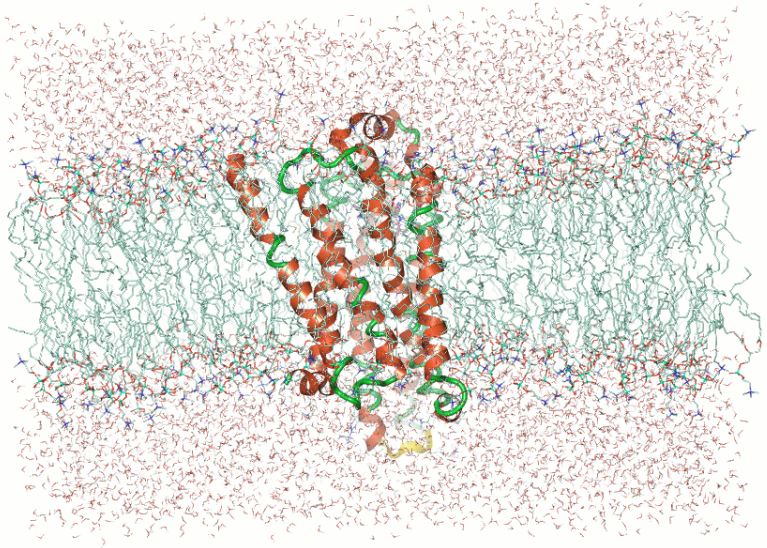
Understand how the system's energy changes upon reactions or movements

Proteins fold to the lowest free-energy state, but this landscape is highly rugged

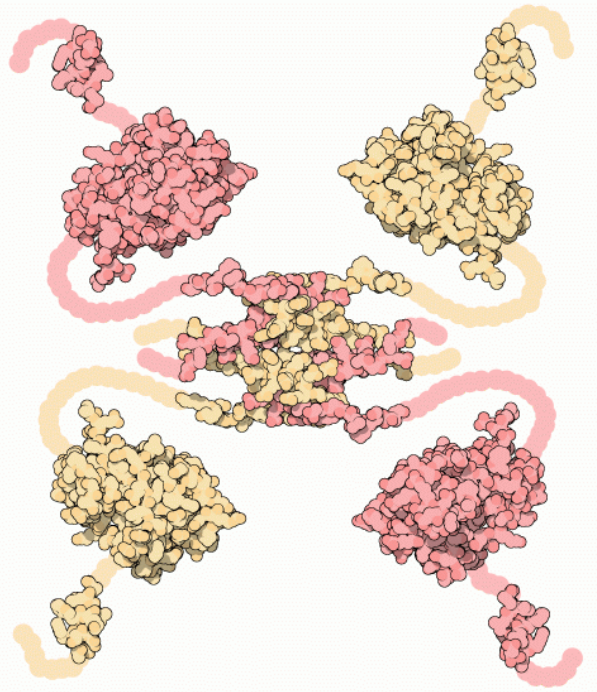
Energy calculations are computationally intensive and depend on accurate force fields



What makes structure prediction hard: **Flexibility and dynamics**



Proteins are not static; they adopt multiple conformations (flexibility) based on their environment or interactions with other molecules



Some proteins or regions do not adopt a fixed 3D structure but remain disordered or flexible under physiological conditions

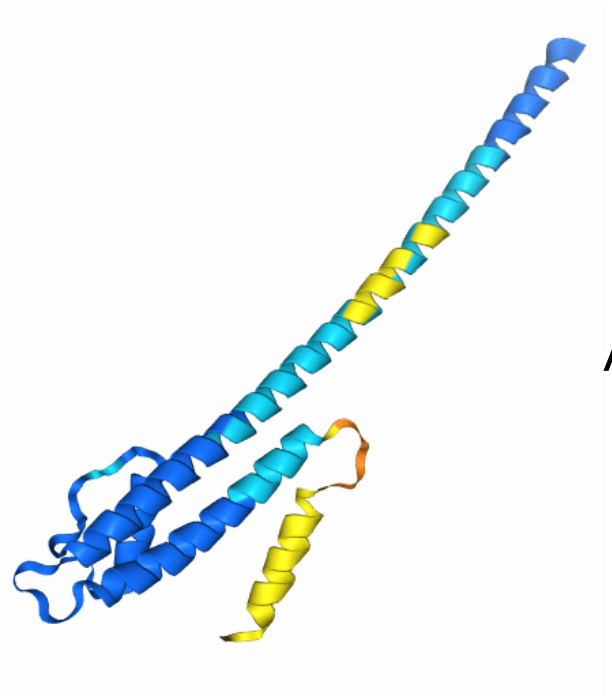
What makes structure prediction hard: **Environmental effects**

Proteins fold differently in different environments

Predictions need to capture interactions with solvent molecules, ions, and cofactors

7MHX

pH-gated K+ channel



AlphaFold 3

Example: Predicting transmembrane protein structures, where the lipid bilayer plays a key role in folding, is particularly complex.

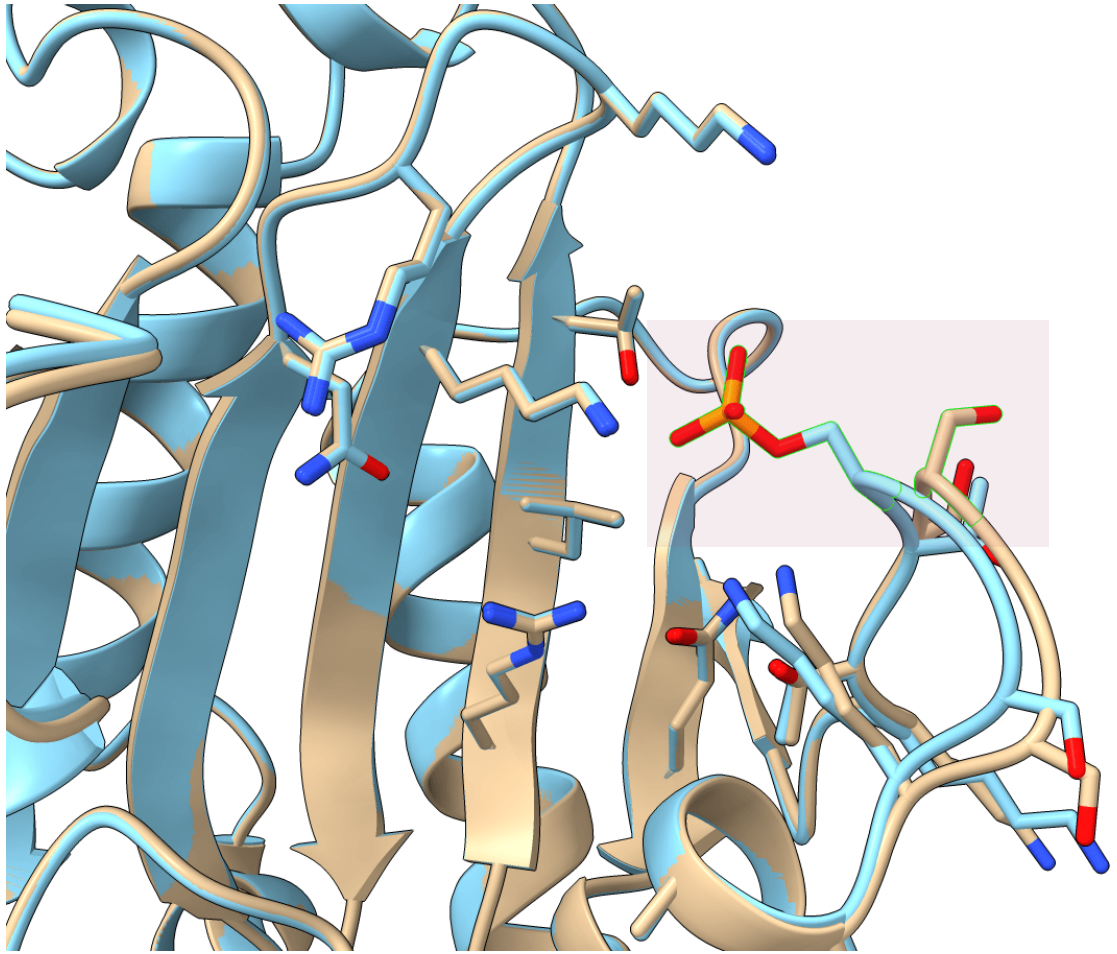
What makes structure prediction hard: **Post-translational modifications**

PTMs such as phosphorylation, glycosylation, and methylation can alter protein folding and function

Example: eIF4E is a eukaryotic translation initiation factor involved in directing ribosomes to the cap structure of mRNAs

Ser209 is phosphorylated by MNK1

AlphaFold 3 accurately predicts these changes when they are already known

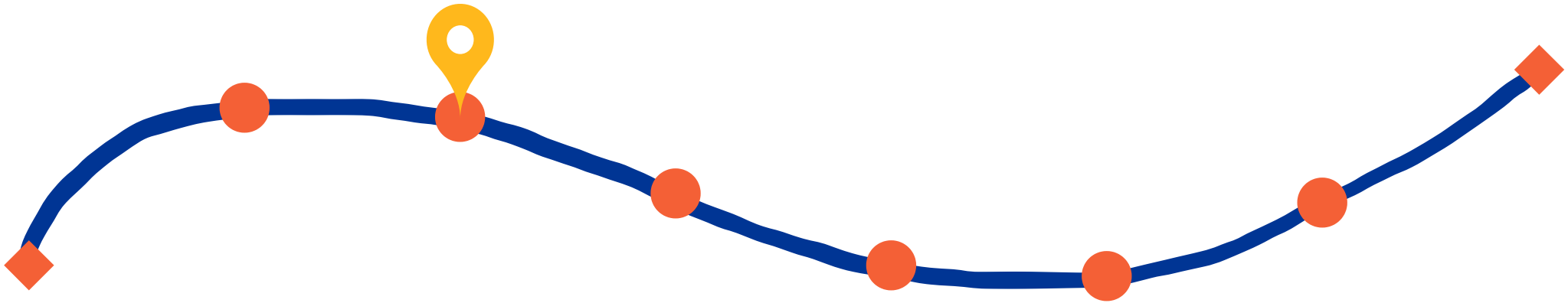


What makes structure prediction hard: **Methods are data driven**

Our predictions **rely on similarity to known structures**, but novel sequences or folds (for which no homologous structures exist) are difficult to predict accurately

Example: AlphaFold has made strides, but predicting **de novo** structures remains challenging, especially for proteins with no templates

After today, you should be able to



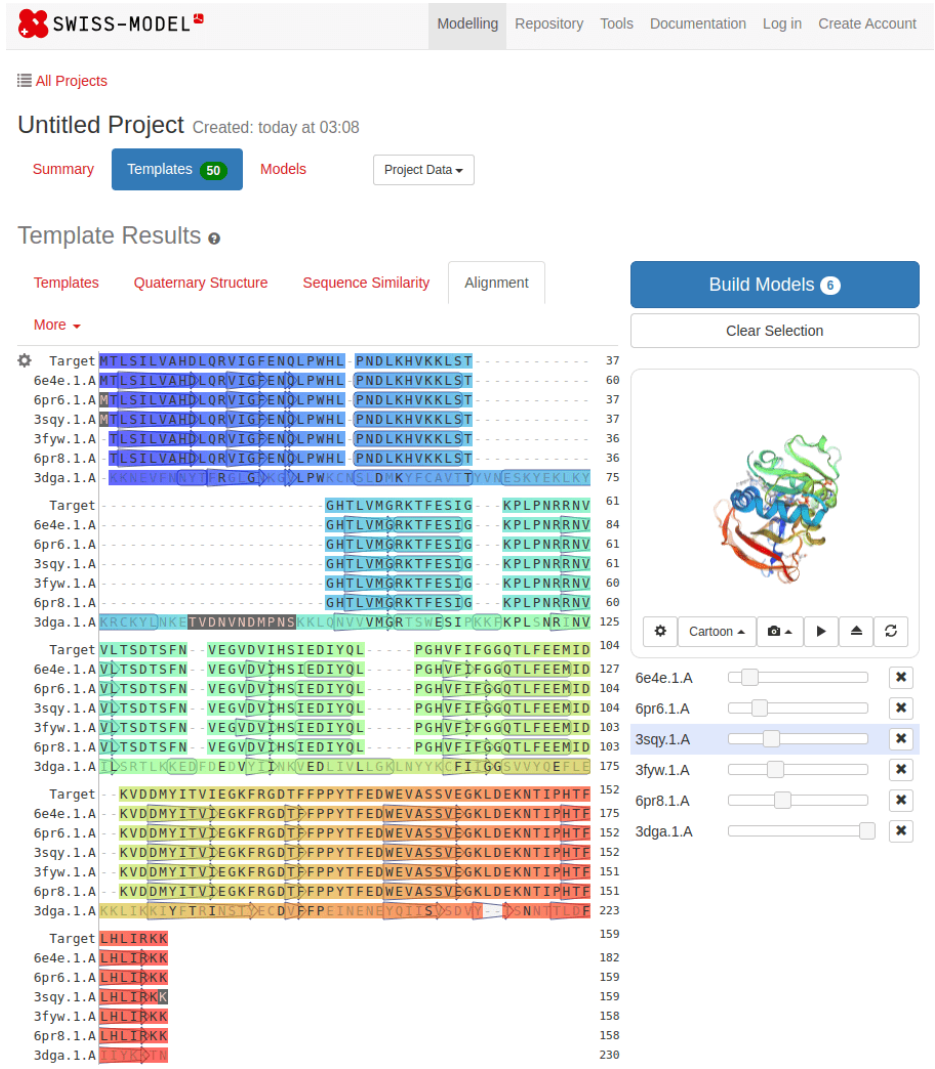
Explain homology modeling

Homology modeling predicts protein structures based on evolutionary relationships

The main principle is that proteins with **similar sequences tend to fold into similar structures**

Common tools for homology modeling include **MODELLER**, **SWISS-MODEL**, and **Phyre2**

Homology modeling is the most accurate when sequence identity to other proteins is high (>30%)



Hidden Markov Models (HMMs) Capture Evolutionary Patterns in Proteins

HMMs are statistical models representing sequences using probabilities for matches, insertions, and deletions

Essentially more robust alignments

Start with a multiple sequence alignment

↓

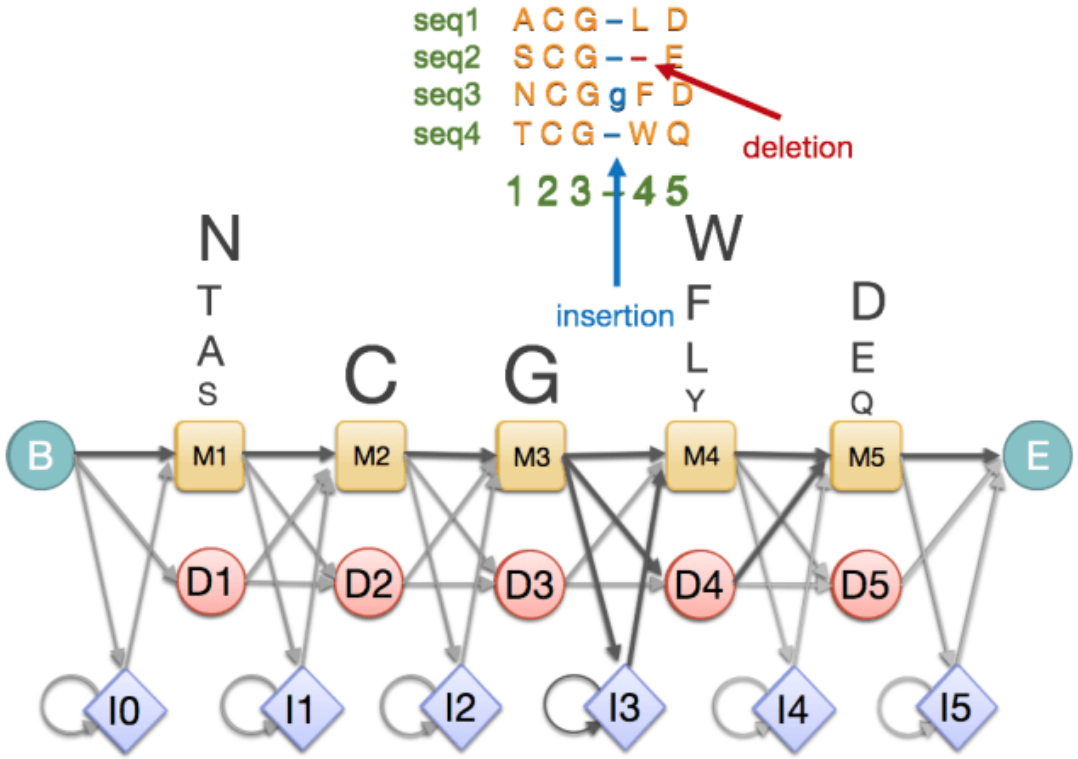
Insertions / deletions can be modelled

↓

Occupancy and amino acid frequency at each position in the alignment are encoded

↓

Profile created



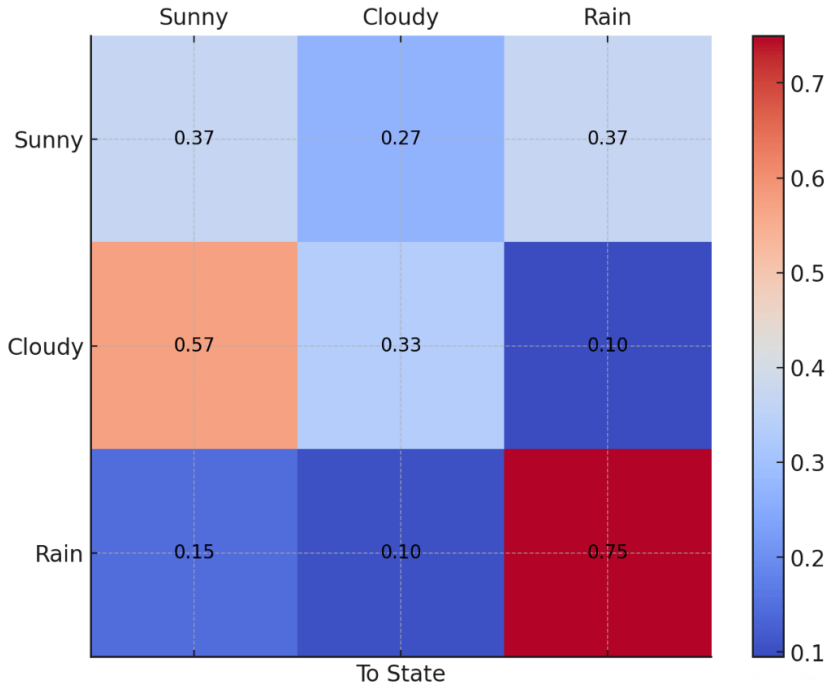
A Markov model predicts outcomes based on transitional probabilities

Suppose I collect weather data in Pittsburgh for the past 30 days: **Sunny**, **Cloudy**, or **Rain**

I want to figure out how to predict tomorrow's weather based on today's

Example: If today is cloudy, there is a 57% chance it will be Sunny tomorrow

Tomorrow's weather

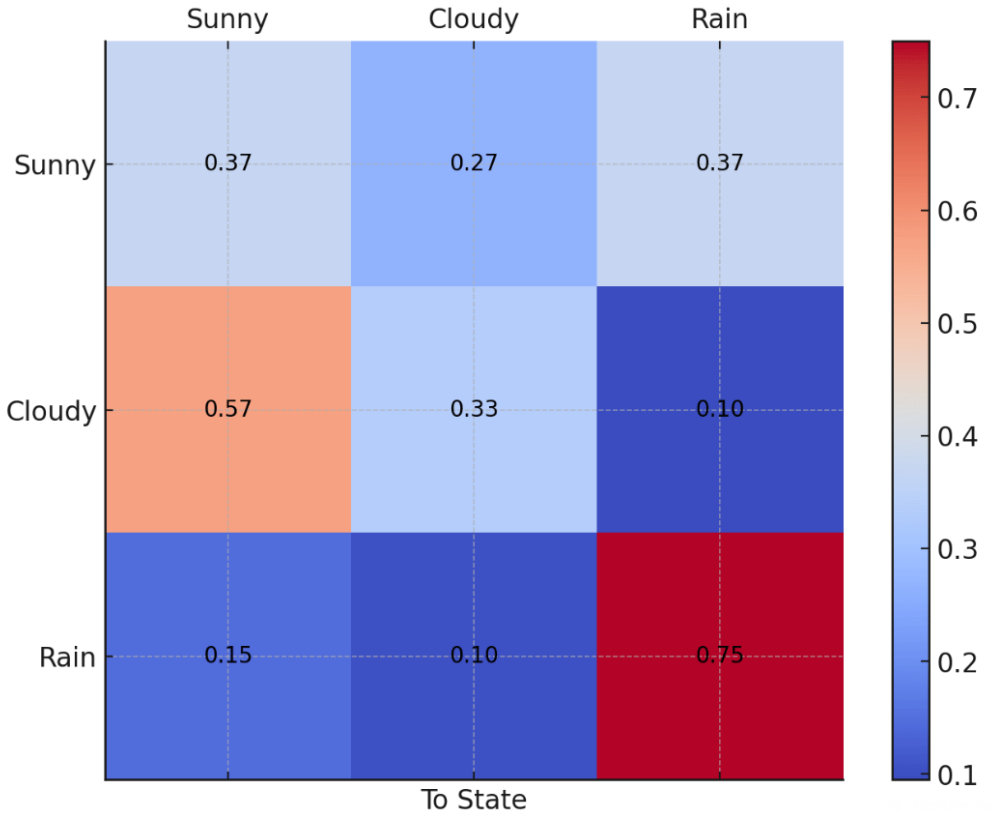


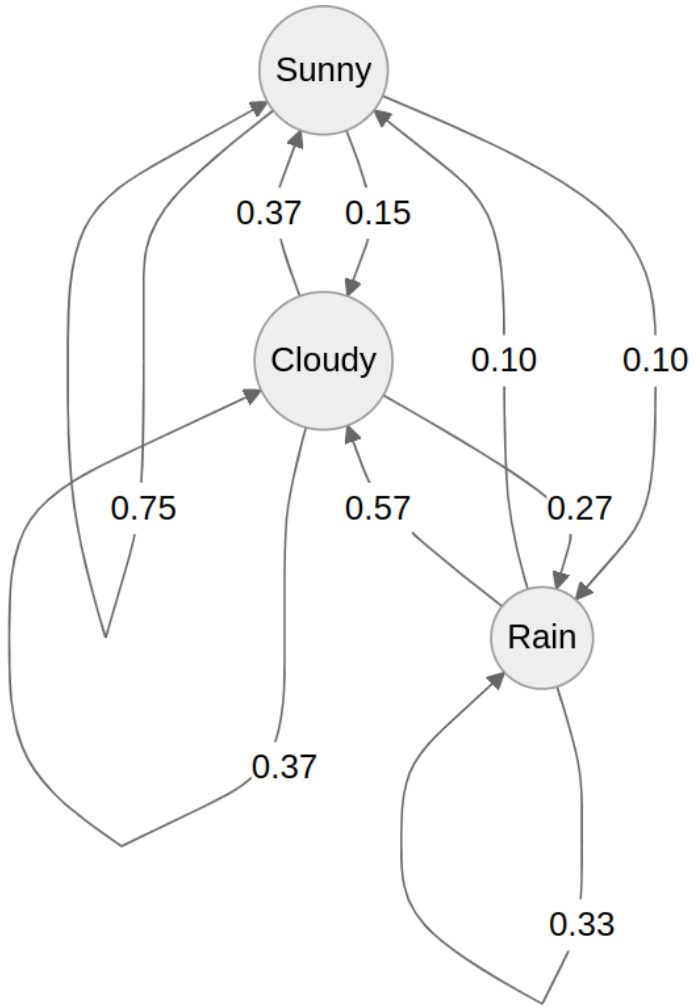
Today's weather

Transition probability

We can represent these states and probabilities as a (cursed?) graph

Each edge represents the probability of transitioning from one state to the next





Hidden Markov models also include additional information in "hidden states"

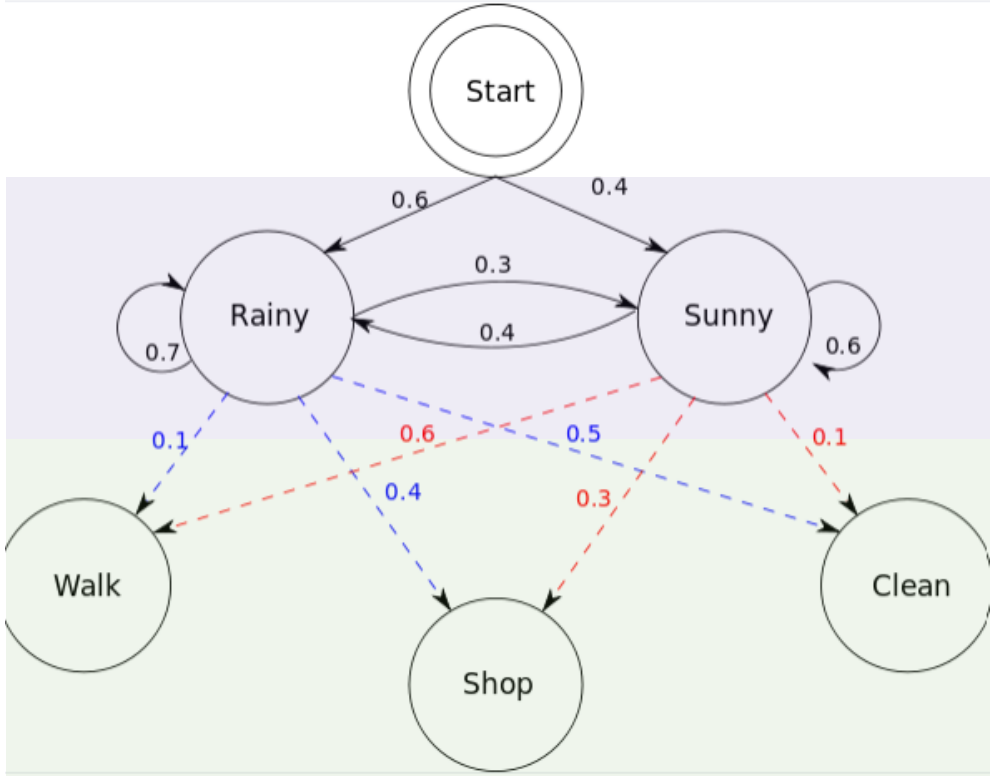
Suppose my friend lives in a remote location where it is either Rainy or Sunny

I cannot look up the weather but I have last year's weathers reports **Hidden states**

My friend can only tell me

- Walking
- Shopping **Obervables**
- Cleaning

We know how weather patterns transitions, but we don't have this information from our friend



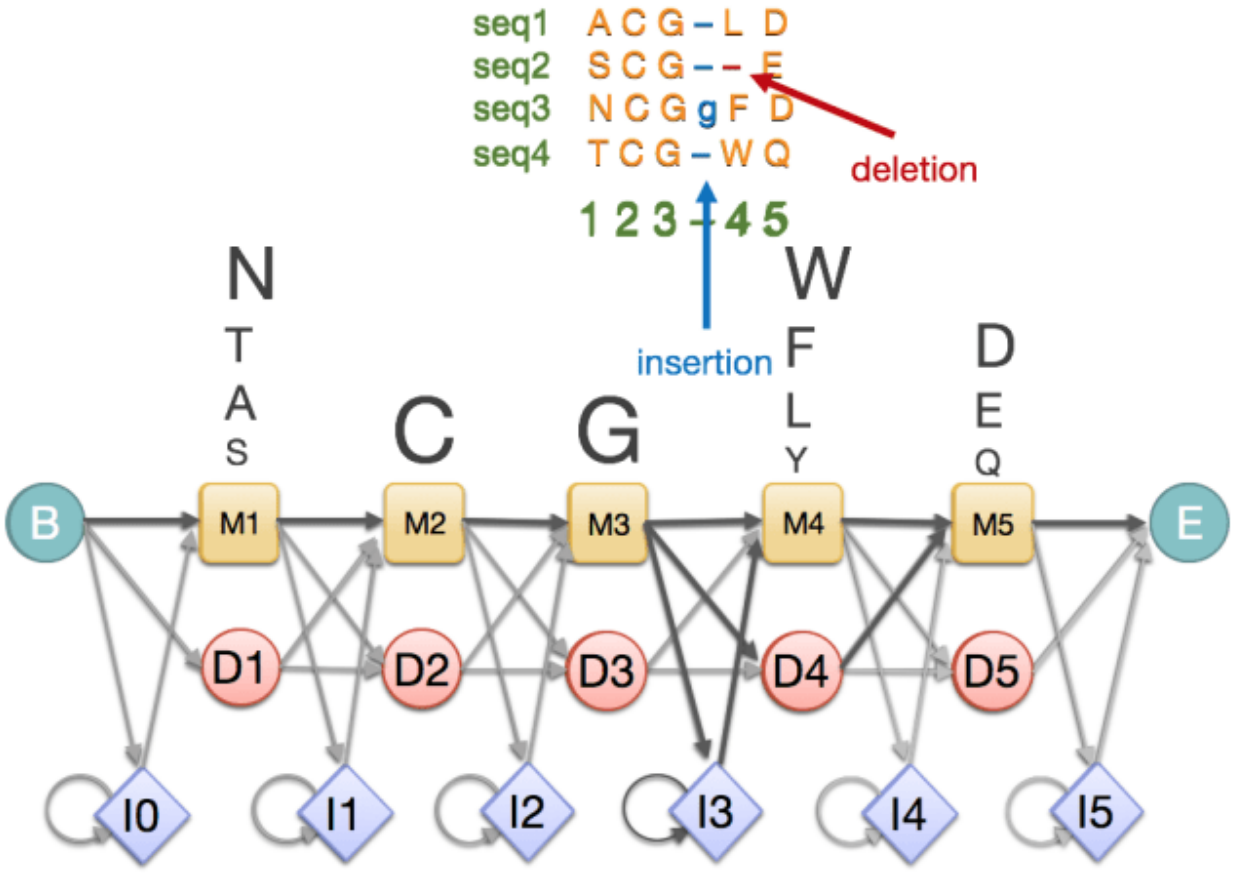
Note: If we had previous observable data, we could fit/learn transition probabilities of hidden states

HMMs Model Protein Sequences as a Series of Probabilistic States

Hidden states represent the underlying biological events that are not directly observable

- **Match states**: conserved positions in the sequence
- **Insertion states**: positions where extra residues are added
- **Deletion states**: positions where residues are missing

Observables are the actual amino acids (residues) in the protein sequence that we can observe



HMMER Uses HMMs to Search Protein Databases for Homology

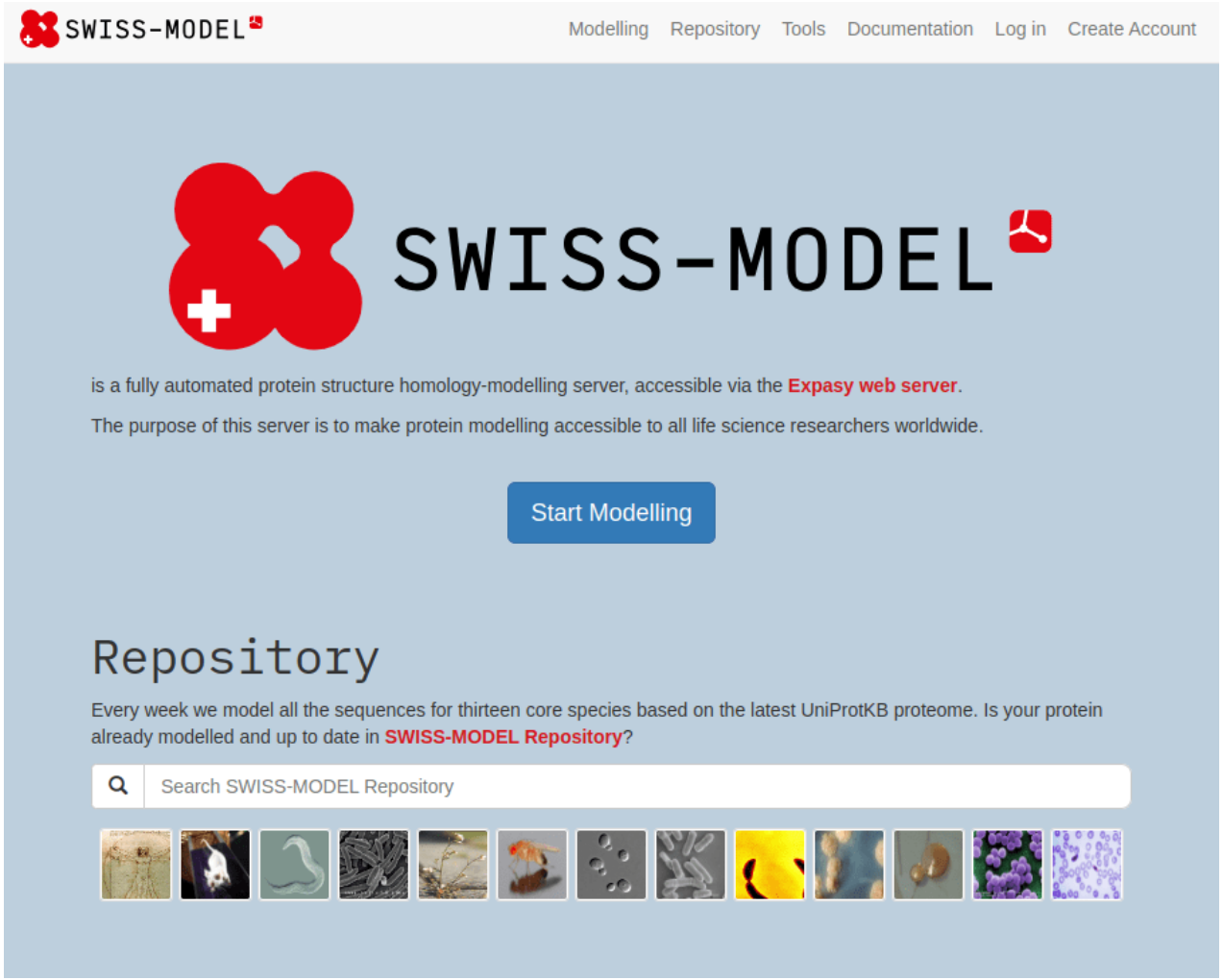
HMMER is a tool that uses HMMs to search databases for sequences that match a given profile HMM

It is used to find homologous sequences, identifying evolutionary relationships across protein families

SWISS-MODEL

```
MTLSILVAHDLQRVIGFENQLPWHLPNDLKHVKKLSTGHTL
VMGRKTFESIGKPLPNRRNVVLTSDTSFNVEGVDVIHSIED
IYQLPGHVFIFGGQTLFEEMIDKVDDMYITVIEGKFRGDTF
FPPYTFEDWEVASSVEGKLDEKNTIPHTFLHLIRKK
```

DHFR (UniProt)



swissmodel.expasy.org

SWISS-MODEL

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All Projects

Untitled Project Created: today at 03:08

Summary | Templates 50 | Models | Project Data

Project Summary

```
Target 1 MTLSILVAHDLQRVIGFENQLPWHLPNDLKHVKKLSTGHTLVMGRKTFESIGKPLPNRRNVVLTSDTSFNVEGVDVIHSI 80
Target 1 EDIYQLPGHVFIFGGQTLFEEMIDKVDDMYITVIEGKFRGDTFFPPYTFEDWEVASSVEGKLDEKNTIPHTFLHLIRKK 159
```

Template Results

A total of 685 templates were found to match the target sequence. This list was filtered by a heuristic down to 50. The top templates are:

Template	Sequence Identity	Biounit Oligo State	Description
6e4e.1	100.00	monomer	Dihydrofolate reductase Crystal structure of dihydrofolate reductase from Staphylococcus aureus MW2 bound to NADP and p218
6pr6.1	100.00	monomer	Dihydrofolate reductase S. aureus dihydrofolate reductase co-crystallized with para-tolyl-dihydropthalazine inhibitor and NADP(H)
3sqy.1	100.00	monomer	Dihydrofolate reductase S. aureus Dihydrofolate Reductase complexed with novel 7-aryl-2,4-diaminoquinazolines
3fyw.1	100.00	monomer	Dihydrofolate reductase Staph. aureus DHFR complexed with NADPH and AR-101
6pr8.1	100.00	monomer	Dihydrofolate reductase S. aureus dihydrofoate reductase co-crystallized with 3,5-dimethylphenyl-dihydropthalazine inhibitor and NADP(H)

Show full template details

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Summary | Templates 50 | Models | Project Data

Template Results

Templates | Quaternary Structure | Sequence Similarity | Alignment | More

Build Models 5

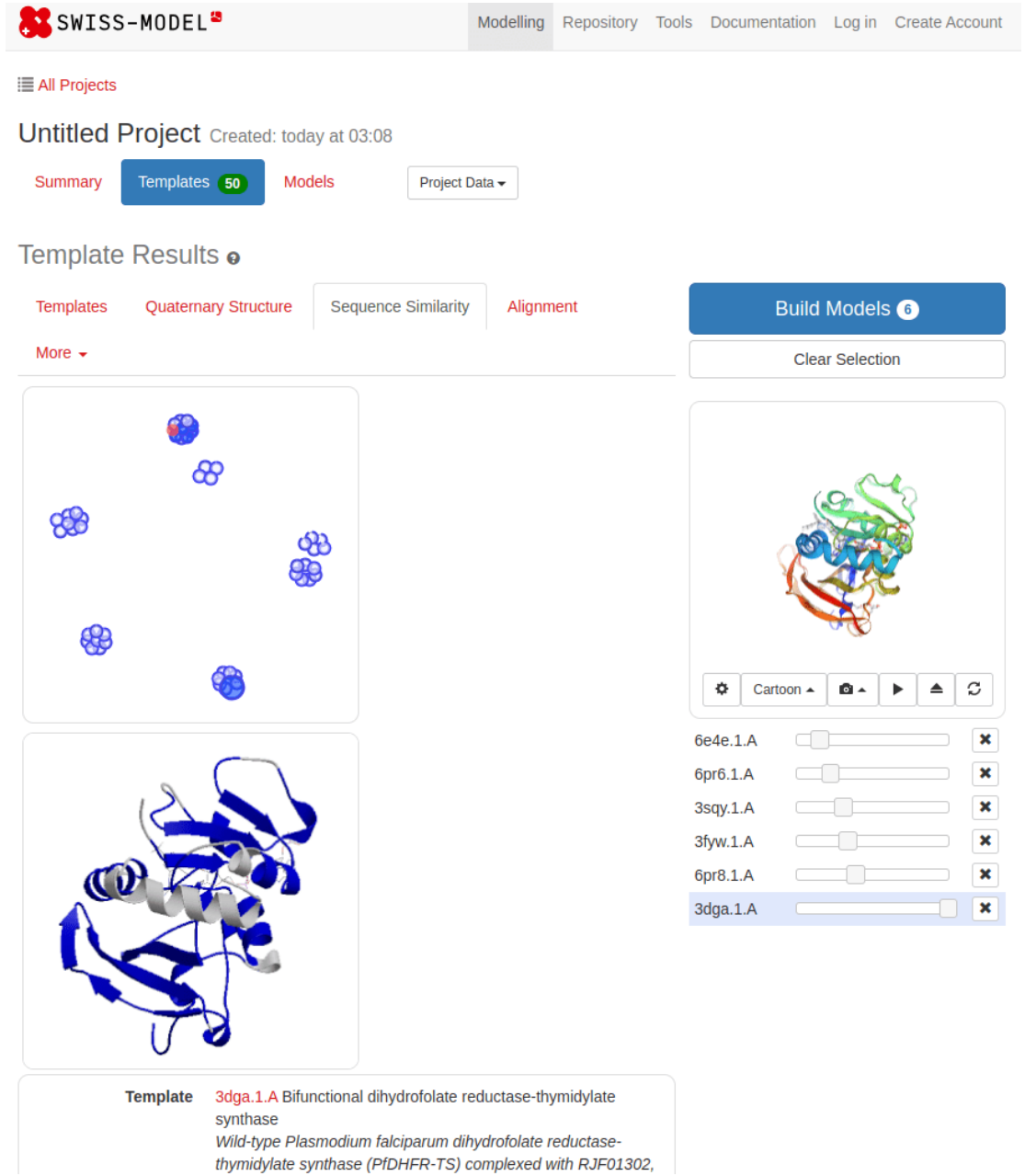
Clear Selection

Sort	Coverage	GMQE	QSQE	Identity	Method	Oligo State	Ligands
☑	6e4e.1.A Dihydrofolate reductase *Crystal structure of dihydrofolate reductase from Staphylococcus aureus MW2 bound to NADP and p218*						
		0.99	-	100.00	X-ray, 1.9Å	monomer ✓	1 x NAP, 1 x MMV
☑	6pr6.1.A Dihydrofolate reductase *S. aureus dihydrofolate reductase co-crystallized with para-tolyl-dihydropthalazine inhibitor and NADP(H)*						
		0.99	-	100.00	X-ray, 2.0Å	monomer ✓	1 x NAP, 1 x OWS
☑	3sqy.1.A Dihydrofolate reductase *S. aureus Dihydrofolate Reductase complexed with novel 7-aryl-2,4-diaminoquinazolines*						
		0.98	-	100.00	X-ray, 1.5Å	monomer ✓	1 x NAP, 1 x Q11
☑	3fyw.1.A Dihydrofolate reductase *Staph. aureus DHFR complexed with NADPH and AR-101*						
		0.98	-	100.00	X-ray, 2.1Å	monomer ✓	1 x NDP, 1 x XCF
☑	6pr8.1.A Dihydrofolate reductase *S. aureus dihydrofoate reductase co-crystallized with 3,5-dimethylphenyl-dihydropthalazine inhibitor and NADP(H)*						
		0.98	-	100.00	X-ray, 2.0Å	monomer ✓	1 x OWJ, 1 x NAP
☐	3sr5.1.A Dihydrofolate reductase *S. aureus Dihydrofolate Reductase complexed with novel 7-aryl-2,4-diaminoquinazolines*						
		0.98	-	100.00	X-ray, 1.7Å	monomer ✓	1 x NAP, 1 x Q12
☐	2w9g.1.A DIHYDROFOLATE REDUCTASE *Wild-type Staphylococcus aureus DHFR in complex with NADPH and trimethoprim*						
		0.98	-	100.00	X-ray, 2.0Å	monomer ✓	1 x TOP, 1 x NDP

Cartoon

6e4e.1.A
6pr6.1.A
3sqy.1.A
3fyw.1.A
6pr8.1.A

SWISS-MODEL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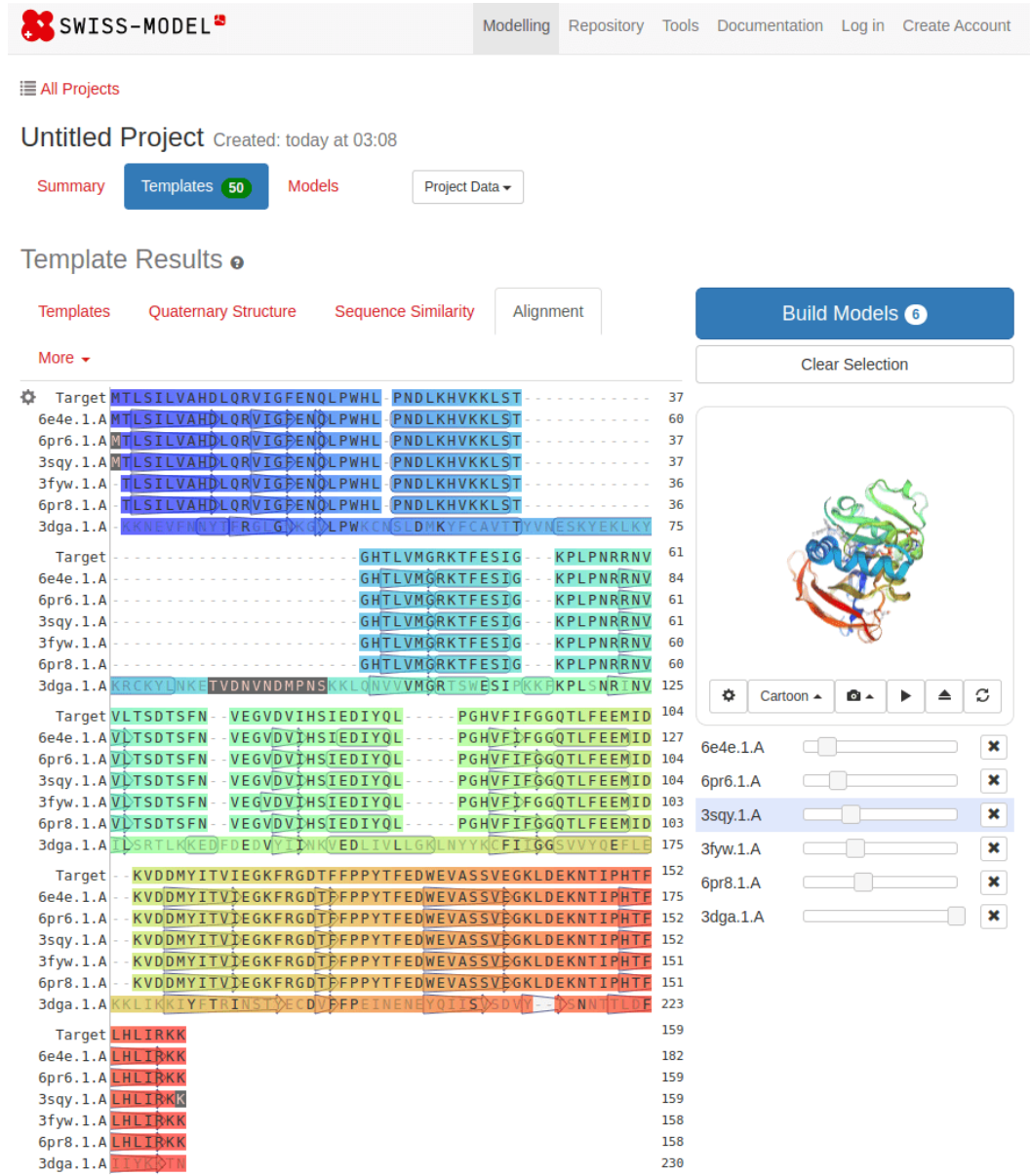
Template Results
Templates
Quaternary Structure
Sequence Similarity
Alignment
More
Build Models 6
Clear Selection
Cartoon
6e4e.1.A
6pr6.1.A
3sqy.1.A
3fyw.1.A
6pr8.1.A
3dga.1.A
Template 3dga.1.A Bifunctional dihydrofolate reductase-thymidylate synthase
Wild-type Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) complexed with RJF01302,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3dga.1.A

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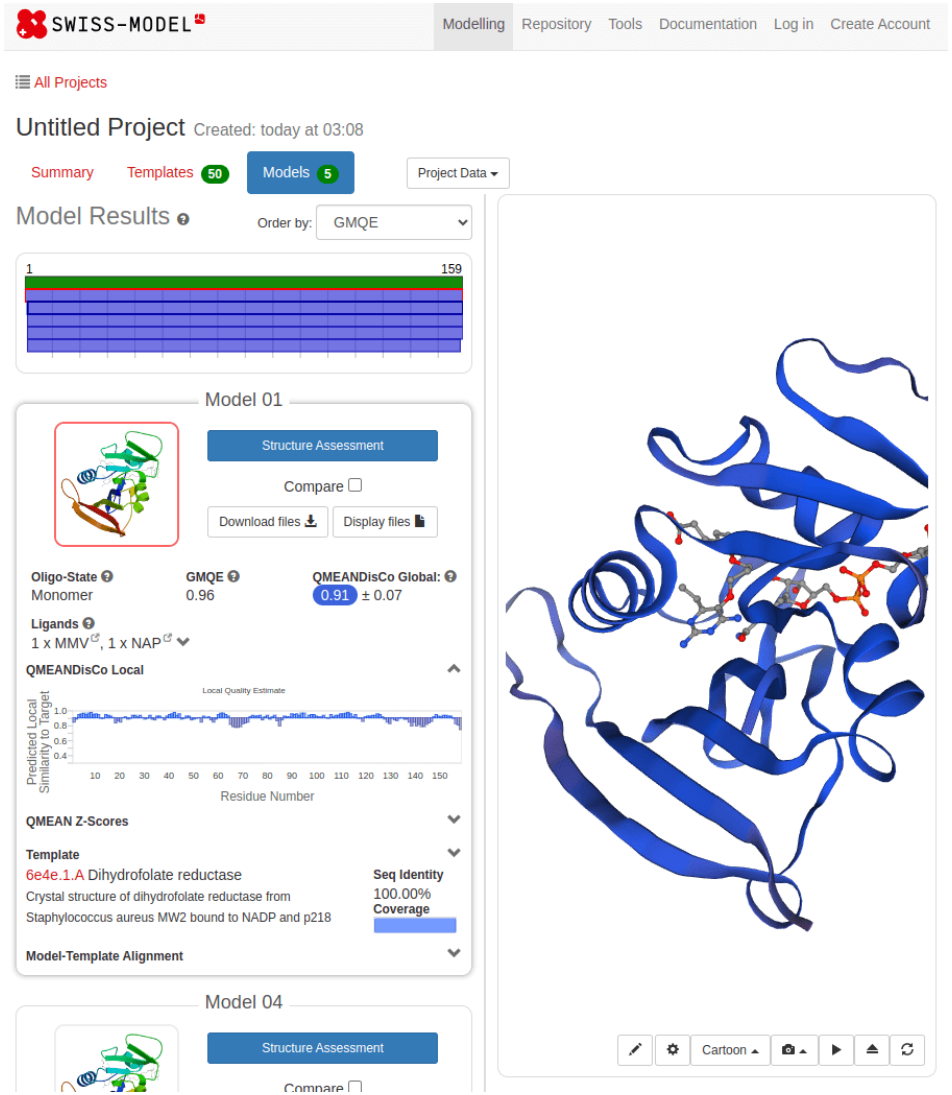
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Project Data
Model Results
Order by: GMQE
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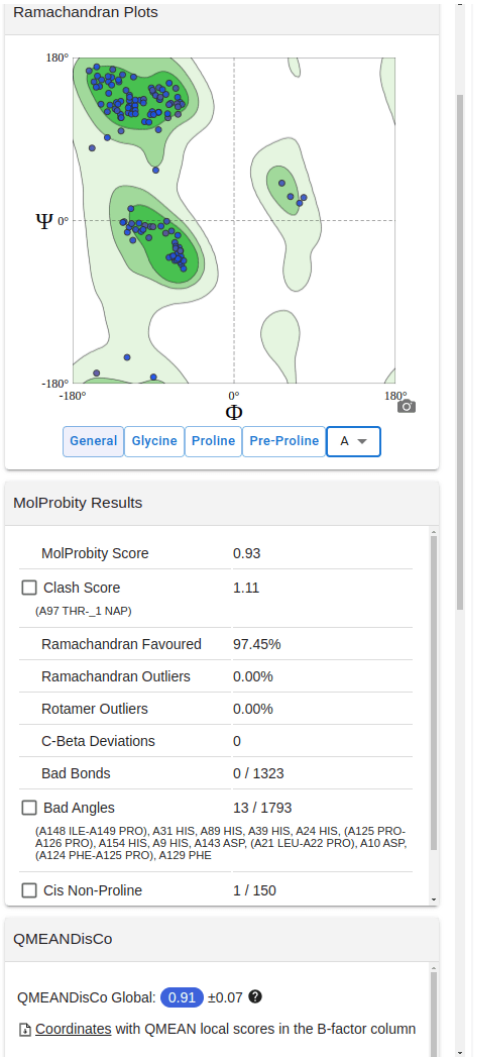
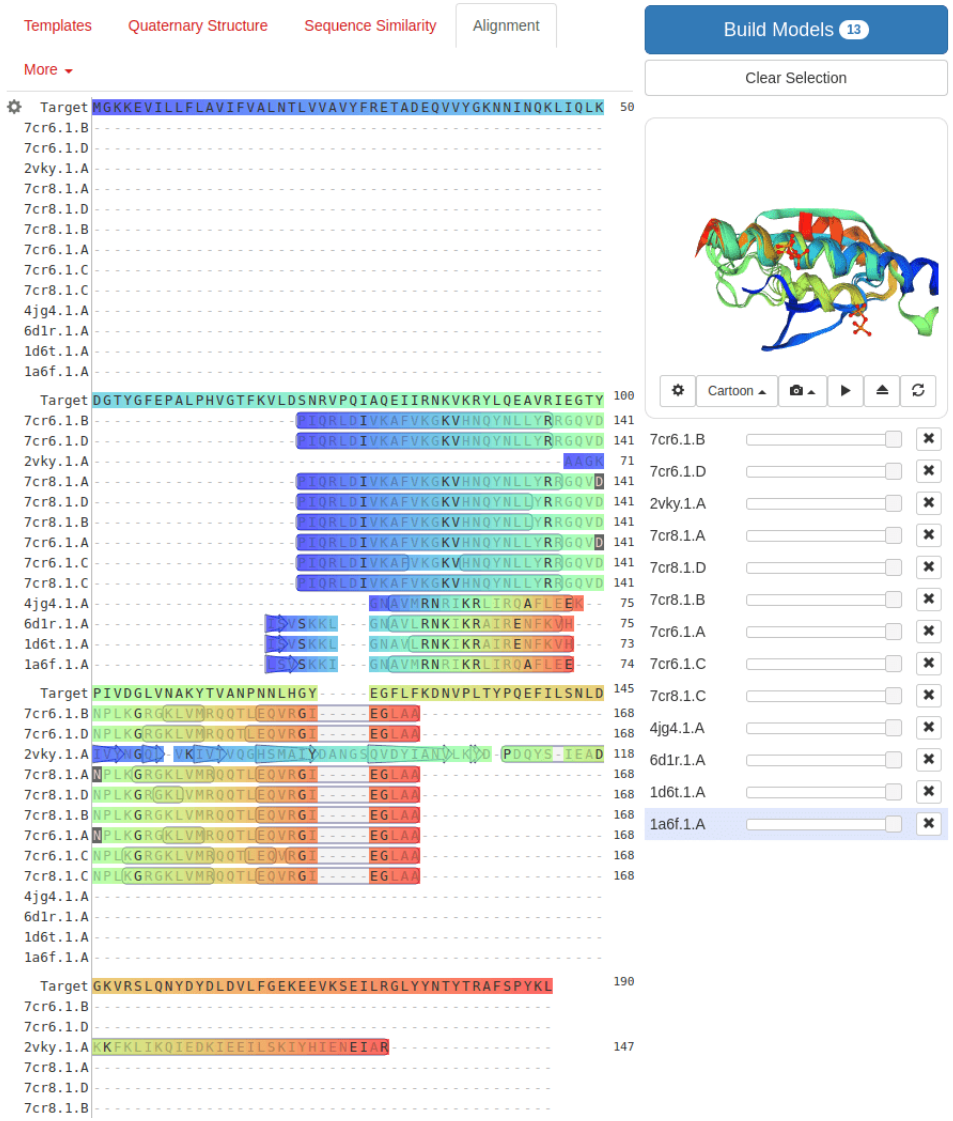
Model 01
Structure Assessment
Compare
Download files
Display files
Oligo-State
Monomer
GMQE
0.96
QMEANDisCo Global:
0.91 ± 0.07
Ligands
1 x MMV, 1 x NAP
QMEANDisCo Local
Local Quality Estimate
Predicted Local Similarity to Target
Residue Number
QMEAN Z-Scores
Template
6e4e.1.A Dihydrofolate reductase
Crystal structure of dihydrofolate reductase from Staphylococcus aureus MW2 bound to NADP and p218
Seq Identity
100.00%
Coverage
Model-Template Alignment
Model 04
Structure Assessment
Compare
Cartoon


Ramachandran Plots
180°
Ψ 0°
-180°
-180°
0°
180°
Φ
General
Glycine
Proline
Pre-Proline
A
MolProbity Results
MolProbity Score 0.93
Clash Score 1.11
(A97 THR-_1 NAP)
Ramachandran Favoured 97.45%
Ramachandran Outliers 0.00%
Rotamer Outliers 0.00%
C-Beta Deviations 0
Bad Bonds 0 / 1323
Bad Angles 13 / 1793
(A148 ILE-A149 PRO), A31 HIS, A89 HIS, A39 HIS, A24 HIS, (A125 PRO-A126 PRO), A154 HIS, A9 HIS, A143 ASP, (A21 LEU-A22 PRO), A10 ASP, (A124 PHE-A125 PRO), A129 PHE
Cis Non-Proline 1 / 150
QMEANDisCo
QMEANDisCo Global: 0.91 ±0.07
Coordinates with QMEAN local scores in the B-factor column

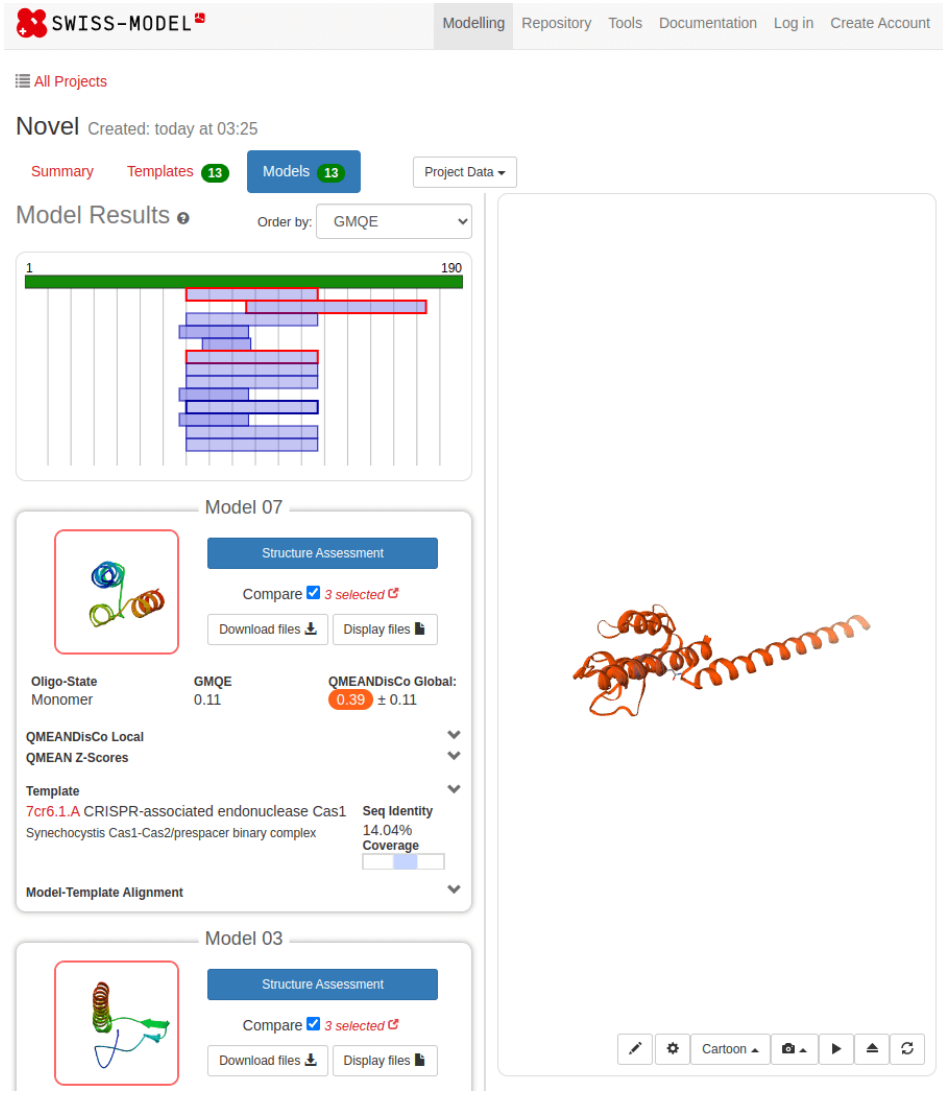
What happens with a novel protein?

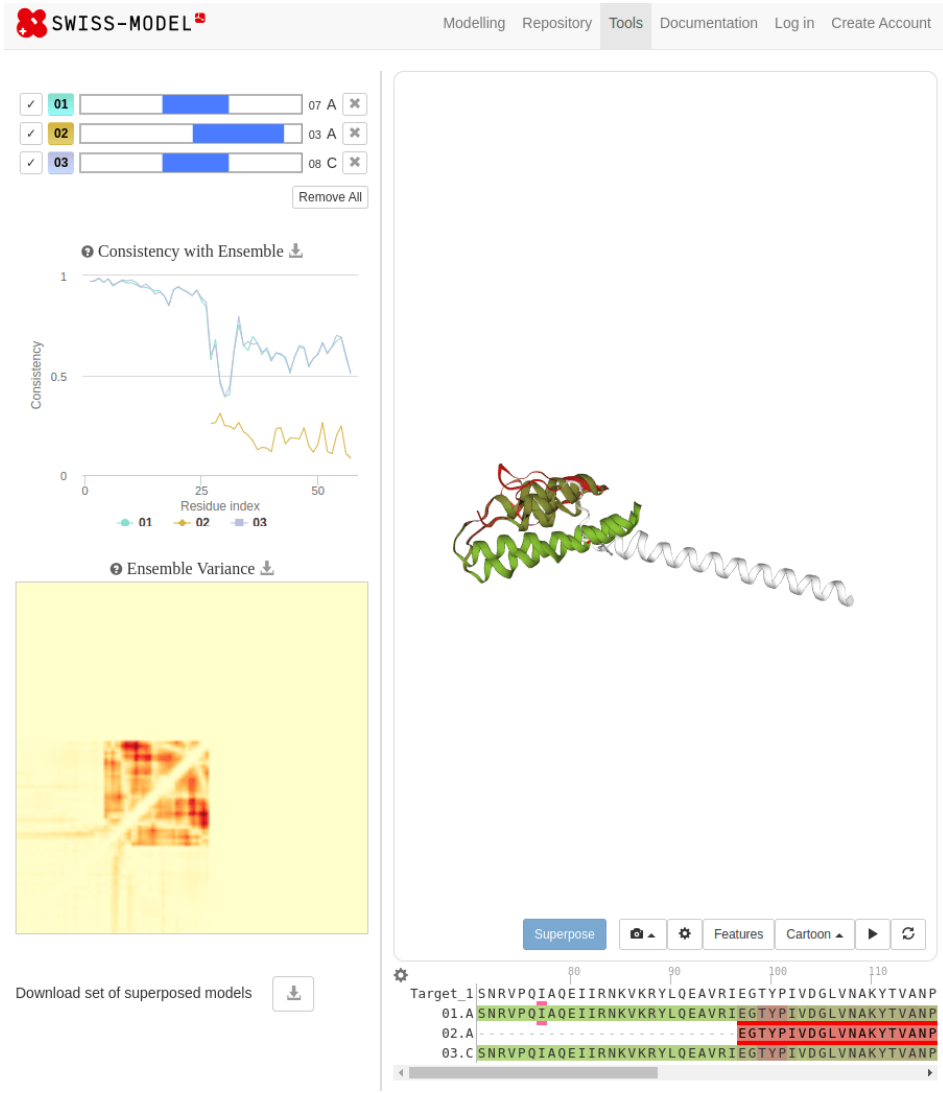
```
MGKKEVILLFLAVIFVALNTLVVAVYFRETADEQVVYGK
NNINQKLIQLKDGTYGFEPALPHVGTFKVLDSNRVPQIA
QEIIRNKVKRYLQEAVRIEGTYPIVDGLVNAKYTVANPN
NLHGYEGFLFKDNVPLTYPQEFILSNLDGKVRSLQNYDY
DLDVLFGEKEEVKSEILRGLYYNTYTRAFSPYKL
```

Novel protein
(ChatGPT)

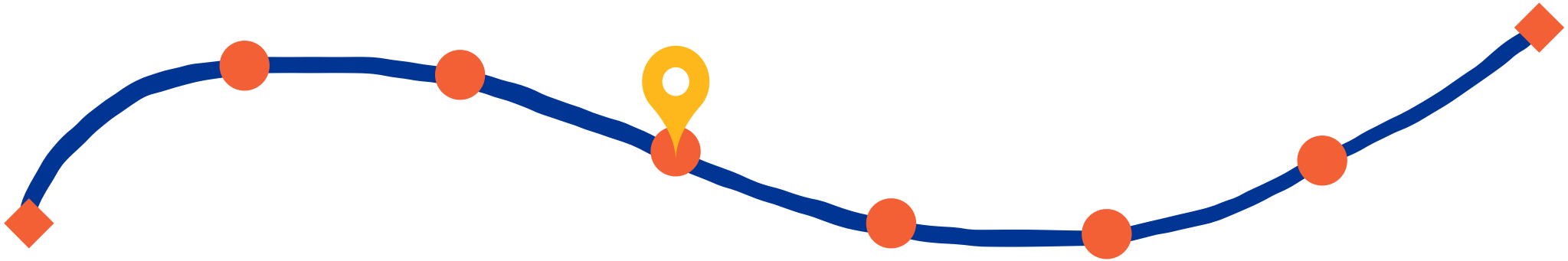


Novel proteins are too challenging





After today, you should be able to



Know when to use threading

instead of homology modeling

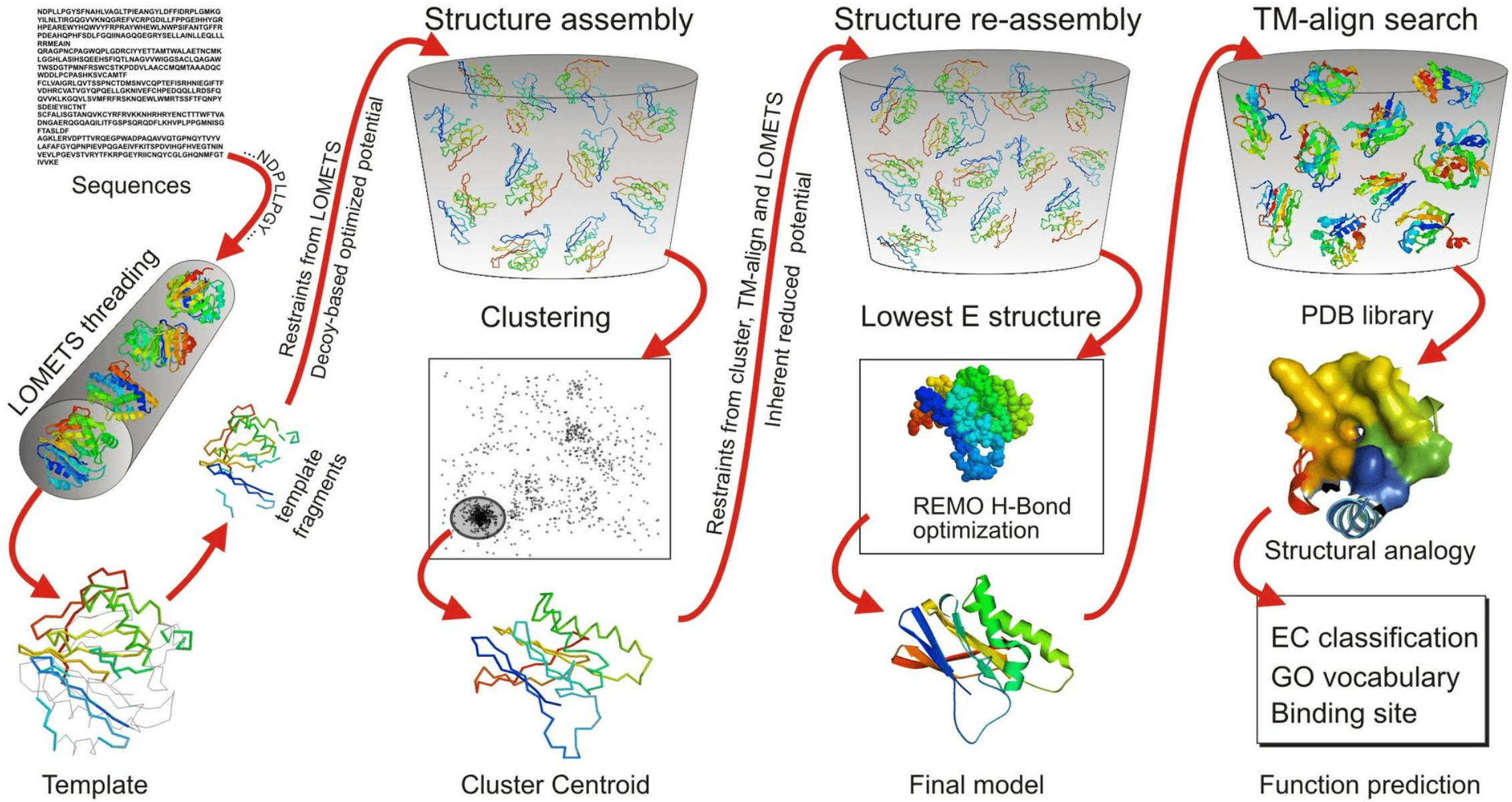
Why Use Threading?

In cases where sequence similarity to known structures is low (< 30%), homology modeling becomes unreliable

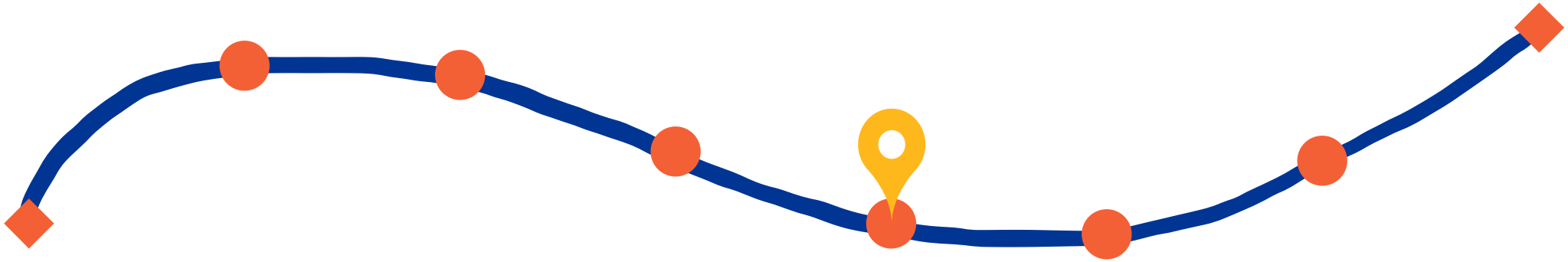
Threading matches sequences to known structural folds based on structural rather than sequence similarity

Phyre2, **RaptorX**, **MUSTER**, and **I-TASSER** are commonly used for threading and takes much longer than homology modeling

Identifying the Right Fold



After today, you should be able to



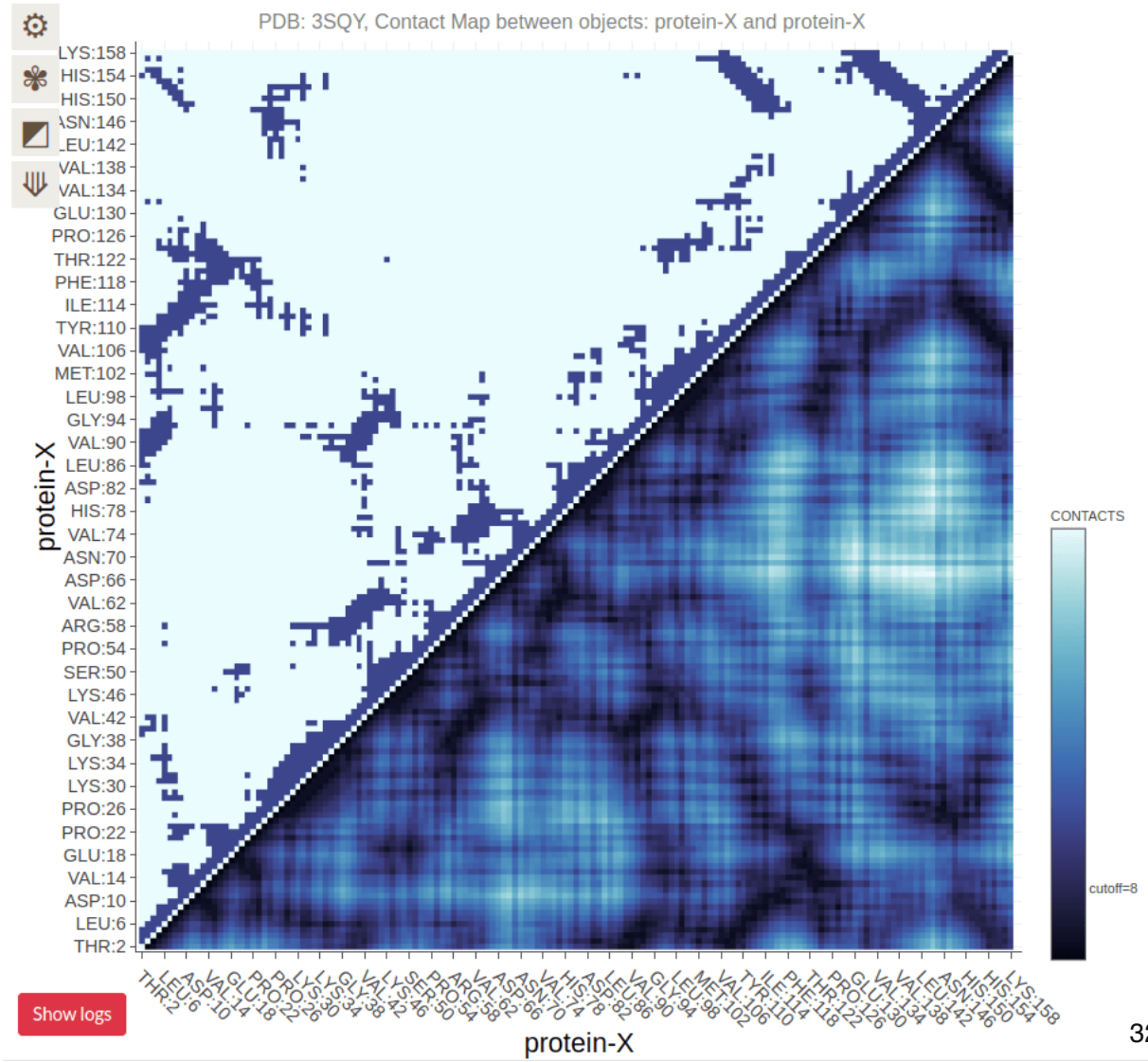
Interpret a contact map for protein structures

Contact Maps Visualize Residue Interactions in Proteins

A contact map is a 2D representation of which residues are in close proximity

Each point on the map corresponds to two residues that are close in 3D space

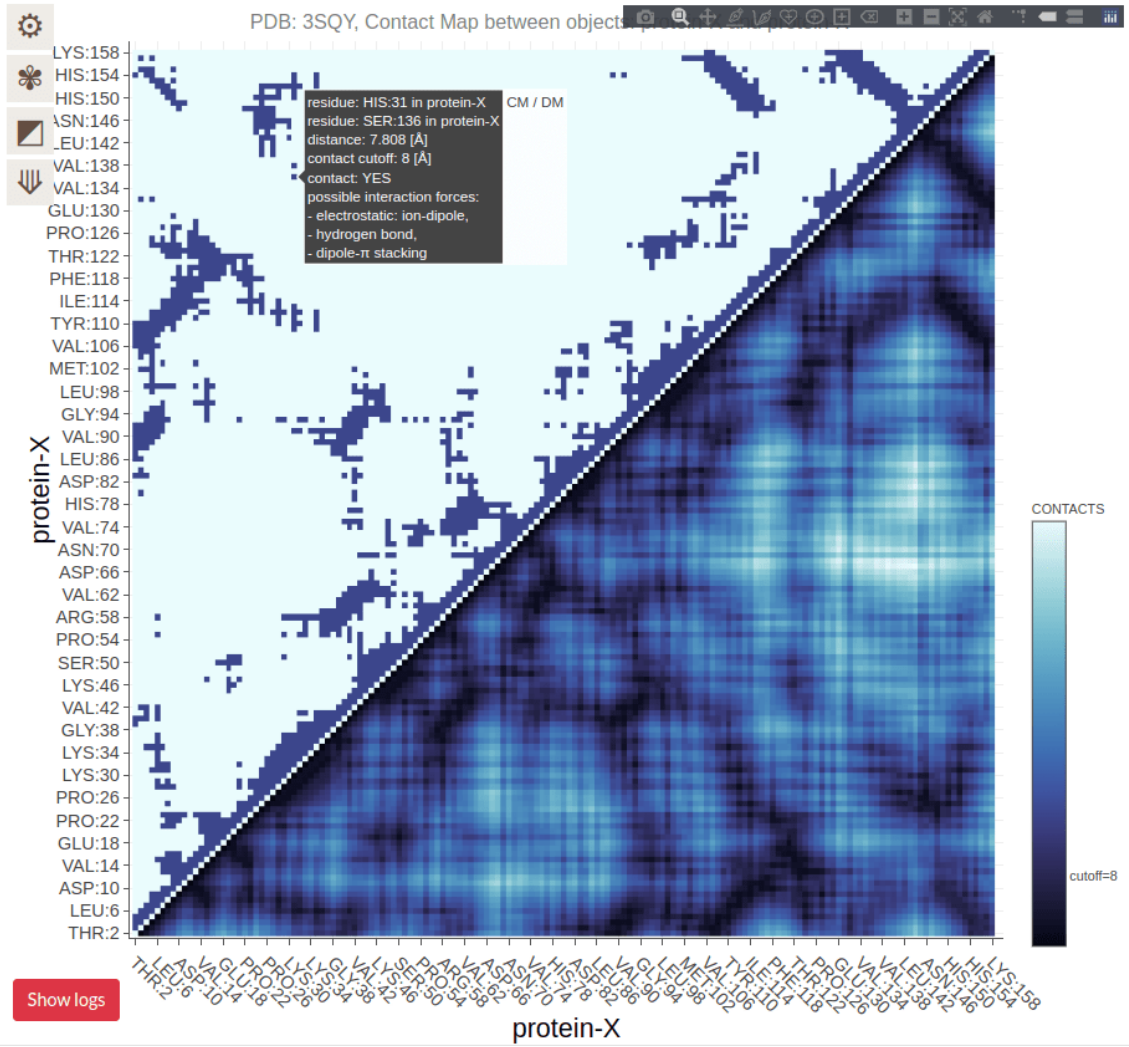
mapiya.lcbio.pl

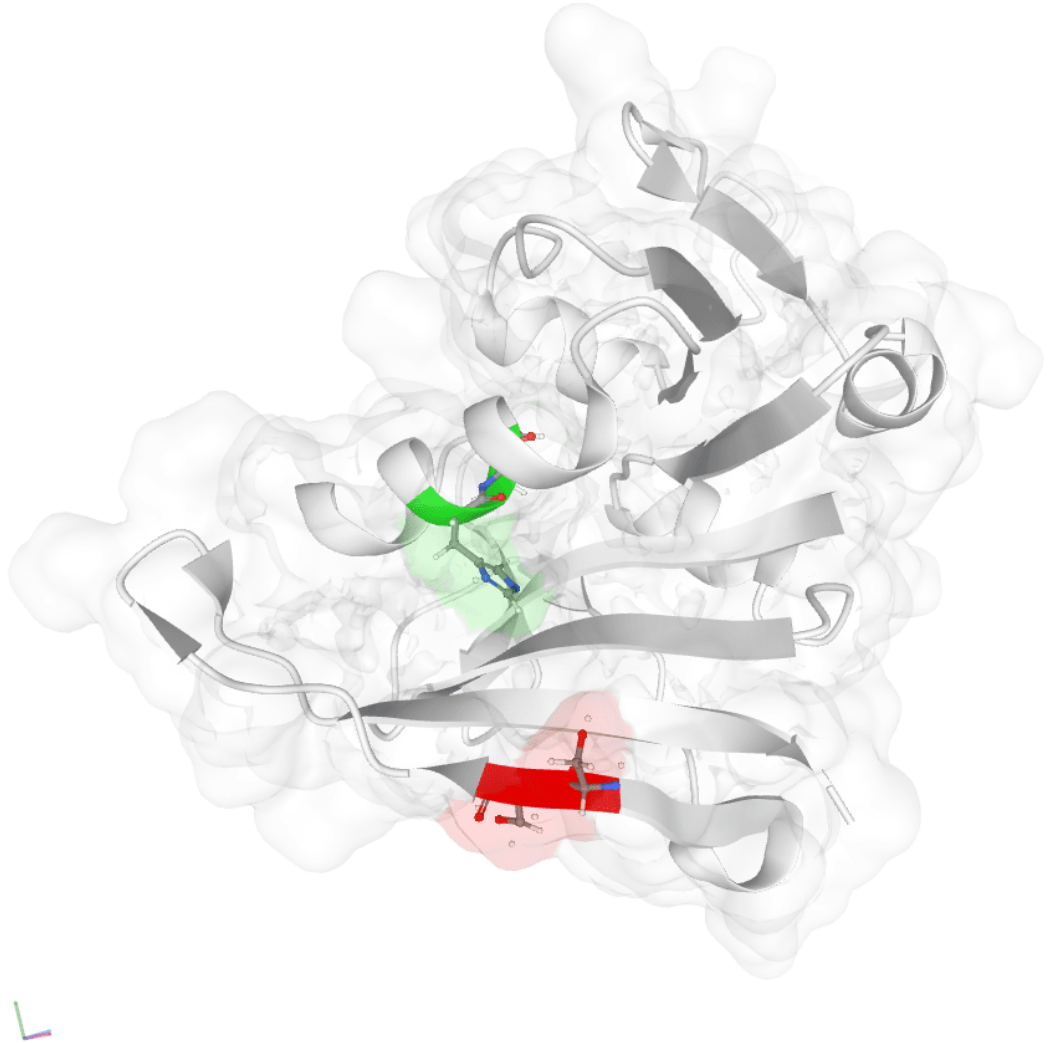


Contact Maps Represent Spatial Proximity, Not Sequence Order

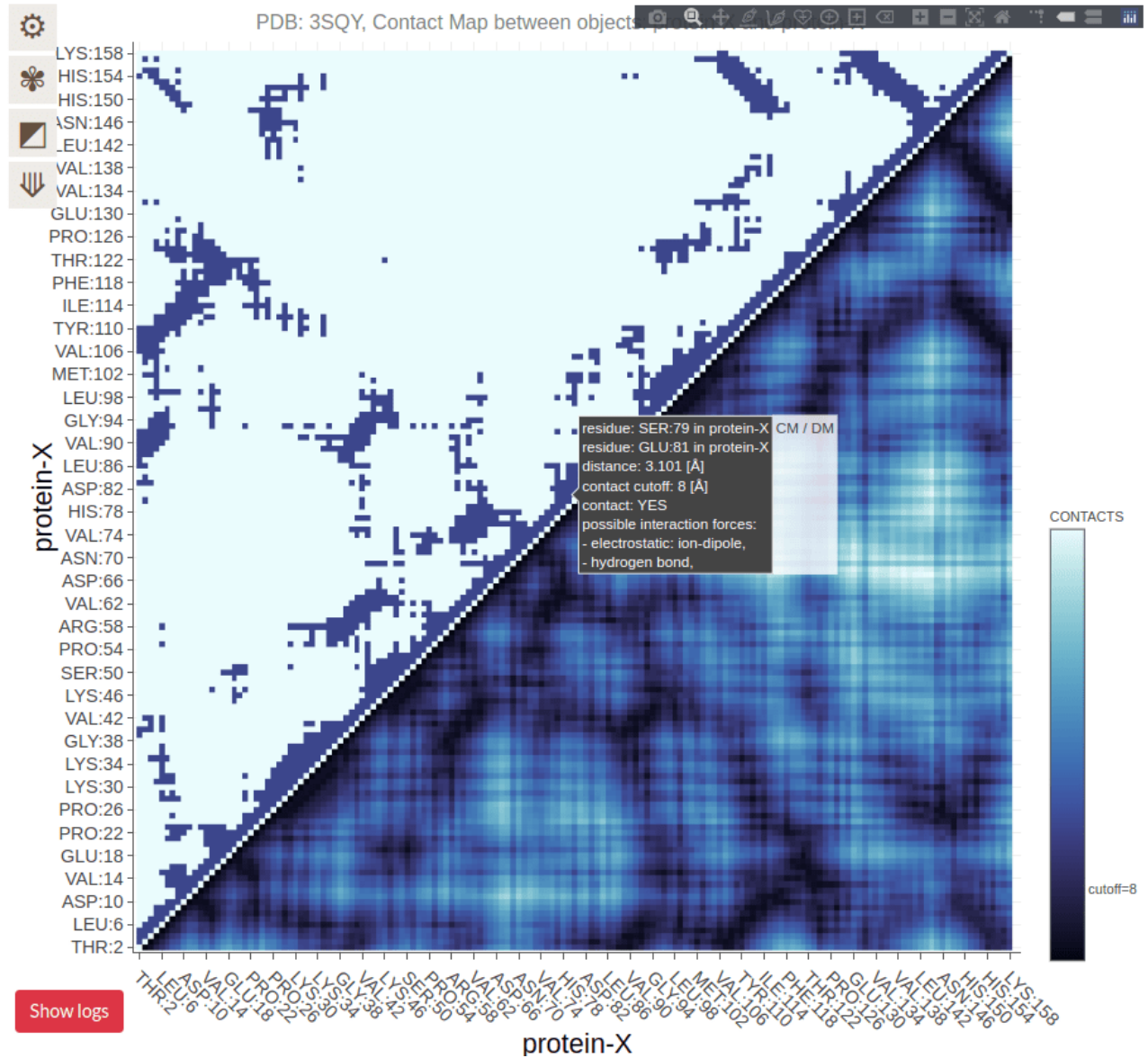
Contacts are determined by spatial proximity, typically within a certain distance threshold

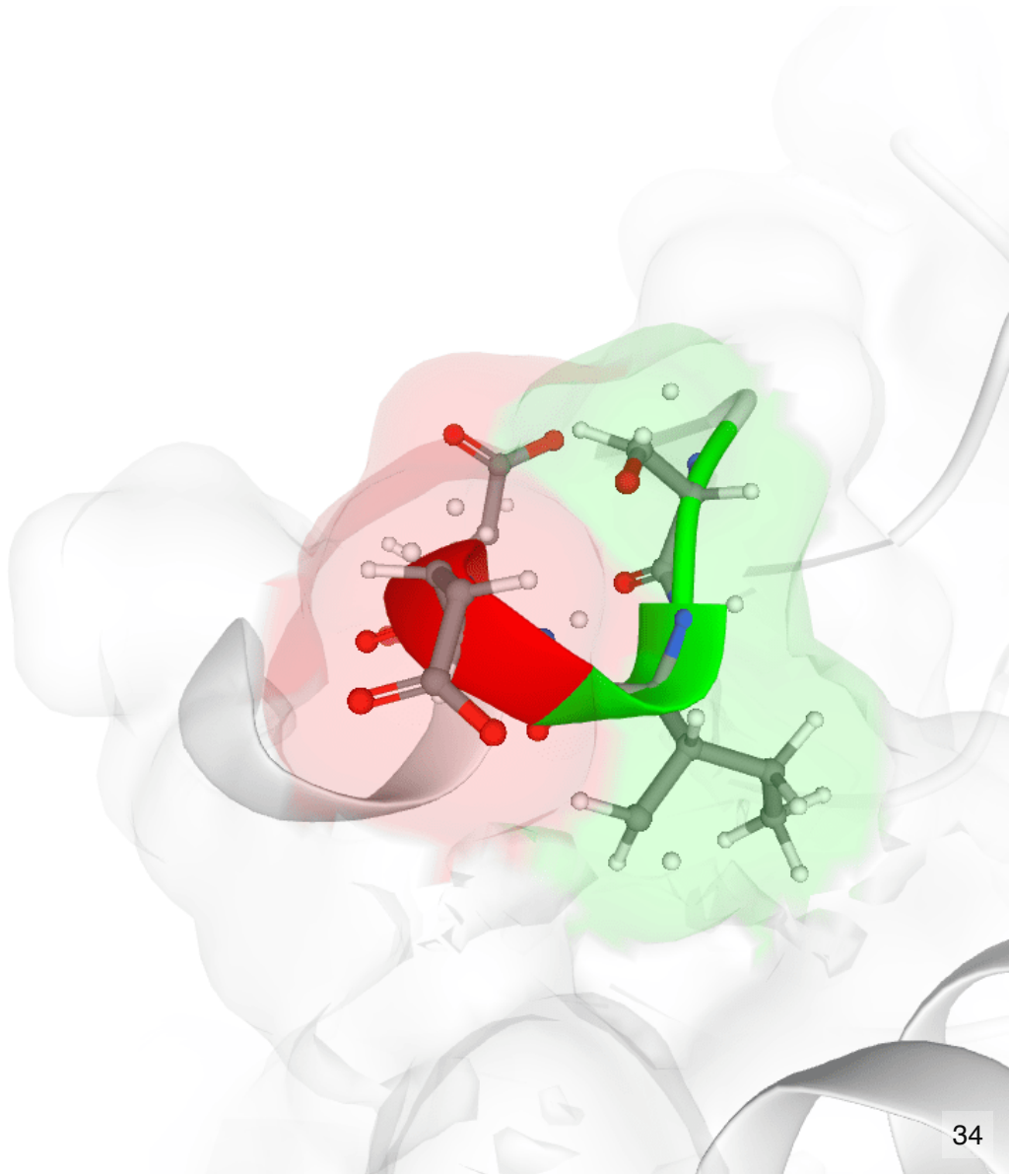
Residues far apart in the sequence can still be close in the 3D structure, reflected in the contact map



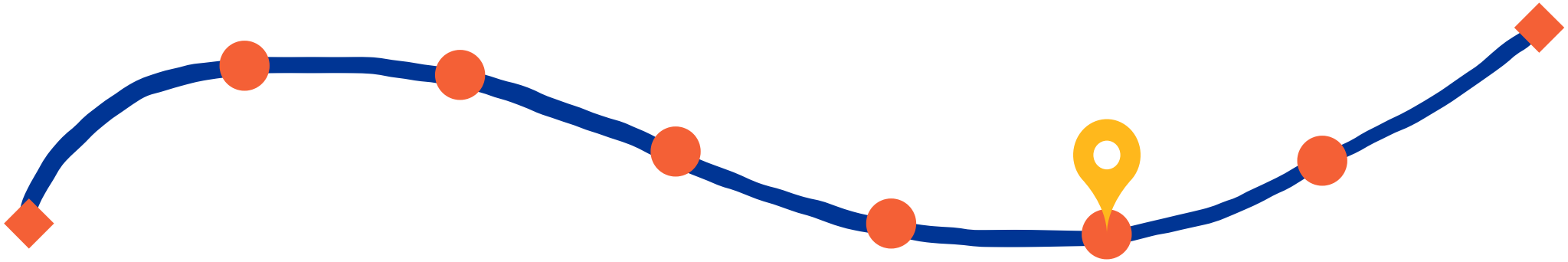


Residues on the diagonal are adjacent in sequence (and spatially)





After today, you should be able to



Comprehend how coevolution provides structural insights

The Rise of Machine Learning in Structural Biology

Traditional methods like **homology modeling** and threading rely on **templates and known structures**

ML predicts 3D structures **only from sequence data**

AlphaFold (DeepMind) and **RosettaFold** (Baker Lab) lead the charge in this area

What is AlphaFold?

Developed by DeepMind, **AlphaFold** predicts protein structures with atomic accuracy by using deep learning models trained on large structural datasets

Breakthroughs

- AlphaFold 2 achieved near-experimental level accuracy in the 2020 **CASP14** competition (Critical Assessment of protein Structure Prediction)
- **AlphaFold 3** (2024) predicts proteins, DNA, RNA, ligands, and post-translational modifications

Coevolving residues mutate in a correlated manner

Mutations in one residue often result in **compensatory mutations** in its interacting partner

This is observed across species through **analysis of homologous protein sequences**

Correlated mutations indicate **functionally significant** residue pairs

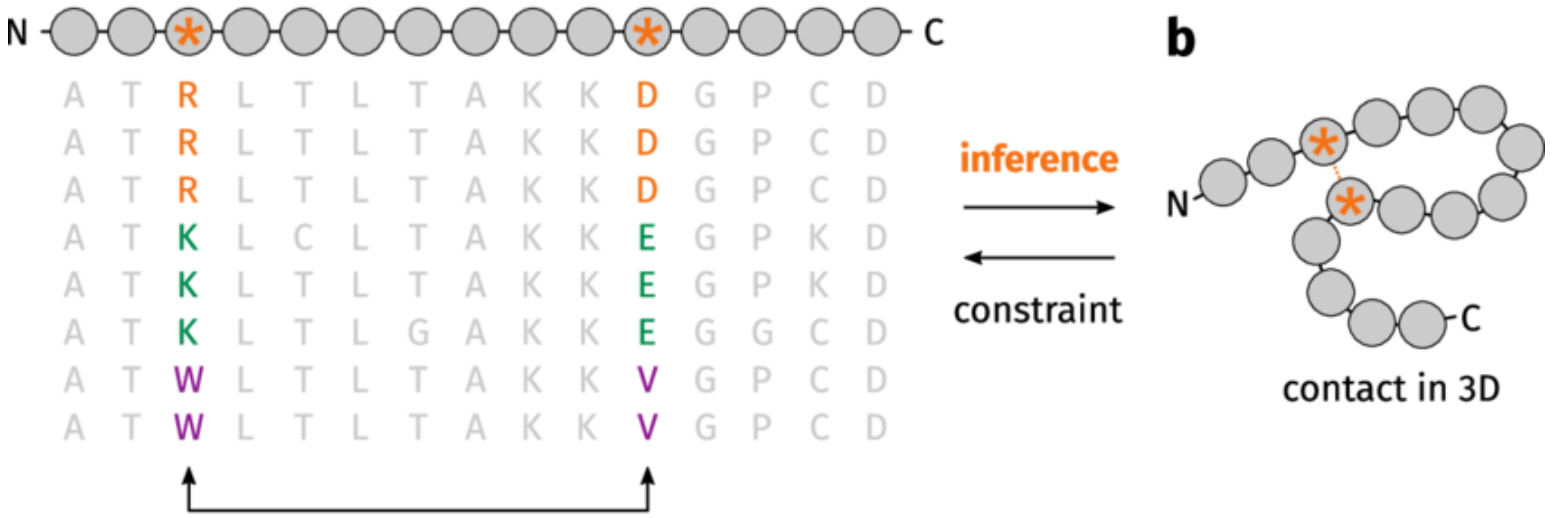
Evolution

Evolutionary Analysis Reveals Structural Insights

Coevolution analysis helps predict which residues are close in the 3D structure

Residues showing correlated mutations are likely to be spatially close in the folded protein

This is particularly useful when no experimental structure is available



Multiple Sequence Alignments Enable Coevolution Detection

Coevolution is detected using large MSAs from homologous proteins

The more diverse the sequences in the MSA, the better the resolution of coevolving residues

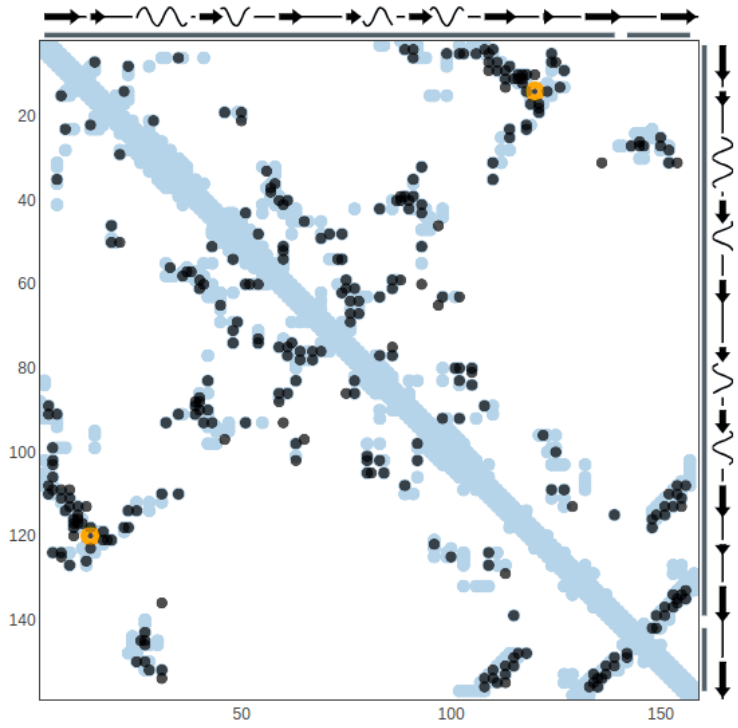
Evolutionary information from MSAs guides predictions for residue-residue contacts

evcouplings.org

Coevolution example: DHFR

Residues with a high Score (i.e., coevolve) are near each other in the protein's structure (i.e., small distance)

Val14 and Gly120 coevolved



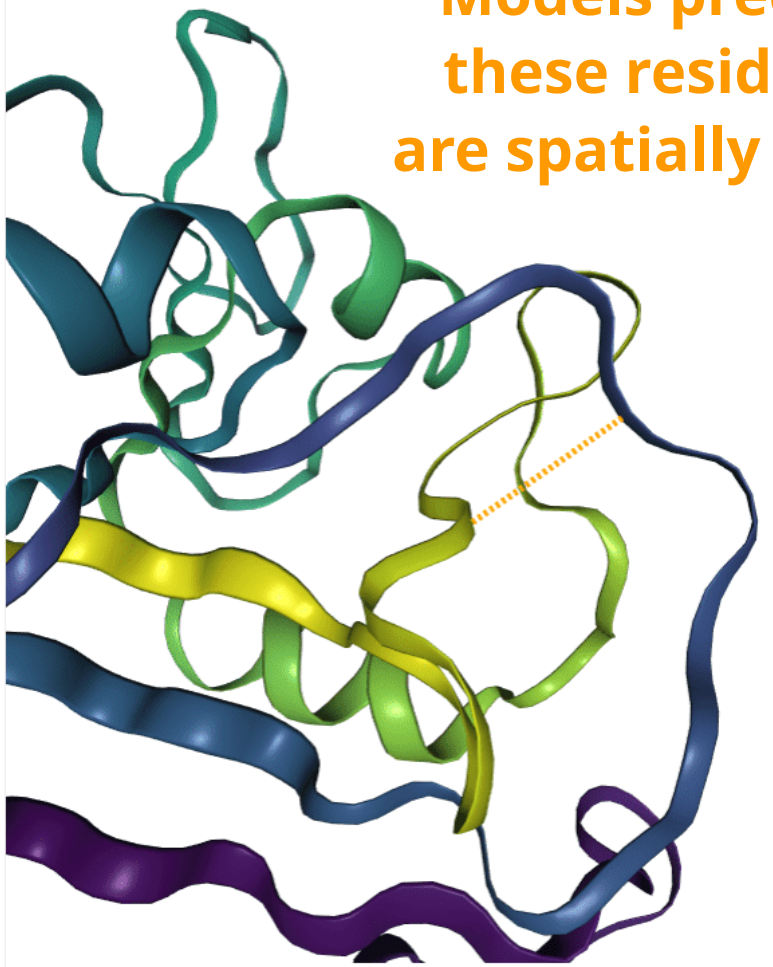
evcouplings.org

Structures | Legend 153 ECs

	i	A_i	j	A_j	Score	Prob	Dist	PPV
1	59	R	75	D	28.14	1.00	2.58	1.00
2	14	V	120	G	25.06	1.00	3.49	1.00
3	77	I	86	L	21.54	1.00	4.85	1.00
4	113	V	153	L	21.54	1.00	3.66	1.00
5	134	V	153	L	21.45	1.00	4.42	1.00
6	11	L	115	E	20.84	1.00	3.27	1.00
7	37	T	57	N	20.63	1.00	3.56	1.00
8	5	I	106	V	20.23	1.00	3.68	1.00
9	80	I	102	M	20.18	1.00	3.77	1.00
10	108	D	154	H	19.81	1.00	2.62	1.00
11	115	E	151	T	17.78	1.00	2.62	1.00
12	39	H	91	F	17.39	1.00	3.73	1.00
13	135	A	156	I	17.11	1.00	3.59	1.00
14	4	S	110	Y	17.04	1.00	3.79	1.00
15	139	E	151	T	16.45	1.00	2.65	1.00
16	137	S	151	T	16.01	1.00	3.30	1.00
17	32	V	93	F	15.74	1.00	3.35	1.00
18	142	L	149	P	15.33	1.00	3.71	1.00
19	14	V	123	F	15.32	1.00	3.49	1.00
20	109	M	127	Y	15.06	1.00	4.03	1.00
21	40	T	59	R	14.60	1.00	3.32	1.00
22	92	I	102	M	14.51	1.00	3.95	1.00
23	4	S	89	H	14.19	1.00	2.63	1.00
24	39	H	89	H	14.19	1.00	3.23	1.00
25	10	D	118	F	13.94	1.00	2.75	1.00
26	11	L	117	K	13.87	1.00	3.74	1.00
27	4	S	108	D	13.85	1.00	2.45	1.00
28	108	D	156	I	13.82	1.00	3.93	1.00
29	31	H	110	Y	13.66	1.00	4.48	1.00
30	137	S	153	L	13.61	1.00	3.27	1.00
31	110	Y	154	H	13.29	1.00	3.32	1.00
32	12	Q	117	K	13.04	1.00	3.48	1.00
33	35	L	110	Y	12.87	1.00	3.42	1.00
34	9	H	113	V	12.78	1.00	3.27	1.00
35	136	S	154	H	12.54	1.00	2.93	1.00
36	75	D	86	L	12.45	1.00	6.11	0.97
37	7	V	111	I	12.42	1.00	3.20	0.97
38	27	N	150	H	12.17	1.00	2.87	0.97

Legend 153 ECs

Models predict these residues are spatially close



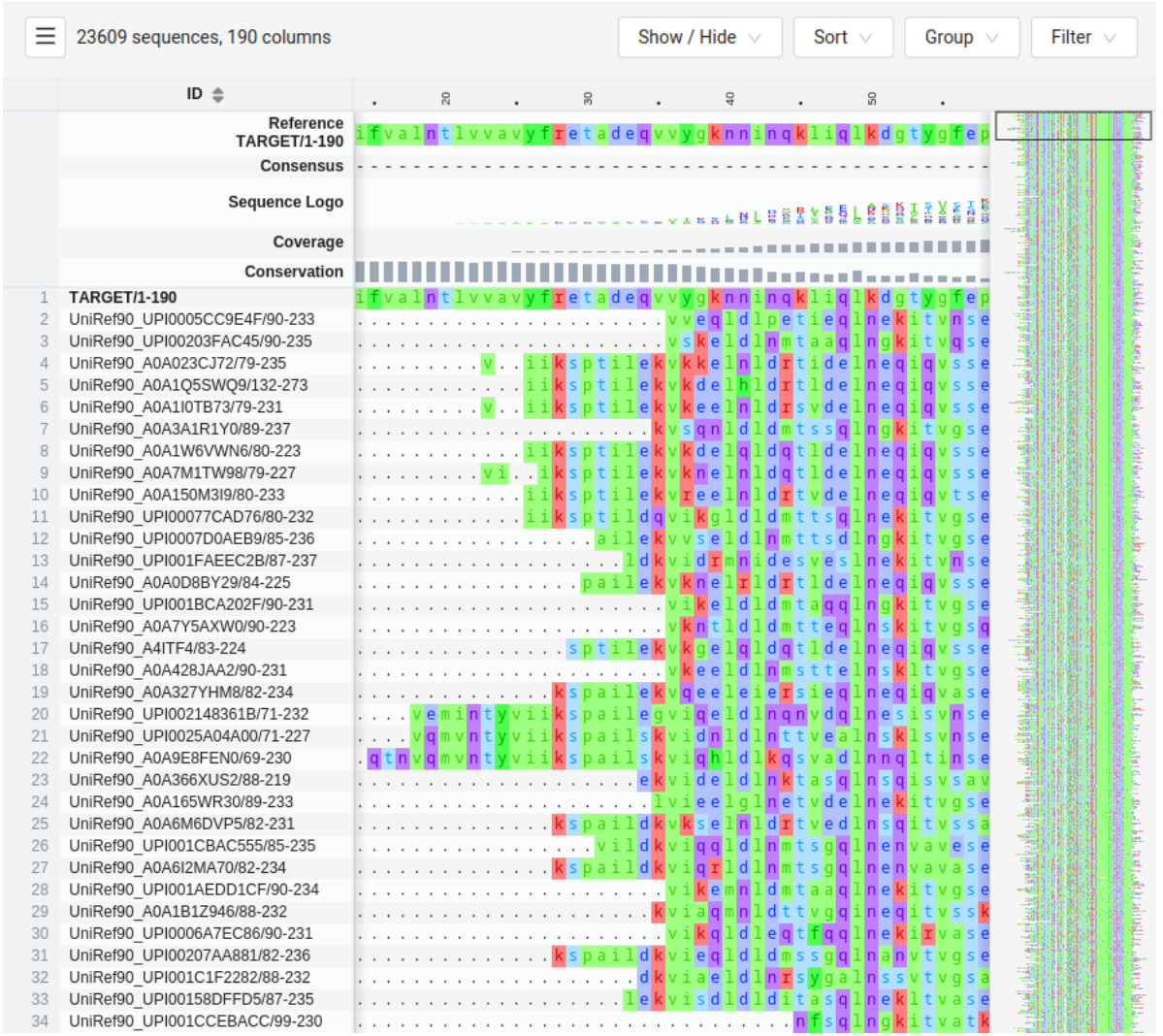
Structures

Coevolutionary signals can be noisy

Not all correlated mutations are due to direct physical interactions; some may be indirect

Noise in the data can come from random mutations or insufficient evolutionary diversity.

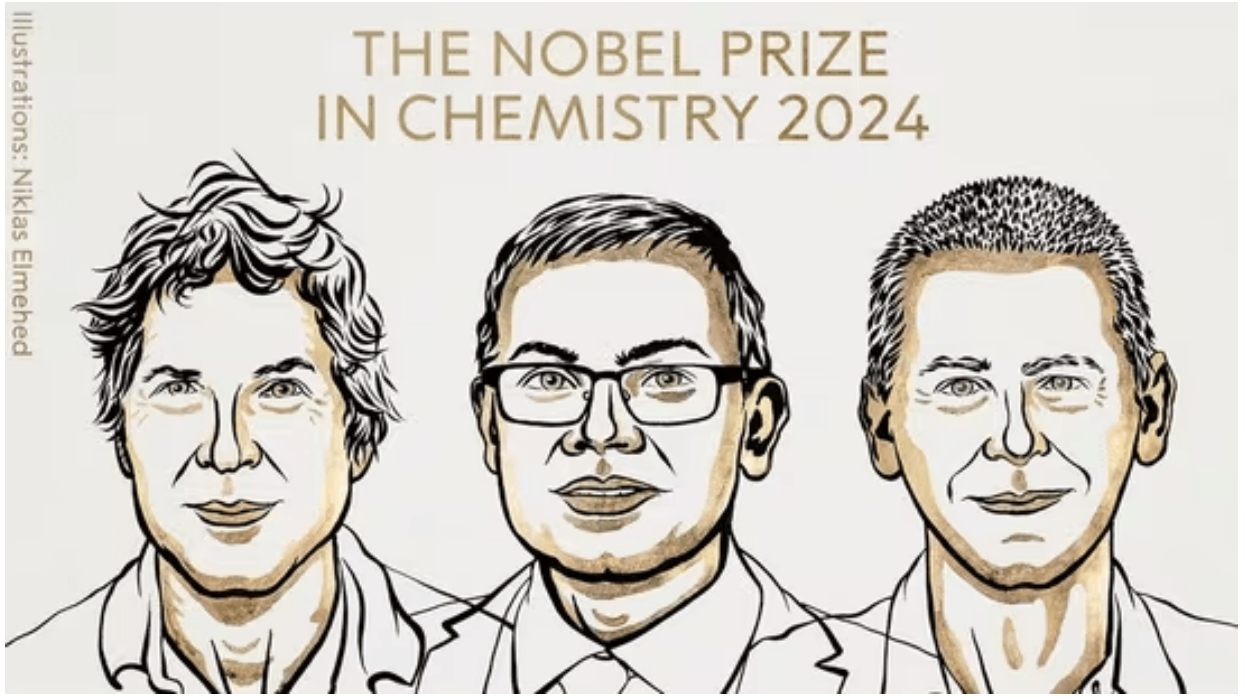
Large and diverse sequence data sets are needed for reliable coevolution predictions.



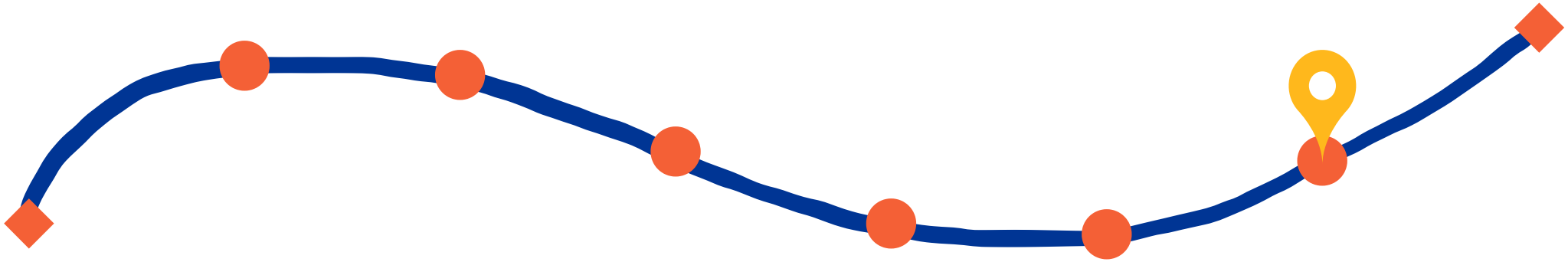
Machine learning leverages coevolution for high-accuracy predictions

AlphaFold and RosettaFold utilize coevolutionary data from MSAs to predict residue interactions

These models incorporate evolutionary information along with structural features, leading to highly accurate predictions



After today, you should be able to



Explain why ML models are dominate protein structure prediction

AlphaFold pipeline, simplified

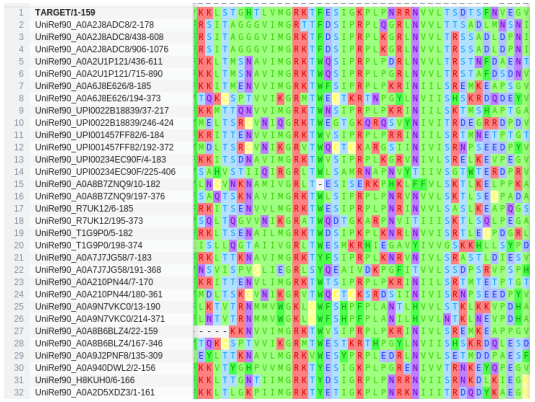
Given the following data

Predict

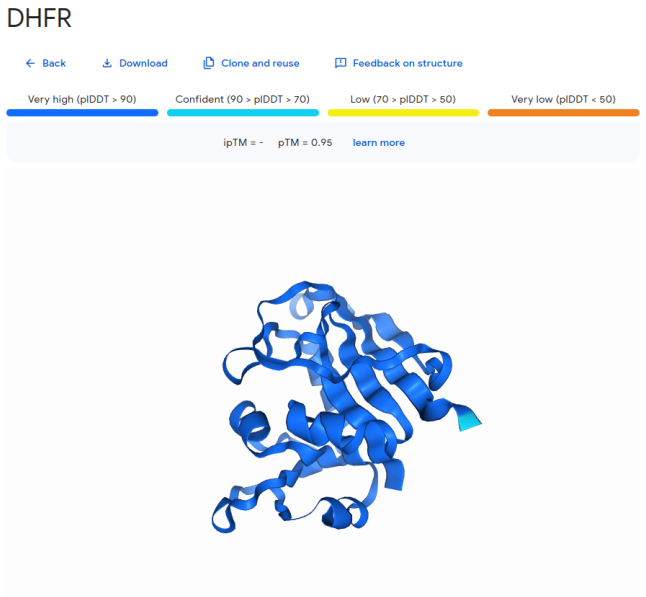
Input sequence

```
MTLSILVAHDLQRVIGFENQLPWHLPNDLKHVKK
LSTGHTLVMGRKTFESIGKPLPNRRNVVLTSDTS
FNVEGVDVIHSIEDIYQLPGHVFIFGGQTLFEEM
IDKVDDMYITVIEGKFRGDTFFPPYTFEDWEVAS
SVEGKLDEKNTIPHTFLHLIRKK
```

Multiple Sequence Alignment



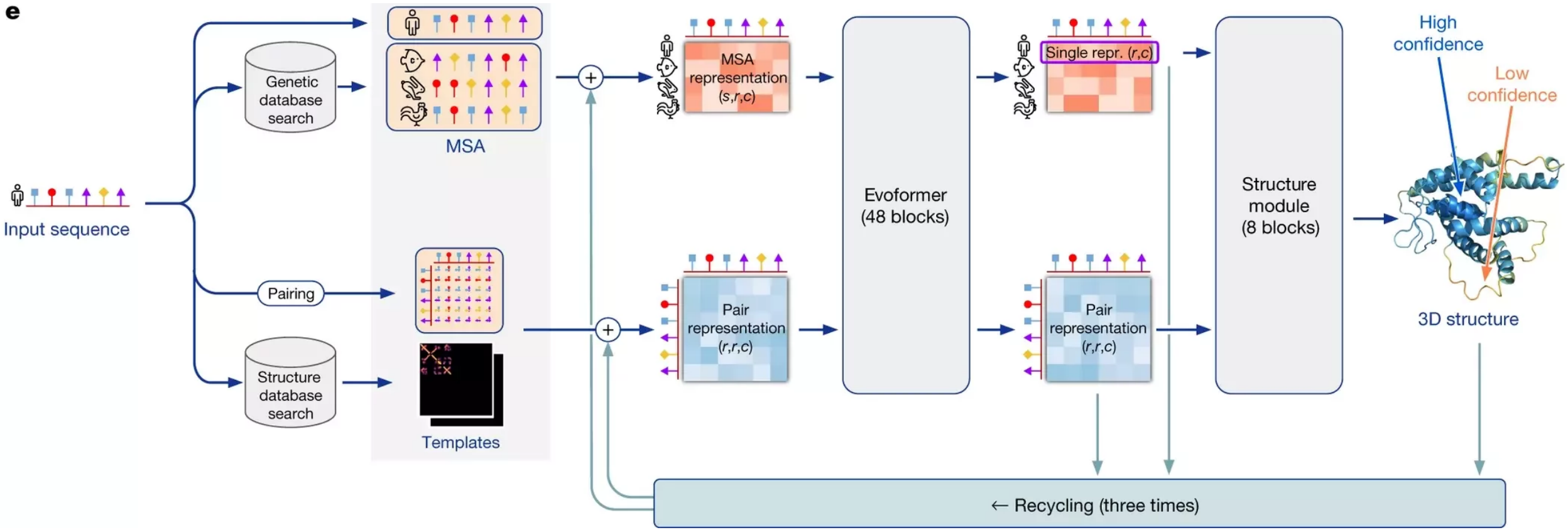
ML models



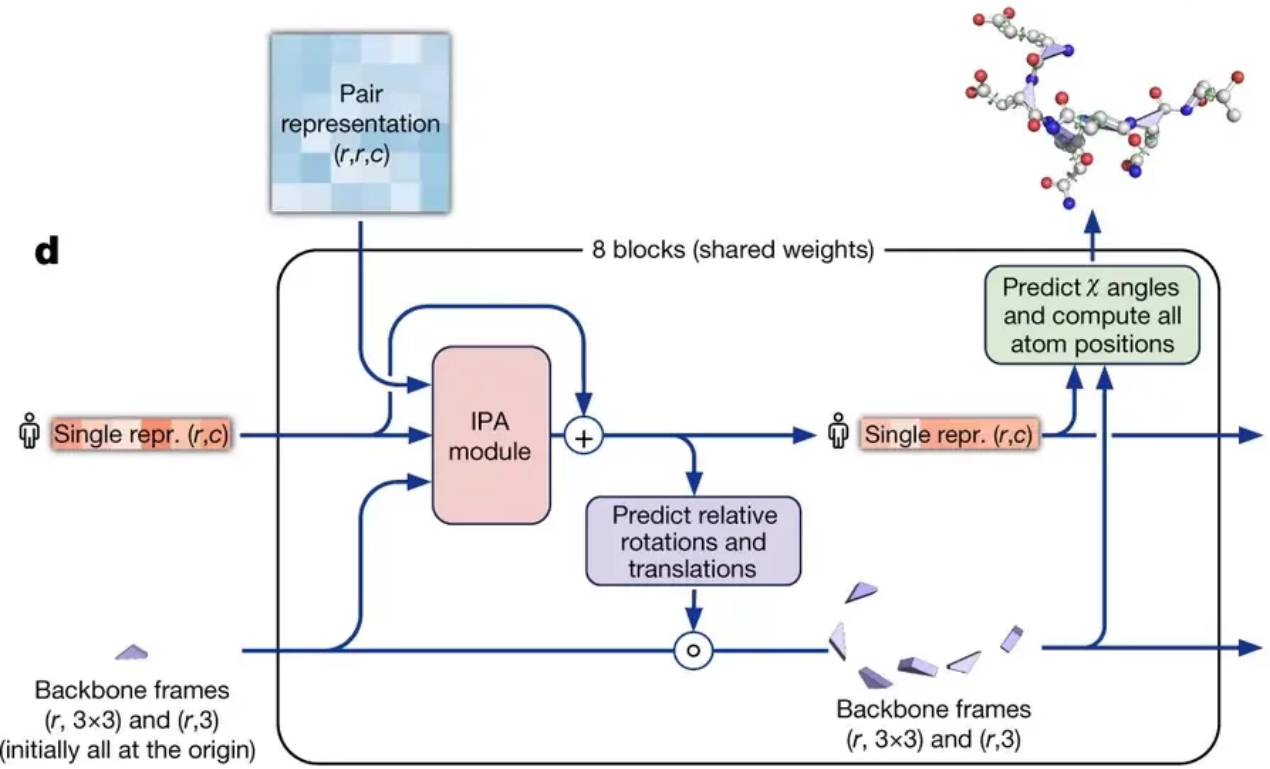
Atomistic structure

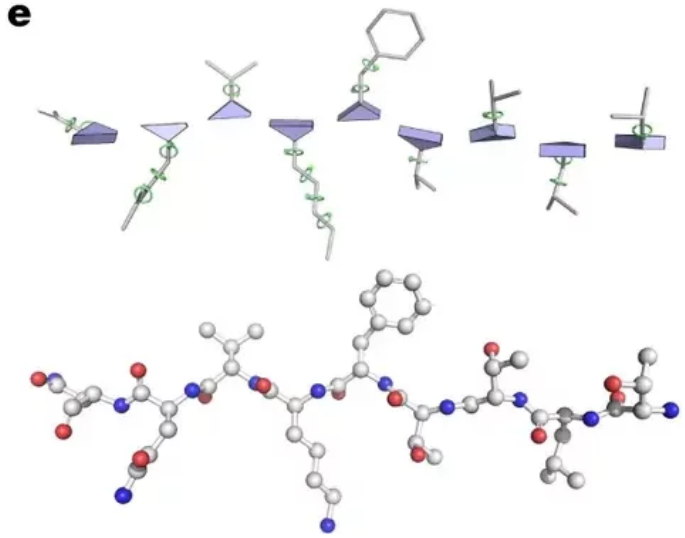
AlphaFold 2 pipeline: **Evoformer**

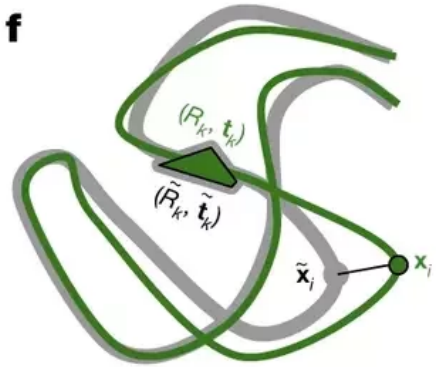
Using MSAs and contact maps, DeepMind trained a model to predict protein structures

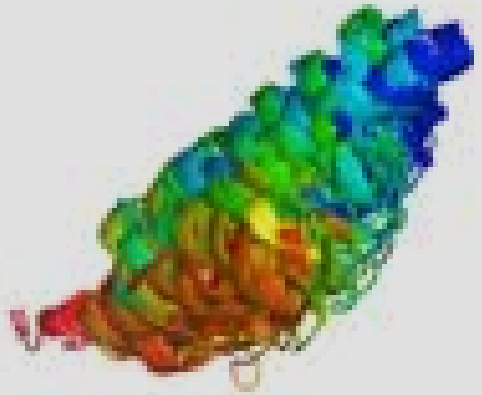


Contact maps are converted into dihedral angles







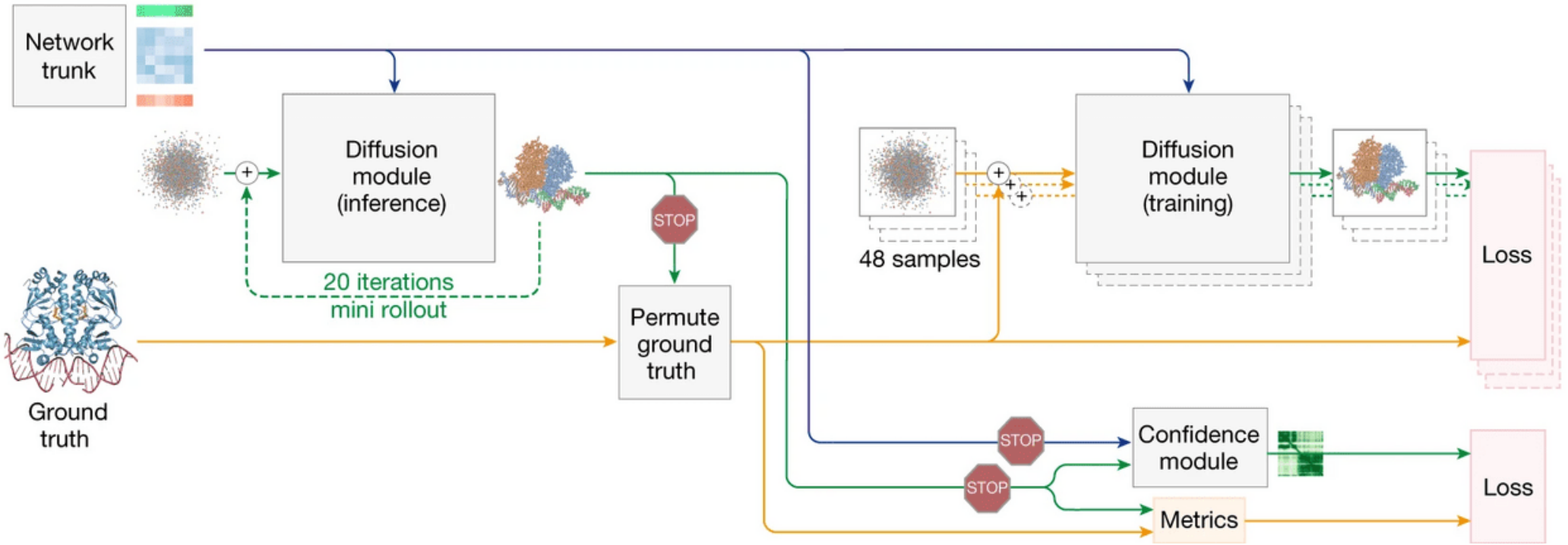


Recycling iteration 0, block 01
Secondary structure assigned from the final prediction

What is new in AlphaFold 3?

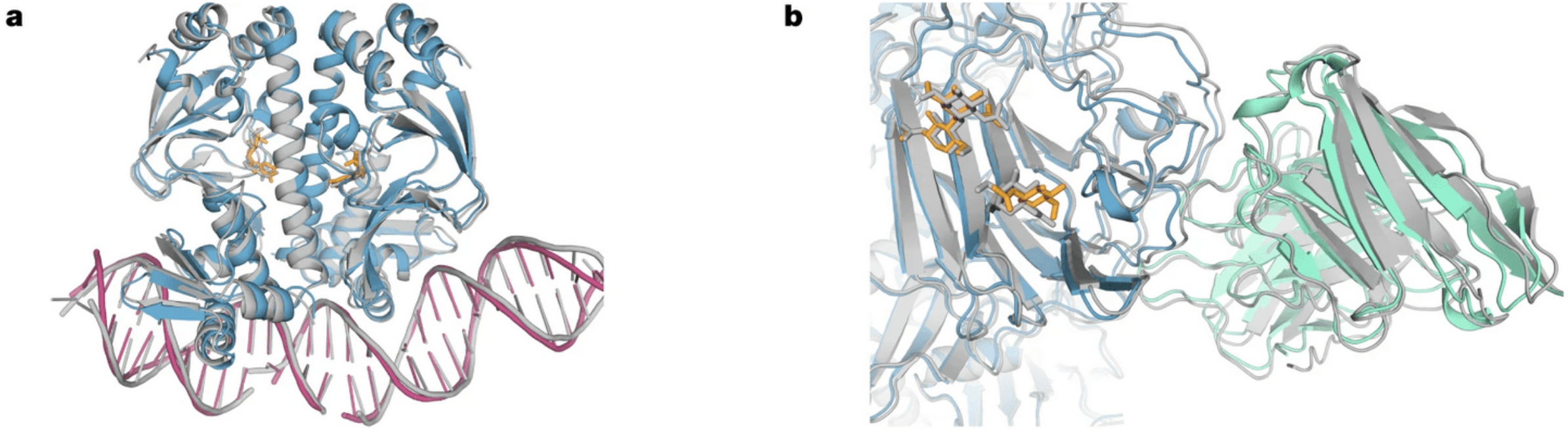
Biggest change is the use of a **diffusion model**

Diffusion models essentially learn to **unscramble atoms into a structure**



AlphaFold 3 is supercharged for any biomolecule

Proteins, DNA, RNA, ligands, PTMs, protein-proteins, etc.



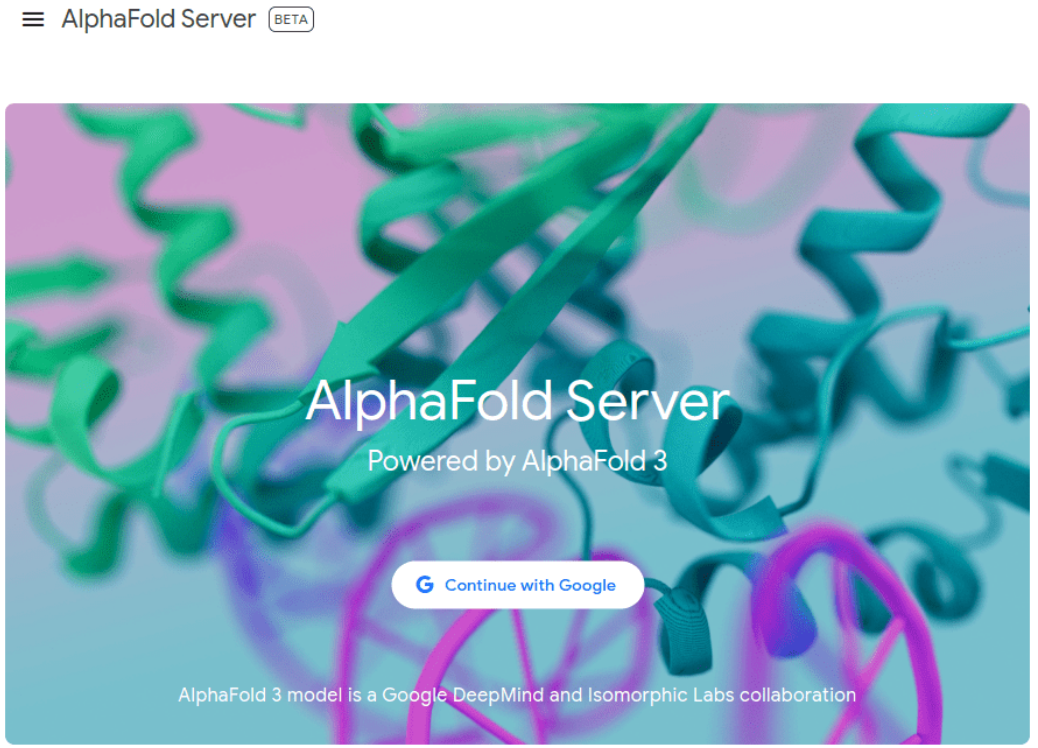
AlphaFold 3

```
MTLSILVAHDLQRVIGFENQLPWHLPNDLKHVKKLSTGHTL
VMGRKTFESIGKPLPNRRNVVLTSDTSFNVEGVDVIHSIED
IYQLPGHVFIFGGQTLFEEMIDKVDDMYITVIEGKFRGDTF
FPPYTFEDWEVASSVEGKLDEKNTIPHTFLHLIRKK
```

DHFR (UniProt)

```
MGKKEVILLFLAVIFVALNTLVVAVYFRETADEQVVYGK
NNINQKLIQLKDGTYGFEPALPHVGTFKVLDSNRVPQIA
QEIIRNKVKRYLQEAVRIEGTYPIVDGLVNAKYTVANPN
NLHGYEGFLFKDNVPLTYPQEFILSNLDGKVRSLQNYDY
DLDVLFGEKEEVKSEILRGLYYNTYTRAFSPYKL
```

Novel protein
(ChatGPT)



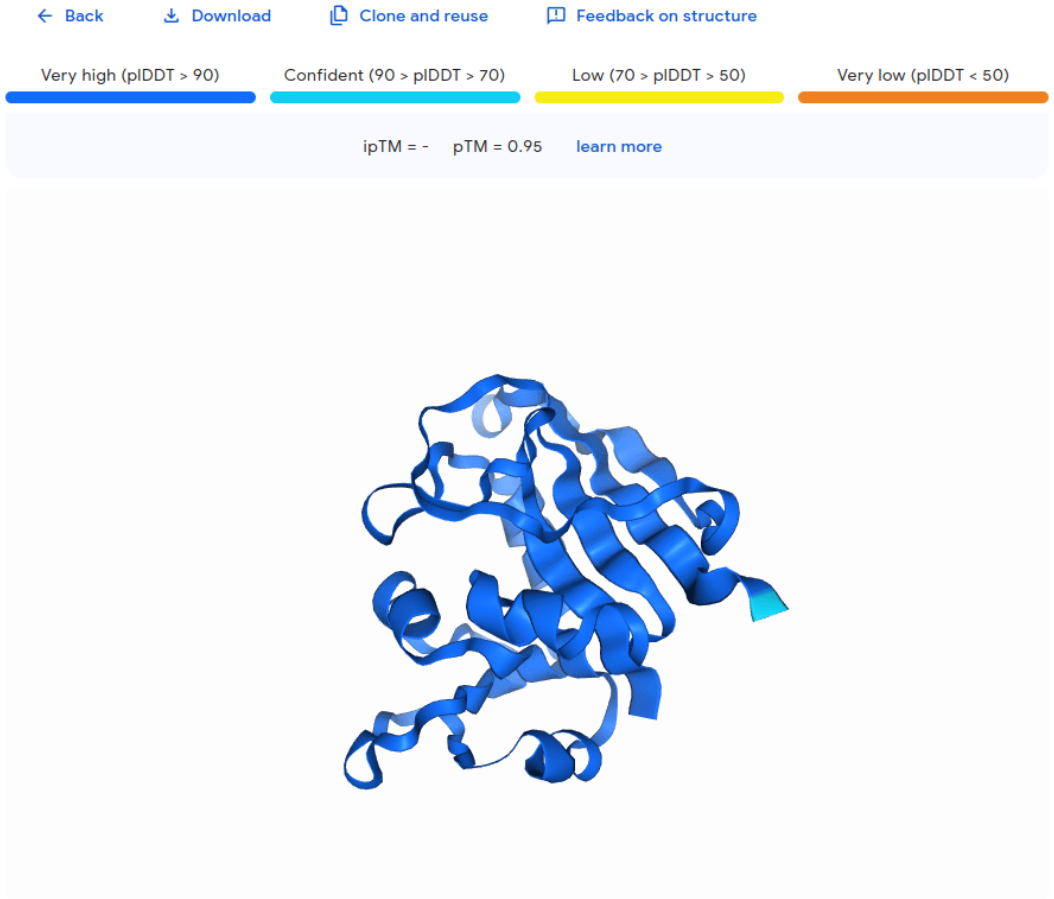
How does AlphaFold Server work?

AlphaFold Server is a web-service that can generate highly accurate biomolecular structure predictions containing proteins, DNA, RNA, ligands, ions, and also model chemical modifications for proteins and nucleic acids in one platform. It's powered by the newest AlphaFold 3 model.

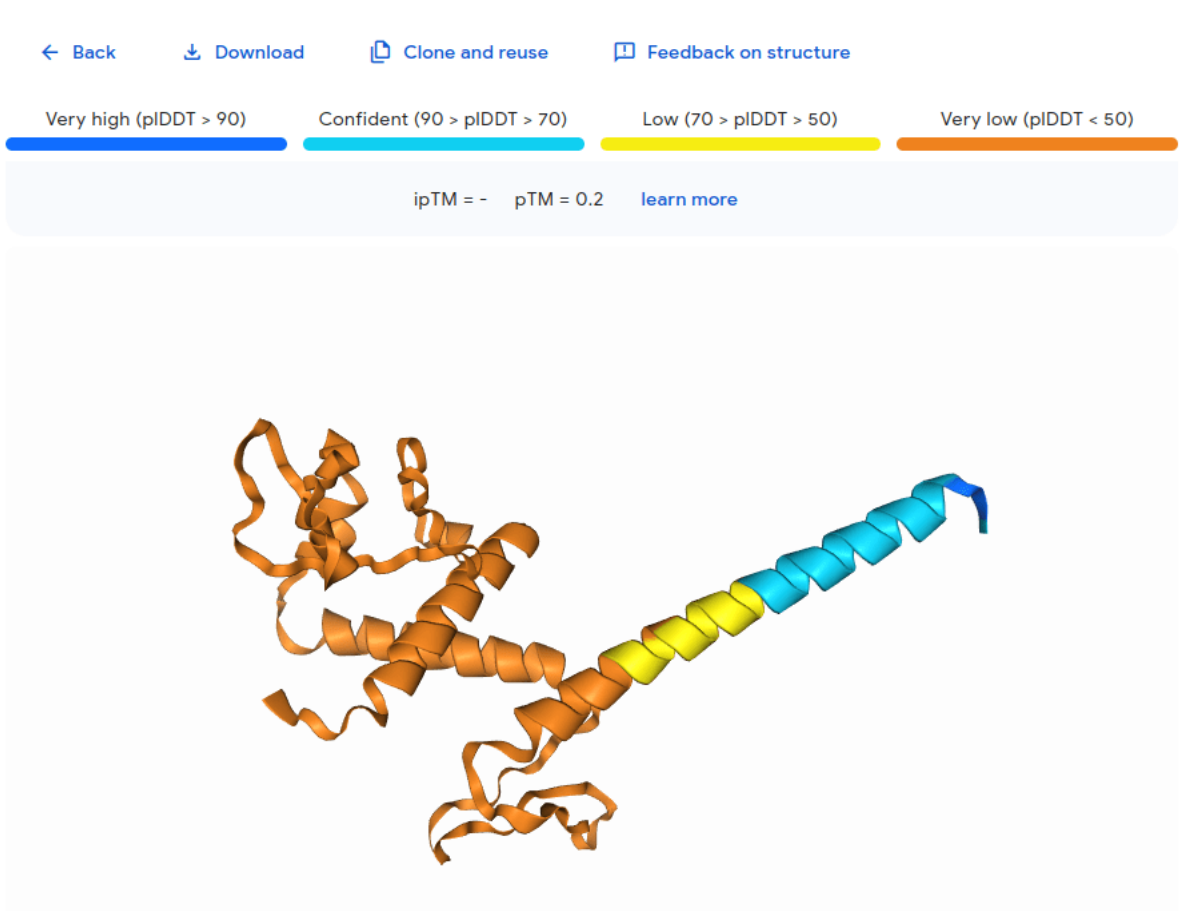
alphafoldserver.com

AlphaFold 3 is a breakthrough, not the final solution

DHFR



Novel



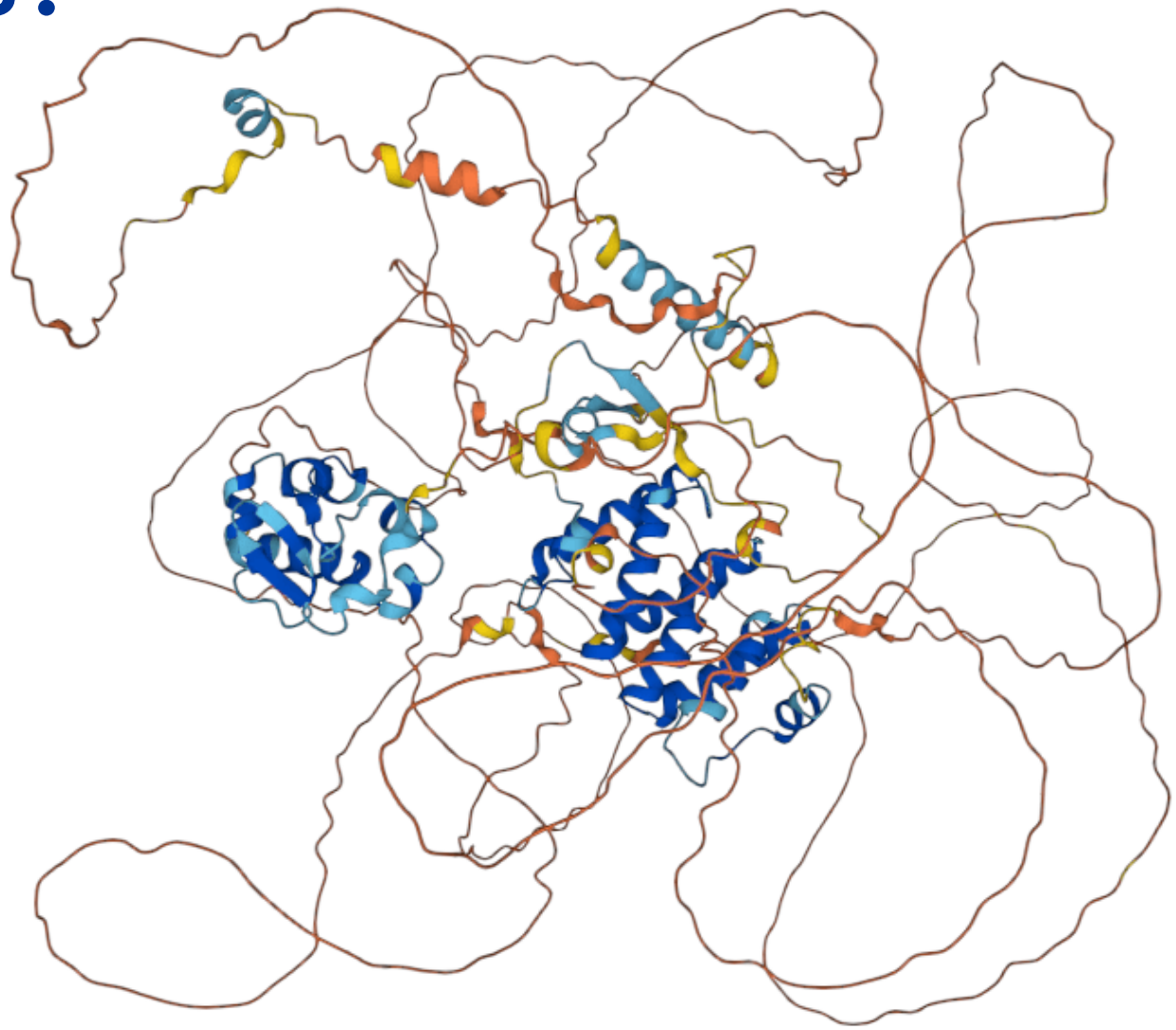
Caveat: Proteins are **dynamic**

https://www.youtube.com/embed/AjcUmxT-QEA?si=qupqTpuV5IvOB_ut&start=43&enablejsapi=1

What about intrinsically disordered proteins?

At least 40% of proteins have disordered regions

AlphaFold (and all other methods) struggle with disordered regions



LARP1

Before the next class, you should

Lecture 12:
Protein structure prediction

Lecture 13:
Molecular simulation princples

Today

Thursday

- Work on A05
- Review material