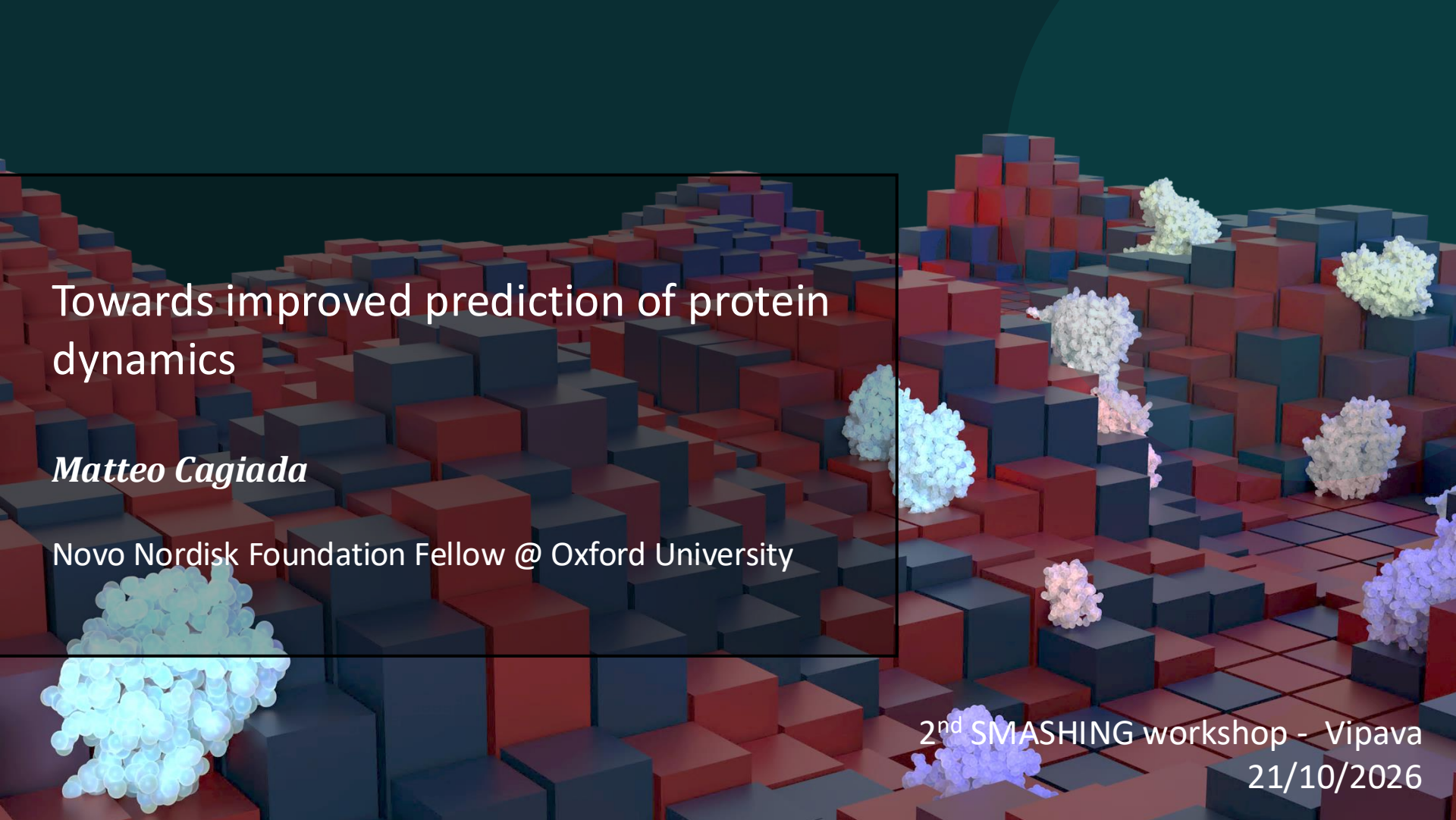
Towards improved prediction of protein dynamics

Matteo Cagiada

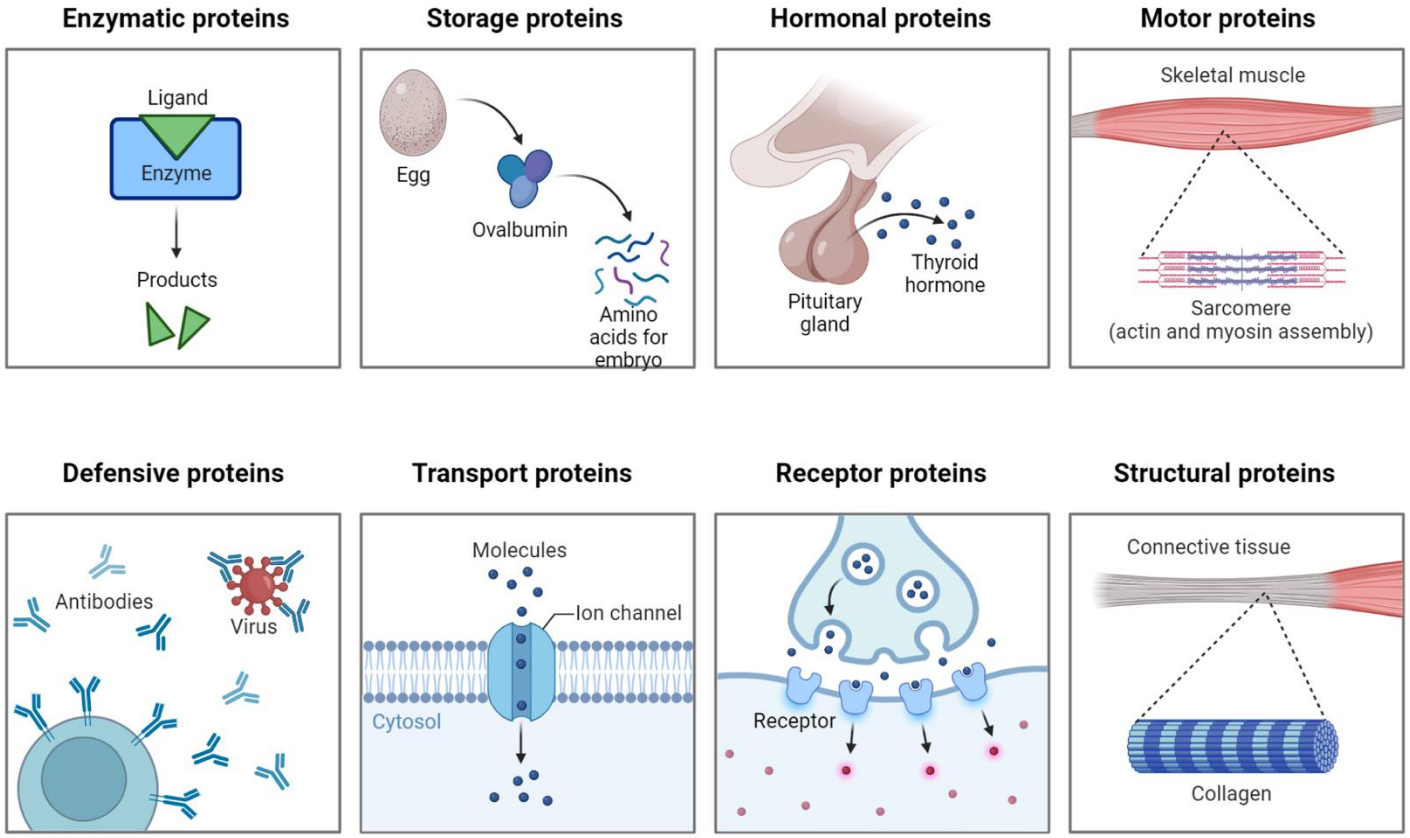
Novo Nordisk Foundation Fellow @ Oxford University



2nd SMASHING workshop - Vipava
21/10/2026

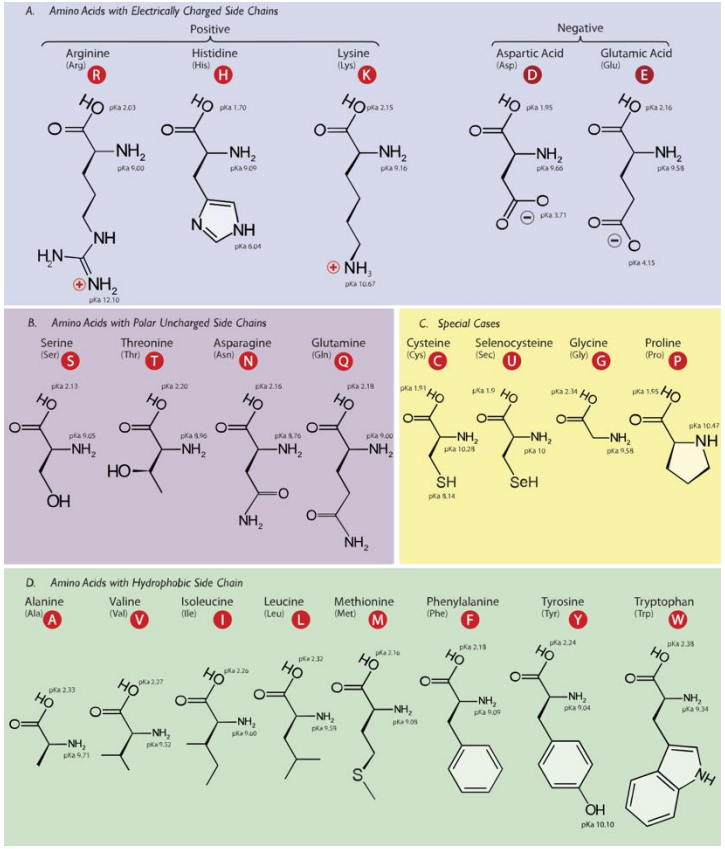
What are proteins?

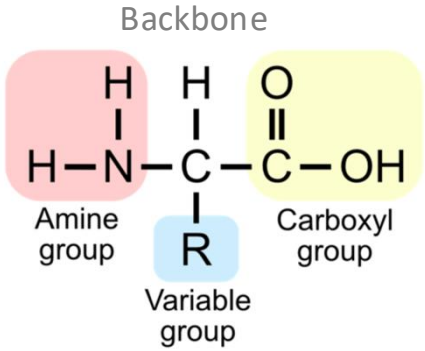
Proteins are the workhorse of organism. They are biopolymers that perform a wide range of functions in all cells and organisms.



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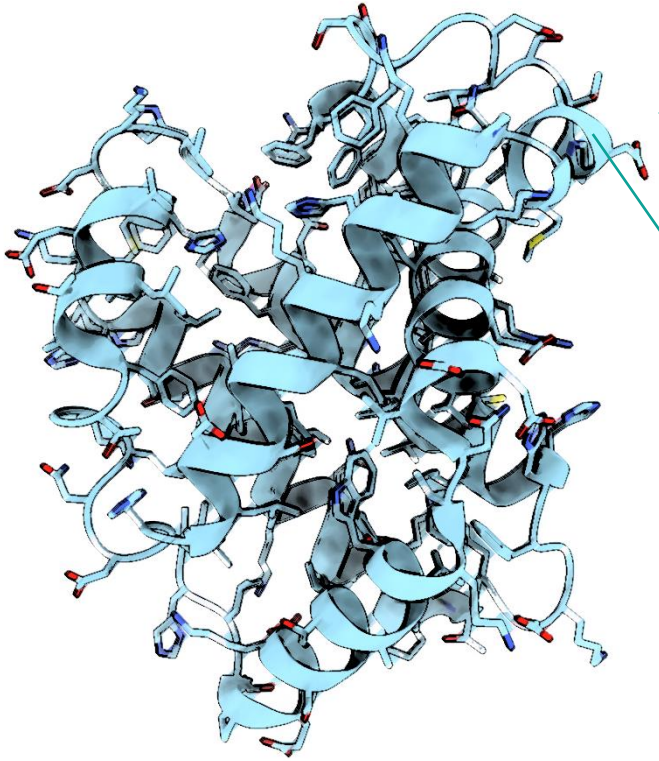


Amino acids

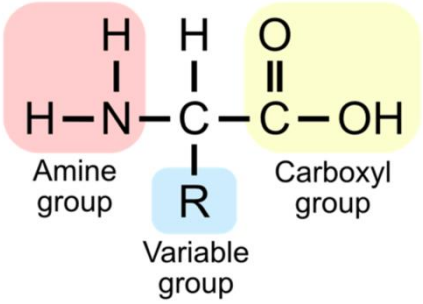
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Structure



Backbone



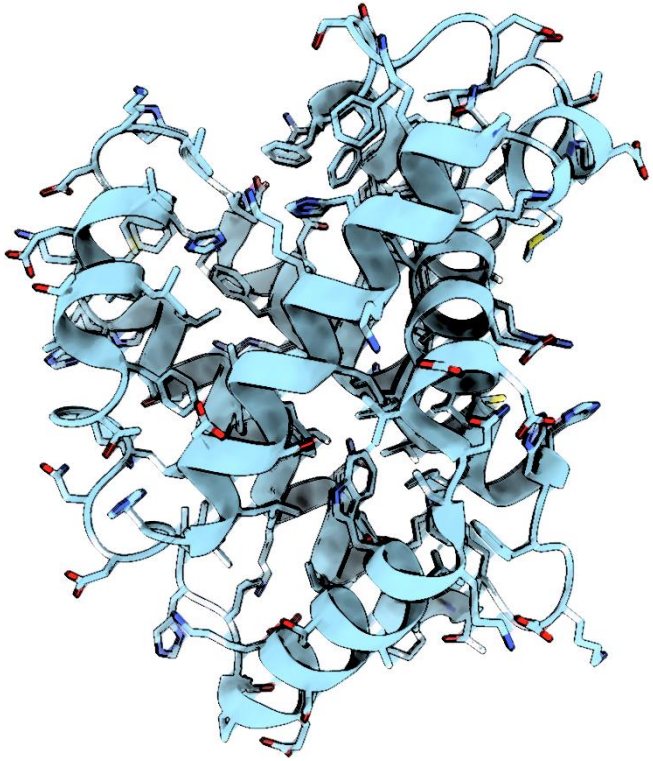
Amino acids

We often refer to amino acids as ‘residues’

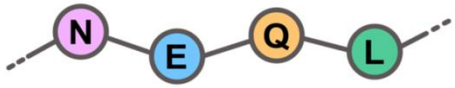
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Proteins are
perform

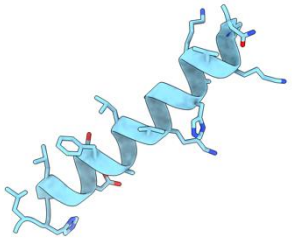
Structure



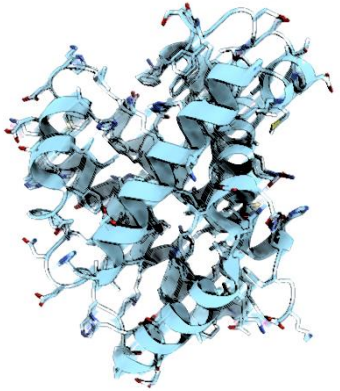
Primary structure (sequence)



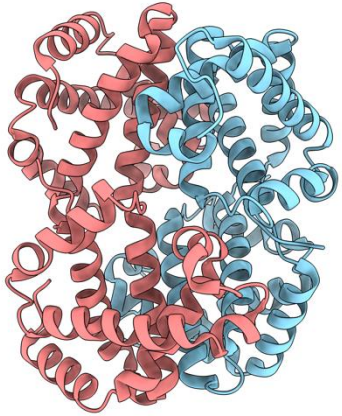
Secondary structure



Tertiary structure



Quaternary structure

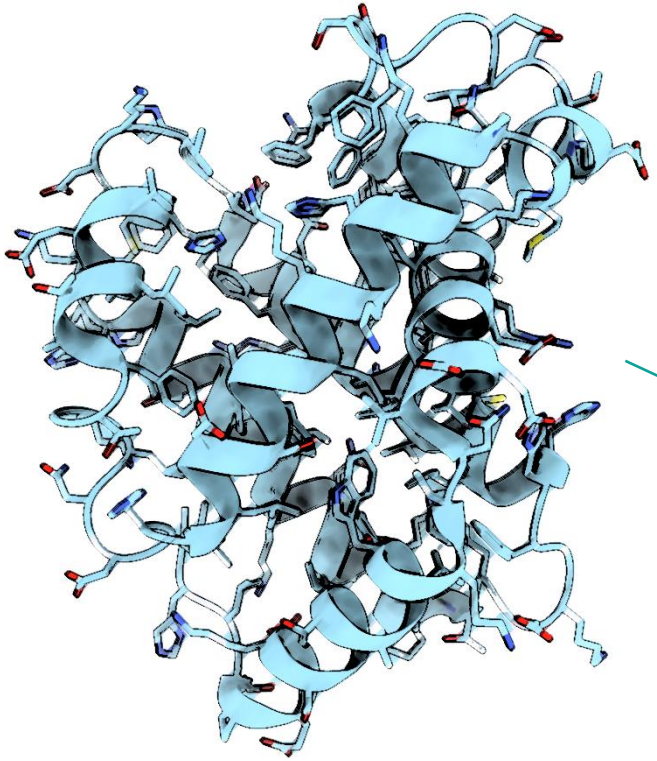


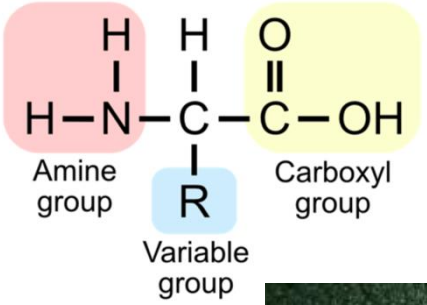
Kaj Ulrik Linderstrøm-Lang (1952)

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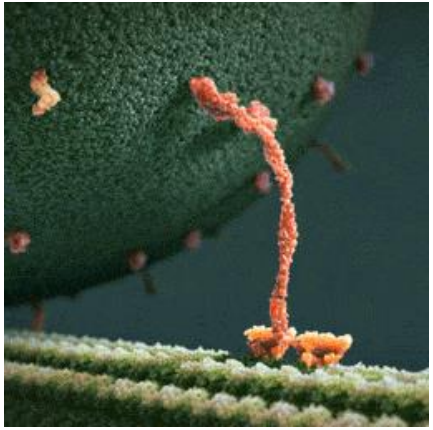
Structure





Amino acids

Function



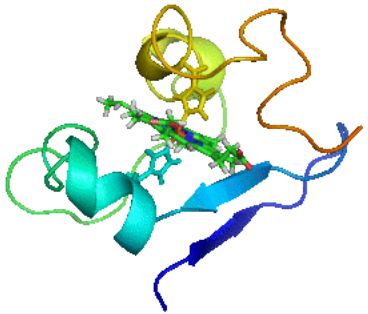
Animated GIF of Kinesin Protein walking.
©John Liebler. All rights reserved.

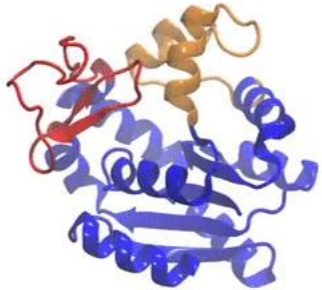
Proteins are not static structures

In a solution or living organism, a protein is dynamic.



Atomic fluctuation | Domain and Loop Movements | Overall Changes





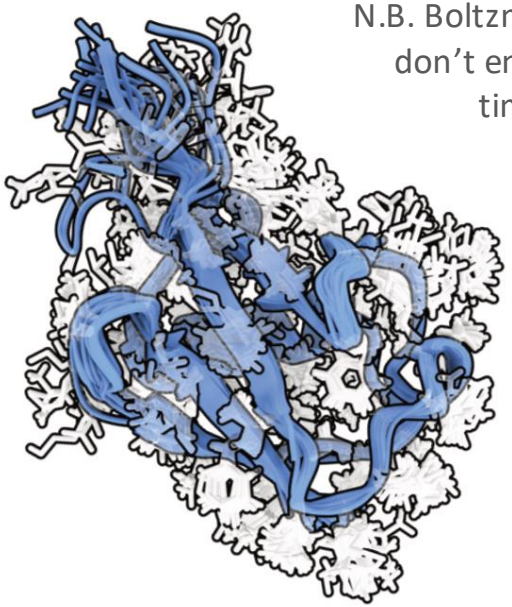
Examples of time-trajectories

Videos from Wikimedia and Baharlab.com

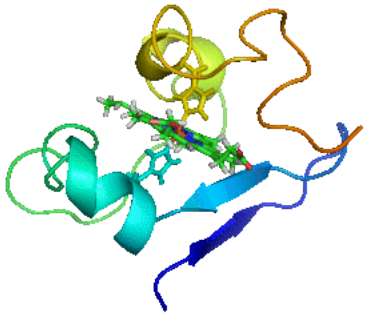
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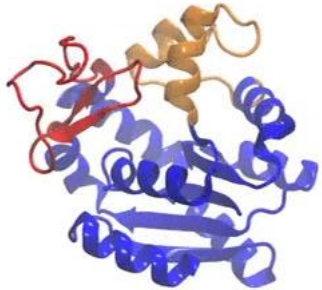
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N.B. Boltzmann ensembles don't encode directly timescale.





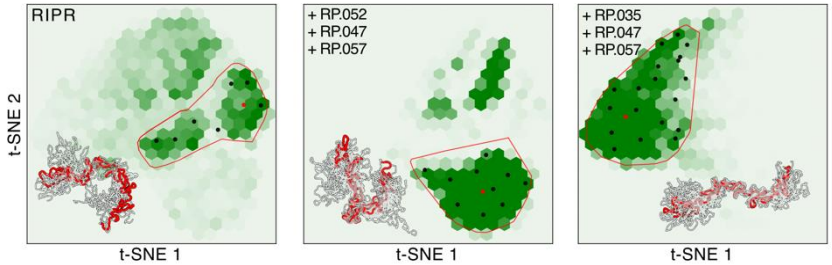
Examples of time-trajectories

Boltzmann-weighted conformational ensemble
which defines all macroscopic properties of a protein, such as stability or binding affinity.

Videos from Wikimedia and Baharlab.com

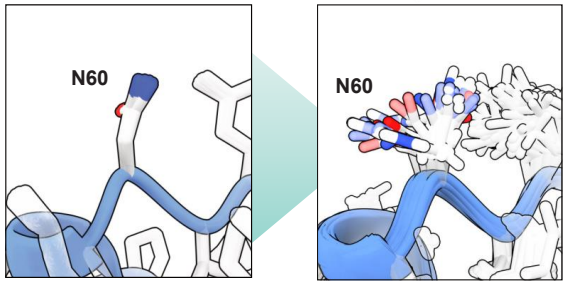
Agenda:

Predicting conformational changes in complex protein system using molecular dynamics



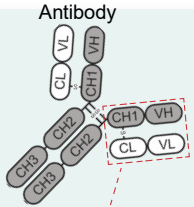
Williams, B. G., Barrett, J. R., Bartholdson Scott, J., Rigby, C. A., Cagiada, M., et al. Immunity (2026) .

Predicting amino acid sidechain conformations



Cagiada, Matteo, F.Emil Thomasen, et al., *bioRxiv* (2025).

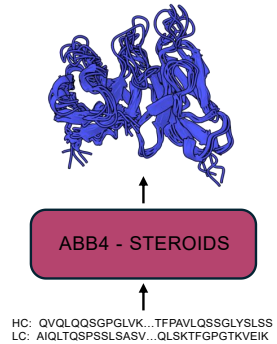
Prediction of conformational variability in immuno-receptors



We generated the largest dataset of molecular dynamics simulation of immuno-receptors (150,000)

FLAbDab & FTCRDab

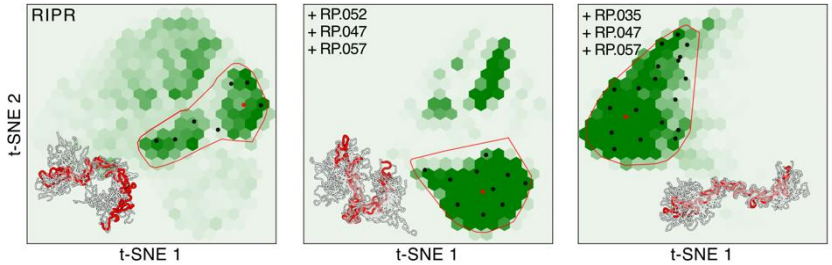
And use it to train the state-of-art predictor generative model for predicting antibody-conformational ensemble.



Cagiada et al, biorvix (2025)
Spoendlin et al, biorvix (2026)

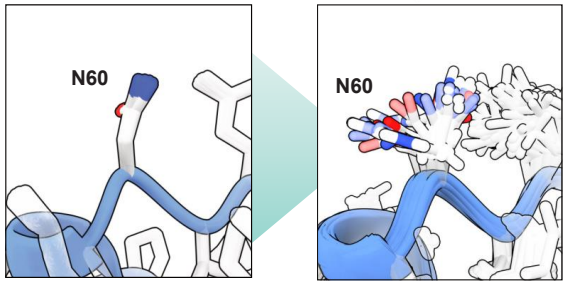
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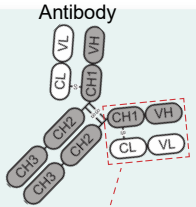
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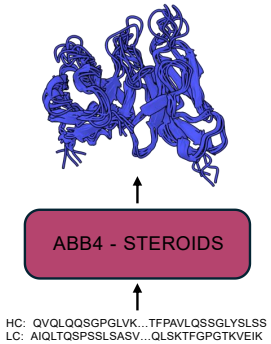
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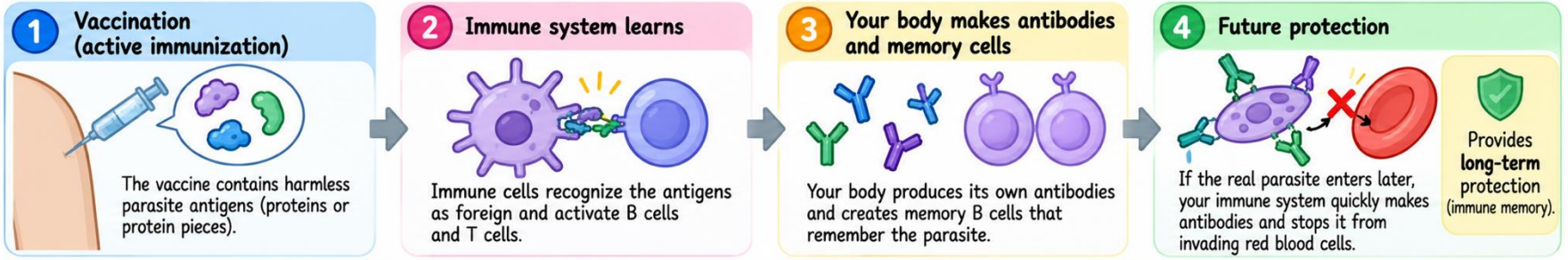


Cagiada et al, biorvix (2025)
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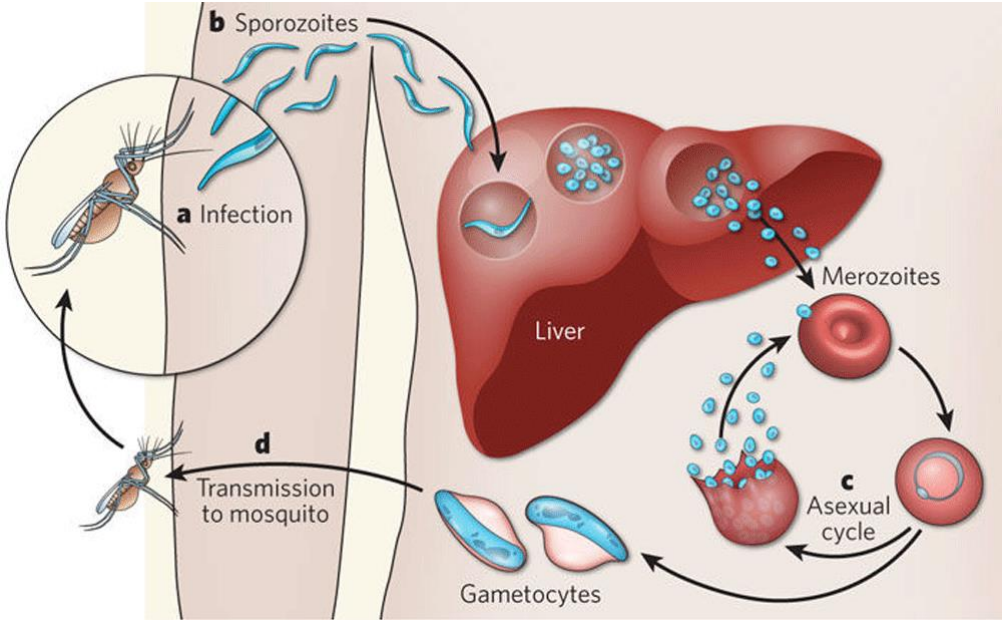
Predicting conformational changes in complex protein system using molecular dynamics

- Classic drugs are often small chemicals or proteins that bind to specific elements connected to the target disease, working as antagonist or agonist.

- Alternative strategies to classic drugs target to use elements of or train our innate immune system



Predicting conformational changes in complex protein system using molecular dynamics



- **Malaria** is caused by *Plasmodium* parasites, mainly *P. falciparum* and *P. vivax*. They spread through the bite of infected female *Anopheles* mosquitoes.
- It causes **fever, chills, headache and tiredness**, and it can be deadly if untreated. It is curable with antimalarial drugs, usually a combination therapy taken by mouth, or by injection for severe cases.

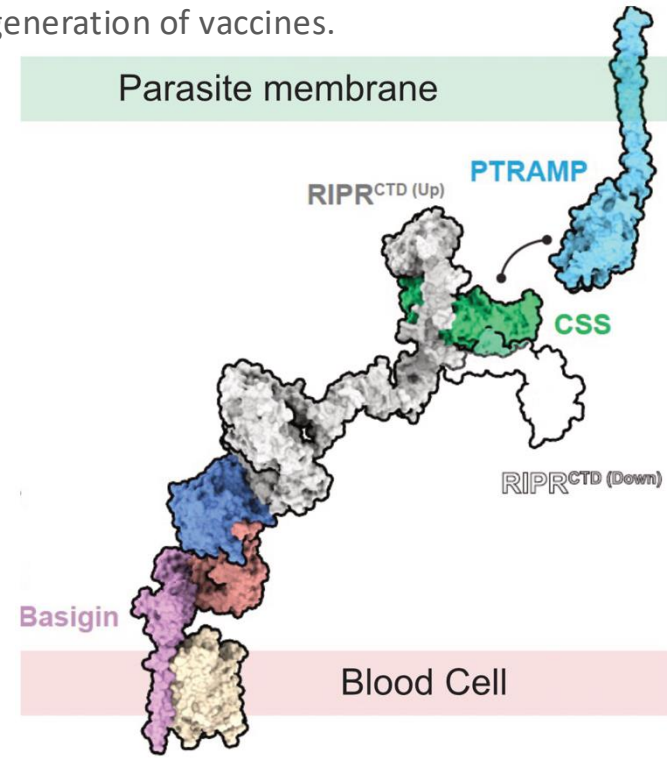
- There are **no** vaccines or antibodies-cocktail drugs for malaria

Figure from nbi.ku.dk

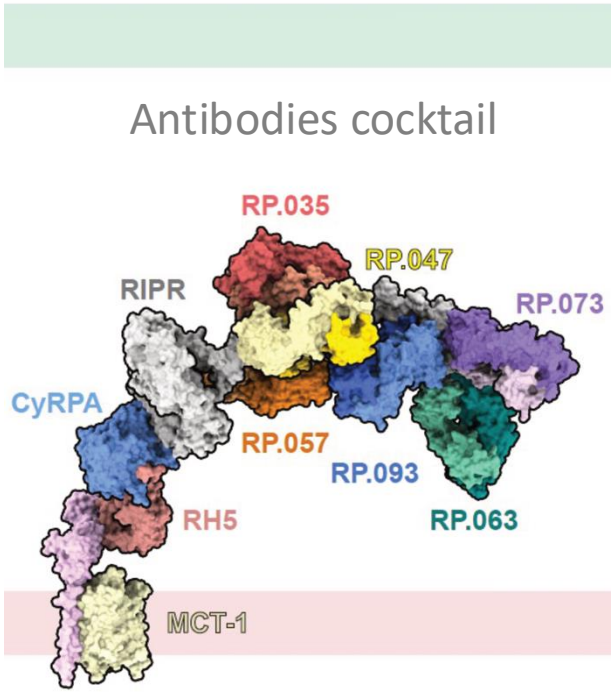
Gates Foundation

Predicting conformational changes in complex protein system using molecular dynamics

In a work lead by the Draper's lab (Biochemistry, Oxford), we worked to understand **the molecular mechanism of RIPR**, which is a protein that forms part of the malaria parasite's invasion complex and is the effect of the new generation of vaccines.



Clinical trial drug

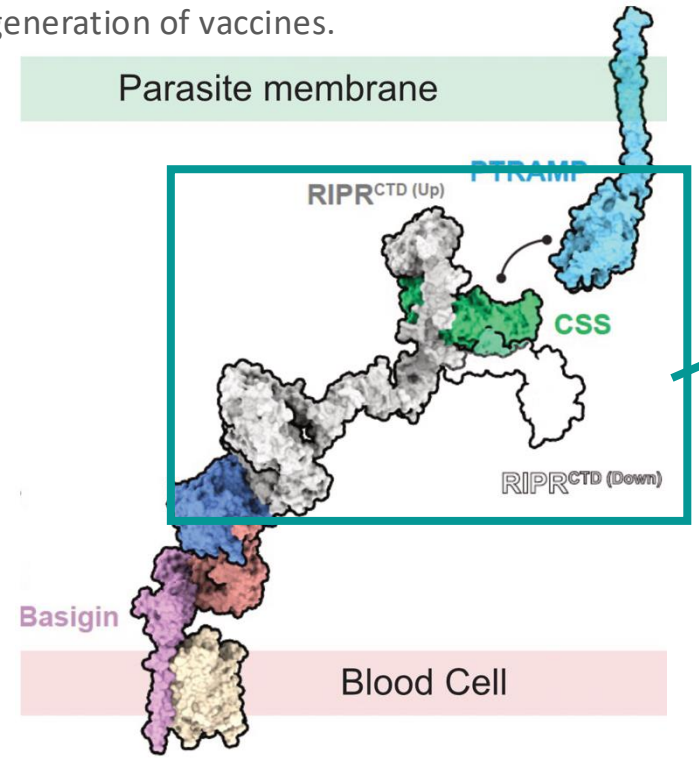


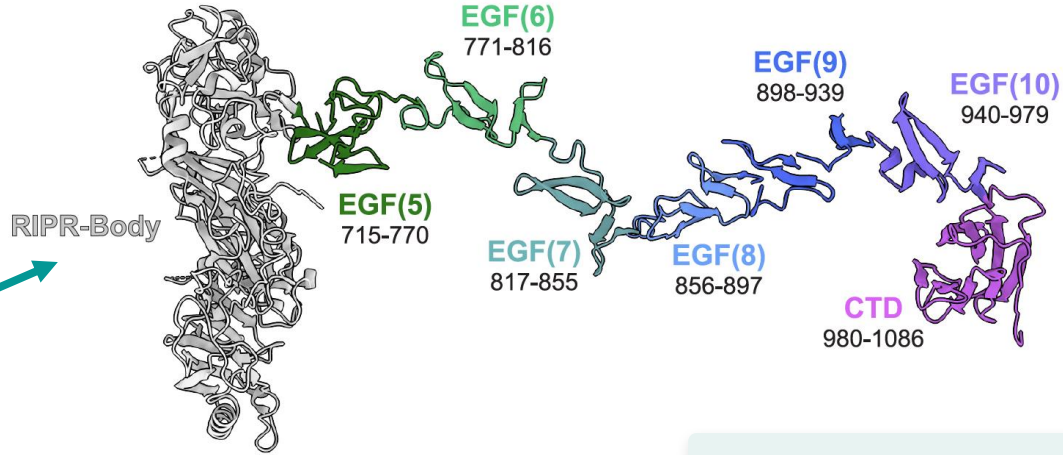
What's the mechanism of action?

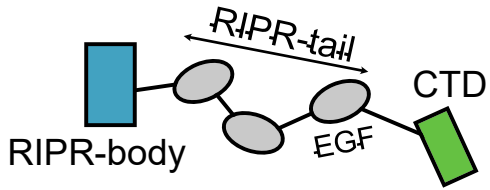
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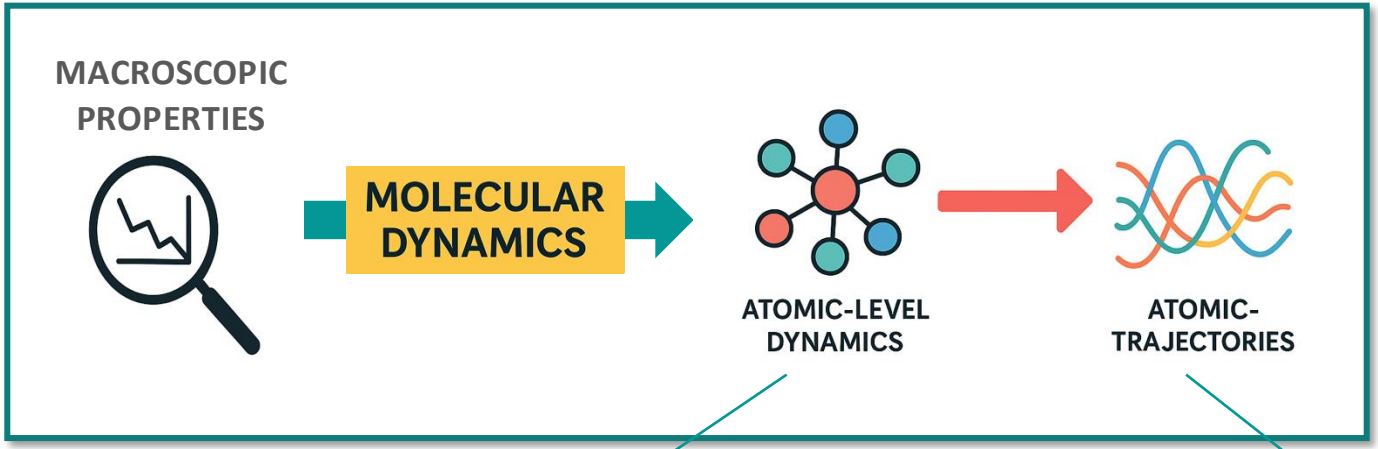


Hypothesis:
Ab mixture impaired the flexibility of the RIPR tail region

Williams, B. G., Barrett, J. R., Bartholdson Scott, J., Rigby, C. A., Cagiada, M., et al. Immunity (2026) .

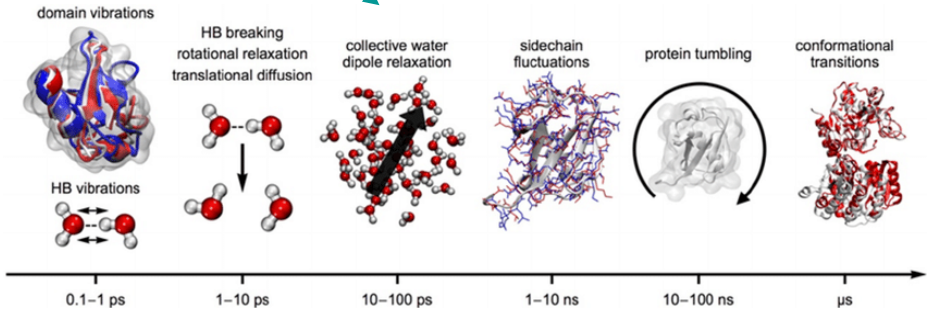
Predicting conformational changes in complex protein system using molecular dynamics

How could we study the impaired conformational landscape of this complex?



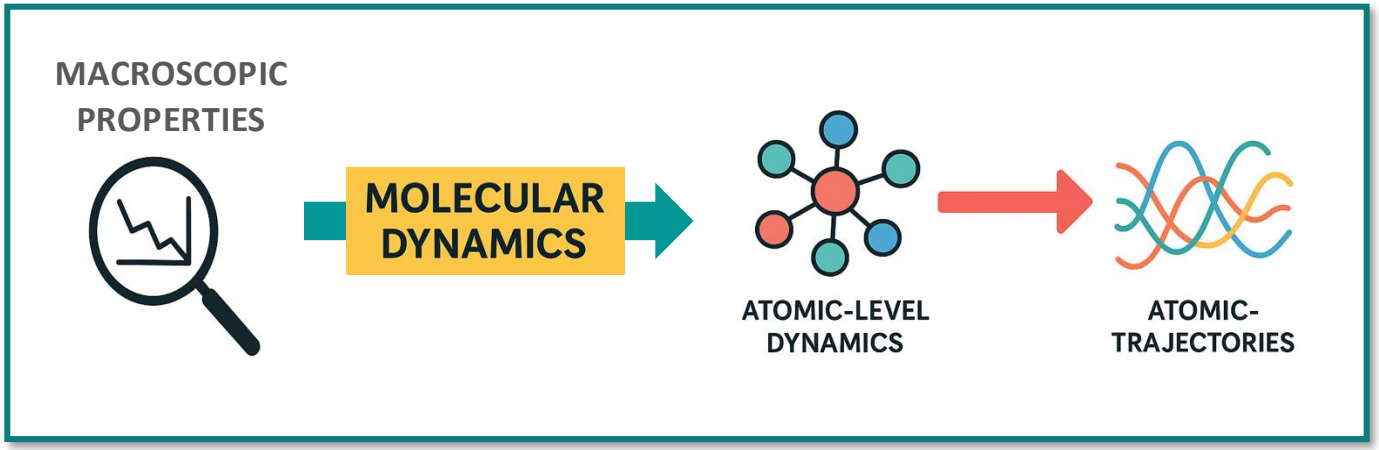
In molecular dynamic simulations estimated forces on atoms are used to update velocities and positions of atoms using Newton's equations

$$\boldsymbol{F}_i = -\frac{\partial U}{\partial \boldsymbol{r}_i} \qquad \frac{d\boldsymbol{r}_i}{dt} = \boldsymbol{v}_i \qquad m_i \frac{d\boldsymbol{v}_i}{dt} = \boldsymbol{F}_i$$



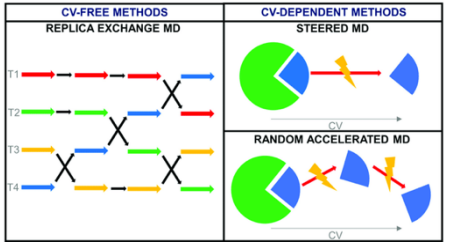
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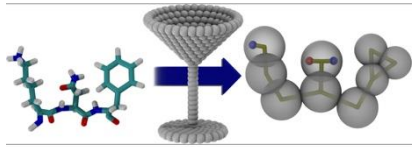


Current solutions

- Enhanced sampling techniques:



- Coarse-grained models:



- Forces evaluation is computationally expensive thus **long-time sampling is limited**, reducing the chances of sampling of biological events that happen in larger time scales

- Force fields are empirical parameterisations, and their accuracy may be limited if they are used outside of their original tuning regime.

Predicting conformational changes in complex protein system using molecular dynamics

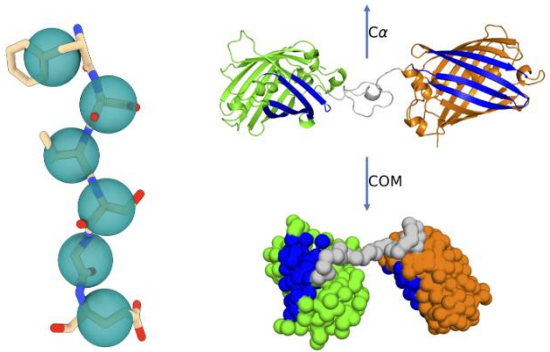
Calvados 3

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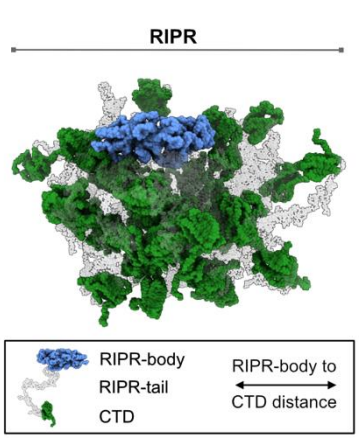
A coarse-grained model for disordered and multi-domain proteins

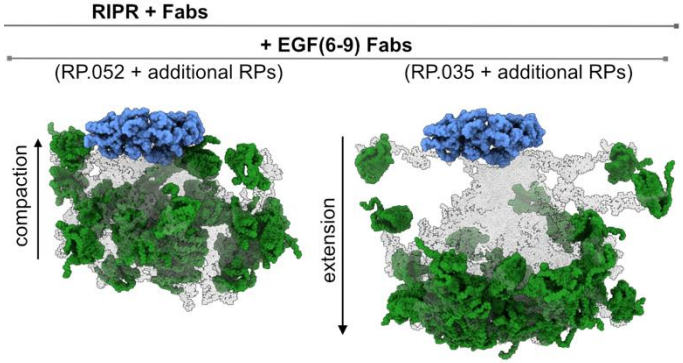
Fan Cao, Sören von Bülow, Giulio Tesei, Kresten Lindorff-Larsen

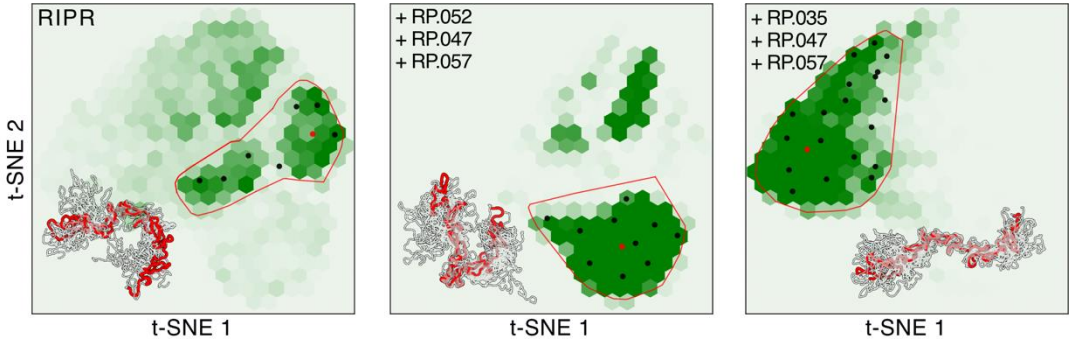
First published: 16 October 2024 | https://doi.org/10.1002/pro.5172 | Citations: 36



We aimed to characterized macromolecular property, the RIPR tail flexibility, both in isolation and in complex with mAbs



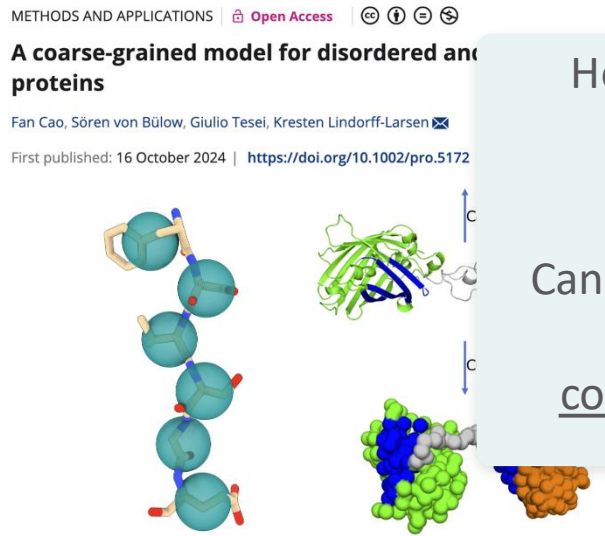




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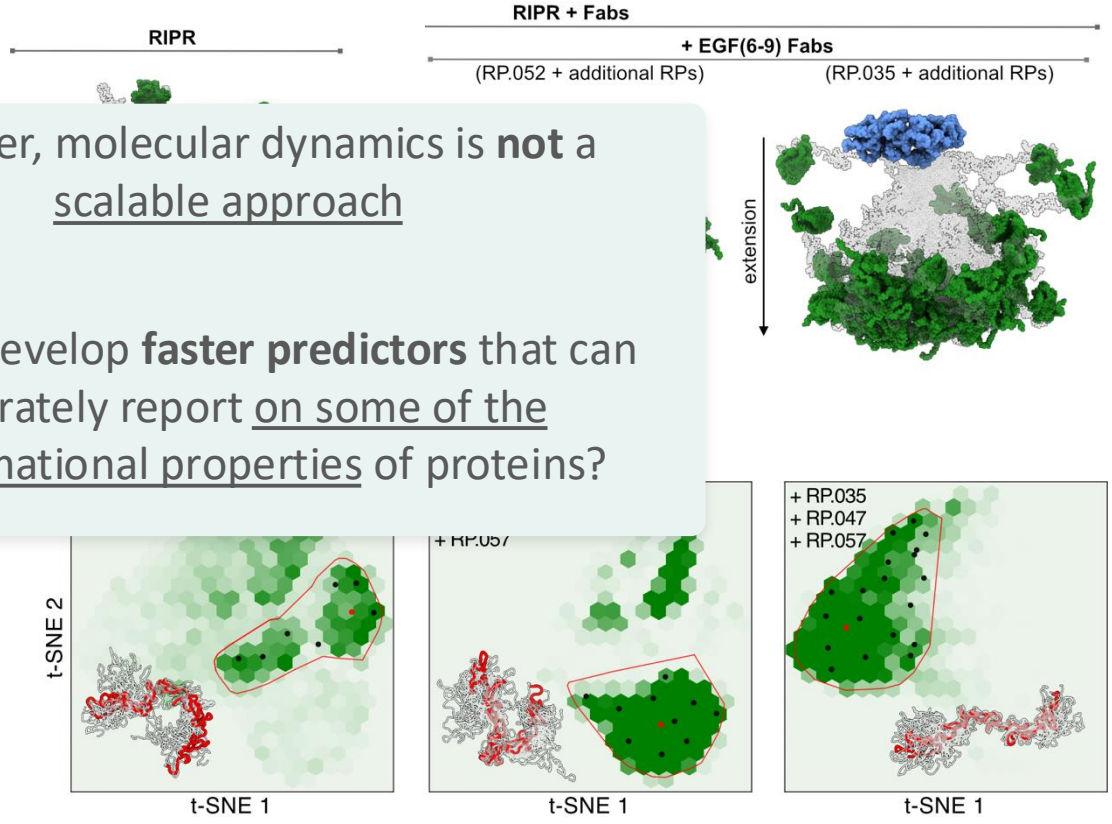
Predicting conformational changes in complex protein system using molecular dynamics

Calvados 3



However, molecular dynamics is **not** a scalable approach

Can we develop **faster predictors** that can accurately report on some of the conformational properties of proteins?

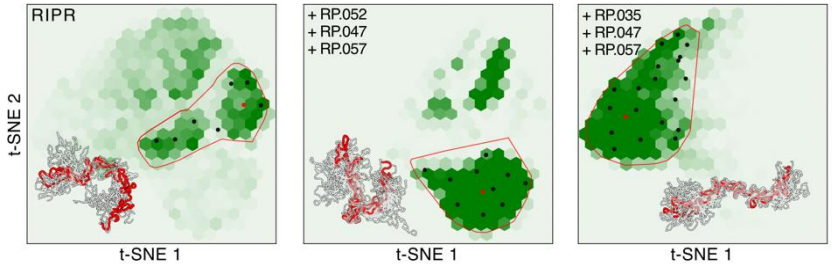


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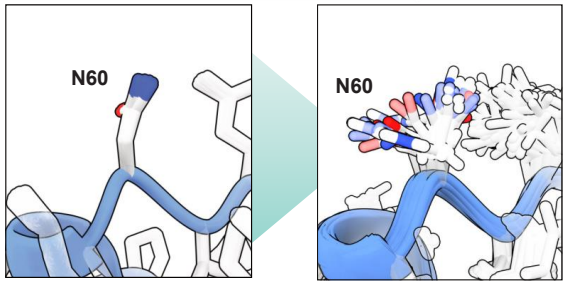
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Predicting conformational changes in complex protein system using molecular dynamics



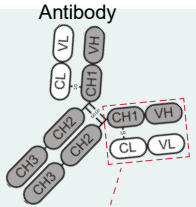
Williams, B. G., Barrett, J. R., Bartholdson Scott, J., Rigby, C. A., Cagiada, M., et al. Immunity (2026) .

Predicting amino acid sidechain conformations



Cagiada, Matteo, F.Emil Thomasen, et al., *bioRxiv* (2025).

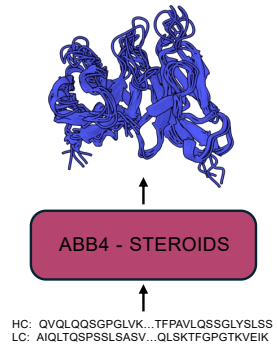
Prediction of conformational variability in immuno-receptors



We generated the largest dataset of molecular dynamics simulation of immuno-receptors (150,000)

FLAbDab & FTCRDab

And use it to train the state-of-art predictor generative model for predicting antibody-conformational ensemble.



Cagiada et al, biorvix (2025)
Spoendlin et al, biorvix (2026)

Did AI help the field of structural biology?

On certain aspects yes..

Article | Open access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold

John Jumper ✉, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman, Ellen Clancy, Michal Zielinski, ... Demis Hassabis ✉

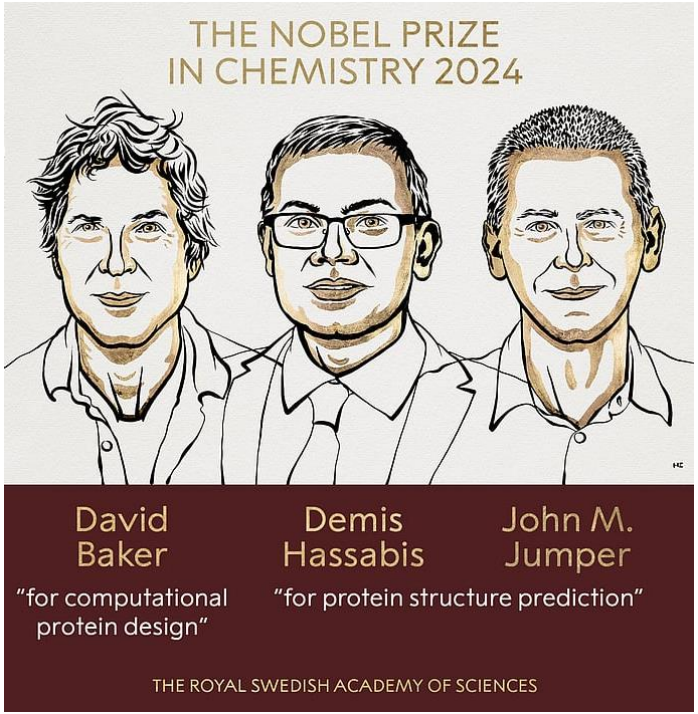
+ Show authors

Article | Open access | Published: 08 May 2024

Accurate structure prediction of biomolecular interactions with AlphaFold 3

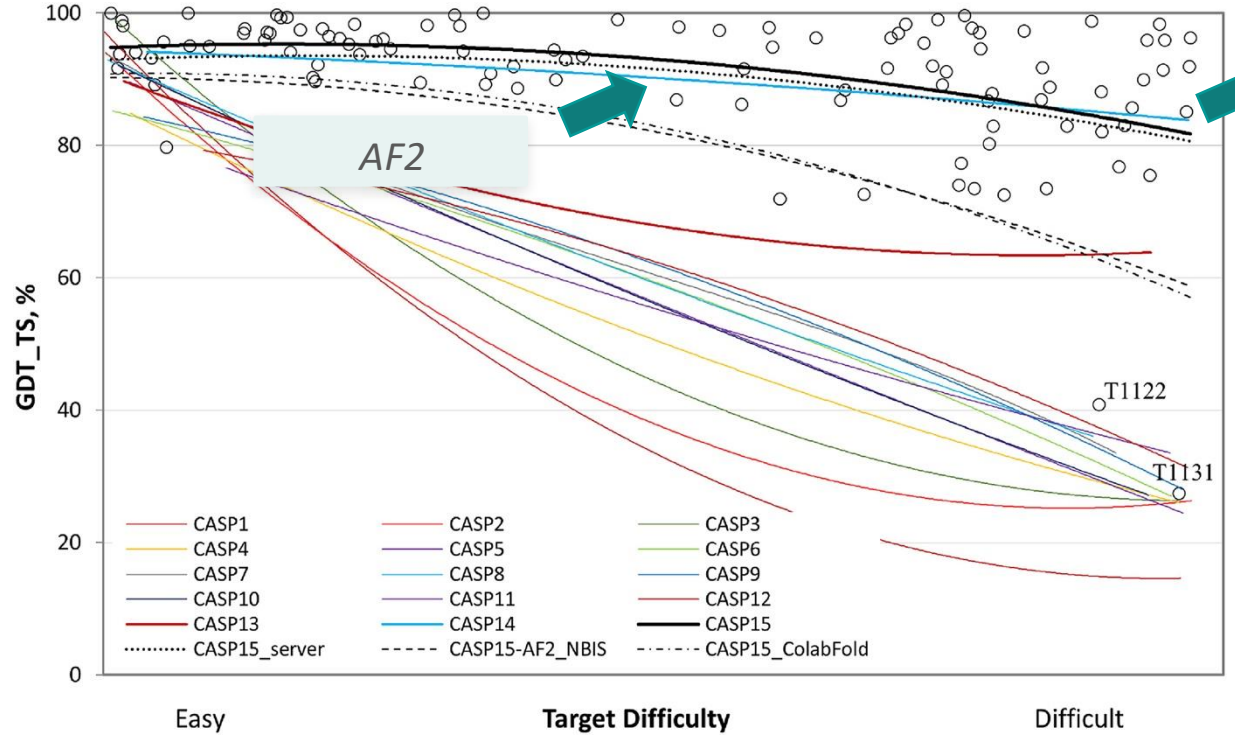
Josh Abramson, Jonas Adler, Jack Dunger, Richard Evans, Tim Green, Alexander Pritzel, Olaf Ronneberger, Lindsay Willmore, Andrew J. Ballard, Joshua Bambrick, Sebastian W. Bodenstein, David A. Evans, Chia-Chun Hung, Michael O'Neill, David Reiman, Kathryn Tunyasuvunakool, Zachary Wu, Akvilė Žemgulytė, Eirini Arvaniti, Charles Beattie, Ottavia Bertolli, Alex Bridgland, Alexey Cherepanov, Miles Congreve, ... John M. Jumper ✉

+ Show authors



Did AI help the field of structural biology?

Critical assessment of structure prediction (CASP) challenge



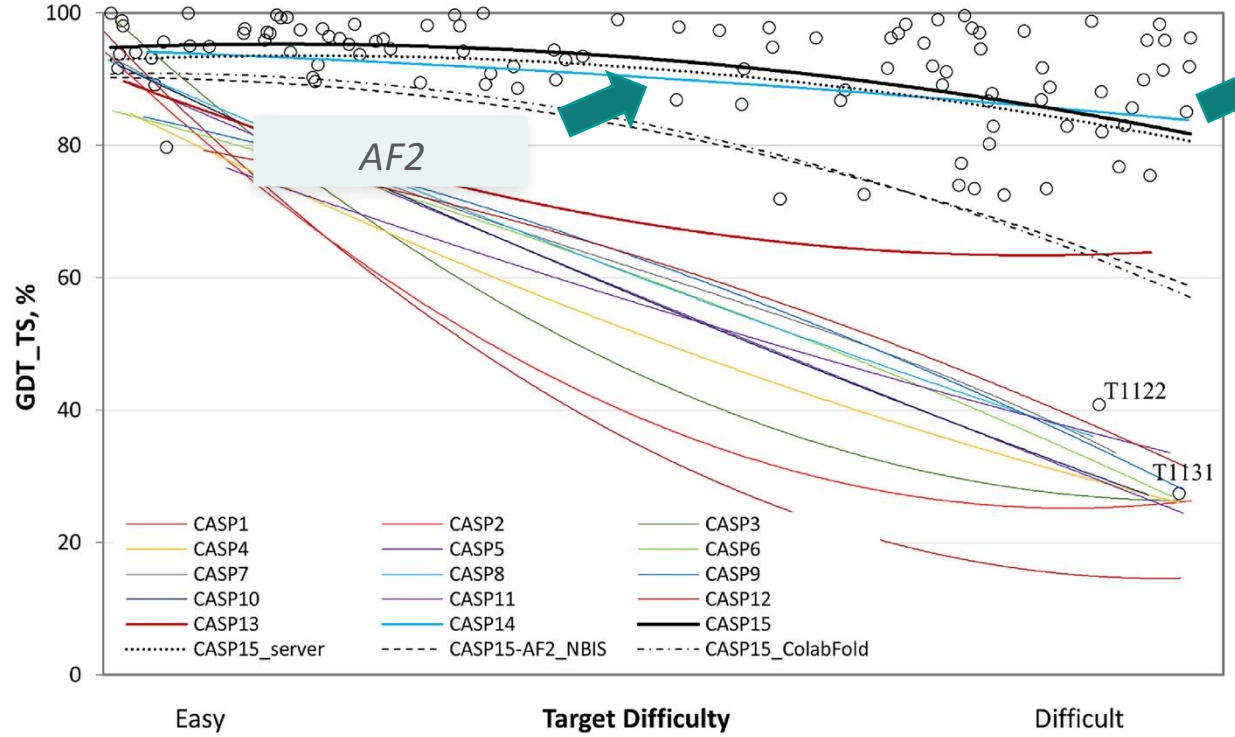




..but can we predict conformational ensembles with similar accuracy?

Did AI help the field of structural biology?

Critical assessment of structure prediction (CASP) challenge



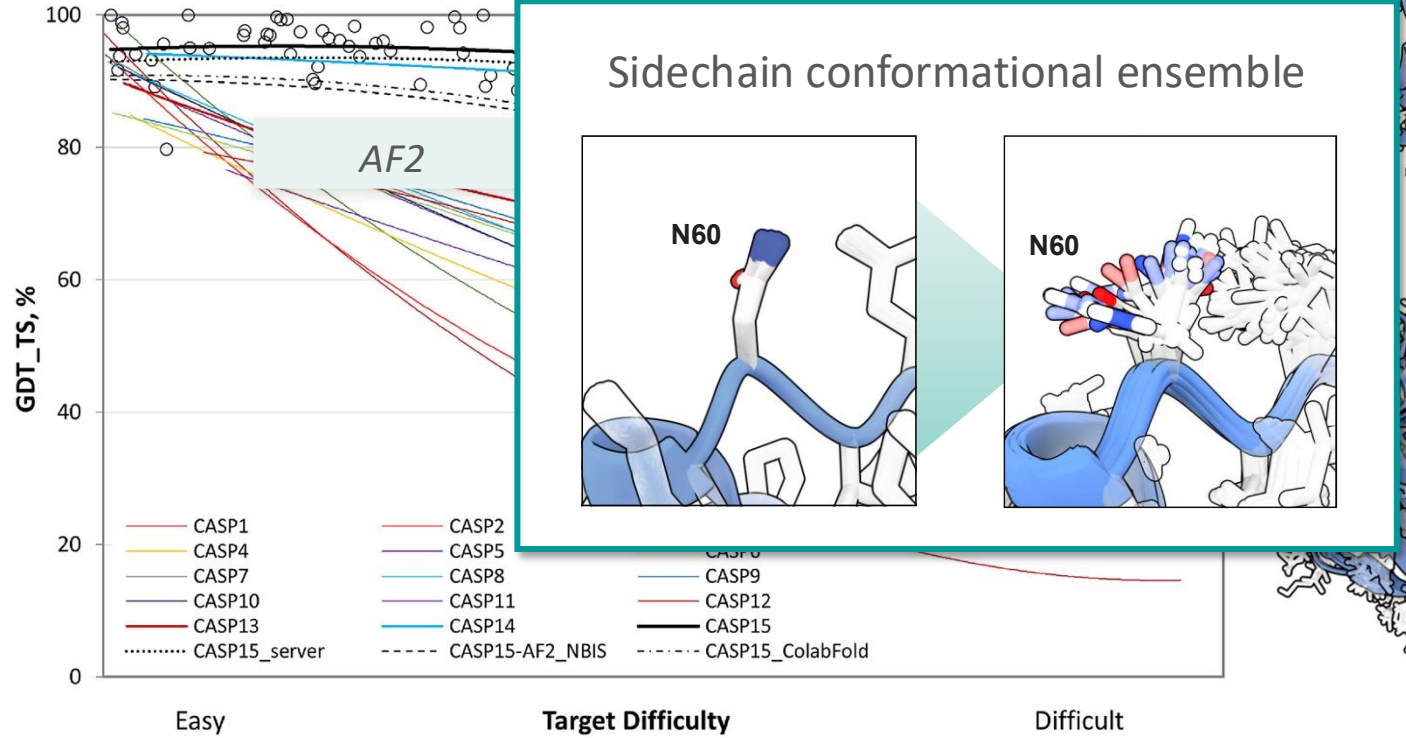


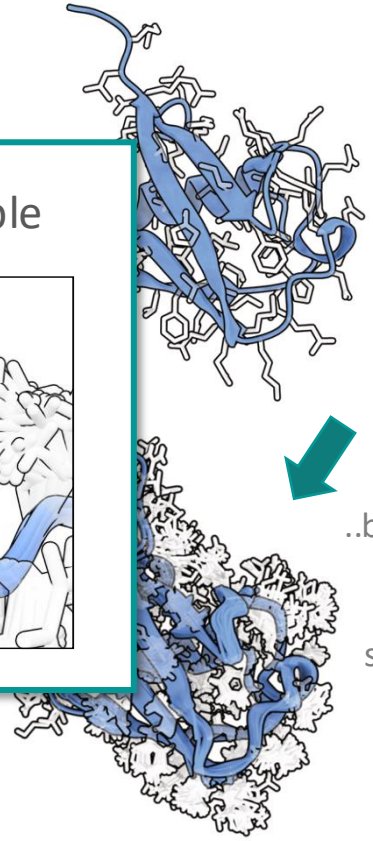


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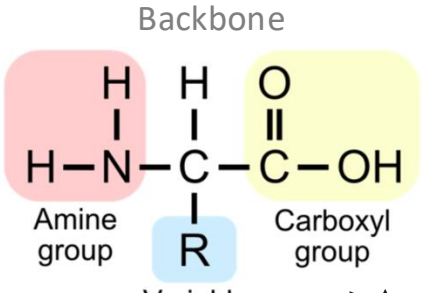
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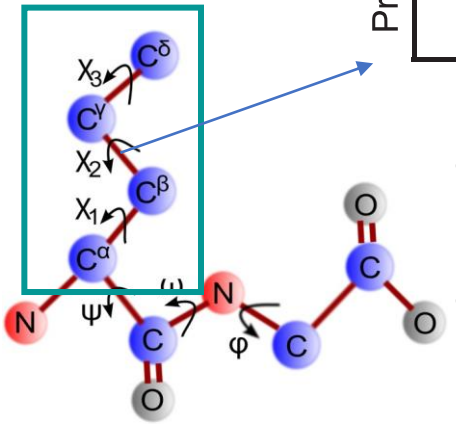


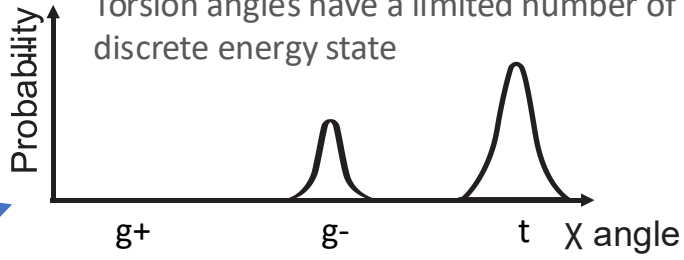


..but can we predict conformational ensembles with similar accuracy?

Improving modelling of protein side-chain conformational ensemble

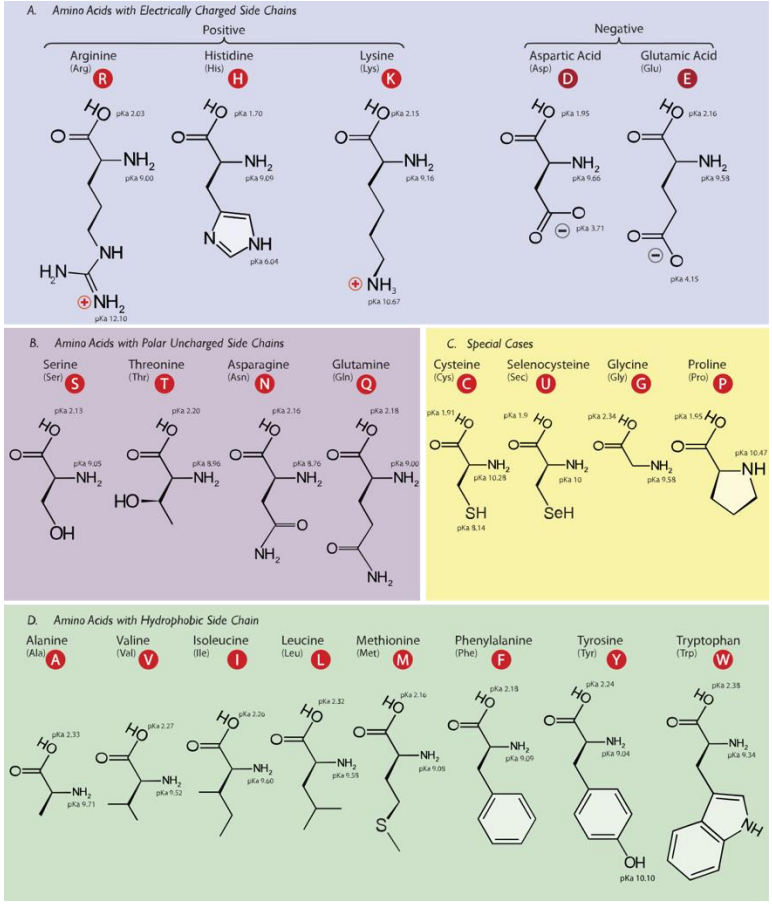




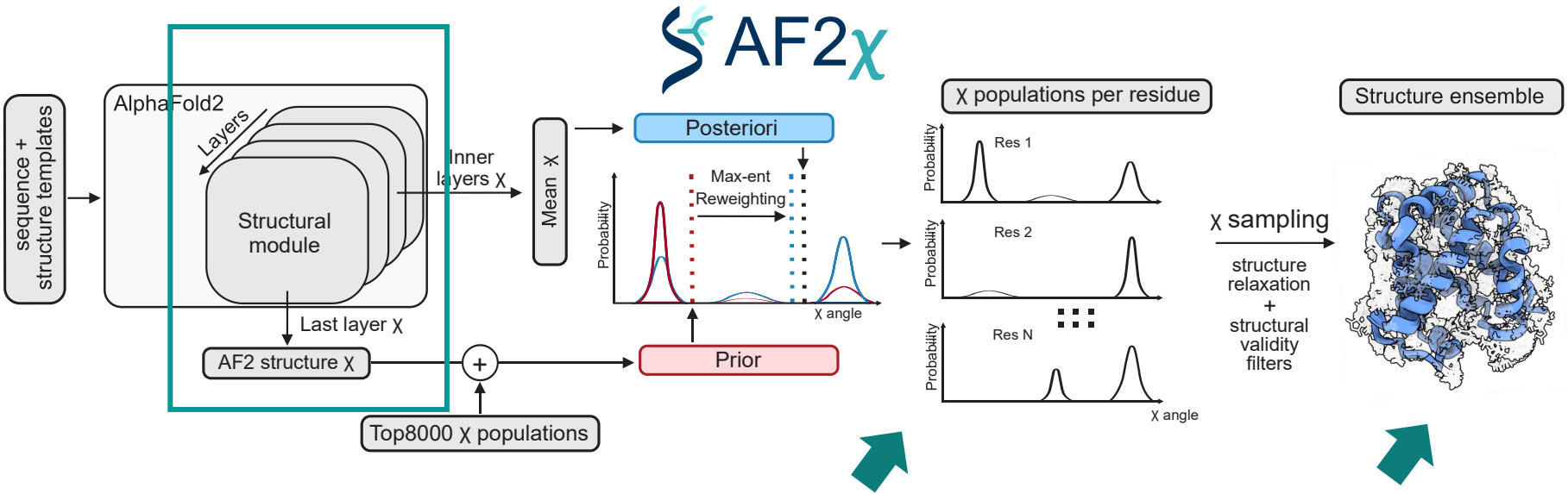


Torsion angles have a limited number of discrete energy state

- Side-chain motions **drive binding, catalysis and allostery**, and they shape stability and design accuracy.
- Their motions range from picoseconds to milliseconds for buried side chains and ring flips.



Improving modelling of protein side-chain conformational ensemble



1. Predict the **χ-angle distributions** for each side chain
2. Generate a set of conformations, i.e. **a structural ensemble**, conditioned on the dihedral distributions.

Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

Improving modelling of protein side-chain conformational ensemble

AF2 architecure

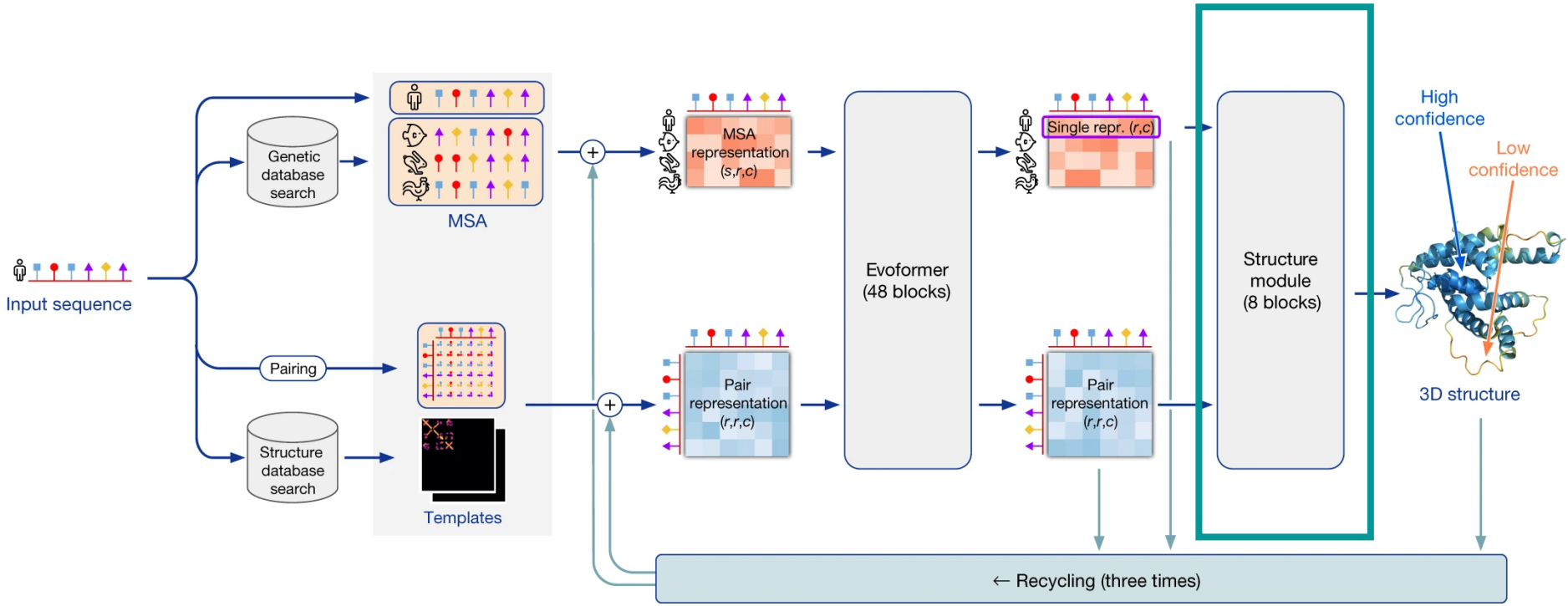


Figure from Jumper, J., et al - *Nature* 596.7873 (2021): 583-5

Improving modelling of protein side-chain conformational ensemble

AF2 Structure module

The side chain prediction unit returns prediction in the form of torsion angles (**χ–angles**).

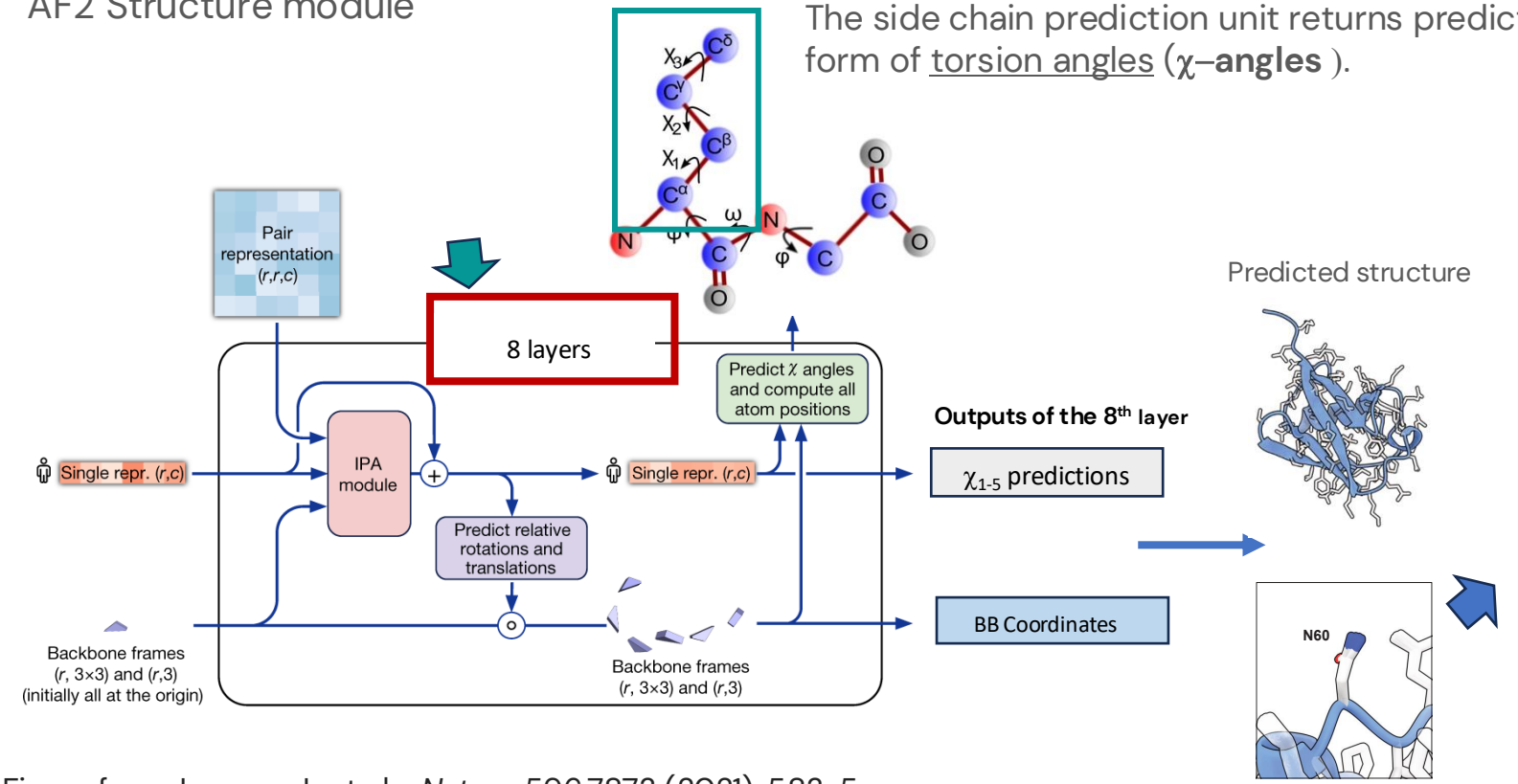
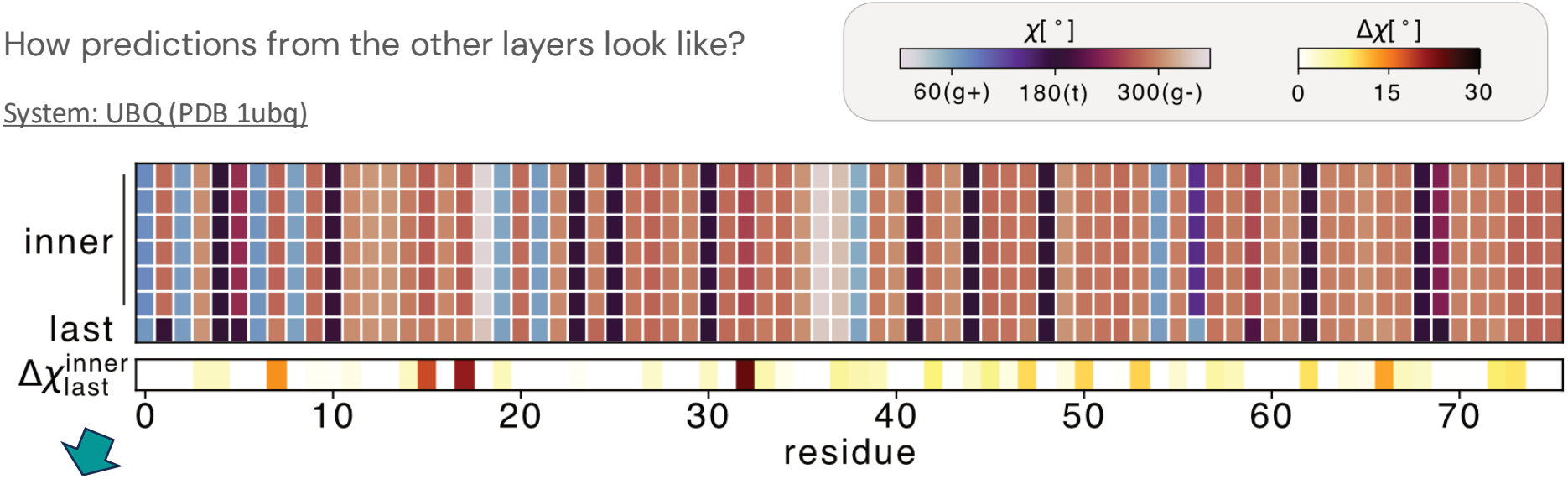


Figure from Jumper, J., et al - *Nature* 596.7873 (2021): 583-5

Improving modelling of protein side-chain conformational ensemble

How predictions from the other layers look like?

System: UBQ (PDB 1ubq)



1. Some inner layer predictions are different from the last (8th layer)
2. If different, inner layer predictions often don't match on χ-angle states

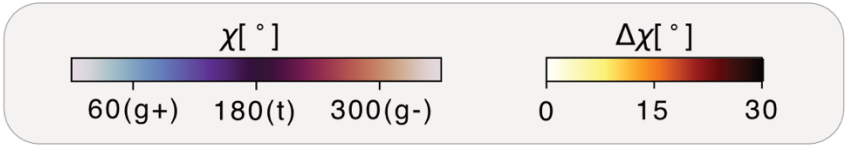
Question #1: why is this happening?

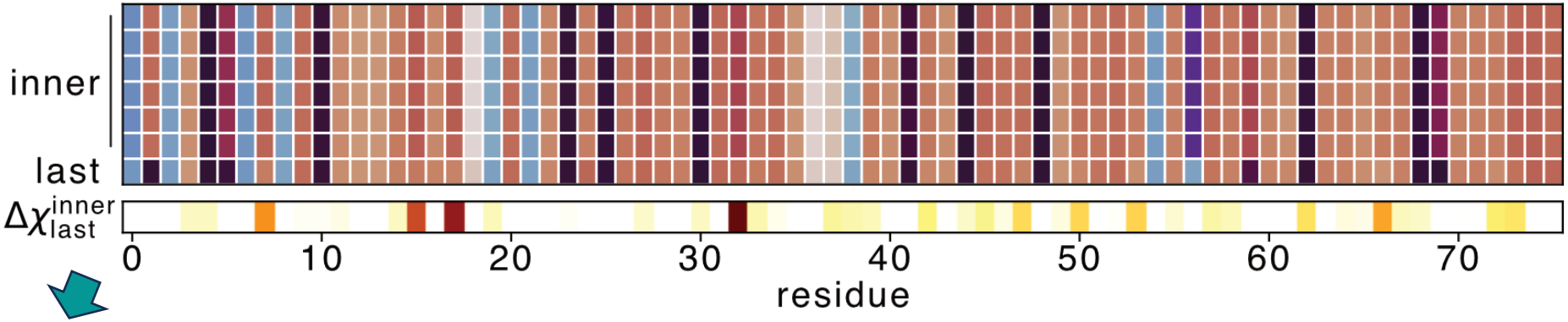
Answer: likely because **torsional losses** (χ-angle) during training were evaluated only on the last-layer values

Improving modelling of protein side-chain conformational ensemble

How predictions from the other layers look like?

System: UBQ (PDB 1ubq)





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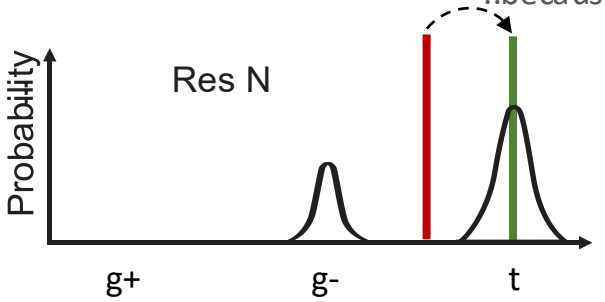
Question #2: what the inner layer values are representing?

Improving modelling of protein dynamics using generative models: AF2χ

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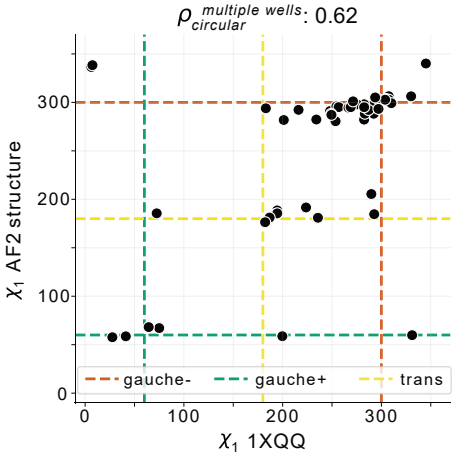
If inner layer predictions differ from last layer, often don't match on **χ-angle energy states**



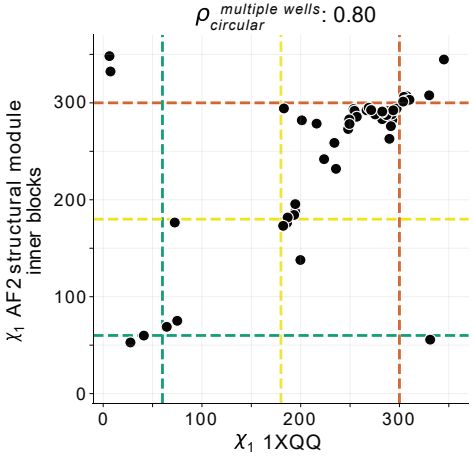


..because of torsion loss in the last layer

Hypothesis: the model may hold information about the full χ-angle distribution in the inner layers



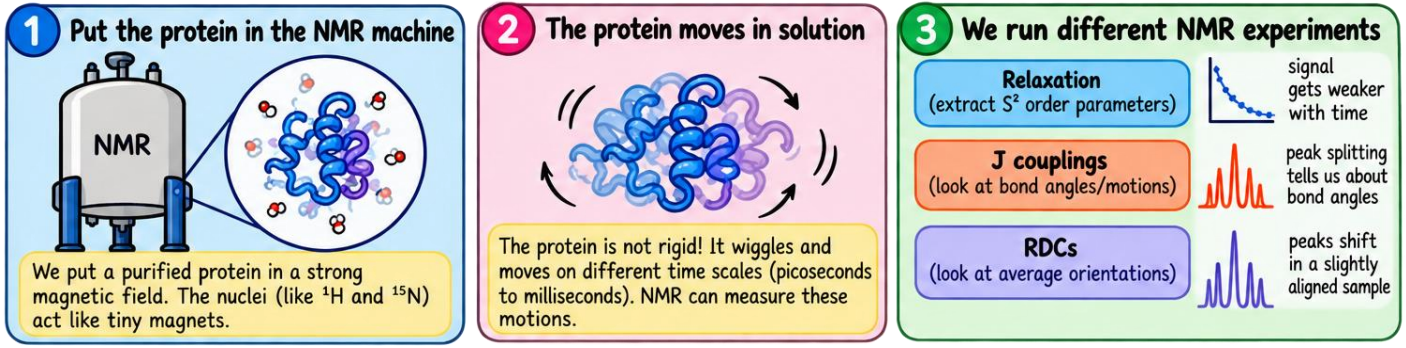
(frame-averages from NMR-constrained ensemble)



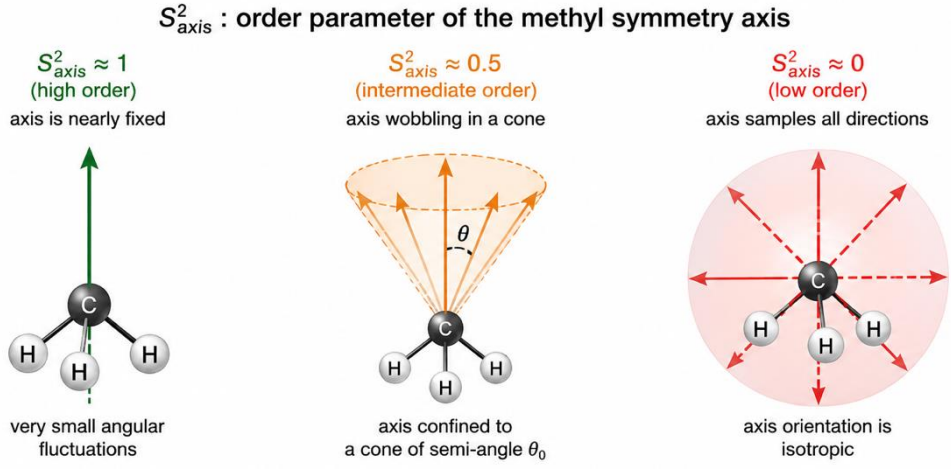
(frame-averages from NMR-constrained ensemble)

Improving modelling of protein dynamics using generative models: AF2χ

Validation on experimental data:

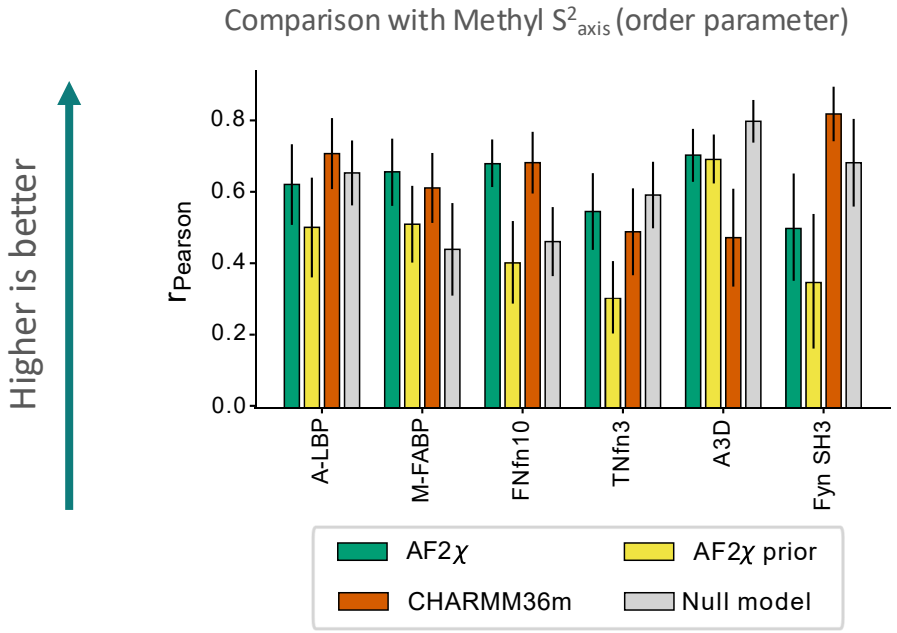


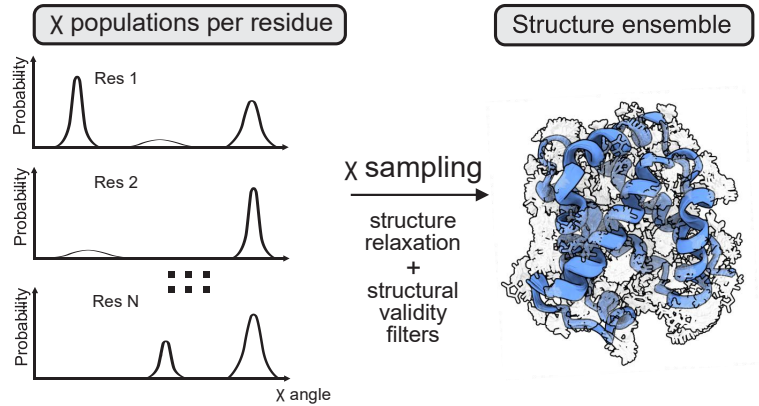
Methyl S^2_{axis} (order parameter): which **quantifies the restriction of the methyl CH_3 symmetry axis** (in this case this measure is proportional to the re-orientation of the covalent bond of the C-CH_3 bond during time *)



Improving modelling of protein dynamics using generative models: AF2χ

AF2χ is as accurate as molecular dynamics in predicting side chains heterogeneity



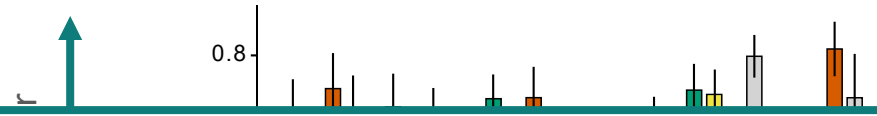


Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

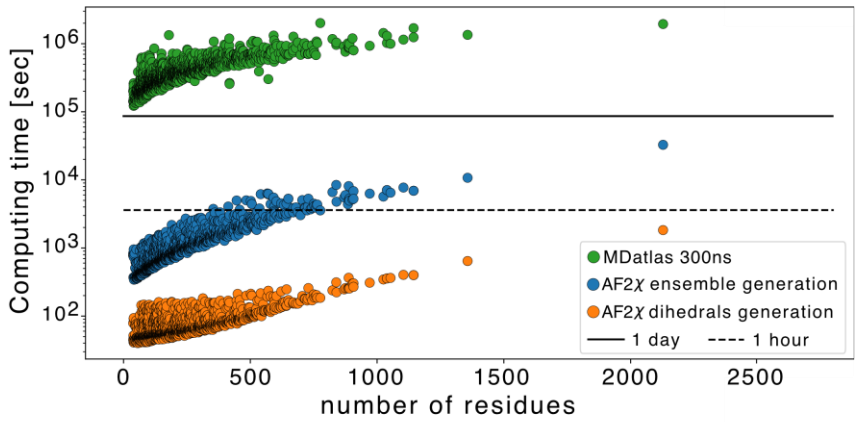
Improving modelling of protein dynamics using generative models: AF2χ

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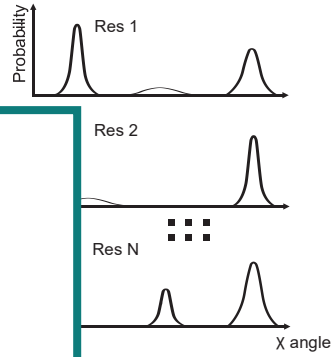
Comparison with Methyl S^2_{axis} (order parameter)



AF2χ is order of magnitude faster than MD



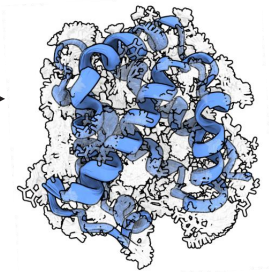
χ populations per residue



χ sampling

structure relaxation + structural validity filters

Structure ensemble



Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

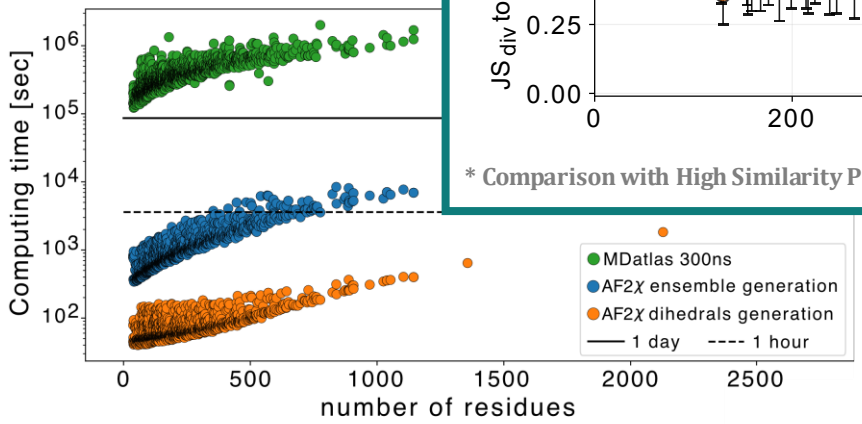
Improving modelling of protein dynamics using generative models: AF2χ

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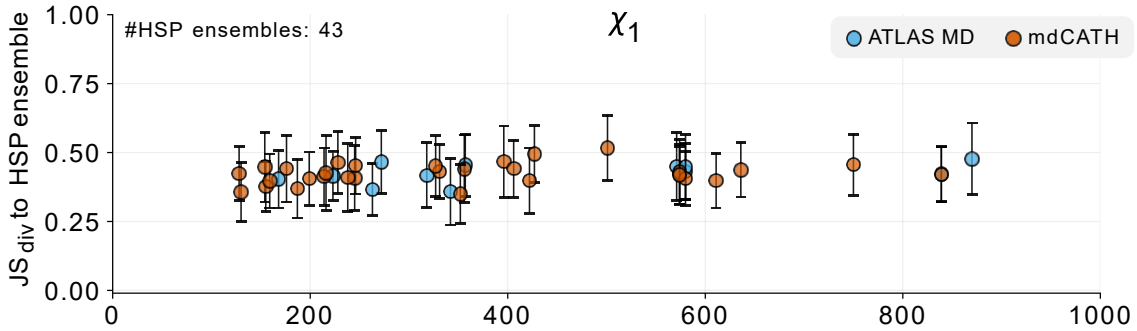
Comparison with Me



AF2χ is order of ma



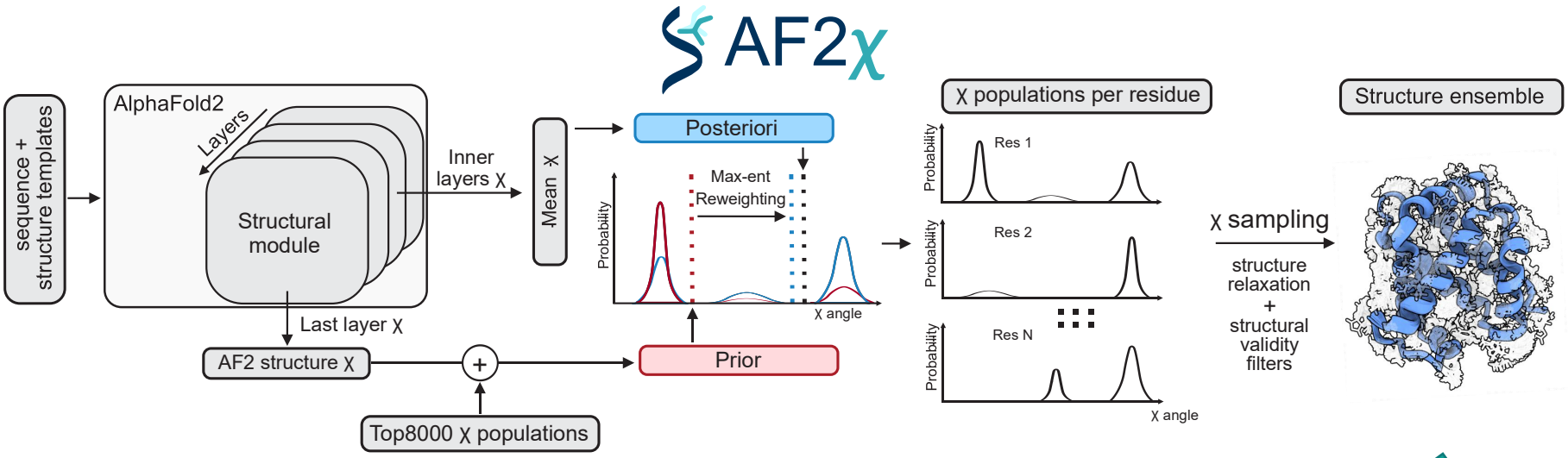
AF2χ is accurate independently of protein fold or size



* Comparison with High Similarity PDB Ensembles, using systems from ATLAS MD and mdCATH

Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

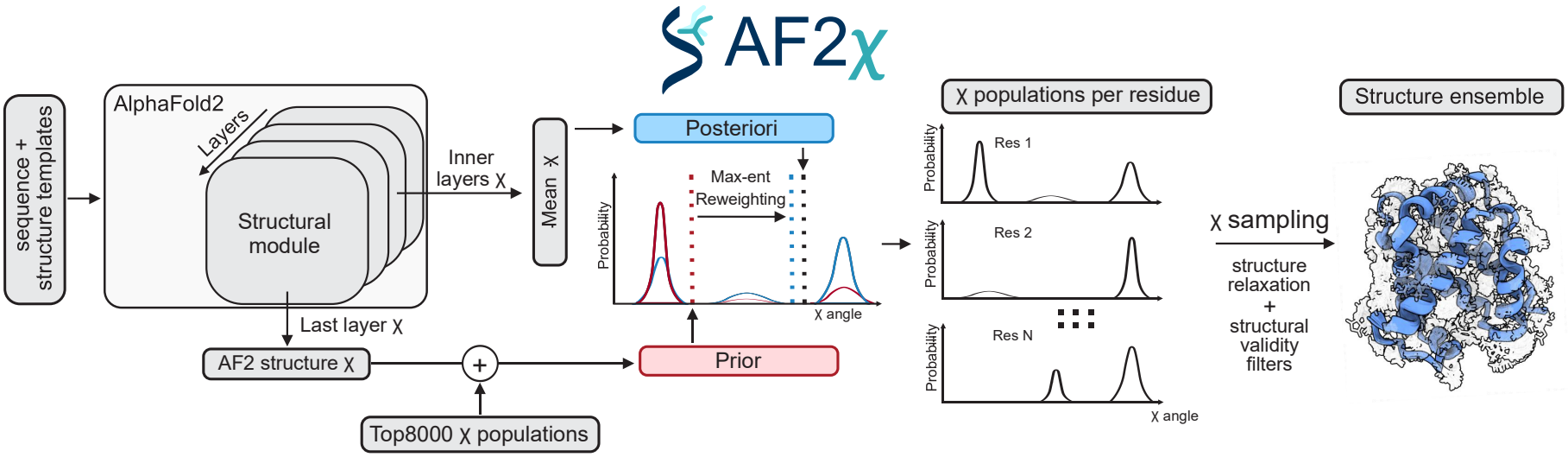
Improving modelling of protein dynamics using generative models: AF2χ



Use **AF2χ conformational ensembles** in design tasks, such as **binding and stability** prediction.

Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

Improving modelling of protein dynamics using generative models: AF2χ



AI Take-home messages:

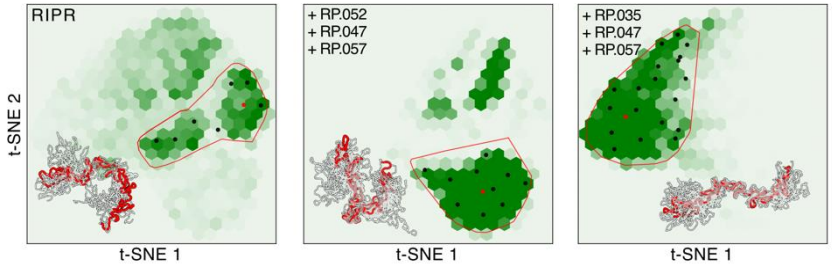
1. If we know where to look (we understand the architecture), we can learn more information from deep learning architectures that **are not the main training objects**.

2. In this case, the model **helped by speeding up the process**.

Cagiada, Matteo, et al. "AF2χ: Predicting protein side-chain rotamer distributions with AlphaFold2." *bioRxiv* (2025).

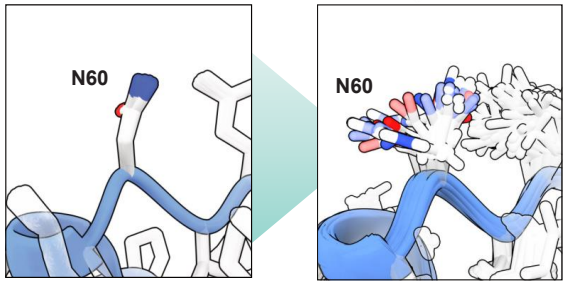
Agenda:

Predicting conformational changes in complex protein system using molecular dynamics



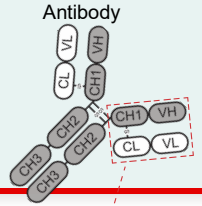
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Predicting amino acid sidechain conformations



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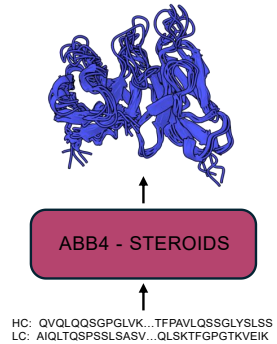
Prediction of conformational variability in immuno-receptors



We generated the largest dataset of molecular dynamics simulation of immuno-receptors (150,000)

FlAbDab & FTCRDab

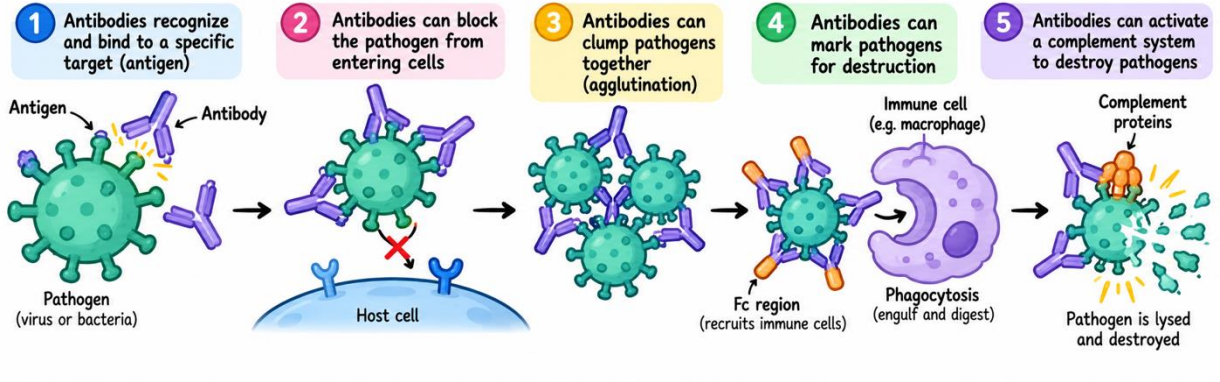
And use it to train the state-of-art predictor generative model for predicting antibody-conformational ensemble.

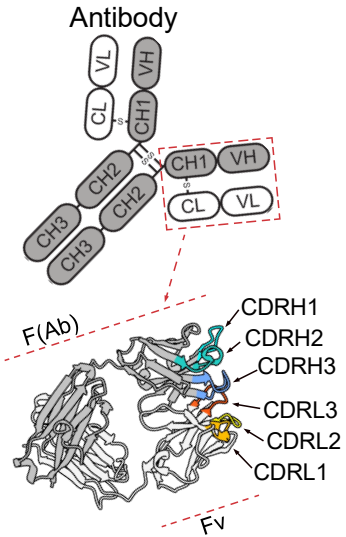


Cagiada et al, biorvix (2025)
Spoendlin et al, biorvix (2026)

Improving modelling of protein dynamics using generative models

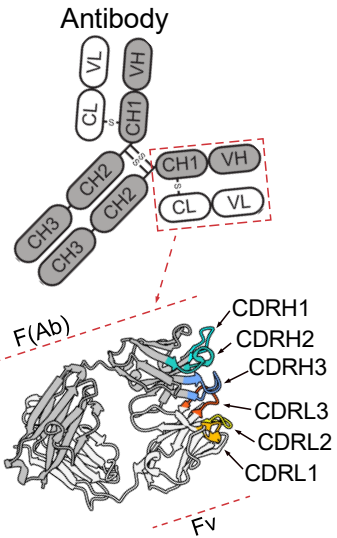
Antibodies are immuno-receptors that recognise and bind specific antigens (pathogens, toxins, or foreign molecules) flagging them for destruction by other immune cells.

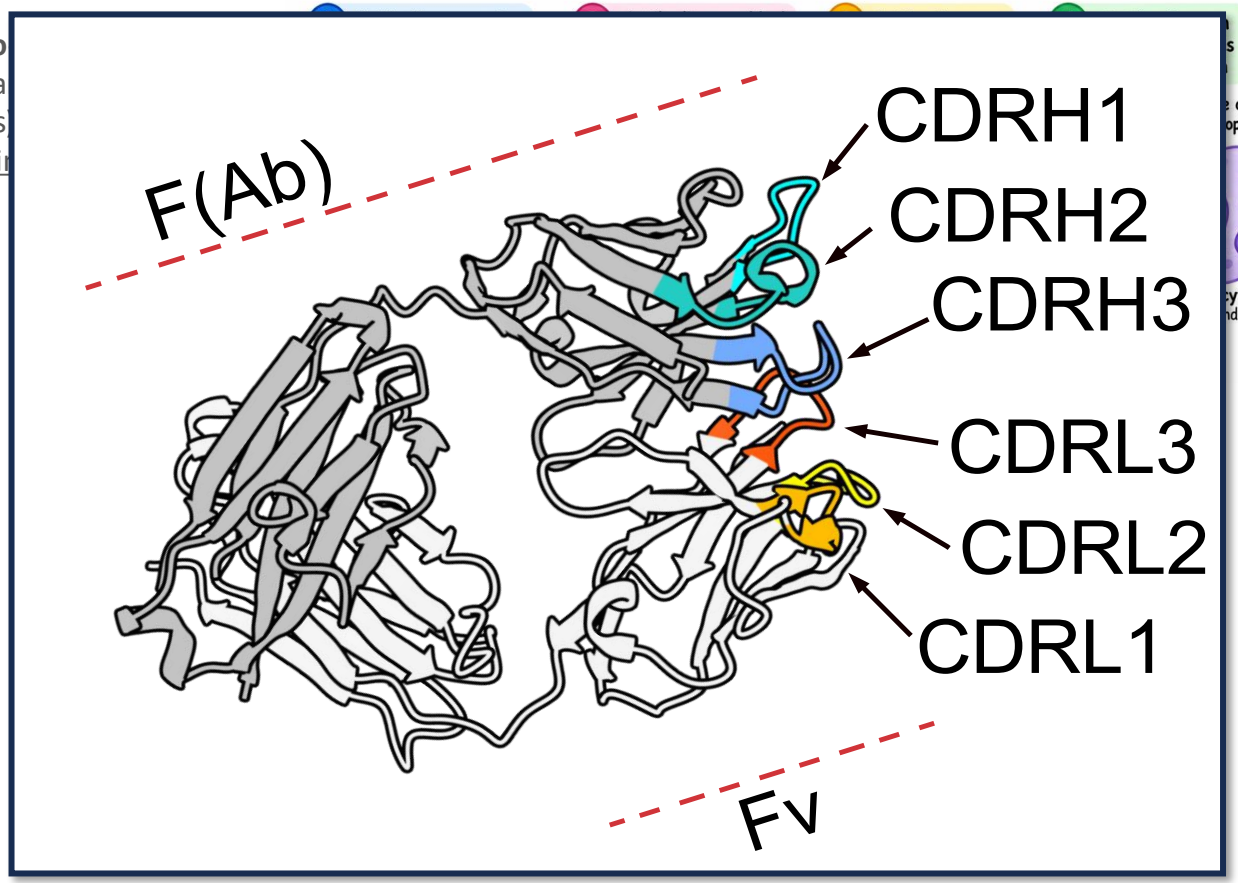


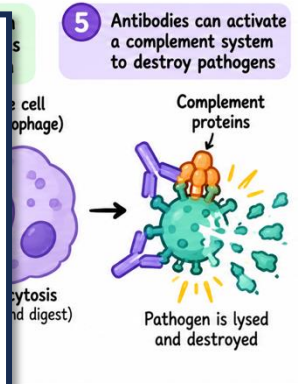


Improving modelling of protein dynamics using generative models

Antibodies are immuno
recognise and bind specific a
toxins, or foreign molecules
destruction by other i





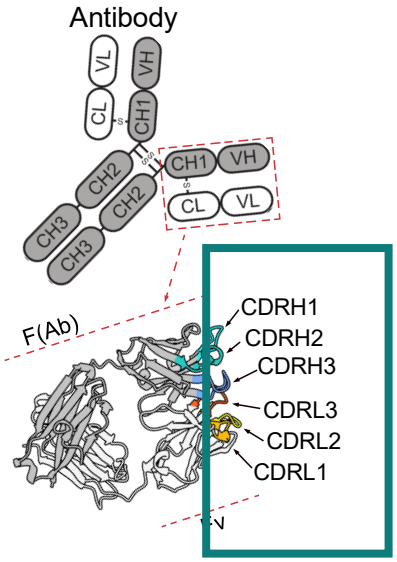


Improving modelling of protein dynamics using generative models

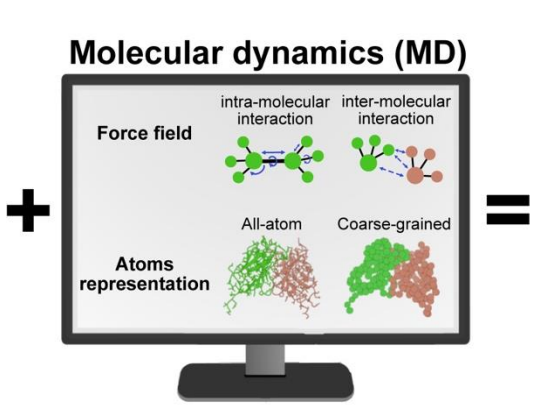
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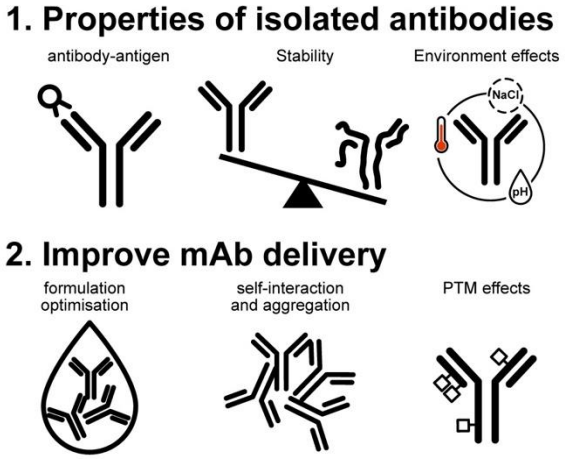
In order to design more efficient antibodies, we need to understand the connection between **sequence and flexibility, particularly with regard to CDRs**.

Generation of conformational ensembles at a large scale



Conformation heterogenicity, especially that of CDR loops, is directly connected to their ability to bind, as well as to other properties such as specificity, stability, propensity to aggregate..





Cagiada M & Deane C.M. - Moving the antibody.. , Current Opinion in Structural Biology (2026)

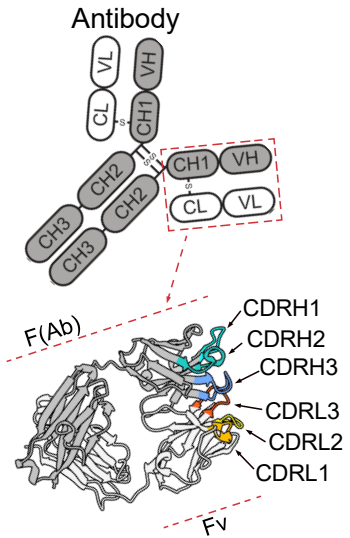
Improving modelling of protein dynamics using generative models: FlAbDab

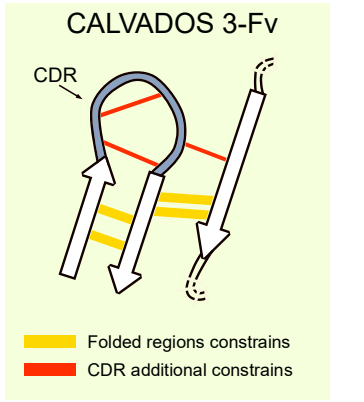
Antibodies are immuno-receptors that recognise and bind specific antigens (pathogens, toxins, or foreign molecules) flagging them for destruction by other immune cells.

In order to design more efficient antibodies, we need to understand the connection between **sequence and flexibility, particularly with regard to CDRs**.

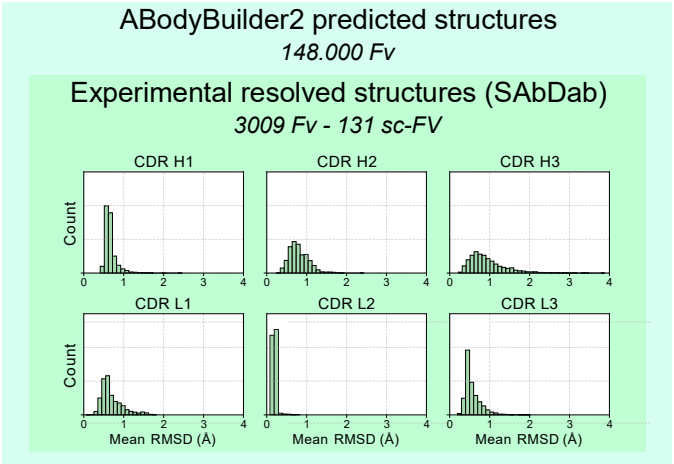
Generation of conformational ensembles at a large scale

FlAbDab & FTCRDab





MD simulations

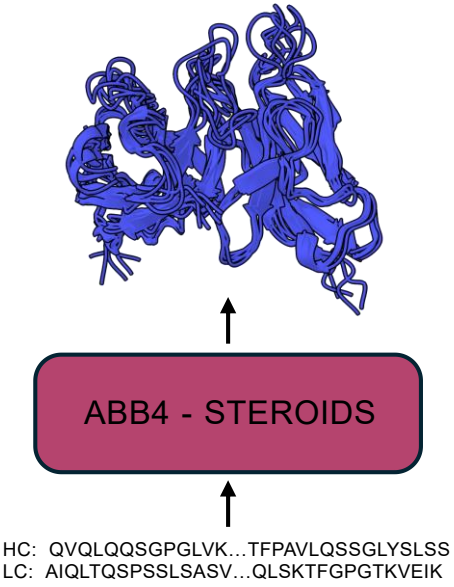


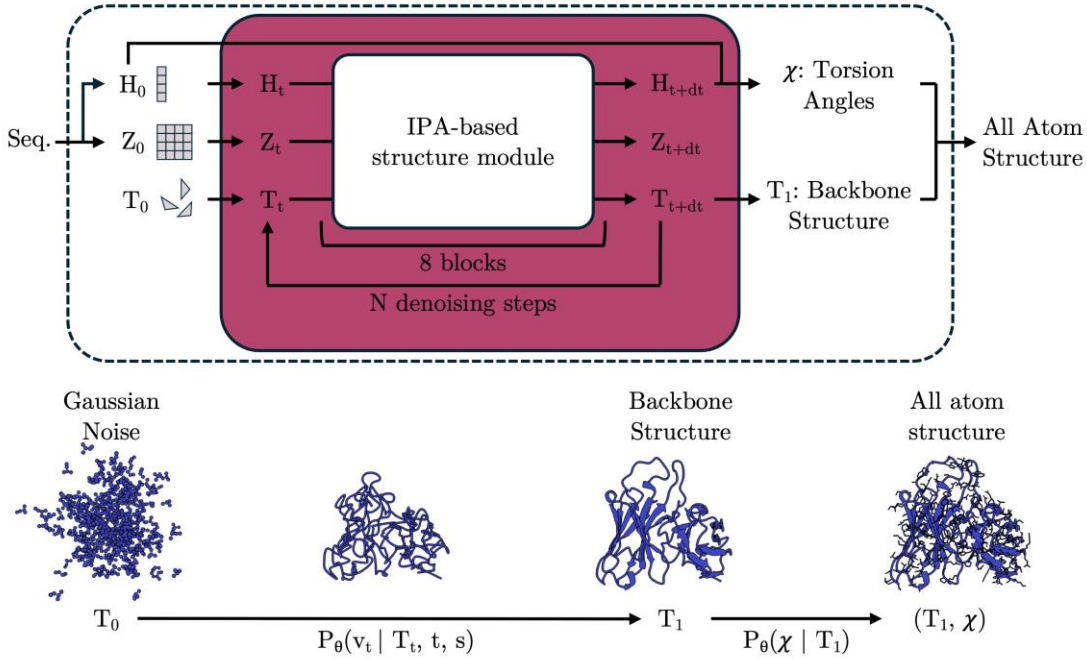
- Investigate the biochemistry behind CDR flexibility and its connection to function.
- **Train new structural predictors**, especially those that aim to generate conformational ensembles.

Cagiada, Spoendlin, et al., biorxiv, (2025)

Improving modelling of protein dynamics using generative models: ABB4-STEROIDS

ABB4-STEROIDS is a generative model using flow matching to generate conformational ensemble of antibodies.



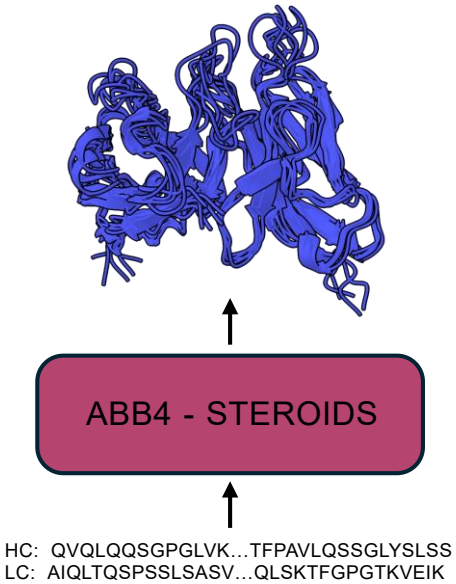


Spoendlin, Cagiada et al., biorxiv, (2026)

Improving modelling of protein dynamics using generative models: ABB4-STEROIDS

ABB4-STEROIDS is a generative model using flow matching to generate conformational ensemble of antibodies.

- We trained the model in using sequential stages:

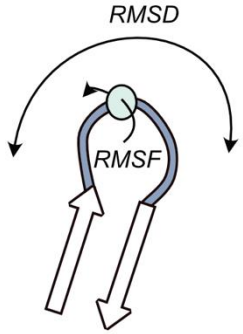


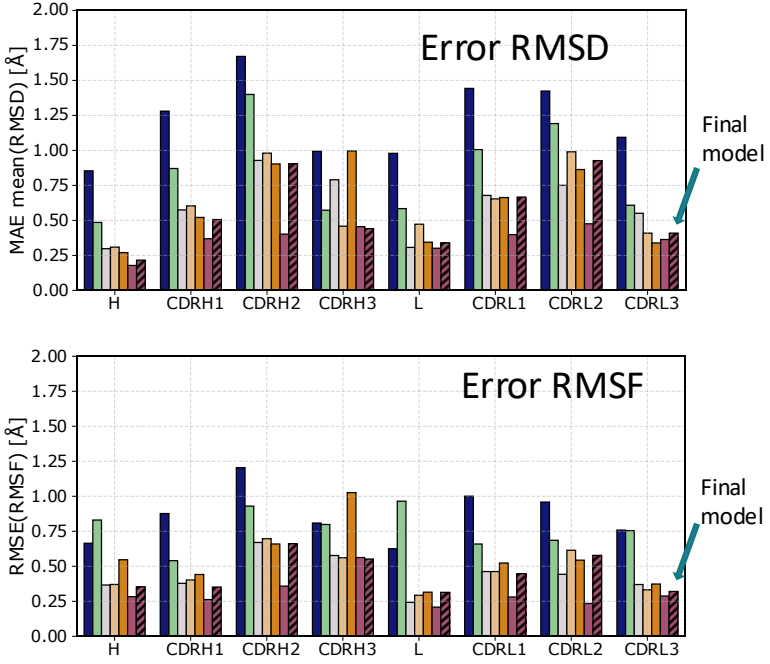
Spoendlin, Cagiada et al., biorxiv, (2026)

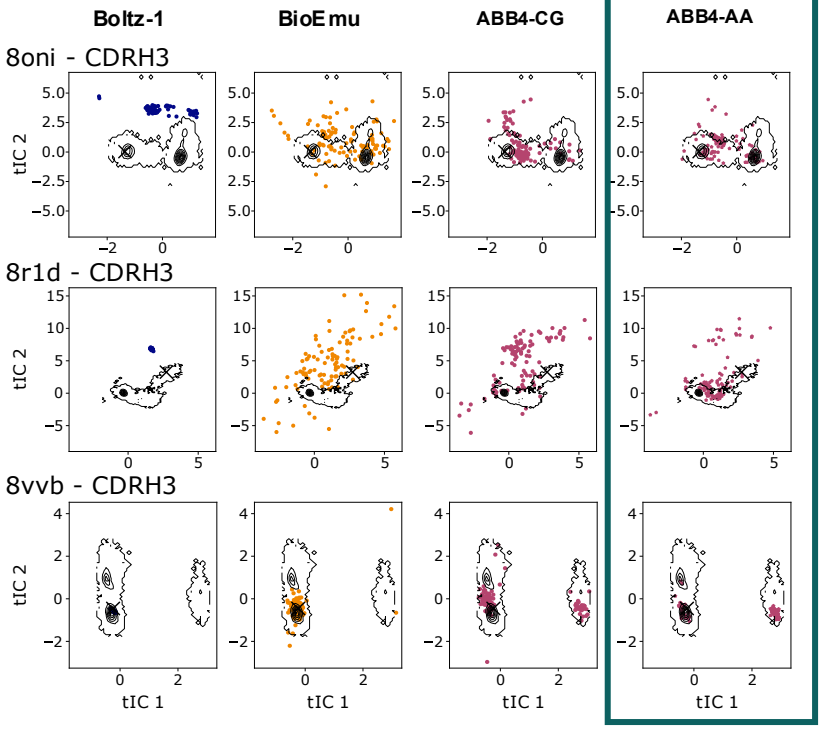
Improving modelling of protein dynamics using generative models: ABB4-STEROIDS

- Overall better agreement with ensemble dynamics metrics, reflecting better sampling

… the final **all-atom training fix the conformation population agreement** when necessary.





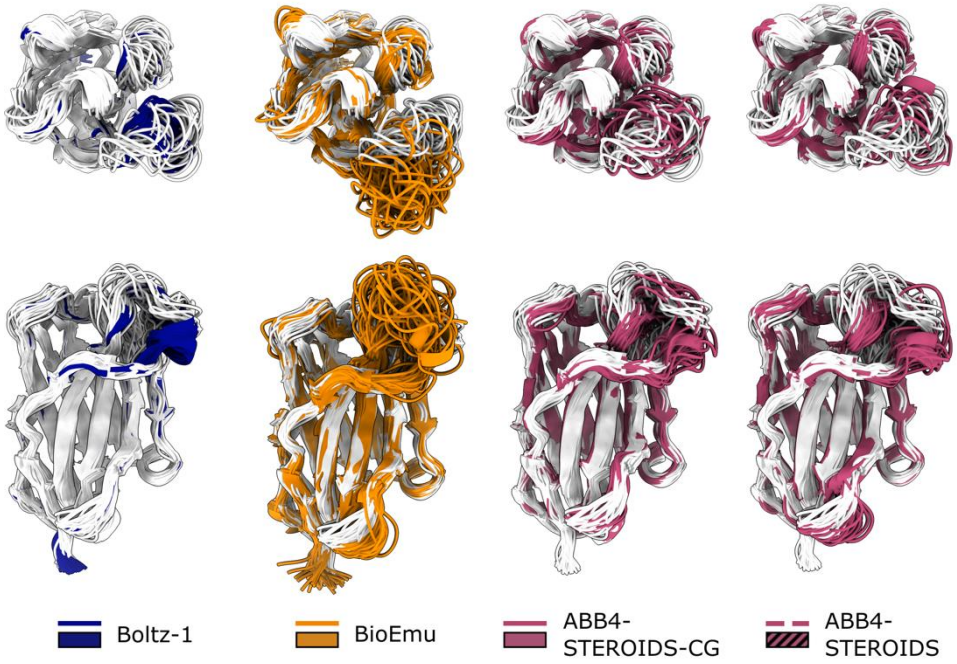


Boltz-1 | AF2 MSA s. | aSAM | AlphaFlow | BioEmu | ABB4-STEROIDS-CG | ABB4-STEROIDS

Spoendlin, Cagiada et al., biorxiv, (2026)

Improving modelling of protein dynamics using generative models: ABB4-STEROIDS

PDB: 9DS1 – Heavy chain



Large NMR experimental validation is on its way

In collaboration with Andrew Baldwin (Oxford – Dept. of Biochemistry)

AI Take-home messages:

- Training models often require a lot of data, but we can generate training data more efficiently.
- New training objectives require us to rethink our training strategies and loss functions.

Spoendlin, Cagiada et al., biorxiv, (2026)

Acknowledgements

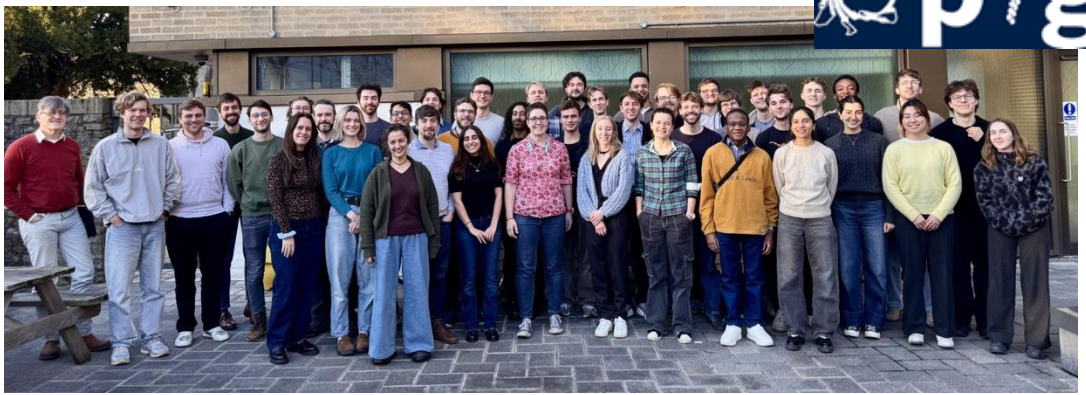
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