Bioinformatics for structural biologists

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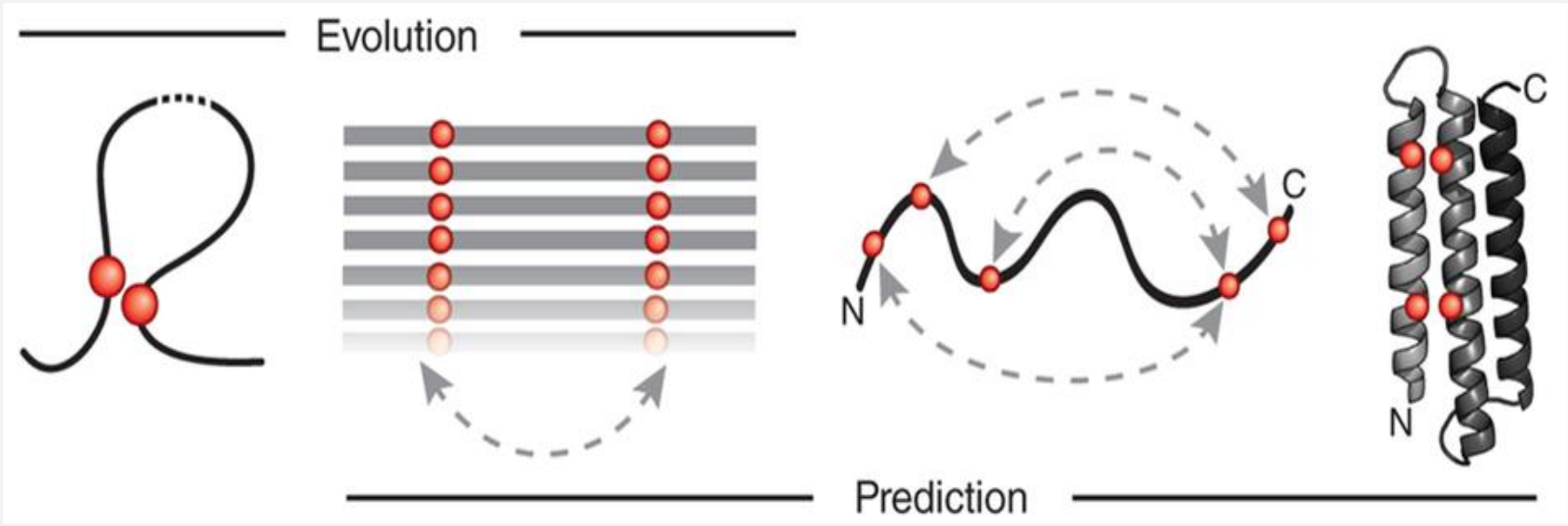
- Introduction
  - Predicting contacts from sequences using evolutionary covariance
  - Deep Learning-based structure prediction eg AlphaFold 2
- Bioinformatics throughout structure determination
  - Predicting domain structure
    - Construct design, experimental strategy
    - Protein engineering
  - ~~Predicting tertiary structure *ab initio* for MR~~
  - Quaternary structure and protein interactions
  - Finalising the structure, validation
  - Structure-based function interpretation
    - Using newer/less well-known data
    - Majoring on easily available servers/predictions
    - Case study from Structural Genomics (if time!)
  - The sequence alignment in your paper…
- Cross-cutting messages
  - Using multiple methods for a task is good
  - CCP4 has many useful options

Introduction: Evolutionary covariance and AF2/RF

Predicting contacts and distances between residues

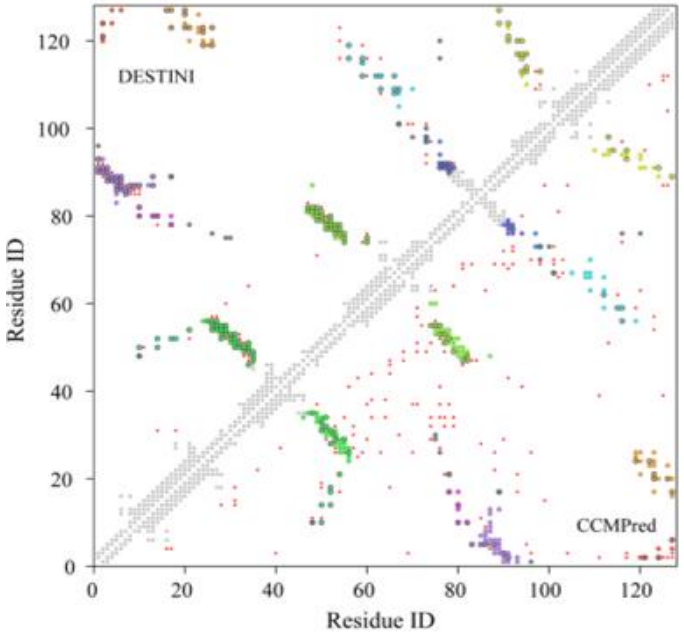
Deep Learning-based structure prediction methods

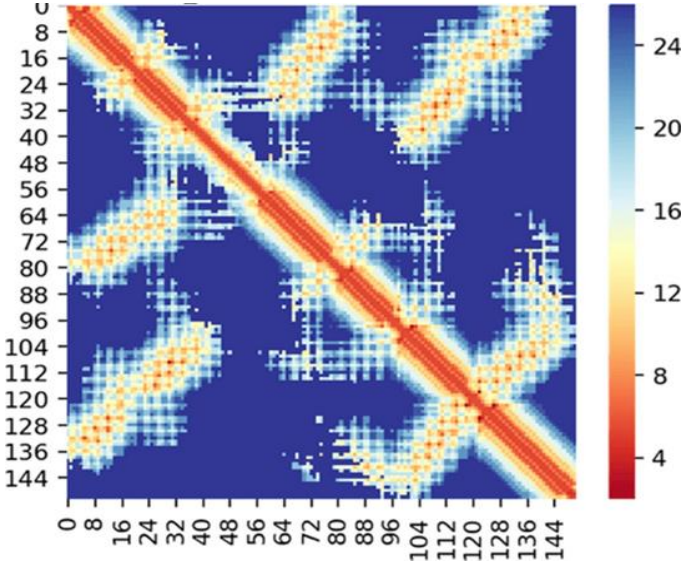
Evolutionary covariance



Marks *et al.* (2012) Nature Biotech 30, 1072

Predicting contact maps and distograms





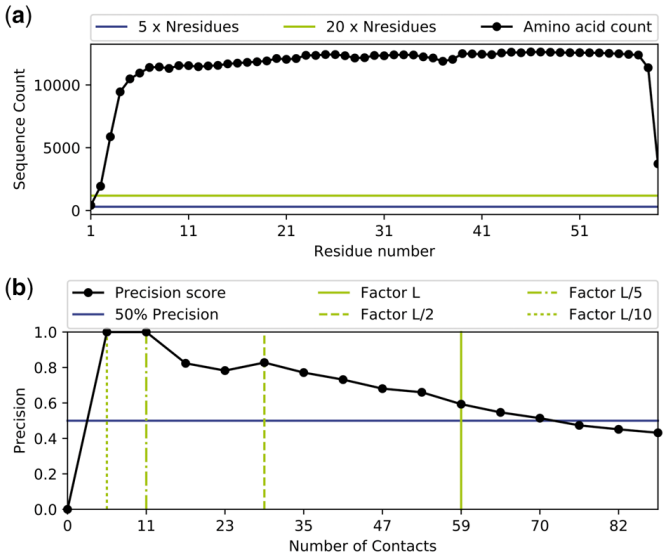
Drove modelling by: EVFold, DMPfold etc

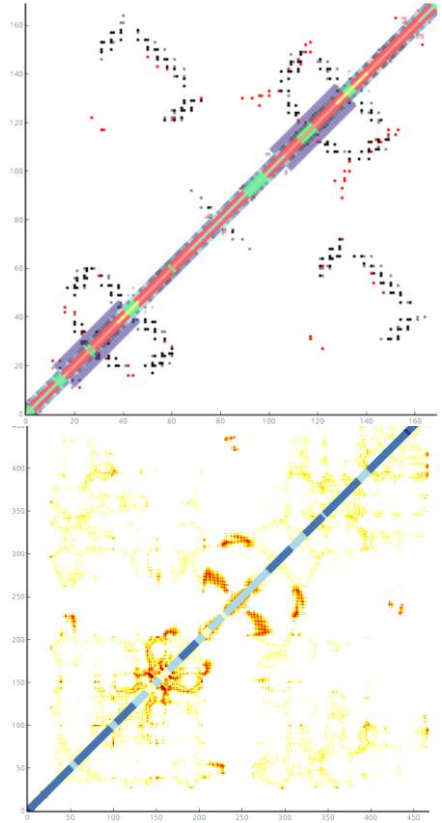
trRosetta, AlphaFold (1)

Gao et al (2019). *Sci Rep* **9,** 3514

Adhikari (2020) *Sci Rep* **10,** 13374

ConKit and ConPlot.org



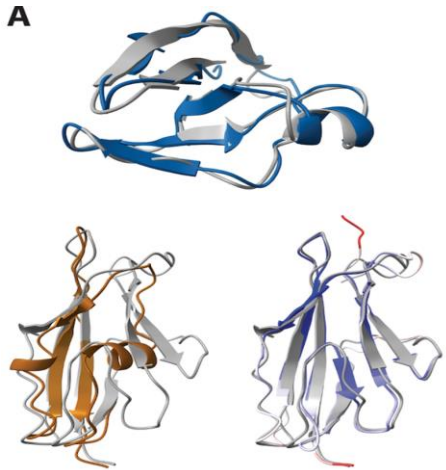


Simkovic *et al.* (2017) Bioinformatics **33** 2209

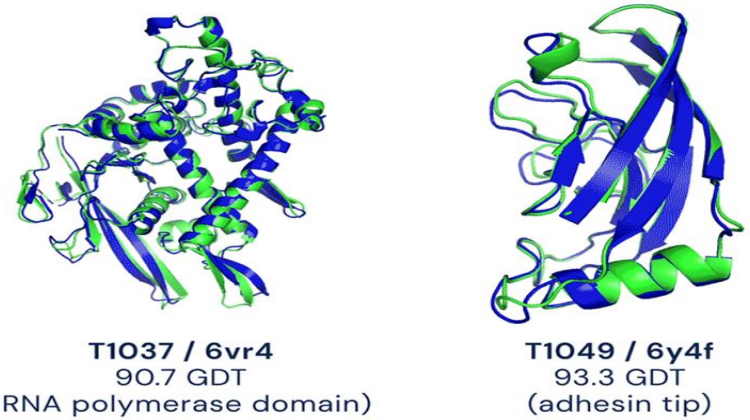
Sánchez Rodríguez *et al.* (2021) Bioinformatics btab049

Multiple methods: RoseTTAFold and AlphaFold 2

- Still use information including covariance from MSAs but networks learn to extract information without imposition of a particular model.
- End-to-end networks produce models directly rather than two separate steps



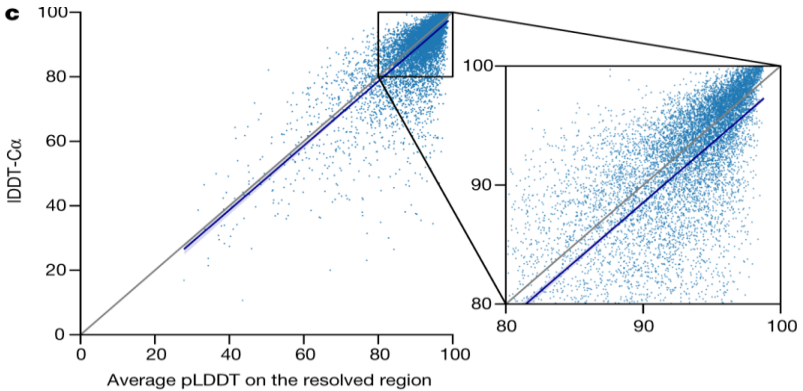
Baek et al (2021)
Science 373,871



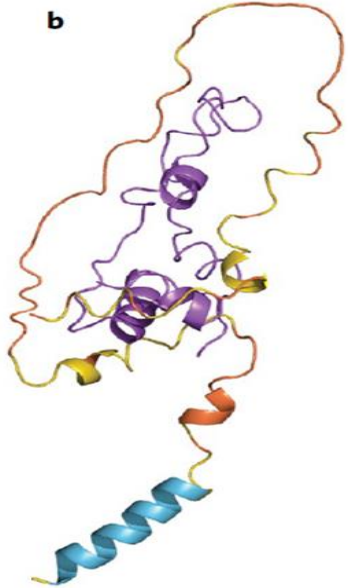
Jumper et al (2021) Nature 596,583

Multiple methods: RoseTTAFold and AlphaFold 2

- Often amazing quality but not always… Need good MSA or template!

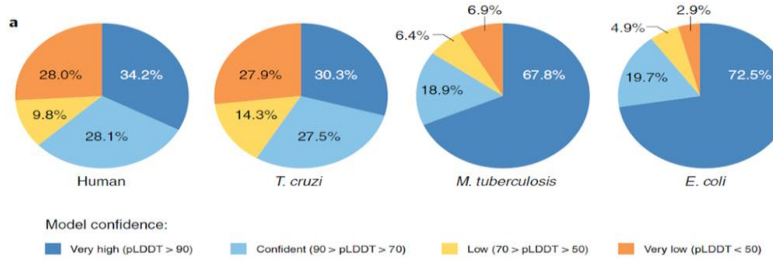


Jumper *et al.* (2021) Nature 596, 583



Phosphatase crystal structure vs AF2 model

Insulin crystal structure vs AF2 model



Thornton *et al.* (2021) Nature Medicine 27. 1666

Domains and construct design

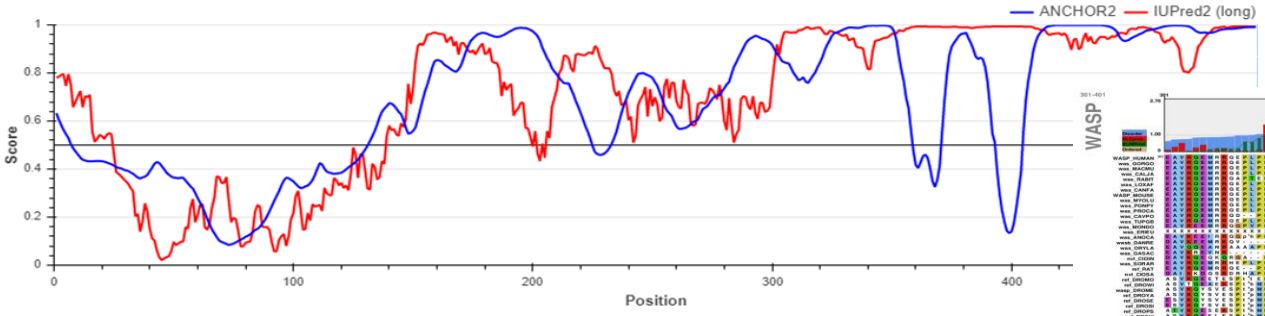
How interesting is your protein?!

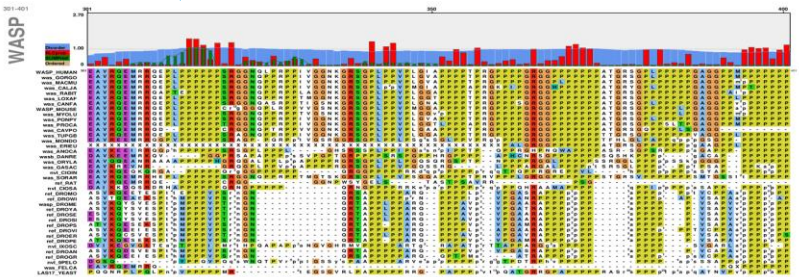
Which part to express for crystallisation?

Which parts of your crystallised protein might enable phasing by MR, or experimentally?

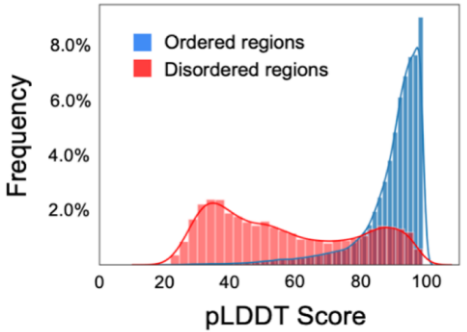
Intrinsic disorder prediction

- Not all proteins and protein regions fold into stable structured domains. ID proteins and regions will not crystallise (alone)
- There are many predictors, all performing roughly equally well
- I recommend IUPred (fast) and MetaDisorder (slow but good)
- Can also look for short interaction motifs in ID regions (ANCHOR, SlimPred)

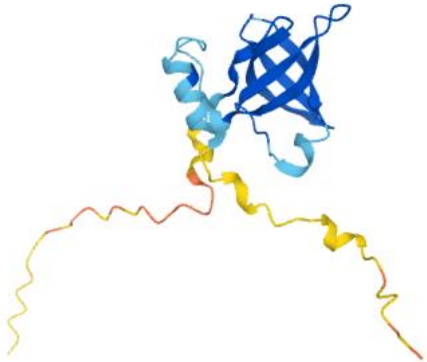


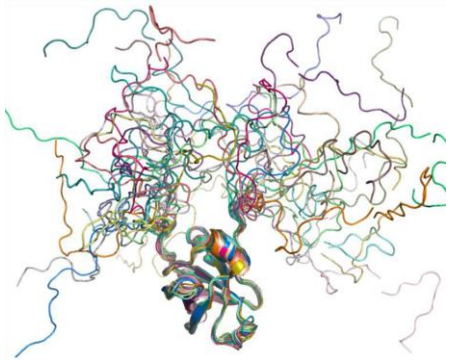


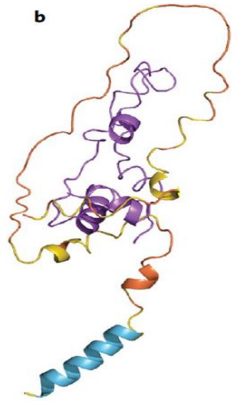
Most low-confidence AF2 regions are disordered but they may just be plain wrong



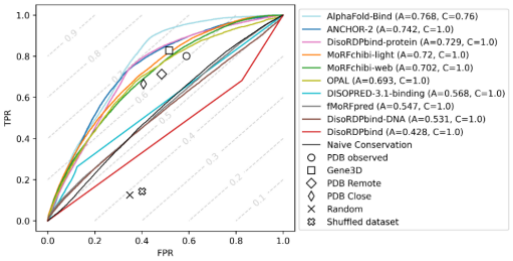
Binder et al (2022) COSB 74, 102372







Relatively high pLDDT regions in AF2 disordered regions may predict interaction motifs



Piovesan *et al.* (2022) BioRxiv https://doi.org/10.1101/2022.03.03.482768

Recognising folded domains in your sequence

- Important to characterise both their limits and relationships to known structures…
- How novel is your protein target? Recognising distant homology might make it less (or more!) interesting
- What related structures are available to serve as MR search models?
- You might get extra ideas about its function to guide lab experiments, co-crystallisation, phasing (eg metal-binding sites)
- You might find the whole protein will not express/stay soluble/crystallise etc and want to deal with only part. You might well design construct to exclude disordered regions anyway.

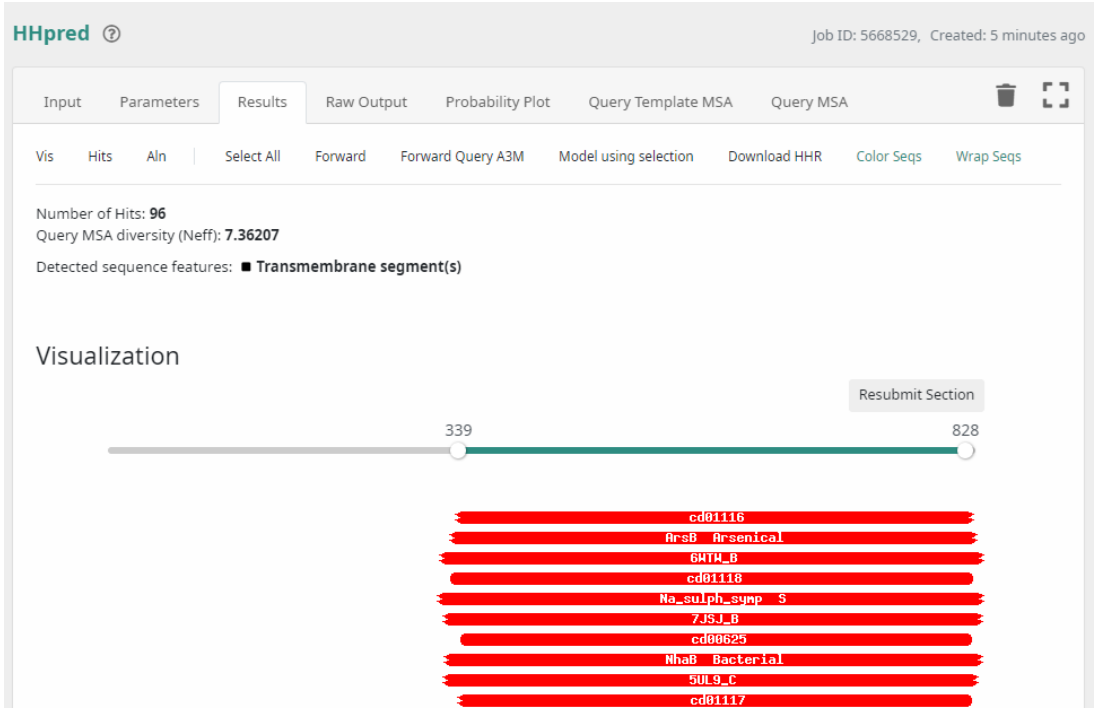
Recognising domains by homology with PDB, SCOP, CATH

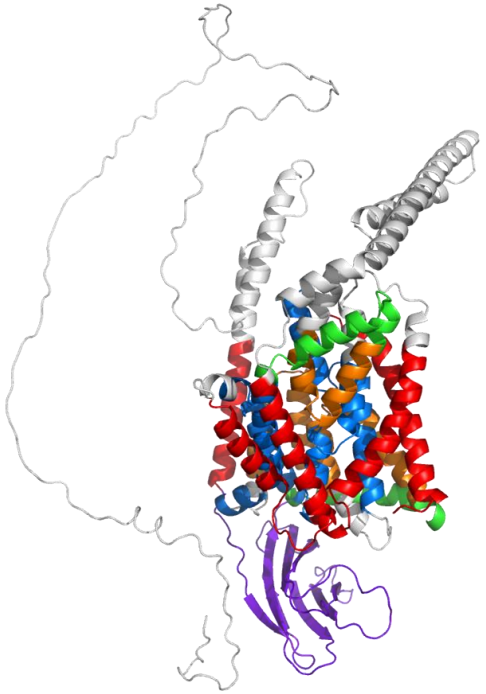
- BLAST vs PDB might work but HHPRED is more sensitive
- HHPRED works by comparing HMMs of alignments, not just single sequences
- Matching of secondary structure also scored
- Used by MrBUMP in MR

HHpred tips and warnings

- Probability (0-100) is generally a good guide….
- …but statistics can mislead for unusual protein sequences eg coiled-coil, low-complexity, Cys-rich
- Consider if the match makes biological sense!
- Look at the matched region
  - Is it a complete domain/structure?
  - If partial, could it reasonably fold?
- If your query contains multiple domains, 'zooming in' on particular regions can improve scores and show results not previously seen since
  - Score contains an element favouring similar lengths
  - Only 250 results are shown: can easily get this number for a common domain, making other results invisible!
- MrBUMP can search for MR search models with HHpred, most easily at CCP4 online or CCP4Cloud

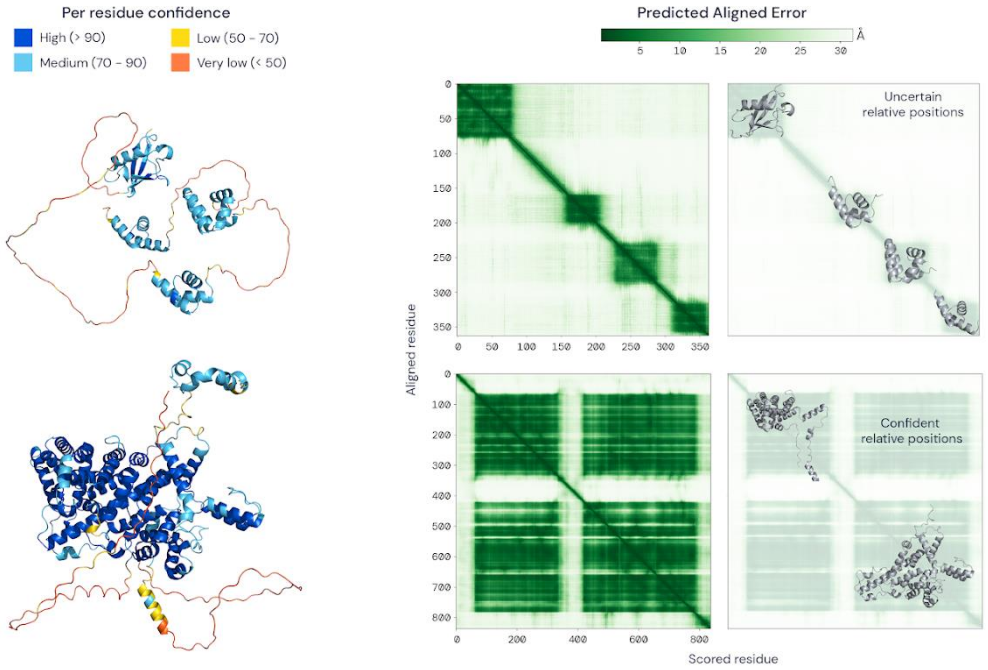
AF2 for domain discovery





Mesdaghi *et al.* (2023) BioSci Rep 43:BSR20230060

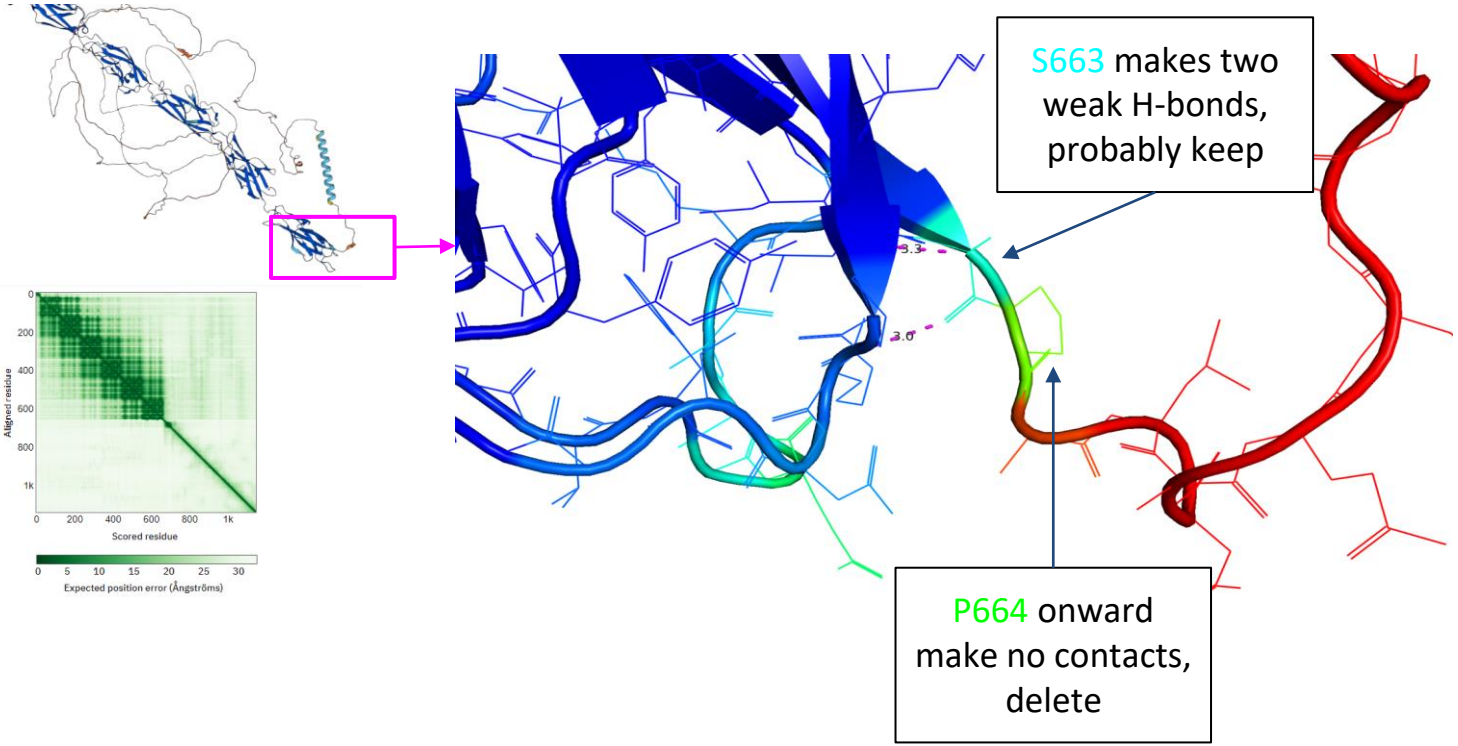
AF2 for domain boundary prediction



Slice'n'Dice uses the PAE or other kinds of clustering to split structures into domains for MR

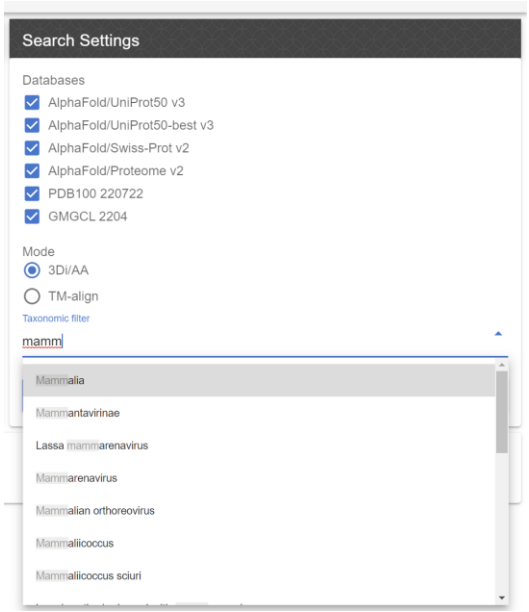
https://deepmind.com/research/publications/2021/enabling-high-accuracy-protein-structure-prediction-at-the-proteome-scale

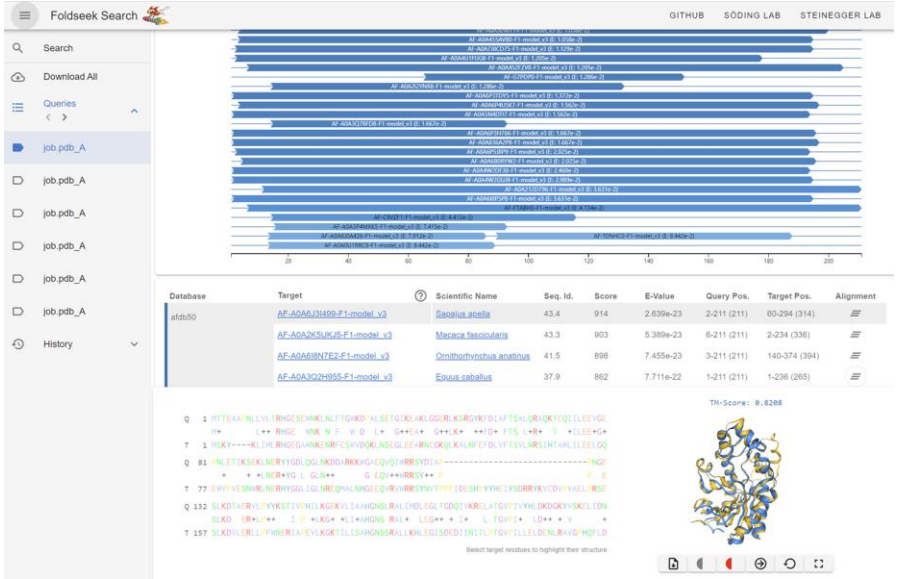
Fine details too, if reliability allows



Recognising homology using a structure

Search PDB, AFDB, ESMAtlas, CATH using a structure in seconds





https://search.foldseek.com/

Van Kempen et al. (2022) Nature Biotech
https://doi.org/10.1038/s41587-023-01773-0

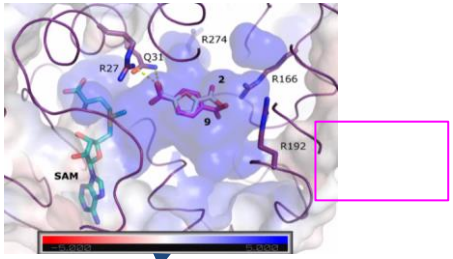
Protein engineering

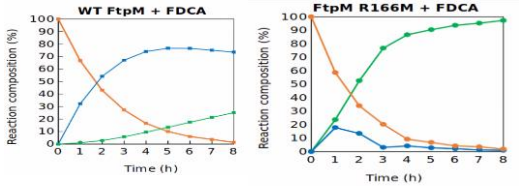
Where you have confidently interpreted a model as reliable then you can treat it almost like a crystal structure…

Rational enzyme engineering using an AF2 prediction

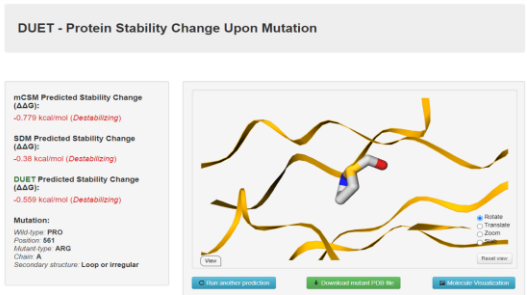
Combining AF2 predictions with other structure-based methods eg for stability prediction

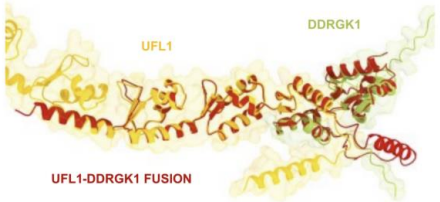
Predicting a stable fusion protein based on analysis of a complex structure prediction





Ward et al (2022) Angewandte Chemie 61,e202117324

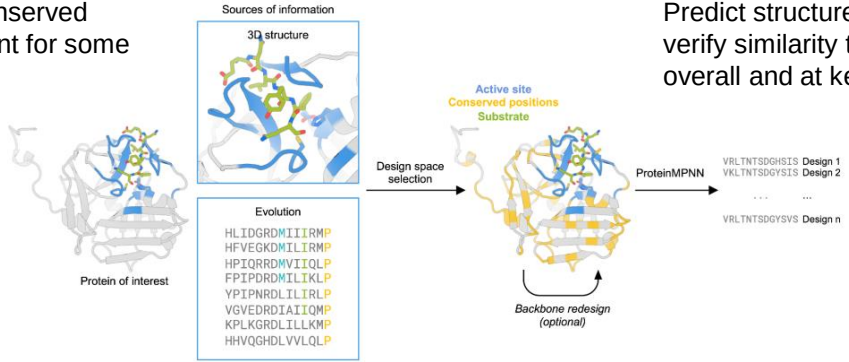




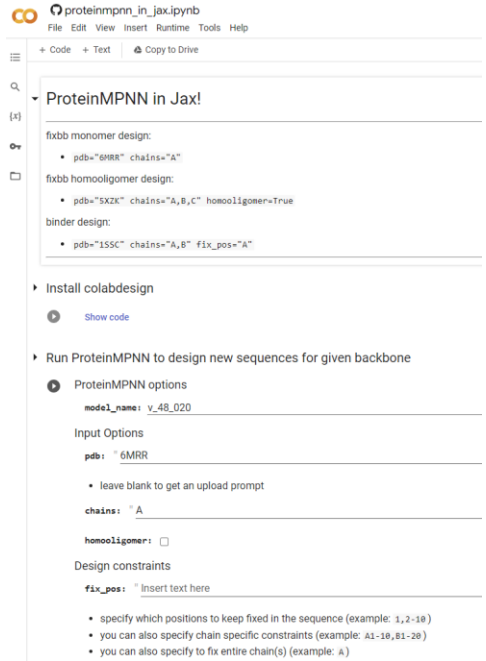
Banerjee et al (2022) BioRXiv https://doi.org/10.1101/2022.09.15.508077

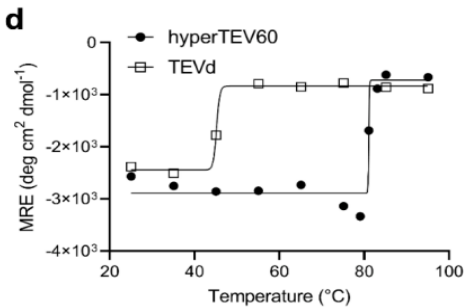
Protein engineering with language models

Identify and retain residues near ligand (functionally important) and conserved positions (important for some reason)

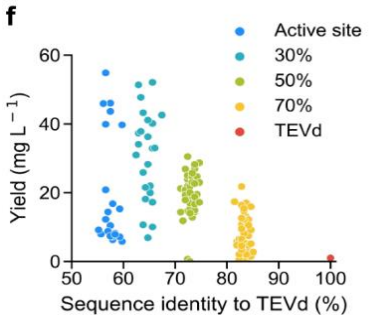


Allow ProteinMPNN to fill in the gaps and design variants. Predict structures with AF2 and verify similarity to starting point, overall and at key positions

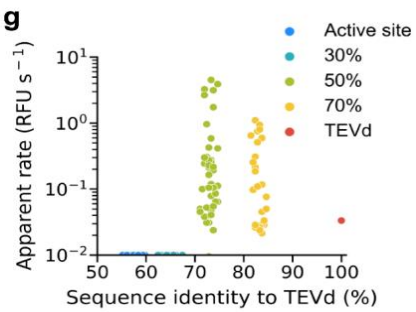




Better thermostability



Better expression



Better activity

Sumida et al (Oct 2023) Improving protein expression, stability, and function with ProteinMPNN bioRxiv doi: 10.1101/2023.10.03.560713

Tertiary structure

AF2 and RF output as search models for MR

AF2/RF and Molecular Replacement

MR *is* a kind of structure prediction so the availability of accurate models of most proteins has impacted structure solution hugely

Similarly, accurate models can be used to interpret cryo-EM maps

- Recall Adam's talk for how to access AF2 models
- See other talks for how to find and deploy AF2 models with MrParse, MrBUMP, Slice'n'Dice, ARCIMBOLDO_SHREDDER etc

Getting diversity in your models

This will be needed for hard cases and for cases where multiple conformations are accessible or sought

Ways to sample conformation more broadly

- Network dropout (eg `num_samples` and/or `is_training` on the advanced colabfold page)

https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/beta/AlphaFold2_advanced.ipynb

- Feed AF2 templates in the 'right' conformation (and maybe ignore MSA features)

de Alamo et al (2022) elife 11:e75751

- Deliberately make the input MSA more shallow

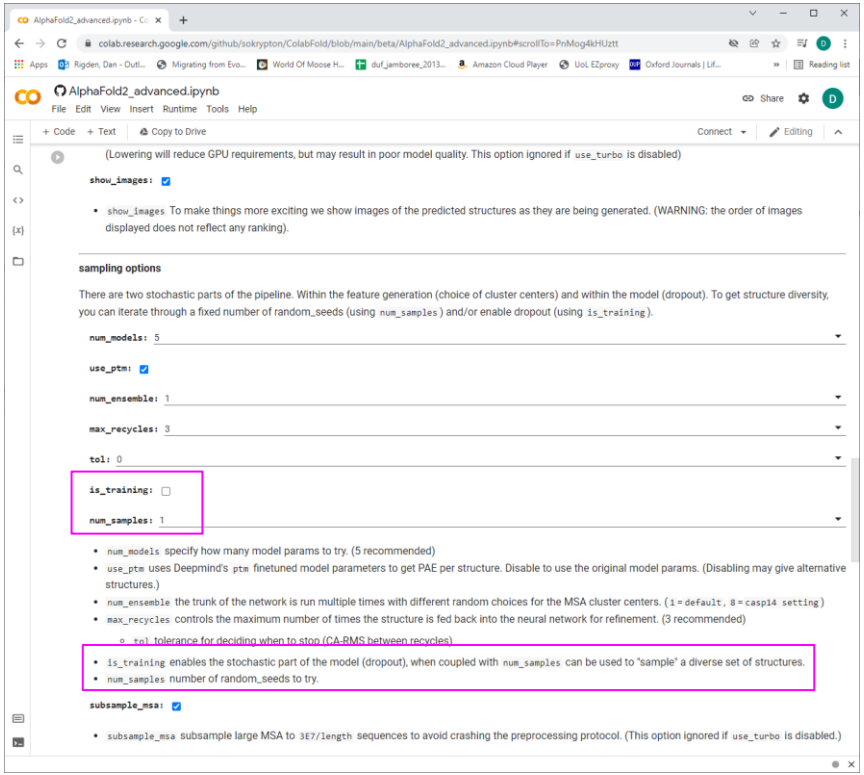
Heo & Feig (2022) PSFB DOI: 10.1002/prot.26382

- Cluster the input MSA and try individual sub-clusters

Wayment-Steele et al (2022) bioRXiv DOI: 10.1101/2022.10.17.512570

- Edit the input MSA to mutate to Ala residue pairs that are driving the 'wrong' conformation

Stein and Mchaourab (2022) PLoS CB 18: e1010483.



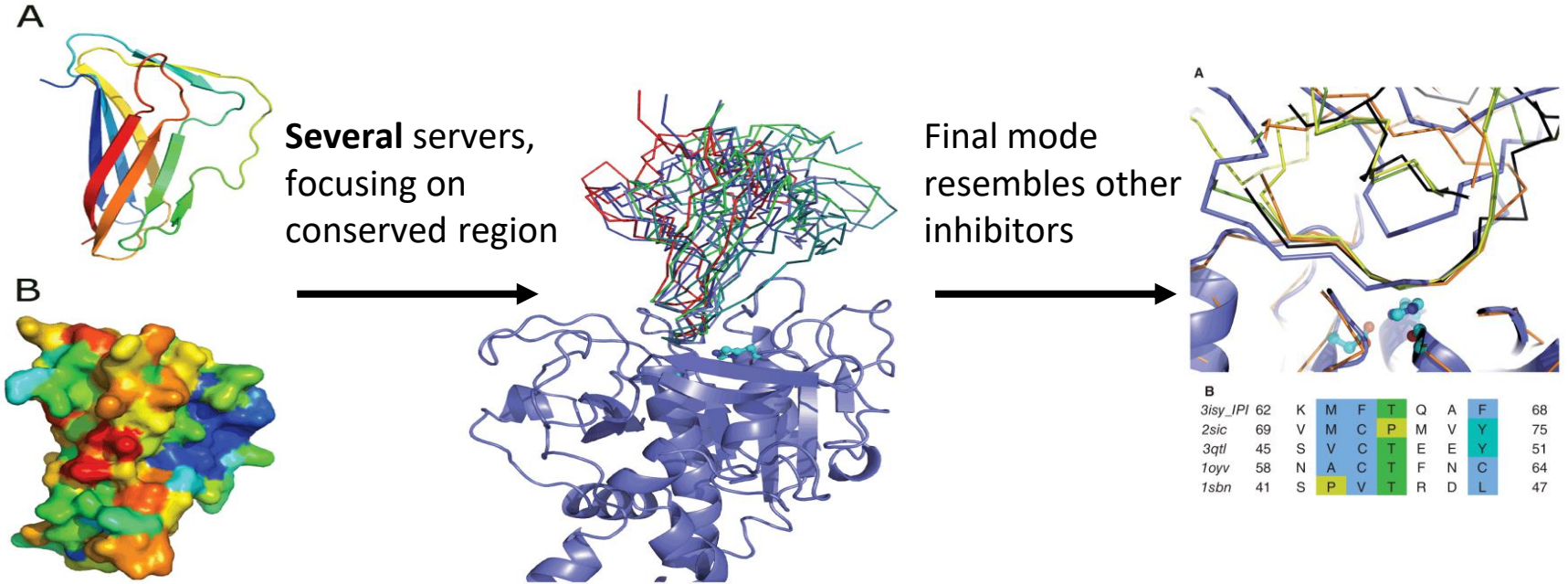
Quaternary structure

Intermolecular interactions

Predicting protein-protein interactions

- Relevant to MR eg proteins A and B are cocrystallised but neither alone solves. An accurately predicted complex, being larger, might solve
- Many docking methods predict complexes based on steric complementarity plus other scoring functions
- Recommendable servers include
  - ClusPro, the best performing docking method
  - Haddock, which has a good server with different modes
  - Each allows inclusion of other information eg known interface residues. RF/AF2 do not (easily, yet)
  - Symmetric docking at ROSIE server. Also unavailable in RF/AF2

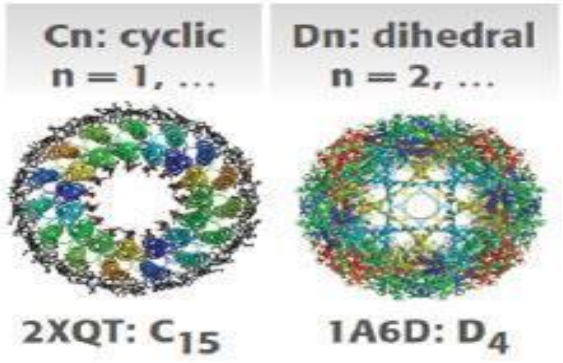
Multiple methods in bioinformatics: *B. subtilis* IPI docking to protease



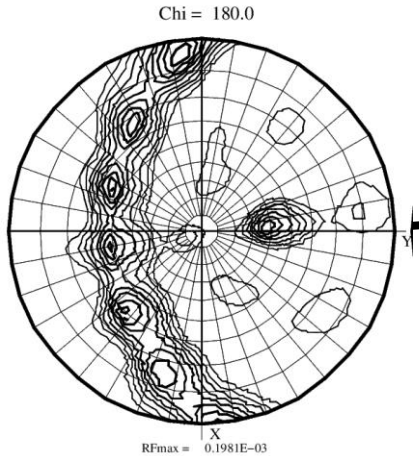
Rigden et al. (2013) F1000Research 2:154

ROSIE symmetric docking for oligomers

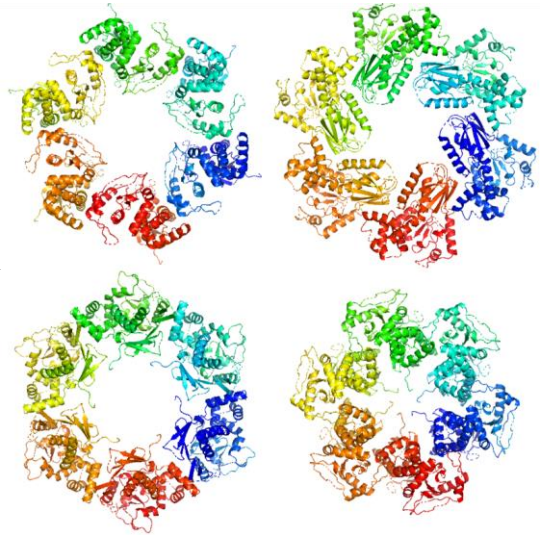
- Only cyclic (C_n) or dihedral (D_n) symmetry at server
- Clues from self-rotation function may be available
- AF2 cannot use this information!



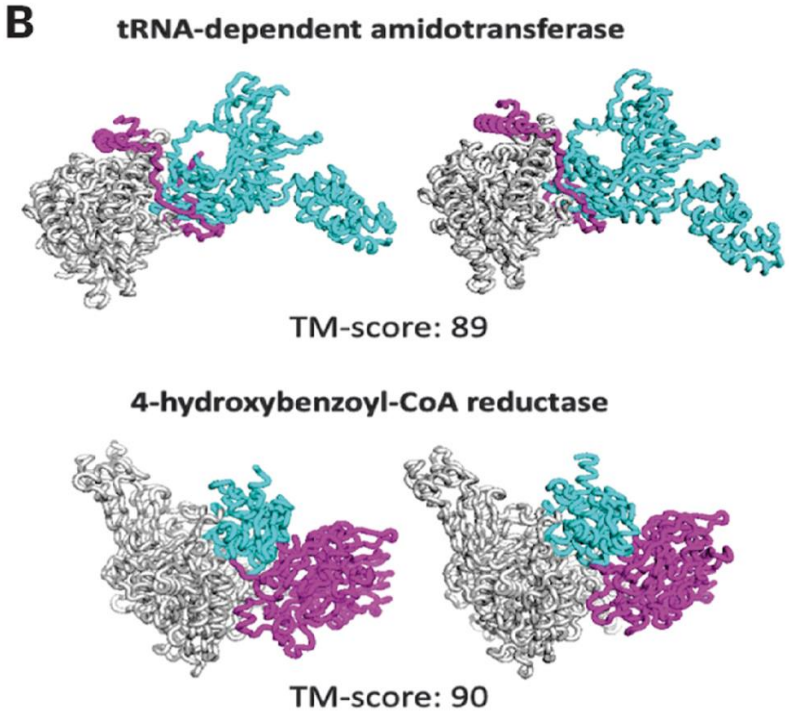
rcsb.org



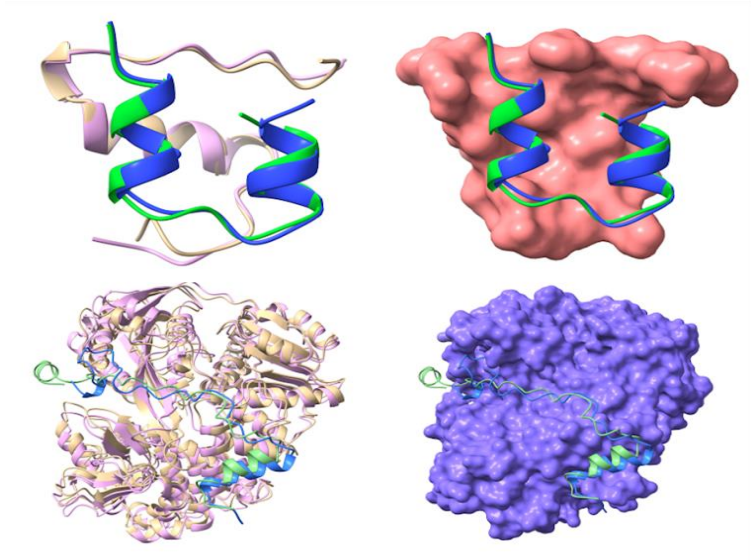
Generate hexamers



RF/AF2 to predict complexes



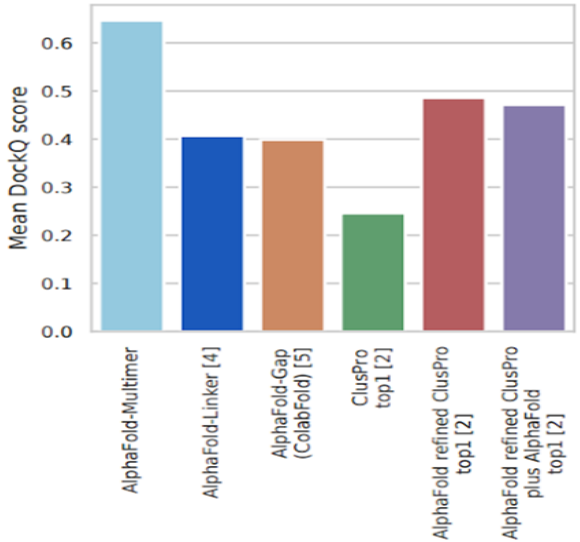
Baek et al (2021) Science 373,871

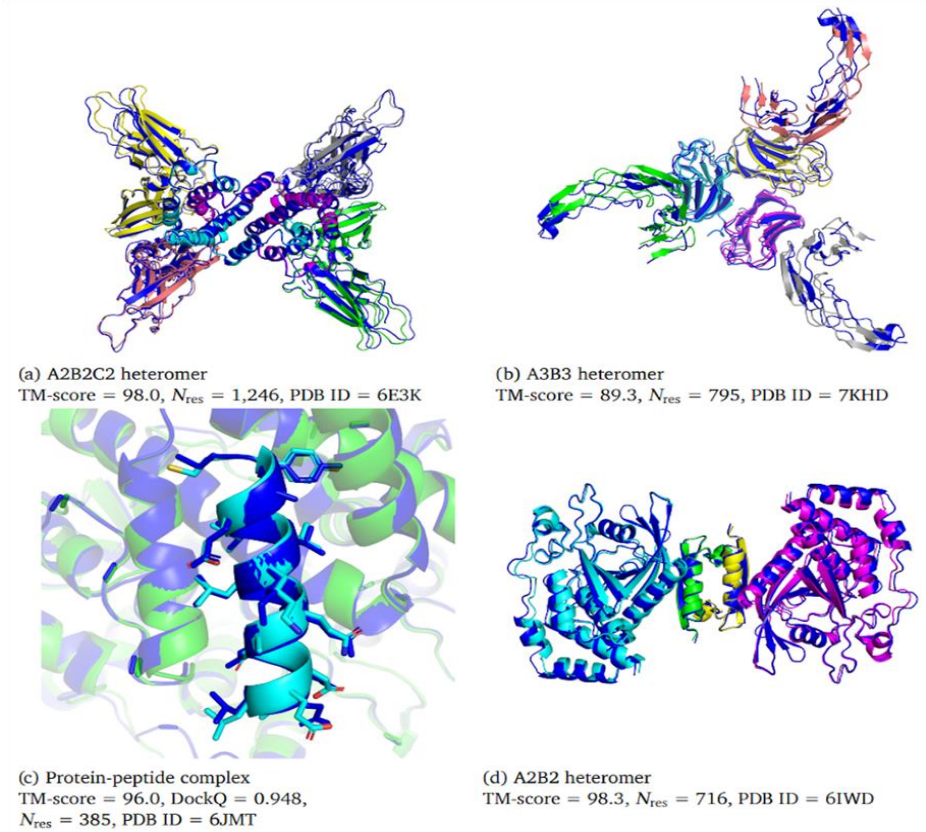


Modelling protein-peptide interactions as separate chains or linked by polyAla are complementary approaches.

Ko and Lee (2021) www.biorxiv.org/content/10.1101/2021.07.27.453972v2

New multimer-trained AF2





(a) A2B2C2 heteromer
TM-score = 98.0, N_{res} = 1,246, PDB ID = 6E3K

(b) A3B3 heteromer
TM-score = 89.3, N_{res} = 795, PDB ID = 7KHD

(c) Protein-peptide complex
TM-score = 96.0, DockQ = 0.948,
N_{res} = 385, PDB ID = 6JMT

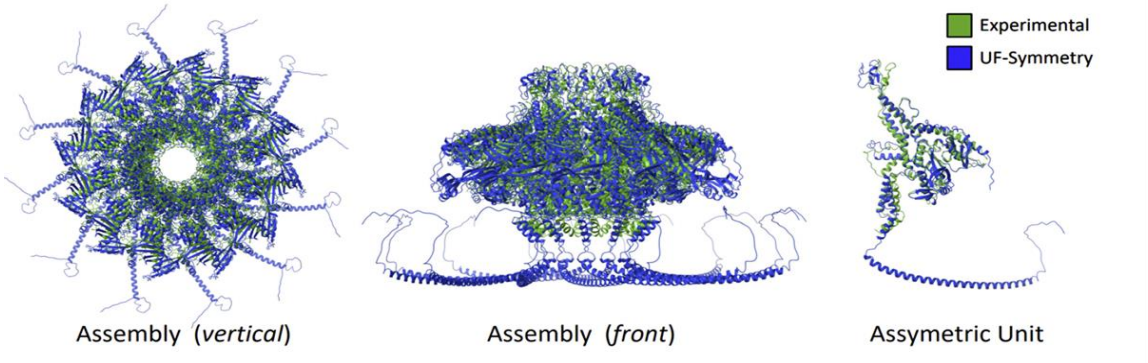
(d) A2B2 heteromer
TM-score = 98.3, N_{res} = 716, PDB ID = 6IWD

AF2 can predict oligomeric state eg asking for 5 copies may produce the natural tetramer + one left over, rather than a pentamer

Evans et al (2021) https://www.biorxiv.org/content/ 10.1101/2021.10.04.463034v1

Uni-Fold symmetry

- Models a single chain with known symmetry to generate oligomer
- Much quicker and slightly better than other methods on oligomers



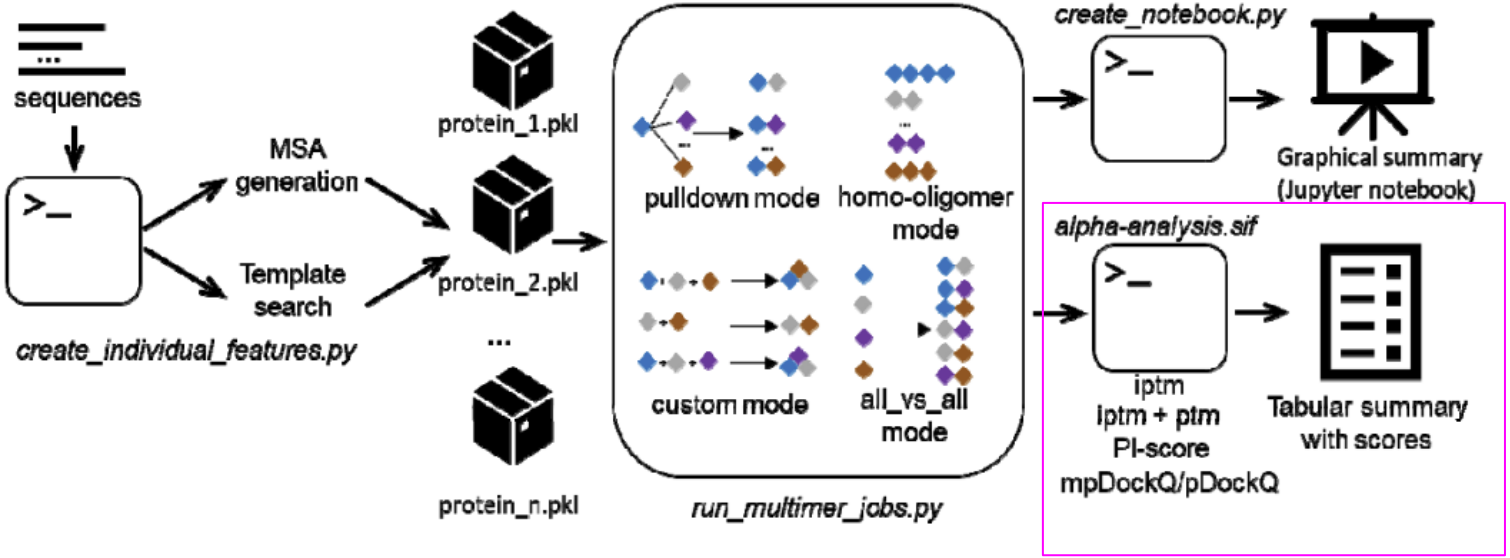
Type	Symmetry	Number of structures
Monomers	–	83,392
Multimers	Asymmetric	27,470
	C2	45,496
	C3	6,037
	C4	1,736
	C5	893
	C6	639
	Larger cyclic	587
	D2	8,571
	D3	2,577
	D4	815
	D5	302
	D6	191
	Larger dihedral	239
	Icosahedral	1,182
	Octahedral	544
	Tetrahedral	475
	Helical	581
	All	98,335
Total	–	181,727

C12 symmetry. AF2 and regular Uni-Fold fail

Li et al (2022) BioRXiv https://doi.org/10.1101/2022.08.30.505833

https://colab.research.google.com/github/dptech-corp/Uni-Fold/blob/main/notebooks/unifold.ipynb

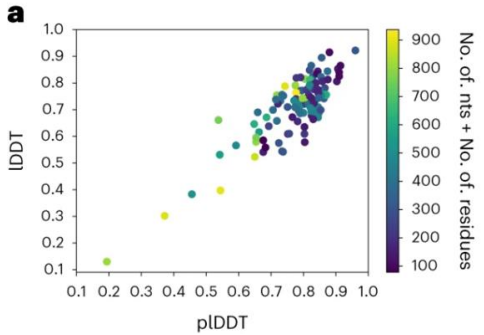
AlphaPulldown for complicated cases

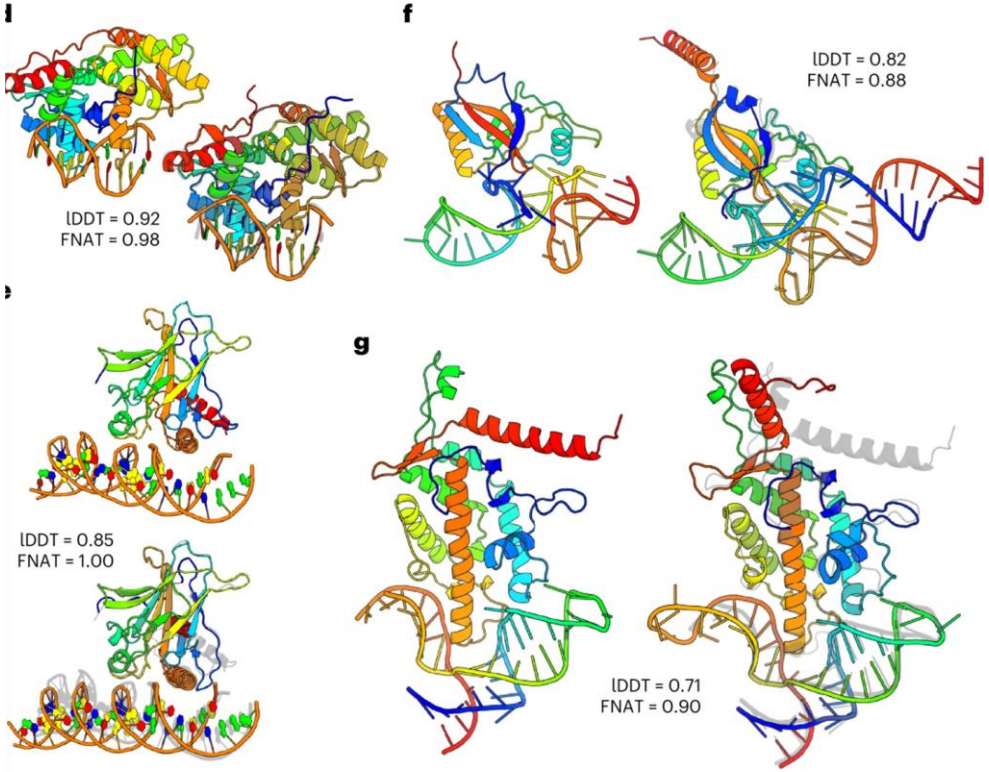


Yue al (2023) Bioinformatics 39,btac749

Protein-NA complexes with RoseTTAFoldNA

As good as AF2 on proteins; as good as DL-based RNA tools on natural RNAs; USP is protein-NA complexes

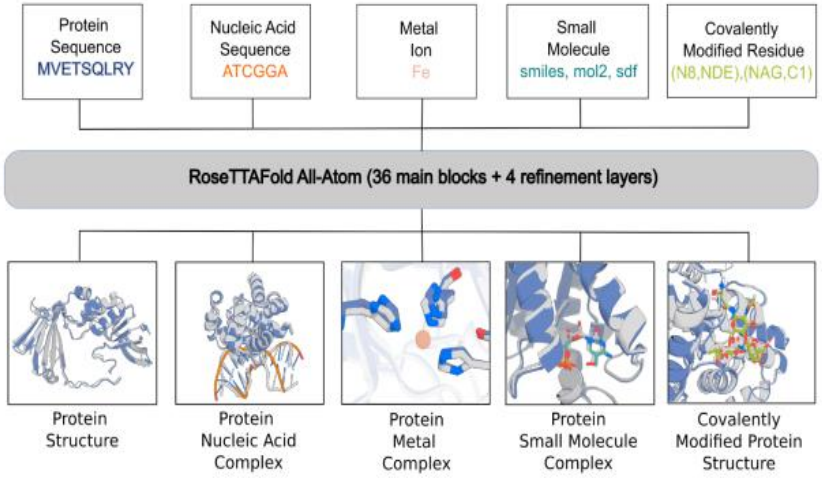




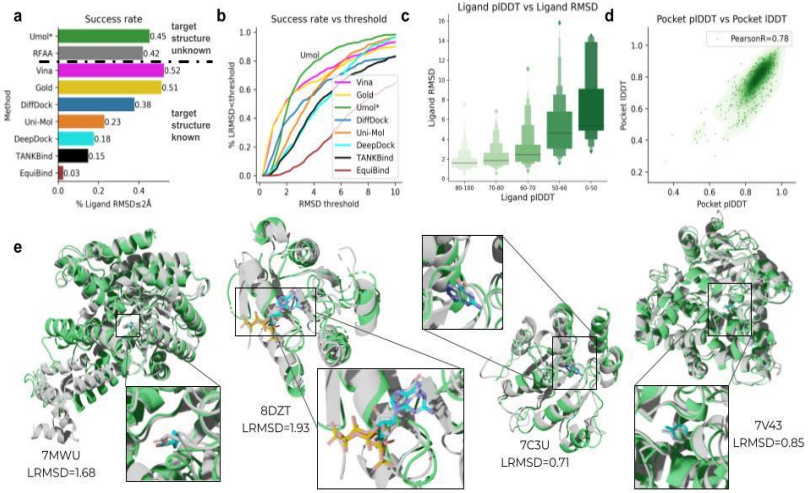
Baek et al (2023) Nature Methods https://doi.org/10.1038/s41592-023-02086-5

Generic protein-ligand prediction

RoseTTAFold All-Atom



Umol



Preprint, no code

Krishna et al (2023) bioRXiv 10.1101/2023.10.09.561603

Preprint, code, Colab page!

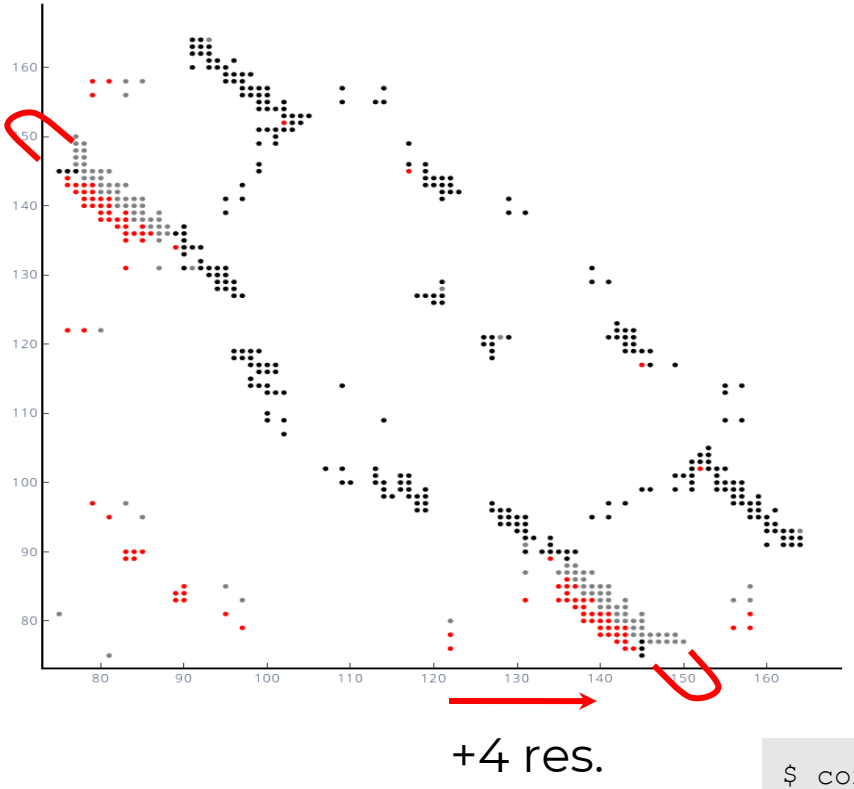
Bryant et al (2023) bioRXiv 10.1101/2023.11.03.565471

Finalising the structure

Does it contain any residual errors?

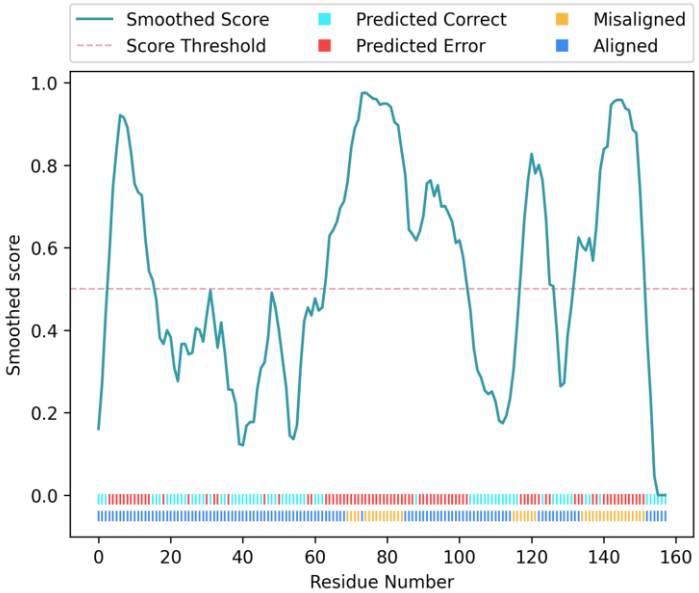
What is the biologically relevant quaternary structure?

New covariance-based metrics for model validation



+4 res.

ConKit



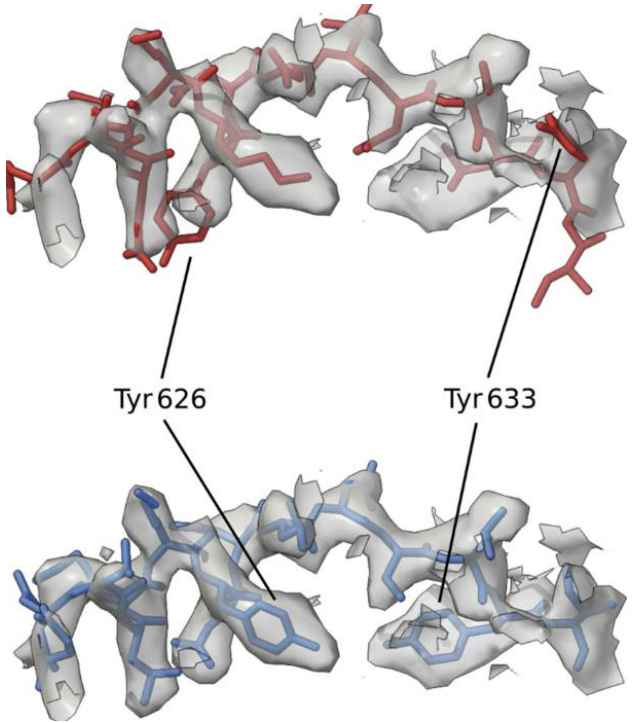

```
$ conkit-validate model.pdb prediction_af2.pkl sequence.fasta
```

Sánchez Rodríguez *et al.* (2022) Acta Cryst D, 78, 1412

New covariance-based metrics for model validation

Particularly powerful for register errors

We estimate at least 10% of 3-5Å deposits in the PDB contain such an error…



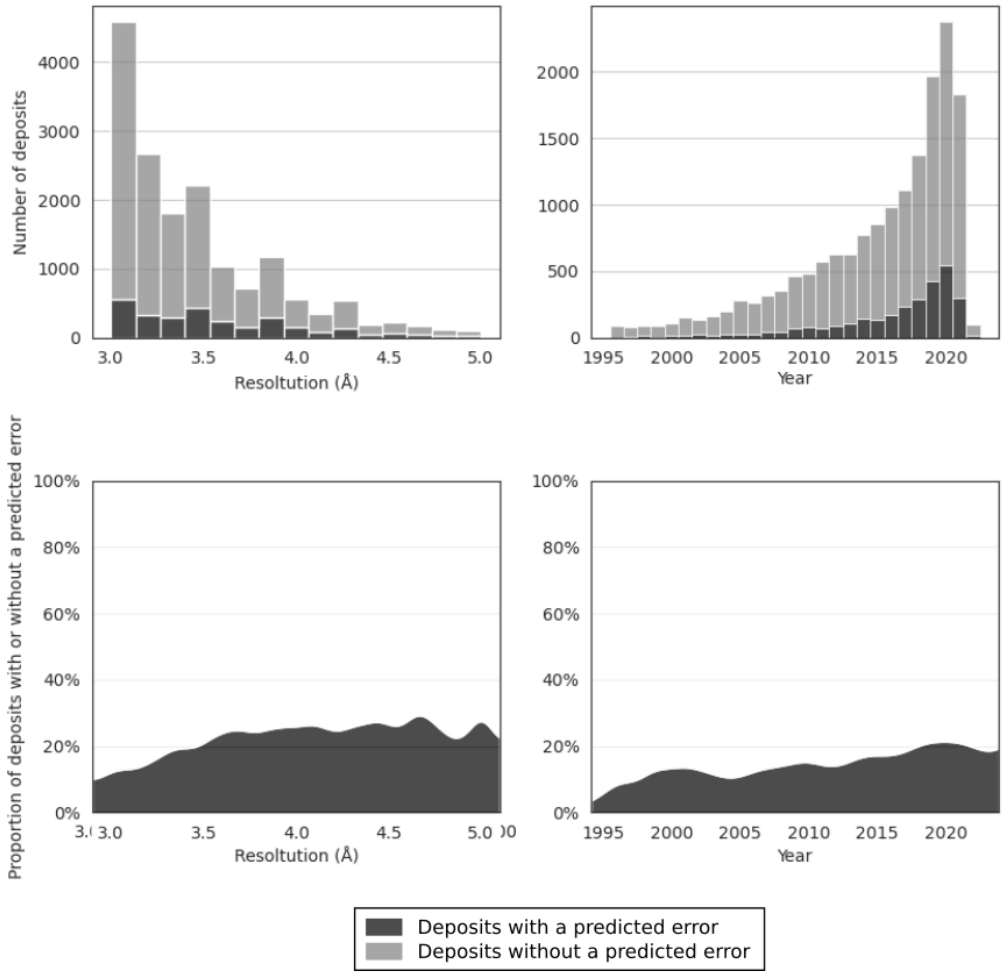
7adkB

Sánchez Rodríguez *et al.* (2022) Acta Cryst D, 78, 1412

PDB-wide screen

Lower resolution structures tend to contain more errors, but dependence is quite modest

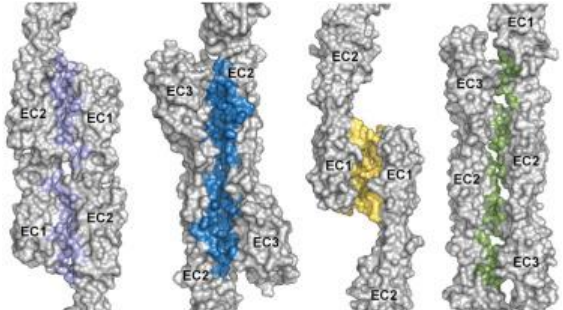
Error rate not declining recently - are experimentalists continually tempted to lower resolutions?

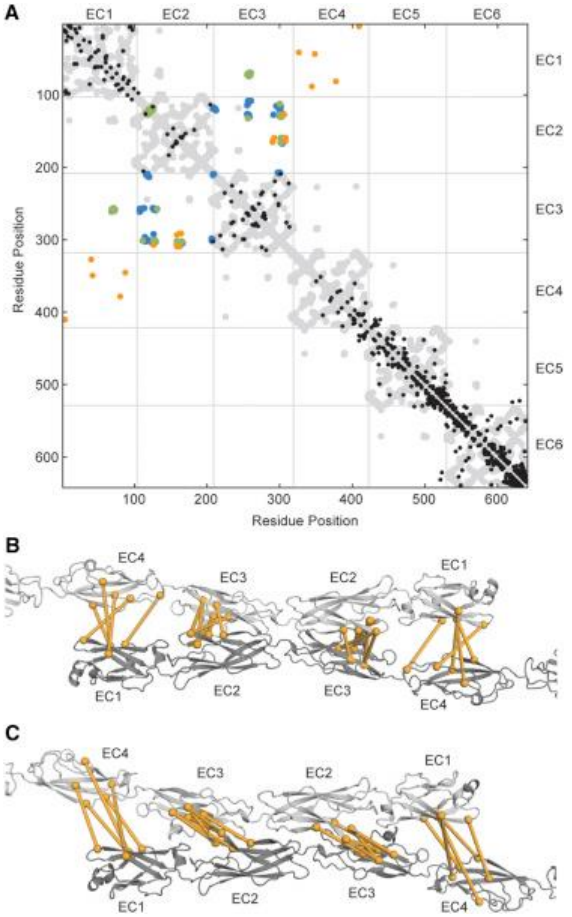


Sánchez Rodríguez *et al.* unpublished

Validating crystal structure contents

- PISA is an excellent general method, but contact predictions help in some cases
- Crystal showed various ways in which protocadherins could interact
- Predicted contacts supported only some of the modes
- PISA-cov is on the way…





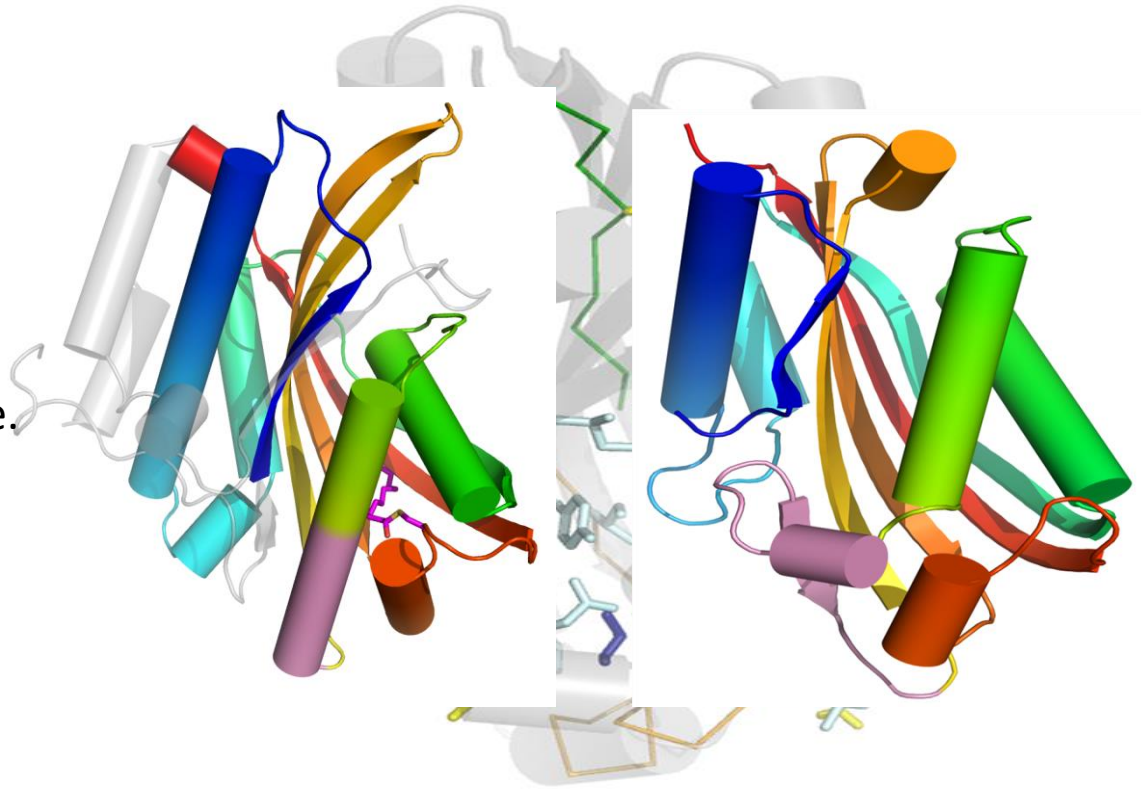
Nicoludis *et al*. (2015) Structure 23, 2087

Structure-based function interpretation

Where are the functional/catalytic sites?

Multiple methods in bioinformatics: Structure comparisons of Evf

- Reported as novel fold...
- ... but in fact related to *Bacillus* toxin structures (DALI)
- Both bind to host insect membranes
- Palmitate seen in Evf structure. Matches conserved region of toxins...
- GESAMT is an excellent CCP4 option
- FoldSeek is great for a quick search of the AFDB, ESMAtlas etc



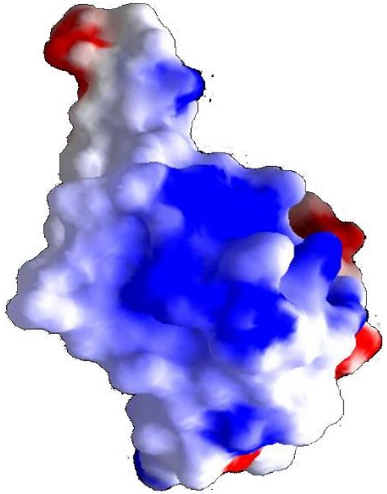
Quevillon-Cheruel et al. (2009) J. Biol. Chem. **35**,2107 Rigden (2009) FEBS Lett. **583**, 1555

Structure-based function annotation

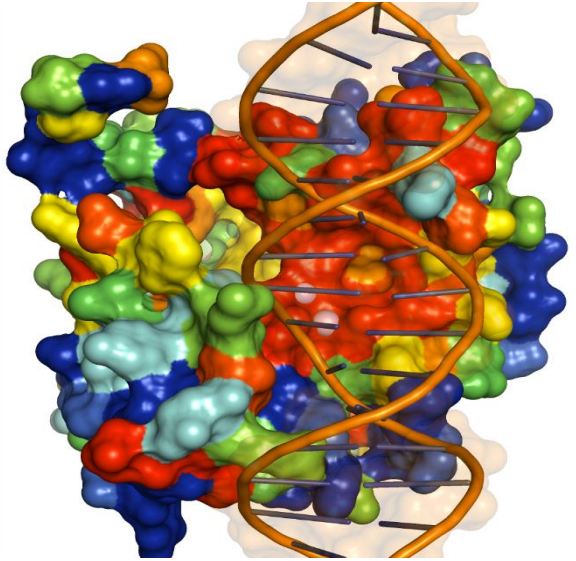
- Finding functional sites is based on their being different somehow to the rest of the protein surface. Important general methods are based on
  - Shape (castP, ProFunc, PyMOL)
  - Electrostatics (PyMOL, APBS)
  - Evolutionary conservation (Consurf)
- Less well-known but valuable characteristics are
  - Statistics of surface atom ‘triangles’ (STP)
  - Probe interaction energetics (ISMBLab)
  - Predicted pKa values (THEMATICS/POOL)

Important general methods

Shape
CastP



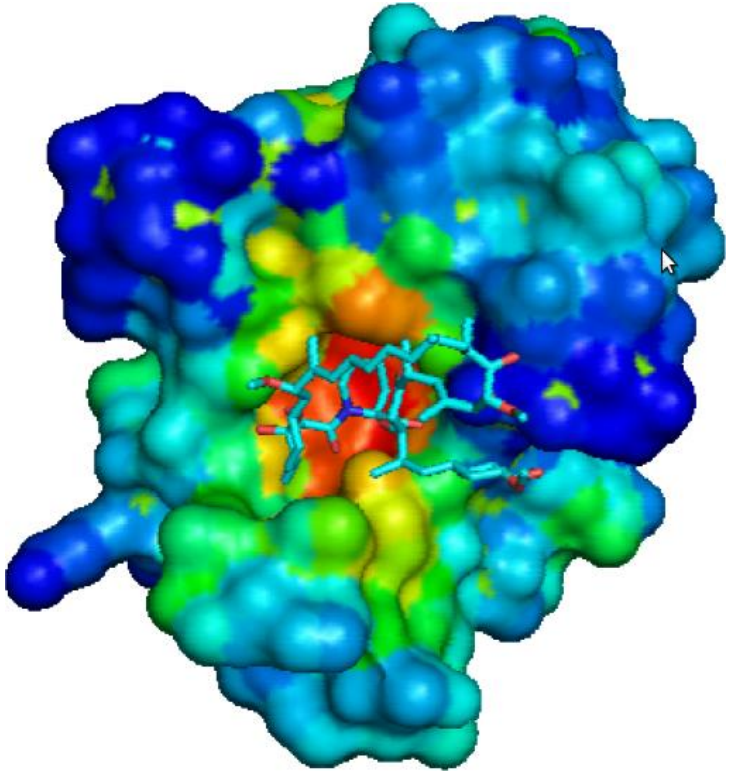
Electrostatics
APBS/PyMOL



Conservation
ConSurf

Binding sites from statistics

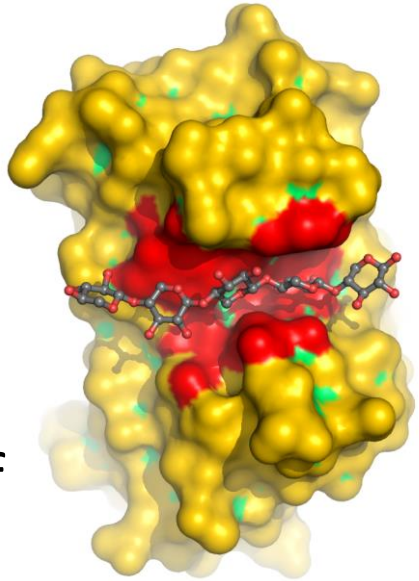
- STP (surface triplet propensities)
- 13 atom types → 455 triplets
- Distribution in binding vs non-binding sites varies
- Designed for small molecules, works on PPIs, including flat surfaces



opus.bch.ed.ac.uk/stp

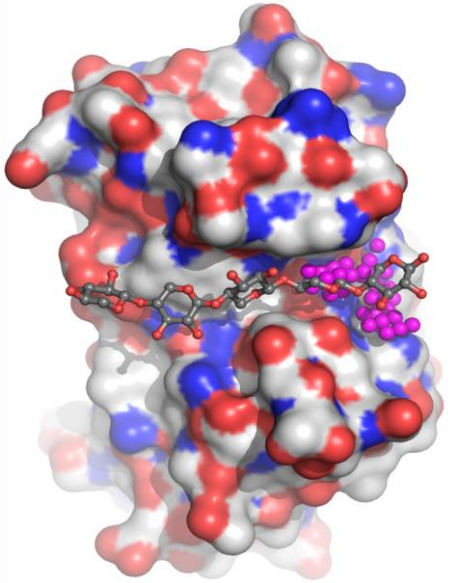
Different probes for different binding sites

- Hydroxyl group can be used to probe for carbohydrate binding sites
- Phosphate oxygen used for binding sites of phosphorylated ligands



ISMBLab

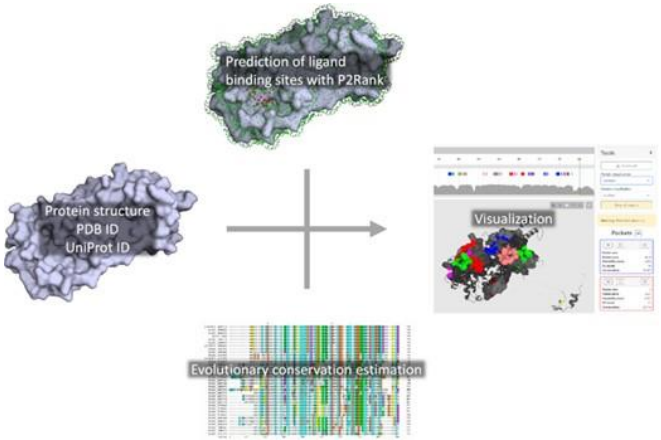
ismblab.genomics.sinica.edu.tw

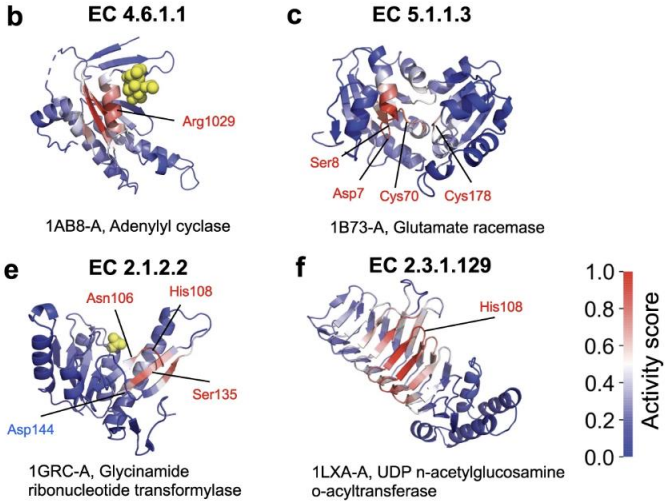


SiteHound

sitehound.sanchezlab.org/

Advanced methods use Machine Learning and/or multiple signals





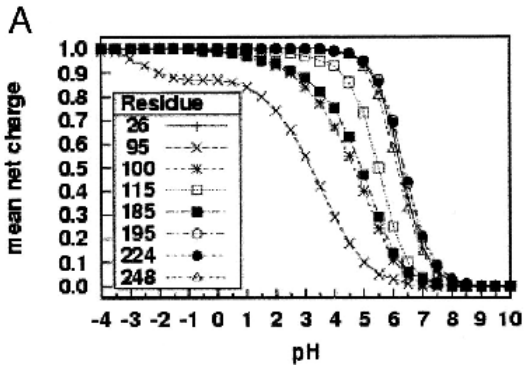
PrankWeb uses structural and physicochemical properties then displays pockets with conservation analysis https://prankweb.cz

Combines LM and contact map features in CNN to predict GO terms and sites

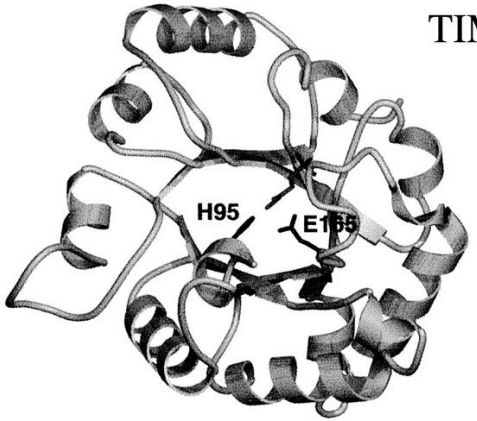
https://beta.deepfri.flatironinstitute.org/

Theoretical microscopic titration

- Computer analysis of a reliable protein structure can predict pKa values for acids and bases. Residues with perturbed pKa values are possible catalytic residues, especially if clustered.



pKa of His95 is atypical compared to other His residues in enzyme



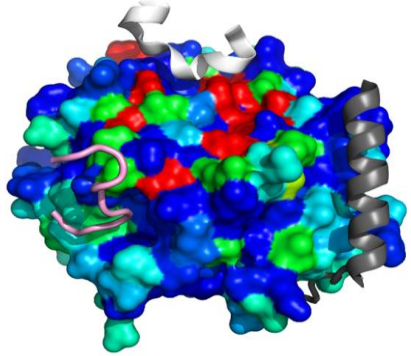
His95 and other residues with atypical pKa cluster at catalytic site

www.pool.neu.edu/wPOOL/index2.jsp

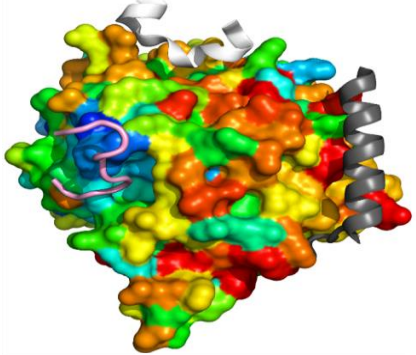
Some servers require thought…

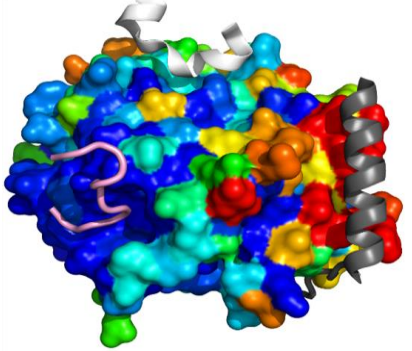
- Consurf maps sequence conservation onto a structure revealing functional sites
- Excellent, general method, but results depend on sequence set chosen for mapping: selecting all or only near relatives gives different results. Either might be more appropriate for you

Mapping 300 homologues mixes different activities so no information on binding sites

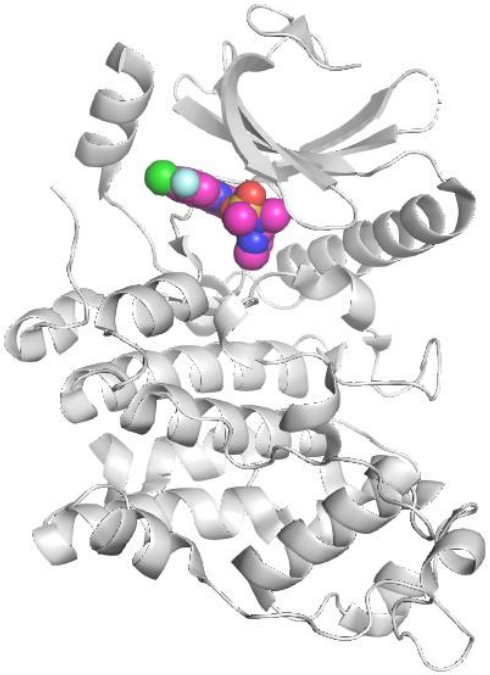


But restricting to a single protein family shows only ‘pink’ site is function in both Diptera and Lepidoptera

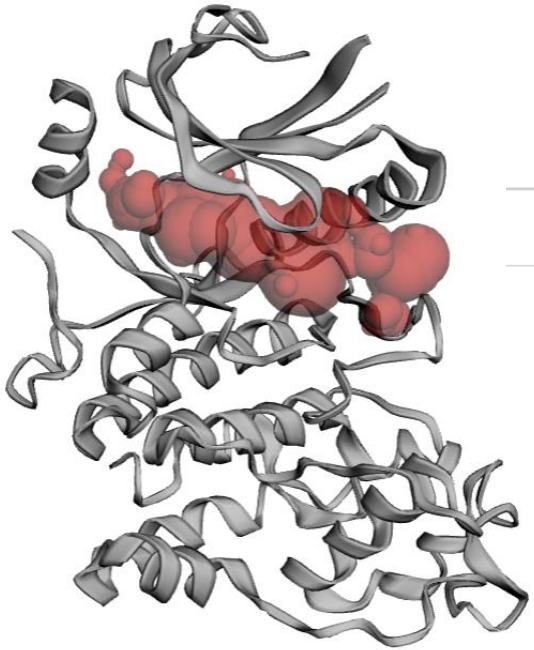




Some servers require thought…



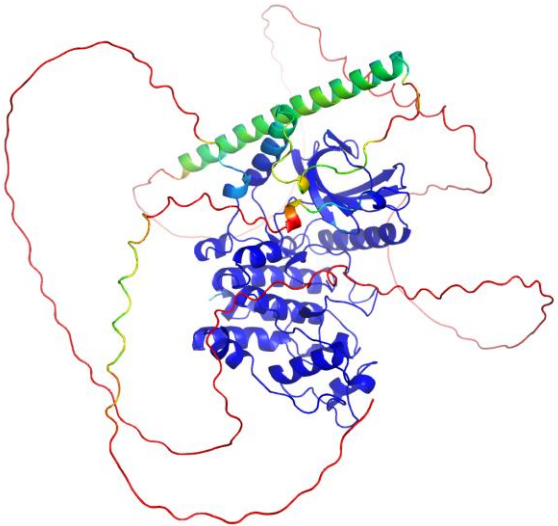
CastP



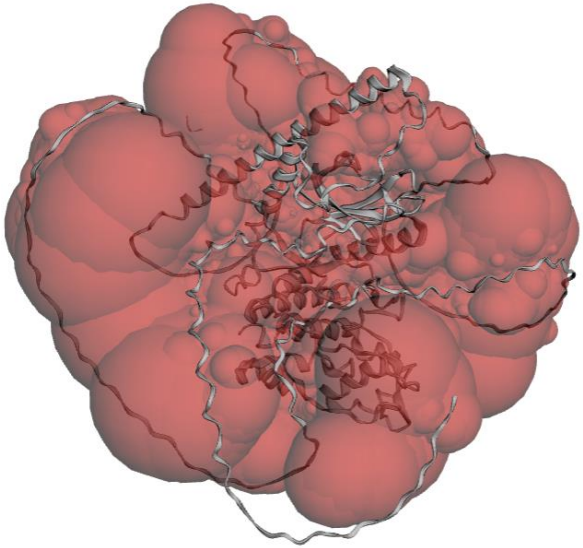
PocID	Area (SA) Å^2	Volume (SA) Å^3
1	762.617	506.394

Human SRSF protein kinase 2, PDB 7zkx

Some servers require thought…



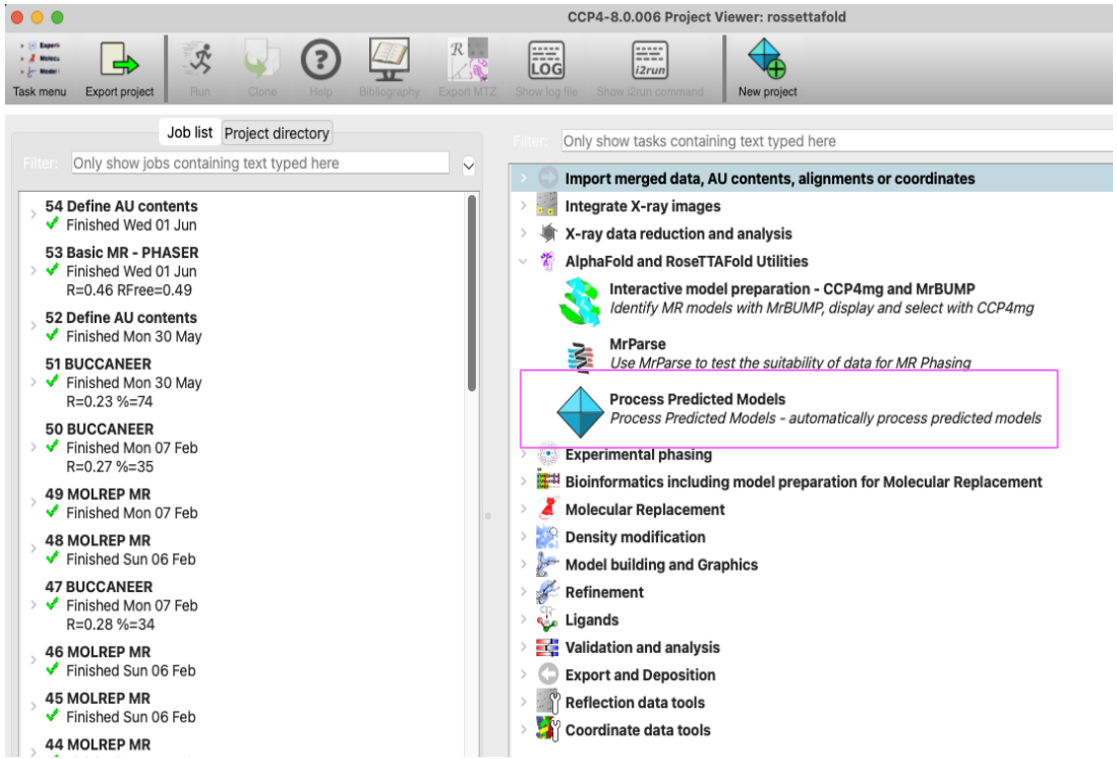
CastP



PocID	Area (SA) Å^2	Volume (SA) Å^3
1	21186.058	159841.419

Human SRSF protein kinase 2, AFDB AF-P78362-F1

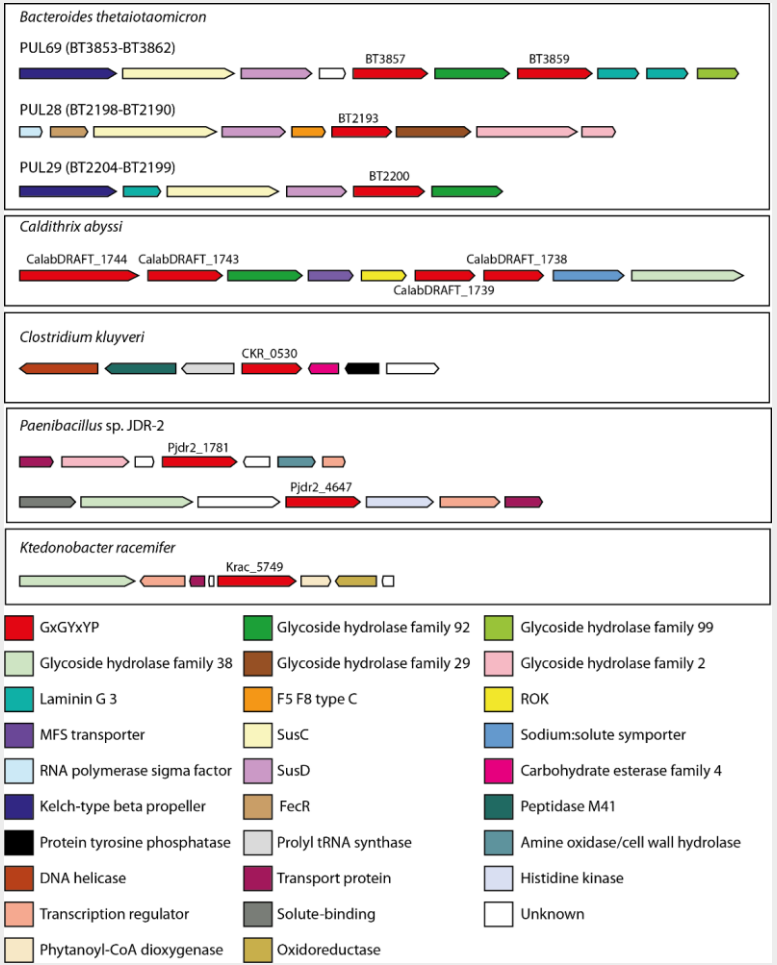
CCP4 i2 can remove the red spaghetti (and convert pLDDT to B-factor)



Case study from Structural Genomics

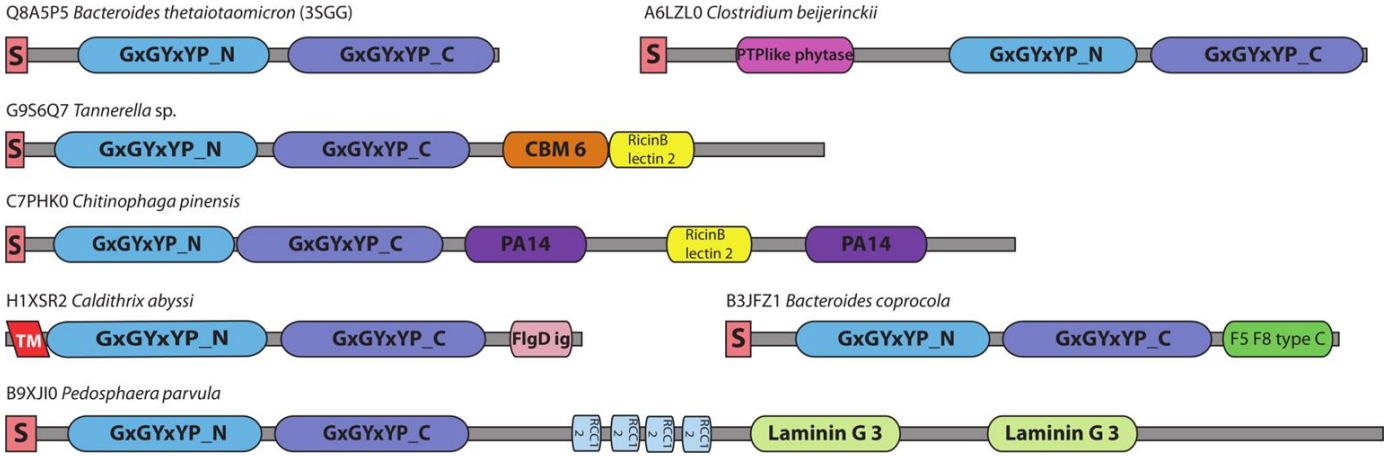
GxGYxYP proteins

- Named for a conserved sequence motif. Molecular function unknown
- Over-represented in gut bacteria
- Found in Polysaccharide Utilization Loci in *Bacteroides thetaiotaomicron*
- *Q: What does the protein do?*



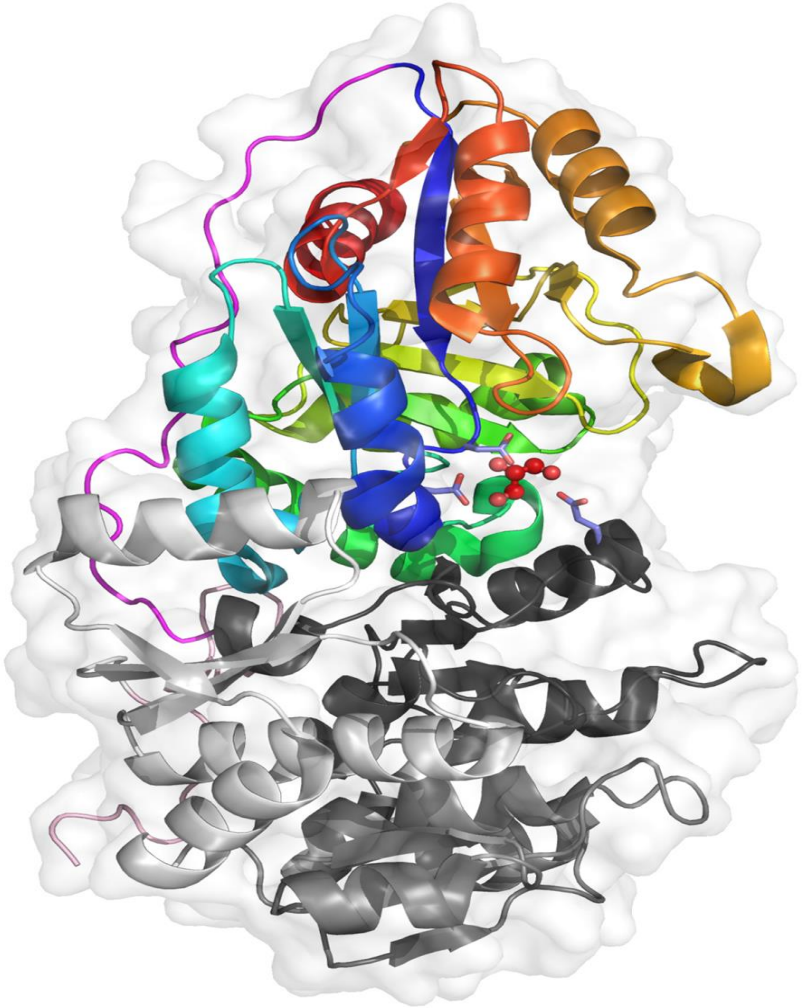
GxGYxYP proteins

- Domain architectures also predict carbohydrate connection



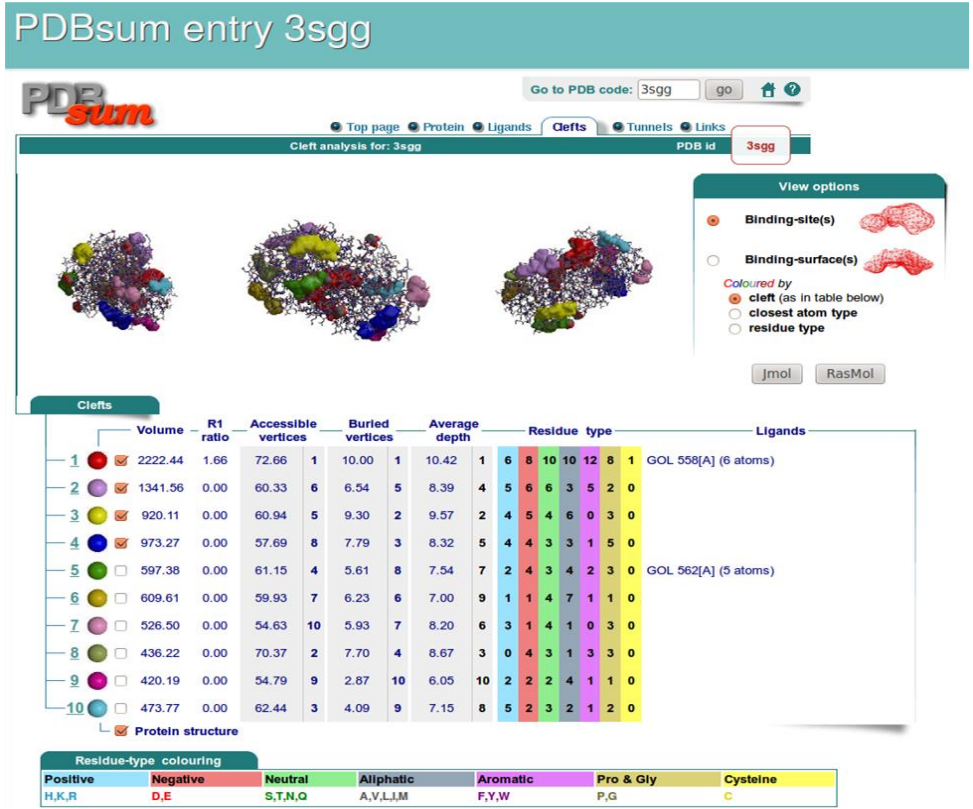
GxGYxYP proteins

- Overall folds don’t help much
- TIM barrel + 3 x novel α+β unit
- TIM barrel DALI Z >13 for
  - Allantoinase
  - Polysaccharide deacetylase
  - Glycosyltransferase



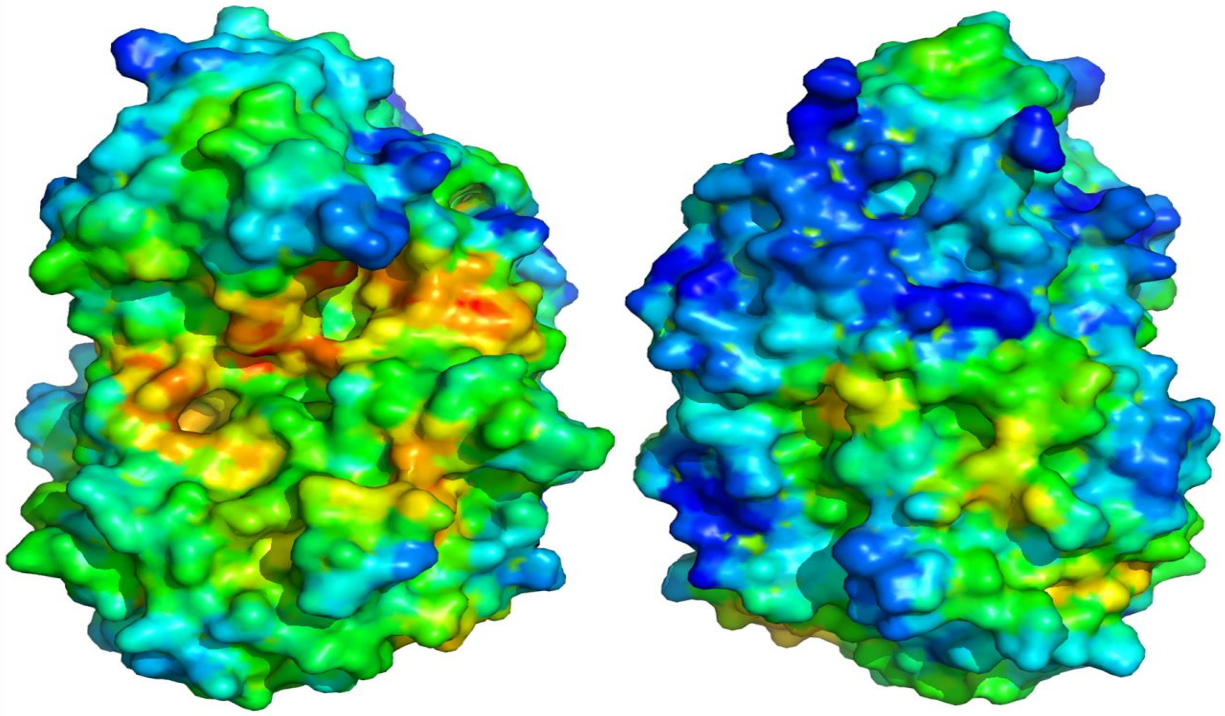
GxGYxYP proteins

- Largest cavity lies between domains
- Glycerol from crystallisation solution in it



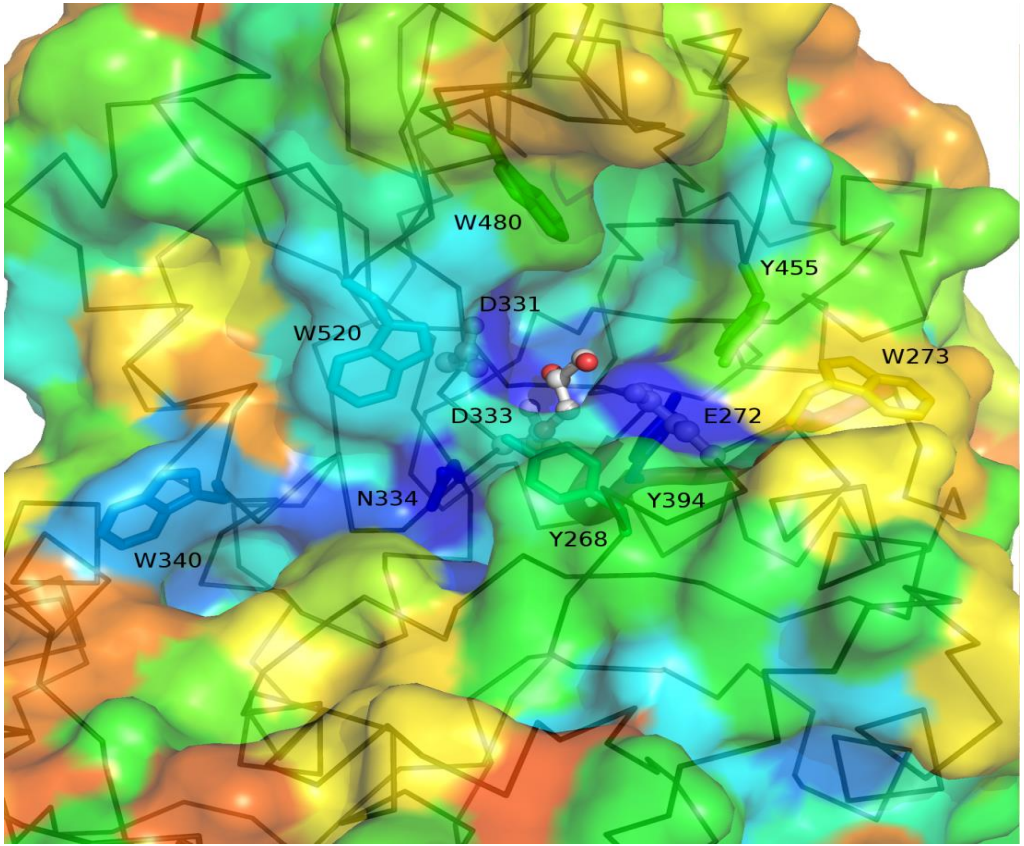
GxGYxYP proteins

- Largest cavity lies between domains
- Glycerol from crystallisation solution in it
- Picked out by non-geometry based STP (surface triplet propensities)



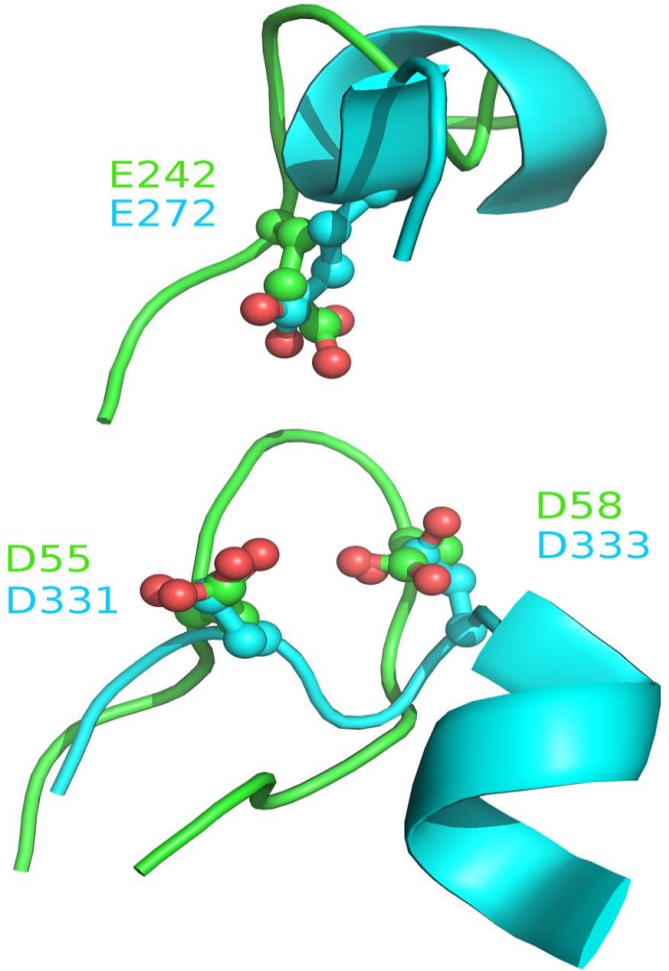
GxGYxYP proteins

- The patch is conserved
- Contains multiple aromatic residues, often surface lying in carbohydrate binding sites…



GxGYxYP proteins

- The patch is conserved
- Contains multiple aromatic residues, often surface lying in carbohydrate binding sites…
- … and acidic residues resembling known glycosidase site… (SPRITE)
- … and with perturbed pKa values (THEMATICS)



cellulase
3sgg

Multiple methods in bioinformatics

GxGYxYP conclusion

- GxGYxYP is a novel Glycoside Hydrolase family
  - Genome context
  - Domain composition
  - Cavity
  - Bound glycerol
  - STP
  - Conservation
  - Match to known GH catalytic site
  - pKa perturbation

Structure-based methods

Rigden et al. (2014) BMC Bioinformatics 15:196

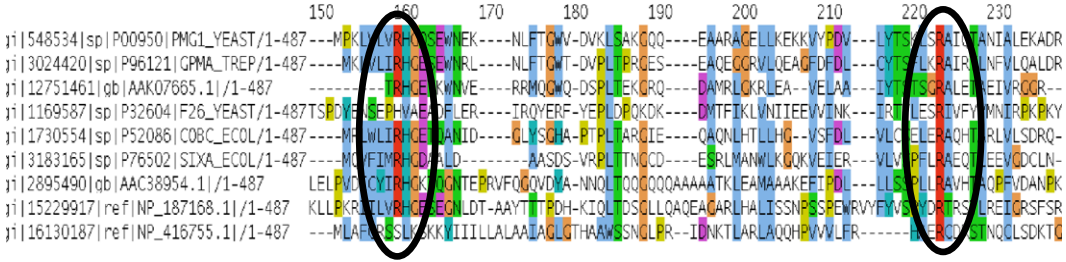
…and finally, you're putting a manuscript together

Calculating and presenting sequence alignments

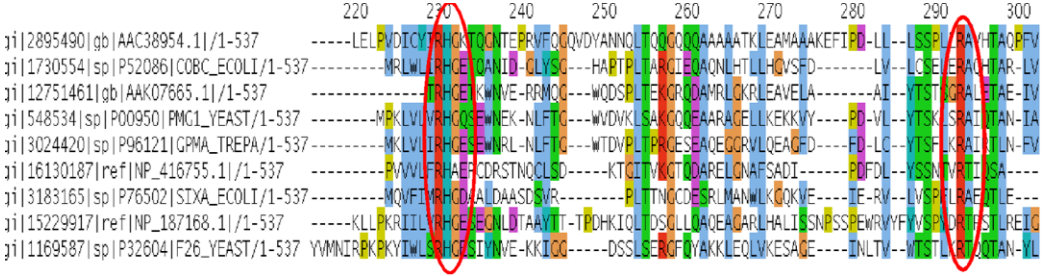
Your sequence alignment

- Don’t use ClustalW! It’s 24 years old! Modern methods like MUSCLE, Probcons and MAFFT are much better

ClustalW misses relatively obvious RHG motif in some of diverse sequence set…

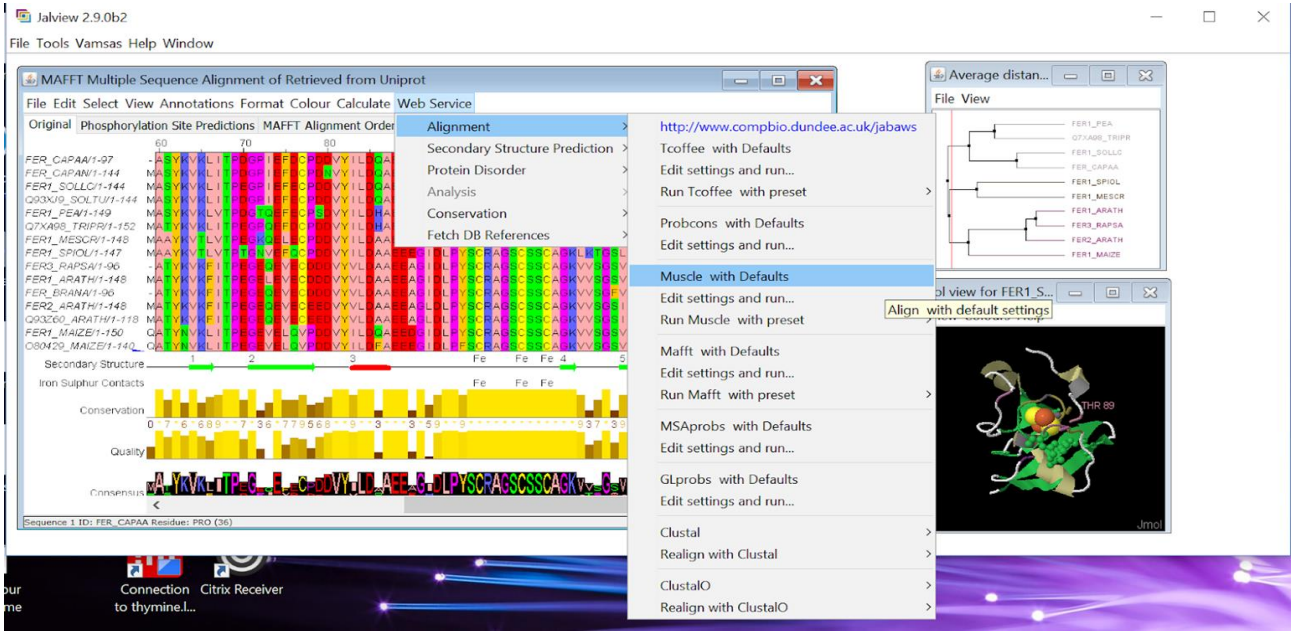


… but MUSCLE gets it



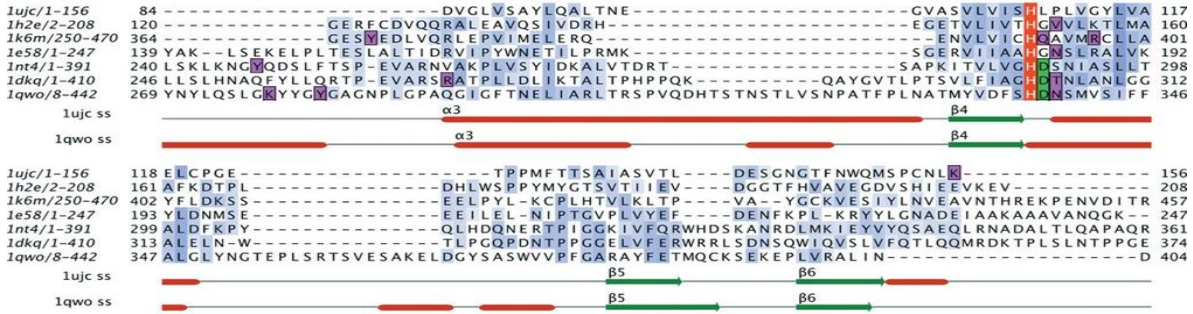
Multiple methods in bioinformatics : Jalview.org

- All these alignment methods and more are available through Jalview on Dundee servers



Jalview

- Also helps you produce figures like this…



- … rather than like this

Don’t forget to cite it (and all your bioinformatics)!

Waterhouse et al (2009), *Bioinformatics* **25**, 1189

Questions?

drigden@liv.ac.uk