

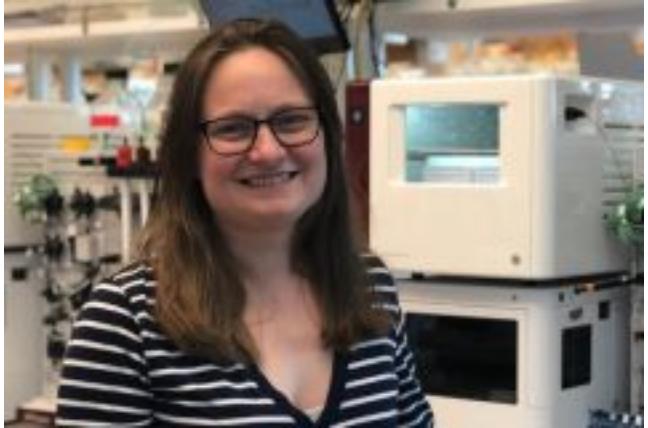
VIB training

Introduction to computational protein design

VUB-VIB Center for structural biology

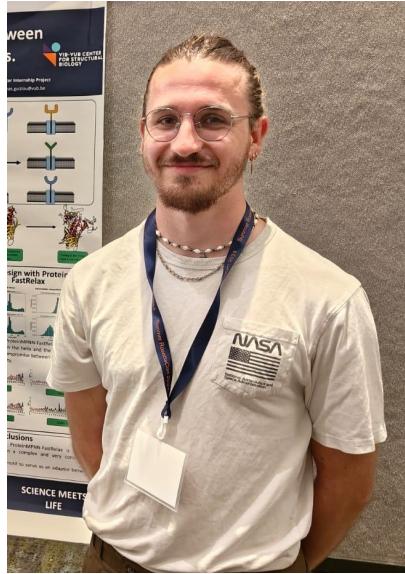
11/09/2024

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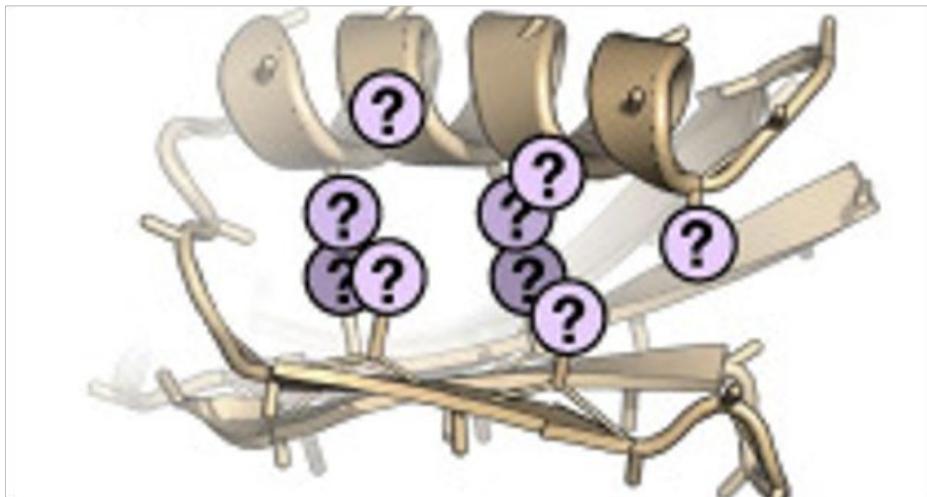
andrei.sokolovskii@vib.be

Training agenda

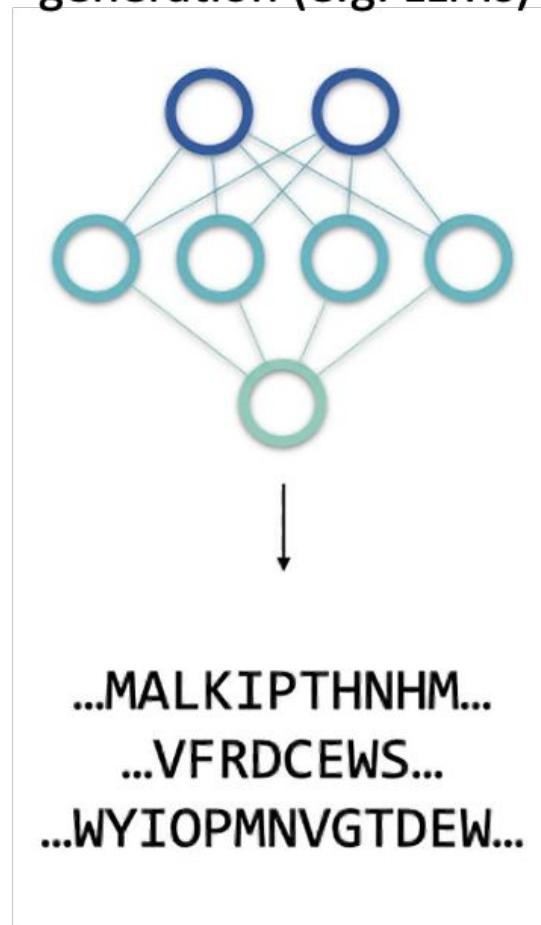
	DAY 1	DAY 2
9:30 – 10:55	Introduction: selecting a PDB template	Introduction to protein design
10:55 – 11:15	Break	Break
11:15 – 12:45	Preparing the PDB	Building backbones with RFDiffusion
12:45 – 13:45	Lunch	Lunch
13:45 – 15:10	Site saturation mutagenesis	Sequence design with ProteinMPNN
15:10 – 15:30	Break	Break
15:30 – 17:00	Combinatorial mutagenesis with PyRosetta	Filtering designs with ColabFold

Structure vs sequence-based protein design

Structure-based design

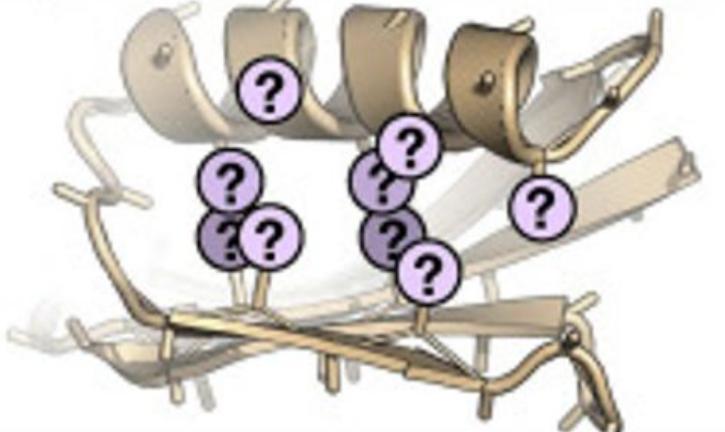


Direct sequence
generation (e.g. LLMs)

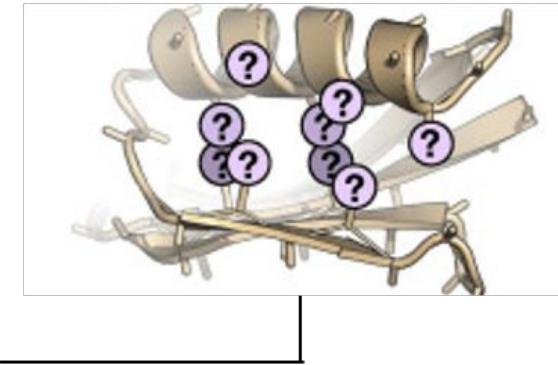
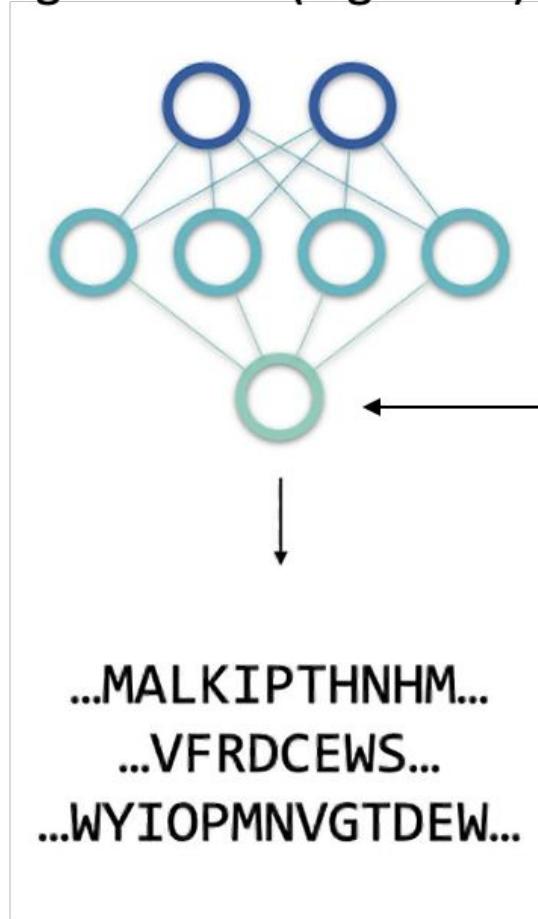


Structure vs sequence-based protein design

Structure-based design



Direct sequence
generation (e.g. LLMs)



DAY 1: Predicting the effect of mutations

1. Selecting your template PDB structure

Biological Assembly 1

7FAO

Top7 surface mutant K42A Q43A K46A K57S K58S, and I68R

PDB DOI: <https://doi.org/10.2210/pdb7FAO/pdb>

Classification: DE NOVO PROTEIN

Organism(s): unidentified

Expression System: Escherichia coli BL21

Mutation(s): No

Deposited: 2021-07-07 Released: 2022-05-18

Deposition Author(s): Ito, Y., Makabe, K.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 1.43 Å

R-Value Free: 0.242

R-Value Work: 0.203

R-value Observed: 0.205

Starting Model: experimental

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree	2	0.242
Clashscore	10	2
Ramachandran outliers	0	0
Sidechain outliers	1.8%	1.8%
RSRZ outliers	6.8%	6.8%

Legend: Worse (red) Better (blue)

Worse Better

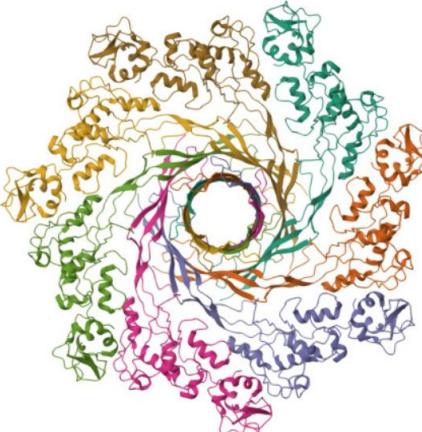
Percentile relative to all X-ray structures

Percentile relative to X-ray structures of similar resolution

EXERCISE: download and visualize the PDB structures to find the issues

Problem: Low resolution structure

Biological Assembly 1 [?](#)
folding_assay folder for storing your documents.



[Explore in 3D: Structure | Sequence Annotations](#)
[| Electron Density | Validation Report |](#)
[Predict Membrane \[?\]\(#\)](#)

Global Symmetry: Cyclic - C7 [?](#) ([Explore in 3D](#))
Global Stoichiometry: Homo 7-mer - A7 [?](#)

[Find Similar Assemblies](#)

[Display Files](#) [Download Files](#) [Data API](#)

5JZT

Cryo-EM structure of aerolysin pore in LMNG micelle

PDB DOI: <https://doi.org/10.2210/pdb5JZT/pdb> EM Map EMD-8187: EMDB EMDataResource

Classification: TOXIN

Organism(s): Aeromonas hydrophila

Expression System: Escherichia coli BL21(DE3)

Mutation(s): No [?](#)

Membrane Protein: Yes [?](#) [OPM](#) [PDBTM](#)

Deposited: 2016-05-17 Released: 2016-07-13

Deposition Author(s): Iacovache, I., Zuber, B.

Funding Organization(s): Swiss National Science Foundation

Experimental Data Snapshot

Method: ELECTRON MICROSCOPY

Resolution: 7.40 Å

Aggregation State: PARTICLE

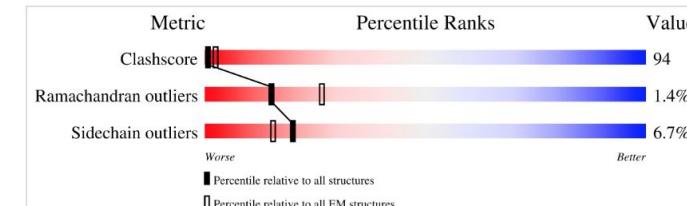
Reconstruction Method: SINGLE PARTICLE

Starting Model: experimental

[View more details](#)

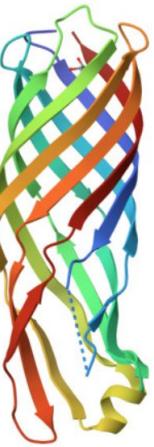
wwPDB Validation [?](#)

[3D Report](#) [Full Report](#)



Problem: Poor refinement

Biological Assembly 1 ?



Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report | Ligand Interaction (GOL) | Predict Membrane ⓘ

Global Symmetry: Asymmetric - C1 ⓘ **Global Stoichiometry:** Monomer - A1 ⓘ

Find Similar Assemblies

Display Files ▾ Download Files ▾ Data API

2F1V

Outer membrane protein OmpW

PDB DOI: <https://doi.org/10.2210/pdb2F1V/pdb>

Classification: MEMBRANE PROTEIN TMB12_3_nb3

Organism(s): Escherichia coli K-12

Expression System: Escherichia coli

Mutation(s): No ⓘ

Membrane Protein: Yes ⓘ OPM PDBTM MemProtMD

Deposited: 2005-11-15 Released: 2006-01-24

Deposition Author(s): van den Berg, B.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.70 Å
R-Value Free: 0.314
R-Value Work: 0.292
R-Value Observed: 0.293

Starting Model: experimental
[View more details](#)

wwPDB Validation ⓘ

3D Report Full Report

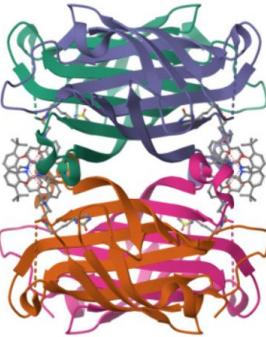
Metric	Percentile Ranks	Value
Rfree	29	0.304
Clashscore	29	
Ramachandran outliers	2.8%	
Sidechain outliers	11.0%	

Worse Better

■ Percentile relative to all X-ray structures
□ Percentile relative to X-ray structures of similar resolution

Problem: Missing loops, alternative conformations (b-factor)

Biological Assembly 1



Explore in 3D: Structure | Sequence Annotations | Validation Report | Ligand Interaction (UFU)

Global Symmetry: Dihedral - D2 (Explore in 3D)
Global Stoichiometry: Homo 4-mer - A4 (Explore in 3D)

Find Similar Assemblies

Biological assembly 1 assigned by authors and generated by PISA (software)

Biological Assembly Evidence: gel filtration

Macromolecule Content

Display Files ▾ Download Files ▾ Data API

8QEX

Streptavidin variant with a cobalt catalyst for CH metal-catalyzed hydrogen-atom-transfer (M-HAT)

PDB DOI: <https://doi.org/10.22110/pdb8QEX/pdb>

Classification: METAL BINDING PROTEIN
Organism(s): Streptomyces avidinii
Expression System: Escherichia coli BL21(DE3)
Mutation(s): No

Deposited: 2023-09-01 **Released:** 2024-07-31
Deposition Author(s): Jakob, R.P., Chen, D., Ward, T.R.
Funding Organization(s): Not funded

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 1.90 Å
R-Value Free: 0.254
R-Value Work: 0.229
R-Value Observed: 0.230

Starting Model: experimental
[View more details](#)

wwPDB Validation i

3D Report Full Report

Metric	Percentile Ranks	Value
Rfree	Worse	0.258
Clashscore	9	0
Ramachandran outliers	0	0
Sidechain outliers	0	0
RSRZ outliers	5.7%	0.258

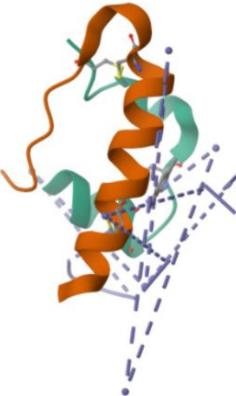
Worse Better
■ Percentile relative to all X-ray structures
□ Percentile relative to X-ray structures of similar resolution

Ligand Structure Quality Assessment i

Worse 0 Better 1
Ligand structure goodness of fit to experimental data

Problem: Ligand modelling

Biological Assembly 1



Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report | Ligand Interaction (CRS)

Global Symmetry: Asymmetric - C1
Global Stoichiometry: Hetero 3-mer - A1B1C1

Find Similar Assemblies

Display Files ▾ Download Files ▾ Data API

7INS

STRUCTURE OF PORCINE INSULIN COCRYSTALLIZED WITH CLUPEINE Z

PDB DOI: <https://doi.org/10.2210/pdb7INS/pdb>

Classification: HORMONE
Organism(s): Sus scrofa
Mutation(s): No

Deposited: 1991-09-03 Released: 1994-01-31
Deposition Author(s): Balschmidt, P., Hansen, F.B., Dodson, E., Dodson, G., Korber, F.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.00 Å
R-Value Observed: 0.194

wwPDB Validation

3D Report Full Report

Metric	Percentile Ranks	Value
Clashscore	85	85
Ramachandran outliers	1.4%	1.4%
Sidechain outliers	22.2%	22.2%
RSRZ outliers	2.0%	2.0%

Worse Better
■ Percentile relative to all X-ray structures
□ Percentile relative to X-ray structures of similar resolution

This is version 1.3 of the entry. See complete history.

This time, download the PDB and the MTZ map. Look at the unknown ligands (UNK) in Pymol

2. Preparing the PDB structure

A. Clean PDB

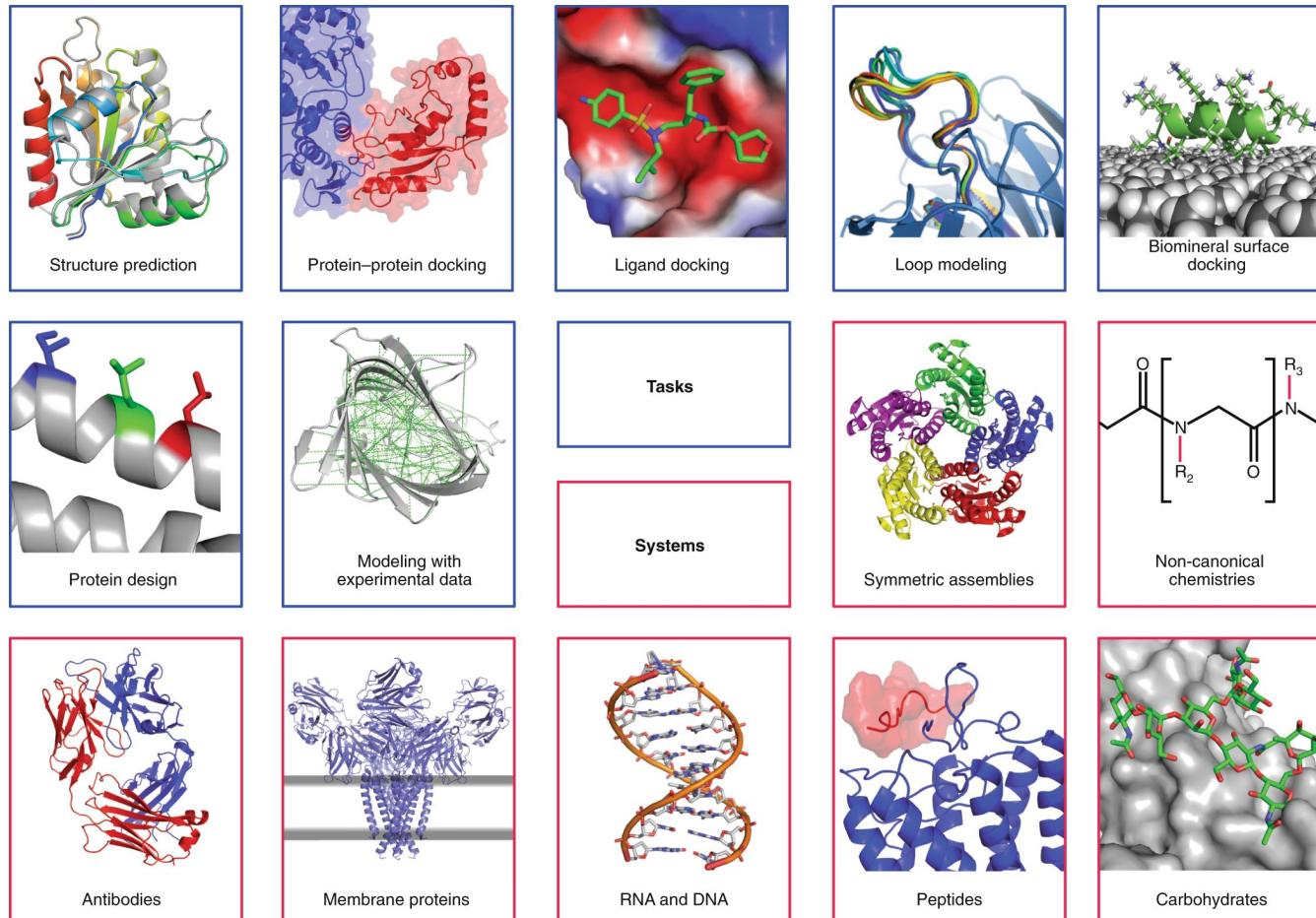


ATOM	8178	CD2	LEU	A	207	135.703	156.448	175.146	1.00	59.65	C
ATOM	8179	N	LEU	A	208	138.542	160.482	176.163	1.00	64.39	N
ATOM	8180	CA	LEU	A	208	139.558	160.550	177.204	1.00	64.39	C
ATOM	8181	C	LEU	A	208	139.523	161.898	177.917	1.00	64.39	C
ATOM	8182	O	LEU	A	208	140.107	162.065	178.988	1.00	64.39	O
ATOM	8183	CB	LEU	A	208	140.944	160.306	176.607	1.00	64.39	C
ATOM	8184	CG	LEU	A	208	141.162	158.912	176.018	1.00	64.39	C
ATOM	8185	CD1	LEU	A	208	142.520	158.825	175.340	1.00	64.39	C
ATOM	8186	CD2	LEU	A	208	141.011	157.842	177.088	1.00	64.39	C
ATOM	8187	OXT	LEU	A	208	138.909	162.851	177.438	1.00	64.39	O
TER	8188		LEU	A	208						
HETATM	8189	O1'	LMT	E	301	138.158	131.617	150.769	1.00	20.00	O
HETATM	8190	C1	LMT	E	301	137.918	131.876	152.147	1.00	20.00	C
HETATM	8191	C2	LMT	E	301	137.062	133.128	152.260	1.00	20.00	C
HETATM	8192	C3	LMT	E	301	137.071	133.678	153.676	1.00	20.00	C
HETATM	8193	C4	LMT	E	301	136.376	135.025	153.714	1.00	20.00	C
HETATM	8194	C5	LMT	E	301	135.935	135.347	155.125	1.00	20.00	C
HETATM	8195	C6	LMT	E	301	135.530	136.801	155.206	1.00	20.00	C
HETATM	8196	C7	LMT	E	301	136.343	137.543	156.242	1.00	20.00	C
HETATM	8197	C8	LMT	E	301	135.478	138.063	157.375	1.00	20.00	C
HETATM	8198	C9	LMT	E	301	136.194	137.846	158.690	1.00	20.00	C
HETATM	8199	C10	LMT	E	301	135.627	138.700	159.797	1.00	20.00	C
HETATM	8200	C11	LMT	E	301	135.963	138.052	161.121	1.00	20.00	C
HETATM	8201	C12	LMT	E	301	135.929	139.084	162.215	1.00	20.00	C



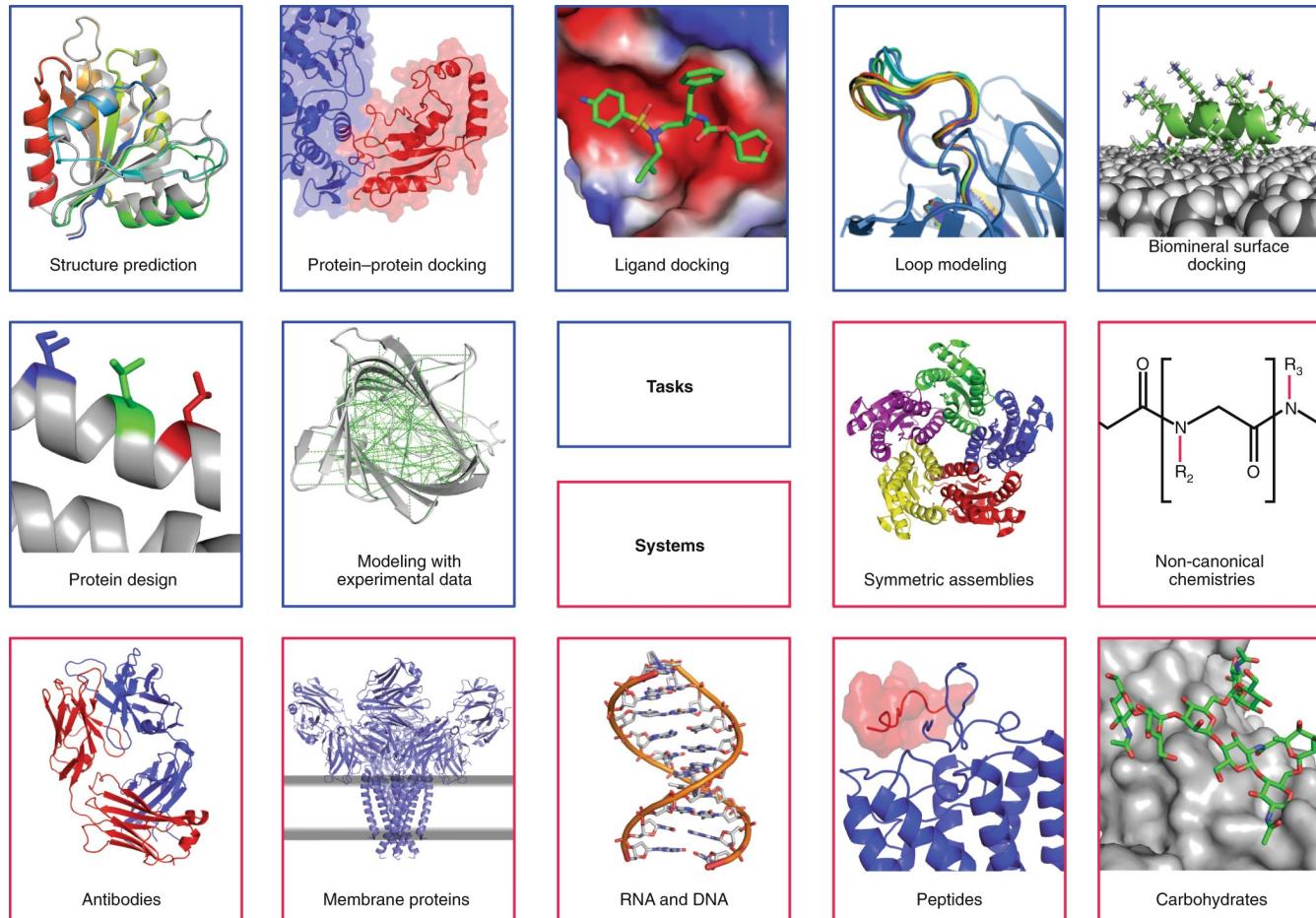
B. Preparing the PDB for Rosetta calculations

What is Rosetta ?

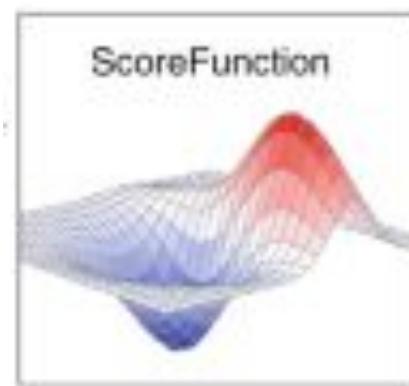
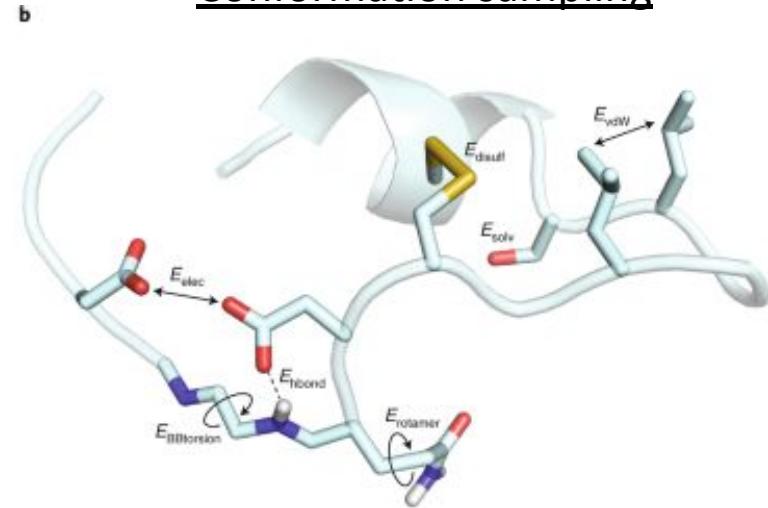


B. Preparing the PDB for Rosetta calculations

What is Rosetta ?



Conformation sampling



Scoring

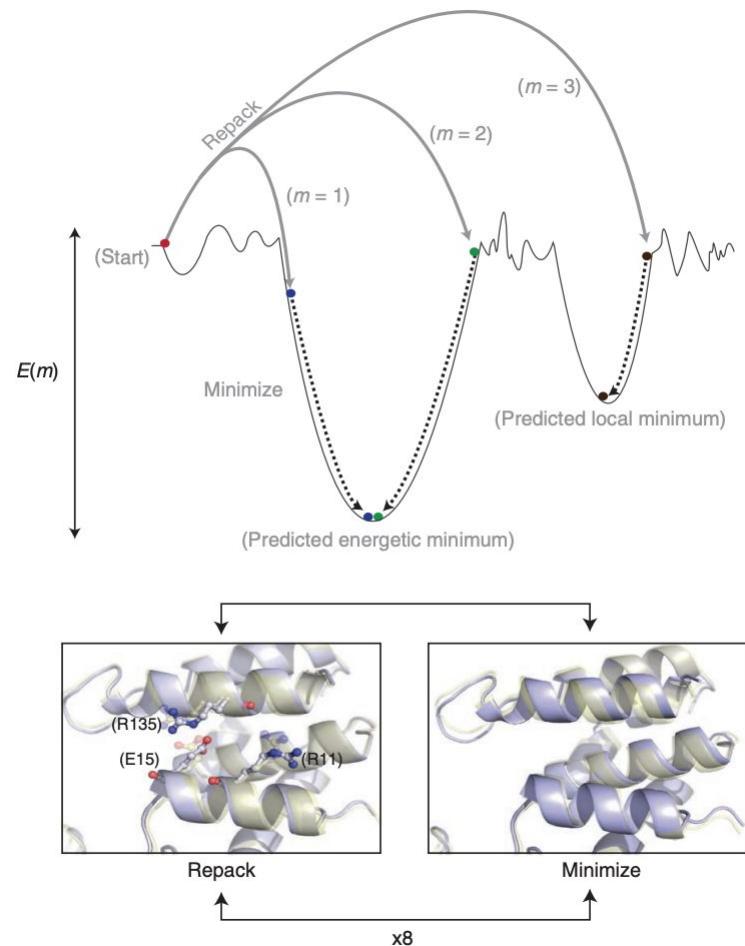
E_{vdW} : Lennard-Jones for attractive or repulsive interaction
 E_{disulf} : Disulfide bonds between cysteines
 E_{solv} : Implicit solvation model penalizes buried polar atoms
 $E_{torsion}$: Backbone torsion preferences from main-chain potential
 $E_{rotamer}$: Side-chain torsion angles from rotamer library
 E_{ref} : Unfolded state reference energy for design

E_{solv} : Implicit solvation model penalizes buried polar atoms
 $E_{torsion}$: Backbone torsion preferences from main-chain potential
 $E_{rotamer}$: Side-chain torsion angles from rotamer library
 E_{ref} : Unfolded state reference energy for design

B. Relaxing the PDB for Rosetta calculations

Rosetta **FastRelax** algorithm

~ simulated annealing



B. Relaxing the PDB for Rosetta calculations

Rosetta FastRelax algorithm

~ simulated annealing

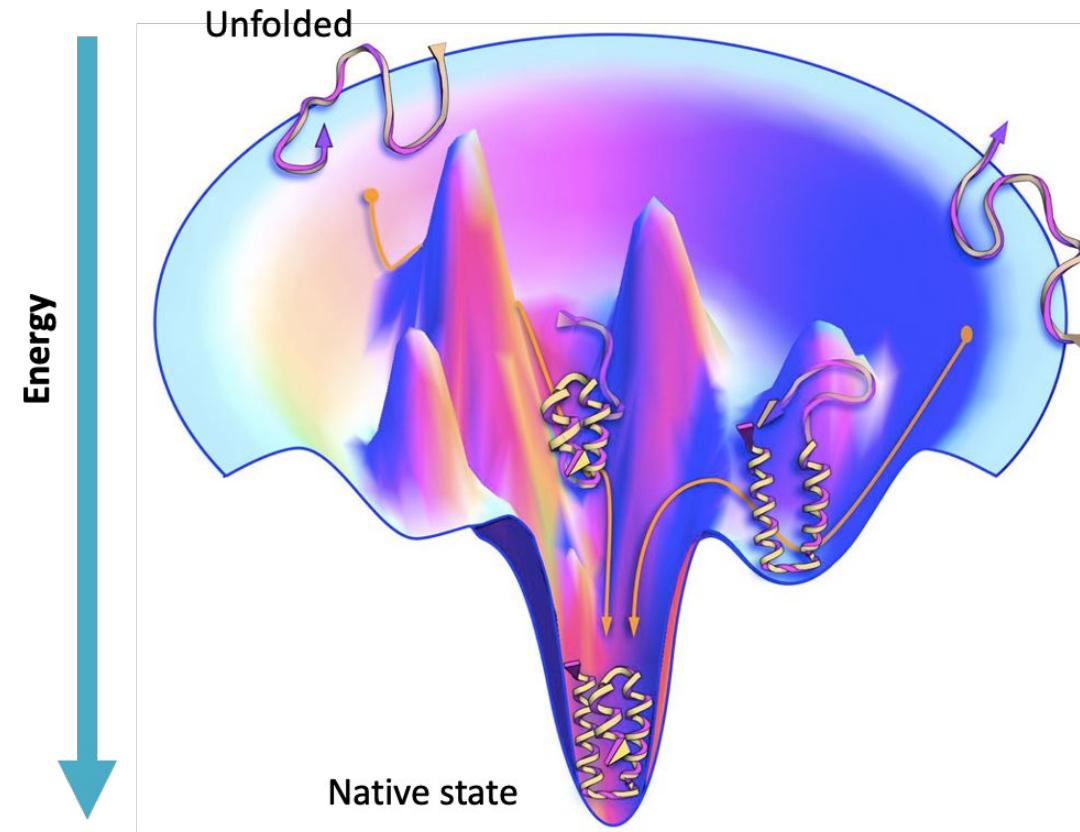
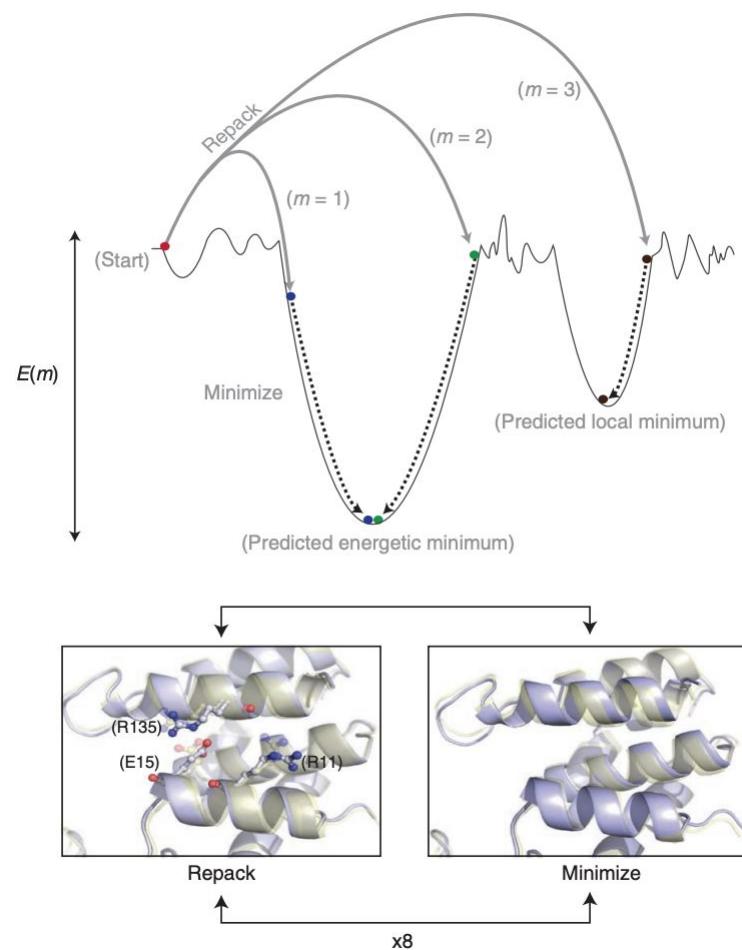
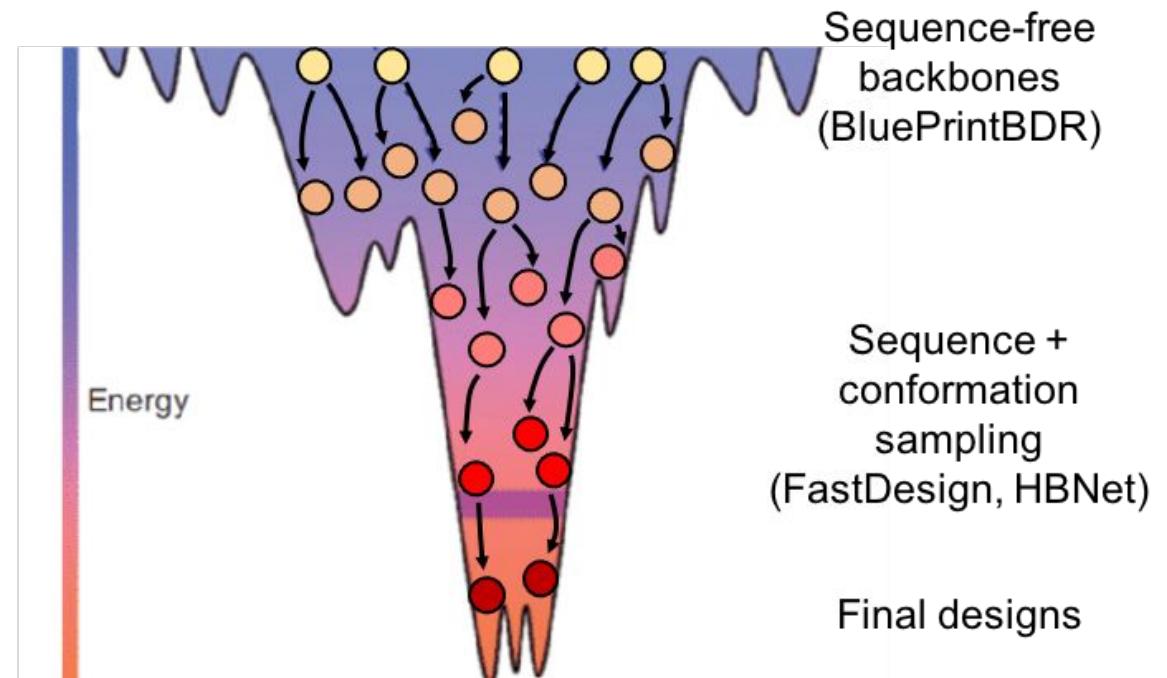
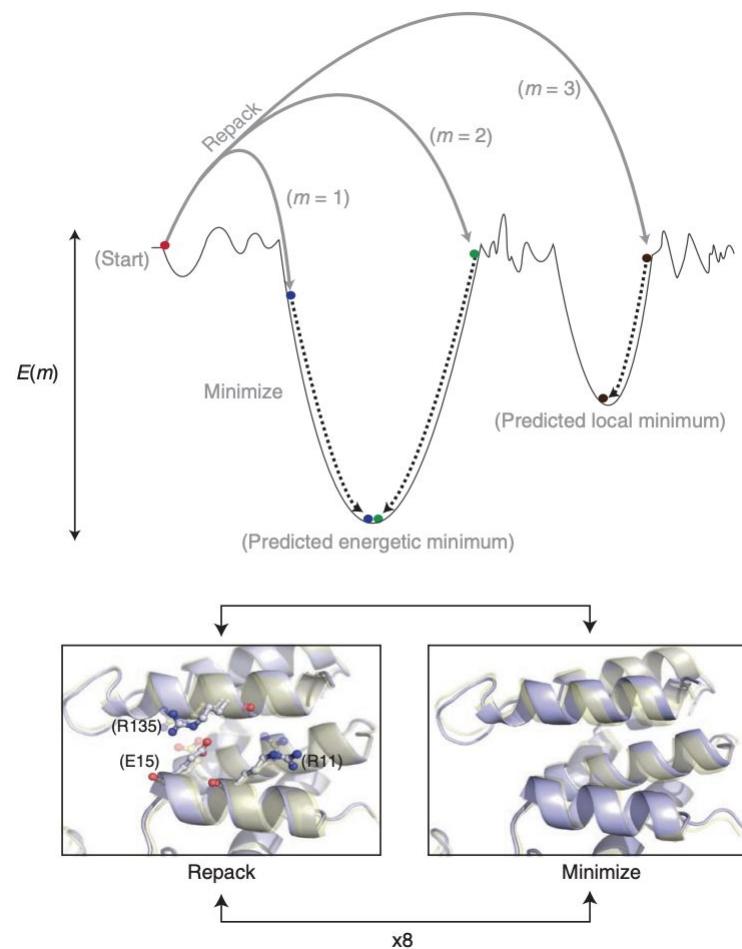


Image from: "The protein-folding problem, 50 years on." science 338, no. 6110 (2012): 1042-1046.

B. Relaxing the PDB for Rosetta calculations

Rosetta **FastRelax** algorithm
~ simulated annealing



Let's connect to your computing environment

1. Connect to the VUB Jupyter hub: <https://notebooks.hpc.vub.be/>

2. Sign-in with your VSC account and authorize Jupyterhub

3. Spawn a server

Reservation: keep empty(automatically *train_comp_prot_d1*)

4. Open a terminal window and create a new directory for the class
mkdir training_day1

5. Go to the directory
cd training_day1/

6. Download the course material:
git clone
https://github.com/AndreiSokolovskii/Protein_ddG_workshop.git
cd Protein_ddG_workshop

7. Look for dependencies on the left and load:

1) PyRosetta/4.release-384-gompi-2022a; 2) SciPy-bundle/2022.05-foss-2022a
3) py3Dmol/2.0.1.post1-GCCcore-11.3.0; 4) Biopython/1.79-foss-2022a; matplotlib/3.5.2-foss-2022a
5) Seaborn/0.12.1-foss-2022a 6) matplotlib/3.5.2-foss-2022a

8. Open the Jupyter notebook that was downloaded

Server Options

Simple	Advanced
Cluster Partition:	<input type="text" value="hydra.skylake"/>
Number of CPUs (--cpus-per-task):	<input type="text" value="5"/> / 40
Total memory (--mem):	<input type="text" value="4"/> / 187GB
Number of GPUs (--gres:<gpu>):	<input type="text" value="0"/> / 0
Job duration (as hh:mm:ss, --time):	<input type="text" value="8:00:00"/>
Launch JupyterLab:	<input checked="" type="checkbox"/>
Jupyter environment:	
Python v3.11.3	
<input type="radio"/> 2023a Default: minimal with all modules available	
<input type="radio"/> 2023a DataScience: SciPy-bundle + matplotlib + dask	
<input type="radio"/> 2023a Molecules: DataScience + nglview + 3Dmol	
<input type="radio"/> 2023a RStudio with R v4.3.2	
<input type="radio"/> 2023a MATLAB	
Python v3.10.4	
<input checked="" type="radio"/> 2022a Default: minimal with all modules available	
<input type="radio"/> 2022a DataScience: SciPy-bundle + matplotlib + dask	
<input type="radio"/> 2022a Molecules: DataScience + nglview + 3Dmol	
<input type="radio"/> 2022a RStudio with R v4.2.1	
<input type="radio"/> 2022a MATLAB	

You're fast ... let's try something else: relax without coordinate constraints

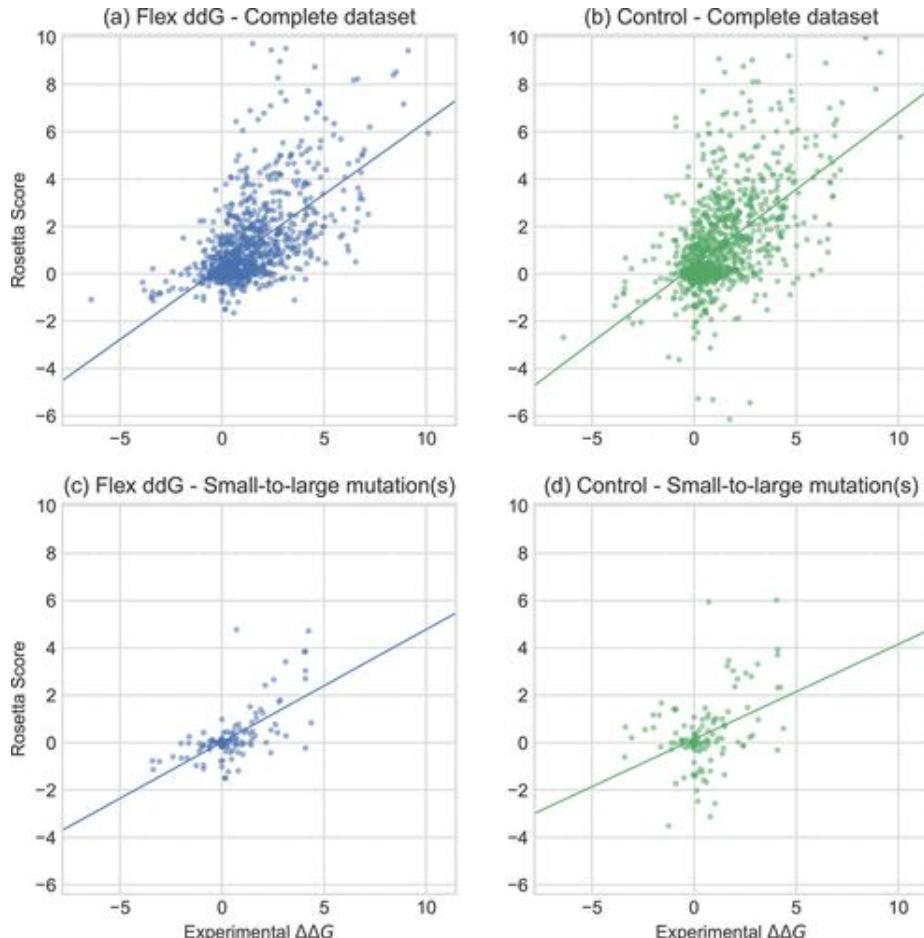
```
xml = pyrosetta.rosetta.protocols.rosetta_scripts.XmlObjects.create_from_string("""  
    <ROSETTASCRIPTS>  
        <SCOREFXNS>  
            <ScoreFunction name="SFX1" weights="ref2015_cart">  
                <Reweight scoretype="coordinate_constraint" weight="1.0"/>  
            </ScoreFunction>  
        </SCOREFXNS>  
        <RESIDUE_SELECTORS>  
        </RESIDUE_SELECTORS>  
        <TASKOPERATIONS>  
        </TASKOPERATIONS>  
        <FILTERS>  
        </FILTERS>  
        <MOVERS>  
            <AtomCoordinateCstMover name="coord_cst" />  
            <FastRelax name="relax" cartesian="true" scorefxn="SFX1" />  
        </MOVERS>  
        <APPLY_TO_POSE/>  
        <PROTOCOLS>  
            <Add mover="coord_cst" /> ← Comment out this line  
            <Add mover="relax" />  
        </PROTOCOLS>  
    </ROSETTASCRIPTS>  
    """).get_mover("ParsedProtocol")  
  
working_dir = os.getcwd()  
output_dir = dest
```

Change **conf**'s *coord_cst* parameter to **False** in a cell below

Look at the models in pymol ... what is the difference?

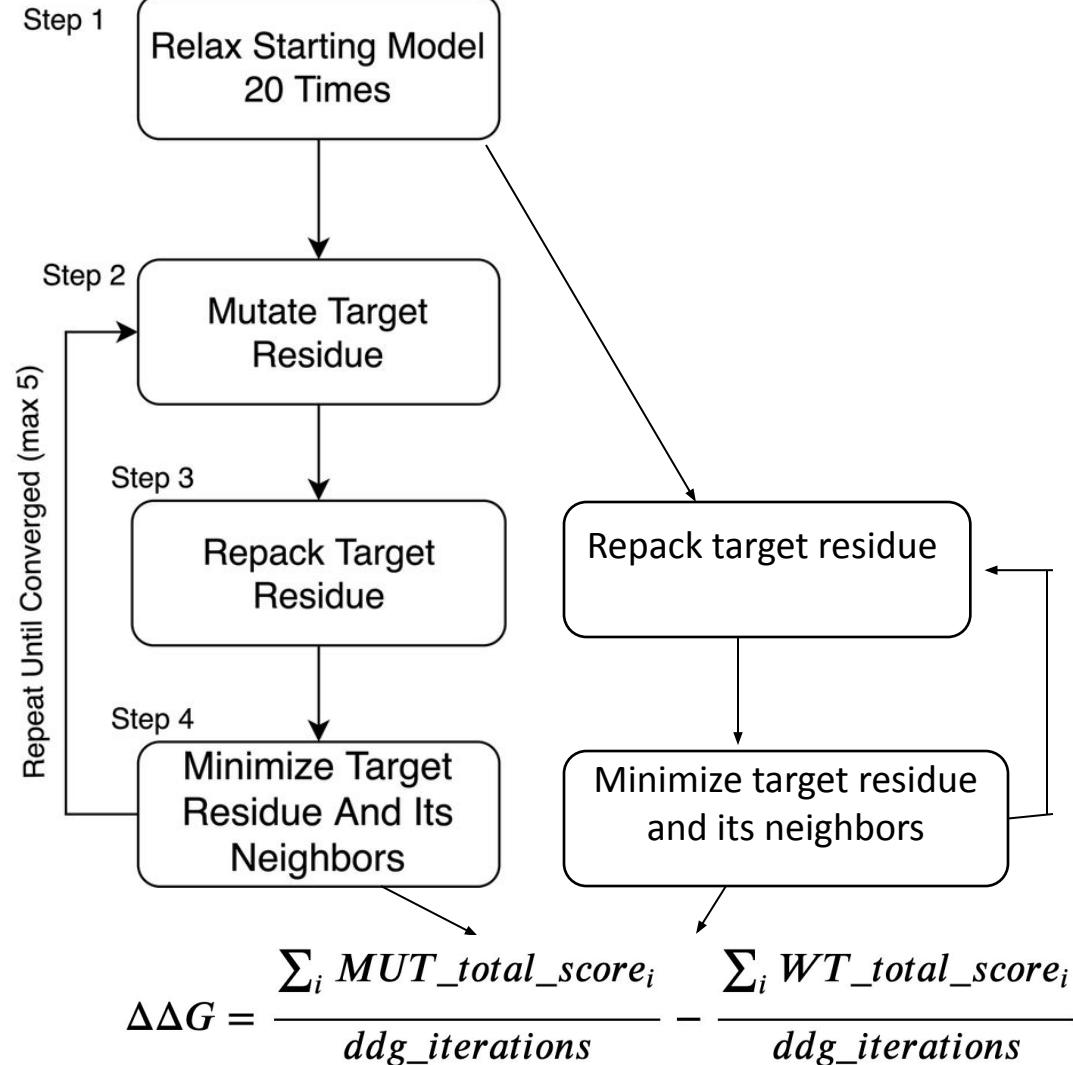
3. Running DDG calculations – Rosetta (energy model)

Rosetta's scorefunction was fitted to experimentally-determined DDGs



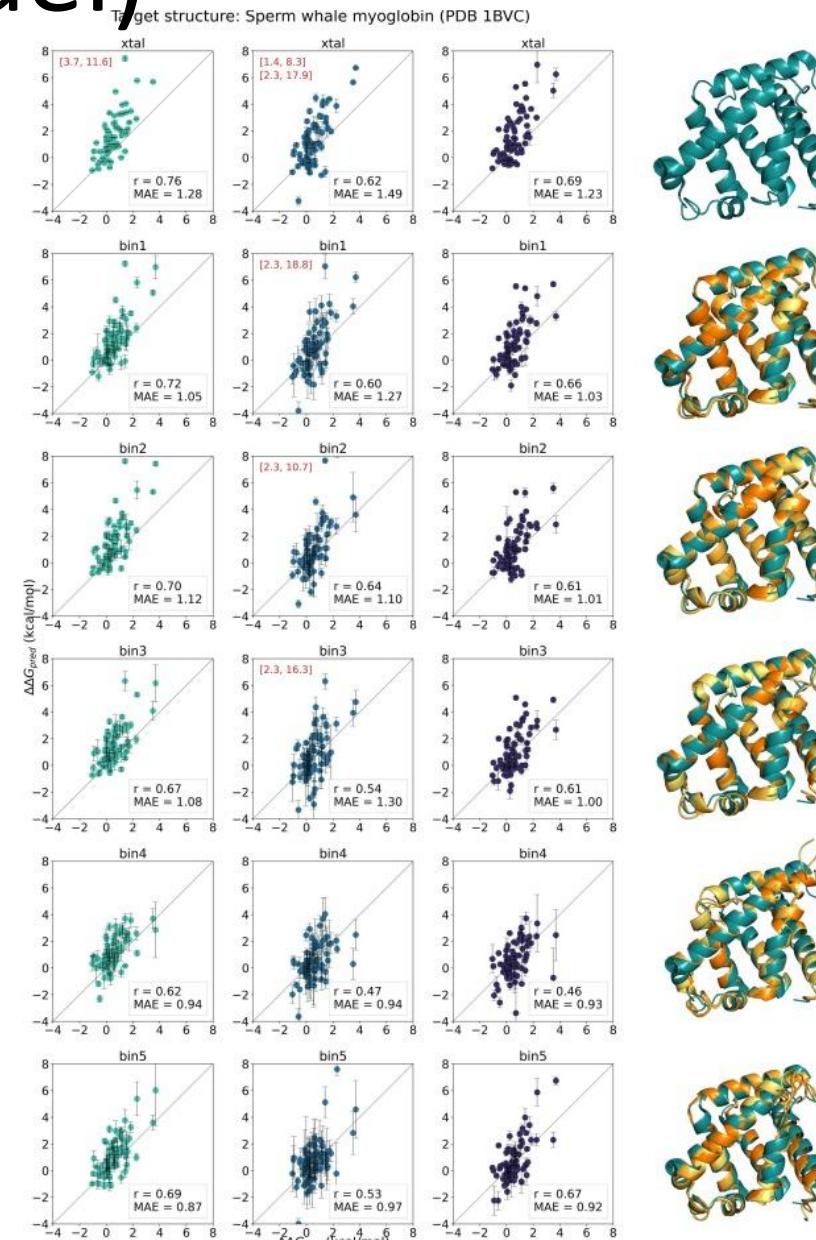
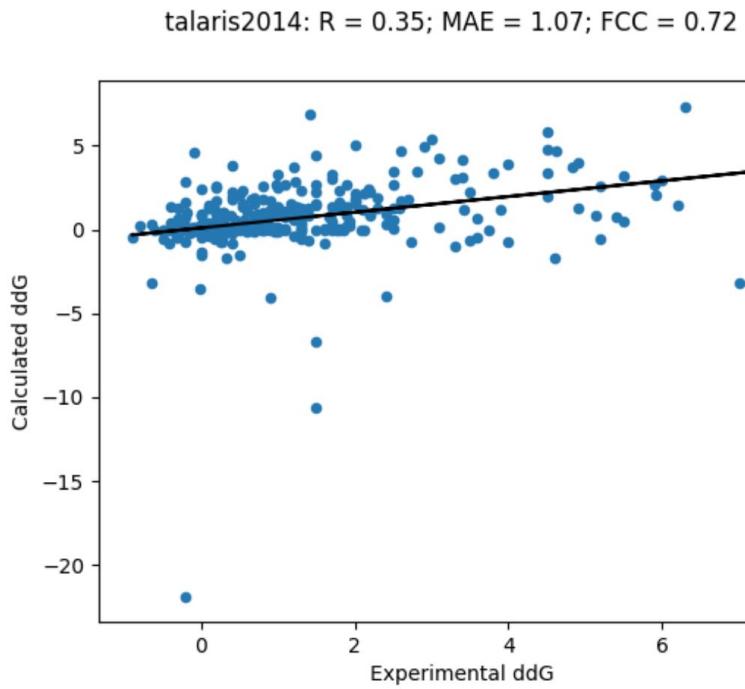
Benchmarks from Kortemme

Cartesian ΔΔG



3. Running DDG calculations – Rosetta (energy model)

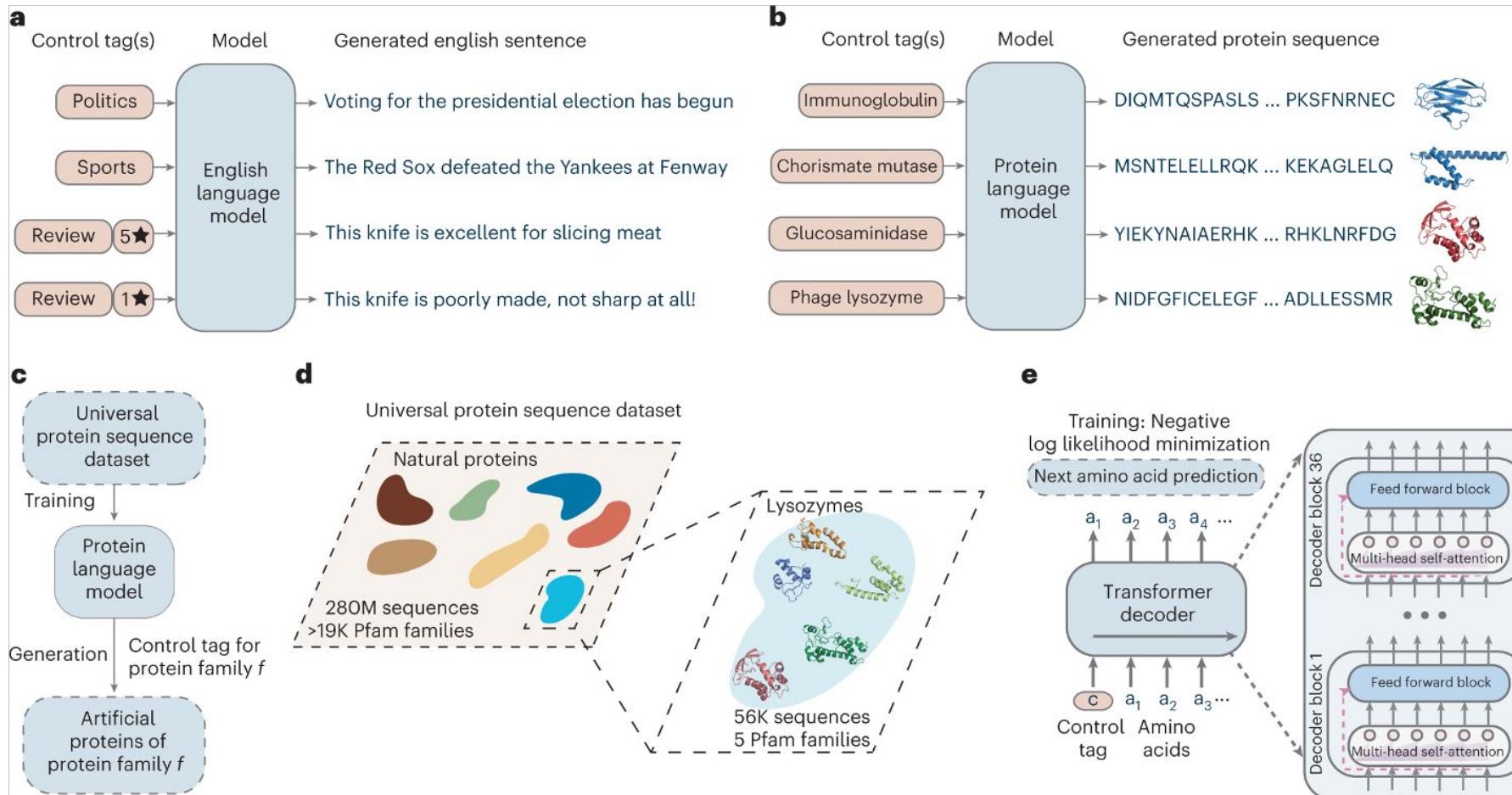
Accurate predictions can be made for alanine scanning, from homology and AlphaFold models



<https://doi.org/10.1016/j.csbj.2022.11.048>

Detailed benchmark: DOI: [10.3389/fbioe.2020.558247](https://doi.org/10.3389/fbioe.2020.558247)

3. Running DDG calculations – LLMs (evolutive models)



A notebook to run a basic LLM fitness prediction will be sent after the class (high memory requirements)

Exercise 1: generate a DDG heatmap with Rosetta

Back to the Jupyter notebook ... Let's start by mutating residues in a small peptide

Remember:

$$\Delta\Delta G = \frac{\sum_i MUT_total_score_i}{ddg_iterations} - \frac{\sum_i WT_total_score_i}{ddg_iterations}$$

Like the color palette? No? Then change it!

Exercise 2: generate an interaction_ddg heatmap

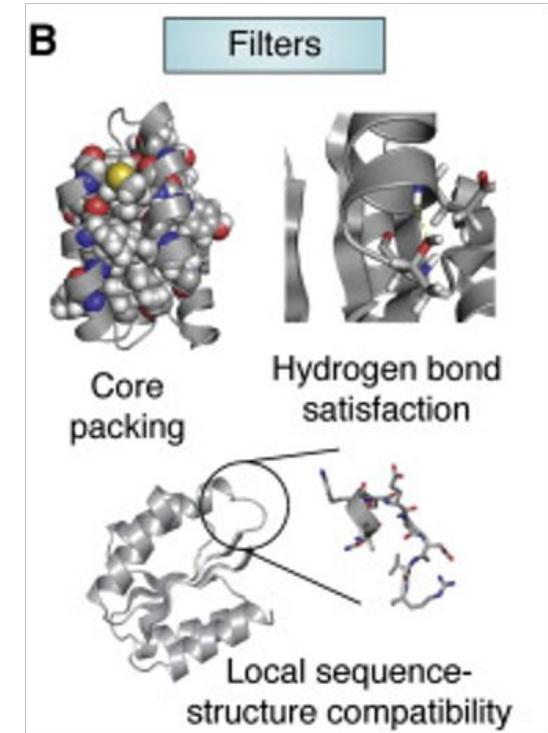
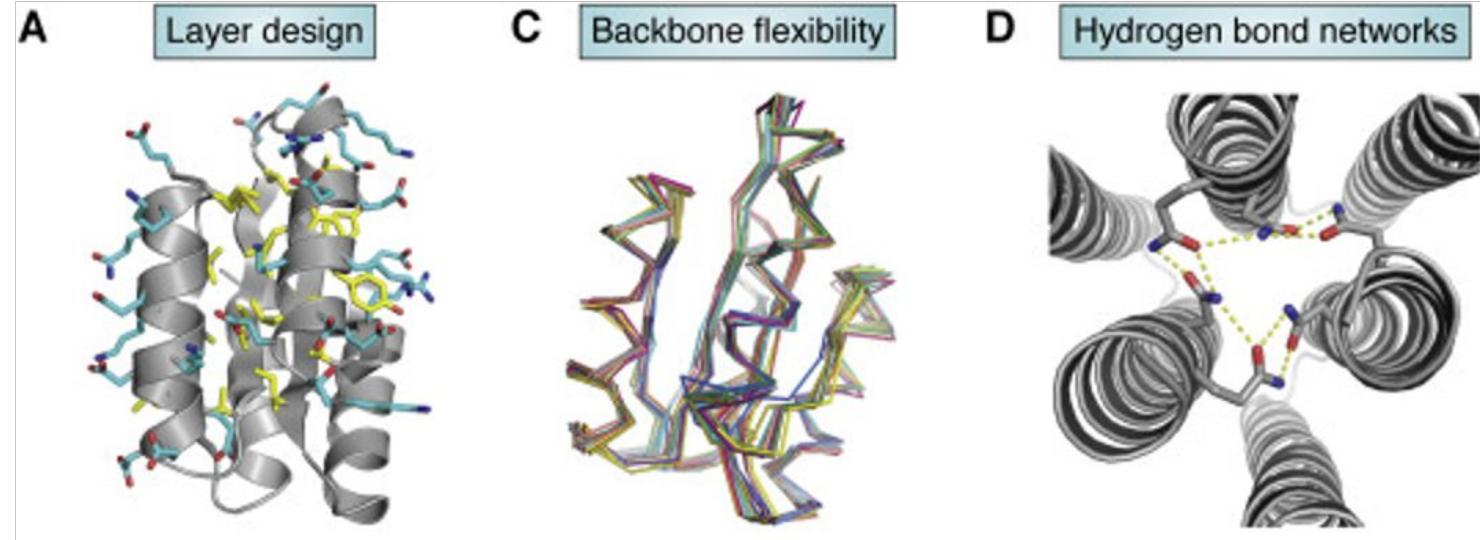
Let's probe the interaction of the alpha-helical peptide BHRF1 with the Bim BH3 domain
(PDB 2WH6)

You'll score the mutations in the complex then separately.

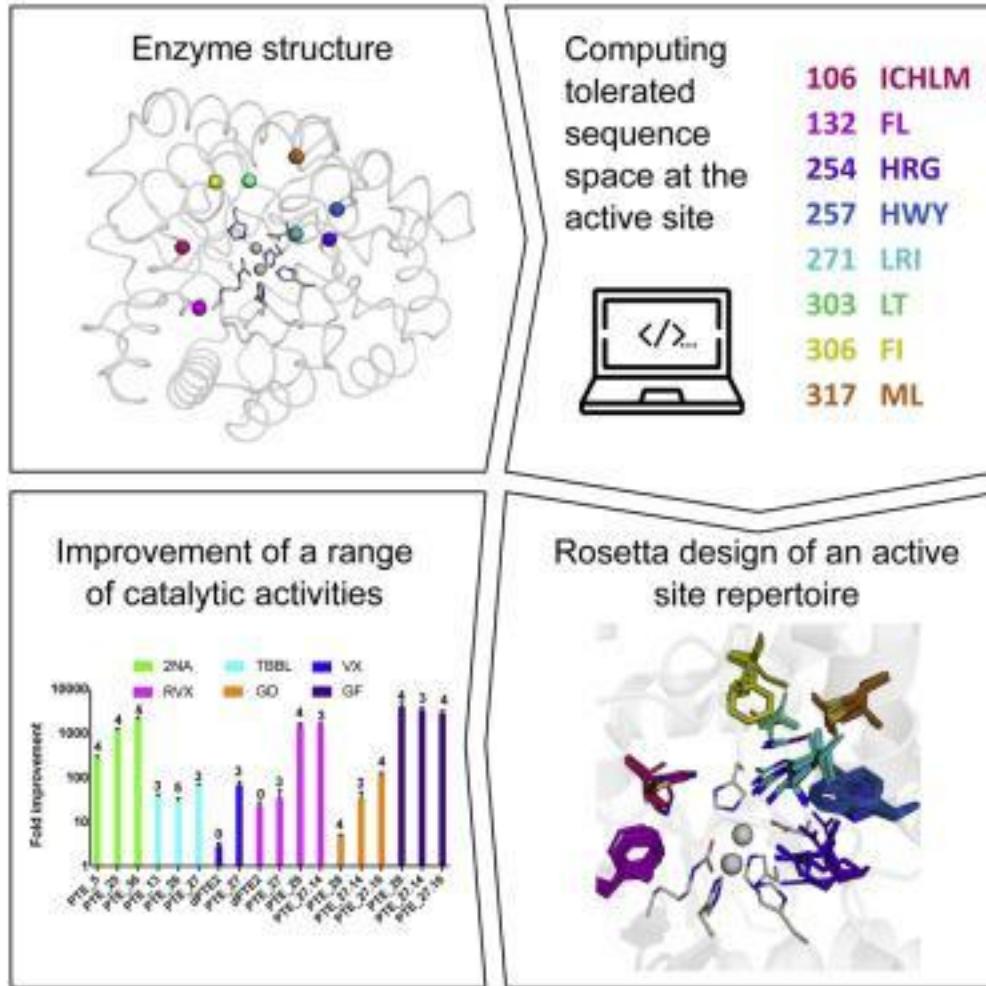
Here a different formula applies:

$$\Delta\Delta G_{interface} = \frac{\sum_{i=0}^n COMPLEX_score_n}{n} - \frac{\sum_{i=0}^n SEPARARATE_score_n}{n}$$

4. Running Combinatorial design – Rosetta

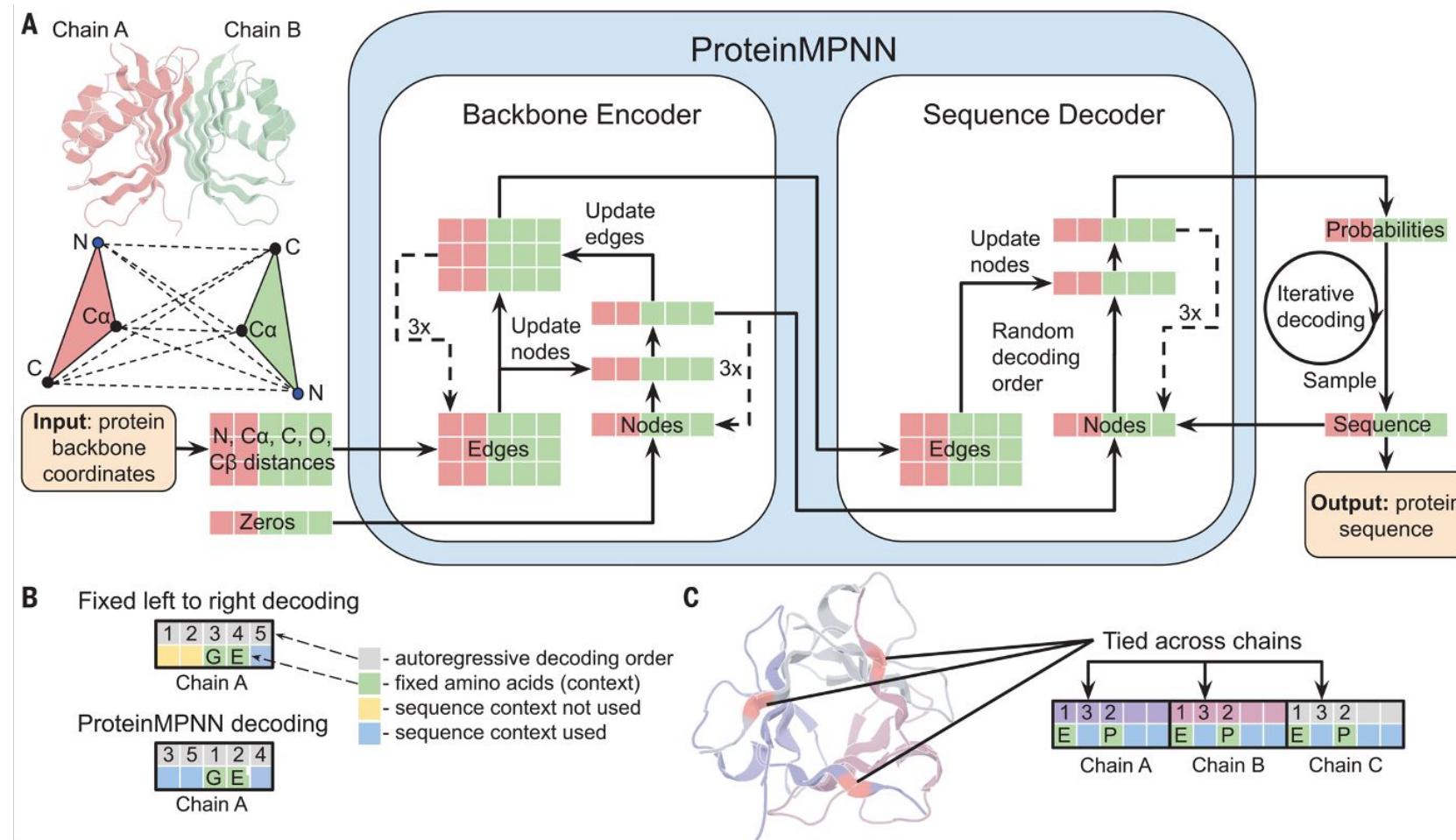


4. Running Combinatorial design - PROSS



Improves bacterial expression, stability and function of many eukaryotic (human) proteins

4. Running Combinatorial design - ProteinMPNN



More about it tomorrow!

Exercise: Run combinatorial design calculations
with Rosetta

DAY 1: Questions?

See you tomorrow!!