Bs”d

Context-dependent design of induced-fit enzymes using deep learning generates well-expressed, thermally stable and active enzymes

Dr. Chen Brestel

Research conducted during my tenure at

ENZYMIT
Designing the bio-molecular machines of tomorrow
The future of biomanufacturing

MEDS
Medical Data Science Israel

Paper: Zimmerman et al. *PNAS* 2024

How to design a novel enzyme?

What are enzymes good for?

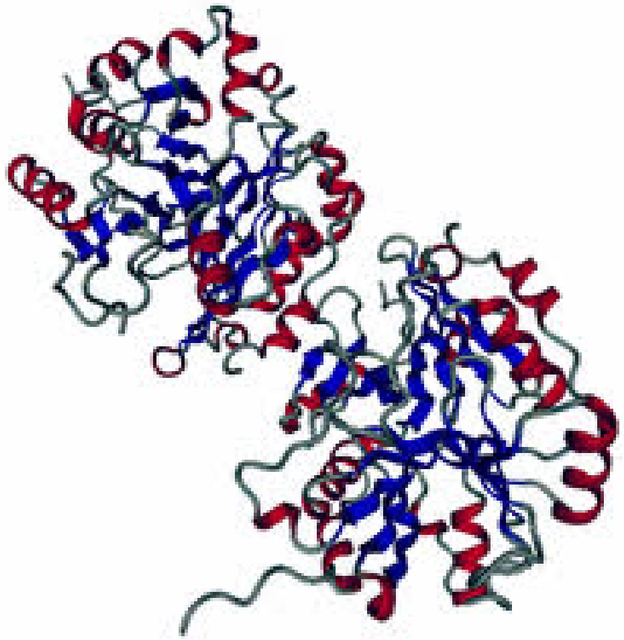
Industrial applications

- Healthcare
- Foodtech
- Energy
- Agtech
- Environment
- Militry/HLS
- etc.

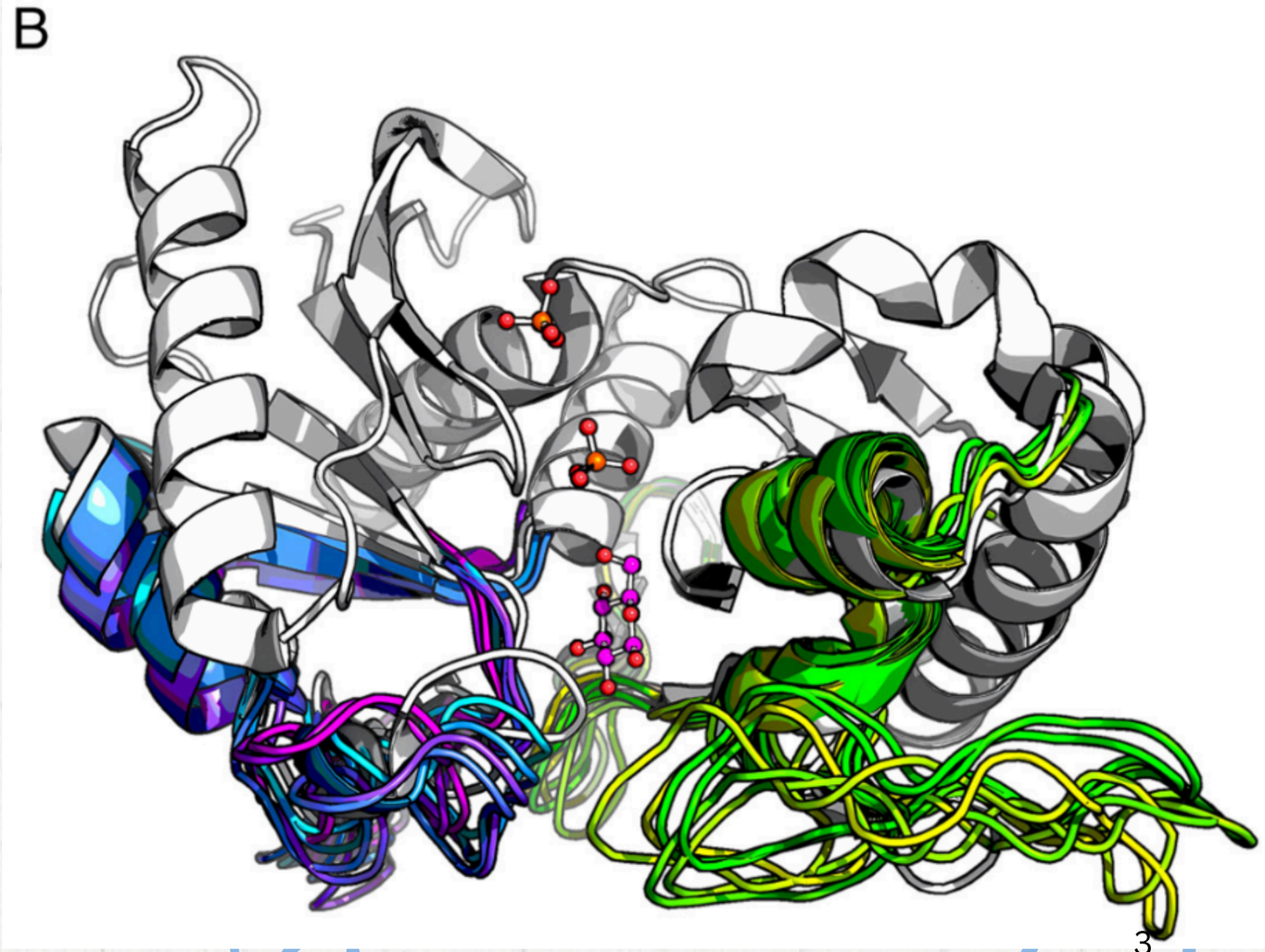
Why we need novel enzymes?

- Different substrate
- Different temprature
- Better activity
- etc.

How does enzymes look?



AndrewGNF



Current Approaches, Fields & Tools

- Fast evolution
- Protein folding
  - Rosetta
  - David Baker, Washington, Lab tools
  - AlphaFold 2 & 3 – Deepmind
- Protein language models
  - ESM2, ESM3 – Facebook
  - ProteinGPT
  - ProGen – Salesforce & Stanford

The Nobel Prize in Chemistry 2024

Ill. Niklas Elmehed © Nobel Prize Outreach

David Baker

Prize share: 1/2

Ill. Niklas Elmehed © Nobel Prize Outreach

Demis Hassabis

Prize share: 1/4

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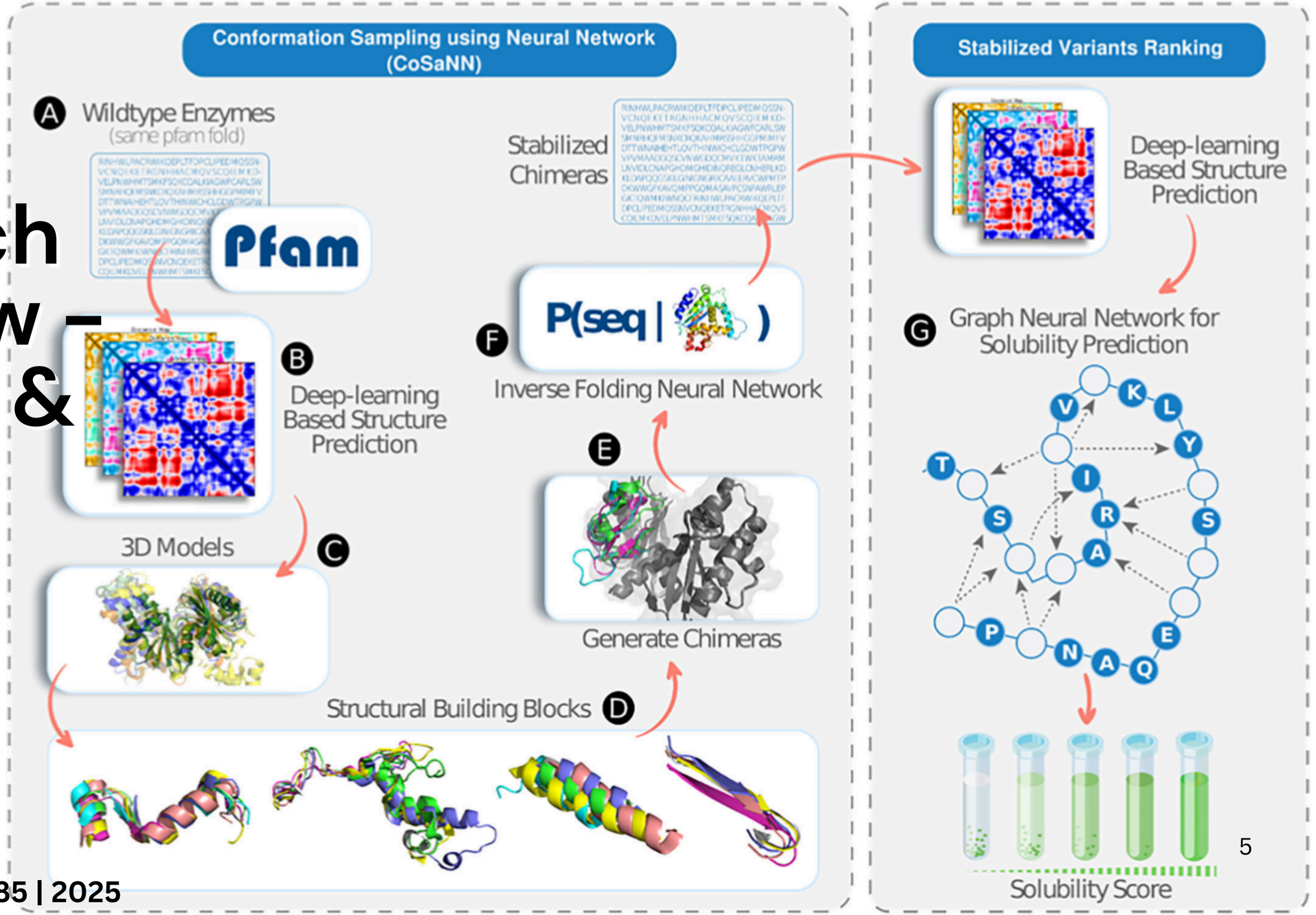
John Jumper

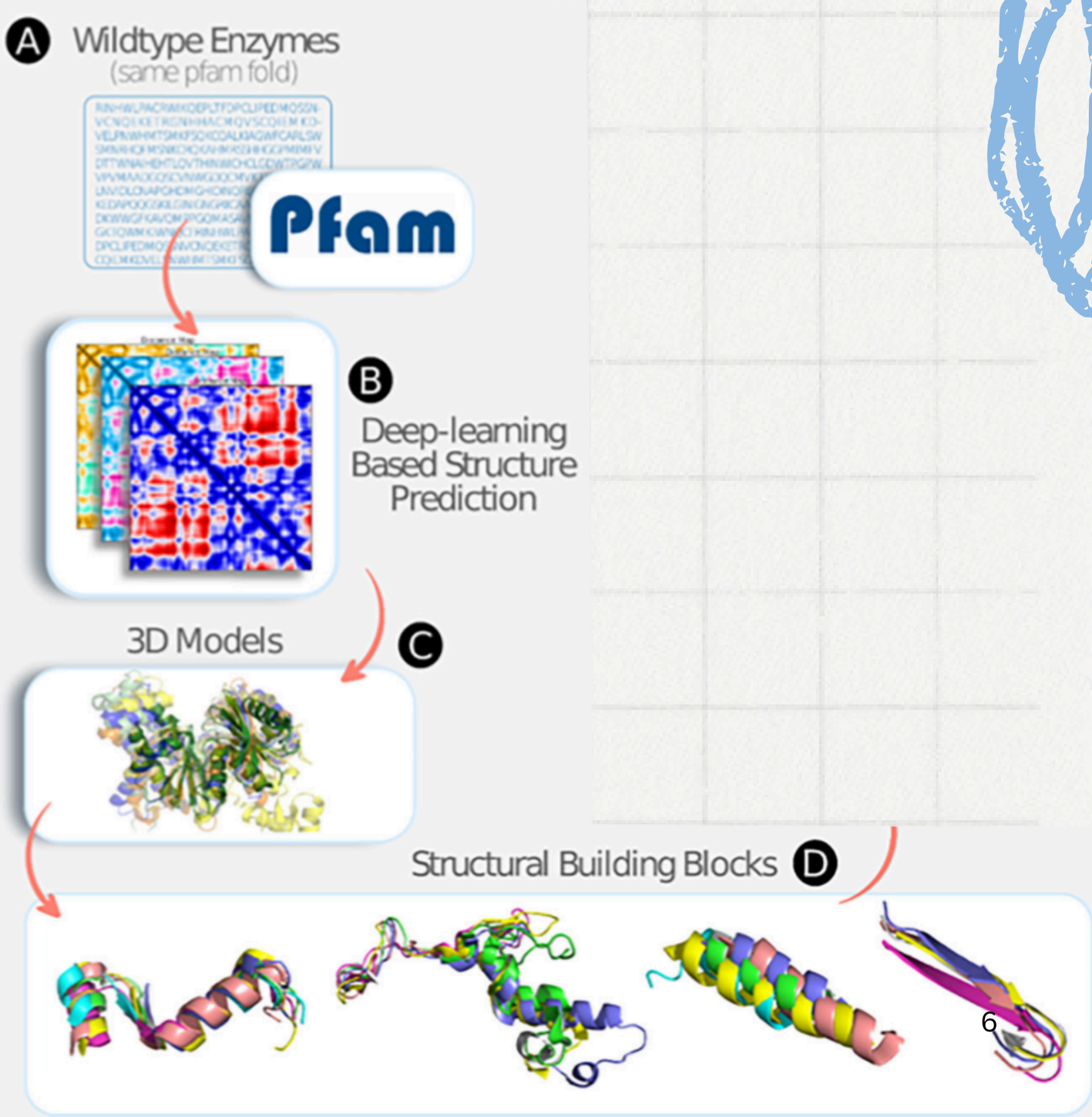
Prize share: 1/4

Approach Overview – CoSaNN & SolvIT



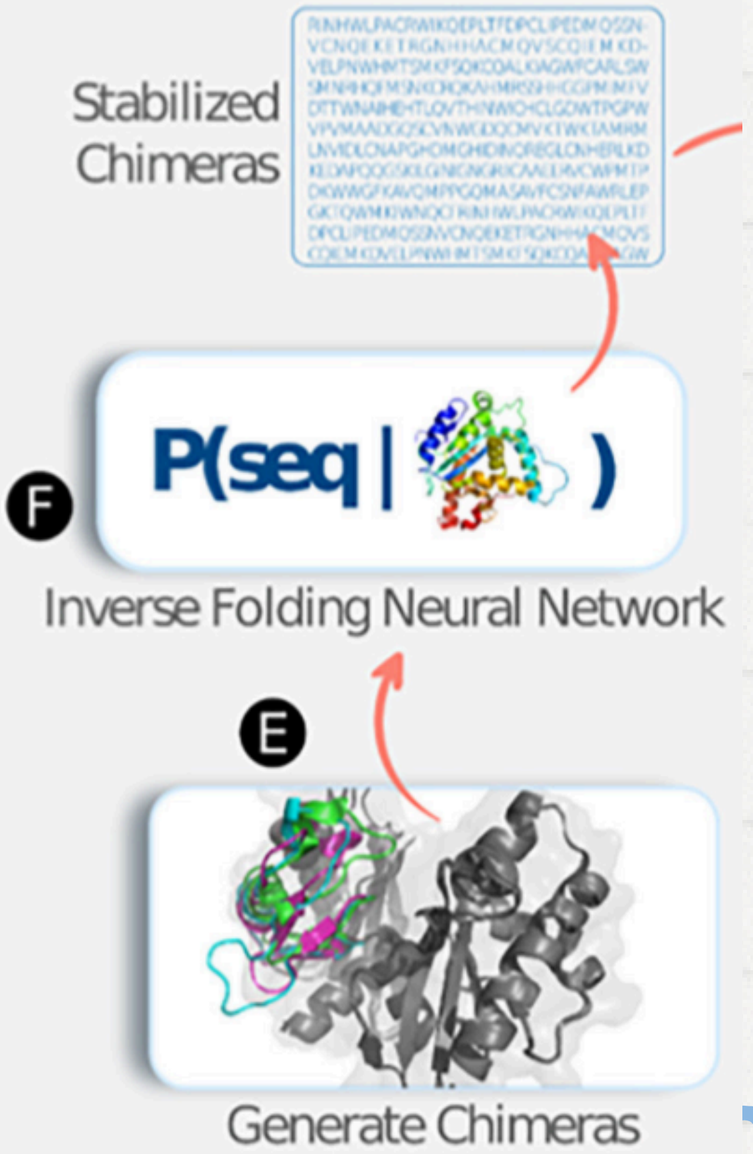
How to find structural building blocks?

- Why blocks?



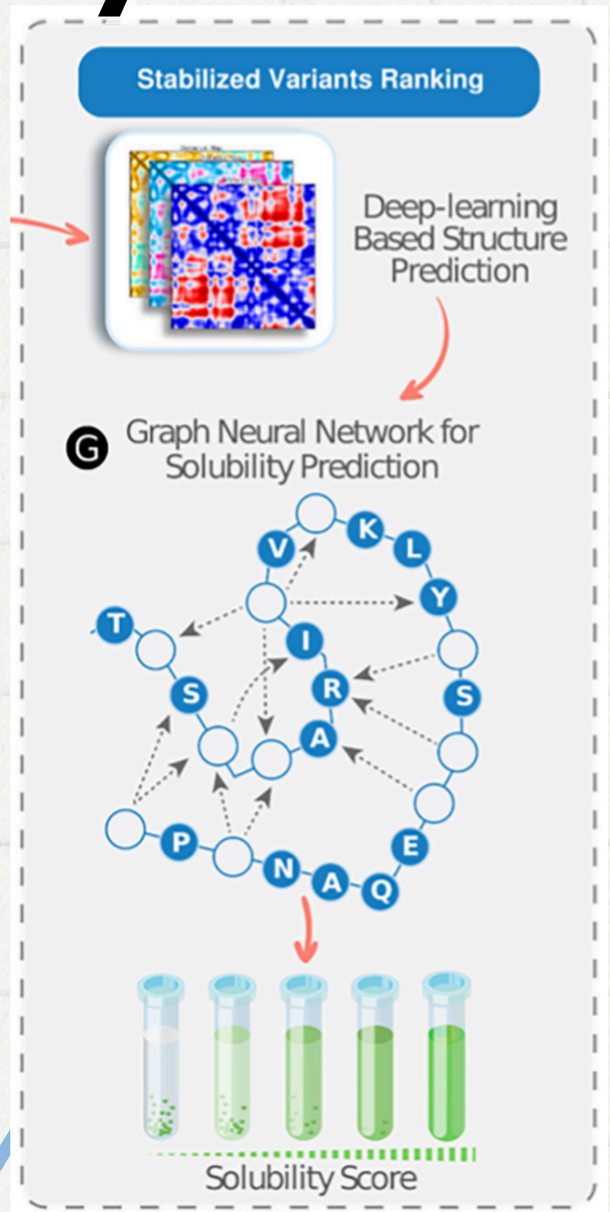
How to generate stabilized chimeras?

- What are chimeras?
- Why chimeras?
- What are satbilized chimeras?
- Why satbilized chimeras?



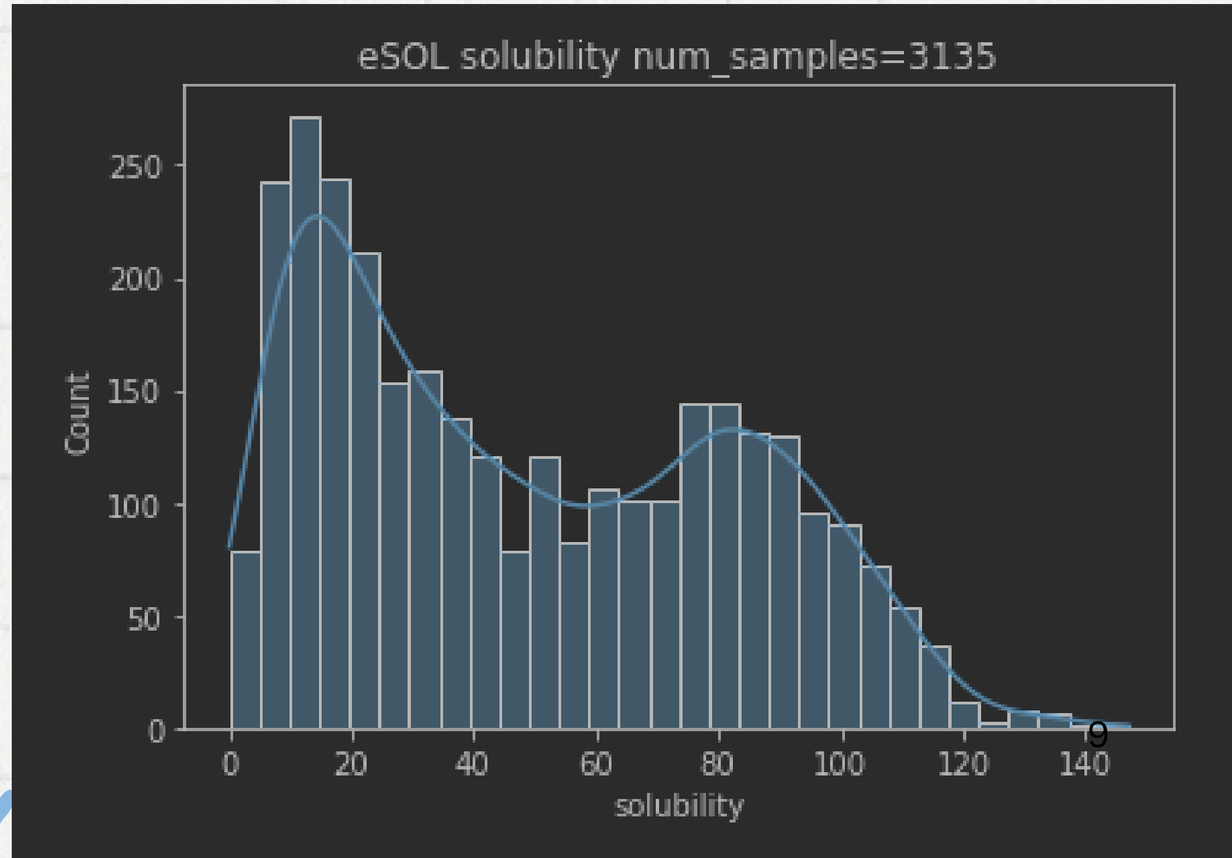
How to get solubility-based ranking

- Why ranking?
- What is solubility-based ranking?



What is the dataset used for training SolvIT?

- What is the target of solubility predictor?
- What is the training purpose?
- eSOL Dataset Niwa2009
- How to clean the data?
- Enzymit Dataset.
- How to ensure the prediction confidence?
- How to ensure the prediction generality?

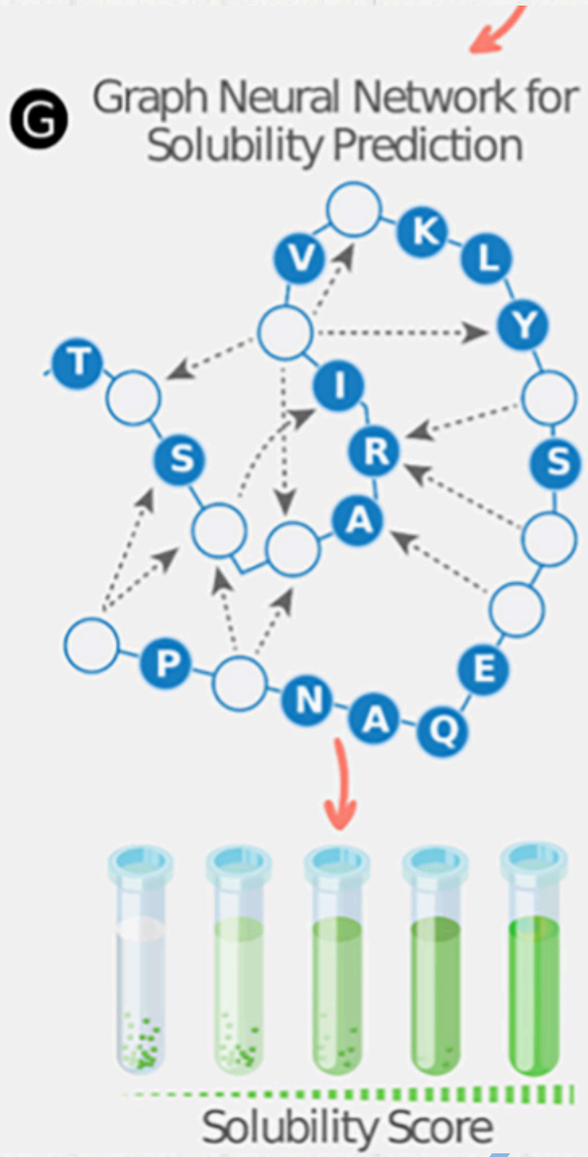


Training experiments

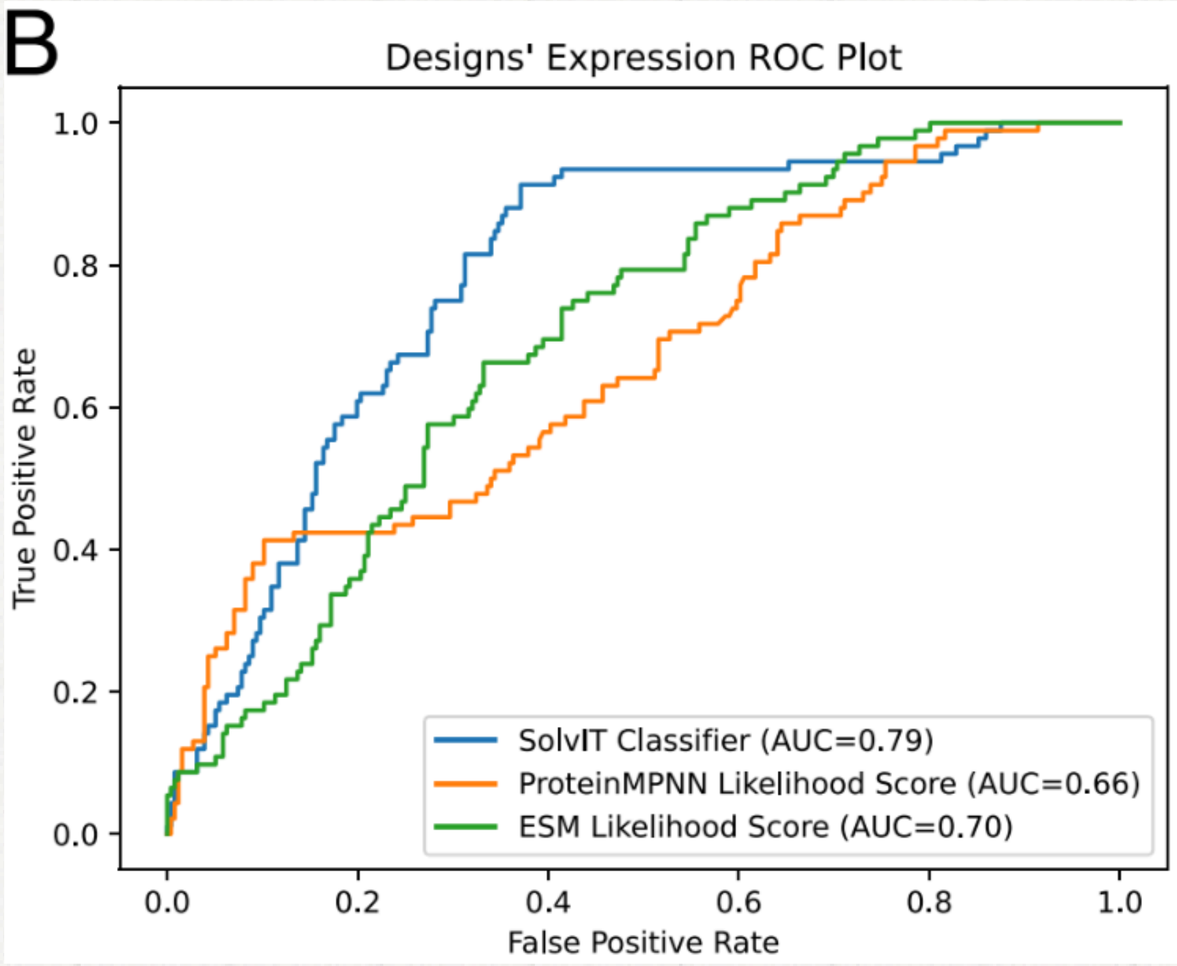
- Why multiple training experiments?
- What parameters were tuned?
  - Heterogeneous/homogeneous GNN
  - Type of convolution layer
  - Type of global pooling layer
  - Number of heads
  - Number of convolution layers
  - Batchnorm
  - Neighbour distance threshold for edges
  - Binary/quantized/float rosetta features
  - ESM features

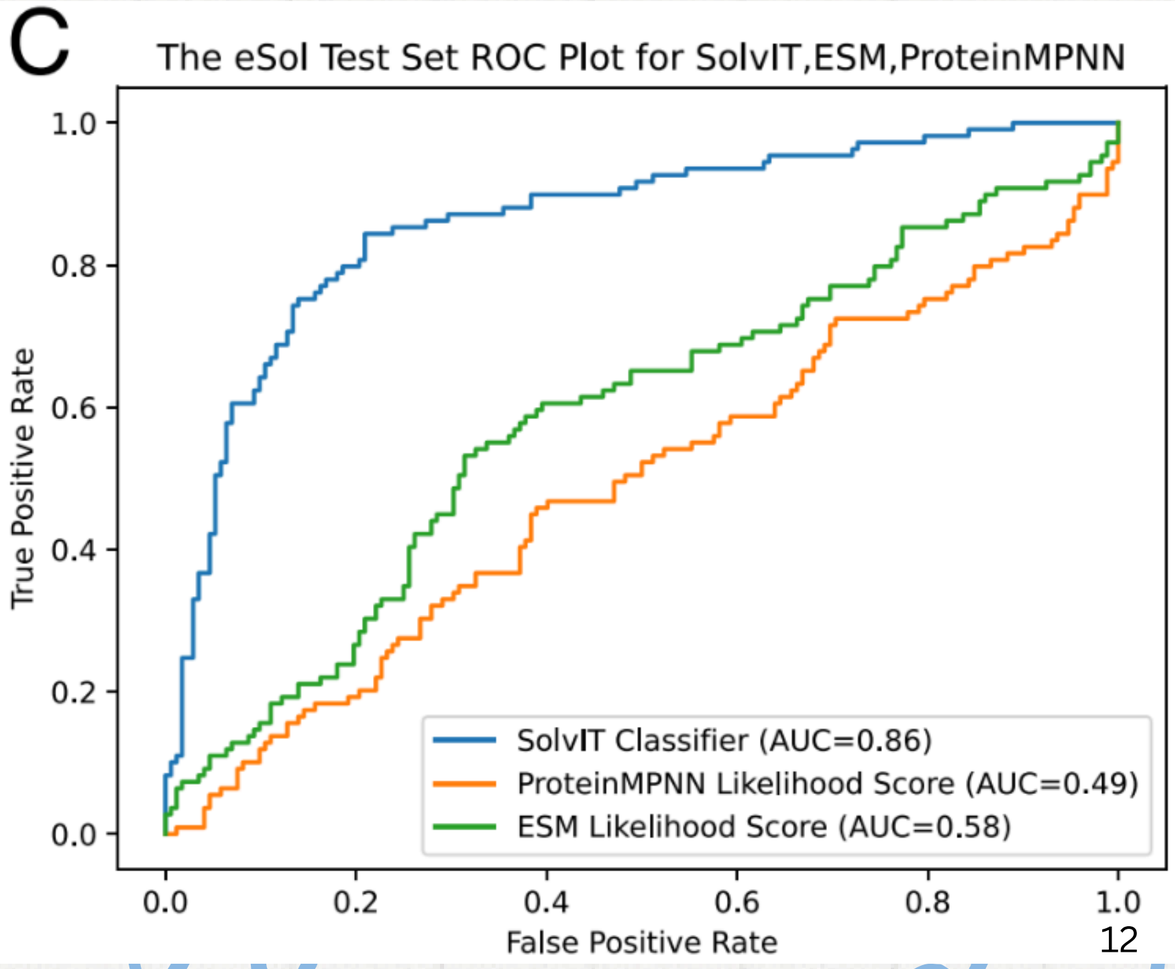
Model architecture

- Graph neural network (GNN)
- Convolution layer: GATv2Conv [Brody2021]
- Global pooling layer: Global Graph Multiset Transformer pooling operator [Baek2021]
- Number of heads: 8
- Number of convolution layers: 2
- Batchnorm: Off
- Neighbour distance threshold for edges: 5 [Å]
- Amino-acid encoding: one-hot
- ESM embedding reduced size: Off
- Model target: binary

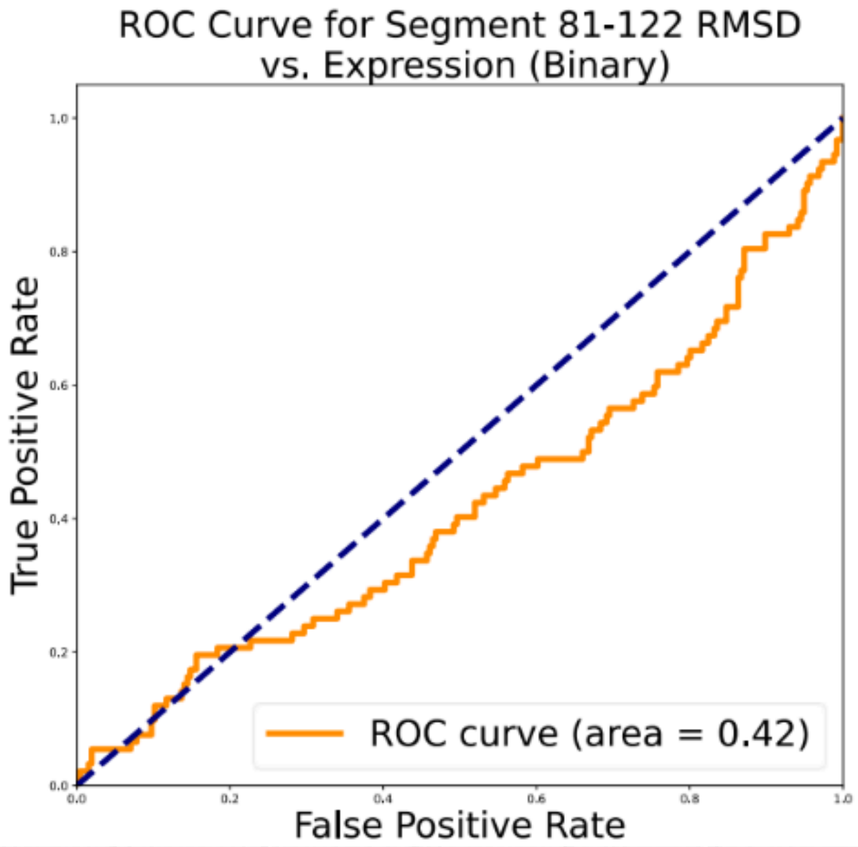


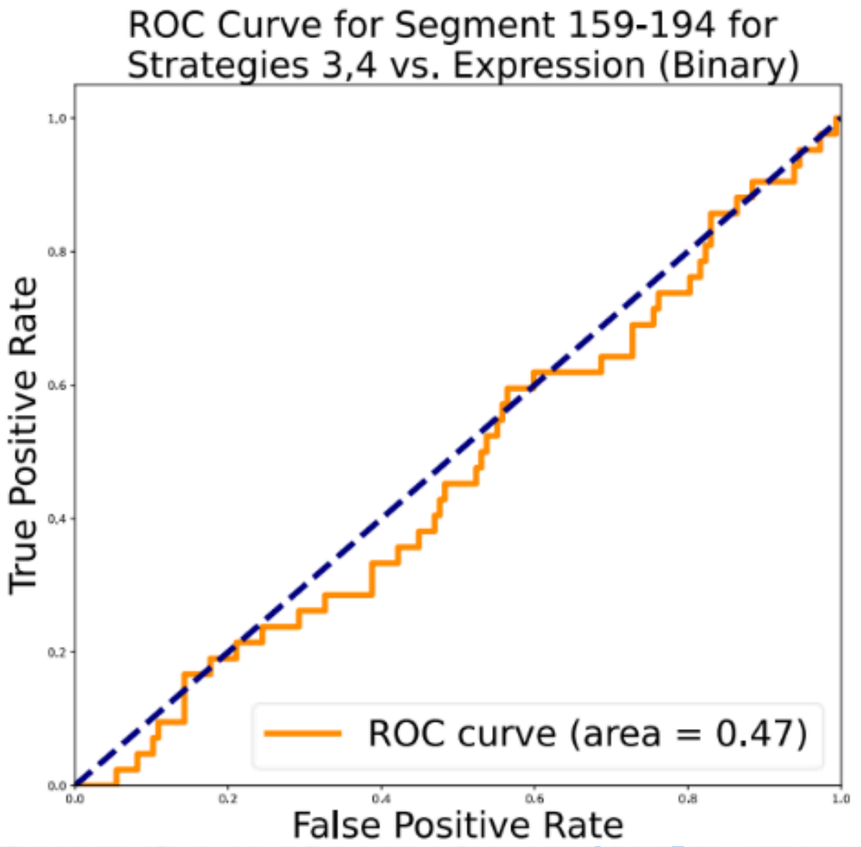
What is the performance of SolvIT?

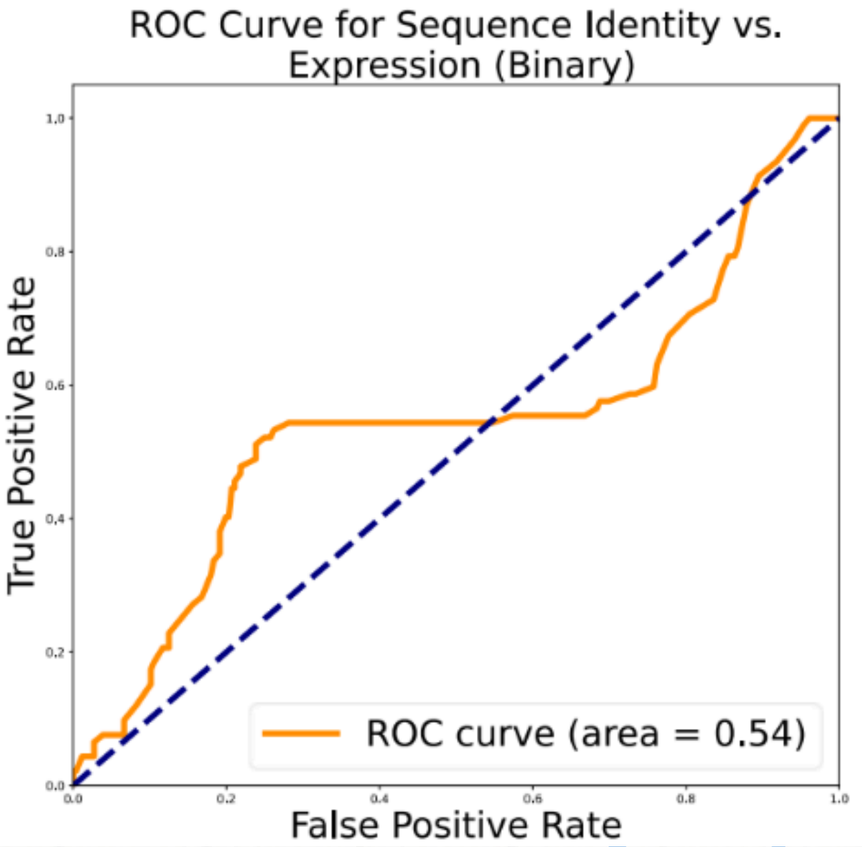




Could we achieve same performance with simpler features?

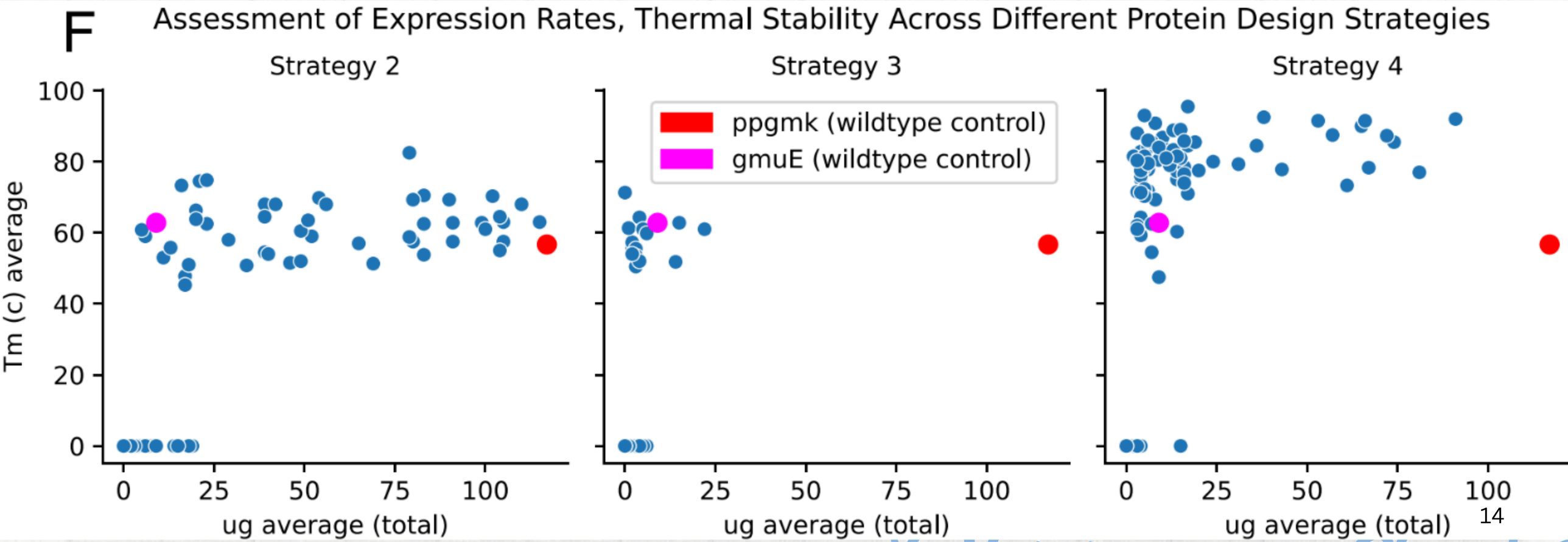






Does the melting temperature change?

- Higher melting temperature – *83%* of novel designs
- Strategy 4 – *78%* of high melting-temp. novel designs

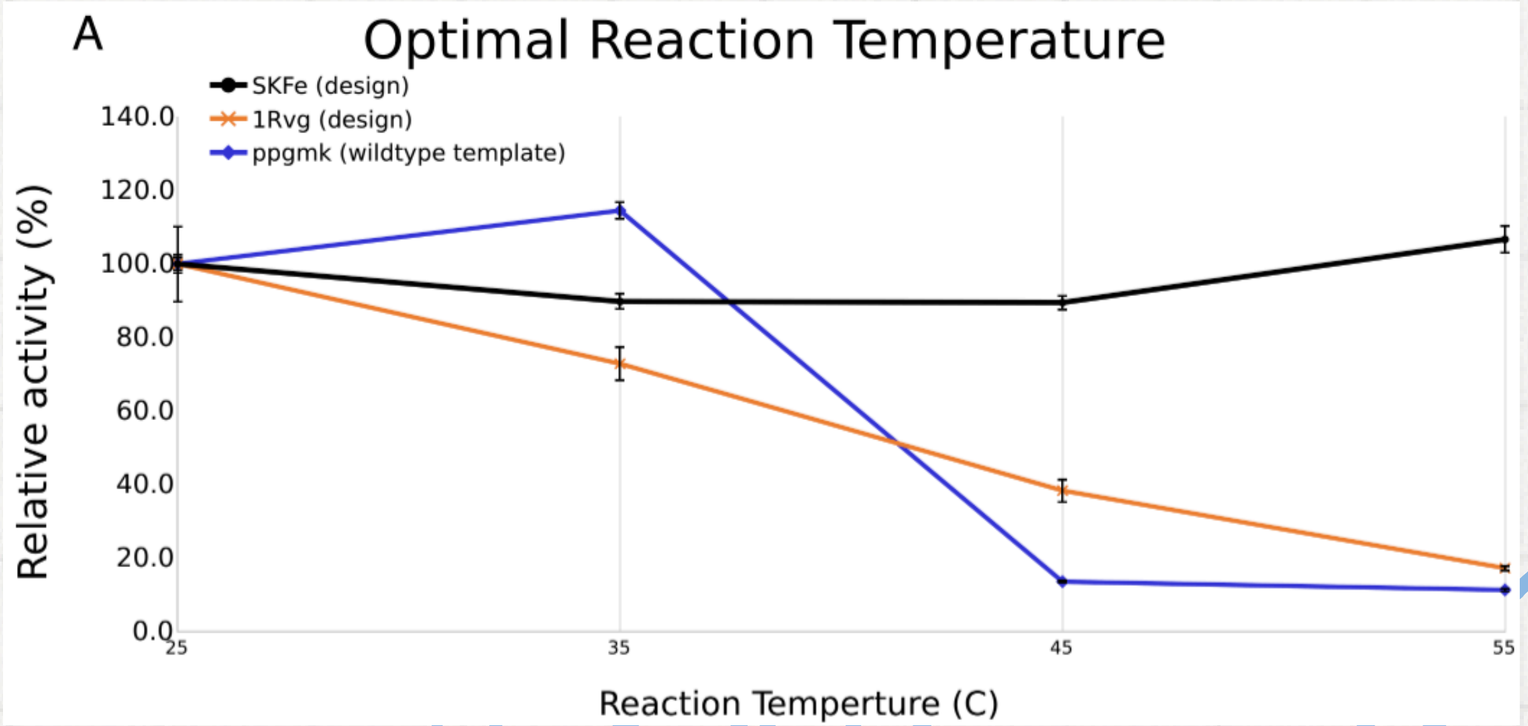


Are the enzymes activite?

- 60 designs active with either hexametaphosphate or ATP
- 8 (13%) designs showed obligatory polyphosphate-dependent activity
- Template enzyme can utilize both ATP an inorganic polyphosphate
- Obligatory polyphosphate activity is not common in natural enzymes. Has been documented in a handful of cases
- Underscores the potential of our method to introduce non-natural enzymatic traits

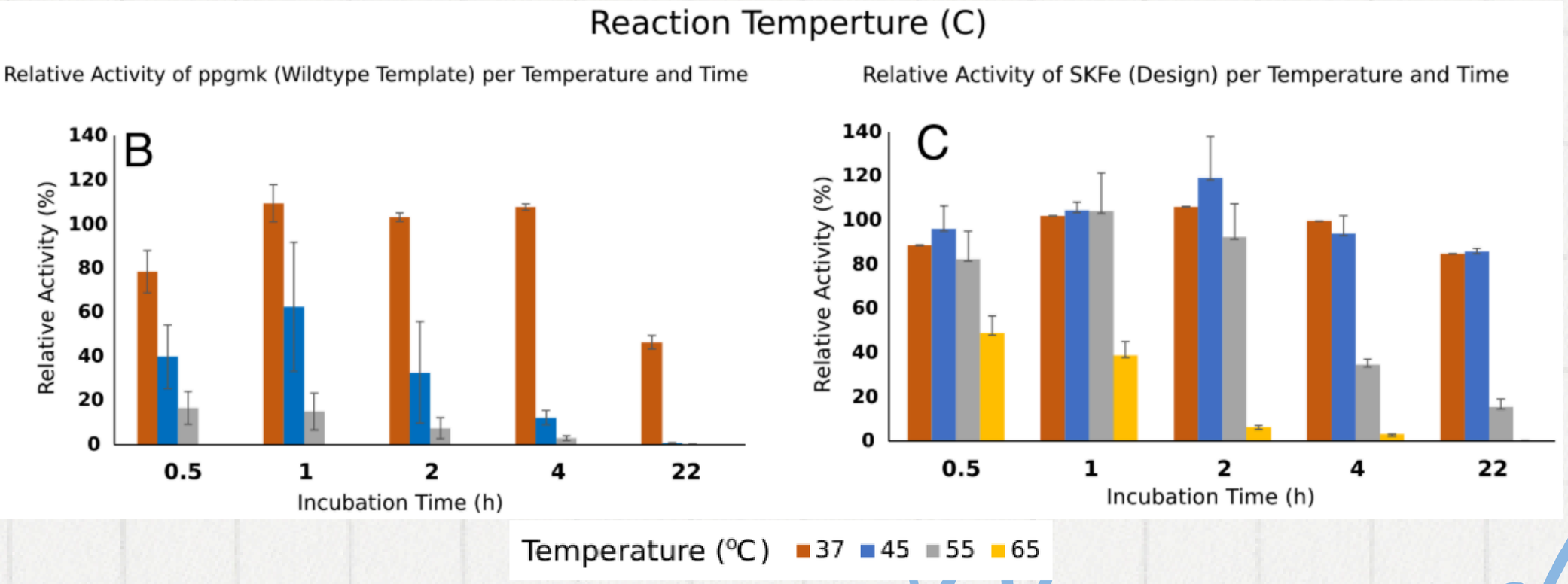
What is the activity of novel enzymes as temp. increase?

- Above 35C novel designs activity is higher
- SKFe retains 40% activity at 95C
- Wildtype has no activity at 95C



What is the activity after prolonged temperature stress?

- 55C: WT non-active after 4h | SKFe 80% activity after 22h
- 65C: WT non-active after 30min | SKFe 40% activity after 30min



Conclusion

- CoSaNN – Enable rapid and robust novel enzyme designs
- SolveIT – Enable high performance solubility prediction
- Tested in wet lab
- Achieve improved novel designs with
  - Higher melting temparature
  - Higher activity
  - Higher robustness to prolonged temperature stress
  - Possible non-natural enzymatic traits

Thank You

Research conducted during my tenure at

ENZYMIT
Designing the bio-molecular machines of tomorrow
The future of biomanufacturing

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