Machine Learning in Rosetta

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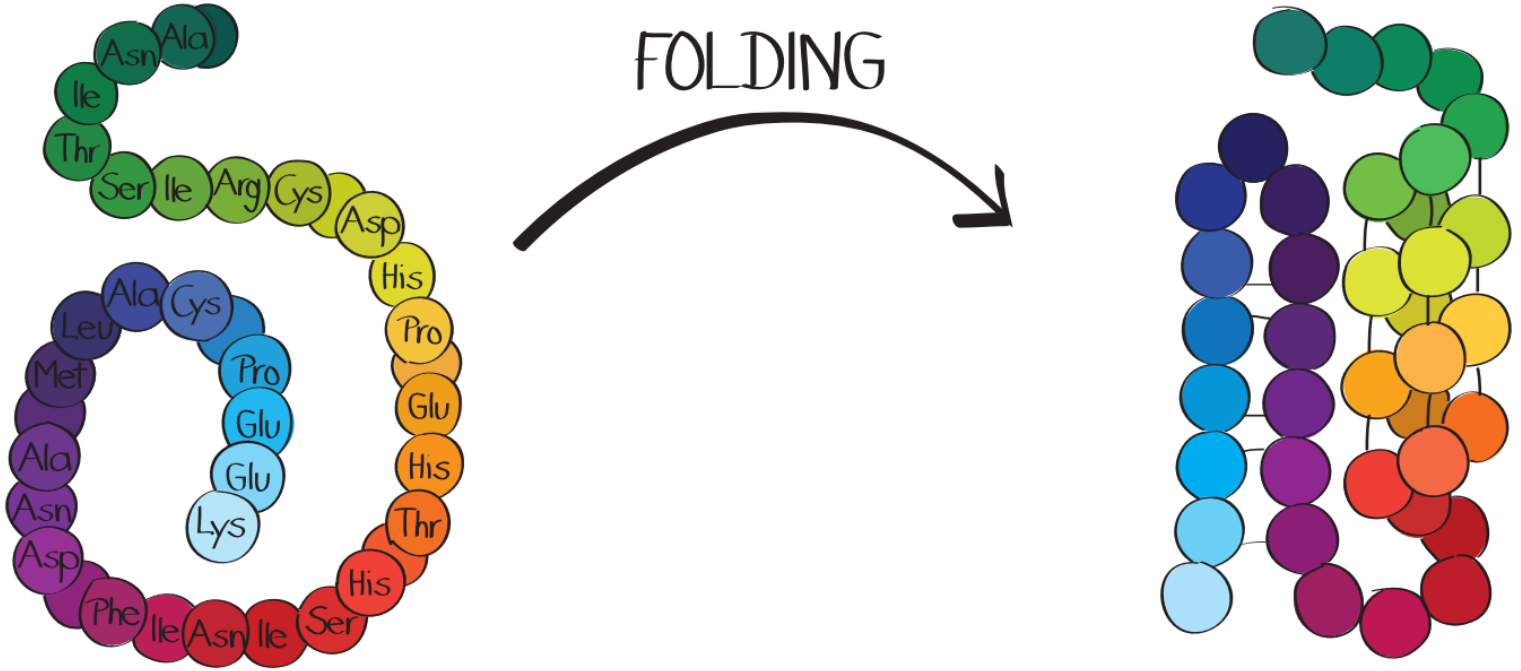
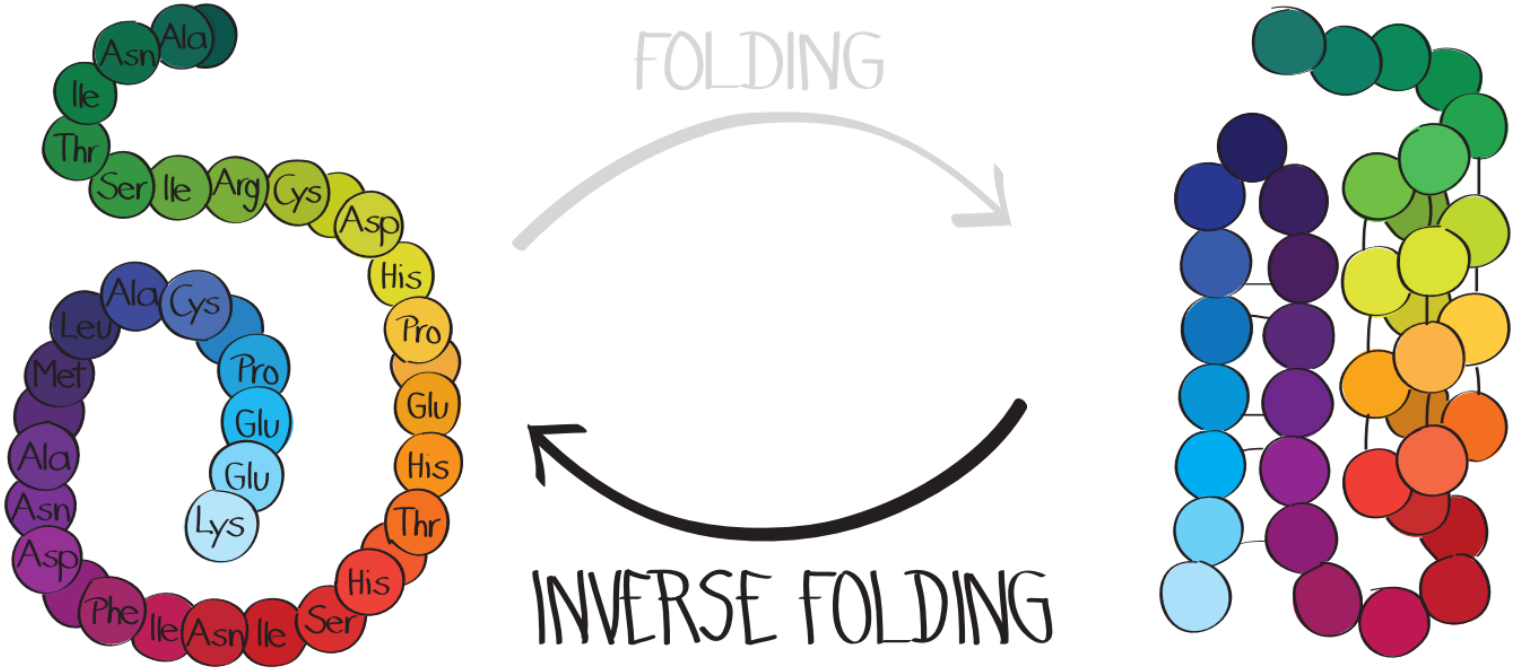
Adapted from: Cristina Elisa Martina
Rosetta Workshop 2024
Meiler Lab

Revolution in Structural Biology:



(Art from Ruth Kellner)

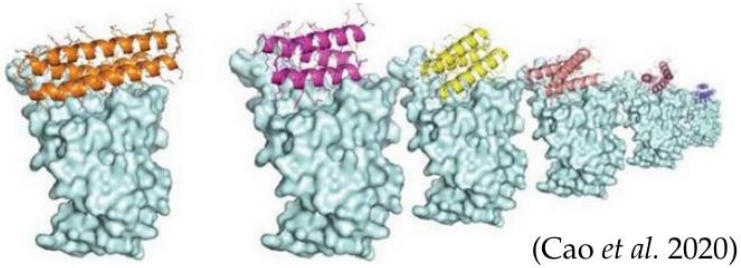
Protein design with ML:



(Art from Ruth Kellner)

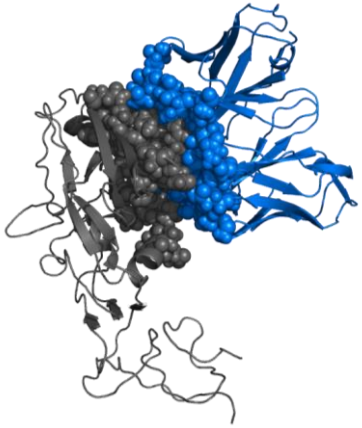
What is the best sequence to:

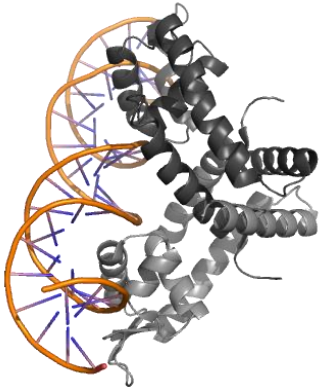
- **fold into this protein scaffold?**
  - new functions
  - new shapes (*de novo* design)



(Cao *et al.* 2020)

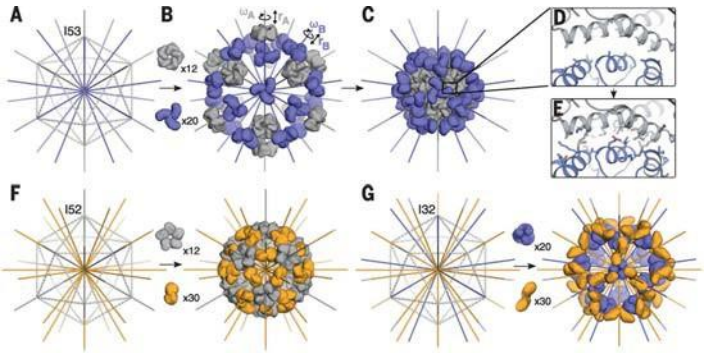
- **increase protein stability?**
  - half-life
  - thermostability
  - crystallizability
  - protein yields





- **increase binding to X?**
  - protein-protein
  - ligand-protein
  - supramolecular assemblies

- **increase enzymatic activity?**
  - activity
  - specificity



(Bale *et al.* 2016)

Computational tools for protein design:

Structure-based methods (*e.g.* Rosetta):

- Starting structure (experimental or model)
- Sampling component
- Scoring component

Machine Learning methods (*e.g.* ProteinMPNN):

- Large dataset for training
- Starting sequences, structures or both
- Very fast
- More accurate

General info on ML:

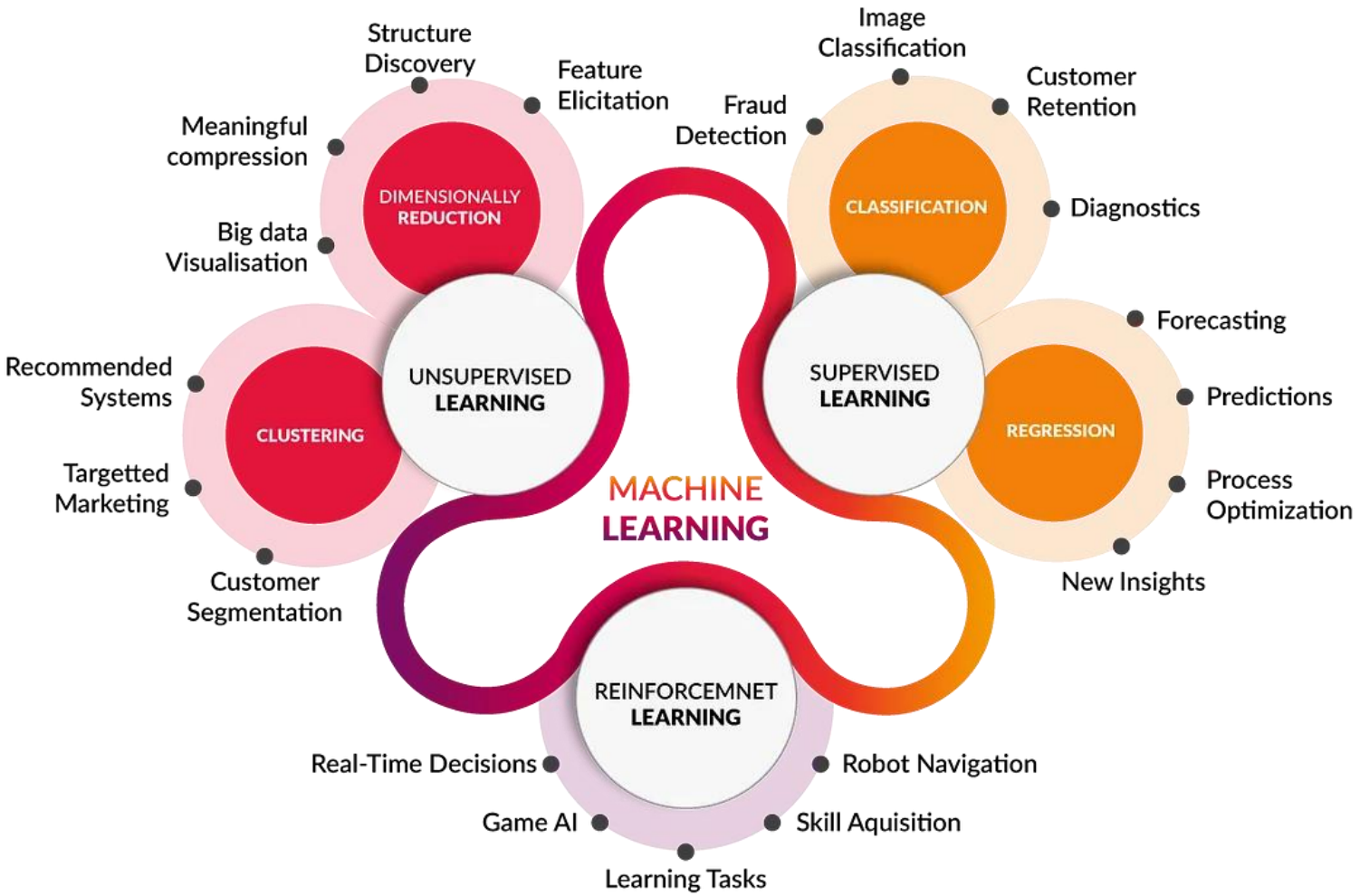


Image source: http://www.cognub.com/index.php/cognitive-platform/

Supervised learning:

Training set labeled!

We define what is a cat and what is a dog in the training set.

Supervised learning:

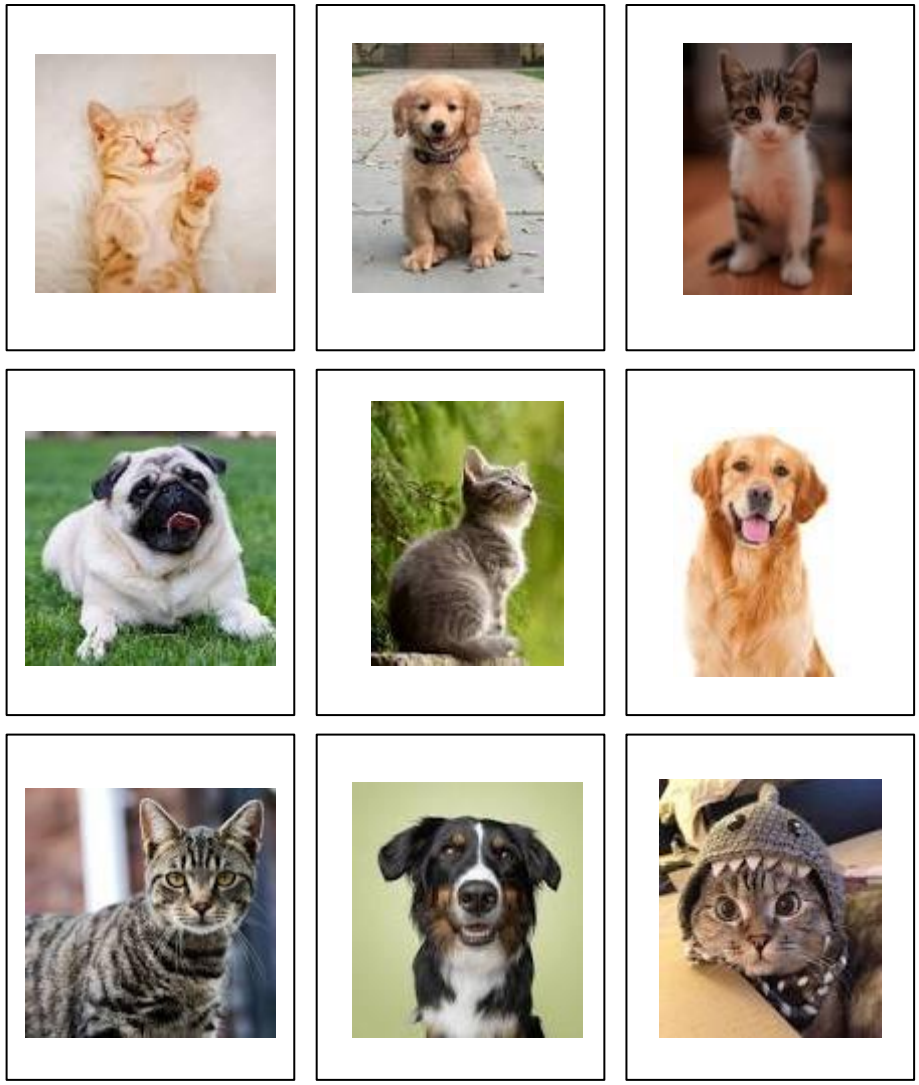
Training set labeled!

We define in the training set what is cat and what is dog.

The model will learn from the dataset and predict correctly with out testing case.

DOG

Unsupervised learning:



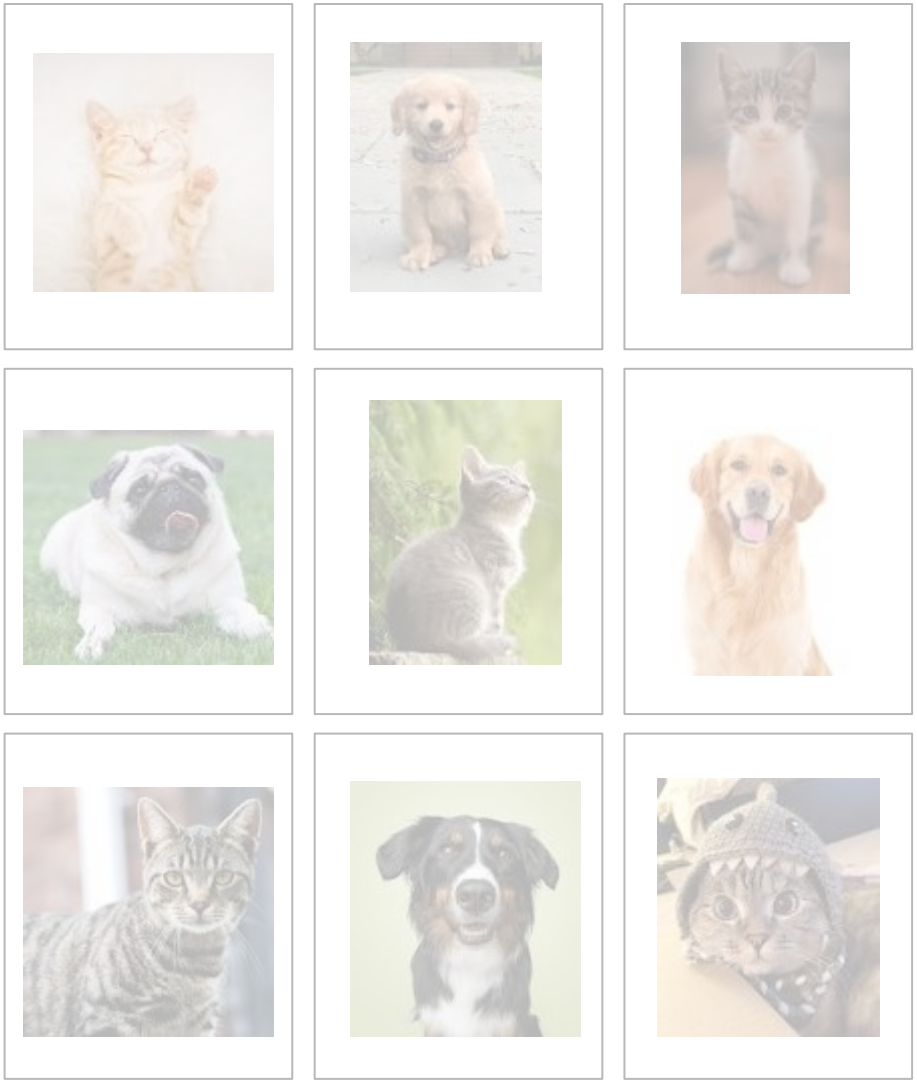
Training set NOT labeled!

Unsupervised learning:

Training set NOT labeled!

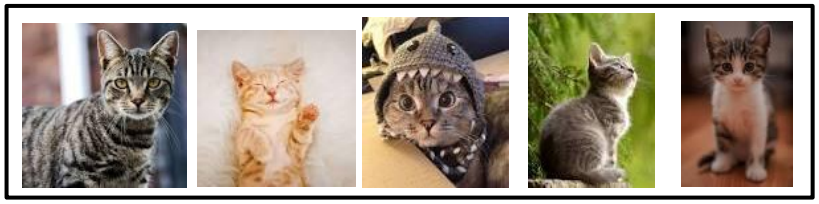
We have a large dataset without labes. The model will learn and cluster from the dataset and predict correctly.

Unsupervised learning:



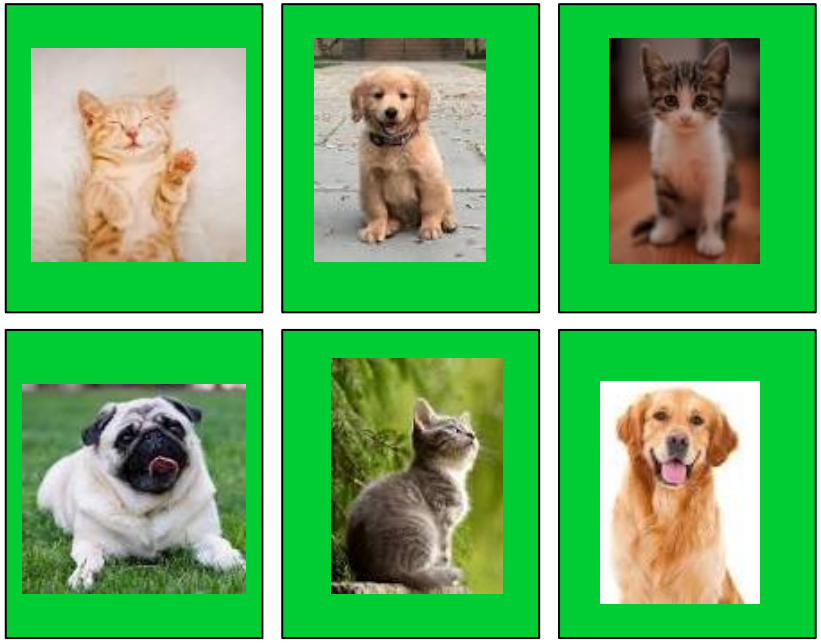
Training set is NOT labeled!

We have a large dataset without labels. The model will learn and cluster from the dataset and predict correctly.



How machines learn:

Training/validation data-set

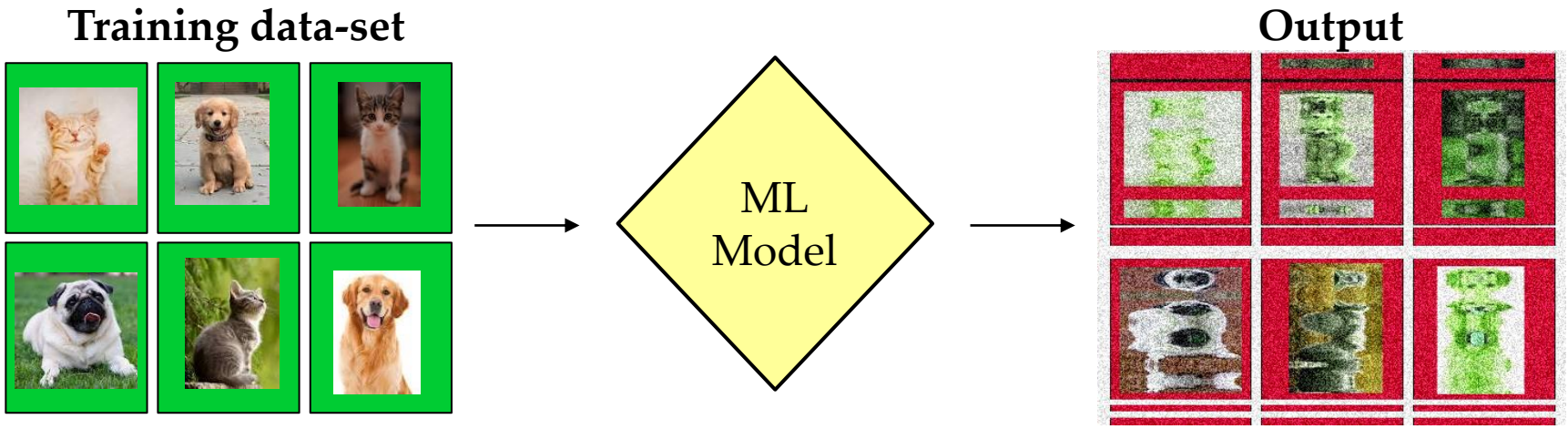


Testing data-set

The data-set is divided into three groups:

- Training data-set (80%)
  - Trains the model (learning)
- Validation (10%)
  - Used to benchmark *during* learning
  - Enables 'fine-tuning'
- Testing data-set (10%)
  - used to evaluate the performances with unseen data *after* learning

How machines learn:



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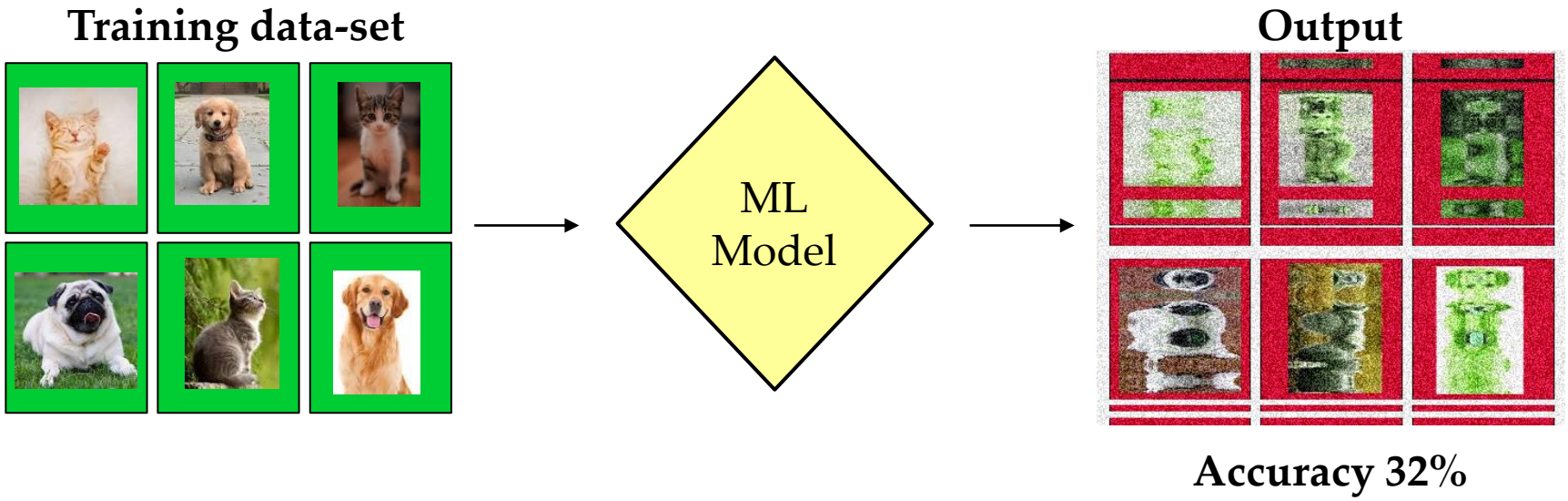

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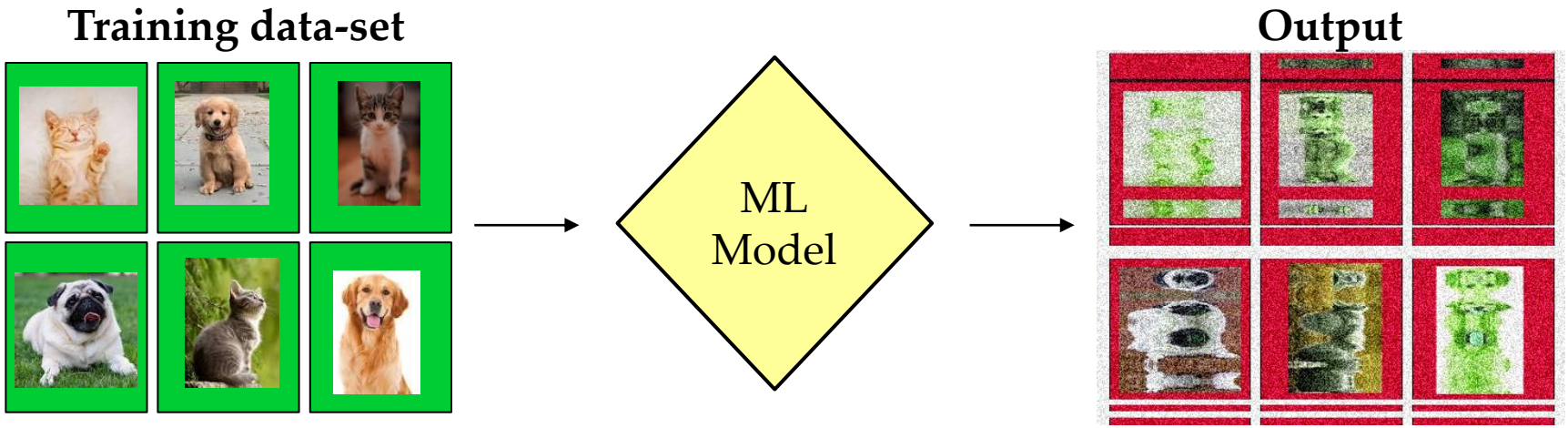
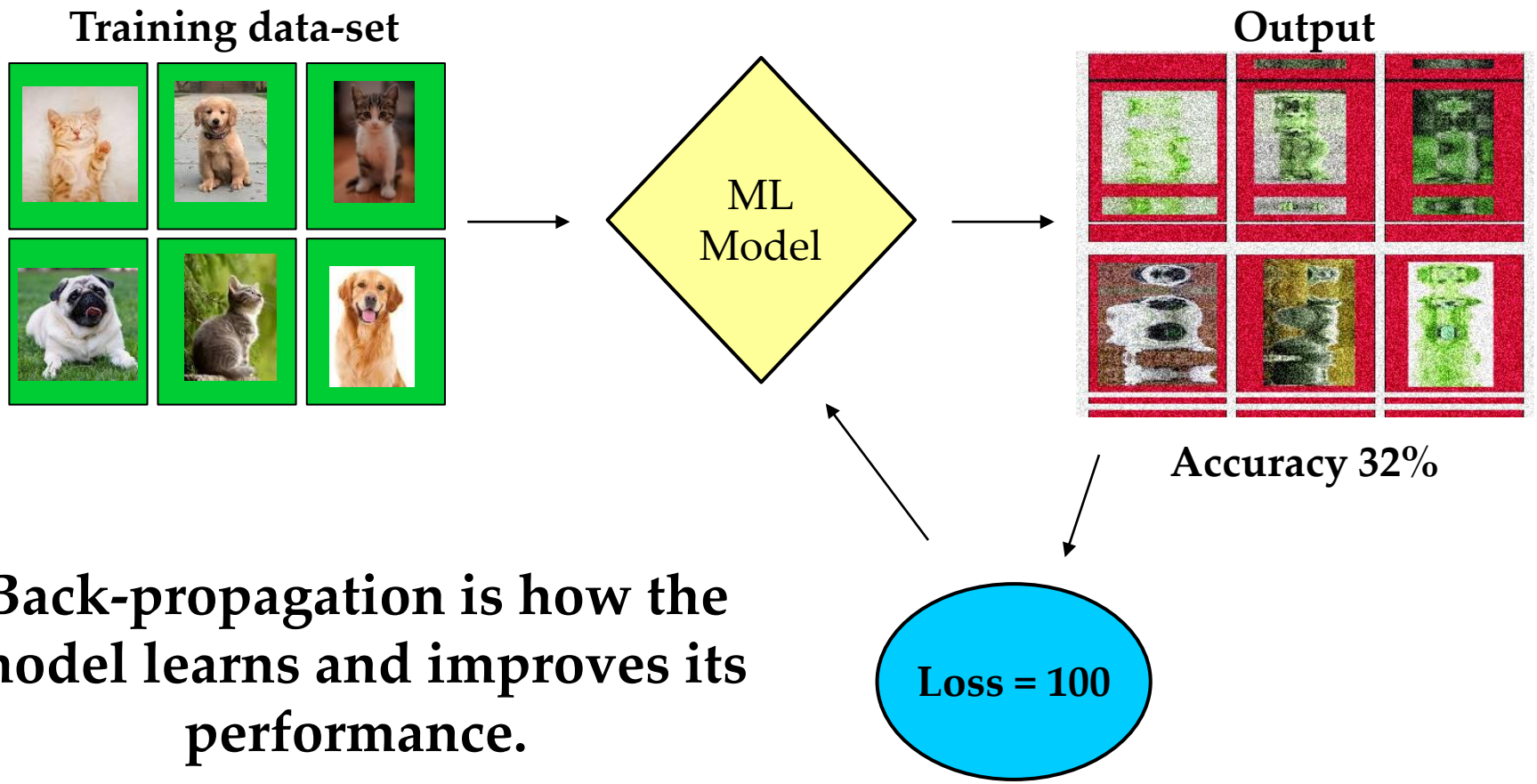
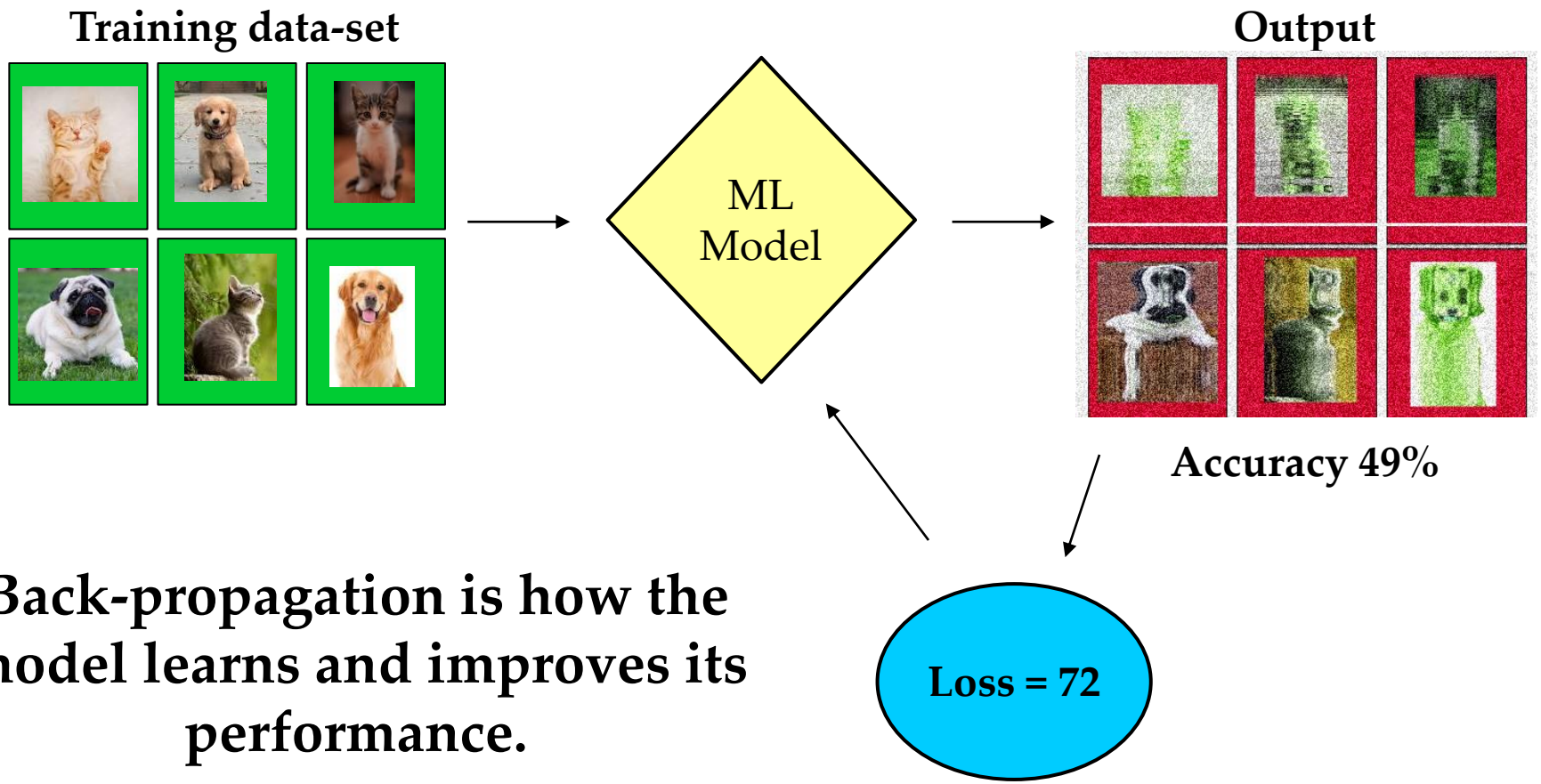


How machines learn:



Back-propagation is how the model learns and improves its performance.

How machines learn:



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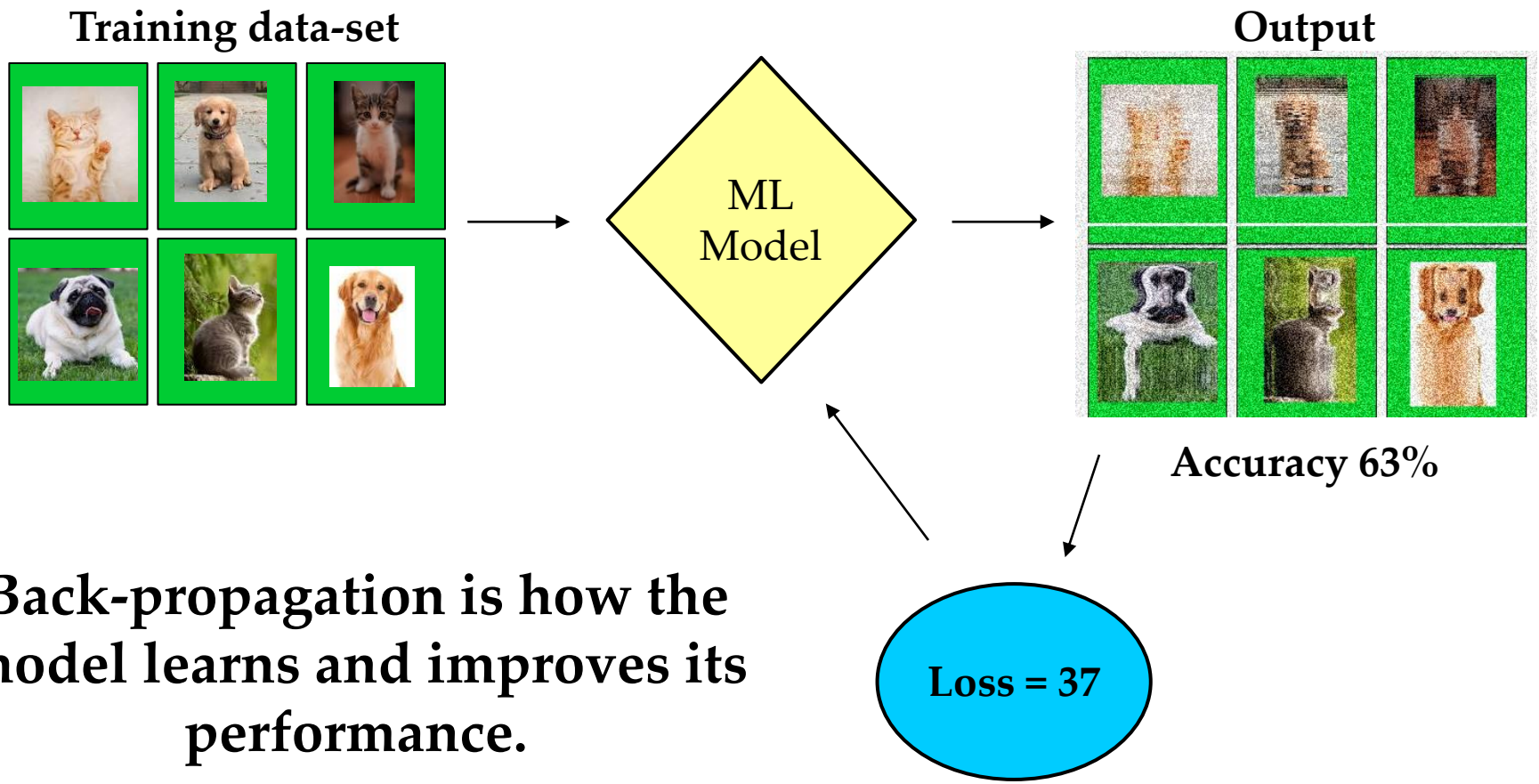

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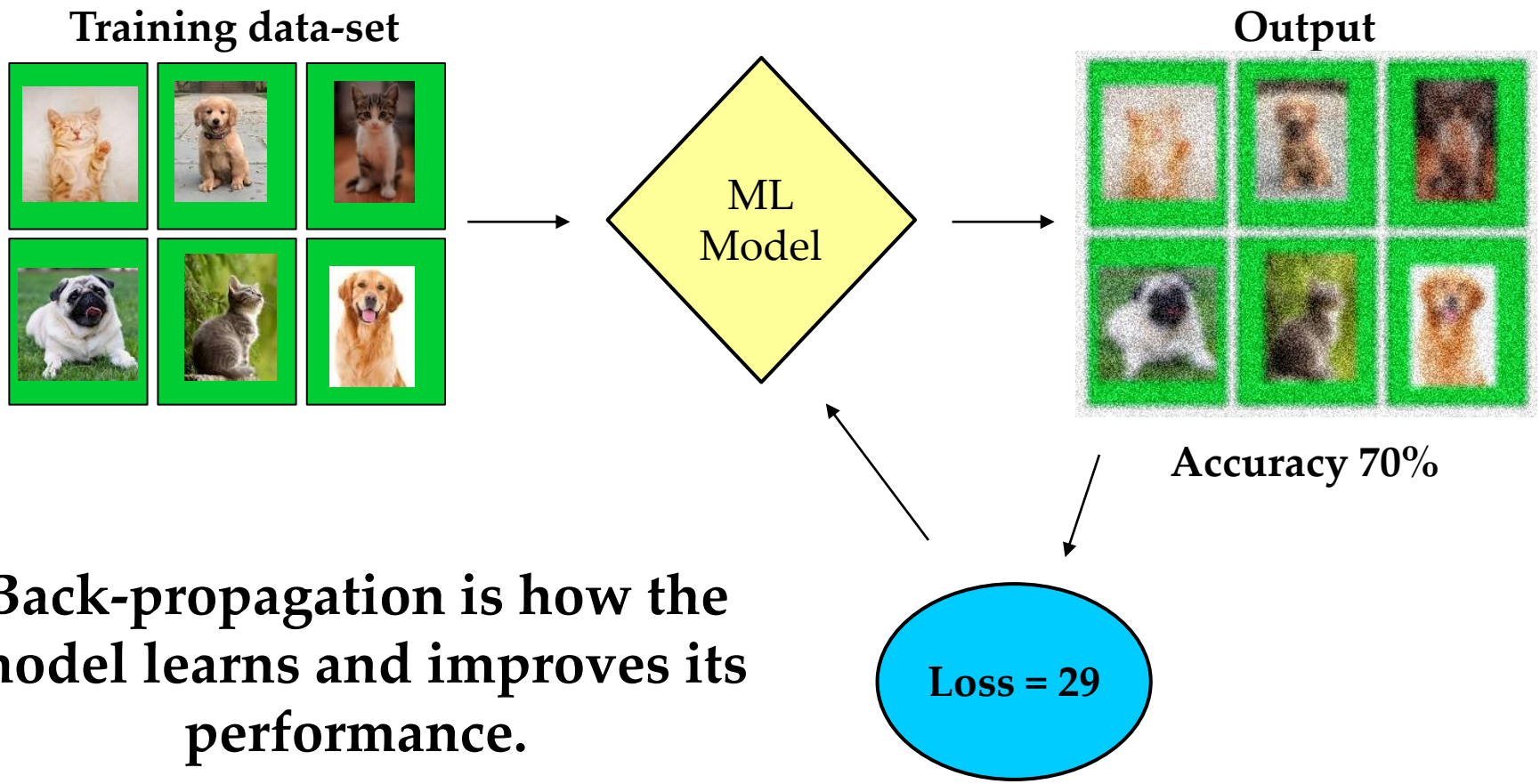

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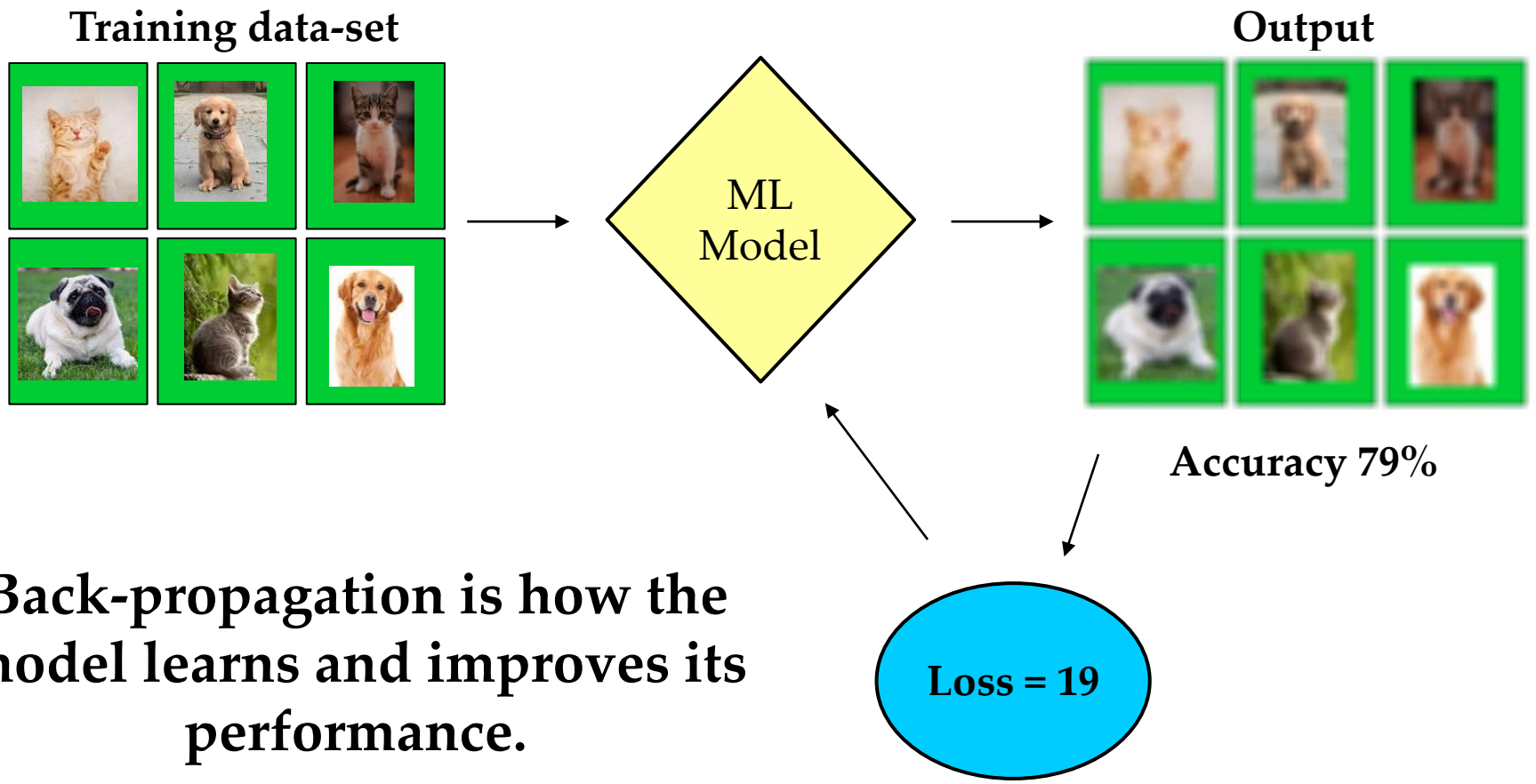

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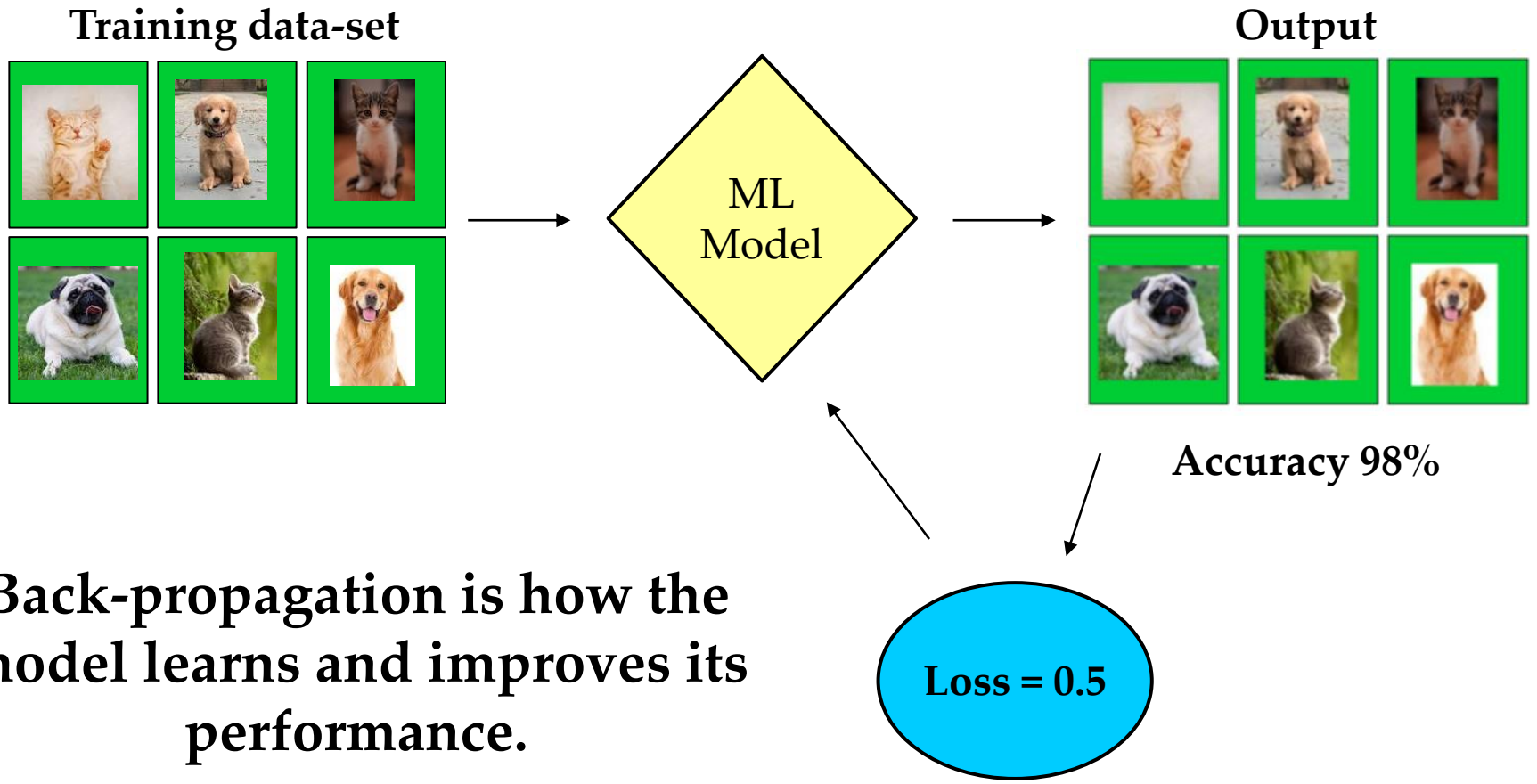

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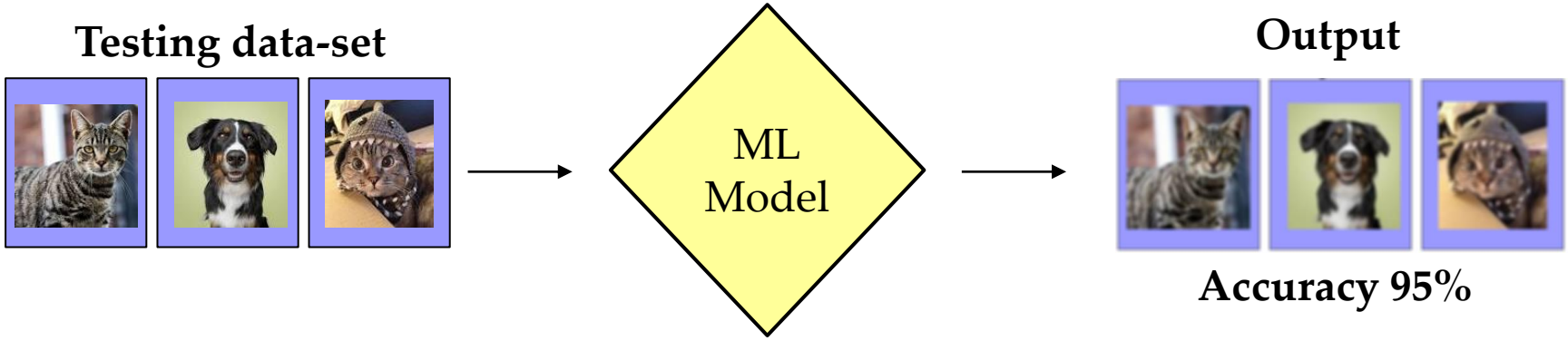


Today's ML methods:

ProteinMPNN

- Dauparas, J. et al. Robust deep learning based protein sequence design using ProteinMPNN. 2022.06.03.494563 Preprint at https://doi.org/10.1101/2022.06.03.494563 (2022).

MIF-ST

- Yang, K. K., Zanichelli, N. & Yeh, H. Masked inverse folding with sequence transfer for protein representation learning. Protein Engineering, Design and Selection 36, gzad015 (2022).

ESM

- Rives, A. et al. Biological structure and function emerge from scaling unsupervised learning to 250 million protein sequences. Proceedings of the National Academy of Sciences 118, e2016239118 (2021).
- Rao, R. M. et al. MSA Transformer. in Proceedings of the 38th International Conference on Machine Learning 8844–8856 (PMLR, 2021).
- Lin, Z. et al. Evolutionary-scale prediction of atomic-level protein structure with a language model. Science 379, 1123–1130 (2023).

ProteinMPNN (Message Passing Neural Network):

Trained on protein structures from RCSB-PDB:

- 19,700 single-chain protein structures

- Further trained on clustered high-res multichain structures

Predict probabilities of each natural aa for each position

Use probabilities to design sequences

Tested *in silico*:

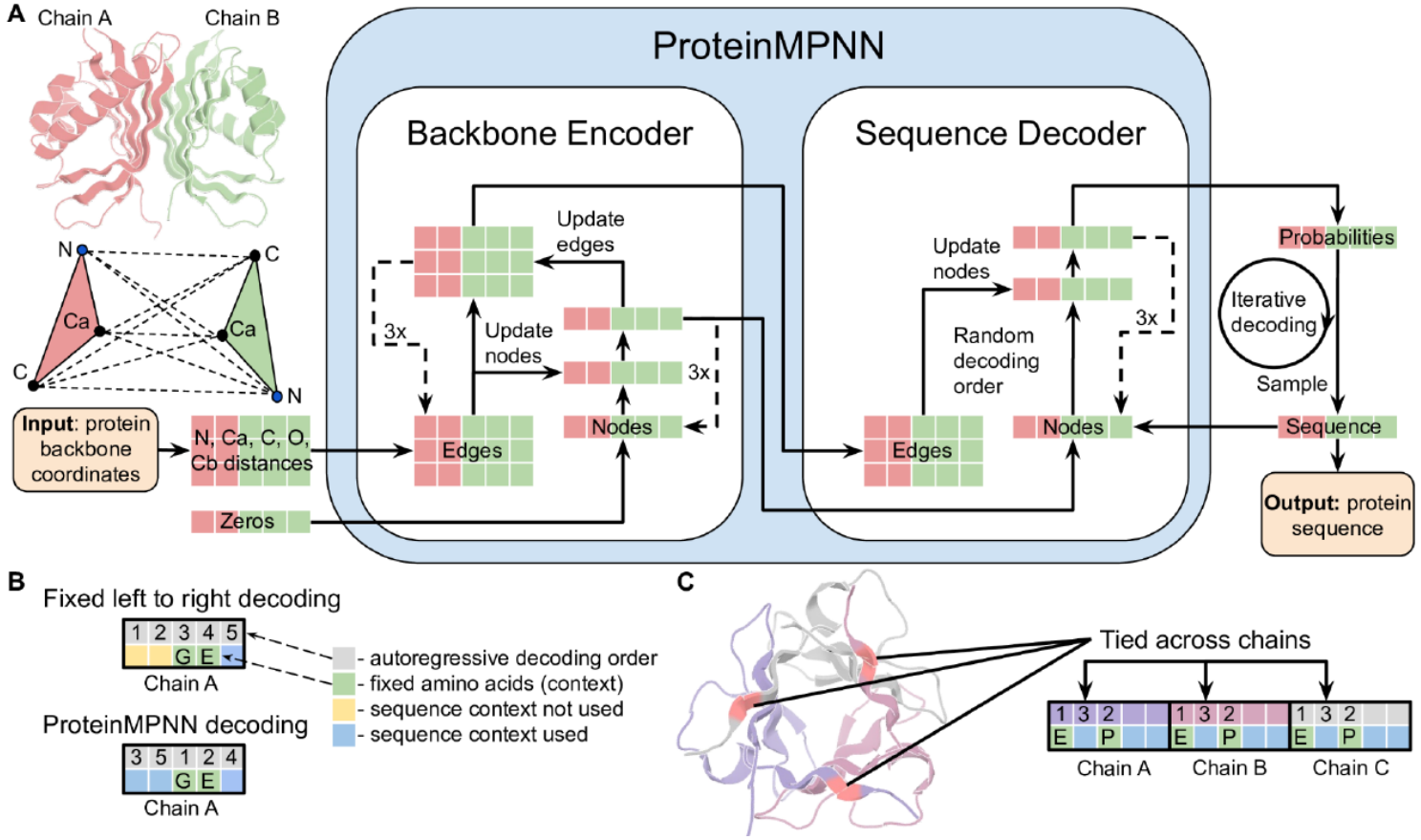
- 690 monomers

- 732 homomers

- 98 heteromers

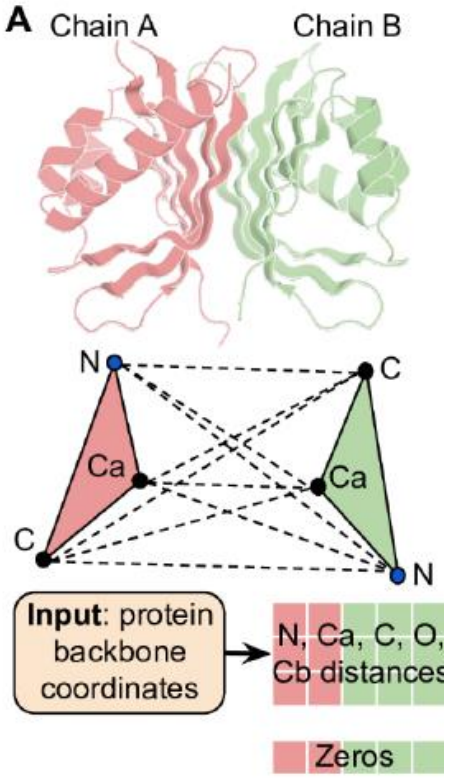
Tested experimentally

ProteinMPNN:



(Dauparas et al., 2022)

ProteinMPNN, inputs:



RCSB-PDB database

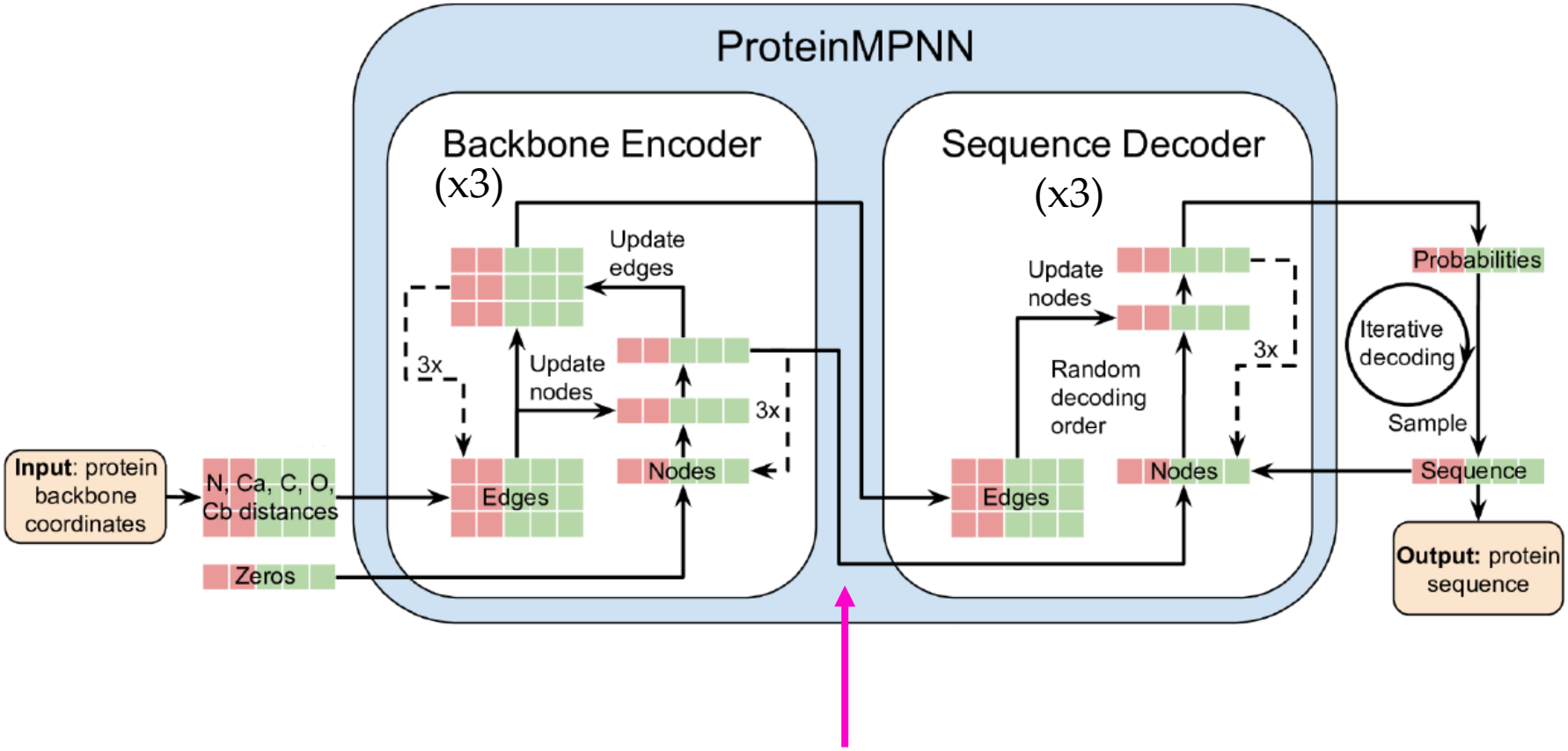
No evolutionary information!

Distances between N, Cα, C, O and virtual Cβ are encoded using graph theory:

- Nodes (atoms)
- Edges (distances)

(Dauparas et al., 2022)

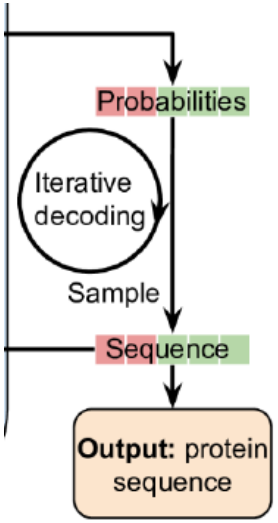
ProteinMPNN, the MPNN:



128 hidden dimensions
(here is where predictions happen)

(Dauparas et al., 2022)

ProteinMPNN, the outputs:

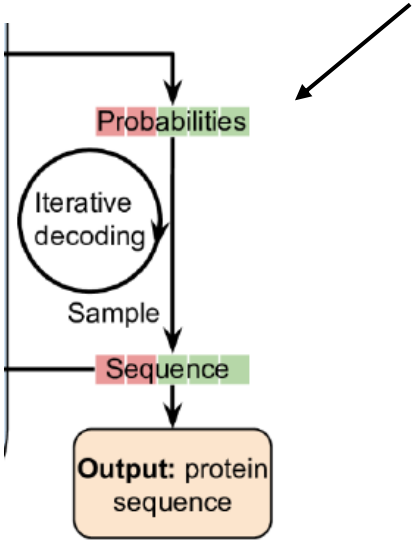


ProteinMPNN outputs re-designed sequences, not structures!

This means that you have predict a designed structure with an alternative method (AF, Rosetta)

(Dauparas et al., 2022)

ProteinMPNN, the outputs:

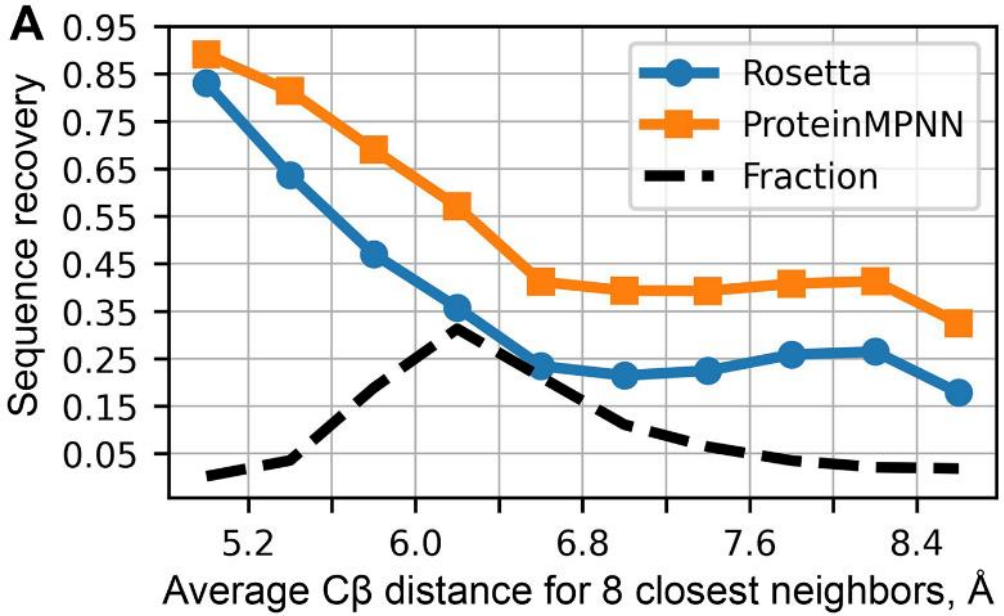


ProteinMPNN in Rosetta takes the probabilities as outputs, and uses it for designing the structure directly!

(Dauparas et al., 2022)

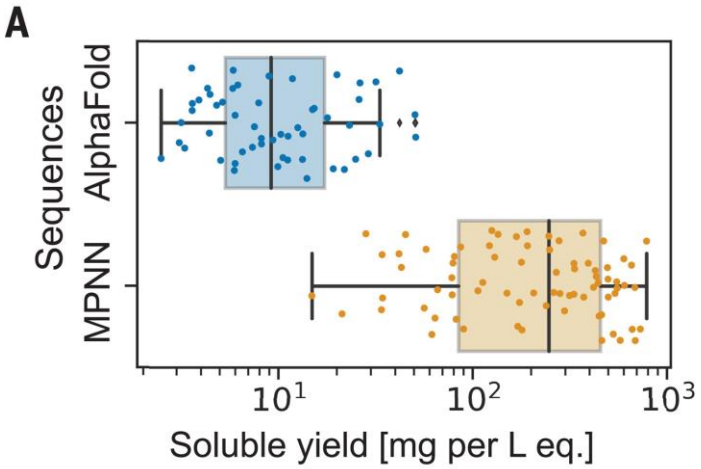
ProteinMPNN, performance:

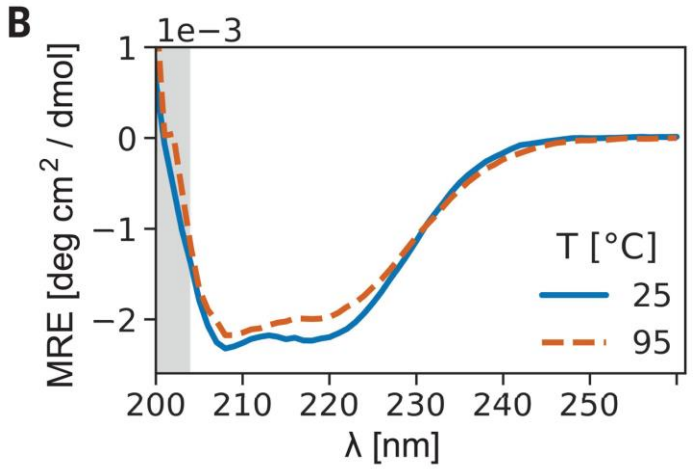
Monomer design (N=408)



(Dauparas et al., 2022)

ProteinMPNN, performance:



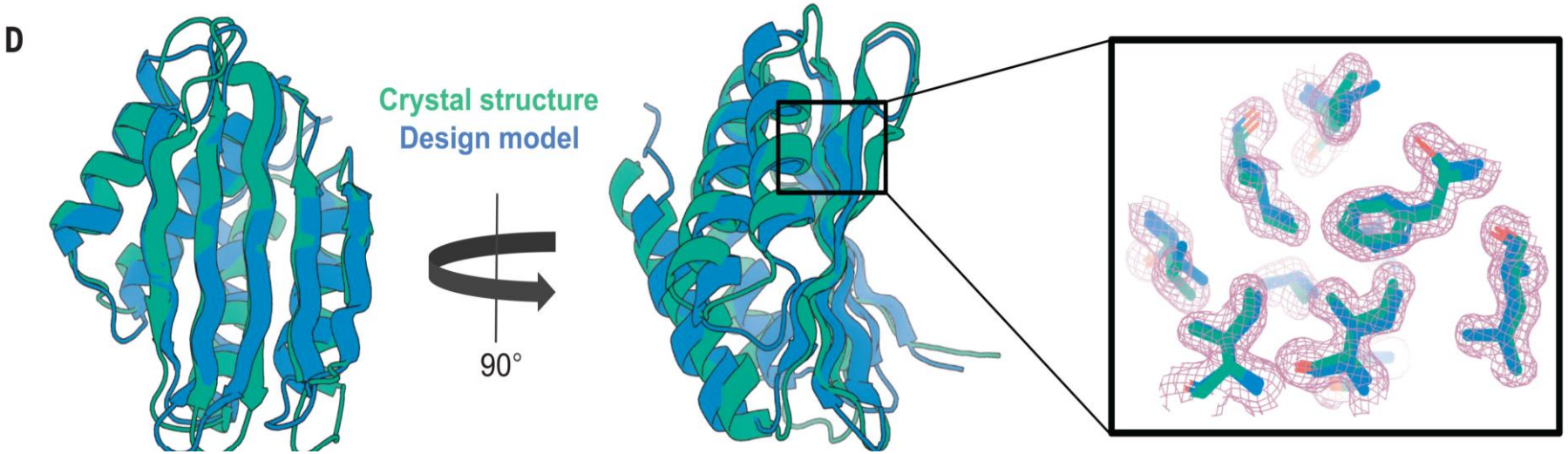


Recovered
solubility

Increased
thermostability

(Dauparas et al., 2022)

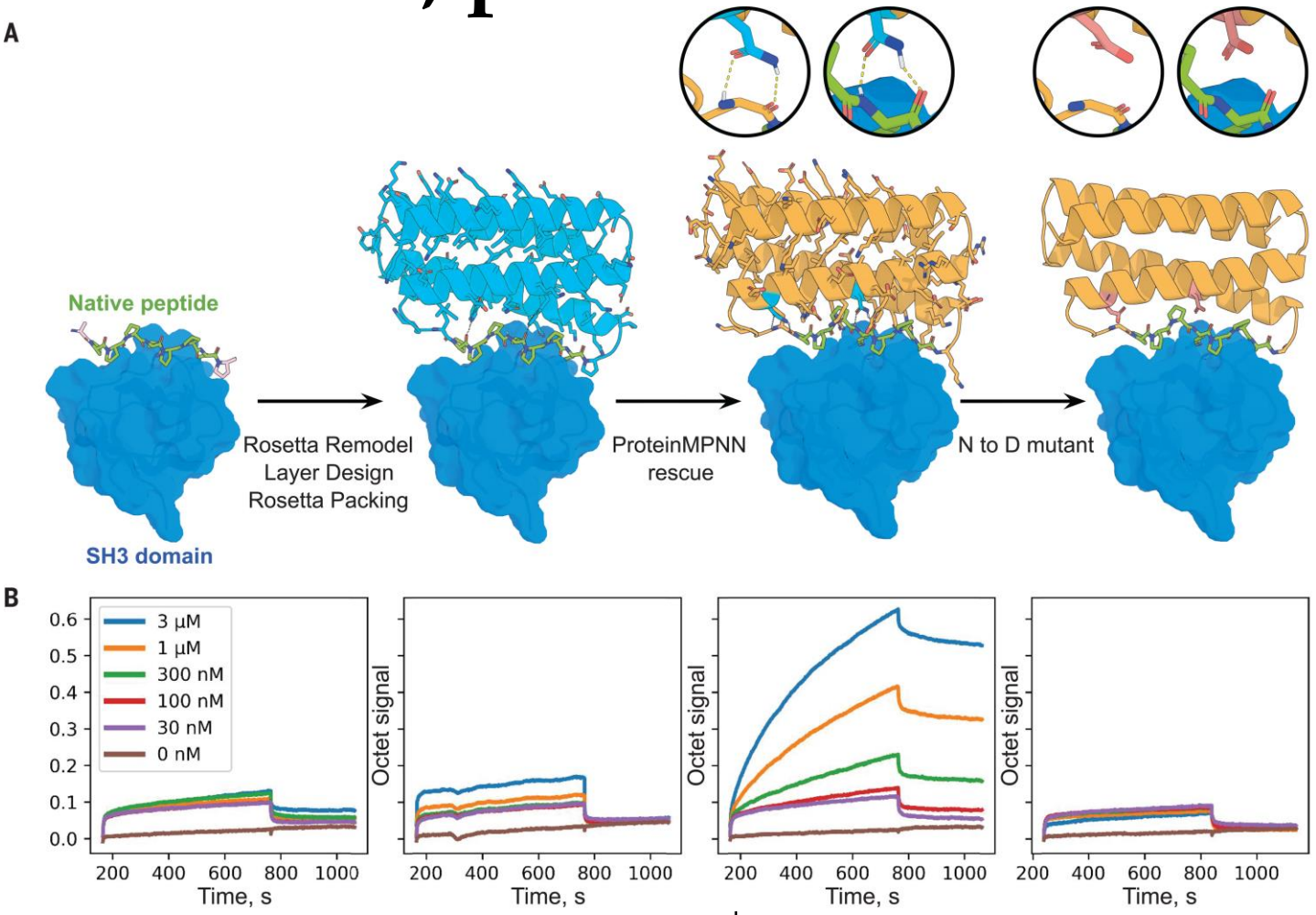
ProteinMPNN, performance:



Increased
crystallizability

(Dauparas et al., 2022)

ProteinMPNN, performance:



Creates
new functions

(Dauparas et al., 2022)

MIF-ST (Masked Inverse Folding with Sequence Transfer):

Pre-trained on both protein structures and sequences:

- 19700 protein structures from RCSB-PDB
- 42 M sequences from UniRef50
- sequences are partially masked
- model must predict masked residues

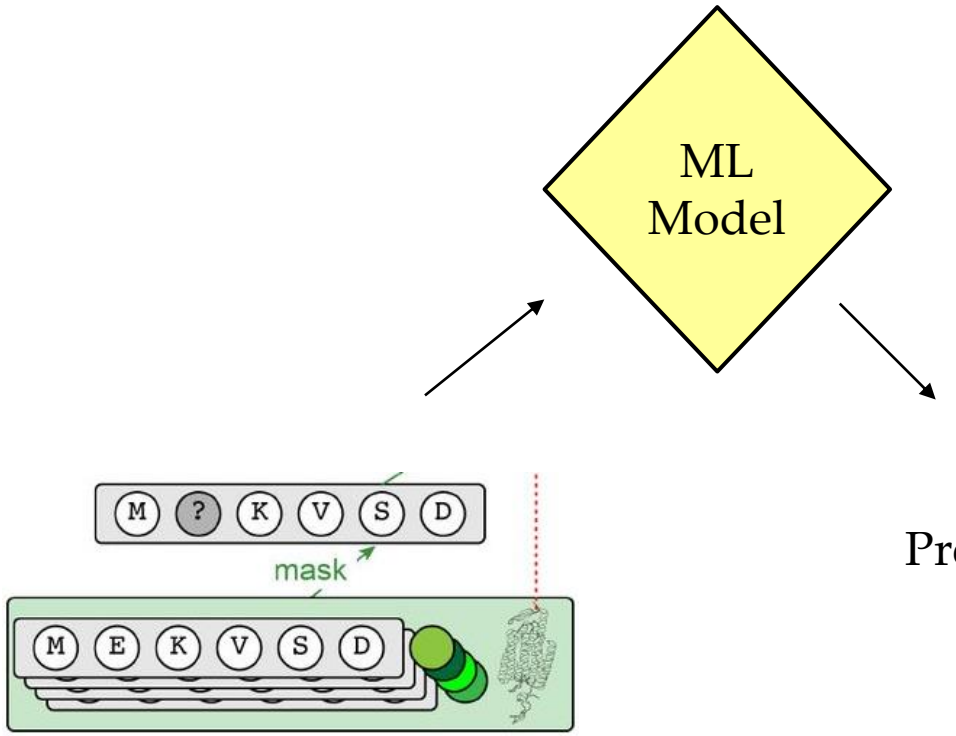
Training for downstream task

- trained on single mutants and predicts multiple mutants
- predict experimental measurements

Tested *in silico* on small and large data-sets:

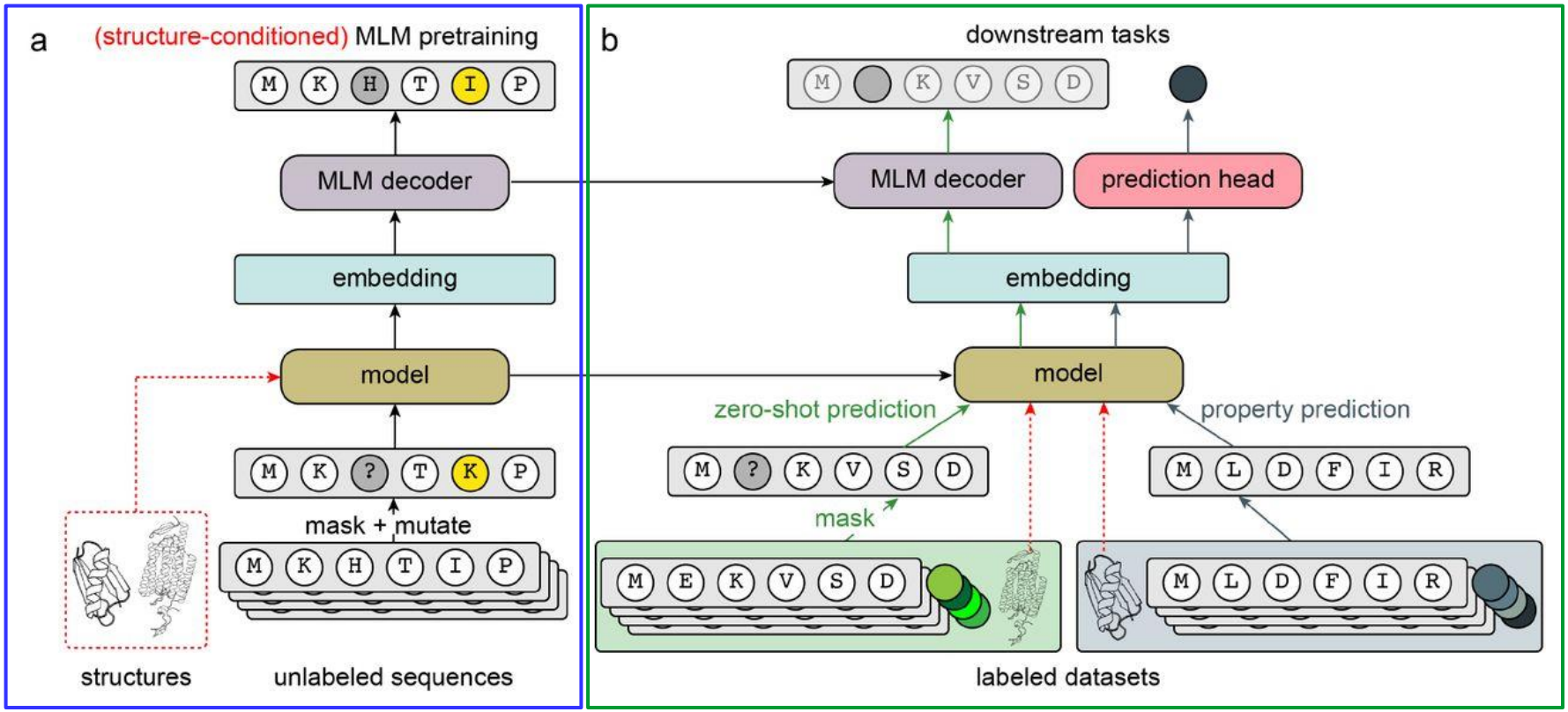
- Deep mutational scans
- Enzymatic activity
- Stability
- Binding

Masking protein sequences in ML:



Prediction: MEKVSD

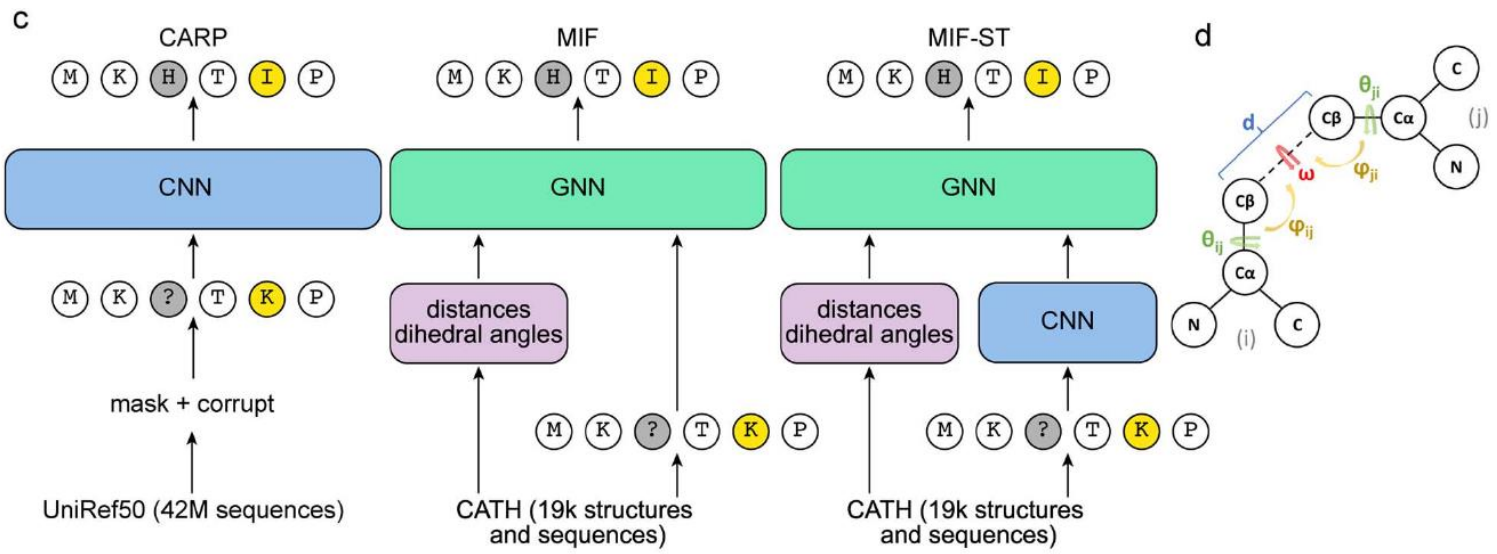
MIF-ST:



Pre-training
(structures, sequences, masking)

Training
(sequences, masking)

MIF-ST:



CNN = Convolutional Neural Network
(ordered data, N to C term of sequence)

GNN = Graph Neural Network
(unordered data, atoms with spatial component)

MIF-ST, performance:

Regime	Model	Parameters	Perplexity	Recovery
Sequence only	CARP-640M	640M	7.06	40.5%
Sequence & structure	MIF-4	3.4M	4.95	49.9%
	MIF-8	6.8M	5.00	46.7%
	GVPMIF	3.5M	4.68	51.2%
+Sequence transfer	MIF-ST	3.4M	**4.08**	**55.6%**
–UniRef50 pretraining	MIF-ST	3.4M	5.70	45.4%

Perplexity:
Model's uncertainty in prediction (lower is better)

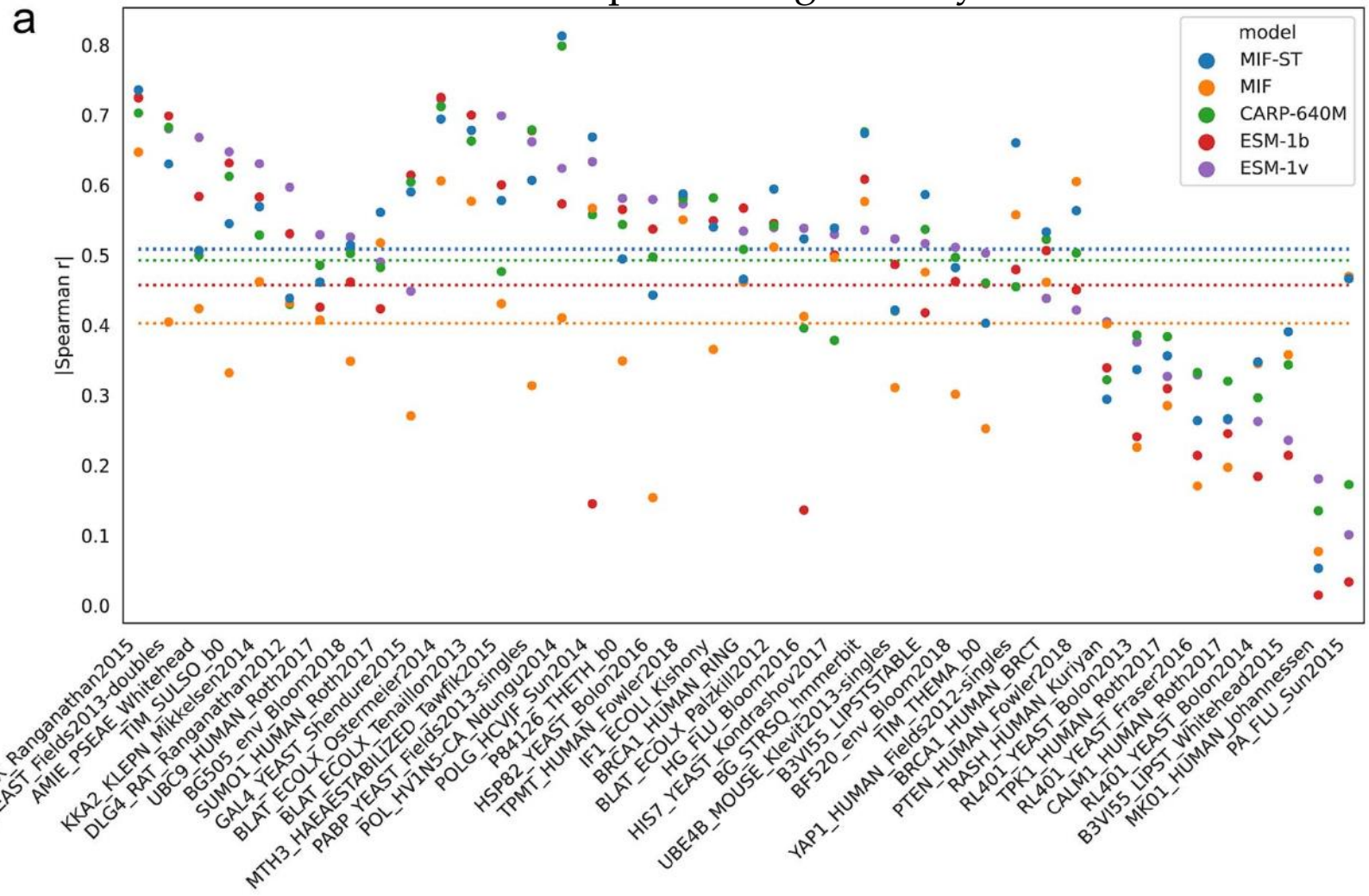
Sequence Recovery:
How well the model recovers native sequences. (higher is better)

(Yang et al., 2023)

MIF-ST, performance:

Predictions on DMS datasets:

MIF-ST is outperforming in many cases.



(Yang et al., 2023)

ESM (Evolutionary Scale Modeling):

Trained on protein sequences:

- 250 M sequences from UniParc
- Also using masking techniques

Evaluated on sequences from UniRef:

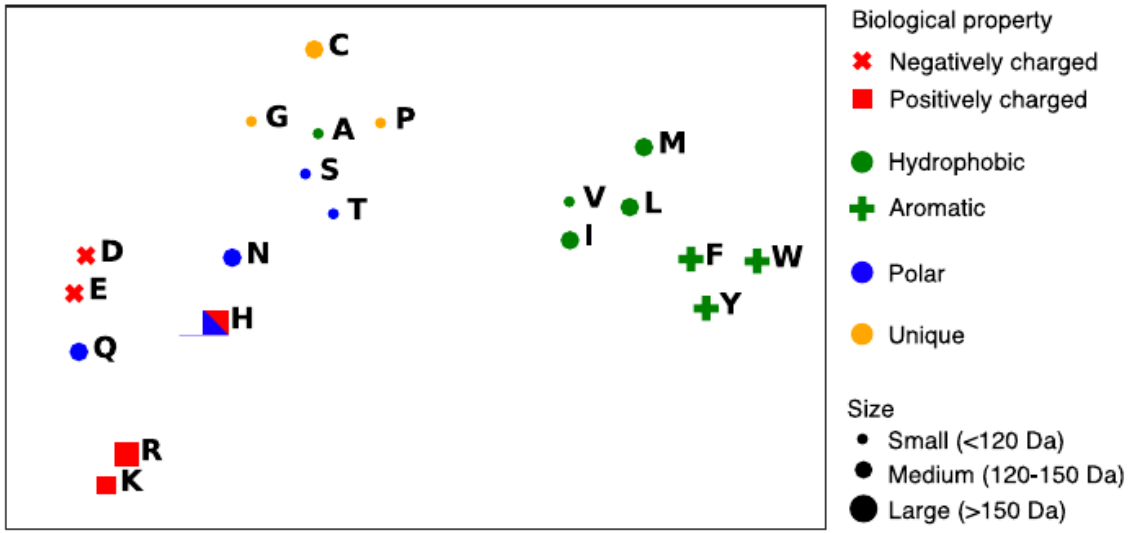
- Low-diversity data-set with UniRef100
- High-diversity sparse data-set with UniRef50 representative
- High-diversity dense data-set with UniRef50 clusters

Tested *in silico* to predict:

- Physio-chemical residue properties
- Biological variation
- Protein homology
- Secondary and tertiary structure (Lin et al., 2023)
- Effects of mutations

Experimental validation (*de novo* design - BioRvix) (Verkuil et al., 2022)

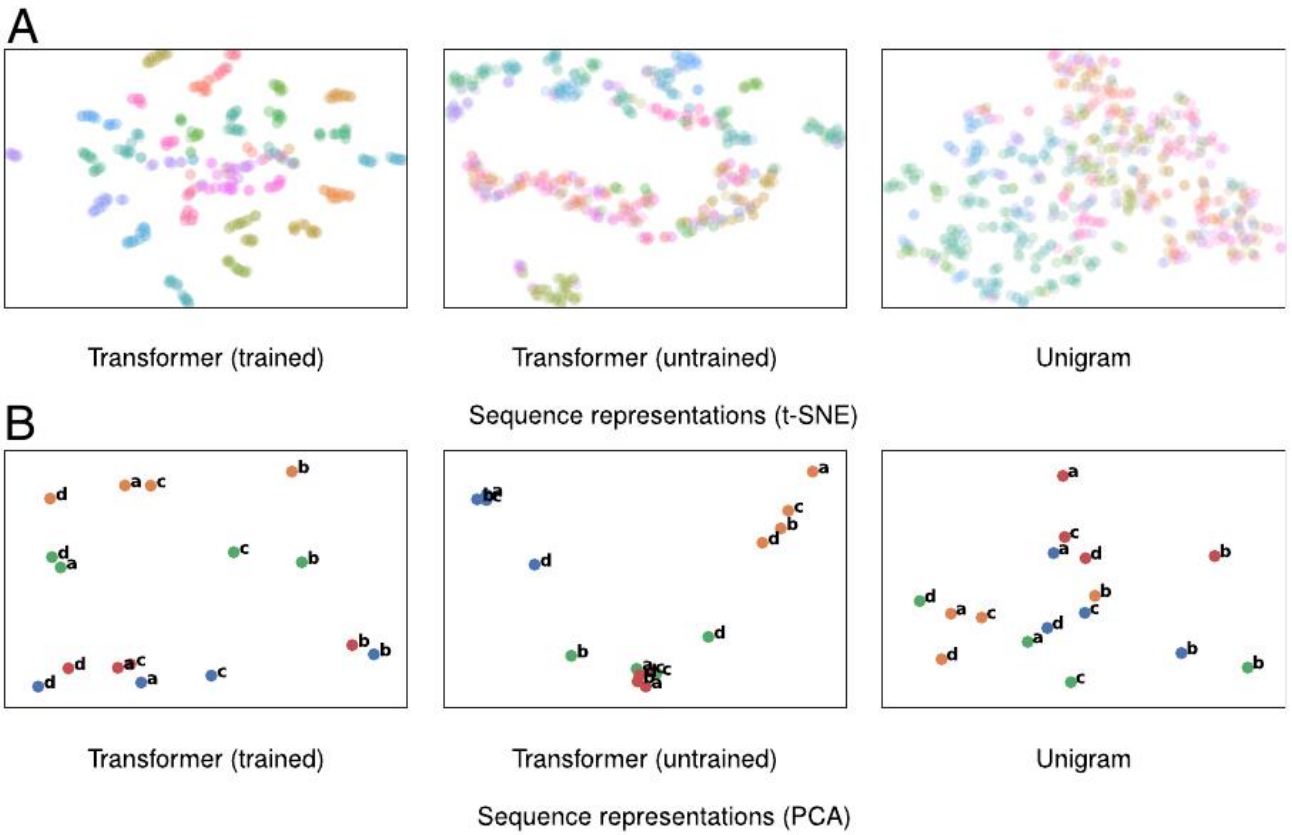
ESM, performance:



Cluster amino acids
by properties

(Rives et al., 2021)

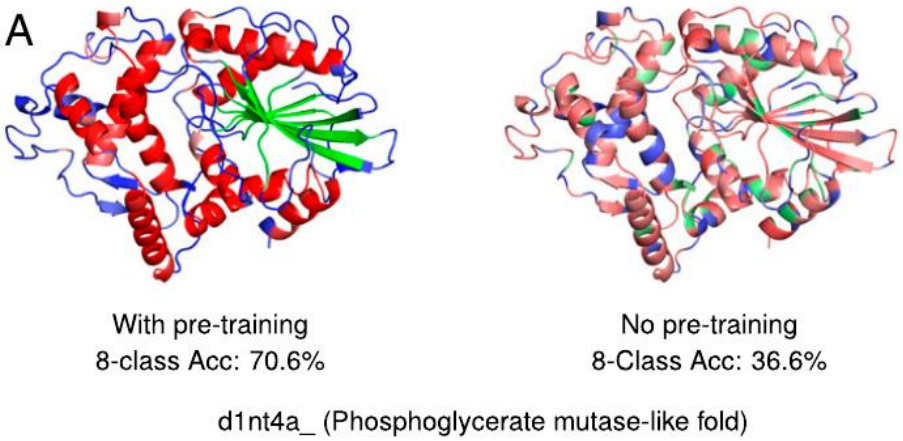
ESM, performance:



Clusters genes by homology and function

(Rives et al., 2021)

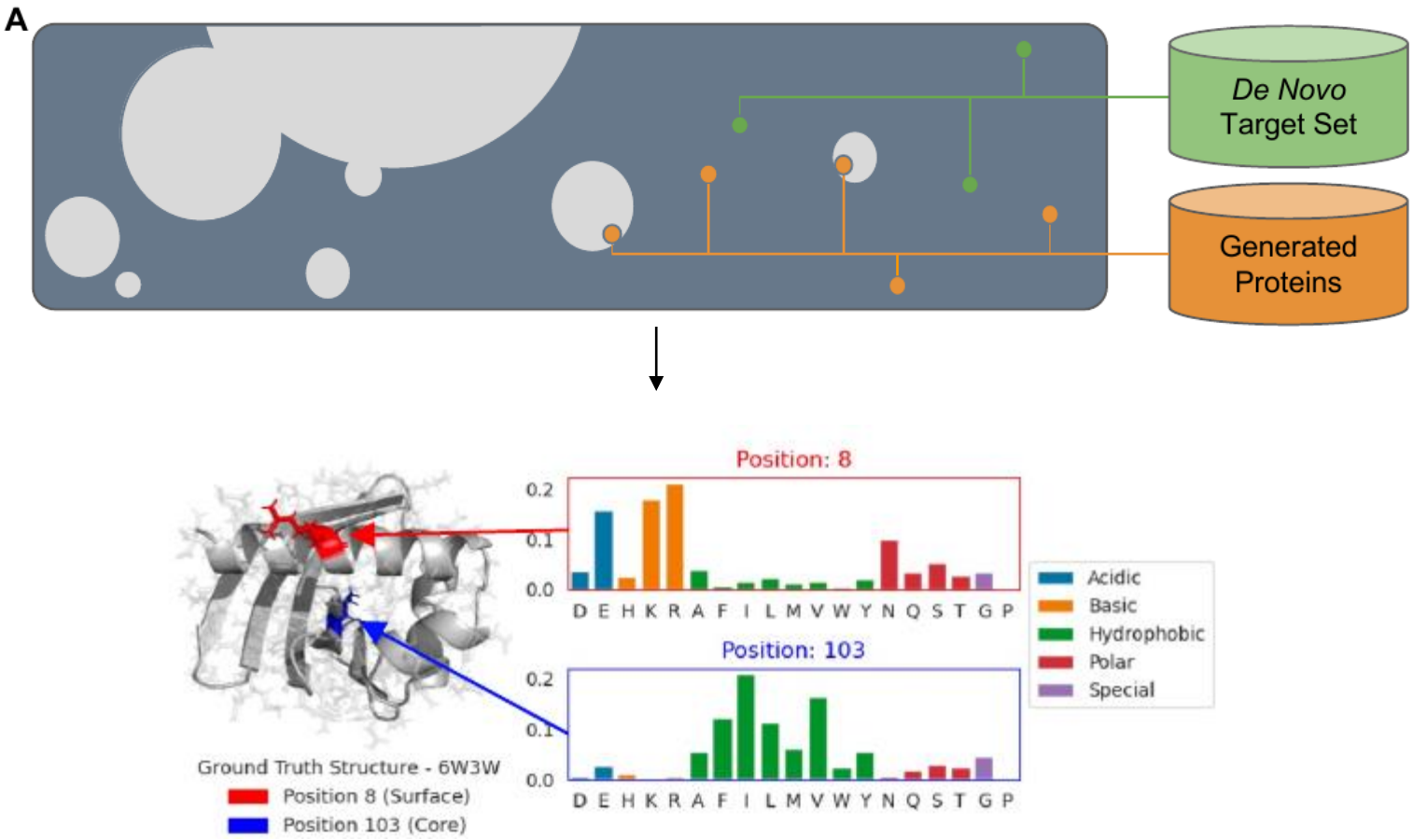
ESM, performance:



→ Predict secondary structures

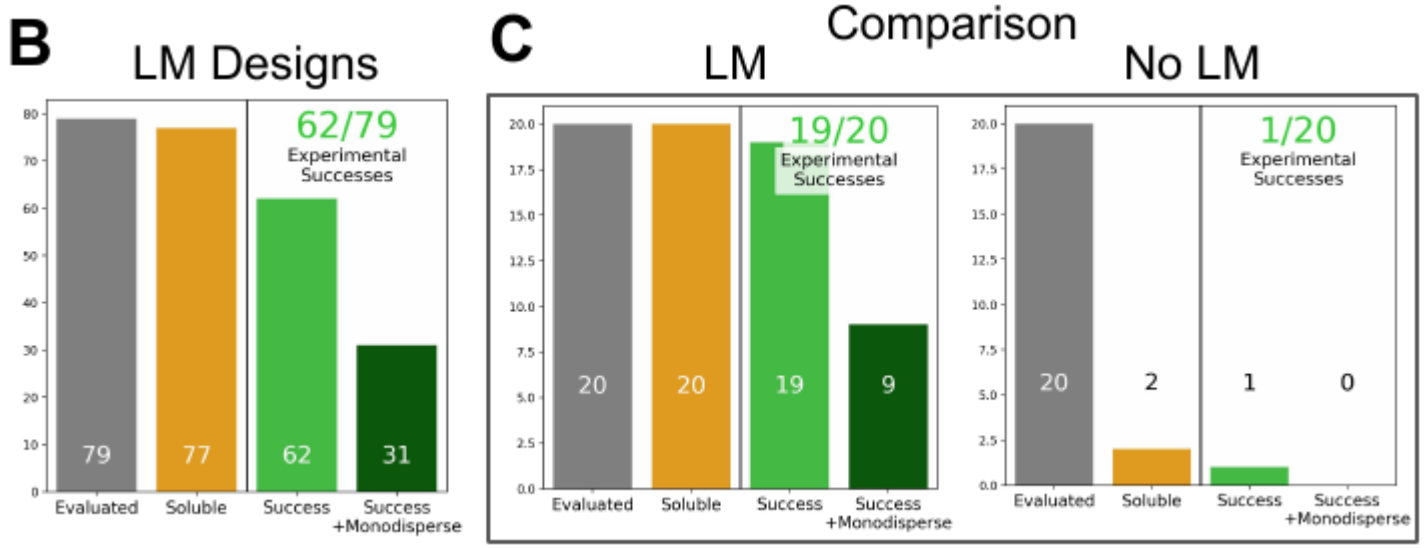
Helices
Strands
Loops

ESM, performance:

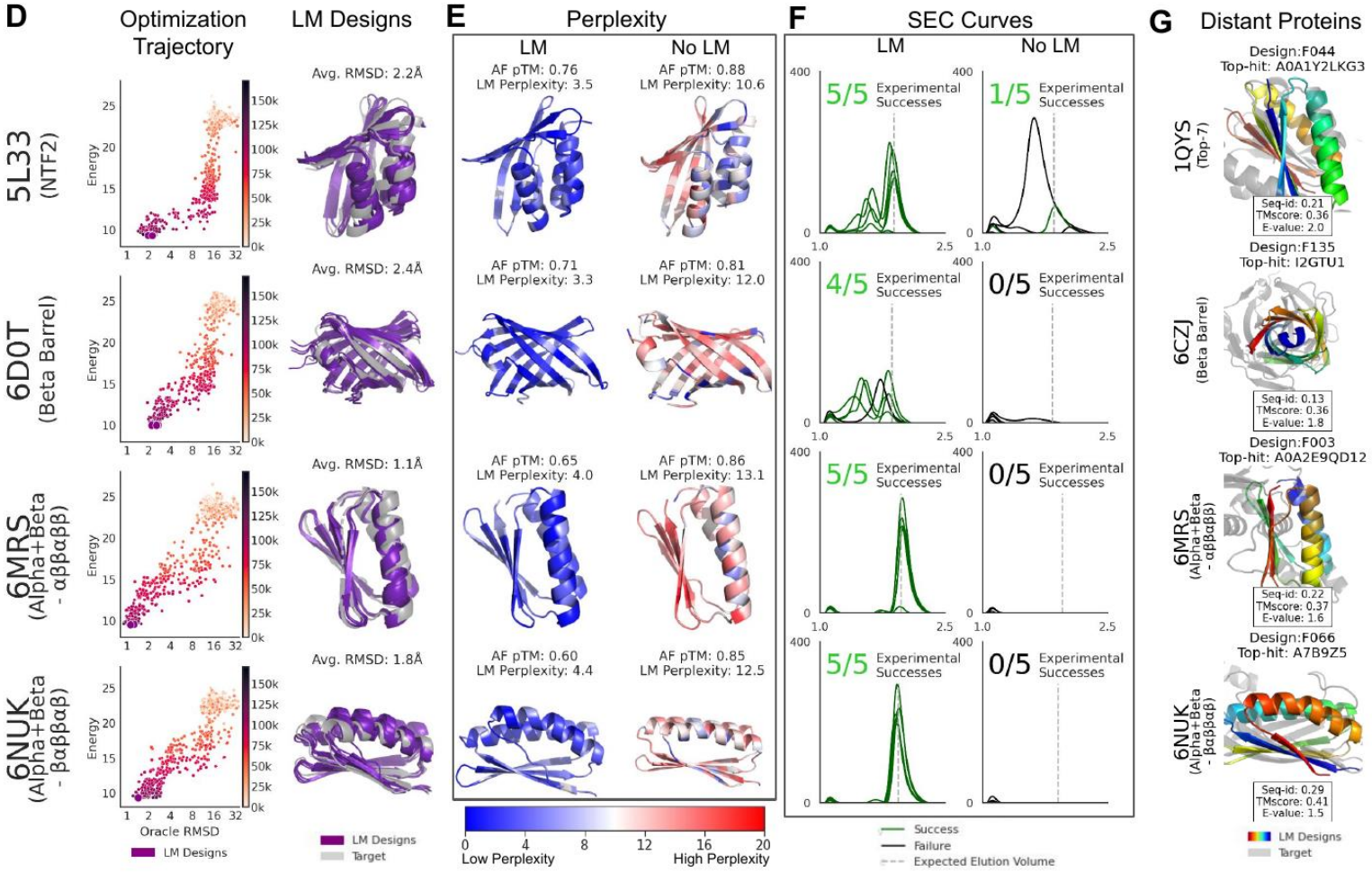


Probabilities!

ESM, performance:



ESM, performance:



(Verkuil et al., 2022)

ML in Rosetta:

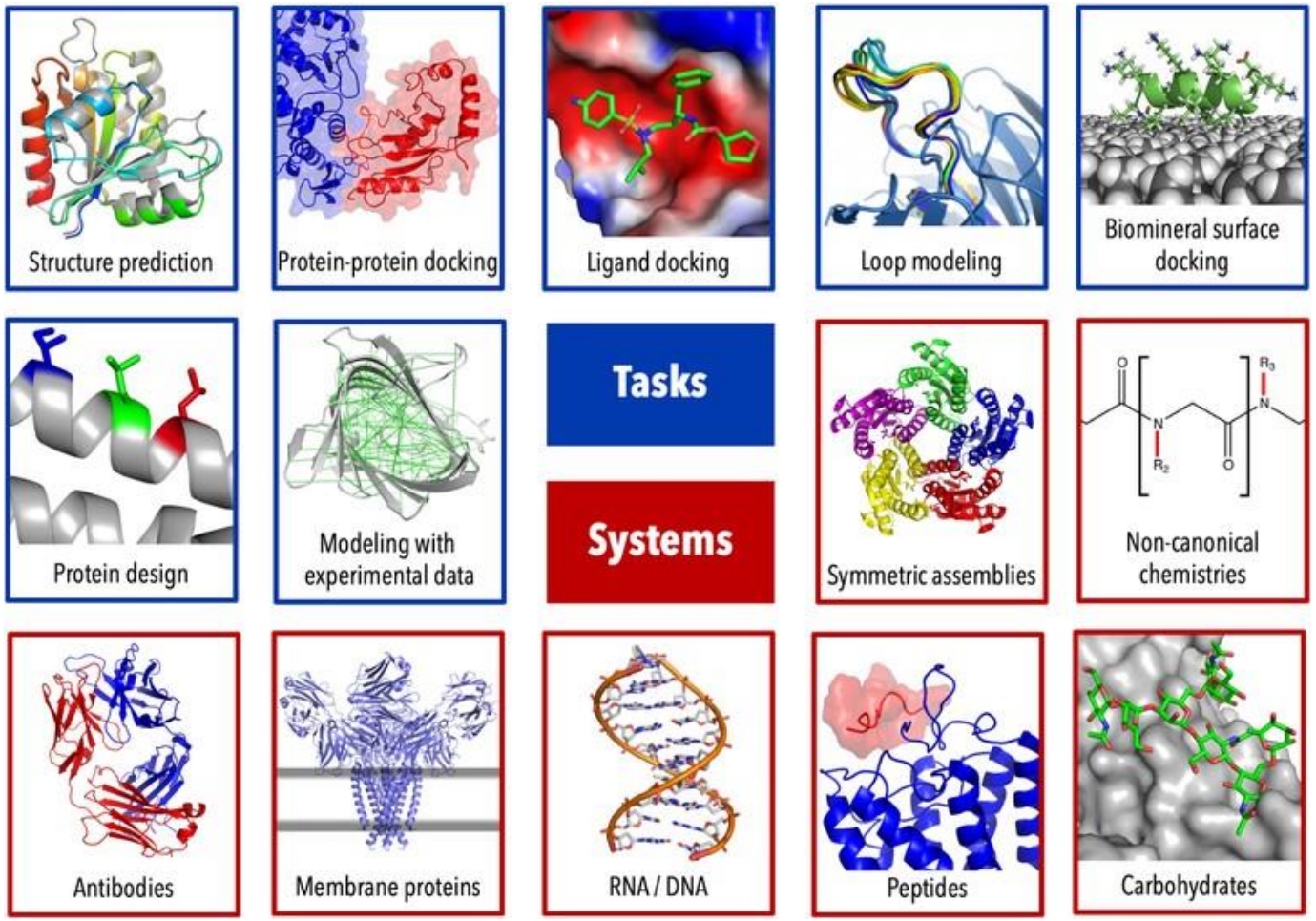
The hero here:

Moritz Ertelt
PhD student in Meiler lab at Leipzig University

Contact:
moritz.ertelt@uni-leipzig.de

ML in Rosetta:

Why integrating protein ML methods in Rosetta?



(Koehler-Lehman *et al.* 2020)

ML in Rosetta:

Why integrating protein ML methods in Rosetta?

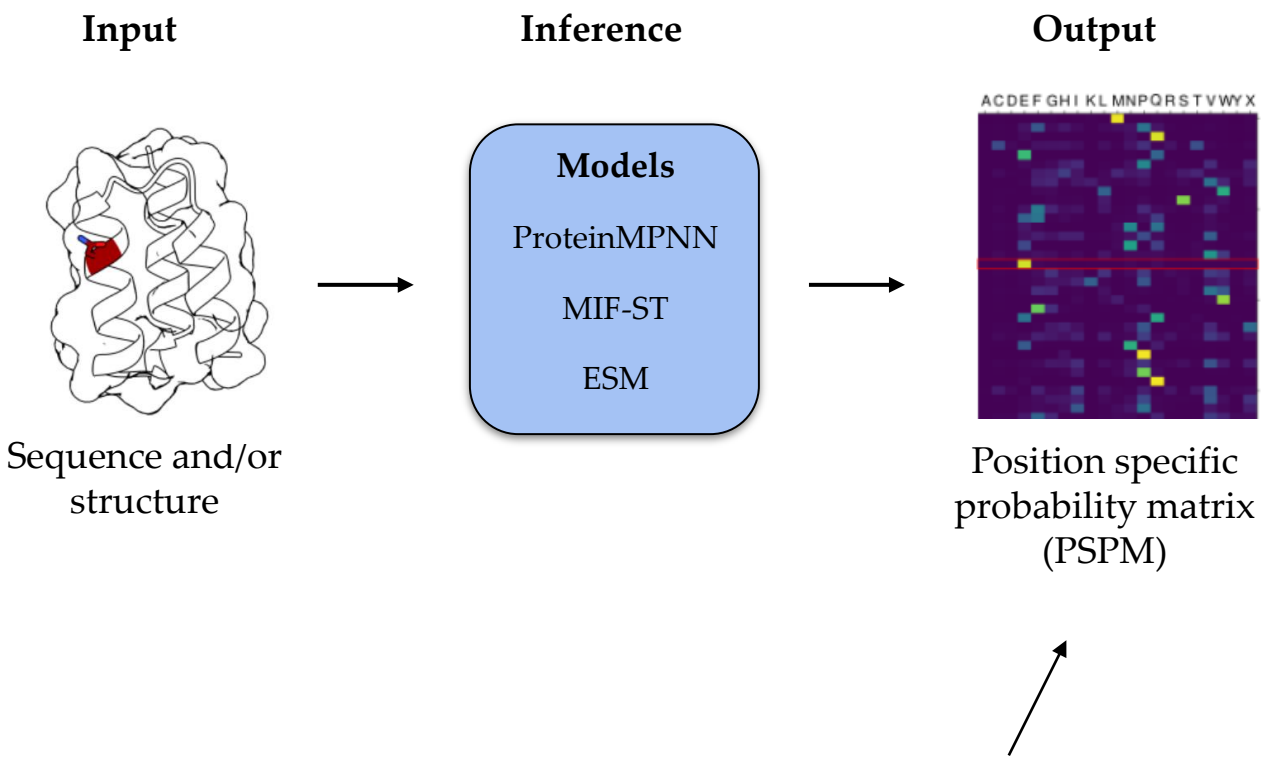
+ Feature calculation is fast in C++
+ No knowledge of Python needed for RosettaScripts
+ Makes it easy to combine ML with Rosetta elements
+ No need to reinvent the wheel for sampling, scoring, etc.
+ Provides an established testing framework

ML in Rosetta, how:

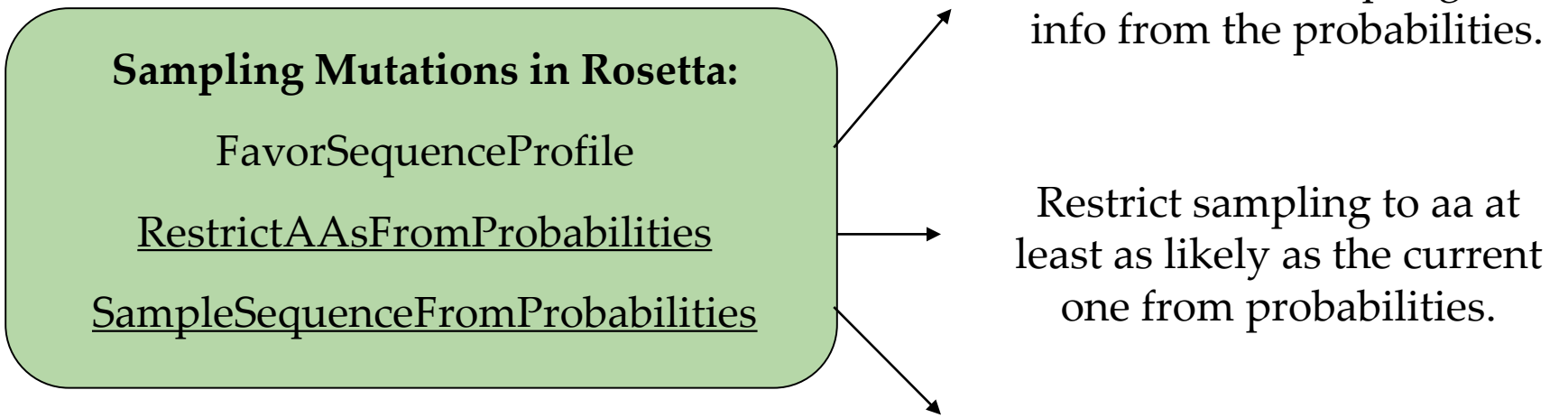
- Link Rosetta against PyTorch/TensorFlow
- Re-create feature calculation & inference in Rosetta
- Standardize output in Rosetta
- Create tools around the standardized output in Rosetta

```
./scons.py -j 14 bin mode=release extras=pytorch,tensorflow
```

ML in Rosetta Design:



ML in Rosetta Design, design tools:



ML in Rosetta Design, design tools:

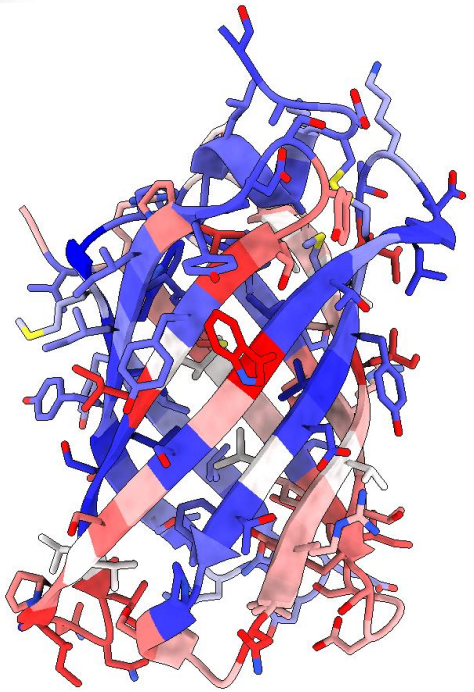
```
<TASKOPERATIONS>
    <ReadResfile name="rrf" filename="./resfile.resfile"/>
</TASKOPERATIONS>
<SIMPLE_METRICS>
    <PerResidueEsmProbabilitiesMetric name="esm" residue_selector="res"
    model="esm2_t33_650M_UR50D"/>
</SIMPLE_METRICS>
<MOVERS>
    <SampleSequenceFromProbabilities name="sample" metric="esm" pos_temp="0.1"
    aa_temp="0.1" prob_cutoff="0.1" delta_prob_cutoff="0.0" max_mutations="10"
    task_operations="rrf" use_cached_data="true"/>
</MOVERS>
```

- Sample 10 positions
  (`max_mutations="10"`)
- Sample aa with p>0.1
  (`prob_cutoff="0.1"`)
- At least as likely as the current aa
  (`delta_prob_cutoff="0.0"`)

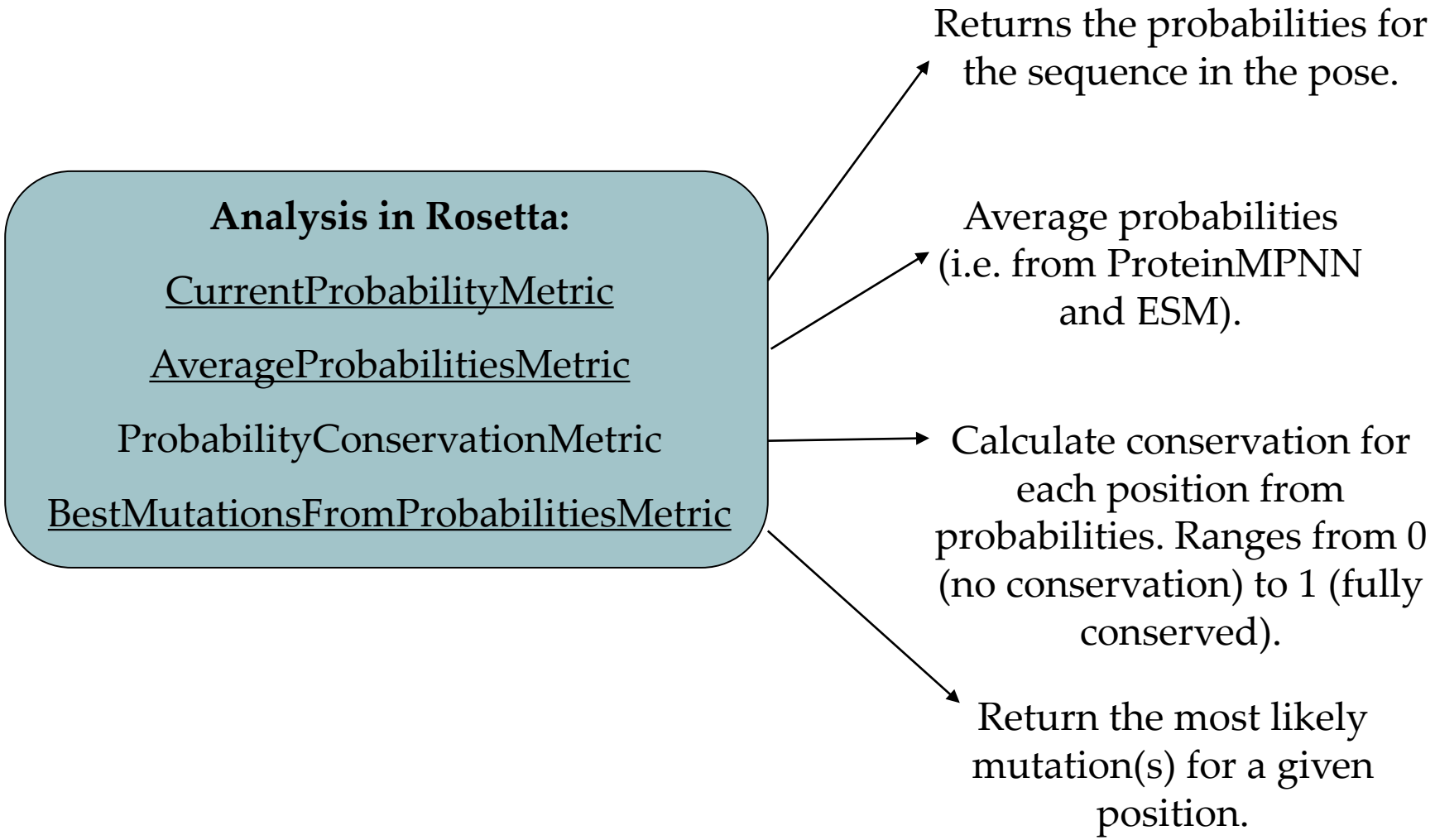
ML in Rosetta Design, analysis tools:

```
<SIMPLE_METRICS>
    <ProteinMPNNProbabilitiesMetric name="prediction"/>
    <CurrentProbabilityMetric name="current" metric="prediction"/>
</SIMPLE_METRICS>
```

The probabilities for the sequence are saved in the b-factor column of the pdb and can be easily visualized with pymol/chimera.



ML in Rosetta Design, analysis tools:



The tutorial:

Monomer

Dimer

Input Preparation:
- Download the pdbs
- Clean the pdbs
- Repack the structure

Calculate probabilities:
- ProteinMPNN, MIF-ST, ESM (independently)
- Get current probability
- Get best mutations

Design:
- Use probabilities to guide design
- Use probabilities to guide scoring
- Design interfaces

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