ML in Rosetta
(part 1)

Cristina Elisa Martina
Rosetta Workshop 2023
Meiler Lab

Revolution in Structural Biology:

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DeepMind

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DeepMind

AlphaFold

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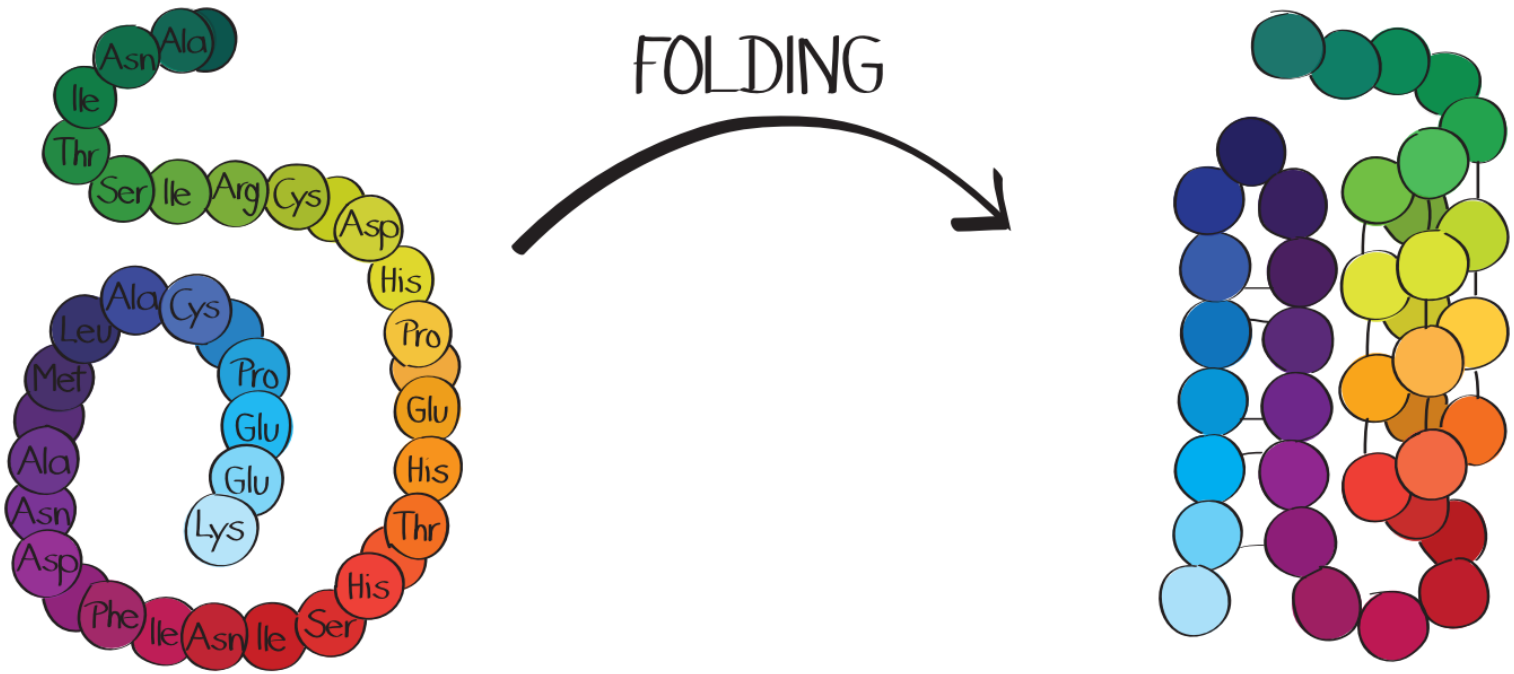
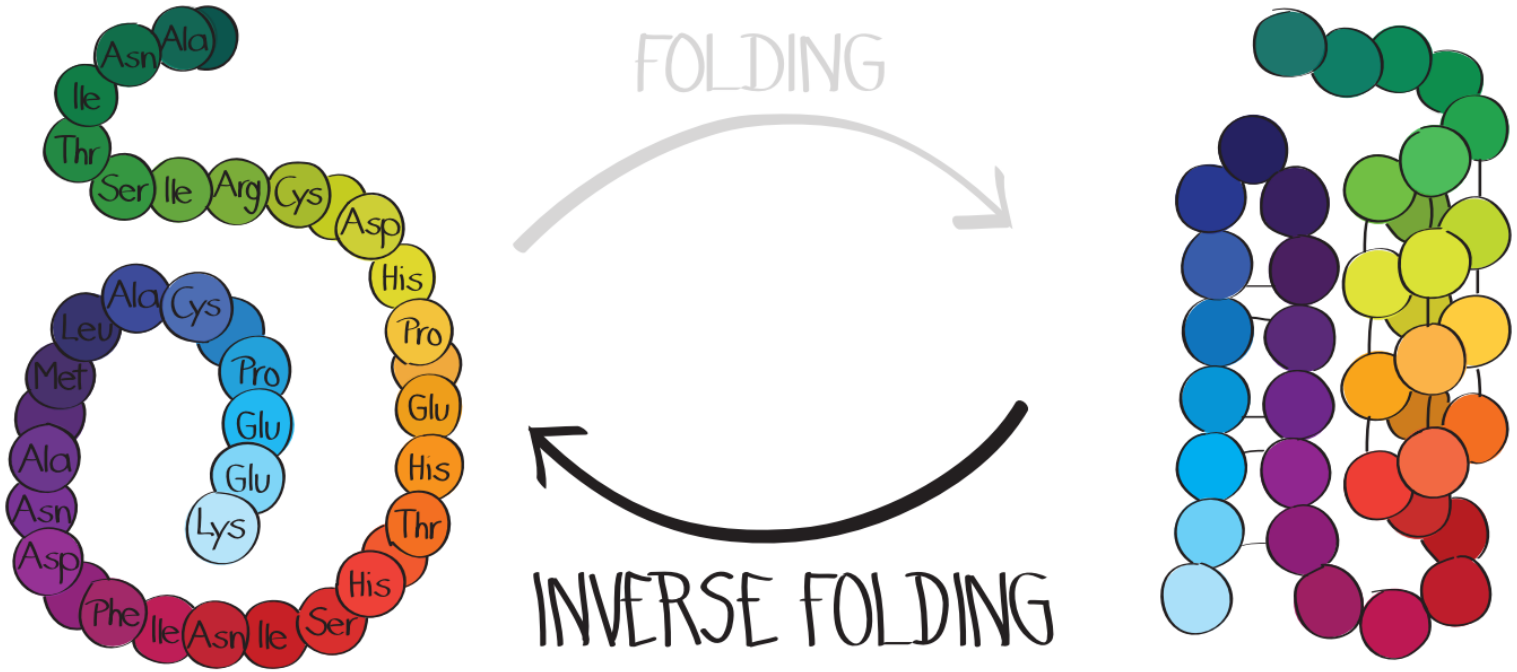
DeepMind

AlphaFold



(Art from Ruth Kellner)

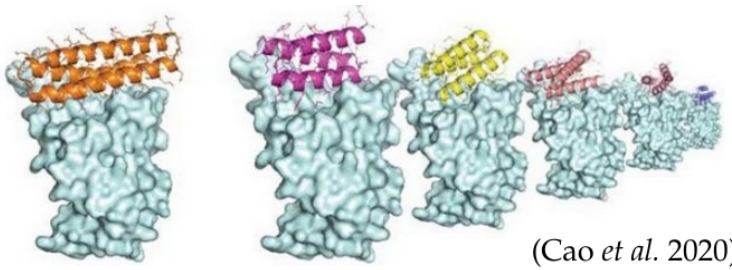
Protein design with ML:



(Art from Ruth Kellner)

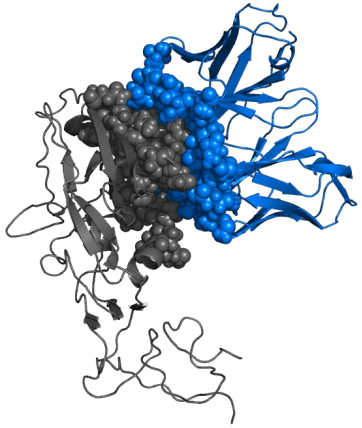
What is the best sequence to:

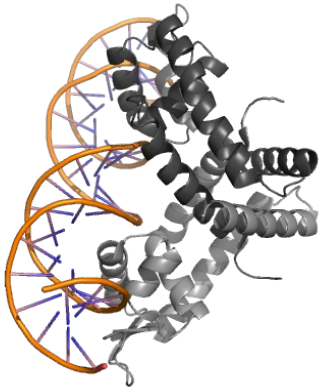
- **fold in this protein scaffold?**
  - new functions
  - new shapes (*de novo* design)



(Cao *et al.* 2020)

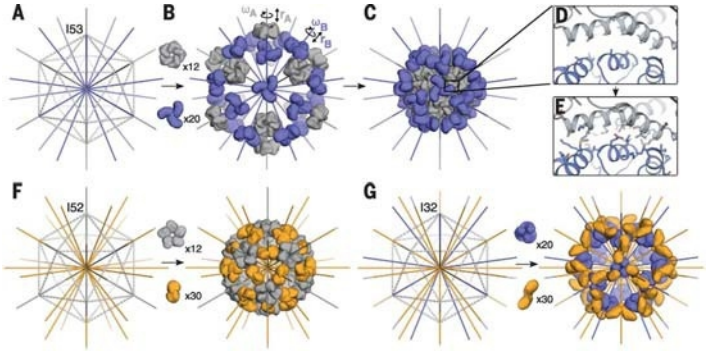
- **increase protein stability?**
  - half-life
  - thermostability
  - crystallizability
  - protein yields





- **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 (Rosetta):

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

Machine Learning methods (Protein MPNN):

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

Today's ML methods:

Protein MPNN

- 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).

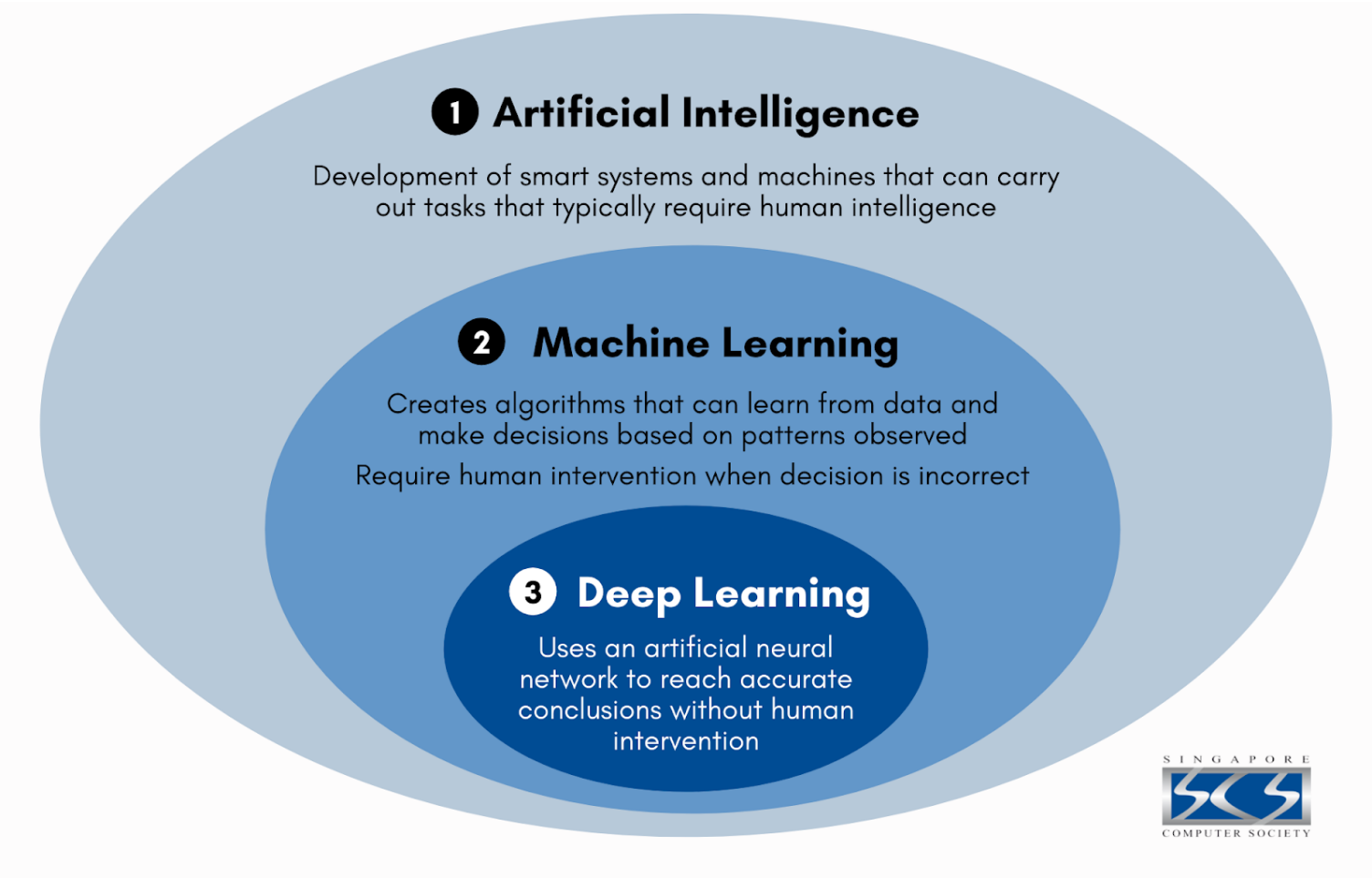
MIF-ST

- Yang, K. K., Zanichelli, N. & Yeh, H. Masked inverse folding with sequence transfer for protein representation learning. Protein Engineering, Design and Selection 36, gzad015 (2023).

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).

General info on ML:

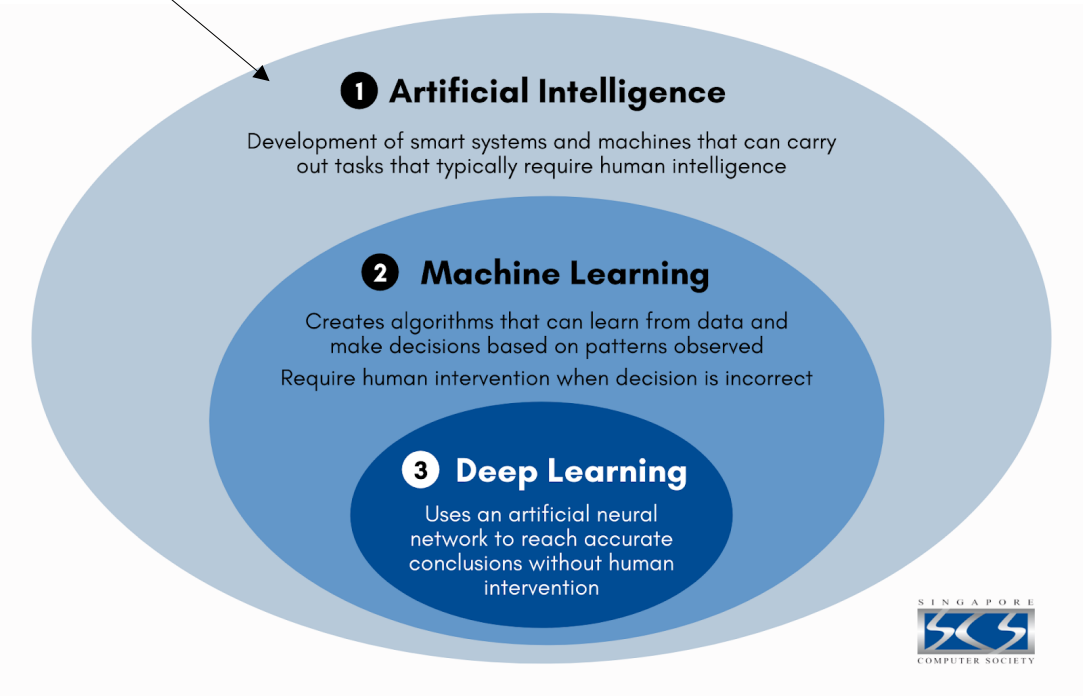


General info on ML:

1- Artificial Intelligence (since 1950)

Computer programs that do something smart:

- Chatbots
- Search engines (Chess)
- Translator (old school)

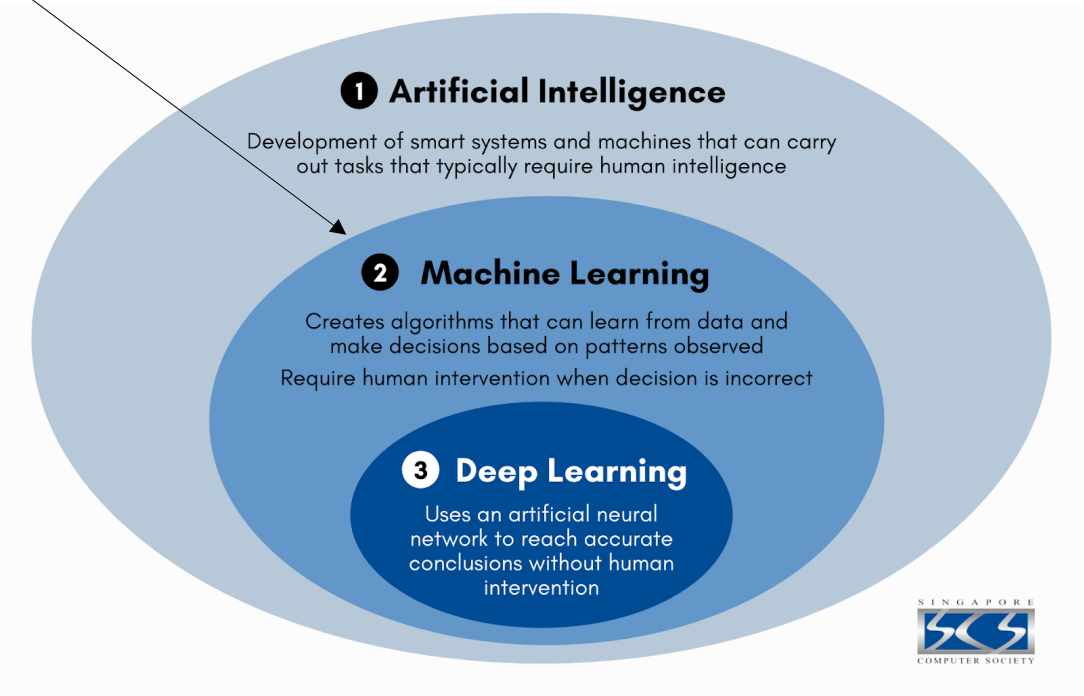


General info on ML:

2- Machine Learning (since 1980)

Computer programs that learn something:

- Personal Assistants (Siri, Alexa)
- Malware filtering
- Translator (new ones)

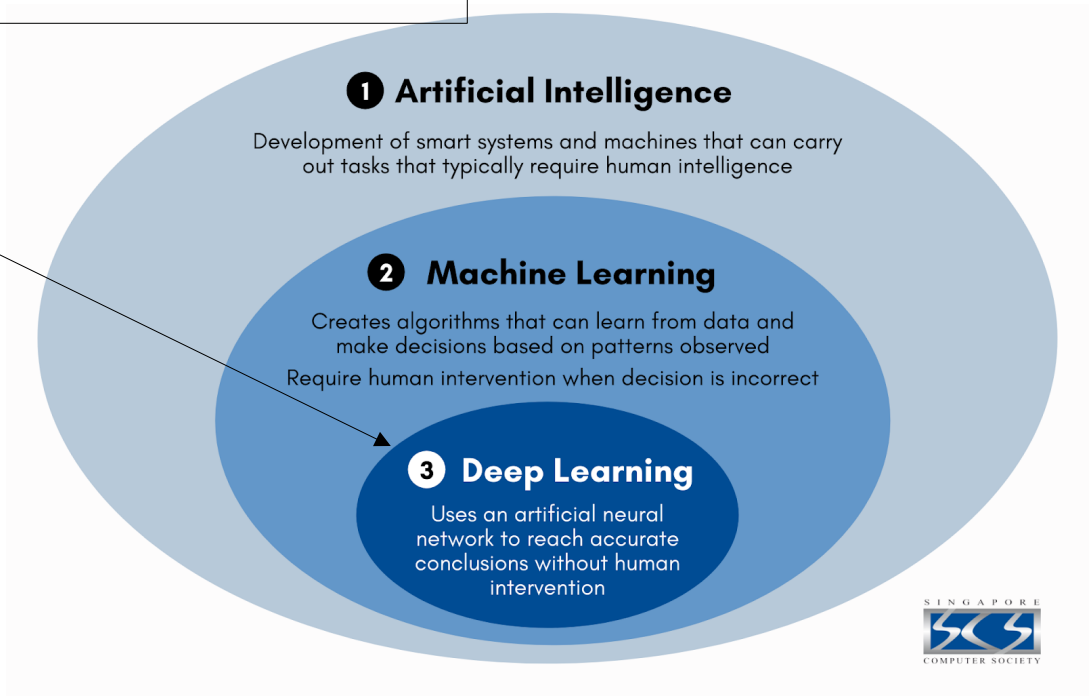


General info on ML:

3- Deep Learning (since 2010)

Computer programs that learn from large unstructured data and use neural networks.

- ChatGPT
- Driveless car
- Google map / Waze



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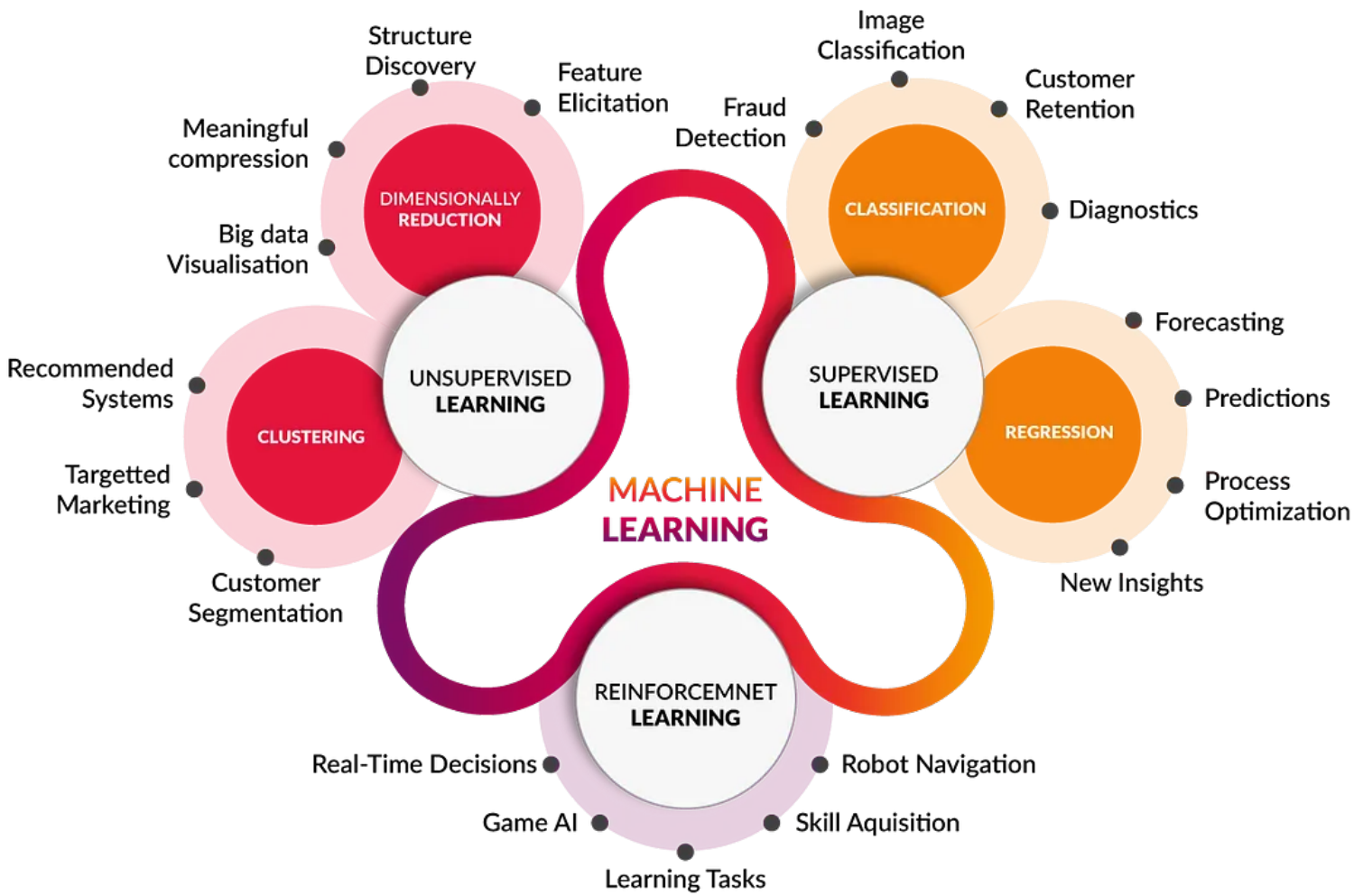


Image source: http://www.cognub.com/index.php/cognitive-platform/

Supervised learning:

Training set labeled!

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

Supervised learning:

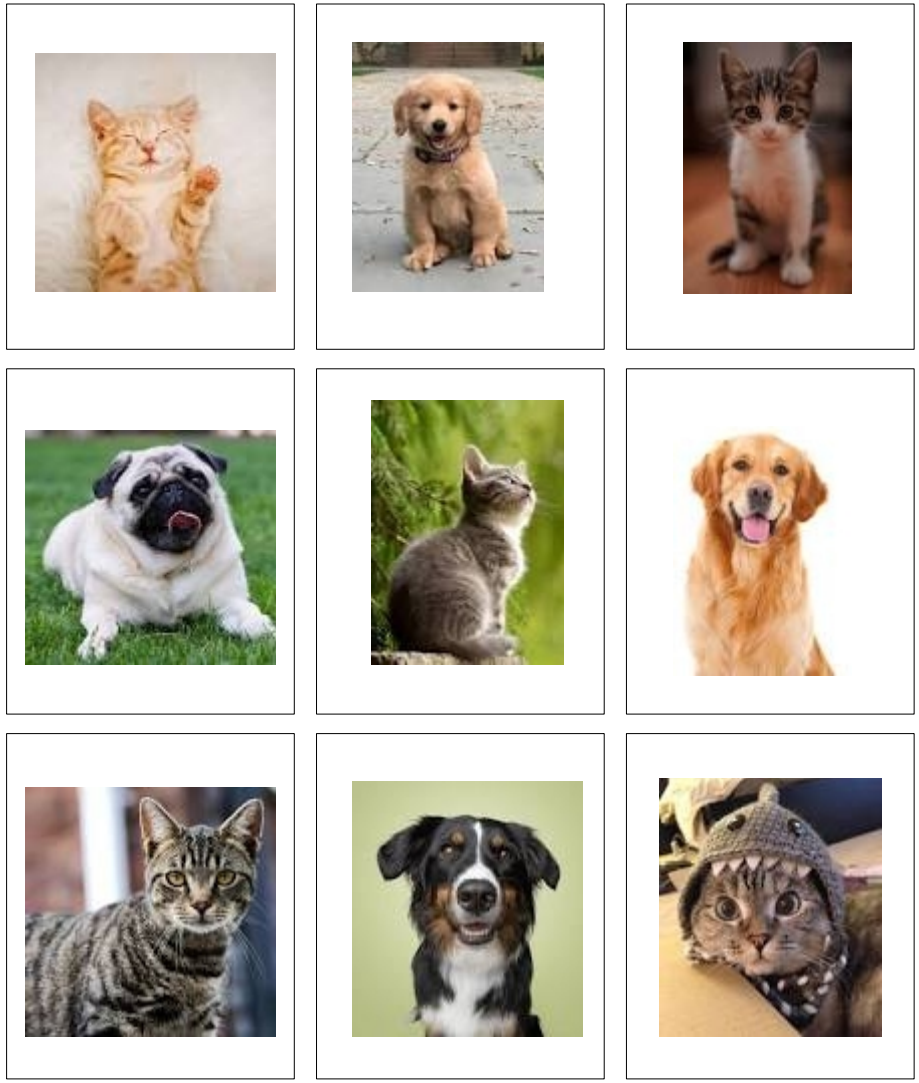
Training set labeled!

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:



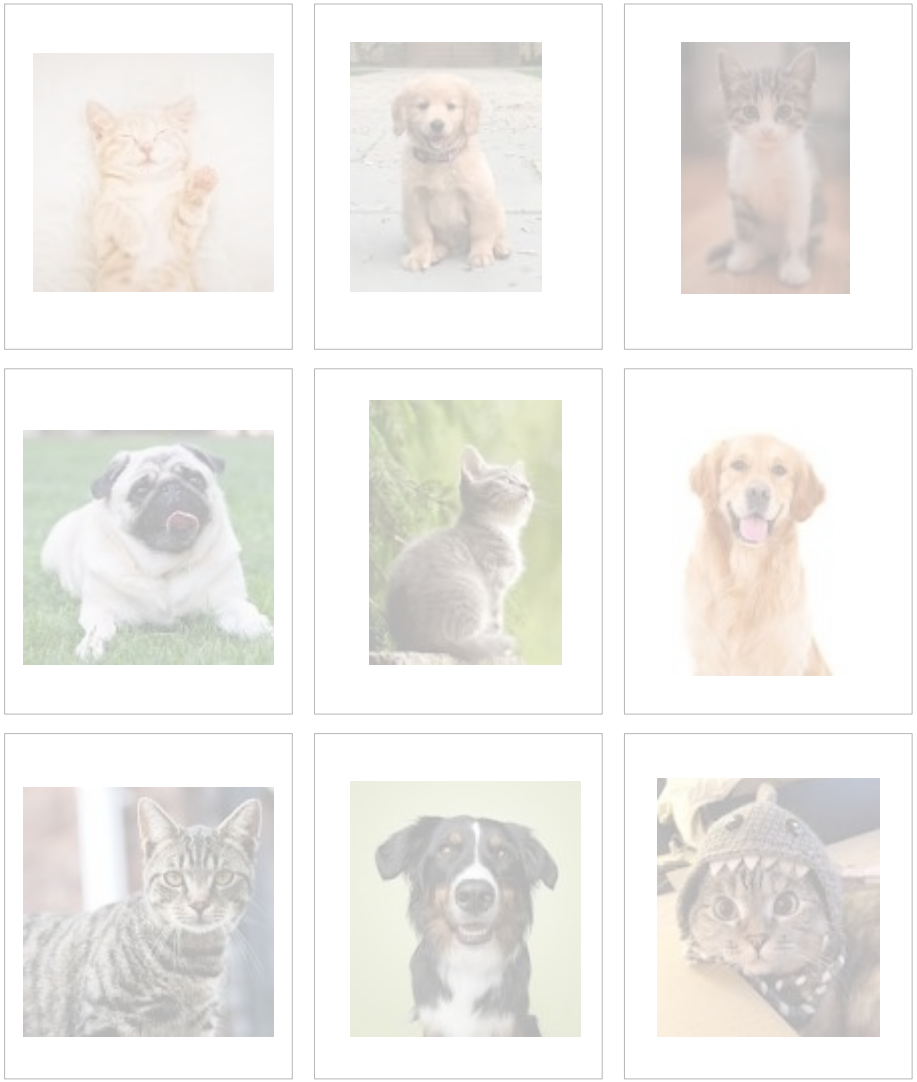
Training set NOT labeled!

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:



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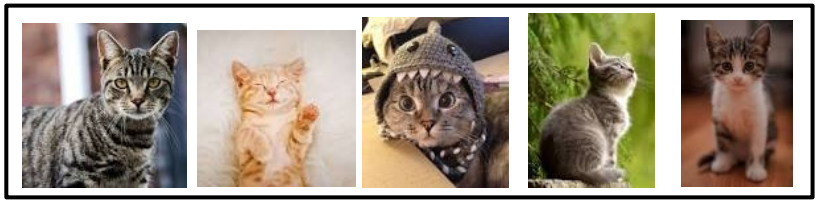


How the learning happens:

Training data-set

Testing data-set

The data-set is divided in two groups:

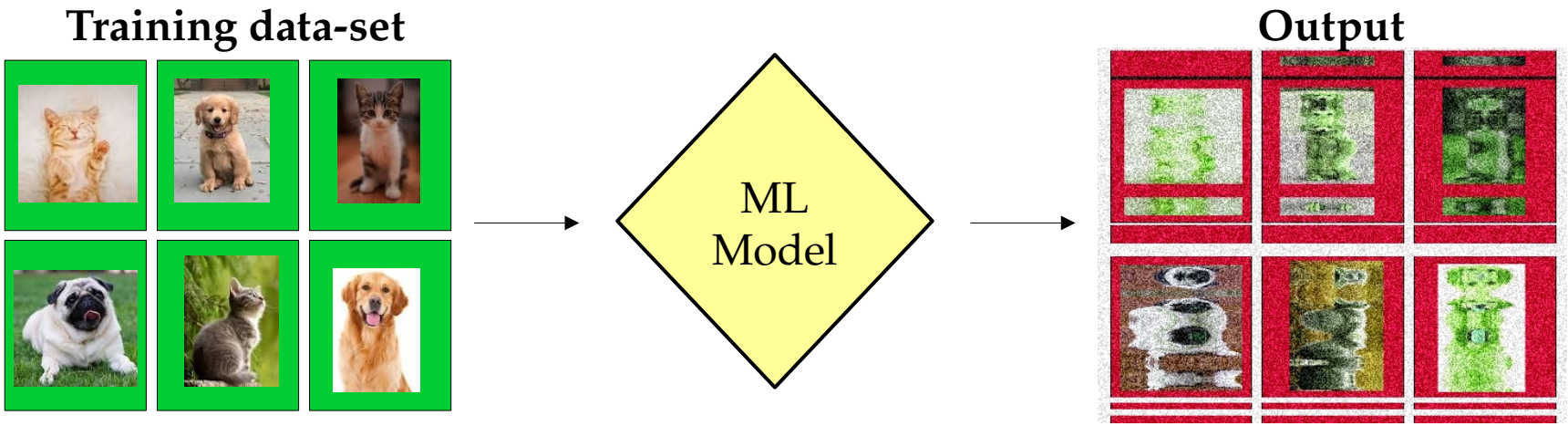
- Training data-set used to train the model (learn)

- Testing data-set used to evaluate the performances with unseen data (predict)

- Generally the training is 80% and the testing is 20% of the full data-set

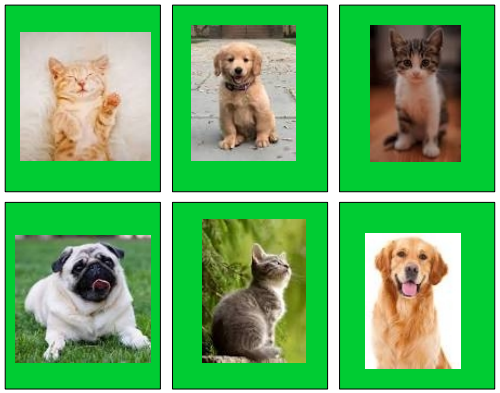
- Performances are obtained from the testing data-set

How the learning happens:



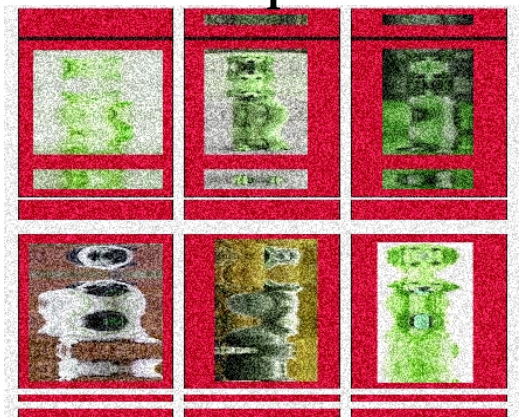
How the learning happens:

Training data-set



ML Model

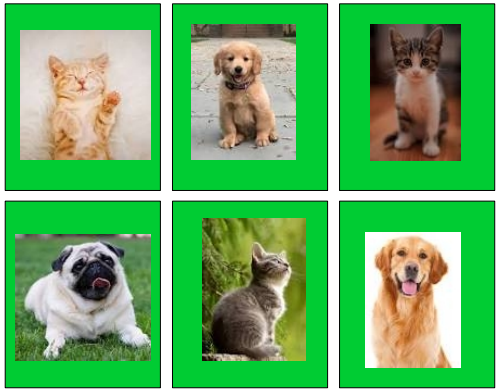
Output



Accuracy 32%

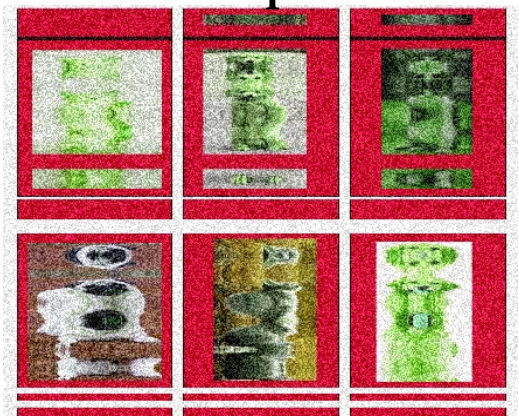
How the learning happens:

Training data-set



ML Model

Output



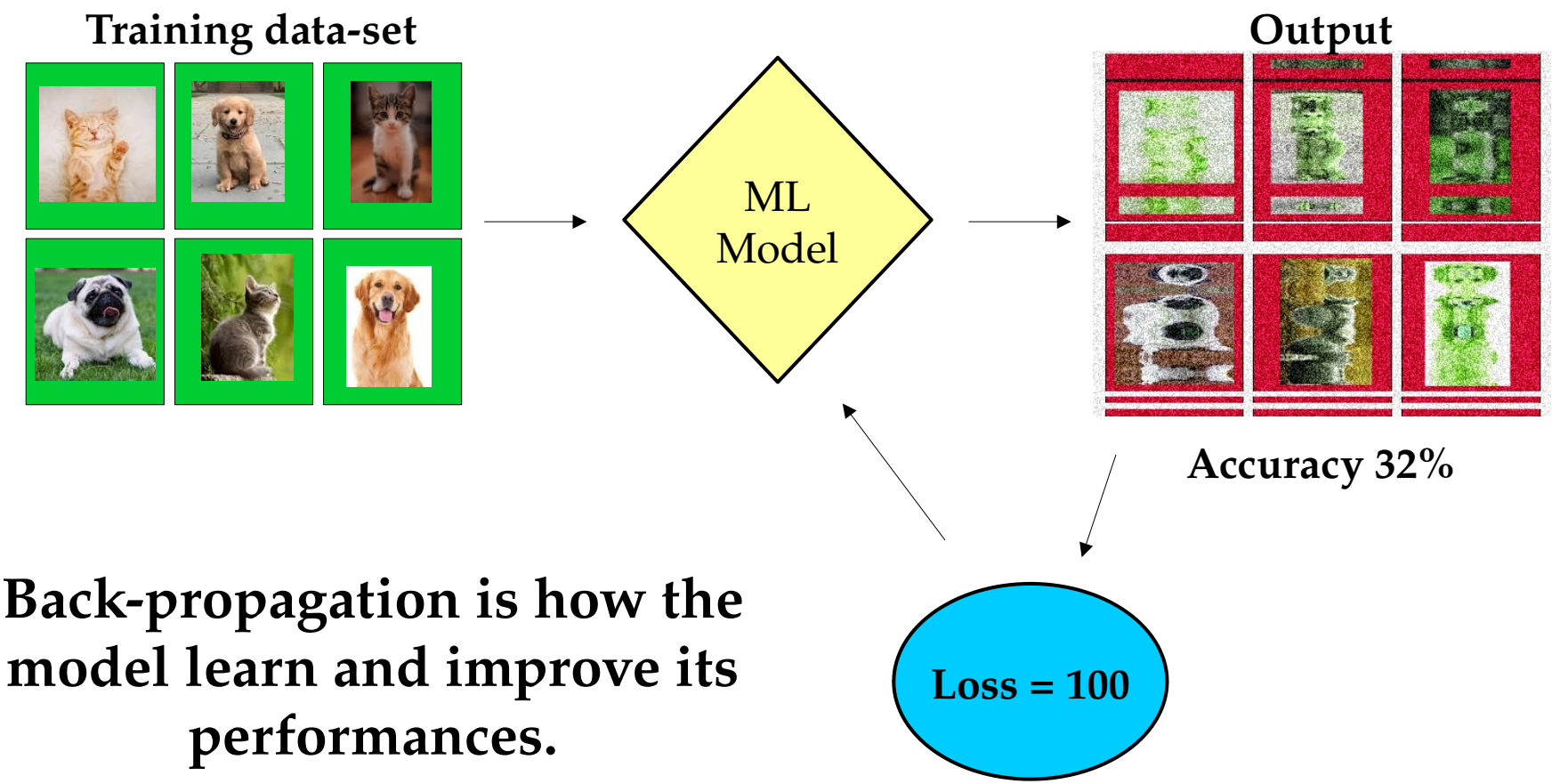
Accuracy 32%

Loss = Real - Predicted

Loss = 100

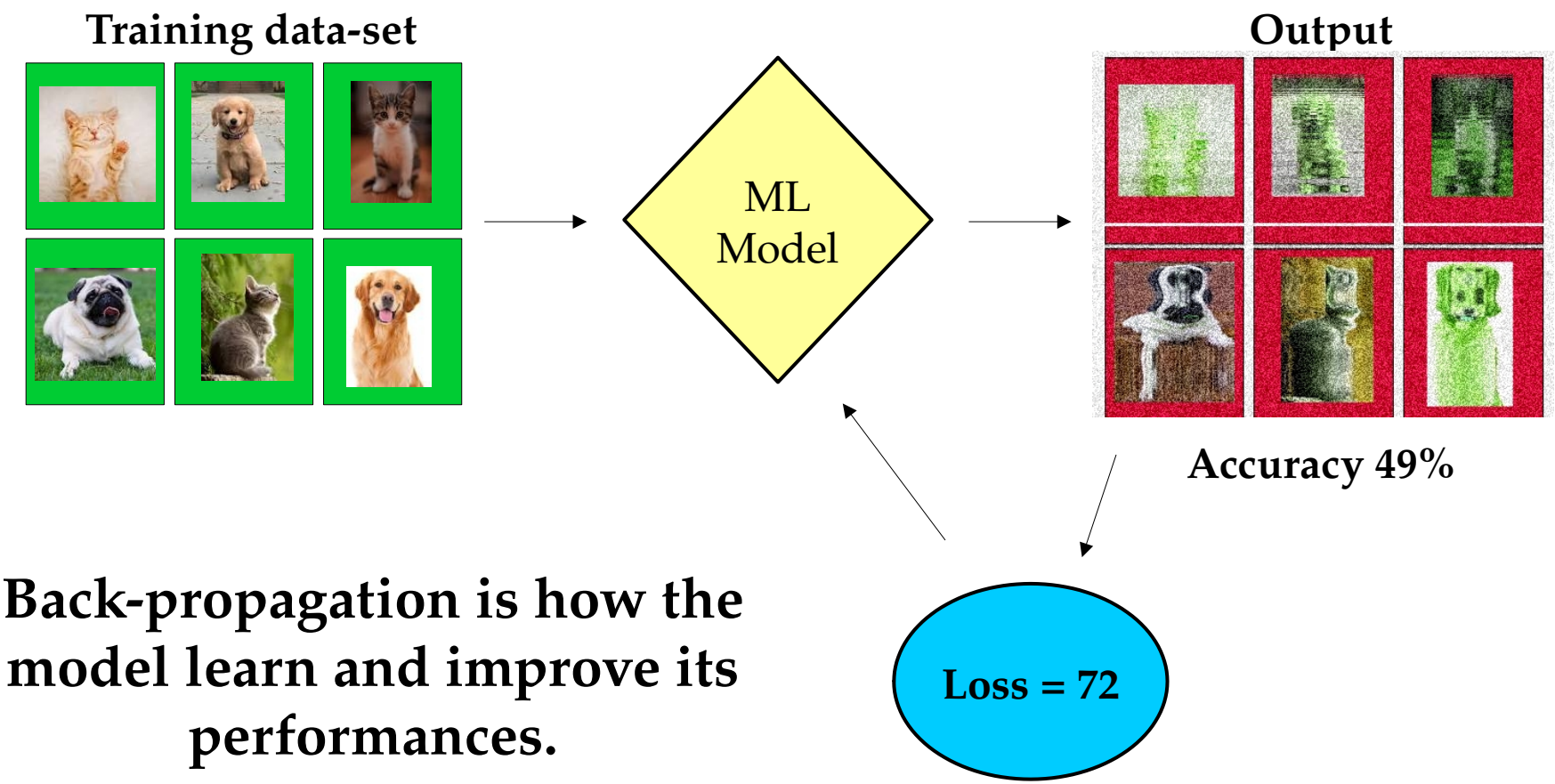
If Loss = 0, the prediction is perfect.

How the learning happens:



Back-propagation is how the model learn and improve its performances.

How the learning happens:



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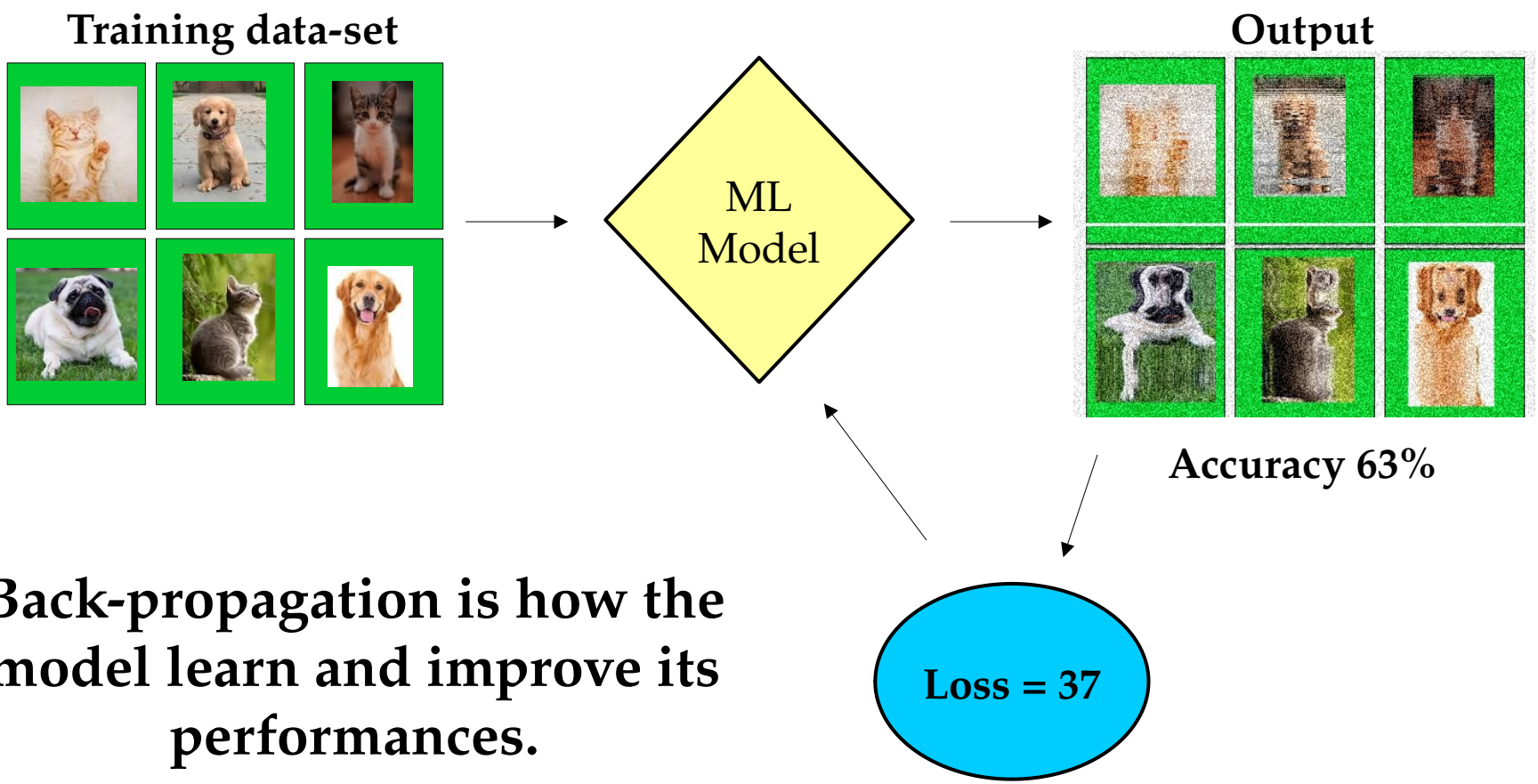

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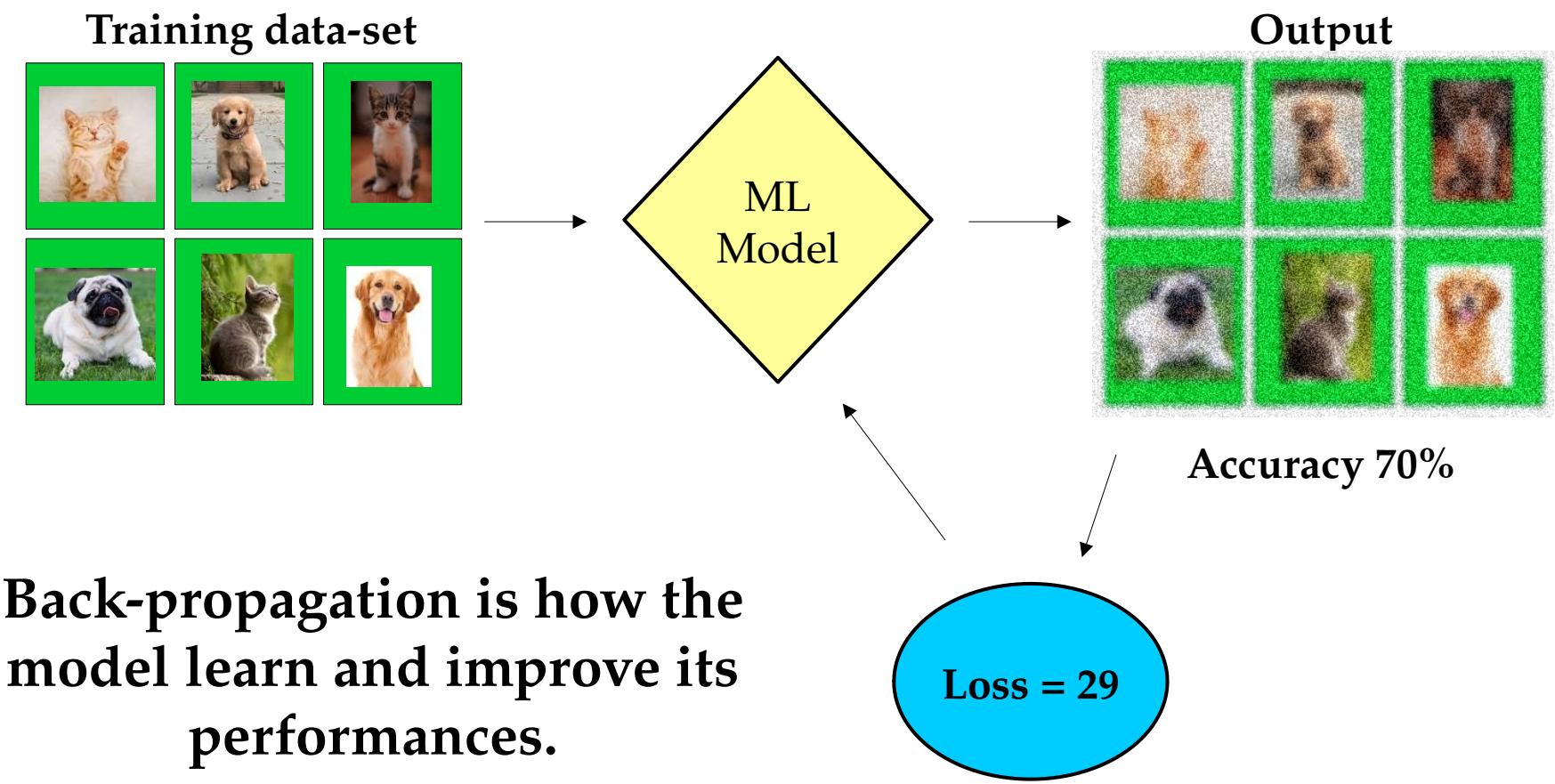

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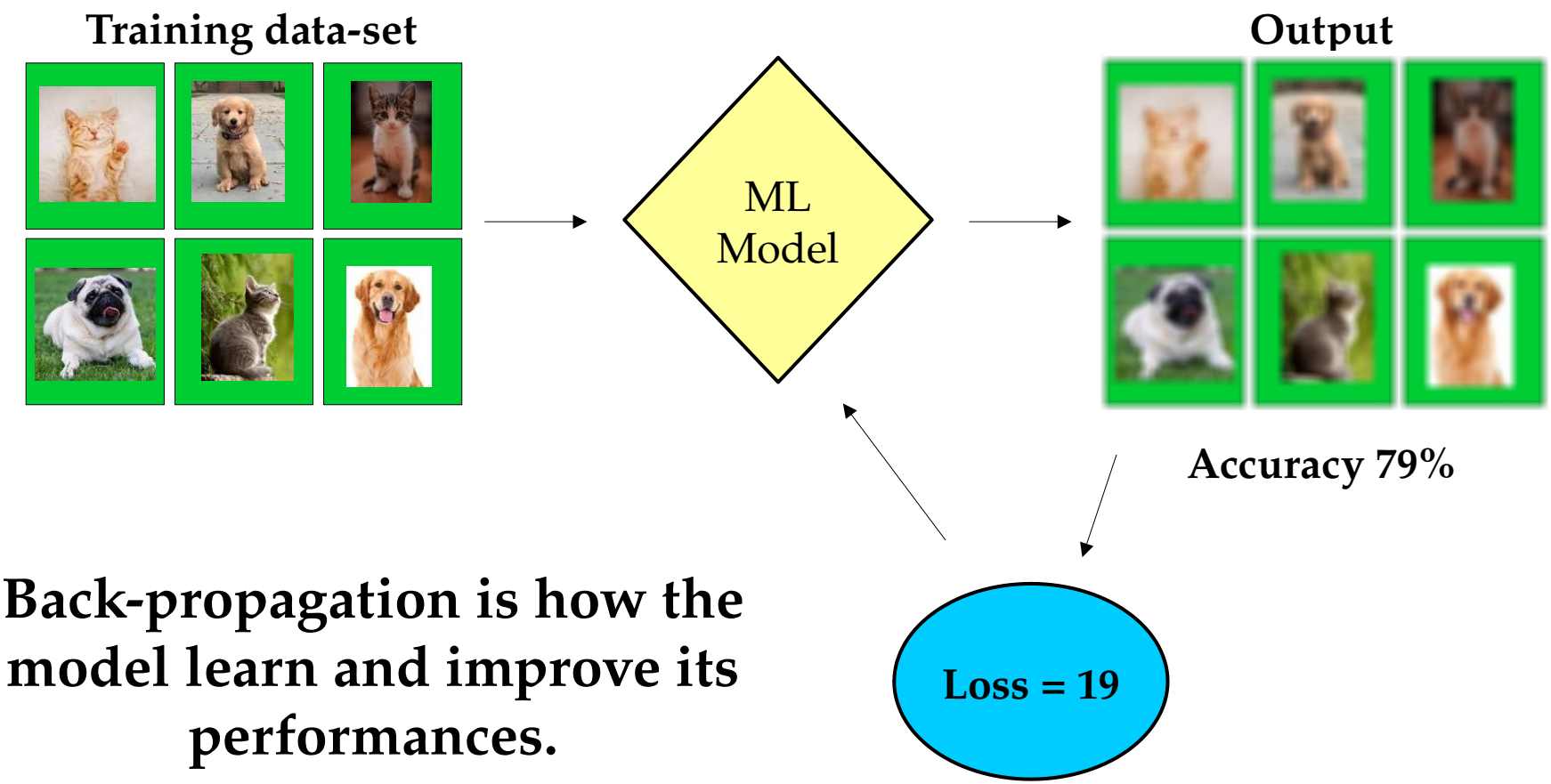

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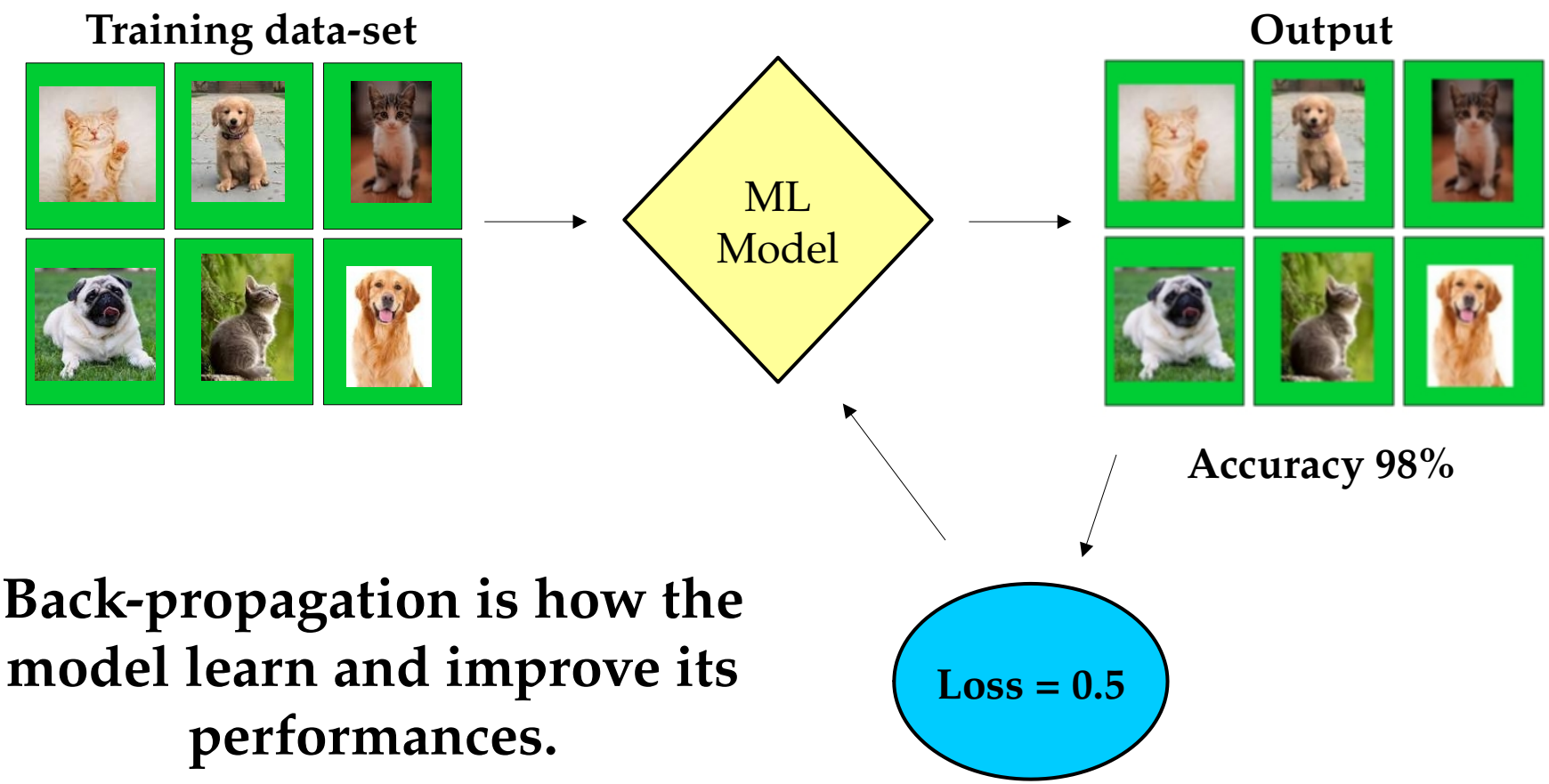

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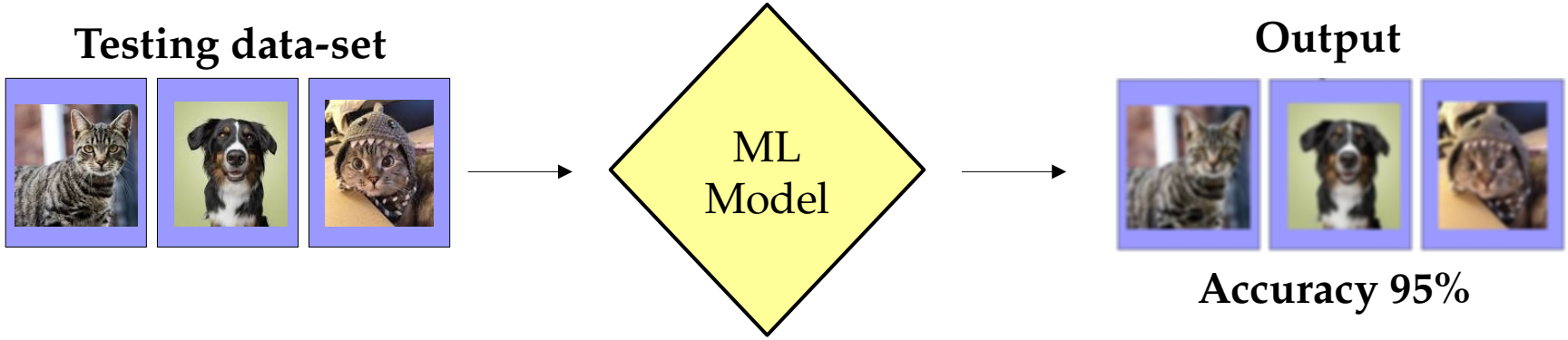


ML in Rosetta
(part 2)

VANDERBILT
UNIVERSITY

Cristina Elisa Martina
Rosetta Workshop 2023
Meiler Lab

Protein MPNN (Message Passing Neural Network):

Trained on protein structures from RCSB-PDB:

- 19700 protein structures
- include complexes (homo- and hetero-oligomers)

Predict probabilities of each natural aa for each position

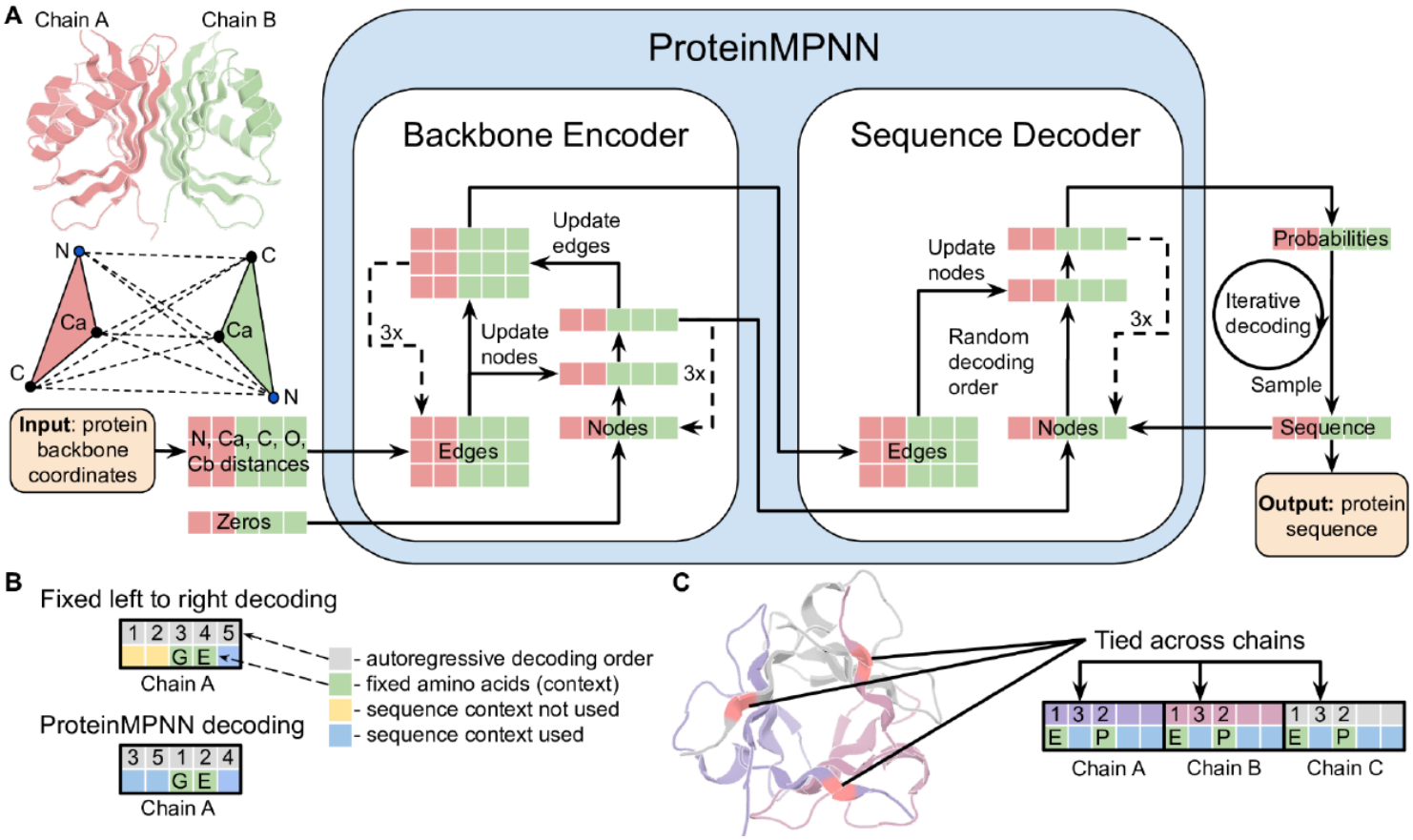
Use probabilities to design sequences

Tested *in silico*:

- 690 monomers
- 732 homomers
- 98 heteromers

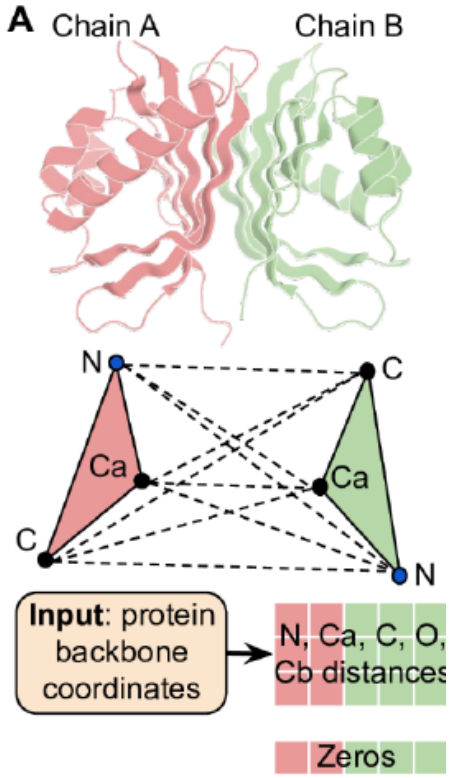
Tested experimentally

Protein MPNN:



(Dauparas et al., 2022)

Protein MPNN, inputs:



RCSB-PDB database (19K)

No evolutionary information!

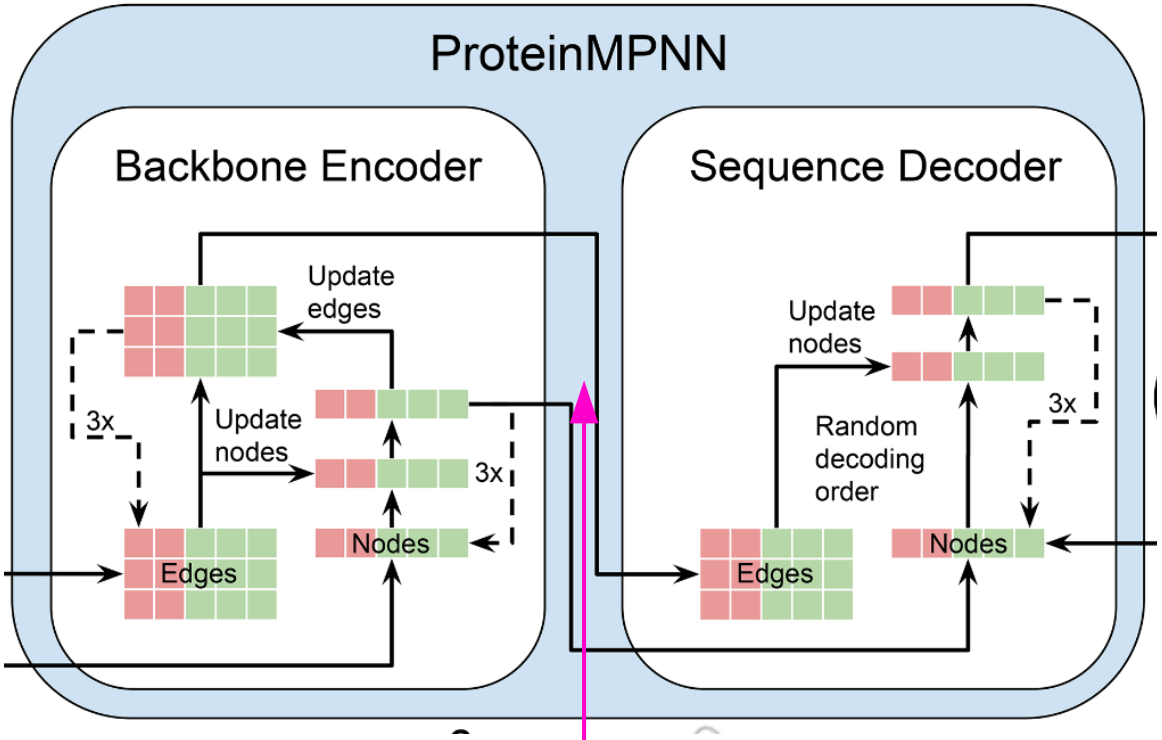
Distances between N, Cα, C, O and virtual Cβ are encoded using graph theory:

- Nodes (atoms)
- Edges (distances)

(Dauparas et al., 2022)

Protein MPNN, the MPNN:

3 encoder layers

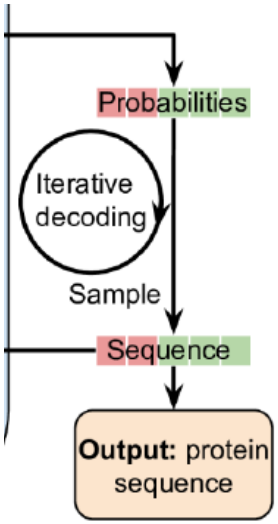


3 decoder layers

128 hidden dimensions
(here is where predictions happen)

(Dauparas et al., 2022)

Protein MPNN, the outputs:

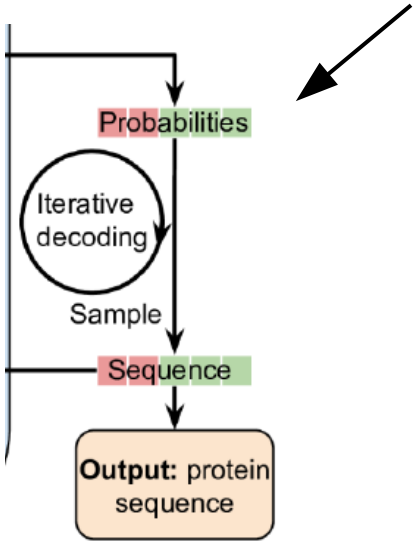


Protein MPNN outputs re-designed sequences, not structures!

This means that then you have to design a structure with an alternative method (AF, Rosetta)

(Dauparas et al., 2022)

Protein MPNN, the outputs:

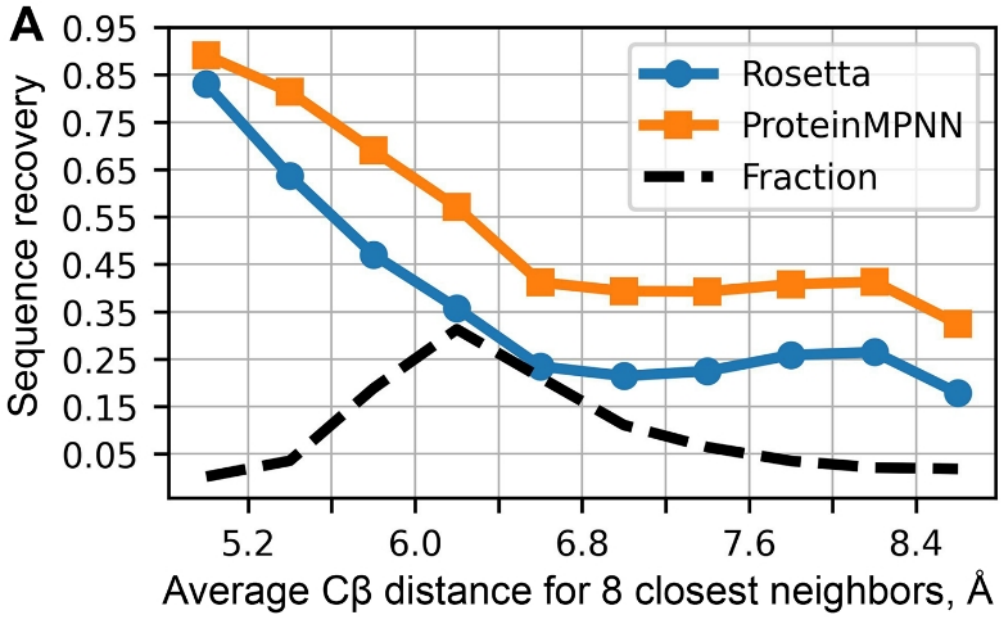


Protein MPNN in Rosetta takes the probabilities as outputs, and uses it for designing the structure directly!

(Dauparas et al., 2022)

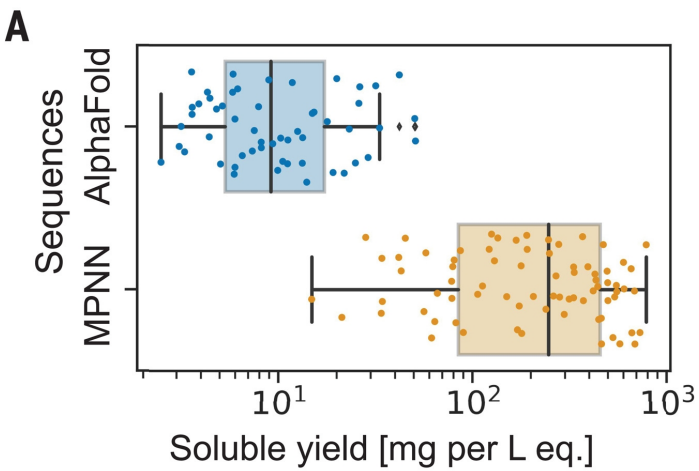
Protein MPNN, performances:

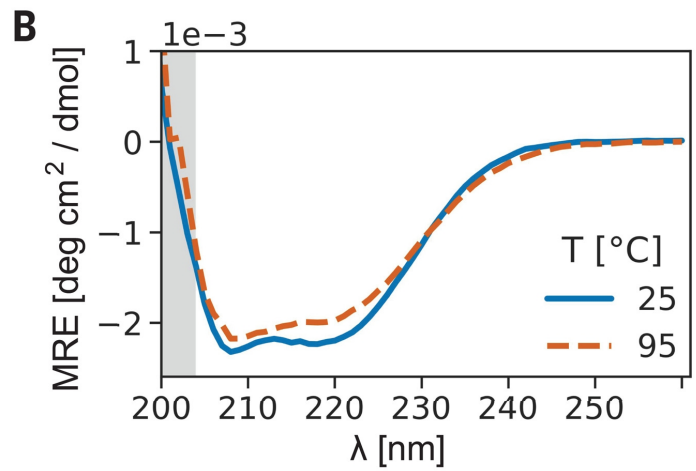
Monomer design (N=408)



(Dauparas et al., 2022)

Protein MPNN, performances:



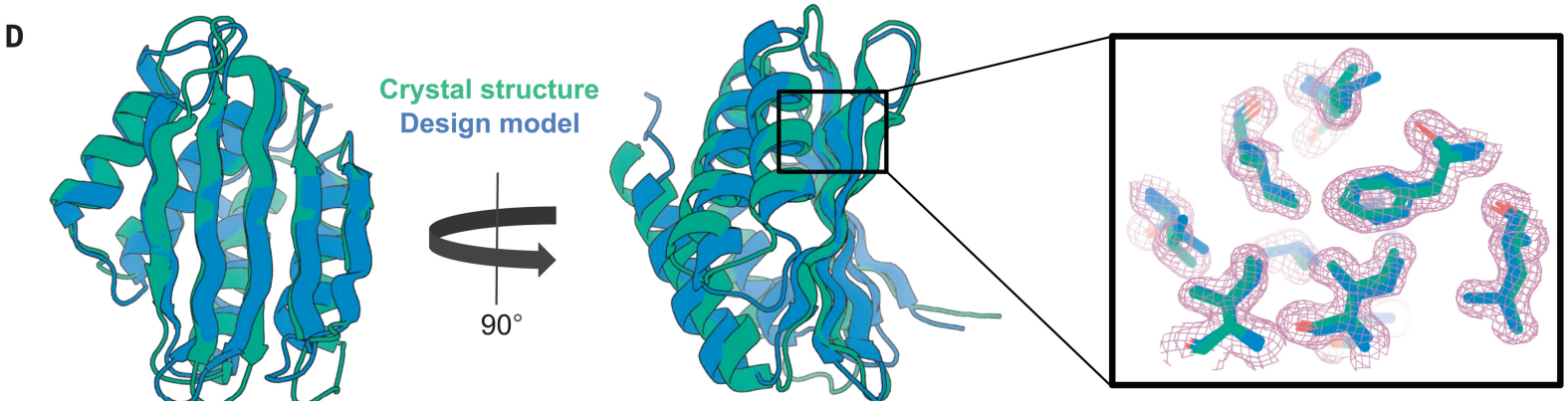


Recovered
solubility

Increased
thermostability

(Dauparas et al., 2022)

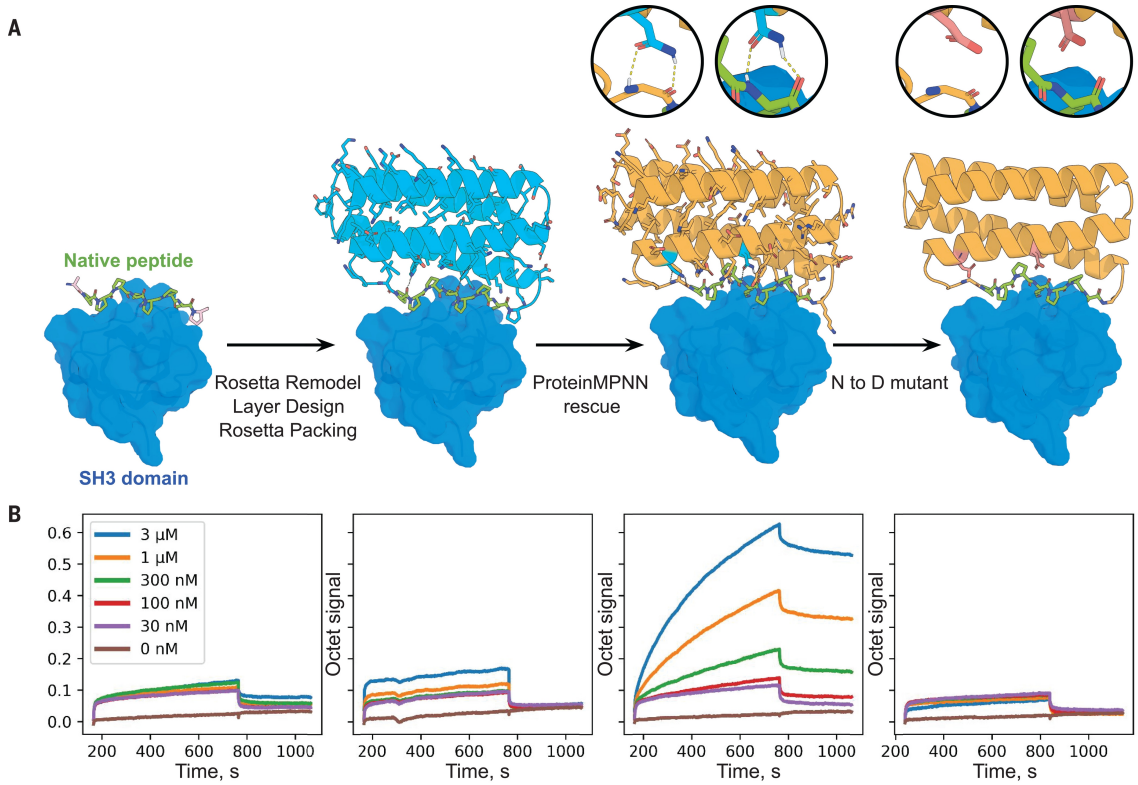
Protein MPNN, performances:



Increased
crystallizability

(Dauparas et al., 2022)

Protein MPNN, performances:



Create
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 masked
- predict masked aa

Training for downstream task

- train on single mutant and predict multi mutants
- predict experimental measurements

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

- Deep mutational scans
- Enzymatic activity
- Stability
- Binding

Masking in ML:

Nucleotides are the building blocks of DNA.

[redacted] are the building blocks of proteins.

Masking in ML:

Nucleotides are the building blocks of DNA.

[redacted] are the building blocks of proteins.

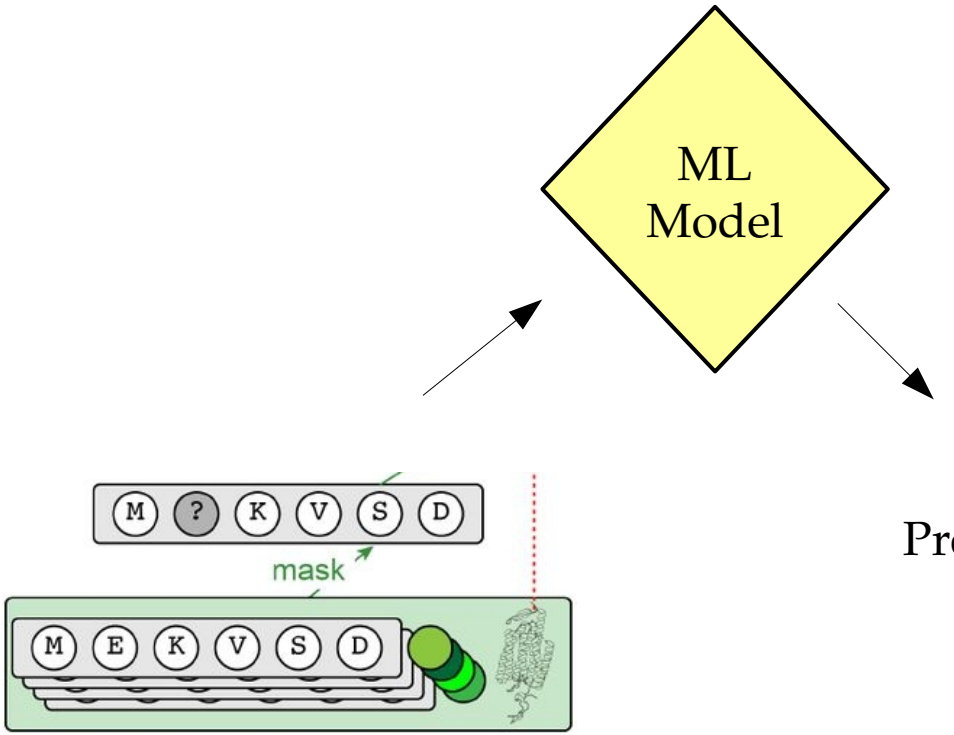
Prediction: [redacted] = Amino acids

Masking in ML:

Nucleotides are the building blocks of DNA.
Amino acids are the building blocks of proteins.

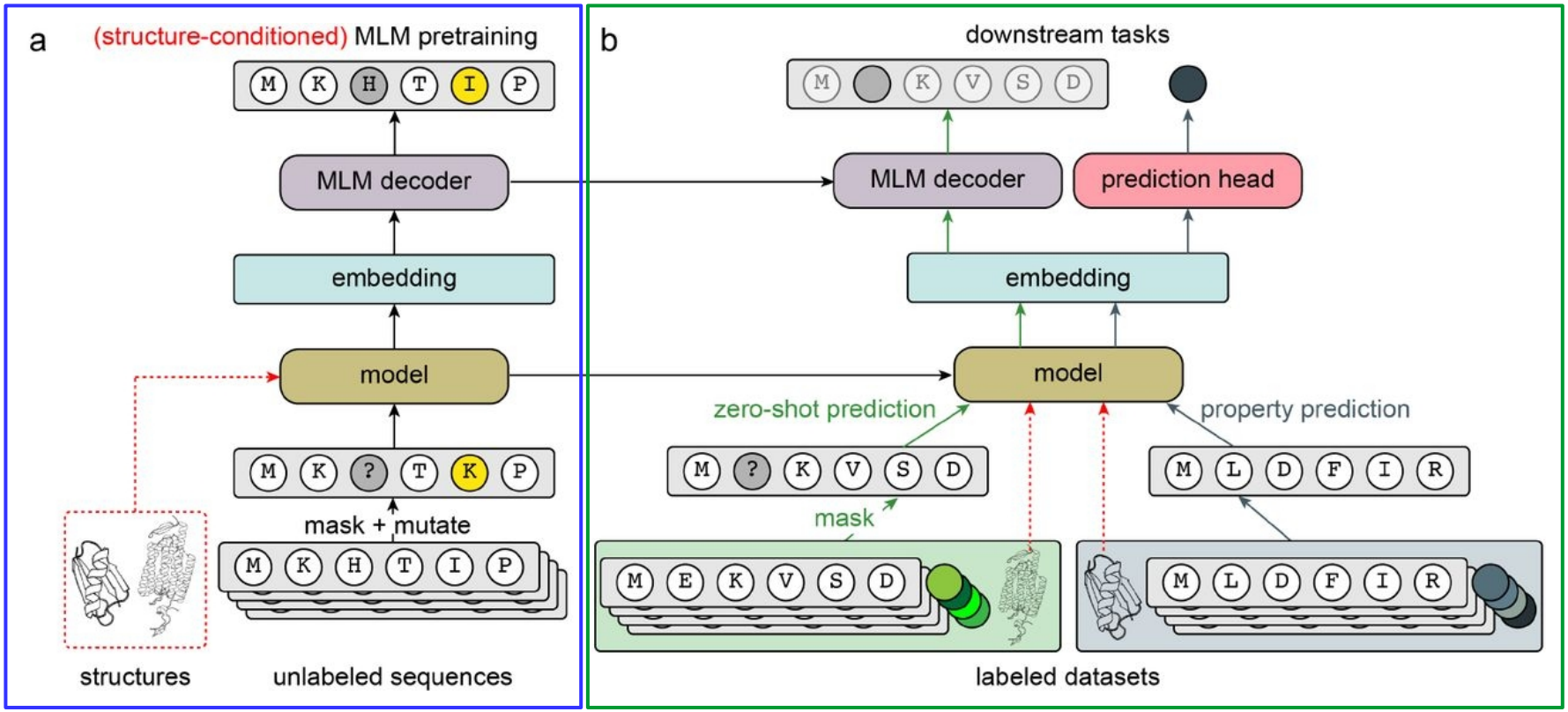
Correct prediction!

Masking protein sequences in ML:



Prediction: MEKVSD

MIF-ST:

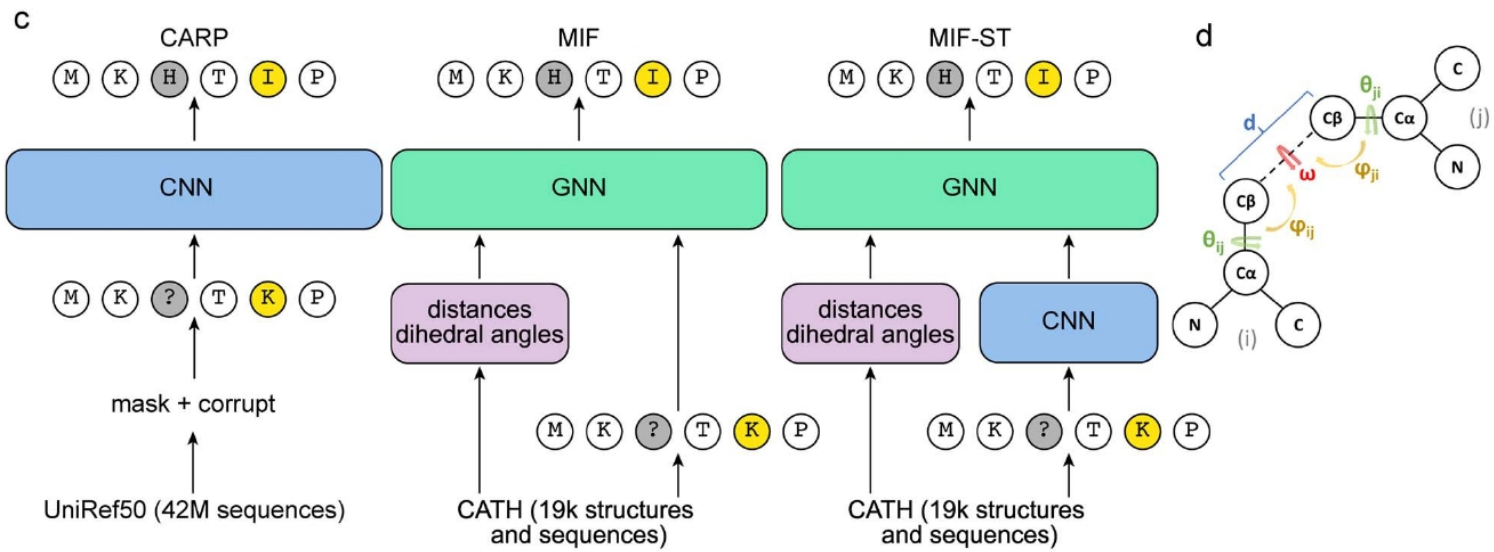


Pre-training
(structures, sequences, maskings)

Training
(sequences, masking)

(Yang et al., 2023)

MIF-ST:



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

GNN = Graph Neural Network
(unordered data, atom in the space)

(Yang et al., 2023)

MIF-ST, performances:

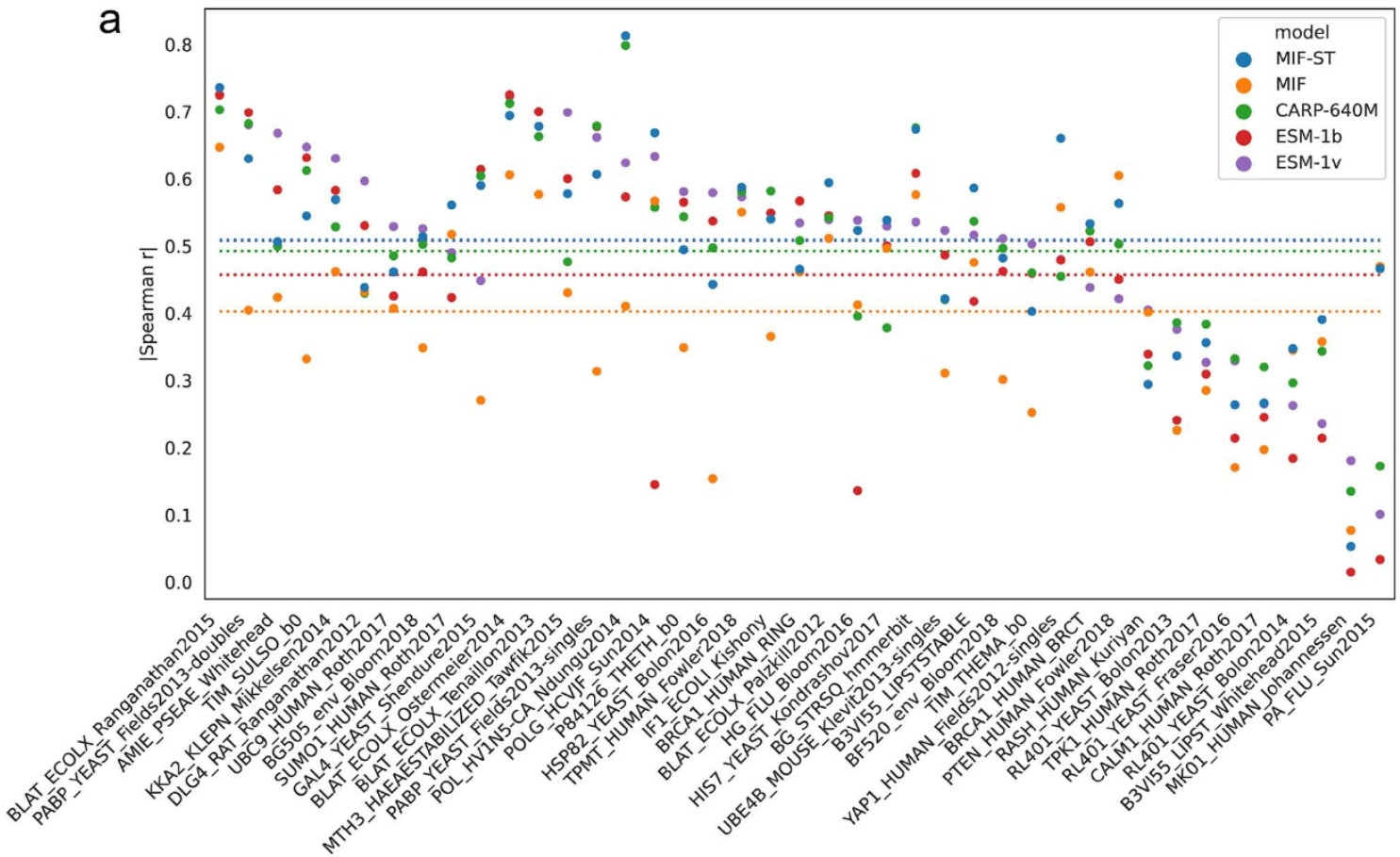
Regime	Model	Parameters	Perplexity	Recovery
Sequence only	CARP-640M	640M	7.06	40.5%
Sequence & structure	MIF-4	3.4M	4.95	49.9%
	MIF-8	6.8M	5.00	46.7%
	GVPMIF	3.5M	4.68	51.2%
+Sequence transfer	MIF-ST	3.4M	**4.08**	**55.6%**
–UniRef50 pretraining	MIF-ST	3.4M	5.70	45.4%

Perplexity:
How good the prediction is.
(lower the better)

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

(Yang et al., 2023)

MIF-ST, performances:



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:

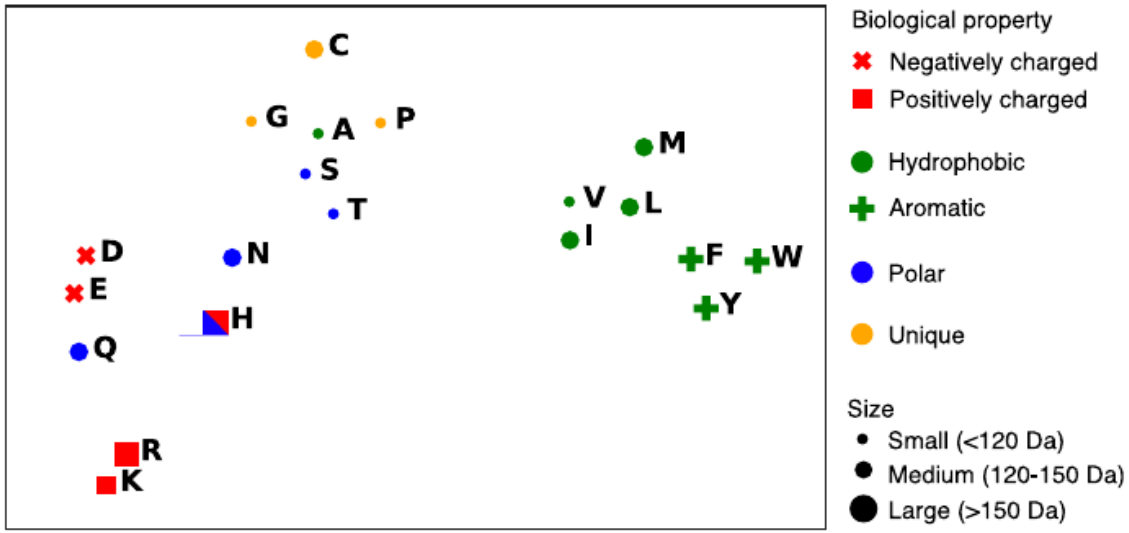
- Low-diversity data-set with UniRef100
- High-diversity sparse data-set with UniRef50 representative
- High-diversity dense data-set with UniRef50 clusters

Tested *in silico* to predict:

- Physio-chemical properties of aa
- Biological variation
- Protein homology and family
- Secondary and tertiary structures → (Lin et al., 2023)
- Effects of mutations

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

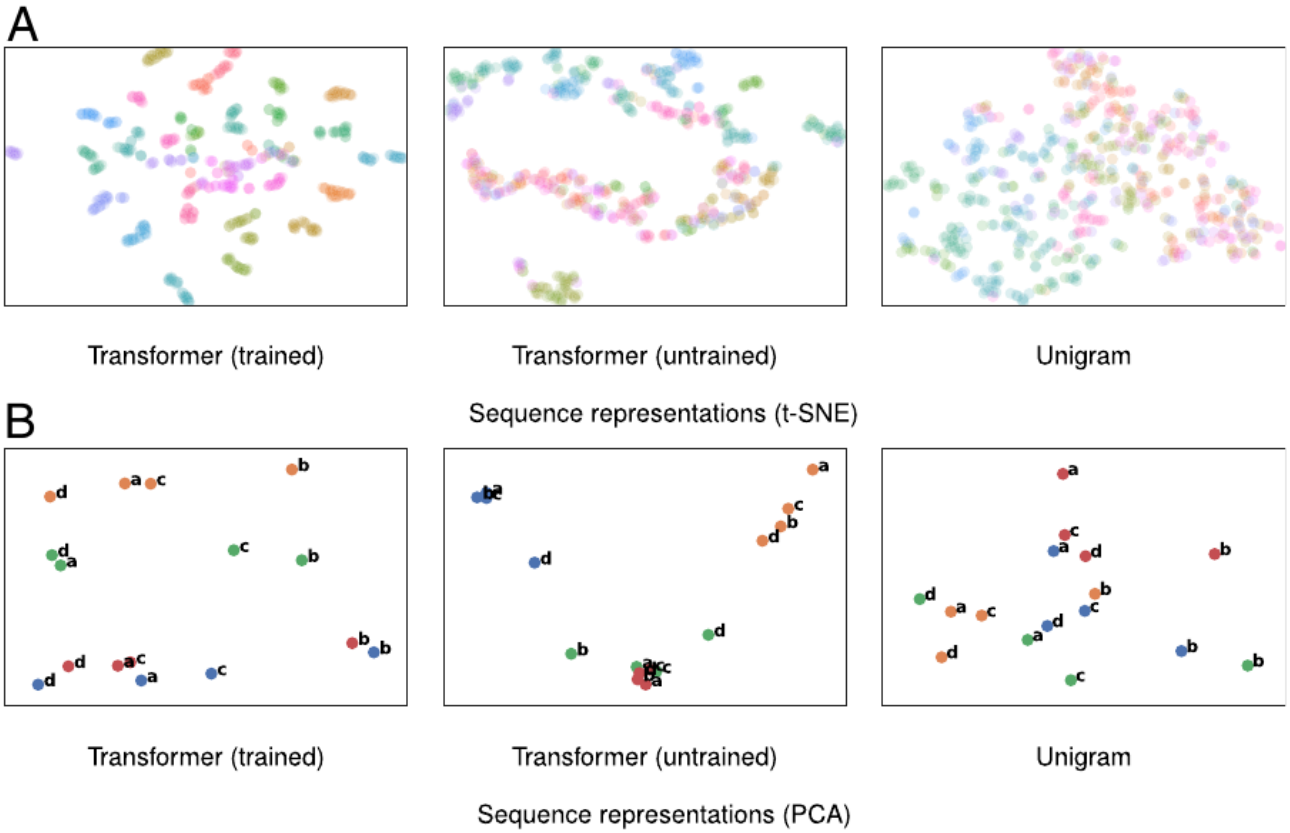
ESM, performances:



Cluster aa by properties

(Rives et al., 2021)

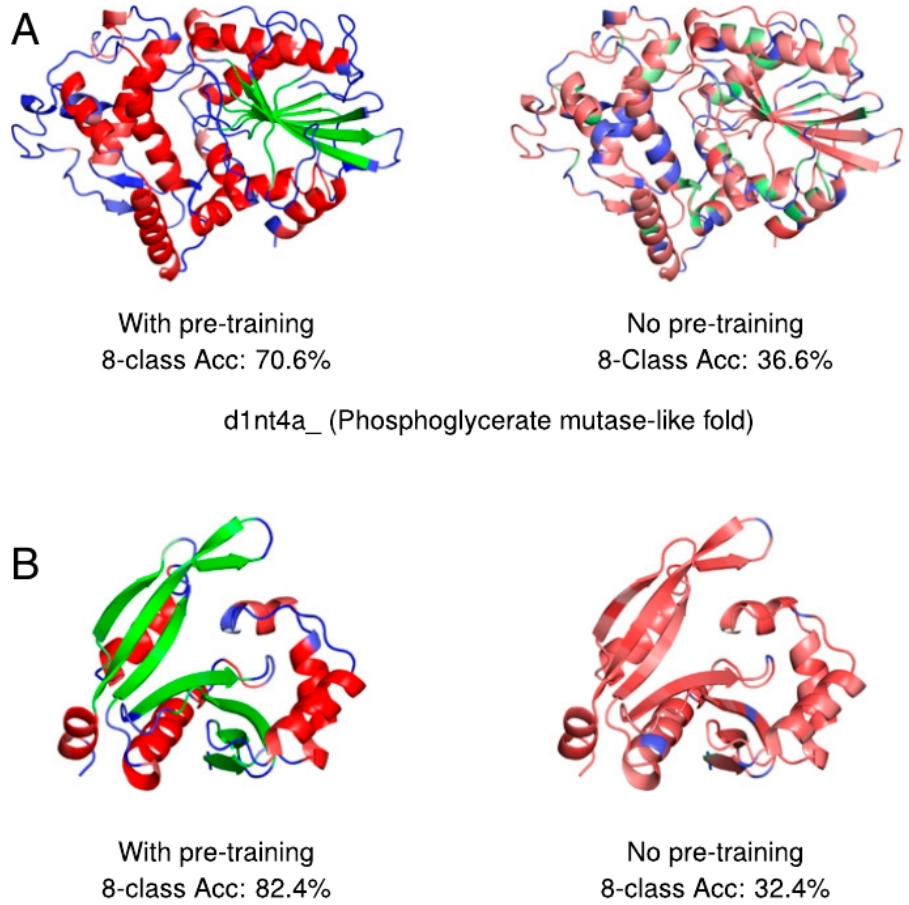
ESM, performances:



Cluster genes by variants

(Rives et al., 2021)

ESM, performances:

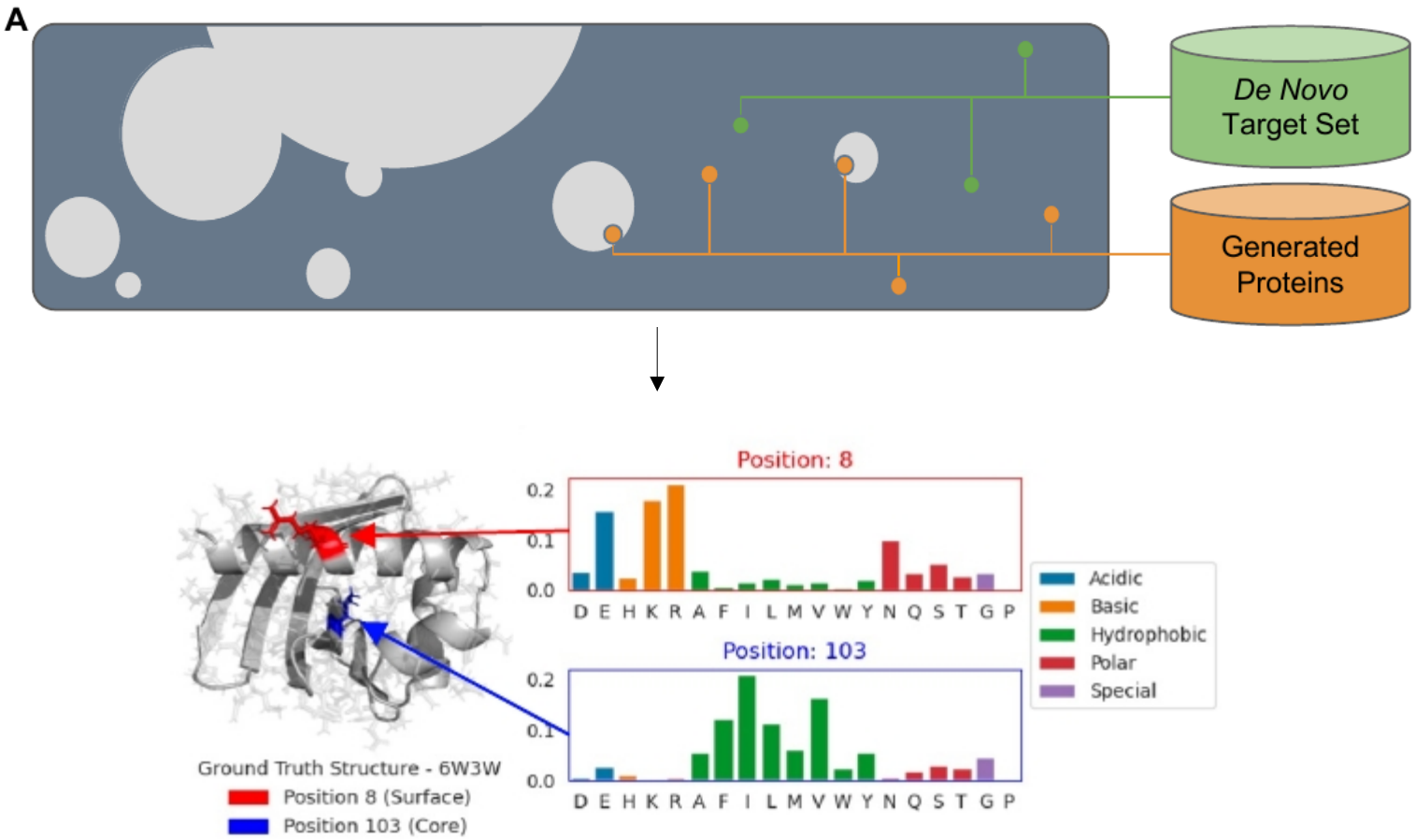


Predict secondary structures

Helices
Strands
Loops

(Rives et al., 2021)

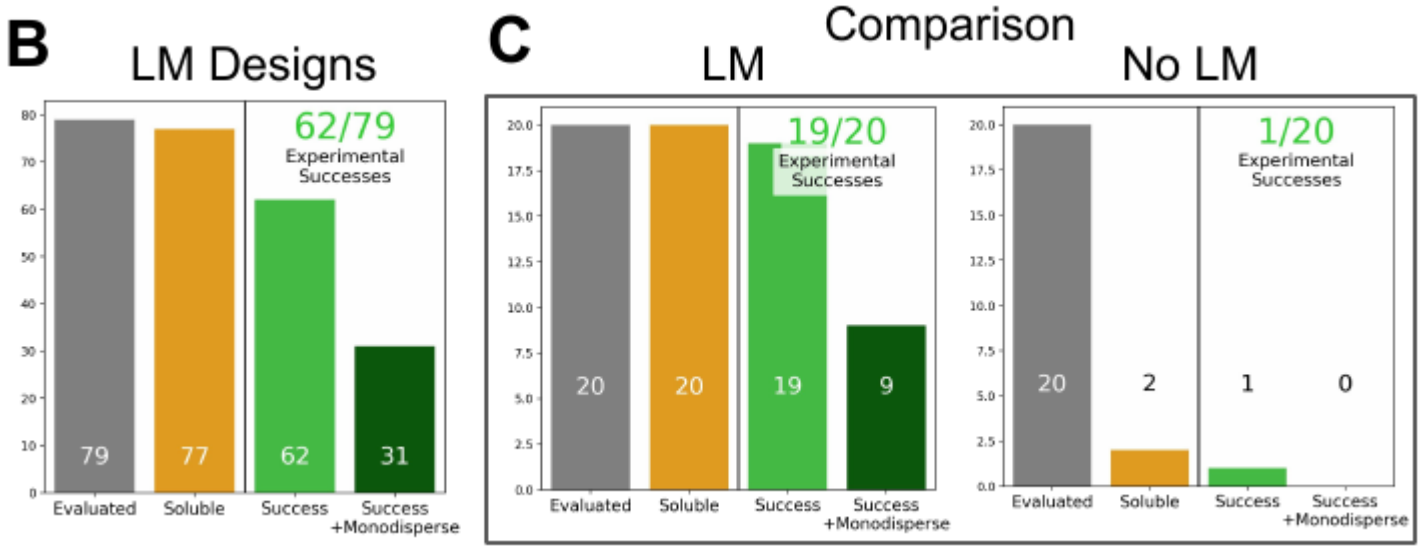
ESM, performances:



Probabilities!

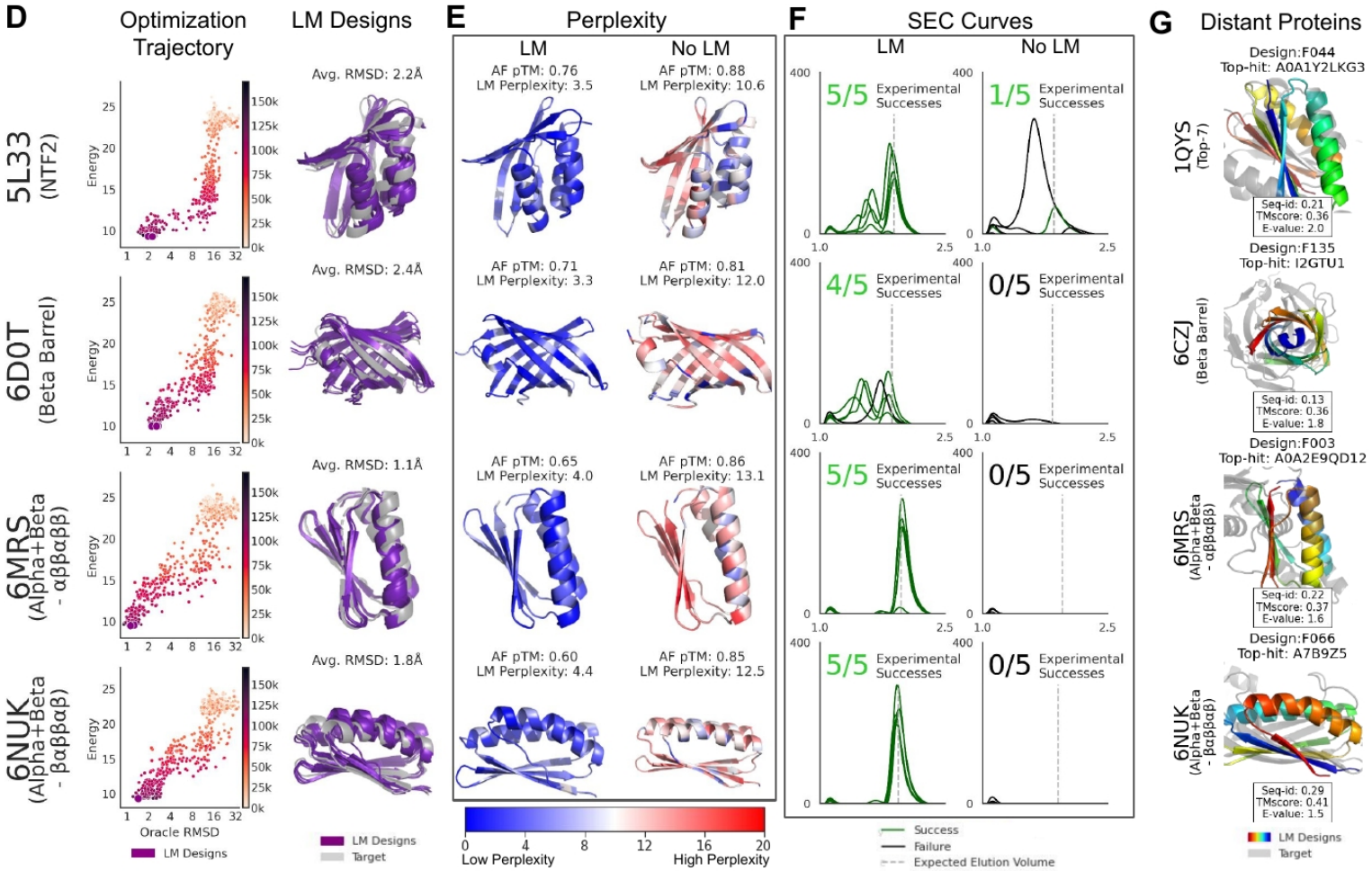
(Verkuil et al., 2022)

ESM, performances:



(Verkuil et al., 2022)

ESM, performances:



(Verkuil et al., 2022)

ML in Rosetta:

The hero here:

Moritz Ertelt
PhD student in Meiler lab at Leipzig University

Contact:
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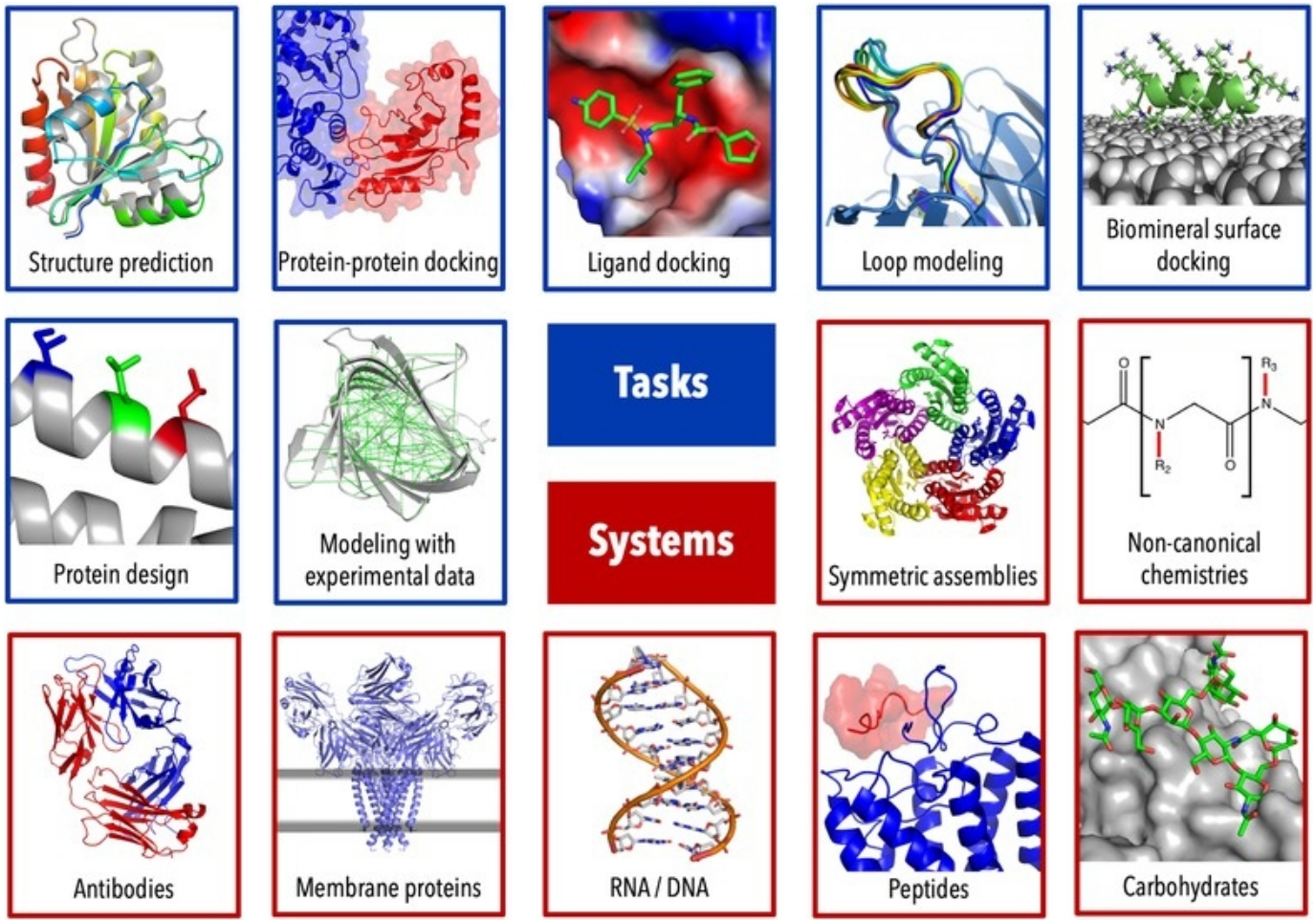
ML in Rosetta:

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:

Input

Sequence and/or structure

Inference

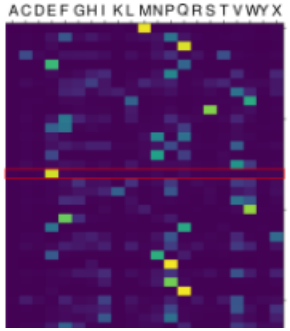
Models

ProteinMPNN

MIF-ST

ESM

Output



Position specific probability matrix (PSPM)

Referred in the tutorial as "Probabilities"

ML in Rosetta Design, analysis tools:

Analysis in Rosetta:

CurrentProbabilityMetric

AverageProbabilitiesMetric

ProbabilityConservationMetric

BestMutationsFromProbabilitiesMetric

Returns the probabilities for the sequence in the pose.

Average probabilities (i.e. from protein MPNN and ESM).

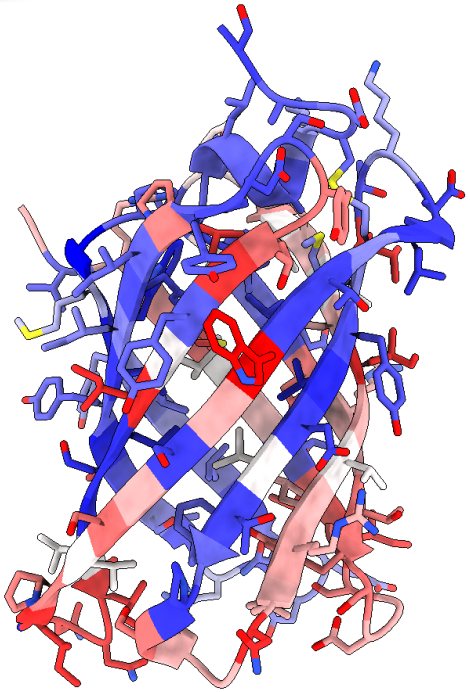
Calculate conservation for each position from probabilities. Ranges from 0 (no conservation) to 1 (fully conserved).

Return the most likely mutation(s) for a given position.

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, design tools:

Sampling Mutations in Rosetta:

FavorSequenceProfile

RestrictAAsFromProbabilities

SampleSequenceFromProbabilities

Constrain the sampling with info from the probabilities.

Restrict sampling to aa at least as likely as the current one from probabilities.

Sample aa from probabilities.

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"`)

The tutorial:

Monomer

Dimer

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

Calculate probabilities:
- Protein MPNN, 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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- Verkuil, R. et al. **Language models generalize beyond natural proteins.** 2022.12.21.521521 (2022).
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- 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).
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- Jumper, J. et al. **Highly accurate protein structure prediction with AlphaFold.** Nature 1–11 (2021) doi:10.1038/s41586-021-03819-2.
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