

UNIVERSITÄT  
HEIDELBERG



# **Evolution and Bioinformatics**

L4, Structural Bioinformatics

**WiSe 2023/24, Heidelberg University**

# Overview

1. Protein Evolution
2. Language Modelling
3. Protein Linguistics: Language Models in Biology
4. Practical Considerations
5. Current Research

# 1. Protein Evolution

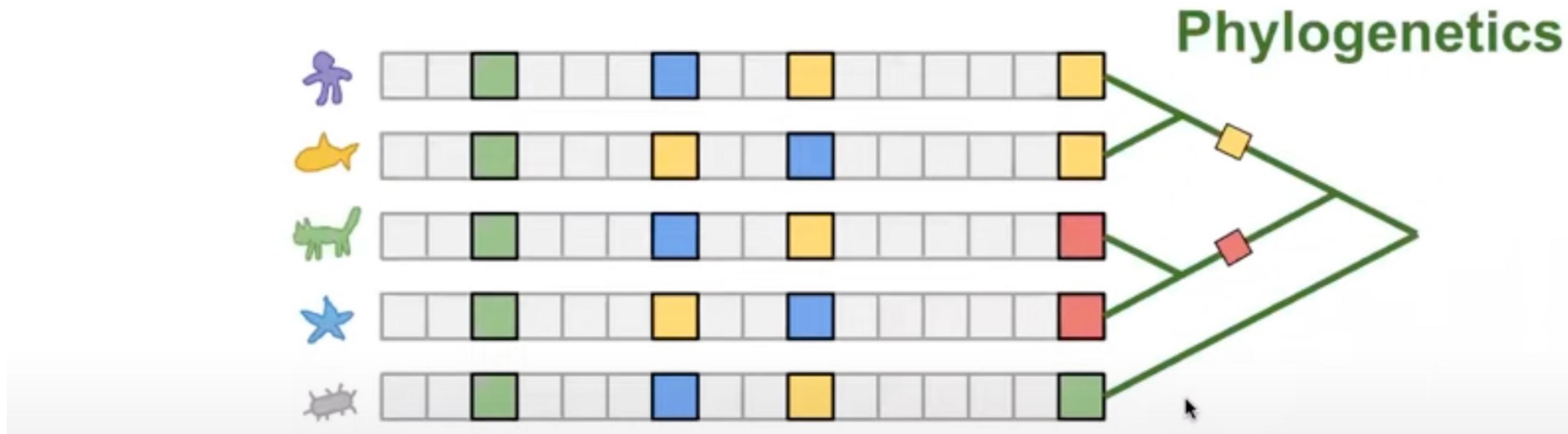
# How do we get function from sequence?

Compare similar proteins across species via alignments

	WALRKTRKRLEEPFGGVKVLLLGDTRQLEPVVPGGEEALYIARTWGGPFFFQAHVWEE--	180
	R R++ +PF GG+++++ GD QL PV G + F FQ+ W+	
	AVARAVRQQ-NKPFGGIQLIICGDFLQLPPVTKGSQPP-----RFCFQSWSKRCV	168
	-VALRVHRLWESQRQREDPLFAELLKRLRQG--DPQALETLNRAAVRPDGGEEPGTLILT	237
	V L + ++W ++ D F LL+ +R G + L A G + L	
	PVTLELTKVW---RQADQTFISLLQAVRLGRCSDEVTRQLQATASHKVG RDGIVATRLC	224
	PRRKEADALNLKRLEALPGKPLEYQAQVKG-EFAET---DFPTTEAALTLLKKGAQVILLRN	293
	+ + N +RL+ LPGK ++A E A T P L LK GAQV+L++N	
	THQDDVALTNERRLQELPGKVHRFEAMDSNPELASTLDAQCPVSQQLQLKLGAQVMLVKN	284
	DPLGE-YFNGDLGWVEDLEAEALAVRLKR--NGRRVVIRPFVWEKIVYTYDSEREEIKPQ	350
	+ NG G V EAE + R G VI W T + ++ +	
	LSVSRGLVNGARGVVVGFEAEGRGLPQVRFLCGVTEVIHADRW-----TVQATGGQLLSR	339
	VVGTFRQVPVRLAWALTIVKAQGLTLDKVHLELGRGLFAHGQLYVALTRVRLQDL	406
	+Q+P++LAWA+++HK+QG+TLD V + LGR +FA GQ YVAL+R R LQ L	
	-----QQLPLQLAWAMSIHKSQGMTLDCVEISLGR-VFASGQAYVALSRARSLQGL	389

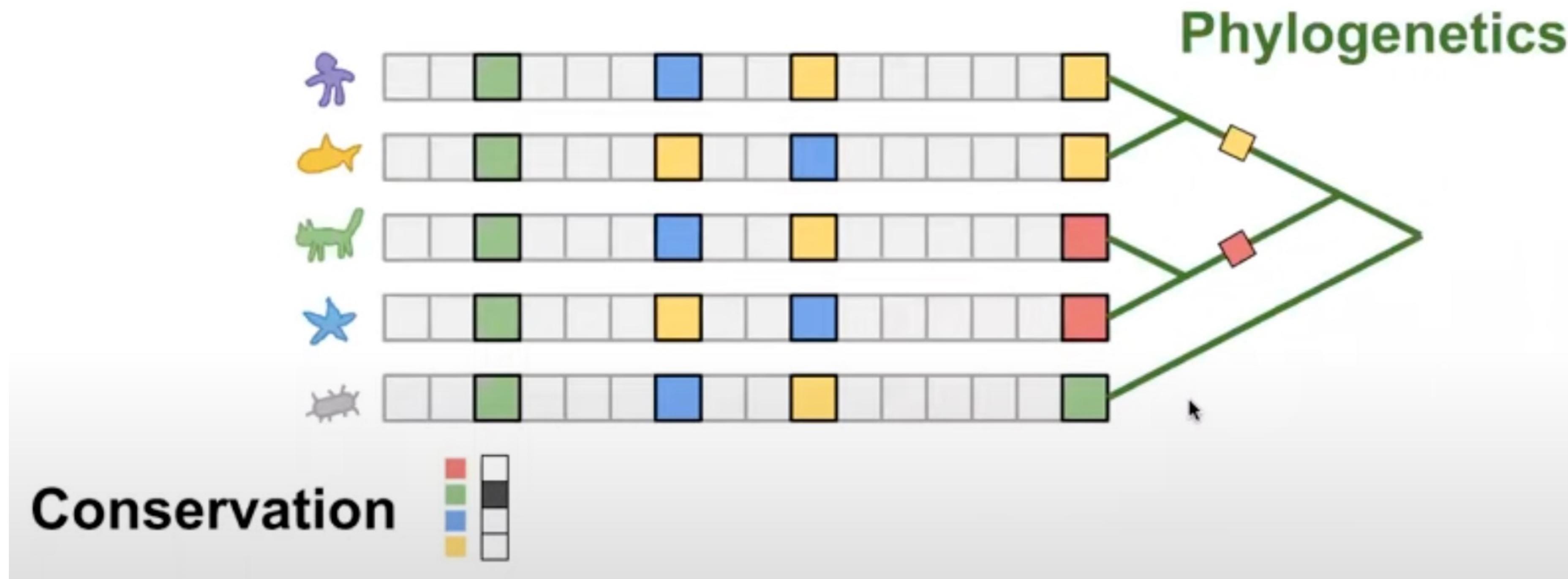
# Evolution can give us hints about function

Phylogenetics: the study of evolutionary history



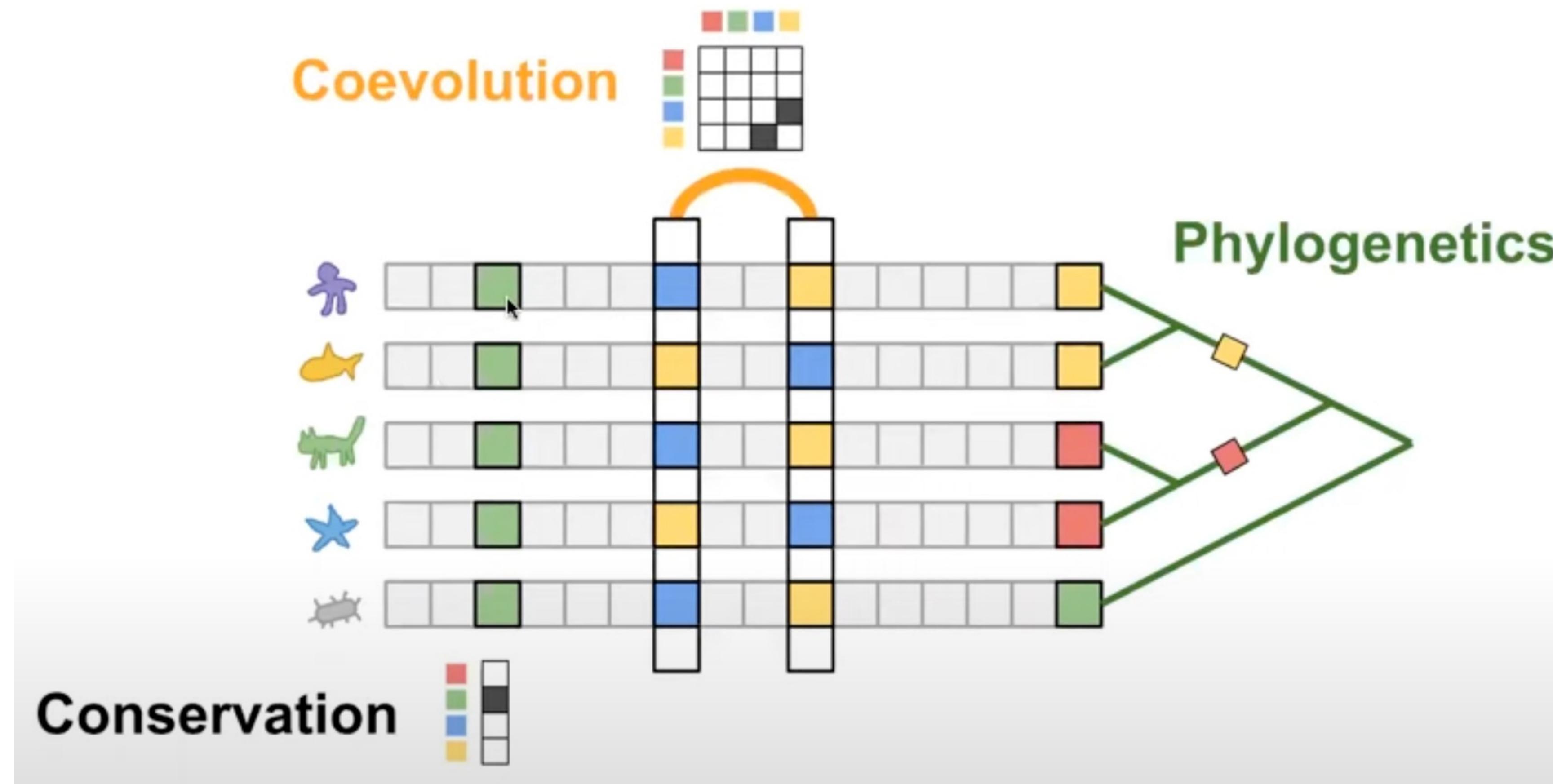
# Evolution can give us hints about function

Conservation: which residues are important?



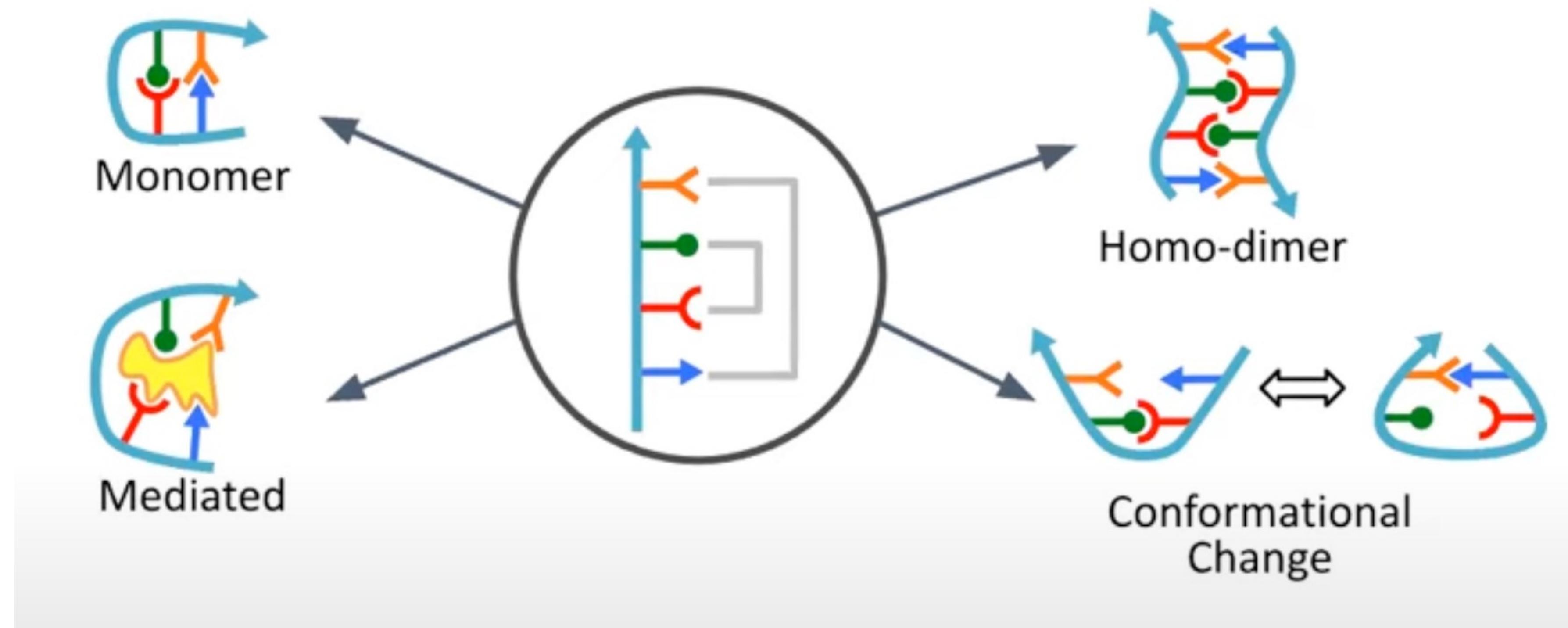
# Evolution can give us hints about function

Coevolution: which residues interact?



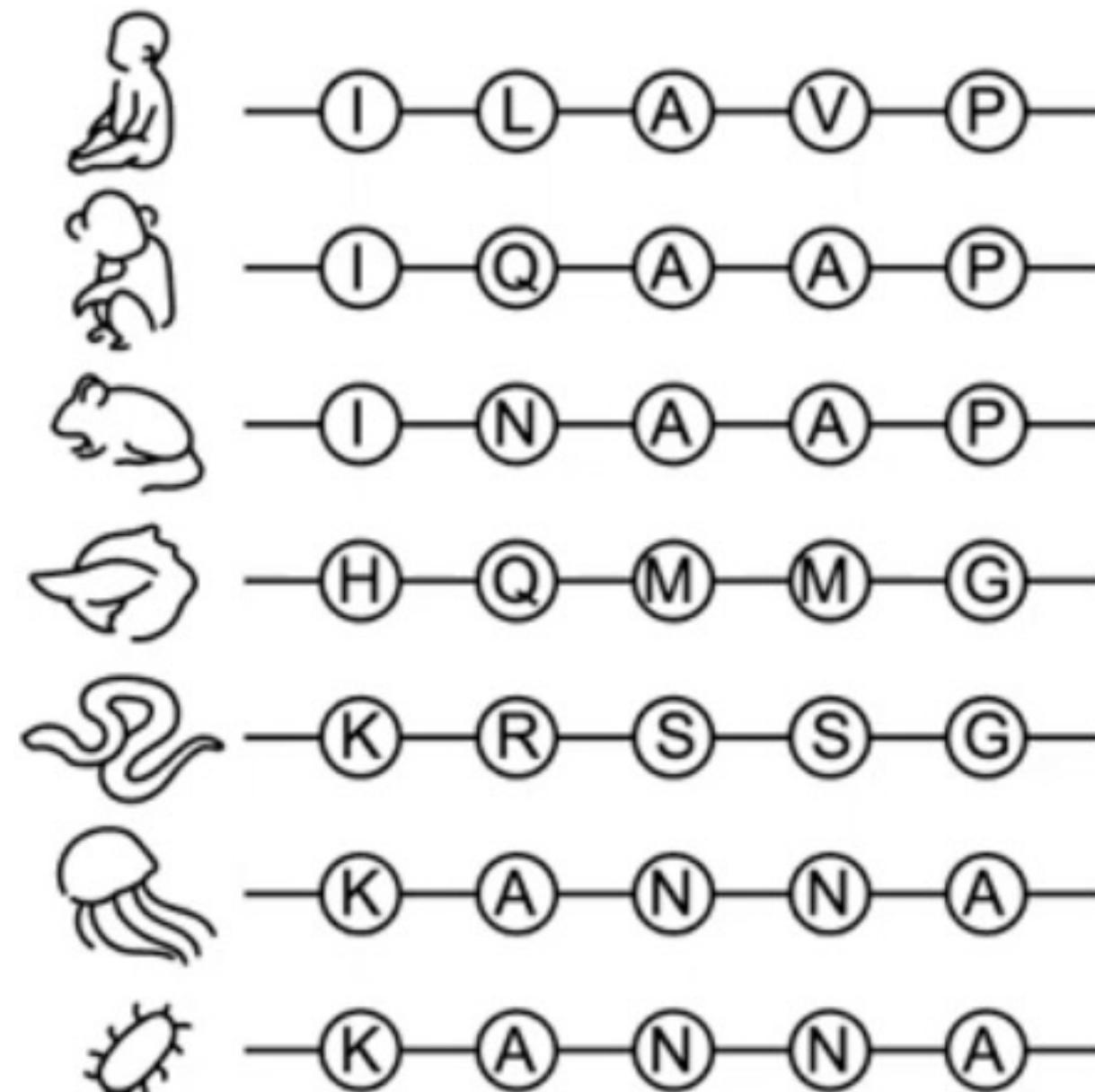
# Evolution can give us hints about function

Coevolution: many types of interactions possible

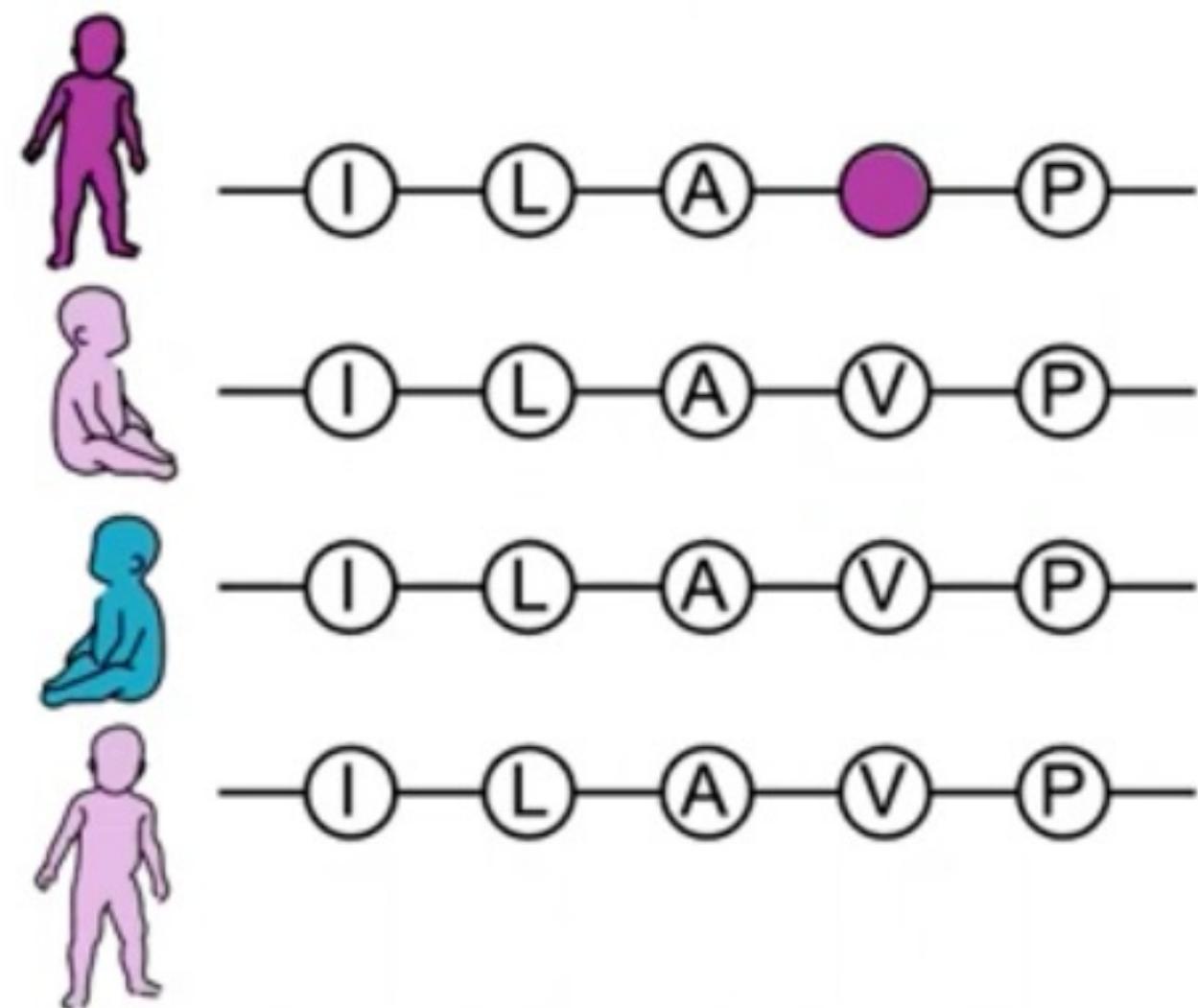


# Fitness Prediction of Variants

How do we model this problem?



$$p(\mathbf{x}|\theta)$$

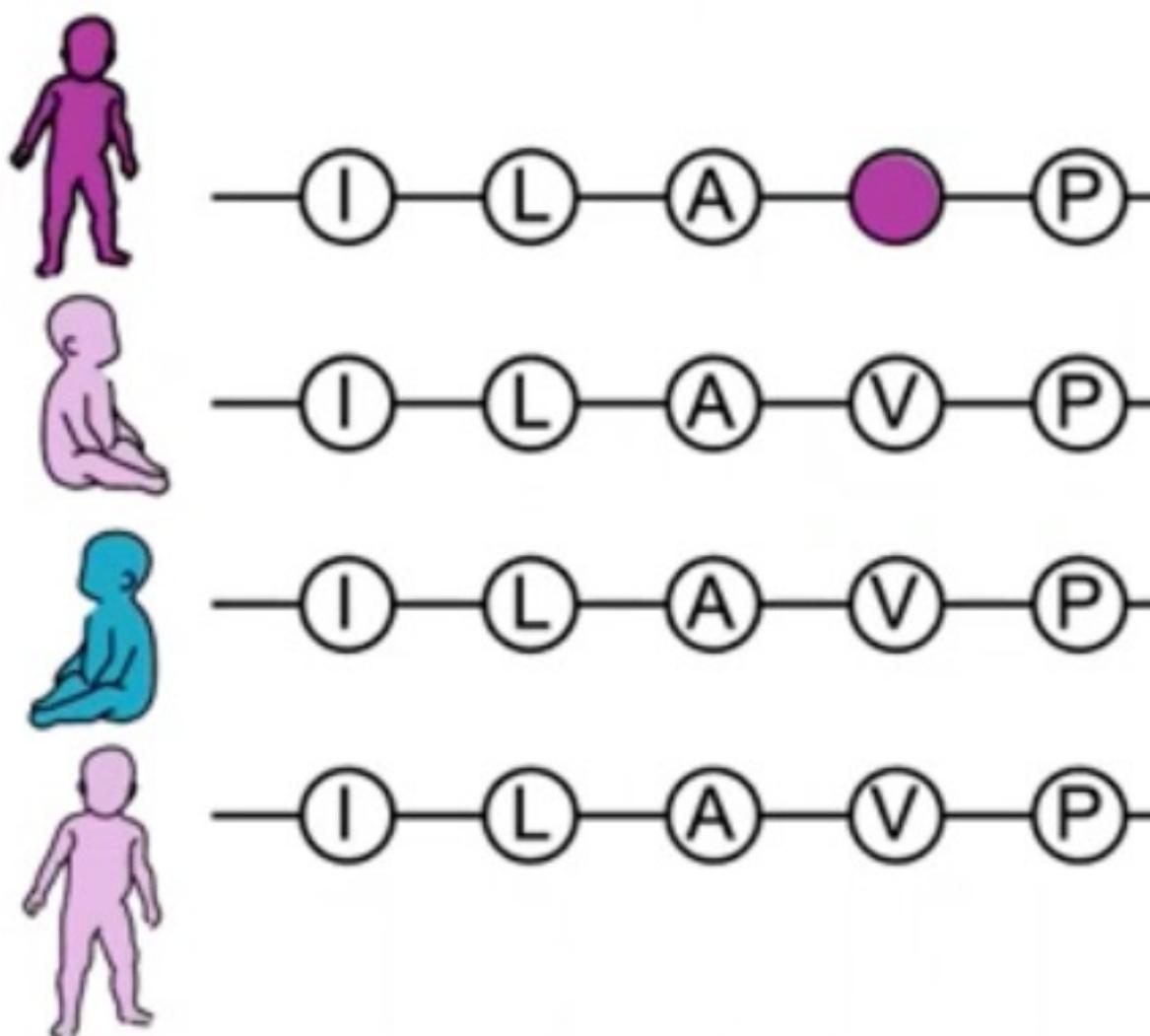


$$\log \left( \frac{p(\mathbf{x}_v|\theta)}{p(\mathbf{x}_{\text{ref}}|\theta)} \right)$$

less probable → less fit

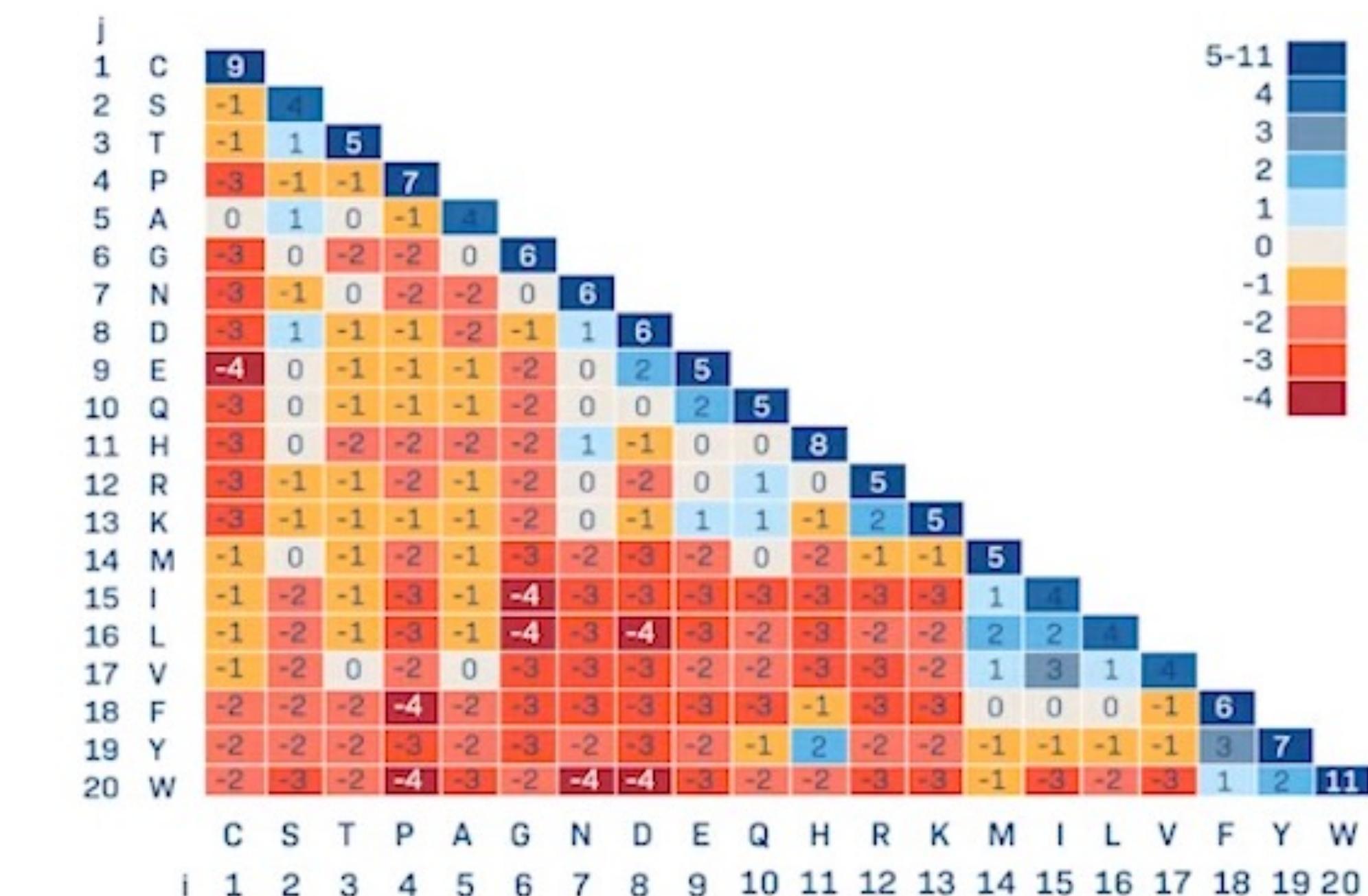
# General Substitution Rules

## Context-independent scores



$$\log \left( \frac{p(\mathbf{x}_v | \theta)}{p(\mathbf{x}_{\text{ref}} | \theta)} \right)$$

less probable → less fit

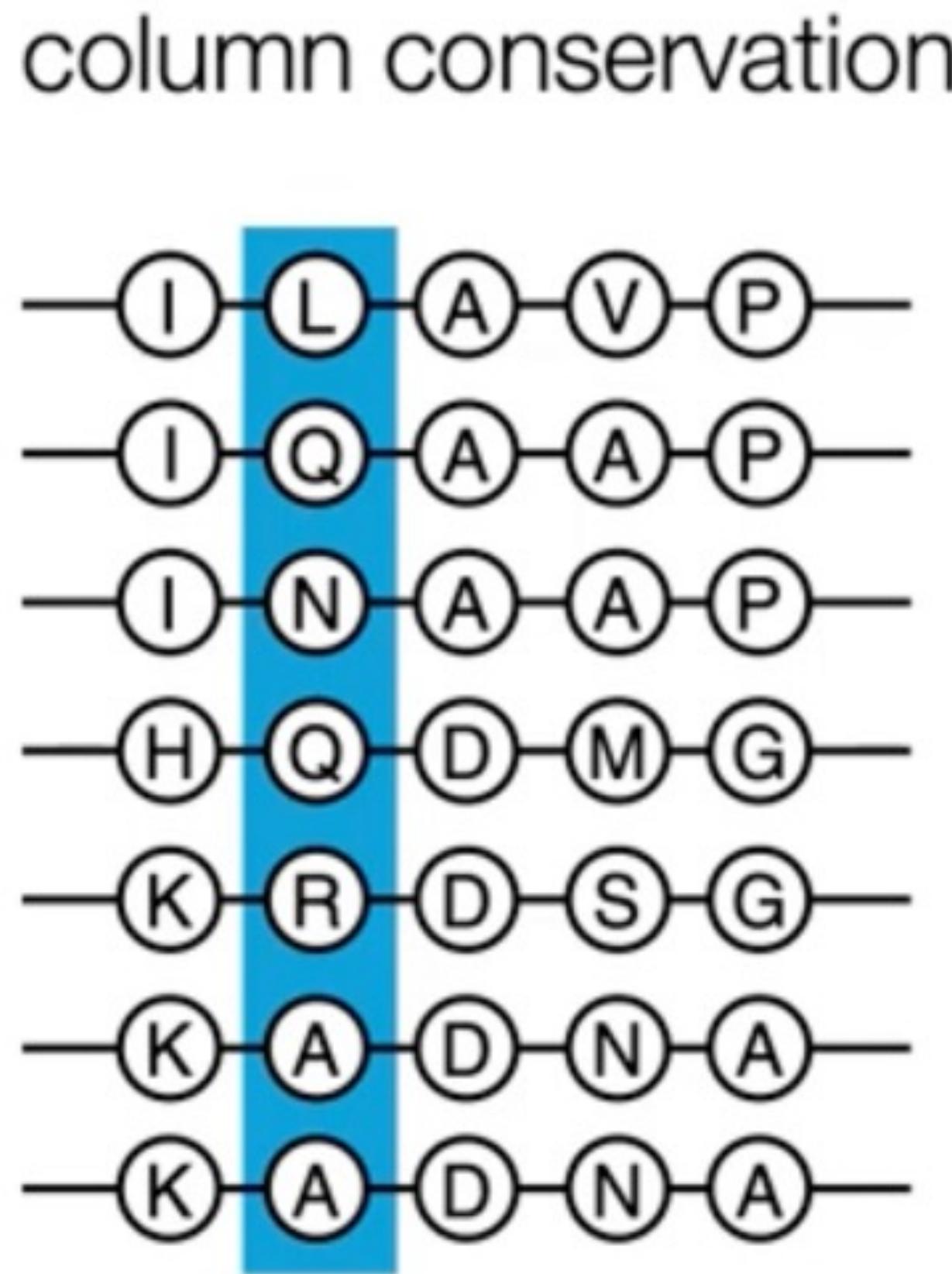


e.g., BLOSUM62

Henikoff and Henikoff, *PNAS*, 1992

# Single-site models: Conservation

Site-independent scores based on single sites



$$E(\mathbf{x}) = \sum_{ij} h_i^j x_i^j$$

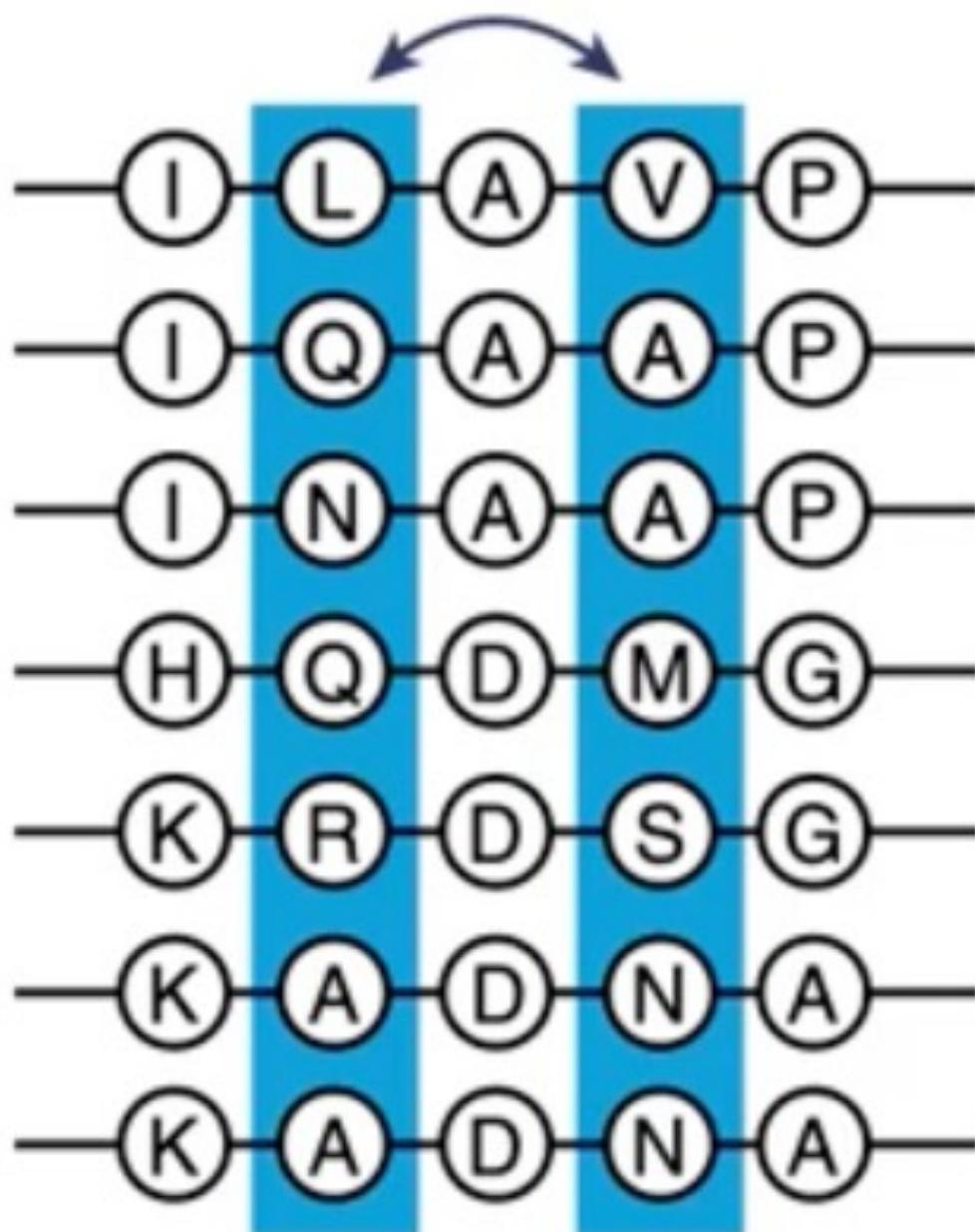
indicator function  
↓  
amino acid  
position

$$p(\mathbf{x}) = \frac{1}{Z} e^{E(\mathbf{x})}$$

# Pairwise models: Coevolution

Interactions captured, but still site-independent

pairwise interactions  
(Potts model)



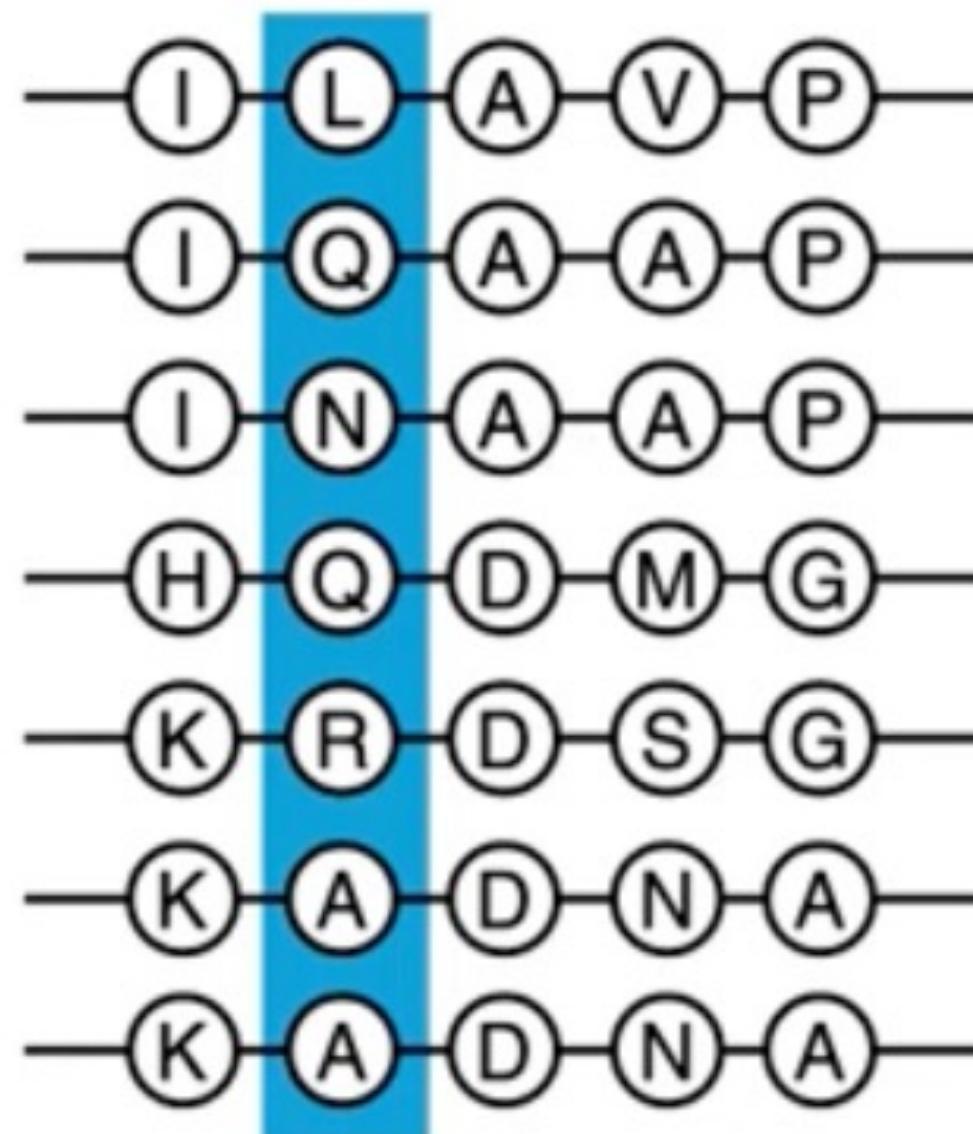
$$E(\mathbf{x}) = \sum_{ij} h_i^j x_i^j + \sum_{ijkl} J_{ij\triangleleft}^{kl} x_i^k x_j^l$$

$$p(\mathbf{x}) = \frac{1}{Z} e^{E(\mathbf{x})}$$

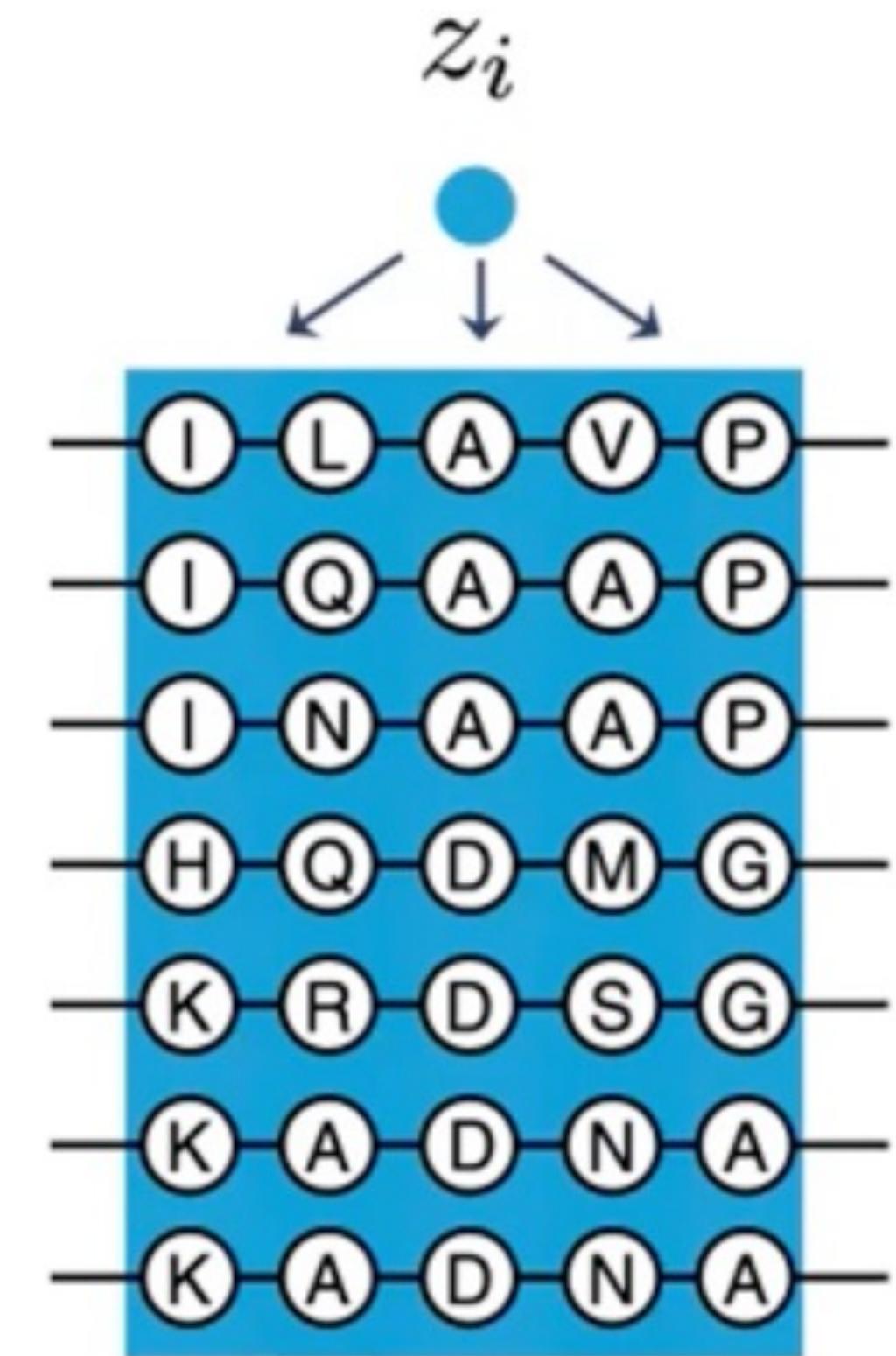
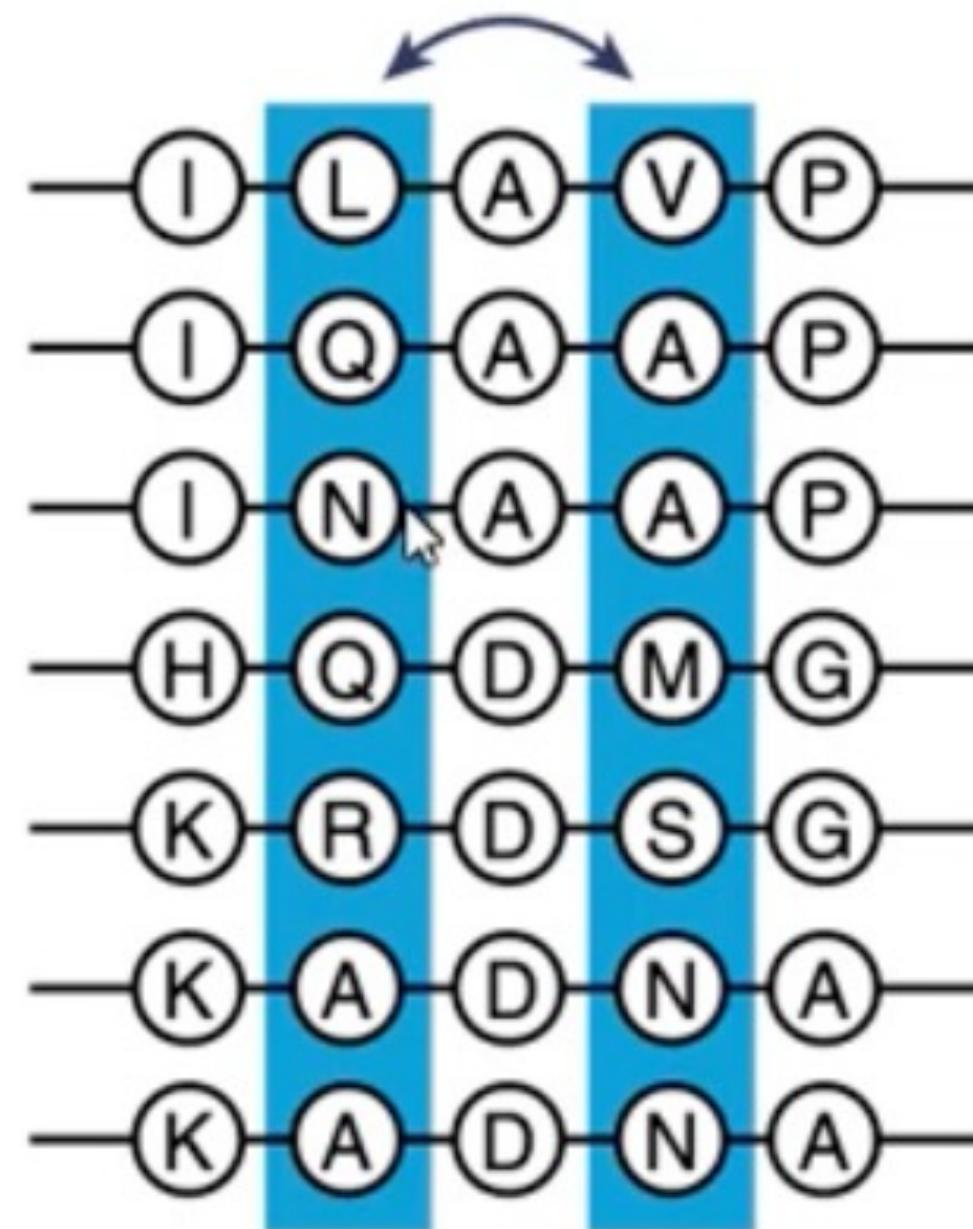
# Higher-order models

Taking context into account

column conservation

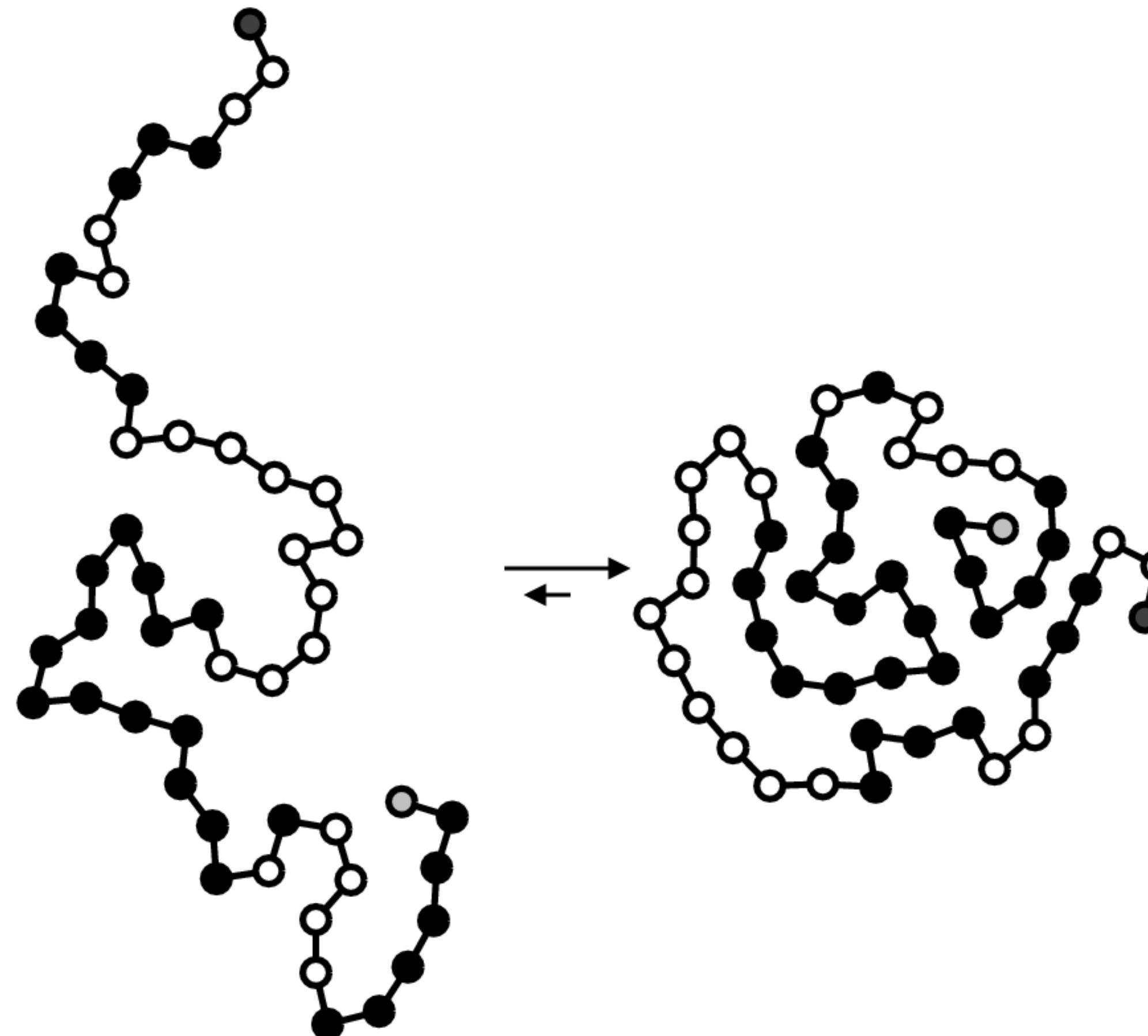


pairwise interactions  
(Potts model)



# Context is everything

Functions can vary depending on the local environment



## 2. Language Modelling

# Evolution of language modelling

A similar story: we start with site-independent models

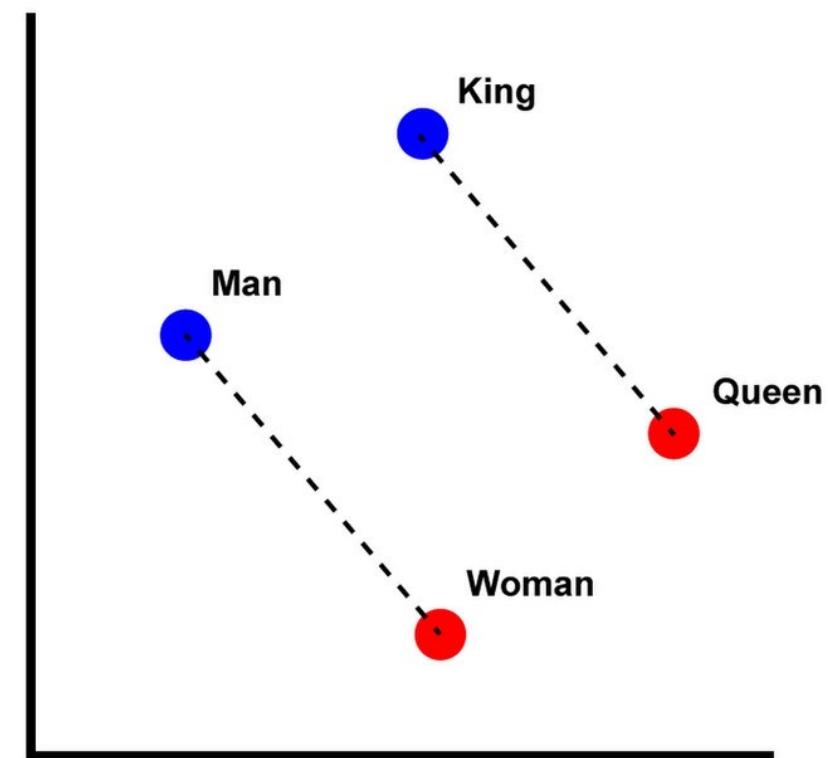
And the king...



[0.2, 0.03, -0.4, ...]

However, the queen...

[0.1, -0.51, 0.2, ...]



# Evolution of language modelling

A similar story: site-independent models (Word2Vec, GloVe, ...)

Along the river **bank** ...

[0.1 0.03, -0.5, ...]



?

The **bank** robber ...

[0.1, 0.03, -0.5, ...]

# Evolution of language modelling

## Solution: contextual representations

Along the river **bank** ...

[0.1 0.03, -0.5, ...]



?

The **bank** robber ...

[0.2, -0.35, 0.4, ...]

# Evolution of language modelling

## Solution: contextual representations

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### Semi-supervised Sequence Learning

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**Andrew M. Dai**  
Google Inc.  
[adai@google.com](mailto:adai@google.com)

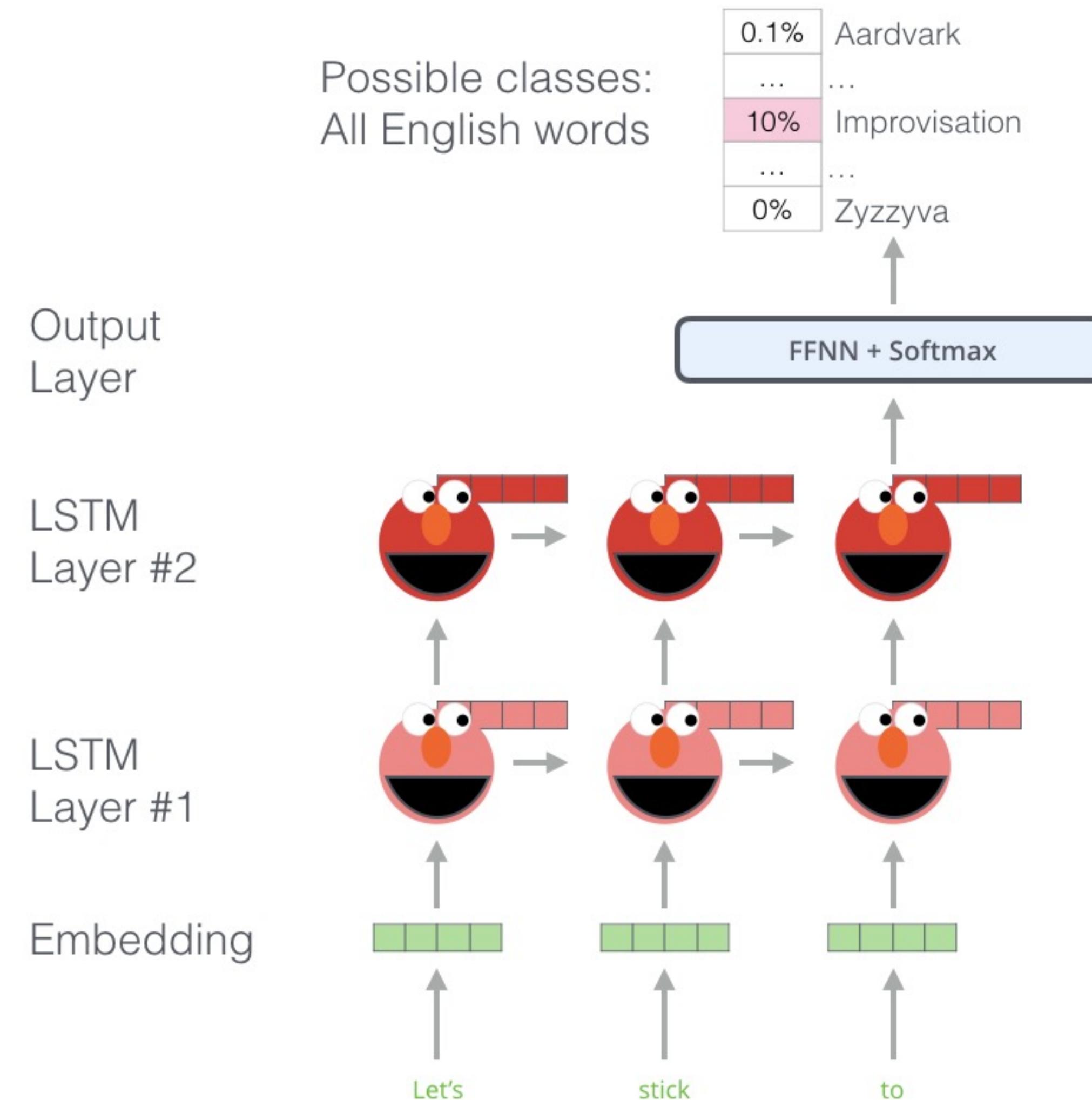
**Quoc V. Le**  
Google Inc.  
[qvl@google.com](mailto:qvl@google.com)

#### Abstract

We present two approaches that use unlabeled data to improve sequence learning with recurrent networks. The first approach is to predict what comes next in a sequence, which is a conventional language model in natural language processing. The second approach is to use a sequence autoencoder, which reads the input sequence into a vector and predicts the input sequence again. These two algorithms can be used as a “pretraining” step for a later supervised sequence learning algorithm. In other words, the parameters obtained from the unsupervised step can be used as a starting point for other supervised training models.

# ELMo: next-word prediction

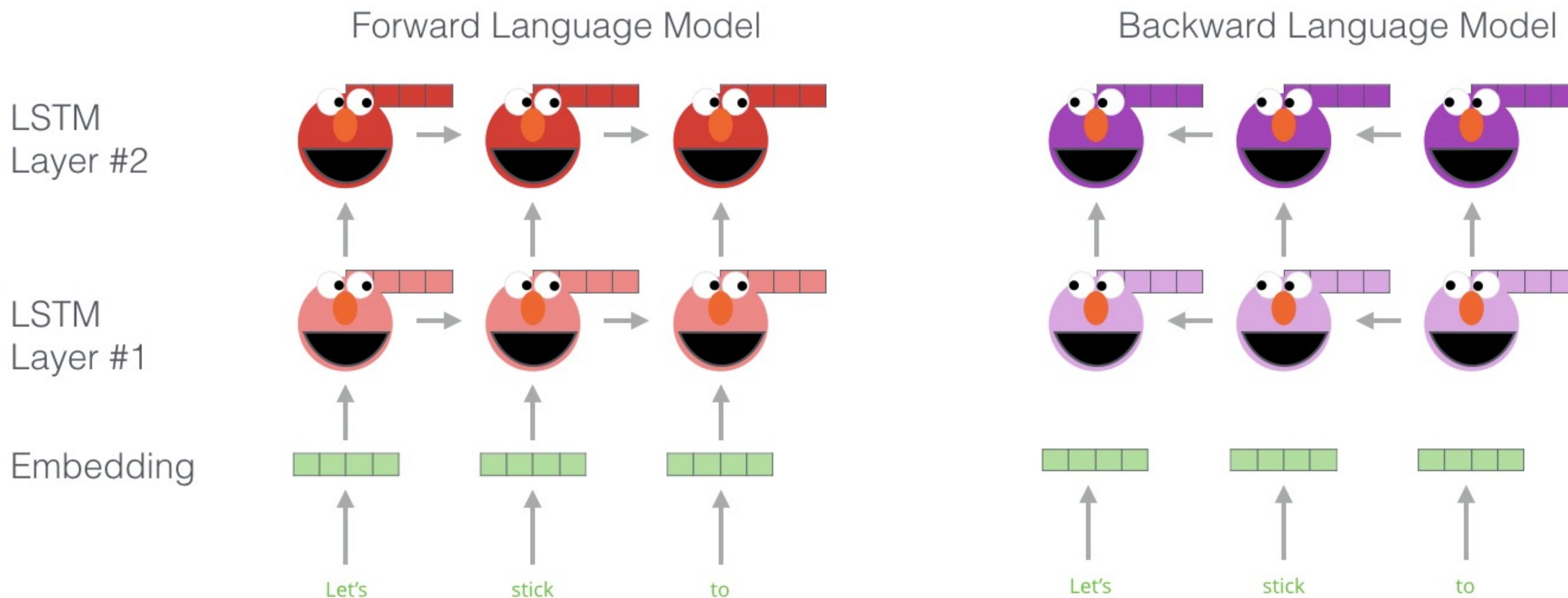
## Solution: contextual representations



# ELMo: forward and backward

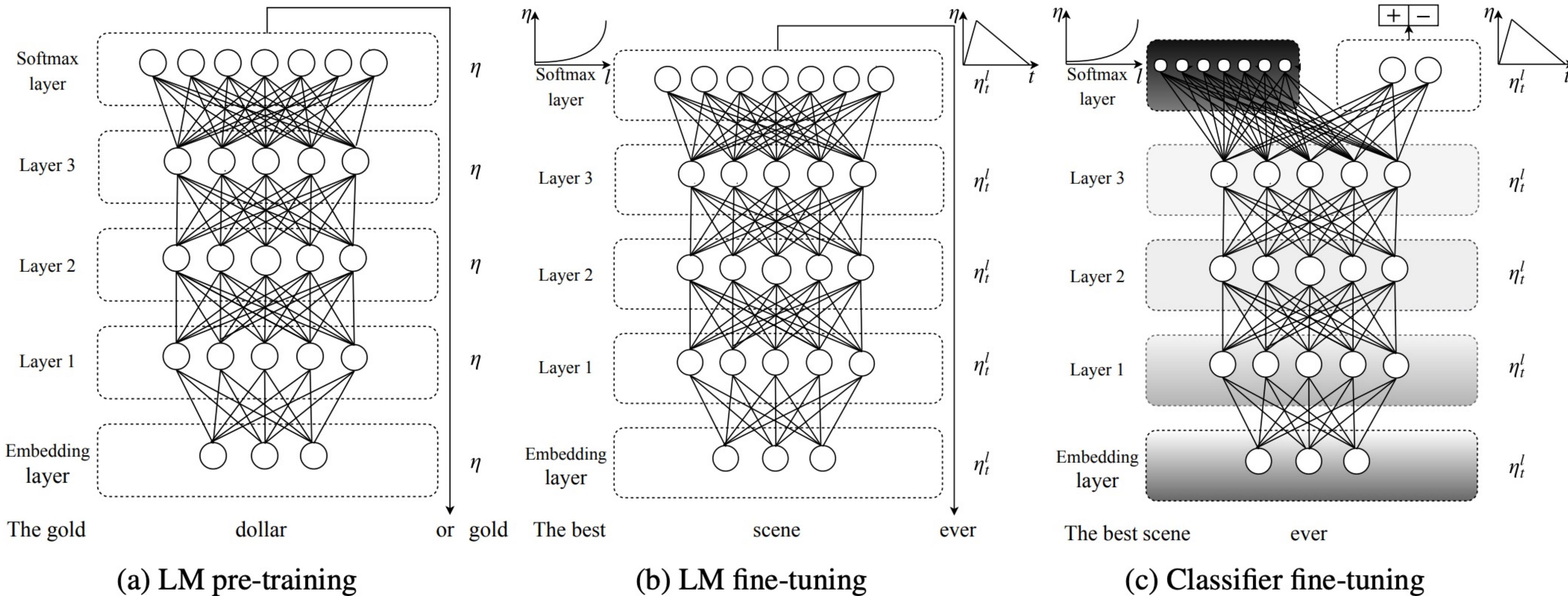
Look at text from both ways

Embedding of “stick” in “Let’s stick to” - Step #1



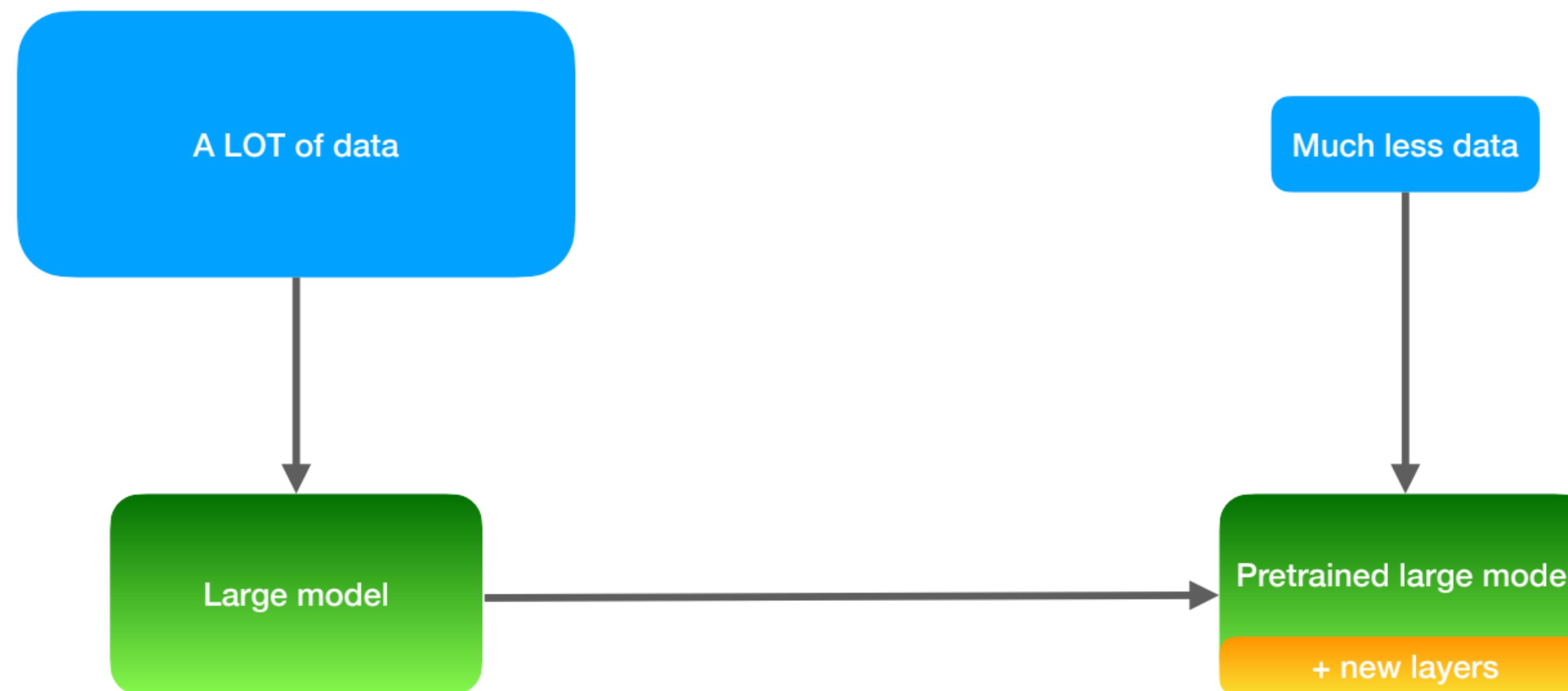
# ULM-FiT: how to do transfer learning in NLP

Use more than embeddings: finetuning for transfer learning



# Transformers are good at Transfer Learning

Use unlabeled data to get better on specific tasks

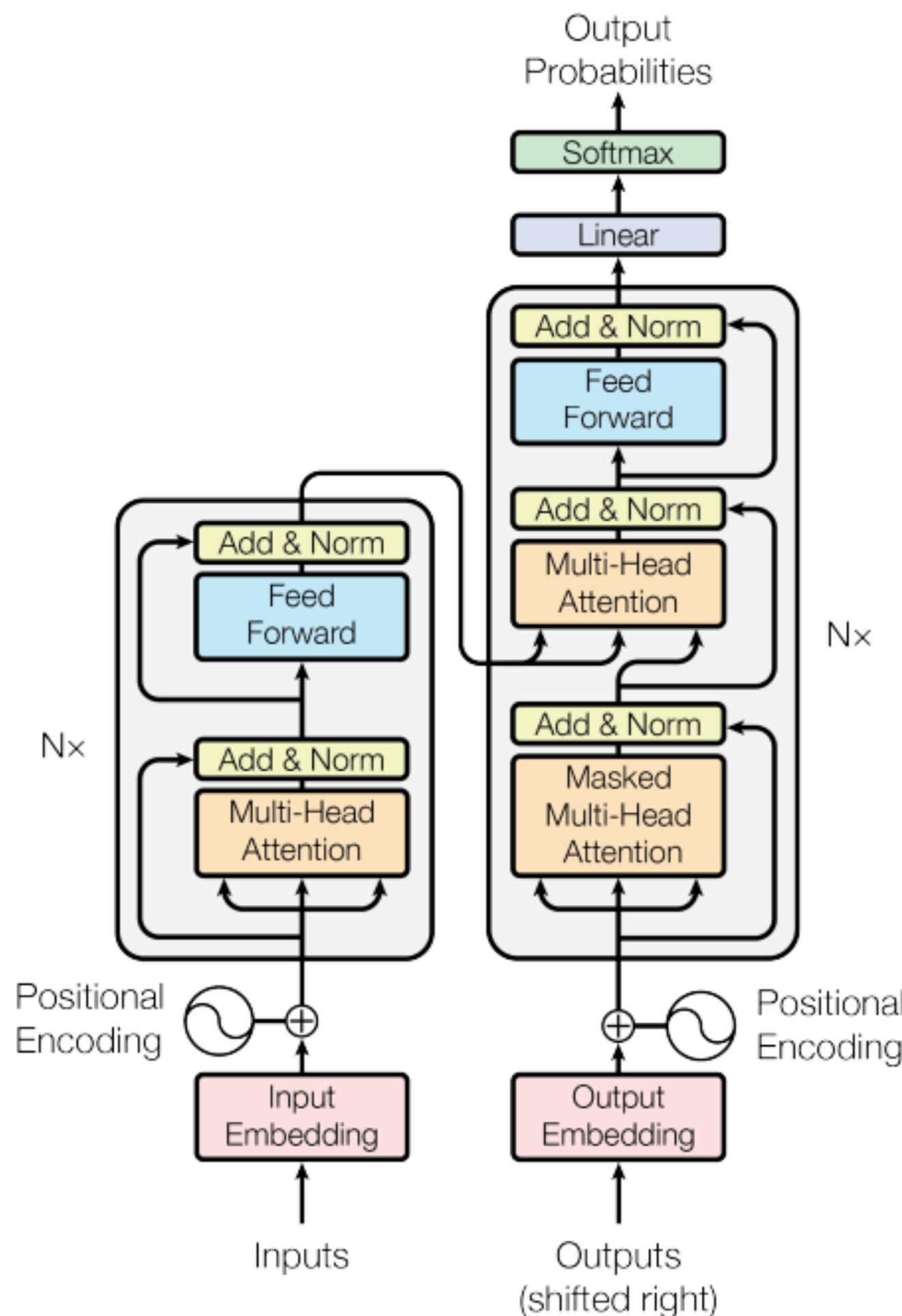


Traditional Machine Learning:  
slow training on a lot of data

Transfer learning:  
fast training on a little data

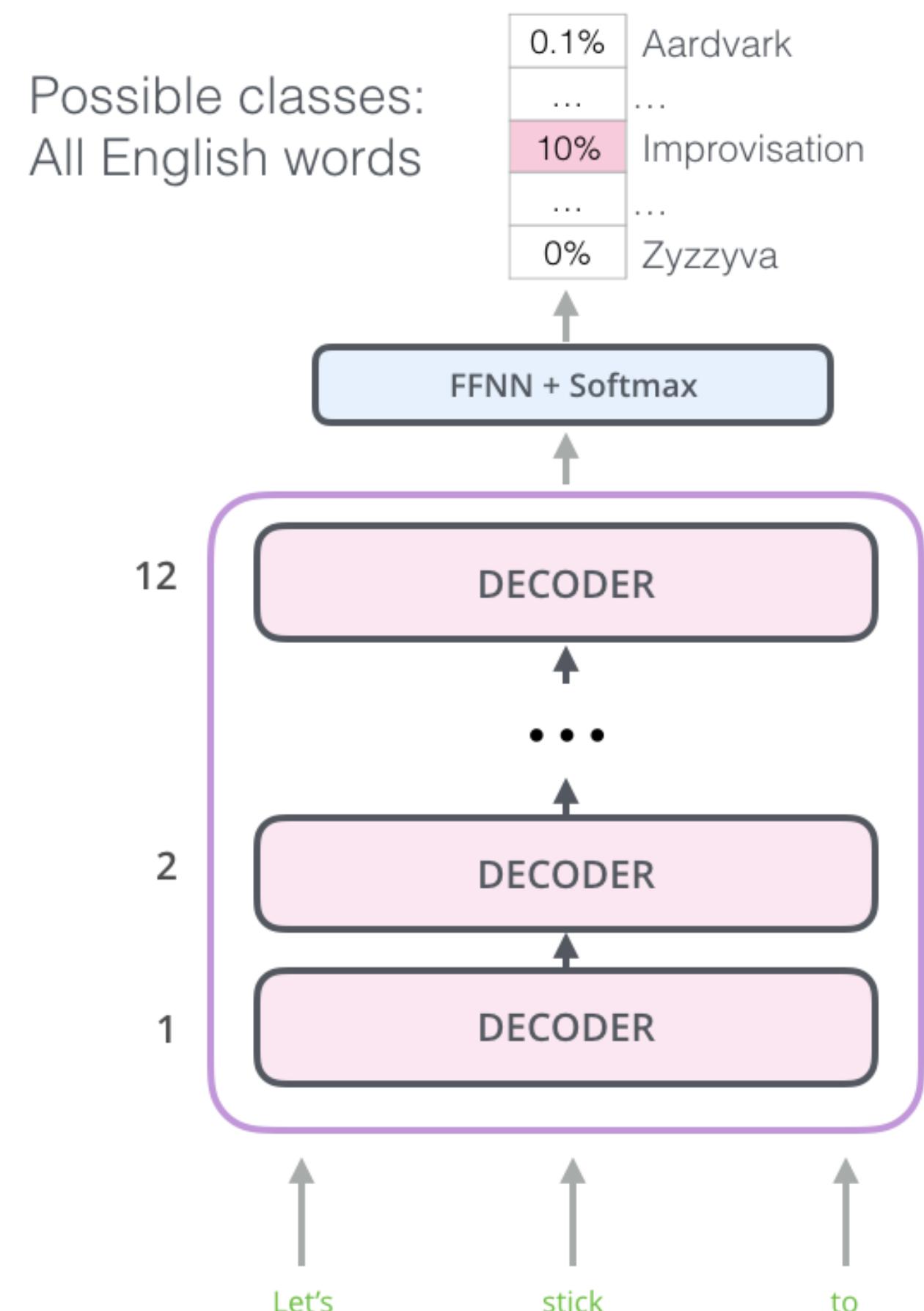
# OpenAI Transformer

Train Deep Transformer LM and fine-tune on final task



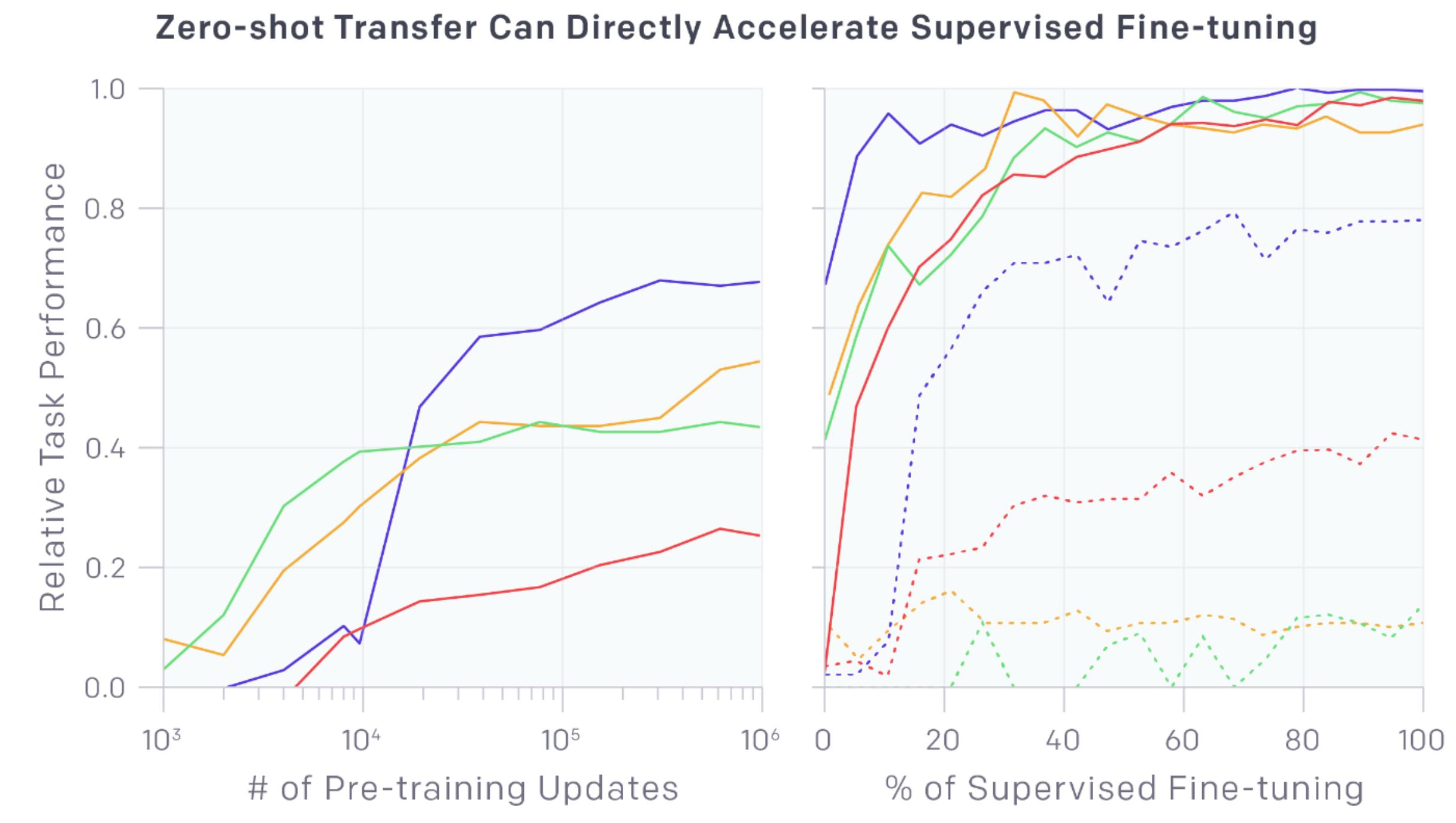
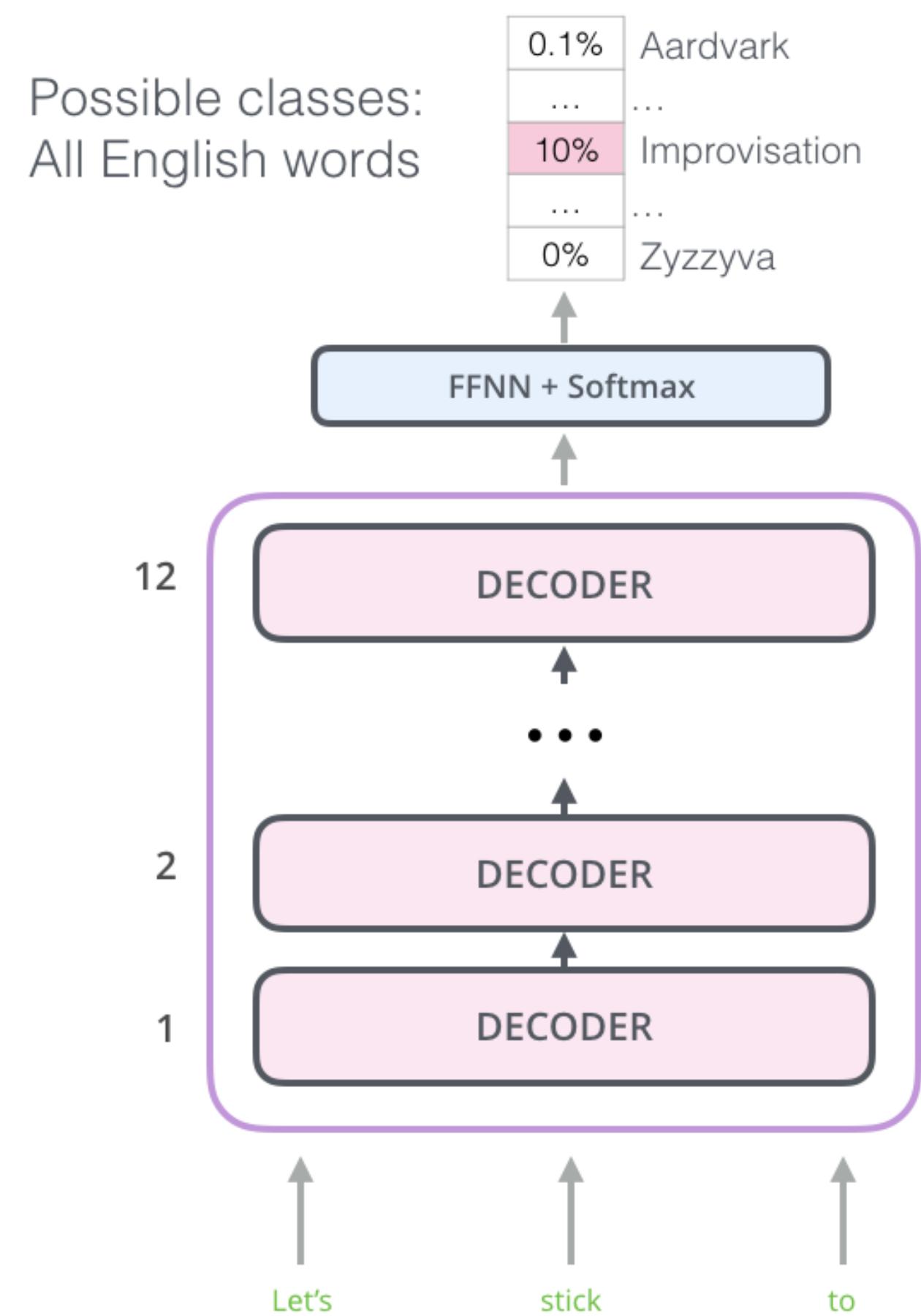
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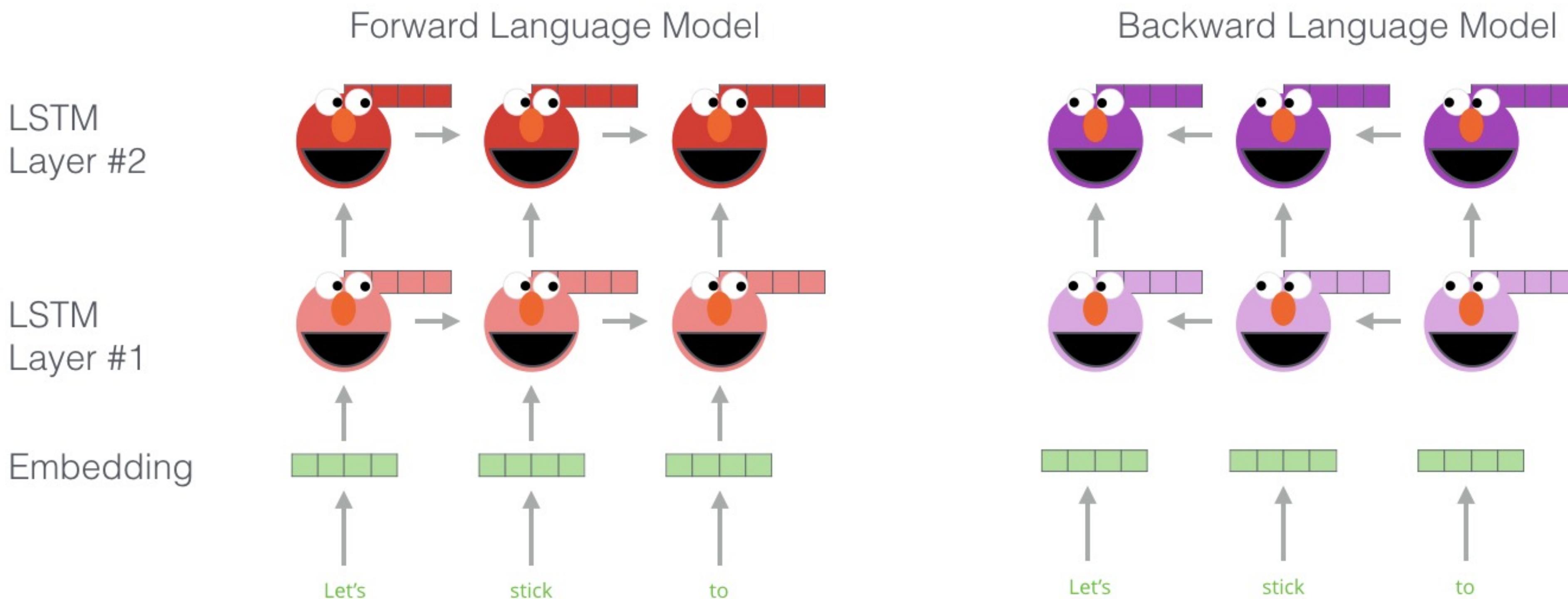
## Train Deep Transformer LM and fine-tune on final task



# Problem: Decoder only

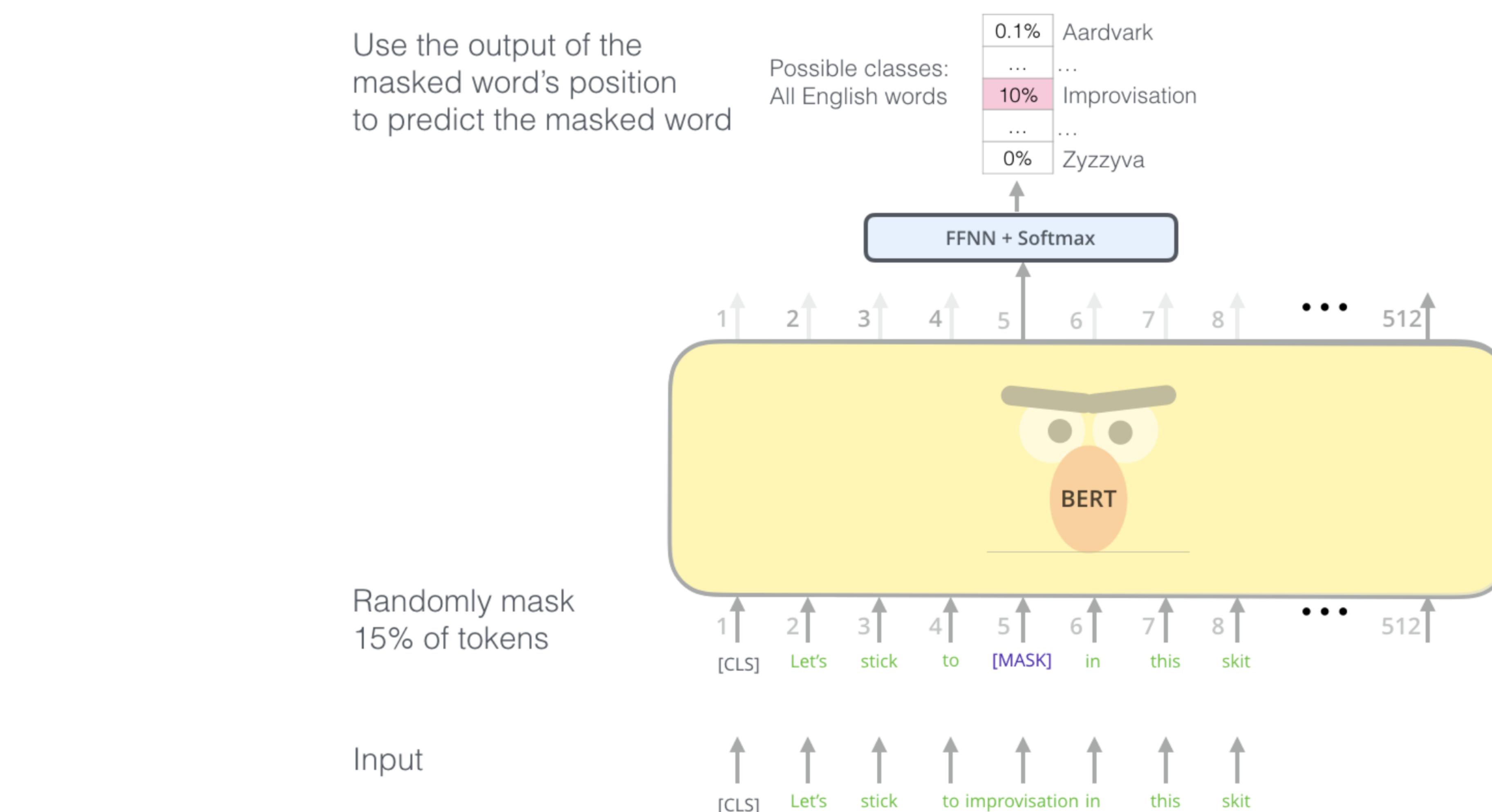
How does our model learn to think backward?

Embedding of “stick” in “Let’s stick to” - Step #1



# BERT

## Just use encoders and mask random tokens



BERT's clever language modeling task masks 15% of words in the input and asks the model to predict the missing word.

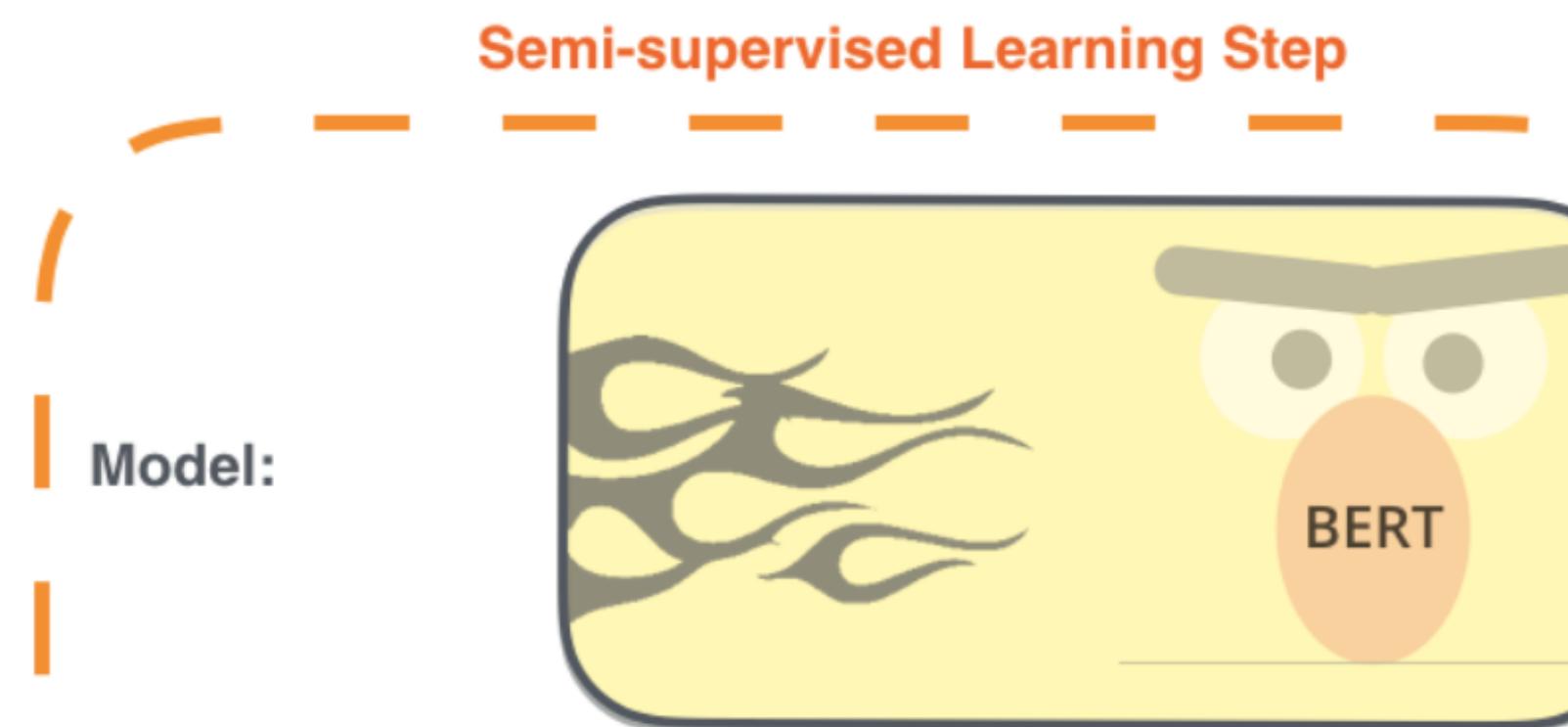
Jay Allamar – Illustrated BERT

# Language Models

## Bigger = Better ?

1 - **Semi-supervised** training on large amounts of text (books, wikipedia..etc).

The model is trained on a certain task that enables it to grasp patterns in language. By the end of the training process, BERT has language-processing abilities capable of empowering many models we later need to build and train in a supervised way.



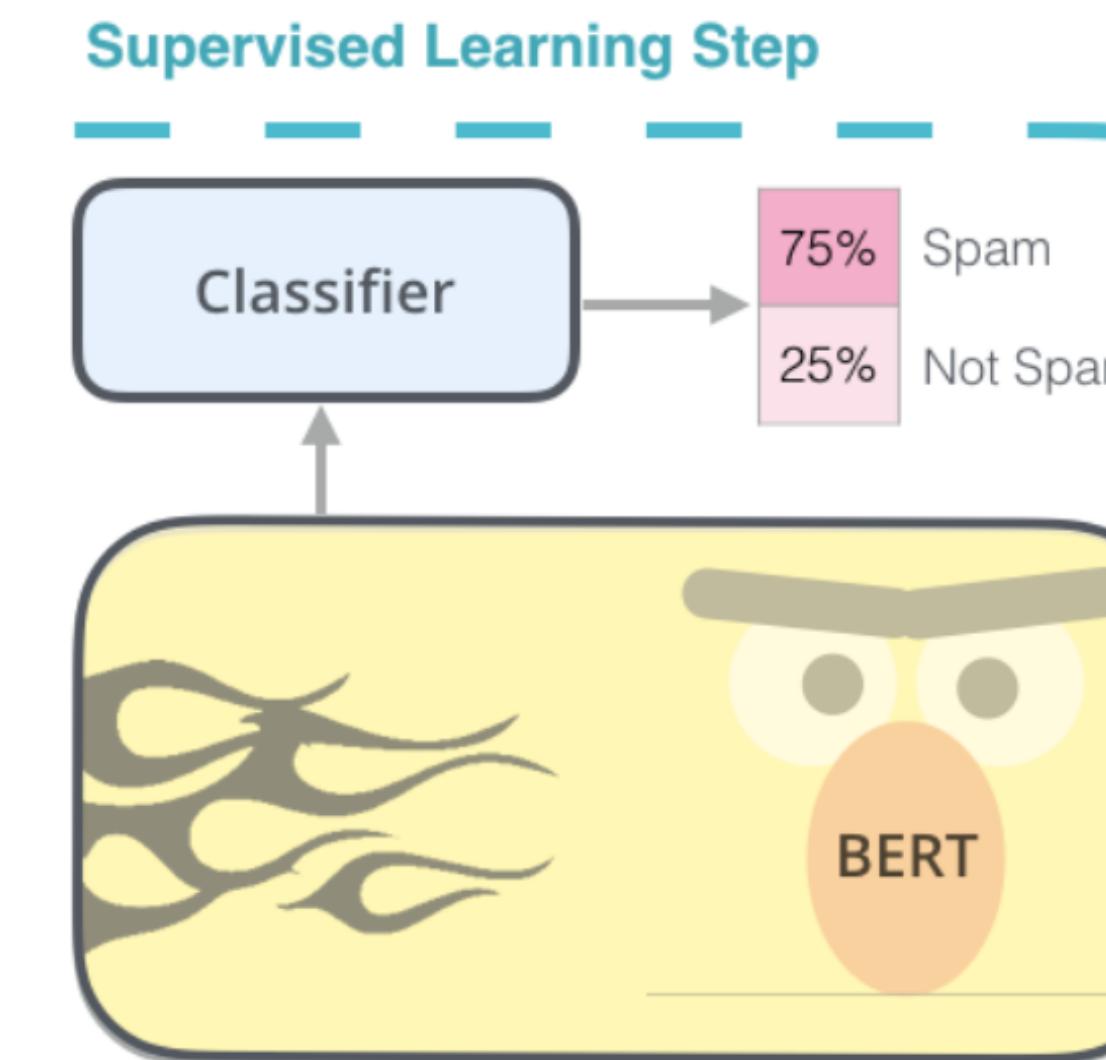
Model:



WIKIPEDIA  
Die freie Enzyklopädie

Predict the masked word  
(language modeling)

2 - **Supervised** training on a specific task with a labeled dataset.



Model:  
(pre-trained  
in step #1)

Email message	Class
Buy these pills	Spam
Win cash prizes	Spam
Dear Mr. Atreides, please find attached...	Not Spam

Dataset:

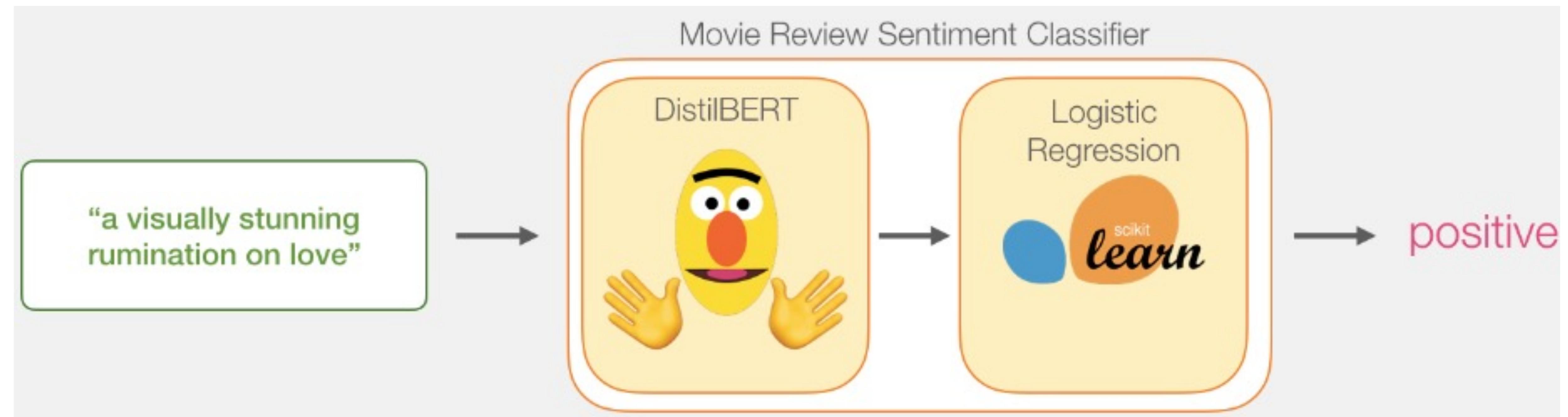
# Transformers are good at Transfer Learning

Pre-Training improves downstream performance



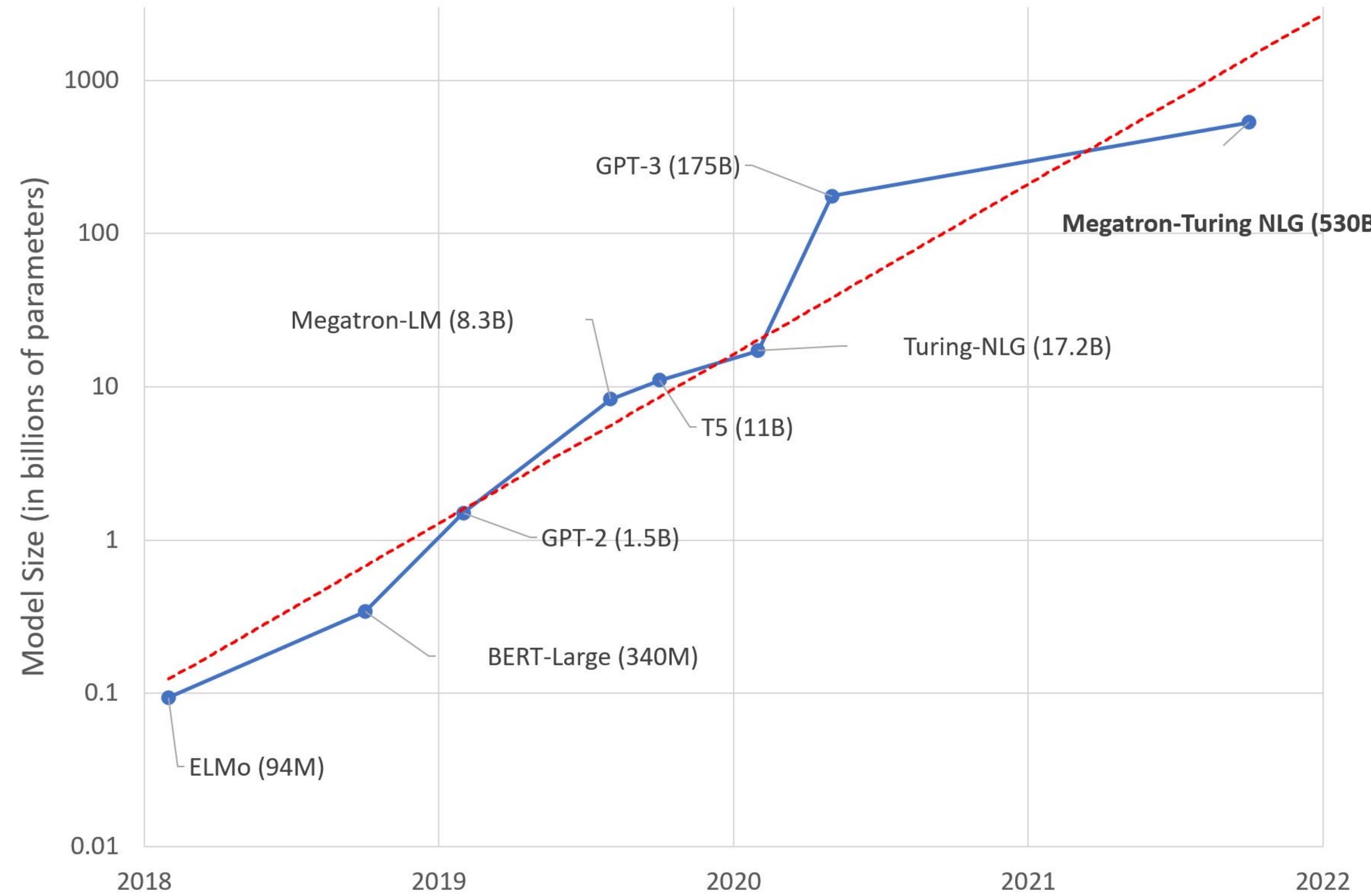
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# Language Models

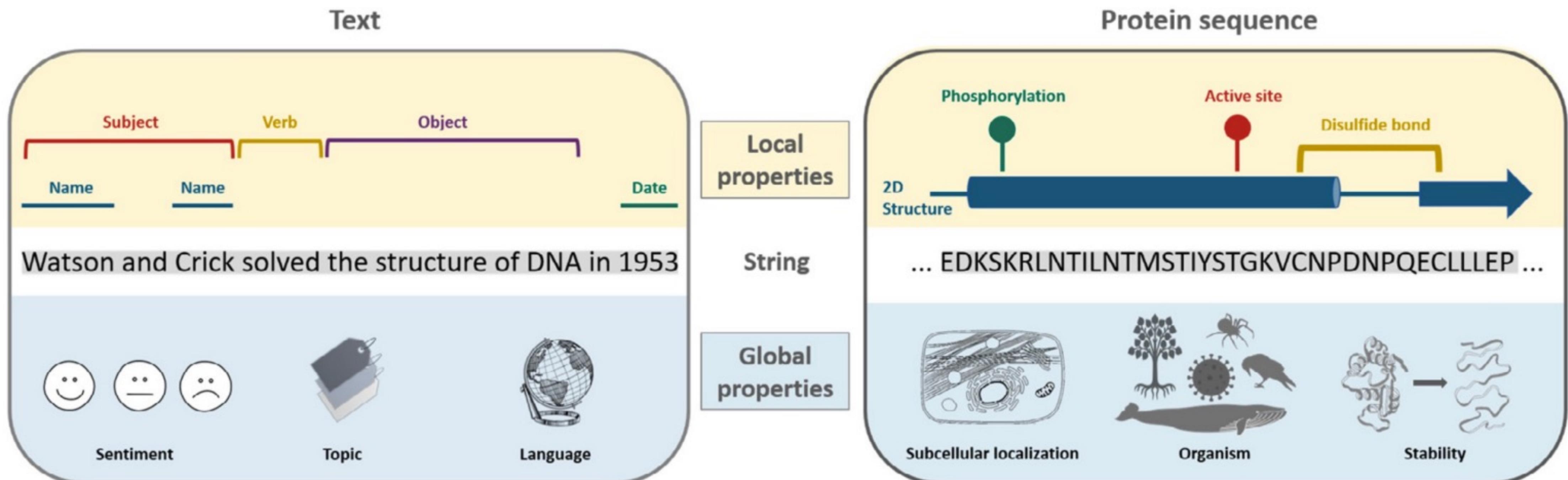
Bigger = Better ?



# 3. Protein Linguistics: Language Models in Biology

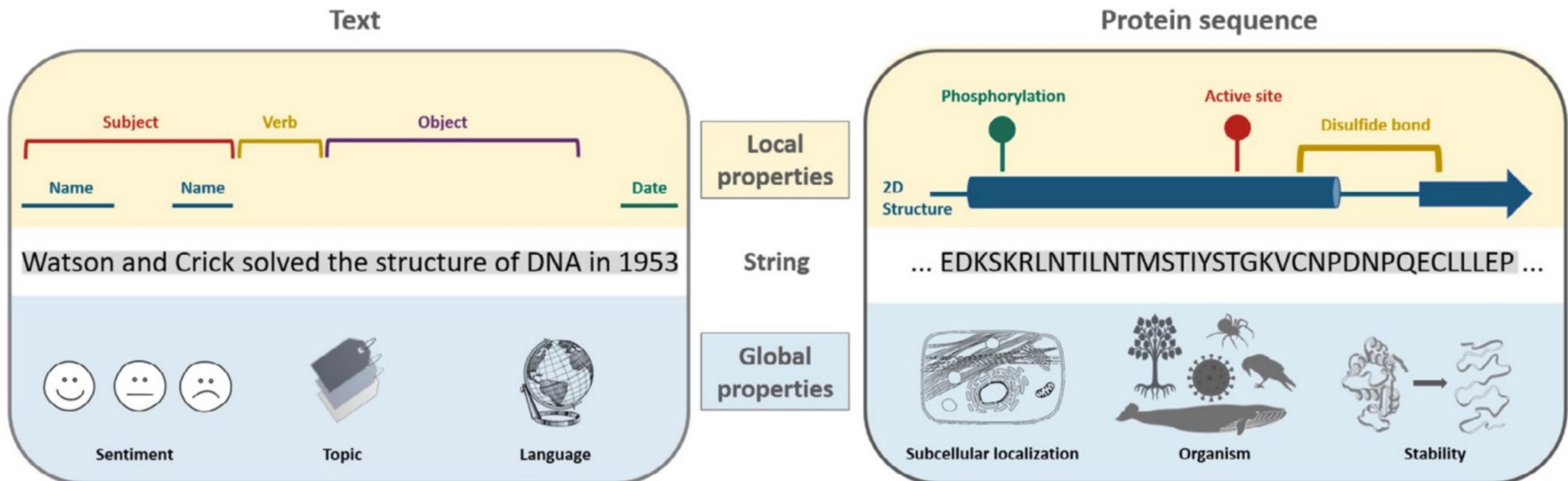
# Proteins vs Sentences: The same?

Similar, but also important differences



# Proteins vs Sentences: The same?

Similar, but also important differences



Can you *read* a protein?

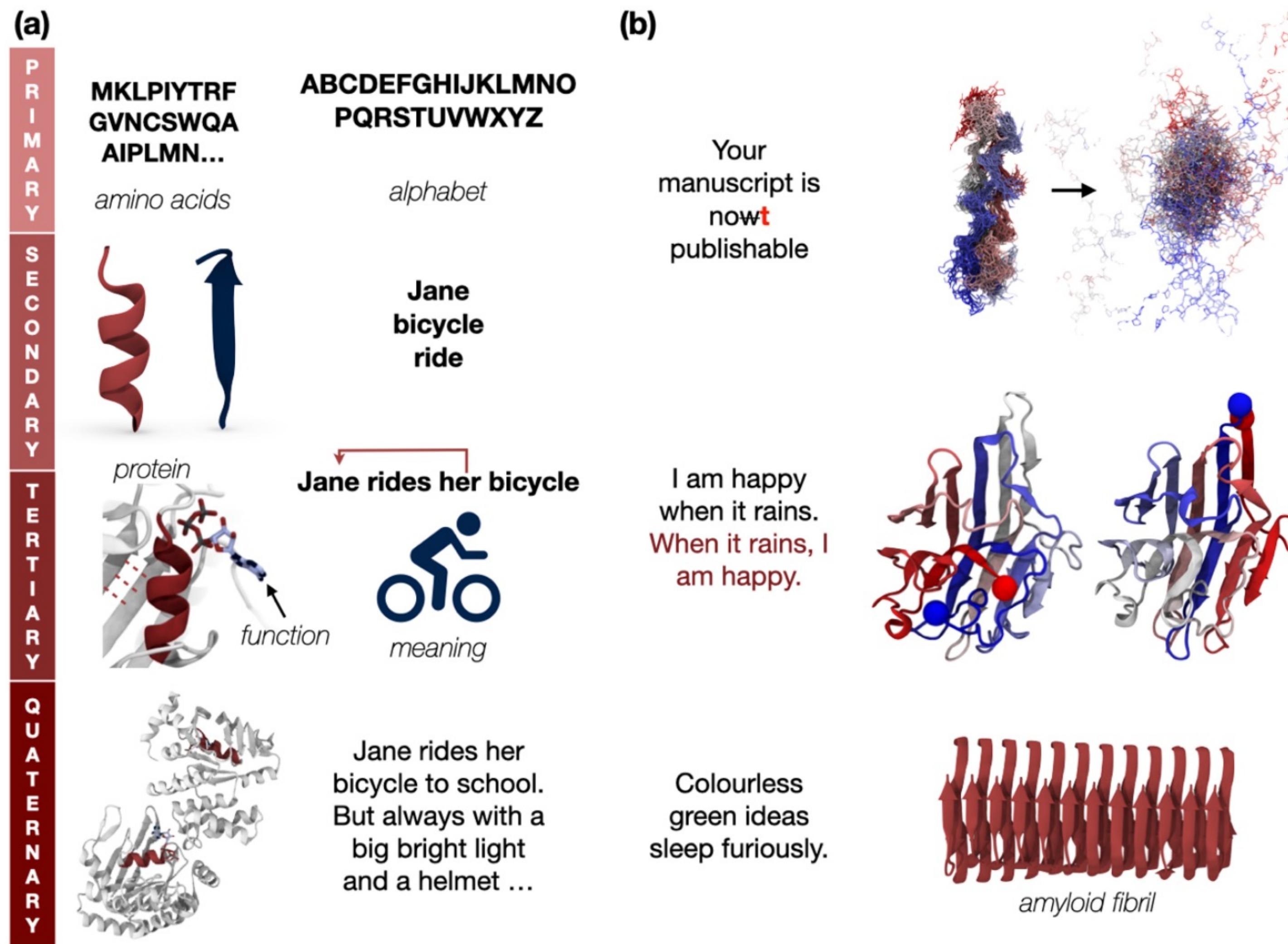
Past and Future tense?

Distant interactions in protein structures

Bias in Sequencing/Research

# Proteins vs Sentences: The same?

Similar, but also important differences



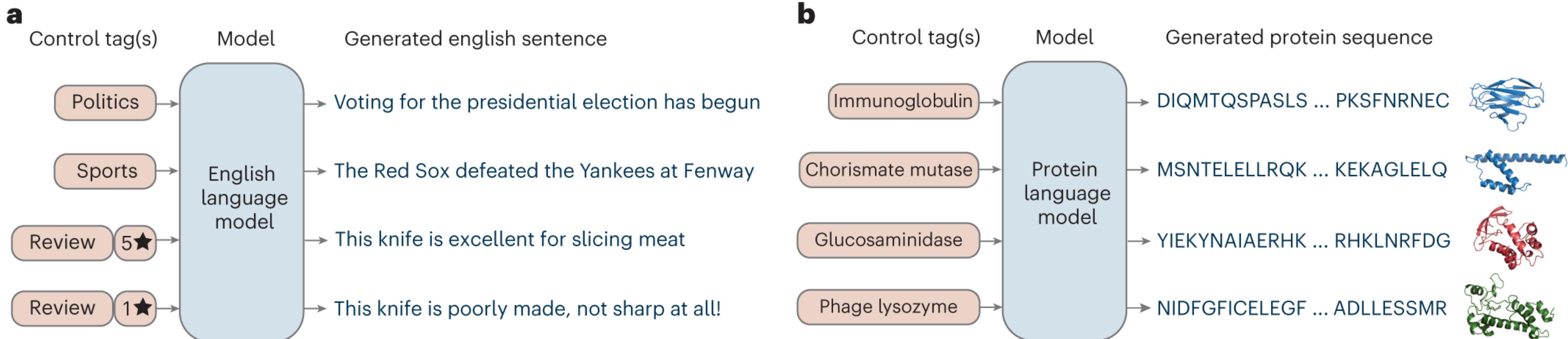
# The linguistic hypothesis

**Did evolution force proteins to develop a “language”?**

- The space of naturally occurring proteins occupies a learnable manifold.
- This manifold emerges from evolutionary pressures that heavily encourage the reuse of components at many scales: from short motifs of secondary structure, to entire globular domains.

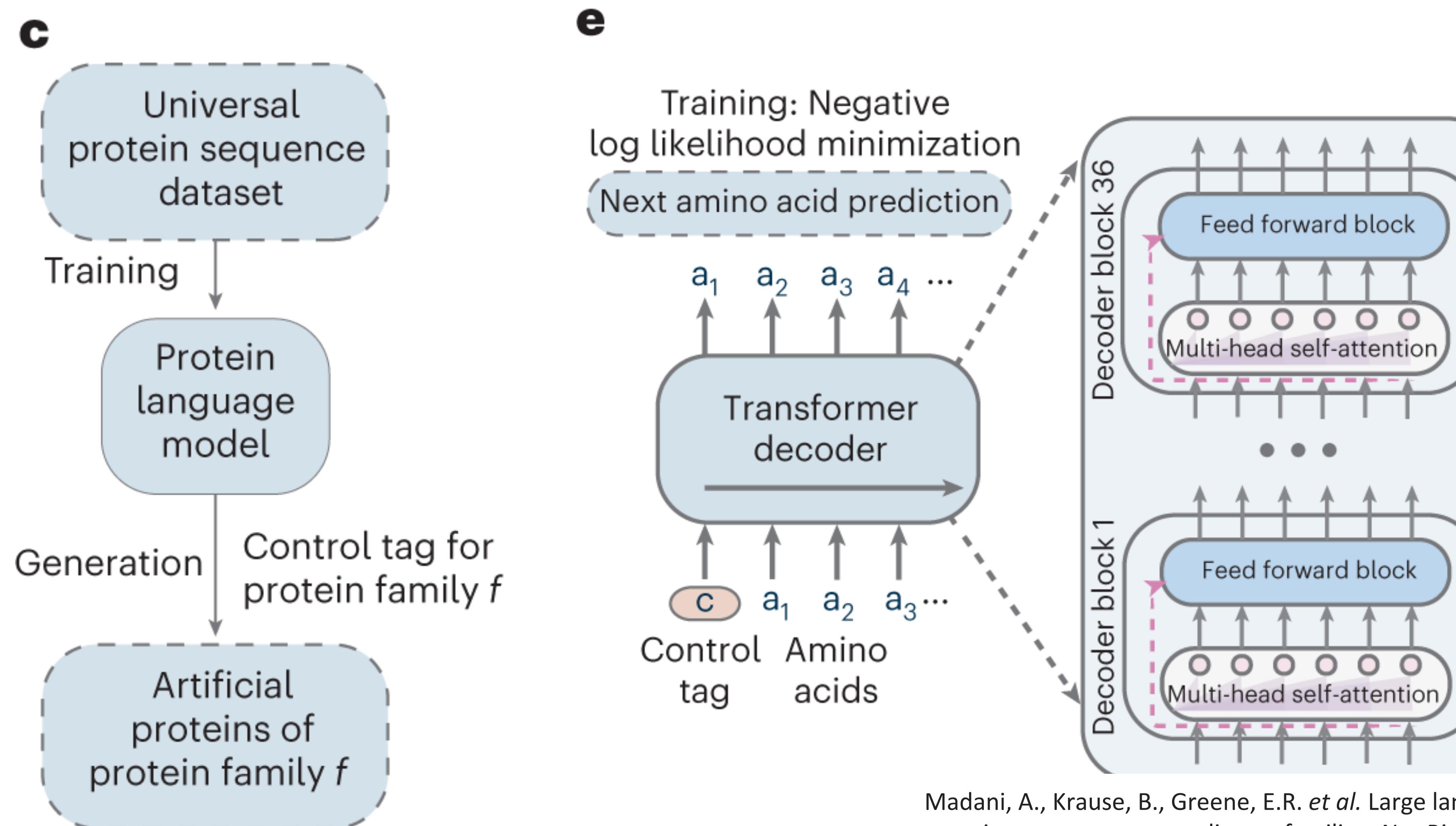
# Protein Language Models

Train a model to understand the language of proteins



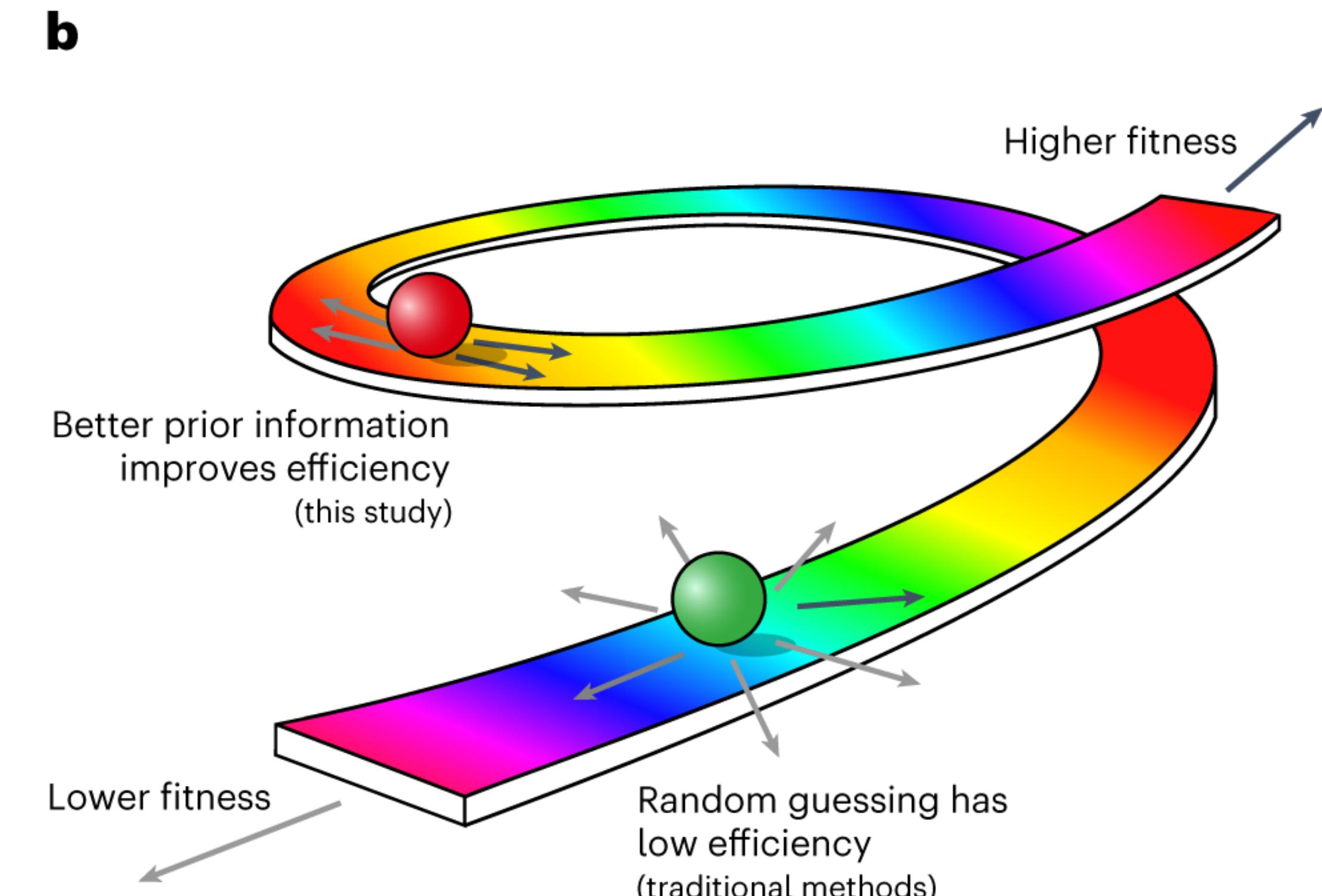
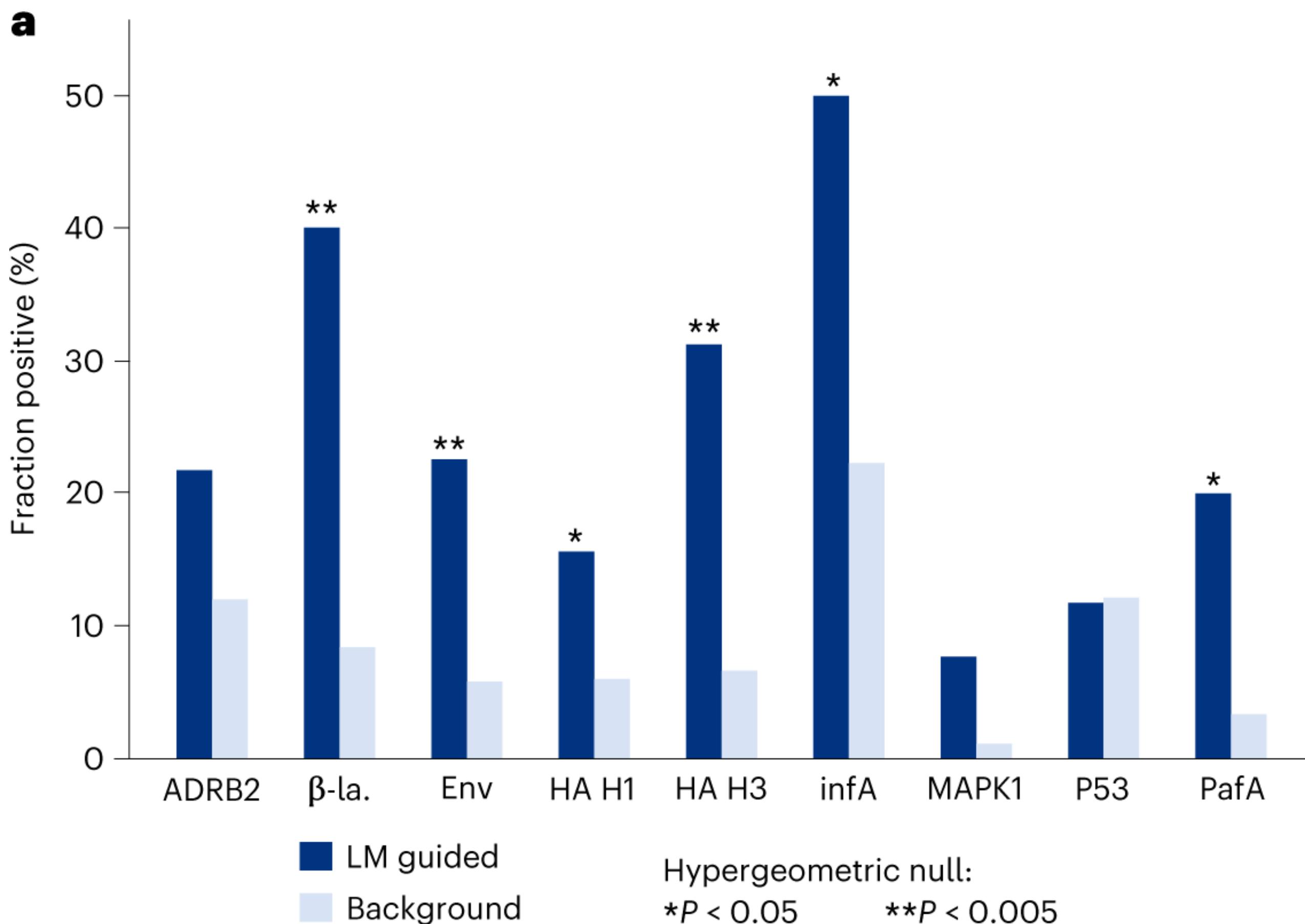
# Protein Language Models

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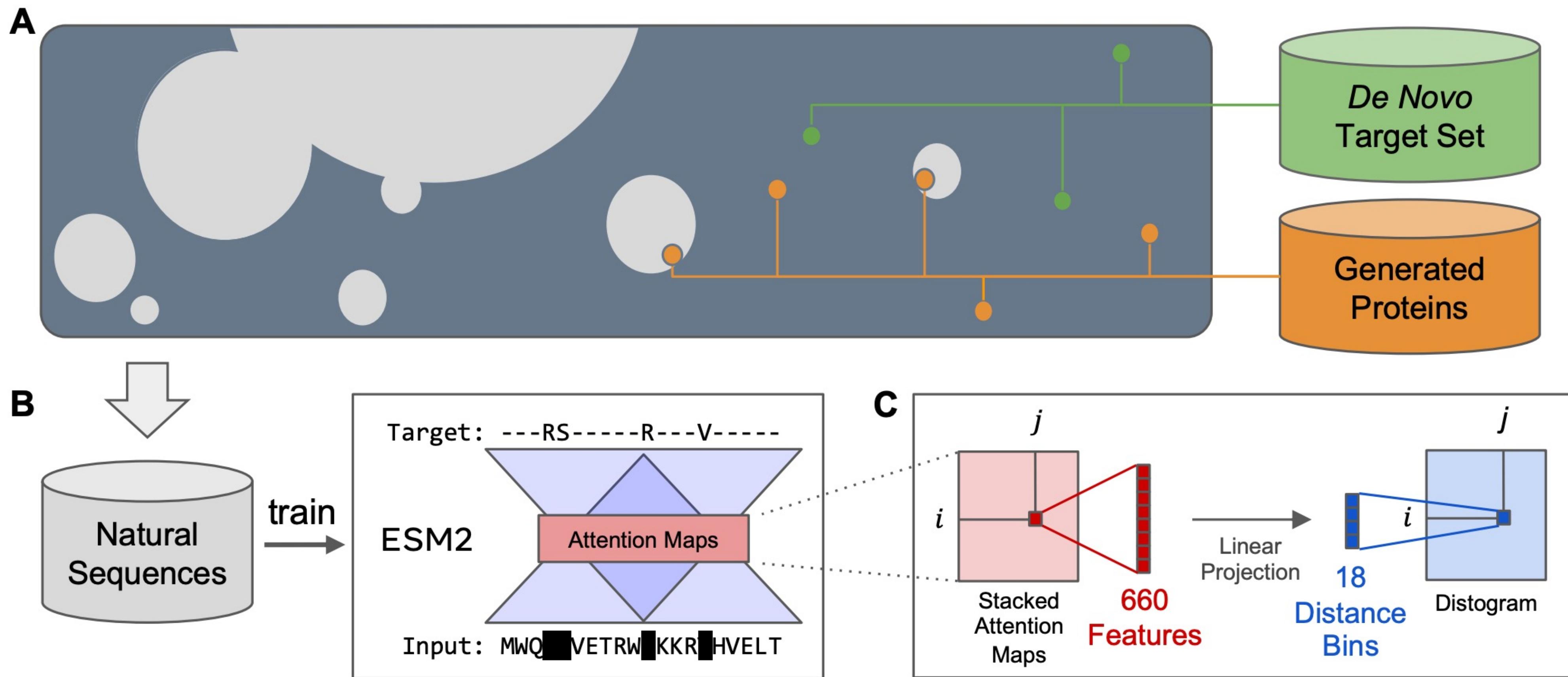
# Applications of PLM

## Efficient Evolution



# Generalisation beyond natural proteins?

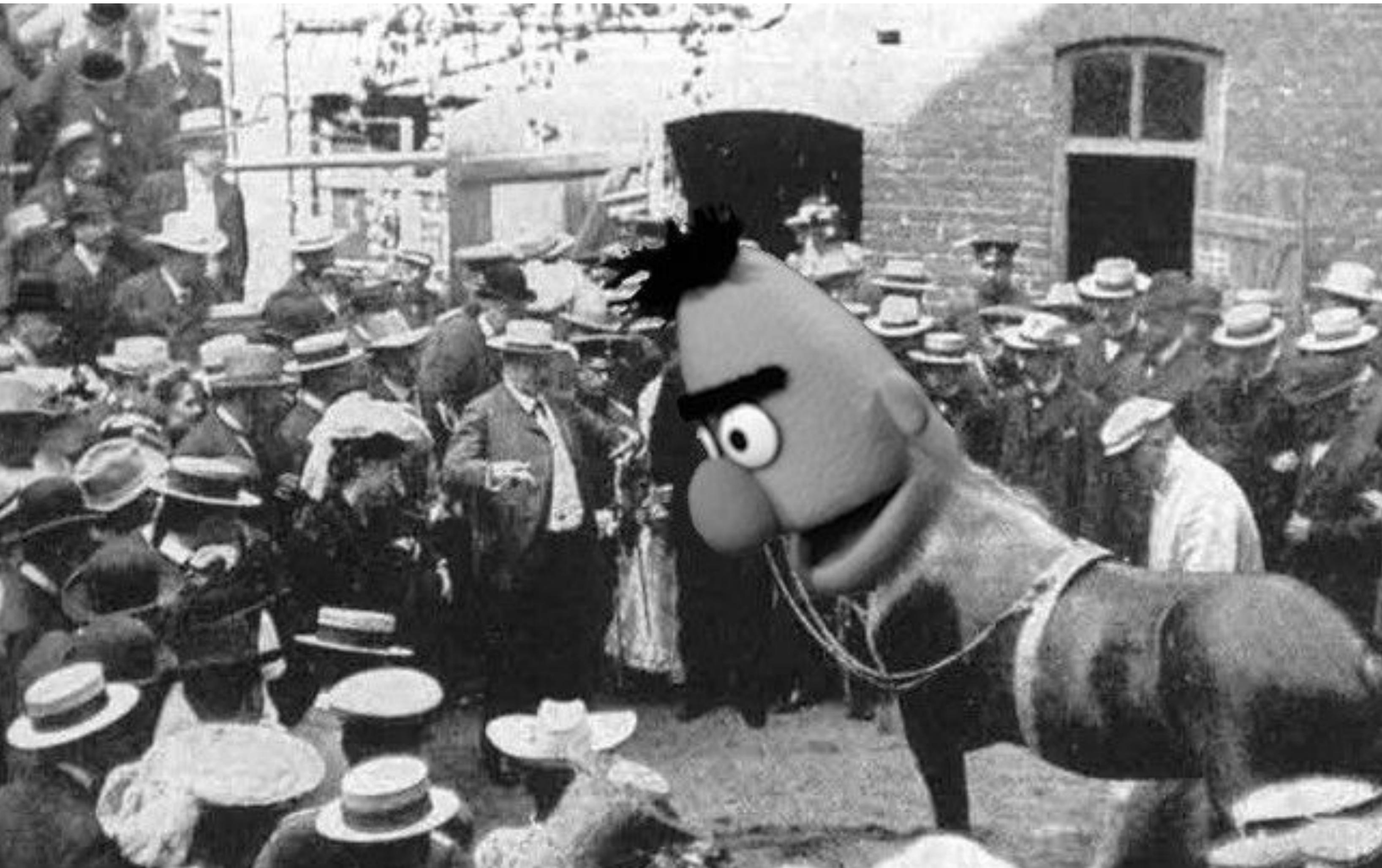
Promising signs, but no consensus yet among researchers



# 4. Practical Considerations

# Clever Hans Moment in NLP

Do our models learn what we want them to learn?



# Clever Hans Moment in NLP

Often they just learn heuristic shortcuts

**Article:** Super Bowl 50

**Paragraph:** “*Peyton Manning became the first quarterback ever to lead two different teams to multiple Super Bowls. He is also the oldest quarterback ever to play in a Super Bowl at age 39. The past record was held by John Elway, who led the Broncos to victory in Super Bowl XXXIII at age 38 and is currently Denver’s Executive Vice President of Football Operations and General Manager. Quarterback Jeff Dean had jersey number 37 in Champ Bowl XXXIV.*

**Question:** “*What is the name of the quarterback who was 38 in Super Bowl XXXIII?*”

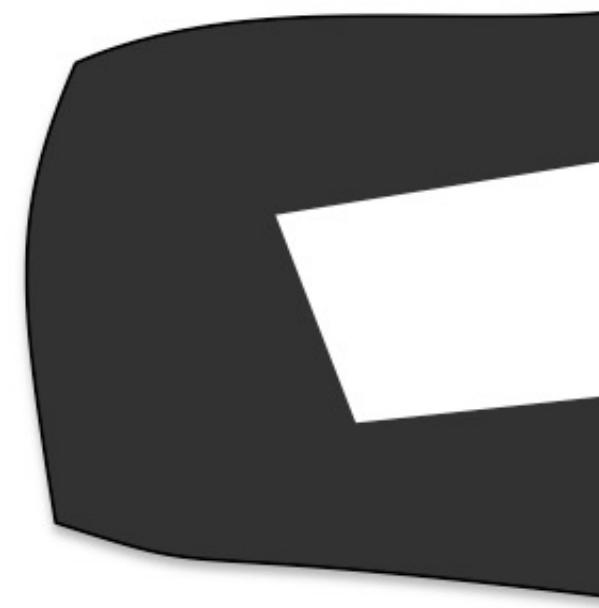
**Original Prediction:** John Elway

**Prediction under adversary:** Jeff Dean

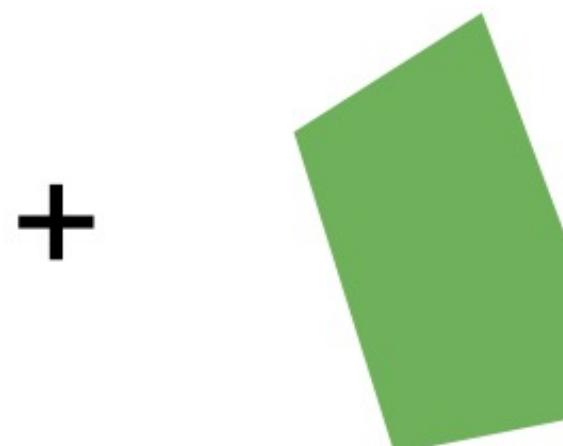
# What can you do?

Spend significant time on good evaluations!

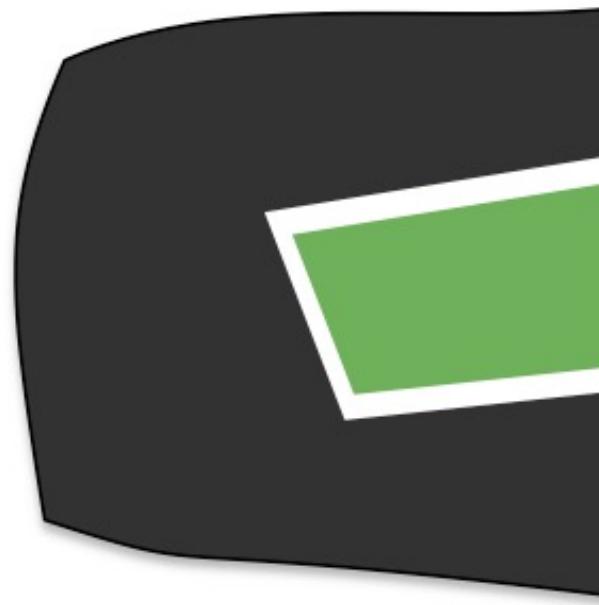
Target



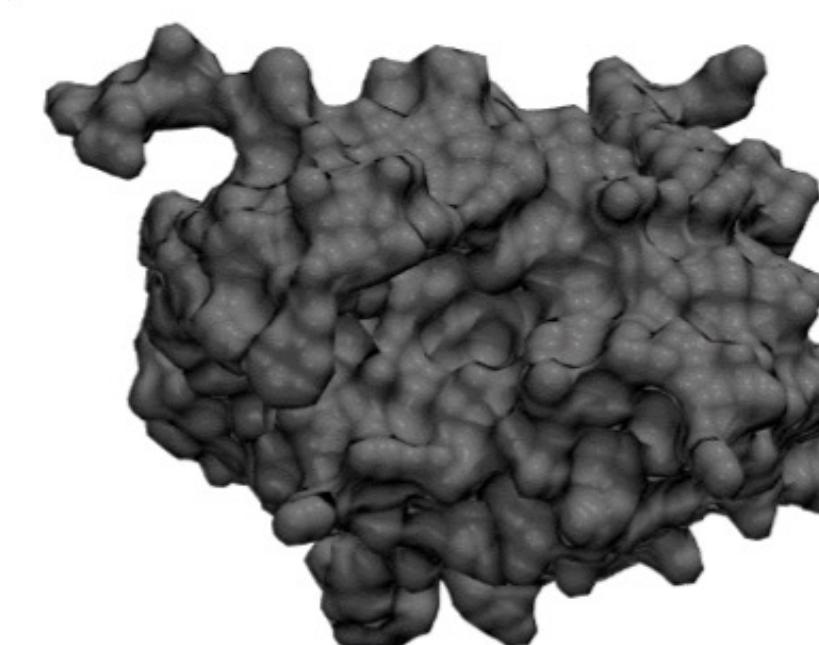
Ligand



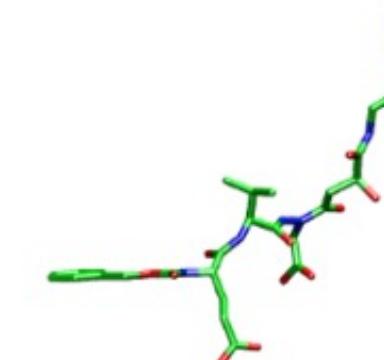
Complex



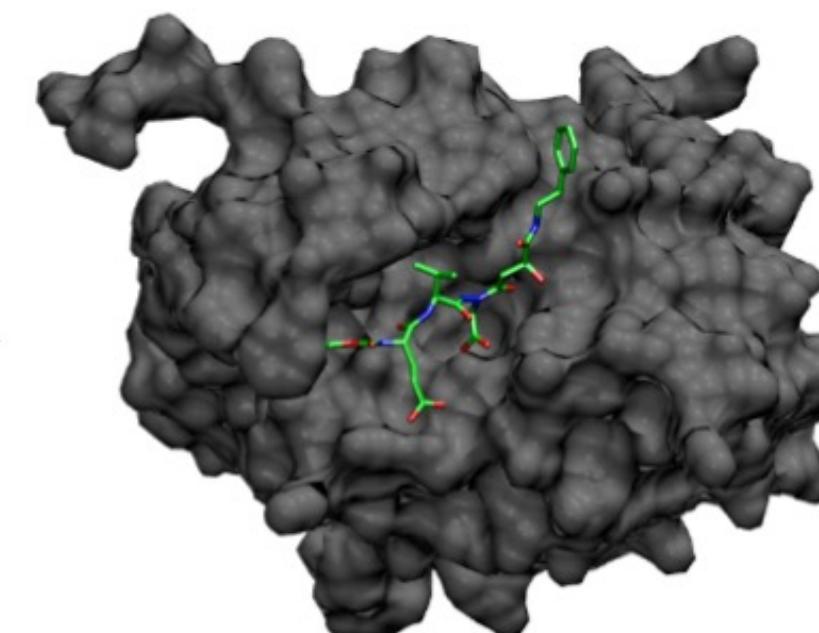
docking



+

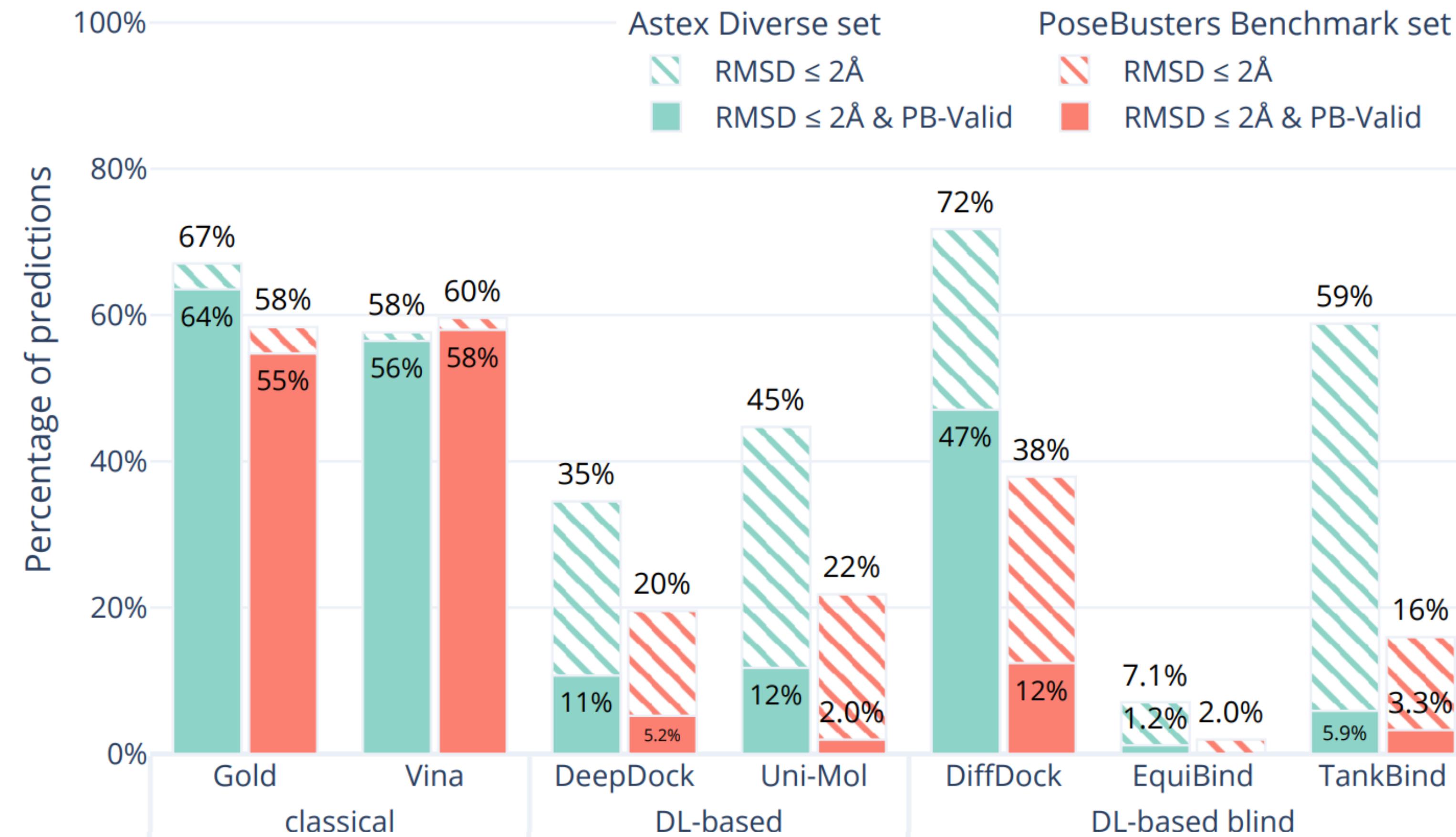


docking



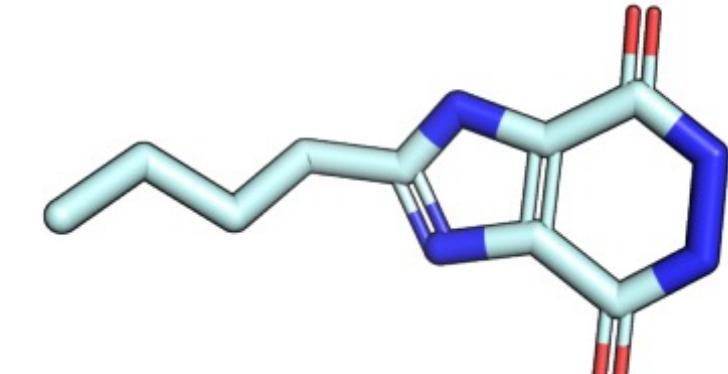
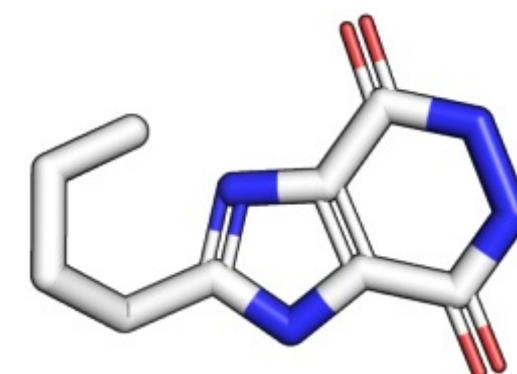
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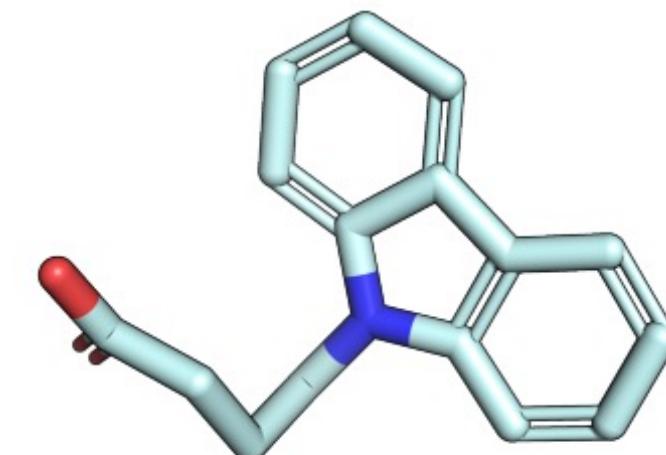
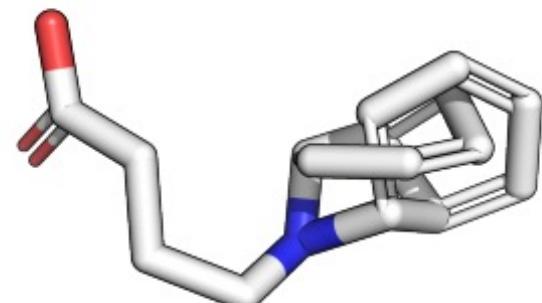


# What can you do?

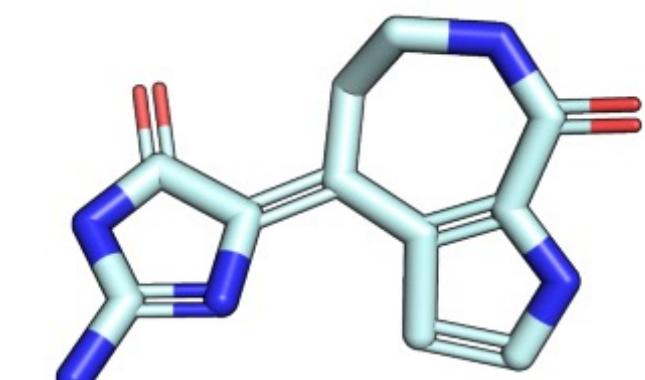
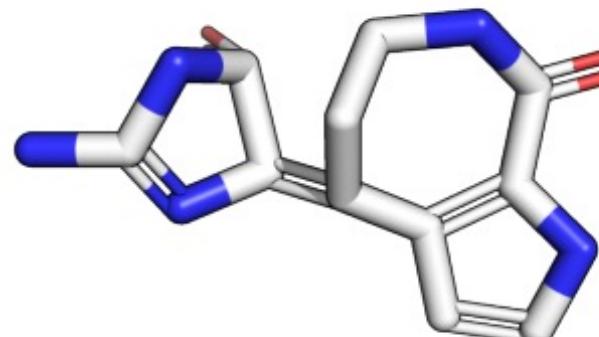
Spend significant time on good evaluations!



(d) Internal clash. DeepDock prediction for ligand BDI of protein-ligand complex 1N2V. RMSD 1.6 Å.



(e) Aromatic rings not flat. TankBind prediction for ligand CRZ of protein-ligand complex 1TOW. RMSD 2.2 Å.

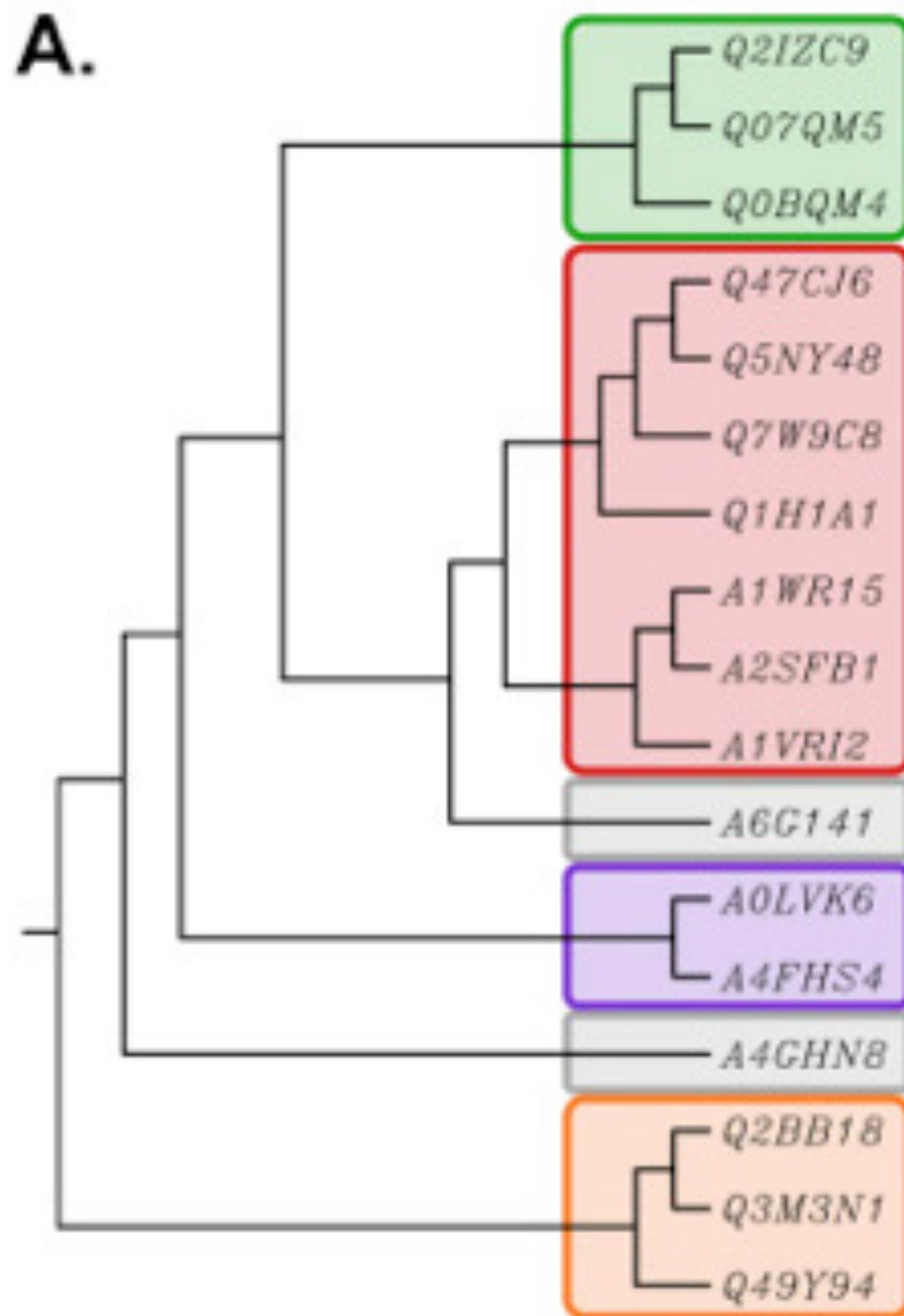


(f) Double bond not flat. TankBind prediction for ligand DBQ of protein-ligand complex 1U4D. RMSD 1.7 Å.

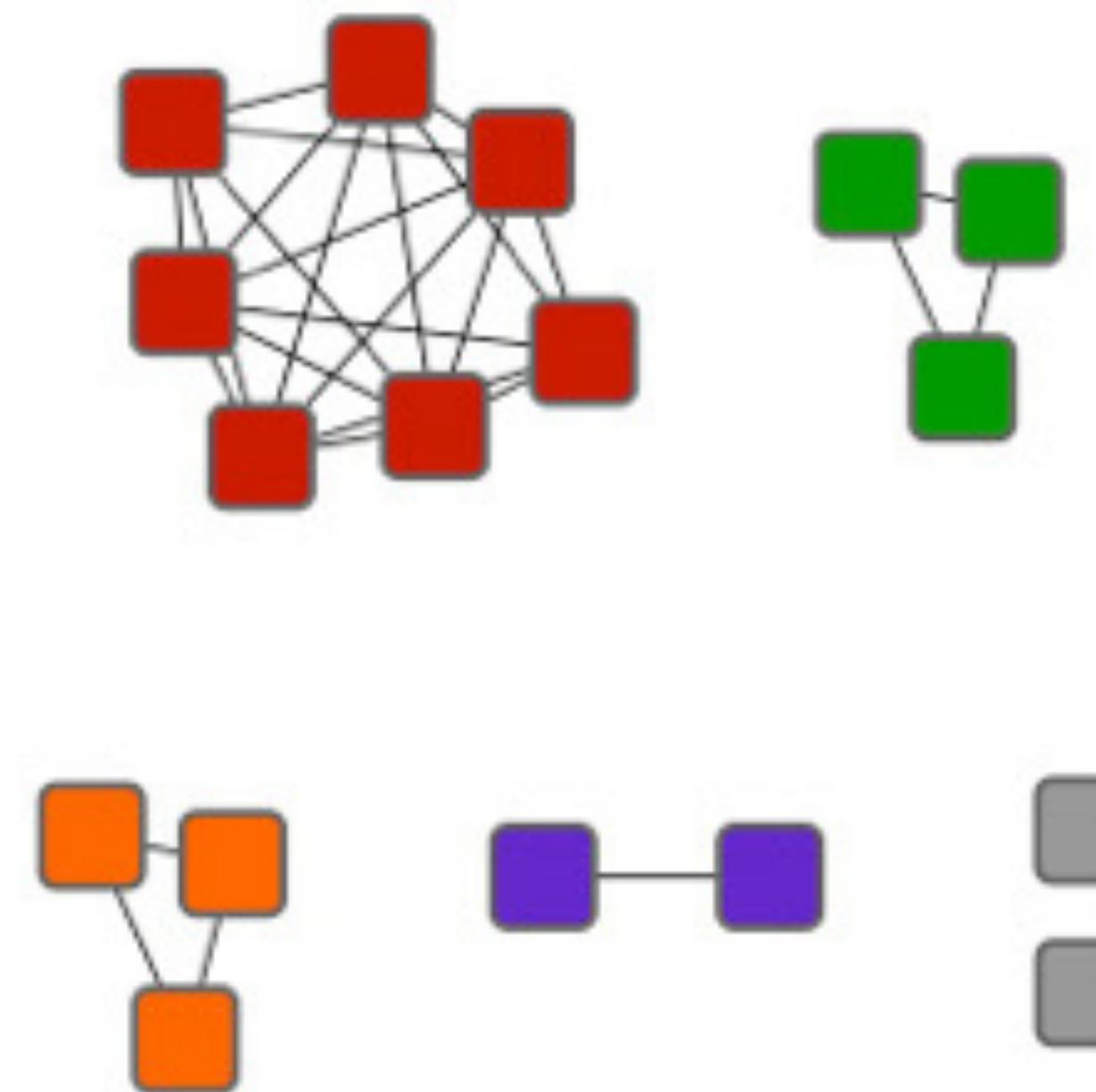
# What can you do?

Prepare your datasets to avoid heuristic short-cuts for models!

A.



B.



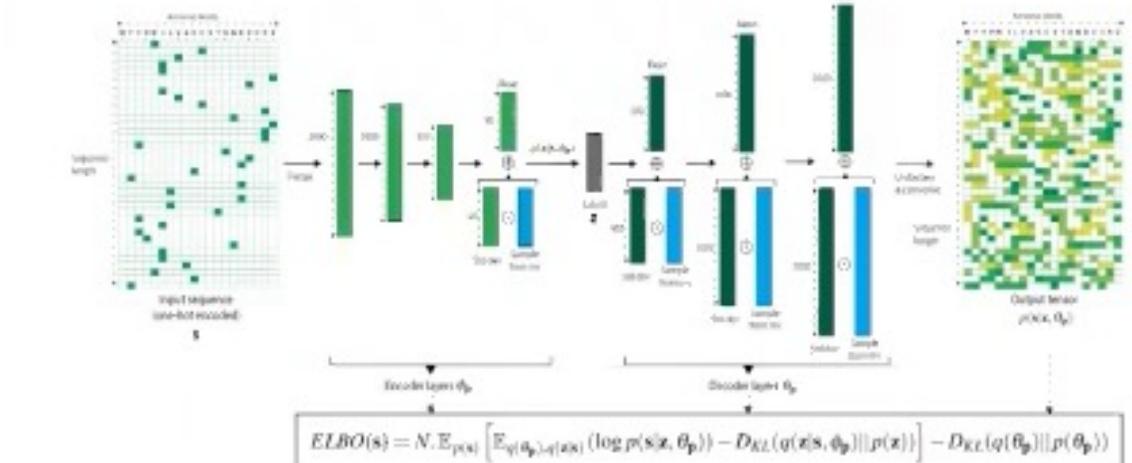
# **5. Current Research**

# Limitations of current approaches

## Both alignments and PLMs have pros and cons

### Alignment-based models

- Learn a distribution from sequences in a **Multiple-Sequence Alignment** (MSA) -- either at **position level** (e.g., Site independent<sup>1</sup>), **pairs of positions** (eg., EVmutation<sup>1</sup>) or **full sequence** (eg., DeepSequence<sup>2</sup>, EVE<sup>3</sup>)
- Limitations:
  - **Unable to score insertions & deletions** ('indels')
  - **Need fairly deep alignments** to learn complex dependencies across positions (certain proteins are difficult to align eg., disordered proteins)
  - **Lack of information sharing** across families (each model is trained from scratch)



### Protein language models

- Train a **(masked) language model** on large quantities of **aligned sequences** (eg., MSA Transformer<sup>4</sup>) or **non-aligned sequences** (eg., ESM-1v<sup>5</sup>) **across protein families**
- Since MLMs **do not learn a proba over full protein sequences**, fitness is approximated via the **masked-marginals heuristic**:

$$\sum_{t \in T} \log p(x_t = x_t^{mt} | x_{\setminus T}) - \log p(x_t = x_t^{wt} | x_{\setminus T})$$

- Limitations (MLMs):
  - **Unable to score insertions & deletions** ('indels')
  - **Approximation for multiple mutations:** ignore dependencies across mutations
  - **Mismatch between training Vs inference:** mask 15% tokens during training Vs 1+ token(s) at inference

# How can we augment our language models?

Give them access to tools and resources in the outside world

## Retrieval



Augment with  
a bigger corpus

## Chains



Augment with  
more LLM calls

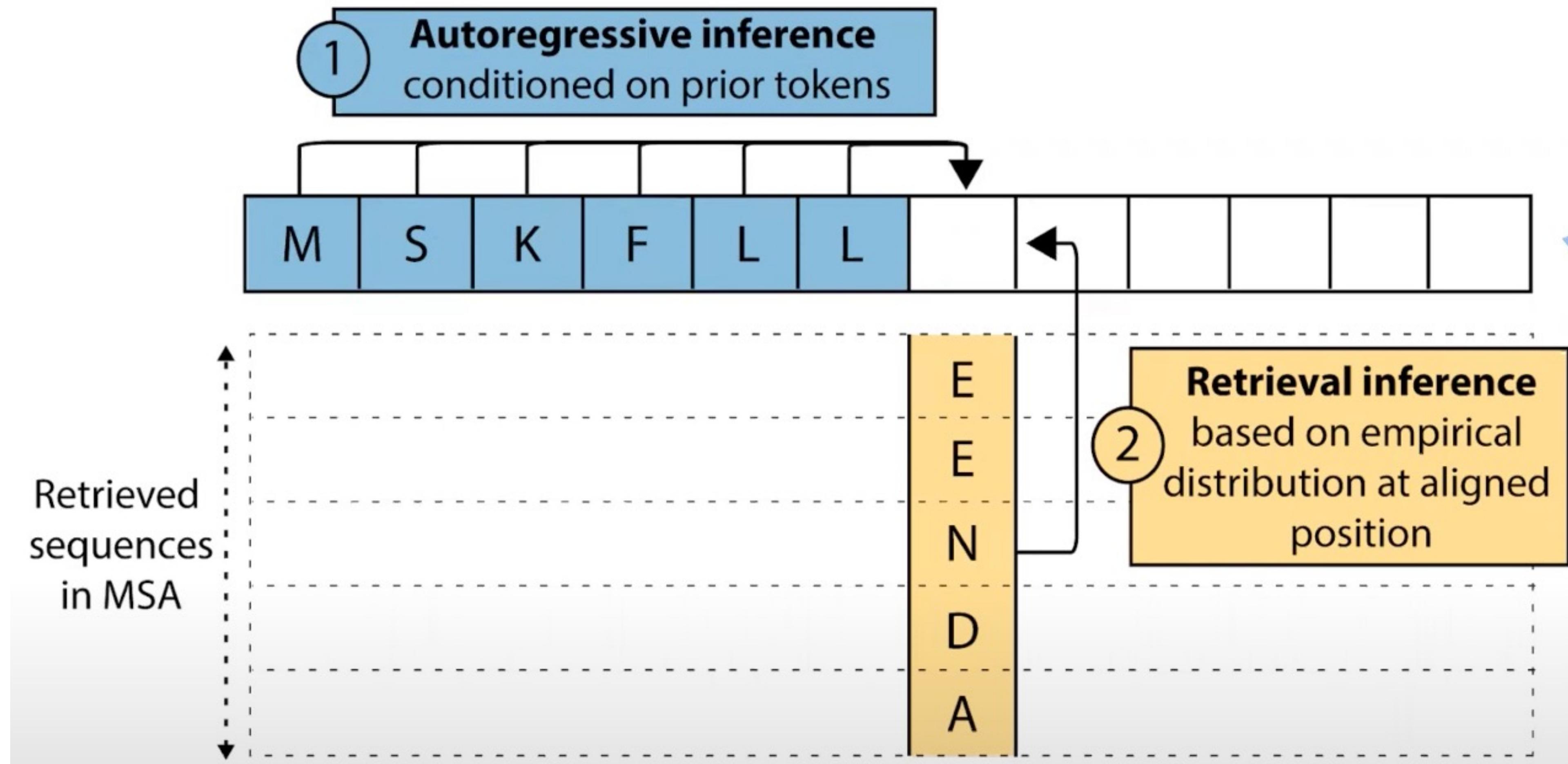
## Tools



Augment with  
outside sources

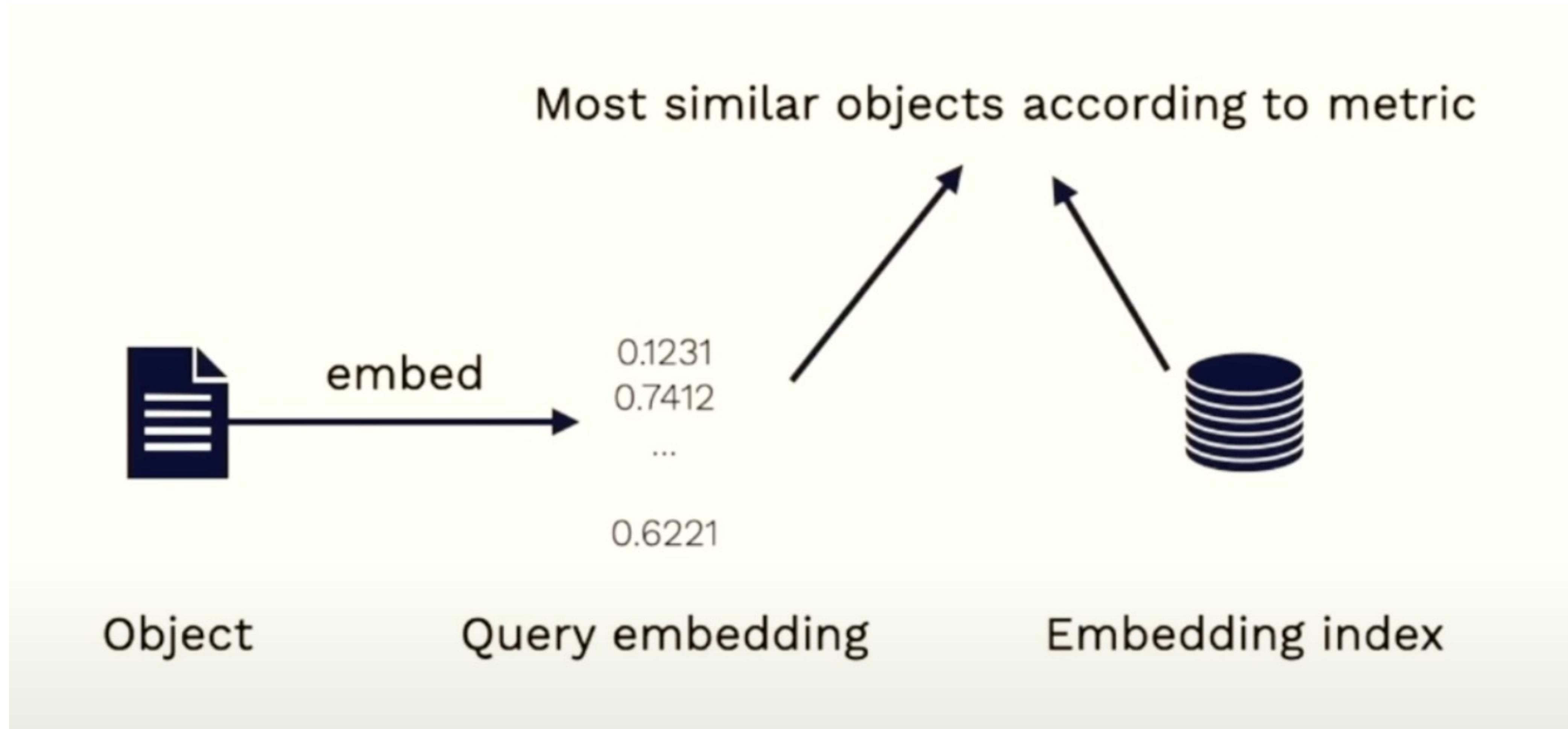
# Tranception: a transformer with retrieval

Combine the best of both worlds



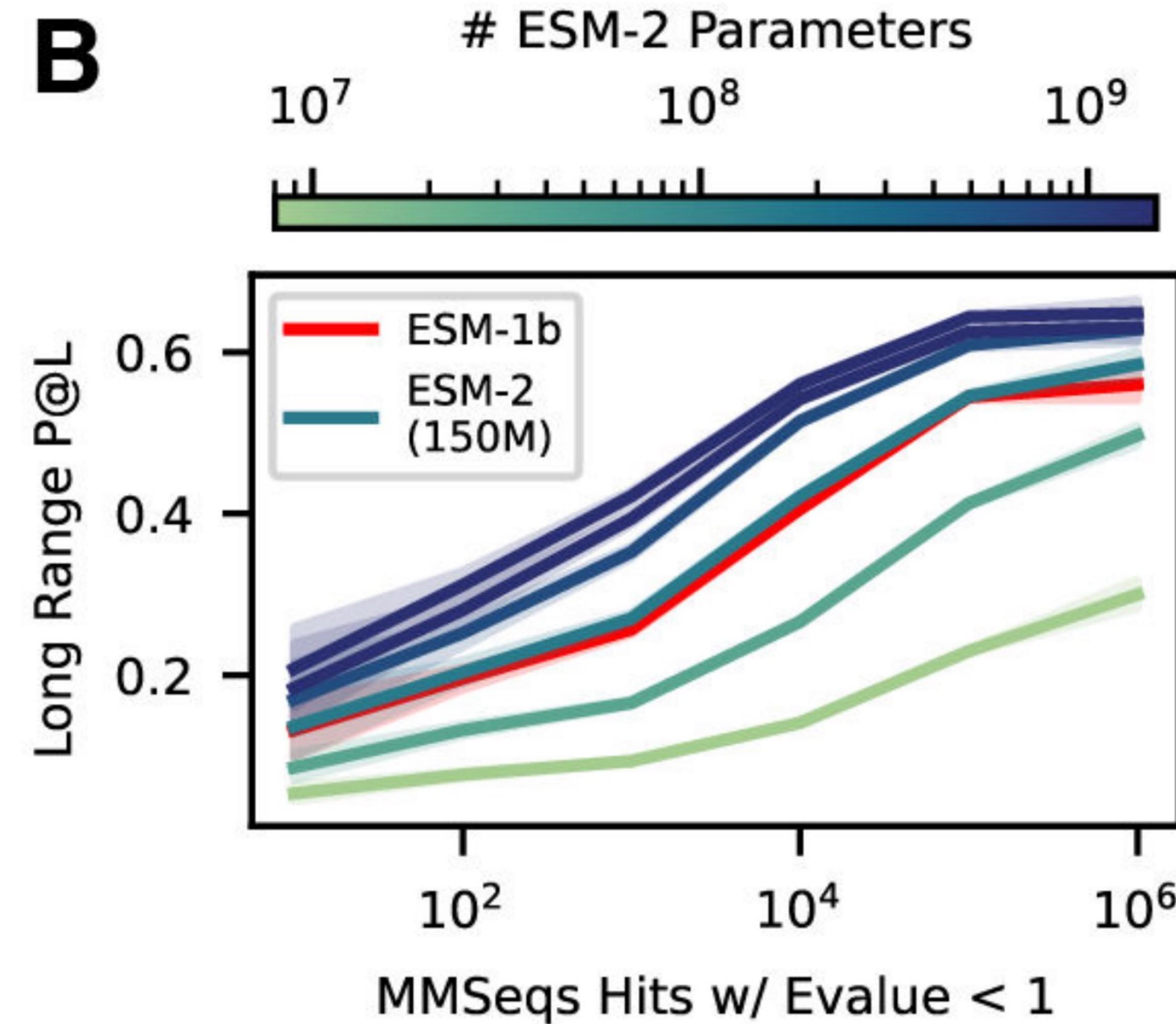
# We can get clever with retrieval

We can use learned embeddings to compare similarities!



# Scaling Laws: Bigger is better?

Are we at the end of scaling? A controversial topic





# Takeaway



While **protein language models** show strong performance on a number of tasks, **relevant and meaningful evaluations** are still an active area of research.