Case Study with deepCDR: Engineering Trastuzumab

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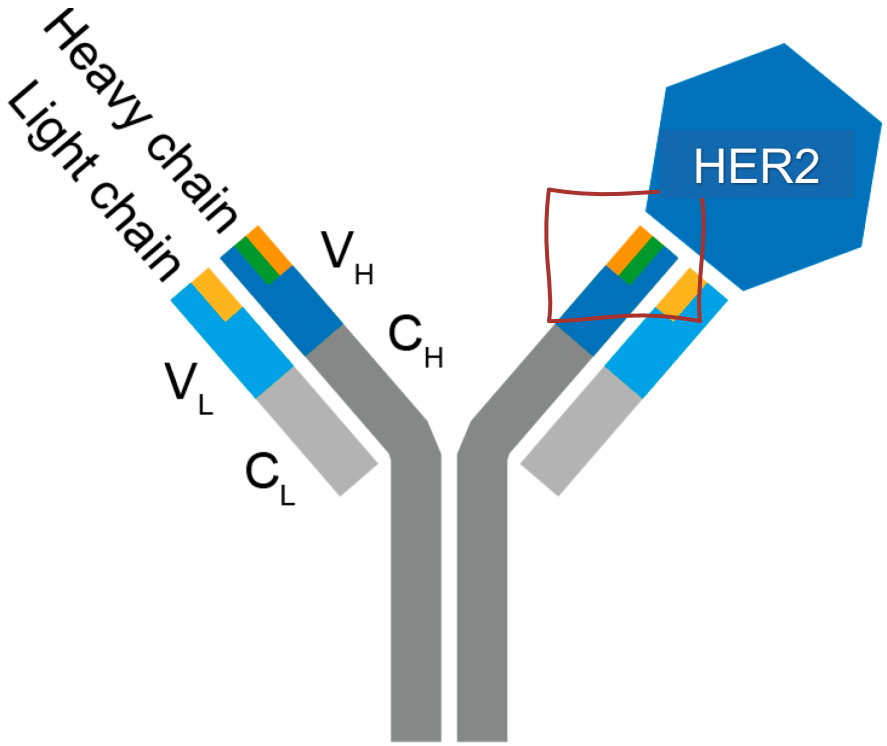
31. August 2021

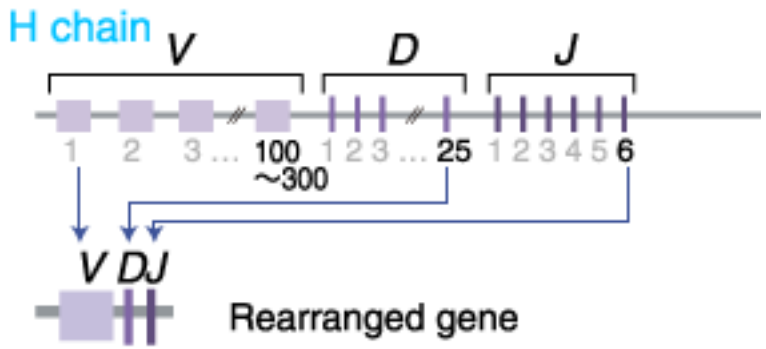
Agenda

- Introduction
- Task 1: Predicting the binding status of novel Trastuzumab Variants
- Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2
- Take-away messages

Diversity in CDR3 regions determines antibody binding specificity



Trastuzumab



Genetic diversity

CSRYGVGGMFPYVYW
CSRFGQAALYAYCYW

.....

CSRWVASGFFVYLYW
CSRYNNGGLYVYRYW

CDR3 Sequence diversity

Strong binders?
Weak binders?
Non-binding?

Functional diversity

[1] Kreer, C et al. *Vaccines* **2020**, *8*, 13. https://doi.org/10.3390/vaccines8010013
[2] https://www.mblbio.com/bio/g/support/method/antibody-role.html

Machine/Deep learning optimizes antibody specificity by in-silico prediction

- Challenges:
  - Conventional bio-assays to measure binding affinity in a large-scale
- Modern solutions :
  - Machine learning models can predict binding affinities to pre-select variants from a large candidates pool

=> Acclerate antiboody discovery and optimization

CDR3 sequences

CSRYGVGGMFPYVYW
CSRFGQAALYAYCYW

.....
CSRWVASGFFVYLYW
CSRYNNGGLYVYRYW

X = sequence, Y = affinity

$$Y = f(X, \beta) + \varepsilon$$

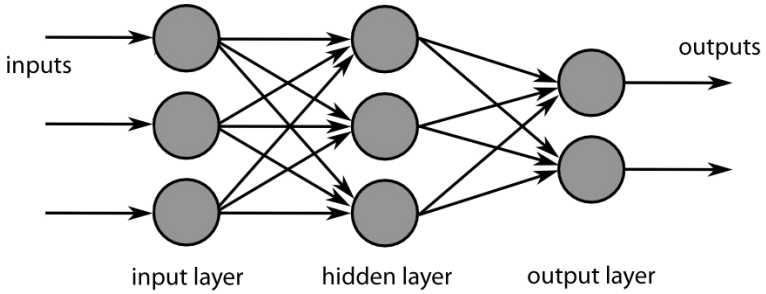
predict

Affinity

Strong binders?
Weak binders?
Non-binding?

Simple: linear regression

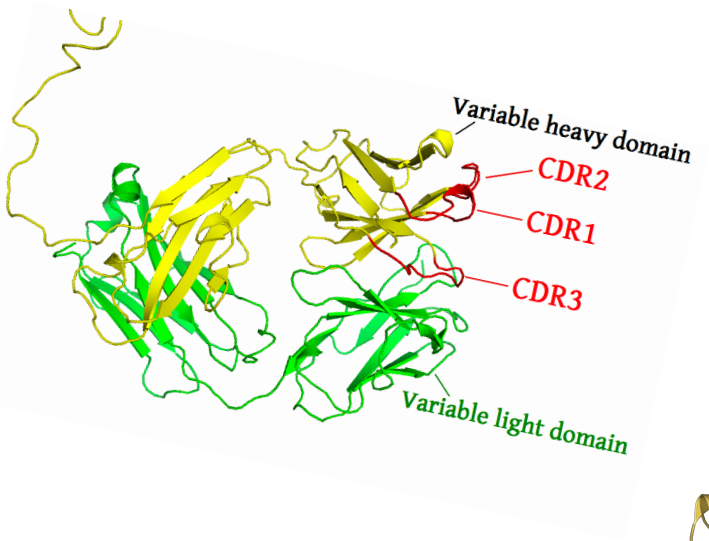
Complex:

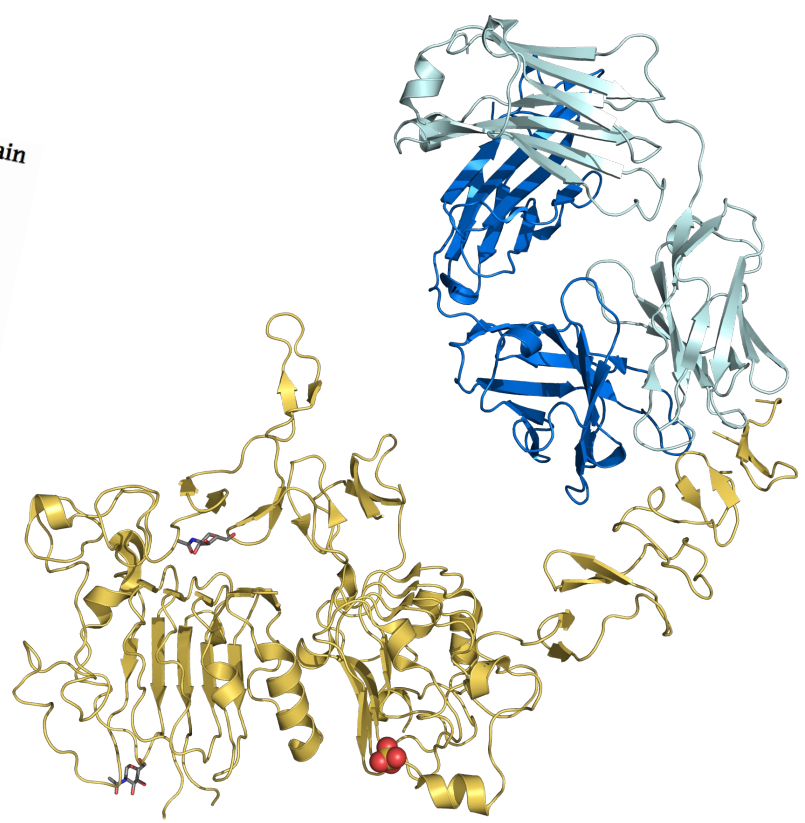


Deep neural network

https://de.wikipedia.org/wiki/Deep_Learning

Task 1: Predicting the binding status of novel Trastuzumab Variants





CDR3 sequences
of Trastuzumab variants

HER2

Binary classification

- Binding?
- Non-binding?

https://en.wikipedia.org/wiki/HER2/neu

Task 1: Predicting the binding status of novel Trastuzumab Variants

Key outlines:

1. Sequencing quality check
2. Sequence diversity analysis

1. Positional One-hot encoding
2. Feature-representation from Pre-trained language model

1. Convolutional neural networks
2. Random forest

Task 1: Predicting the binding status of novel Trastuzumab Variants

Quality control and exploratory data analysis

Data quality check

1. Applied Q score to ensure sequence quality control (QC):
    - Q score with thershold 25
        - Exclude any sequence with any base has a Q score less than 25
        - A rule of thumb cut-off is 20~30, apply a moderate thereshold 25 should guarantee high sequence quality

2. Deleted repetitive binders and non-binders with identical CDR3 amino acid sequence, however with altered CDR3 nucleotide sequence (due to codon degeneracy)

Number of Binder* entries		Number of Non-binder entries	
Before QC	After QC	Before QC	After QC
6146	**3725**	3888	**3620**

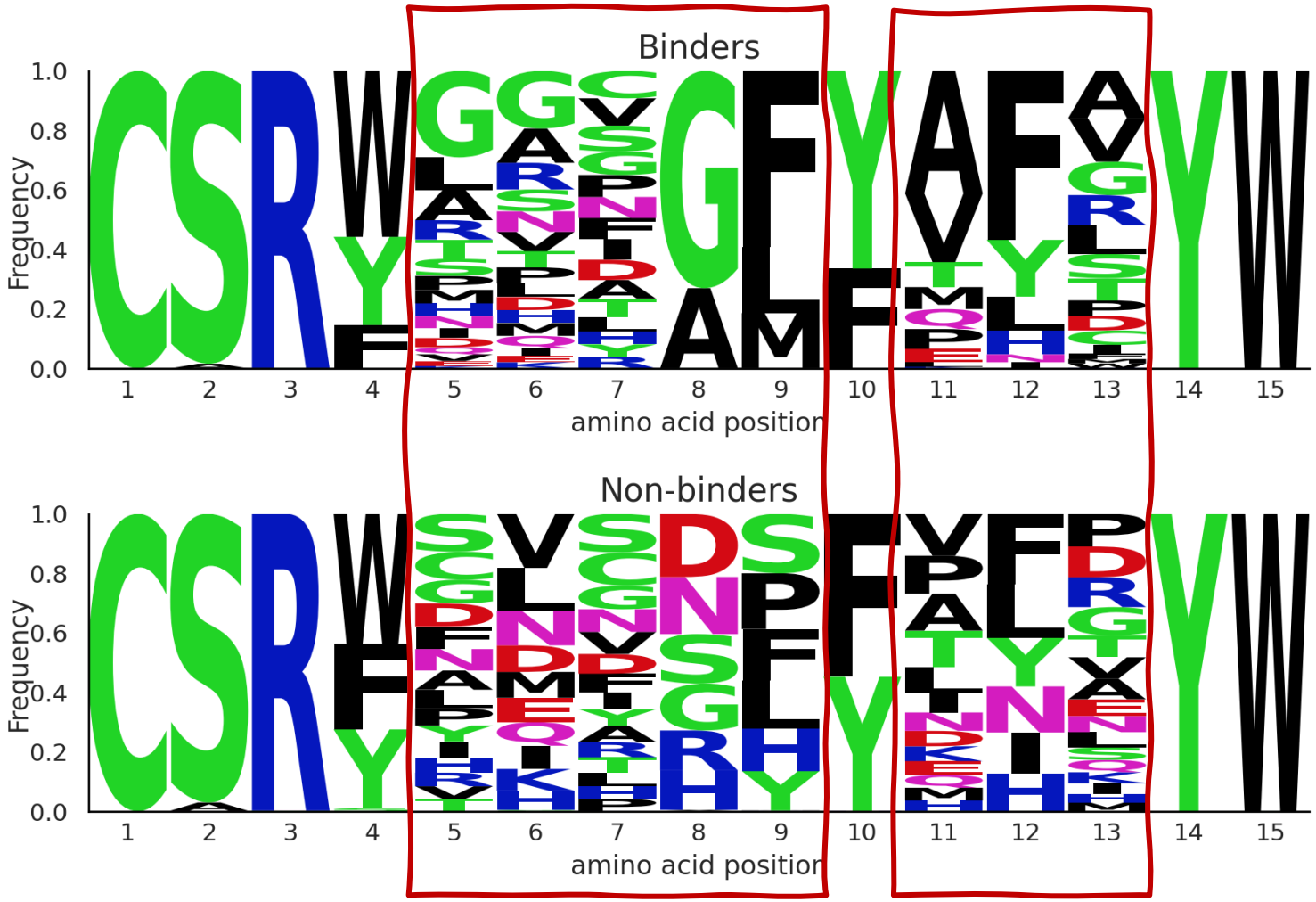
=> After two steps, data was used for downstream analysis

* note: for binders, two dataset was merged and performed quality check

[1] Q-score calculated referencing https://drive5.com/usearch/manual/quality_score.html

Positional amino acids (AAs) diversity analysis in CDR3 sequences

- Length of AA sequences : 15 residues
- **Binders and non-binders differ mainly from position 5-13**
  - Position 1-3 are moslty CSR, ending residues are YW
  - Frequency and compositions of AAs from pos. 5-13 vary



Task 1: Predicting the binding status of novel Trastuzumab Variants

Feature engineering

Feature engineering of CDR3 AA sequences representation

- **Key challenges:**
  1. To transform string-like AA sequences to machine-readable numeric values `CSRYW => 1,2,3,4,5,6`
  2. To reserve positional relationships of AAs
  3. To reserve functional properties of AAs

❖ Select AA sequences rather than nucleotide sequences, as:

- AA impacts protein interaction directly
- More diverse categories (20) to encode

Solutions:

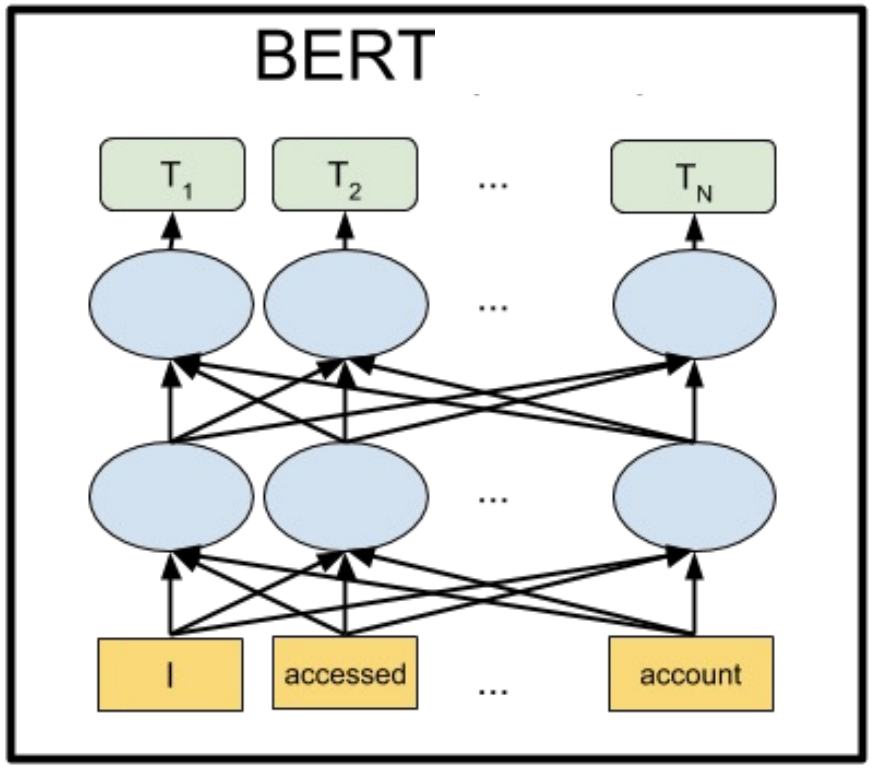
I. **Natural language processing (NLP) approach to learn 1024-vector numeric representation for each sequence**
II. **Positional one-hot encoding deriving 15 X 20 matrix to represent each sequence**

[1] Journal of Cheminformatics volume 5, Article number: 41 (2013)
[2] Mason, D.M., Friedensohn, S., Weber, C.R. *et al.* Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. *Nat Biomed Eng* **5,** 600–612 (2021).

Feature engineering of CDR3 AA sequences

I. Natural language processing (NLP) approach to learn 1024-vector numeric representation for each sequence

- AA sequence is a language with unique syntax and semantics



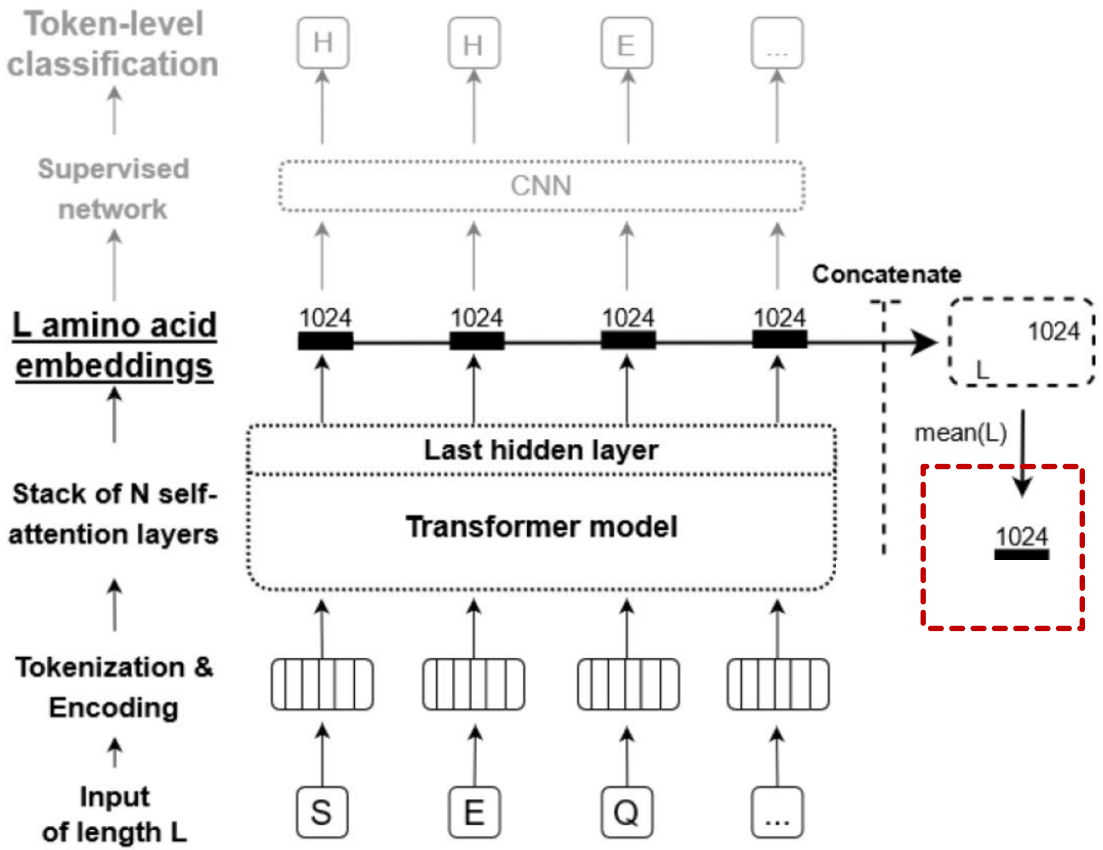
Key ideas:
Leverage BERT (**B**idirectional **E**ncoder **R**epresentations from **T**ransformers) model pretrained with large-scale protein sequences to generate informative representation

- bi-directional learning to gain interconnected context of CDR3 sequences from both left to right context and right to left context simultaneously

[1] A. Elnaggar *et al.*, "ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing," in *IEEE Transactions on Pattern Analysis and Machine Intelligence*, doi: 10.1109/TPAMI.2021.3095381.
[2] https://ai.googleblog.com/2018/11/open-sourcing-bert-state-of-art-pre.html

Feature engineering of CDR3 AA sequences

I. Natural language processing (NLP) approach to learn a 1024-vector numeric representation for each sequence



Leverage BERT (**B**idirectional **E**ncoder **R**epresentations from **T**ransformers) model pretrained with large-scale protein sequences to generate informative representation

Key steps:

1. Tokenize CDR3 sequence and create positional encoding, feed forward to BERT
2. Generate 1024-long vector embedding for each AA
3. Concatenate embeddings and average them into one 1024-vector representation

[1] A. Elnaggar *et al.*, "ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing," in *IEEE Transactions on Pattern Analysis and Machine Intelligence*, doi: 10.1109/TPAMI.2021.3095381.

Feature engineering of CDR3 AA sequences

I. Natural language processing (NLP) approach to learn 1024-vector numeric representation for each sequence

II. Positional one-hot encoding deriving 2-dimensional 15 X 20 matrix to represent each sequence

Example sequence

C	S	R	Y	G	V	G	G	M	F	P	Y	V	T	W

=>

A	C	D	E	F	G	B	J	MLQRS	T	V	W	G
0	1	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	0	1	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	1	0	0
0	0	0	0	0	1	0	0	...	0	0	0	0
0	0	0	0	0	1	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	1	0	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	0
0	0	0	0	0	0	0	0	...	0	0	0	1
0	0	0	0	0	0	0	0	...	0	1	0	0
0	0	0	0	0	0	0	0	...	0	0	0	1
0	0	0	0	0	0	0	0	...	0	0	1	0

Key ideas:

- each row is a 20-d vector with one-hot encoding represents presence of one AA given 20 natural AAs
- 15 rows presents AA positions in the whole sequence
- Reserve positional relationships and diversity representation of AAs

[1] Mason, D.M., Friedensohn, S., Weber, C.R. *et al.* Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. *Nat Biomed Eng* **5**, 600–612 (2021).

Task 1: Predicting the binding status of novel Trastuzumab Variants

Classification

Classification with NLP-learned & one-hot encoded representations: Positional one-hot encoding (OHE) outperforms NLP-learned representations

Dataset:
- 3725 binding sequences
- 3620 non-binding sequences
  train:test = 80:20

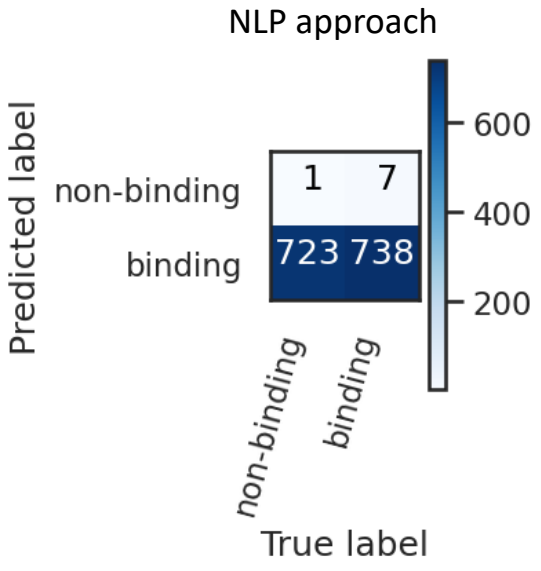
Input:
- 1024-dimensional feature vector
- 15 X 20 positional encoded matrix
  - Flattened to 300-d vector for algorithmic convenience

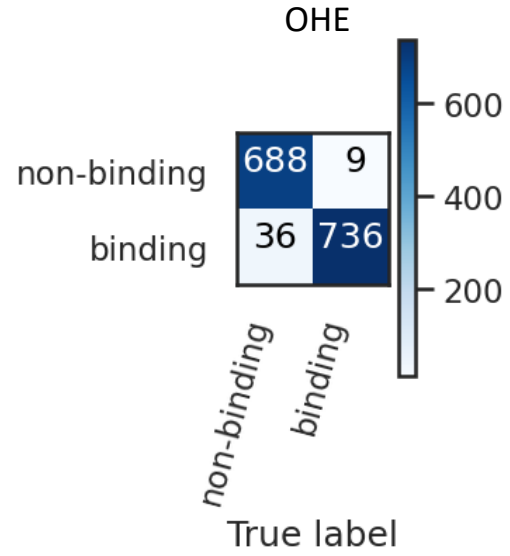
Model:
- Random forest classifier
  - Parameters tuned by grid-search

Performance evaluation
- 10-fold cross-validated
- f1, accuracy score

Test set result

	Test - F1 score	Test - accuracy
NLP	0.669	0.503
OHE	**0.970**	**0.968**

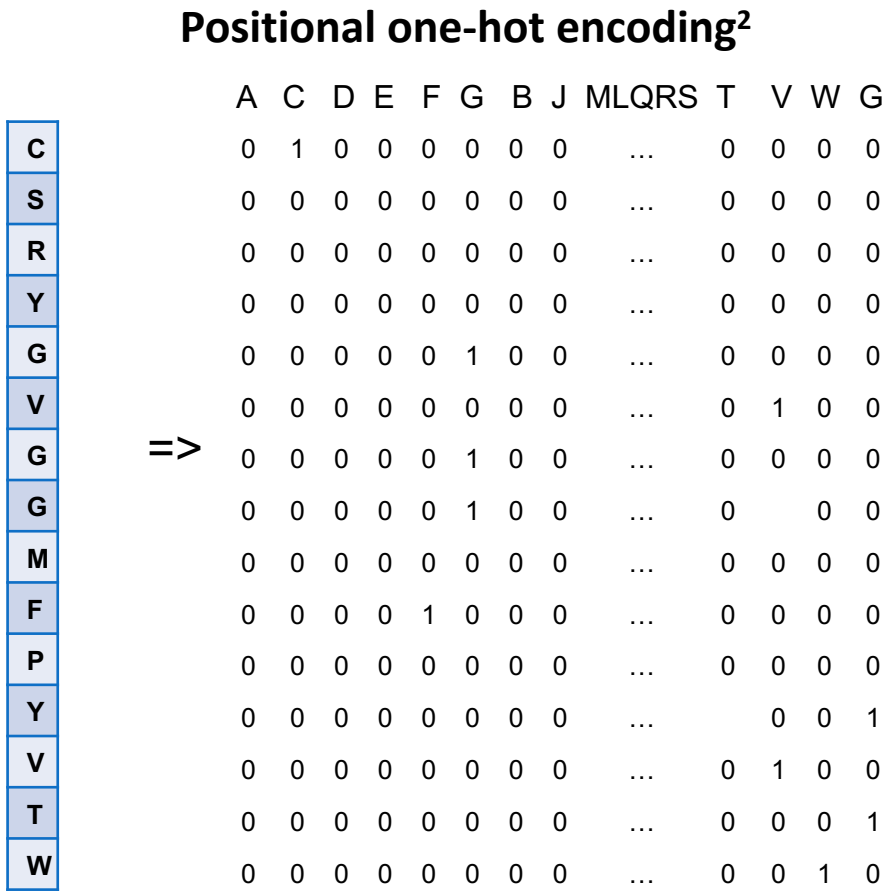




Therefore, positional one-hot encoder was seleceted for Further experiments

Learning with positional one-hot encoding

- Positional one-hot encoding deriving 2-dimensional 15 X 20 matrix to represent each sequence:

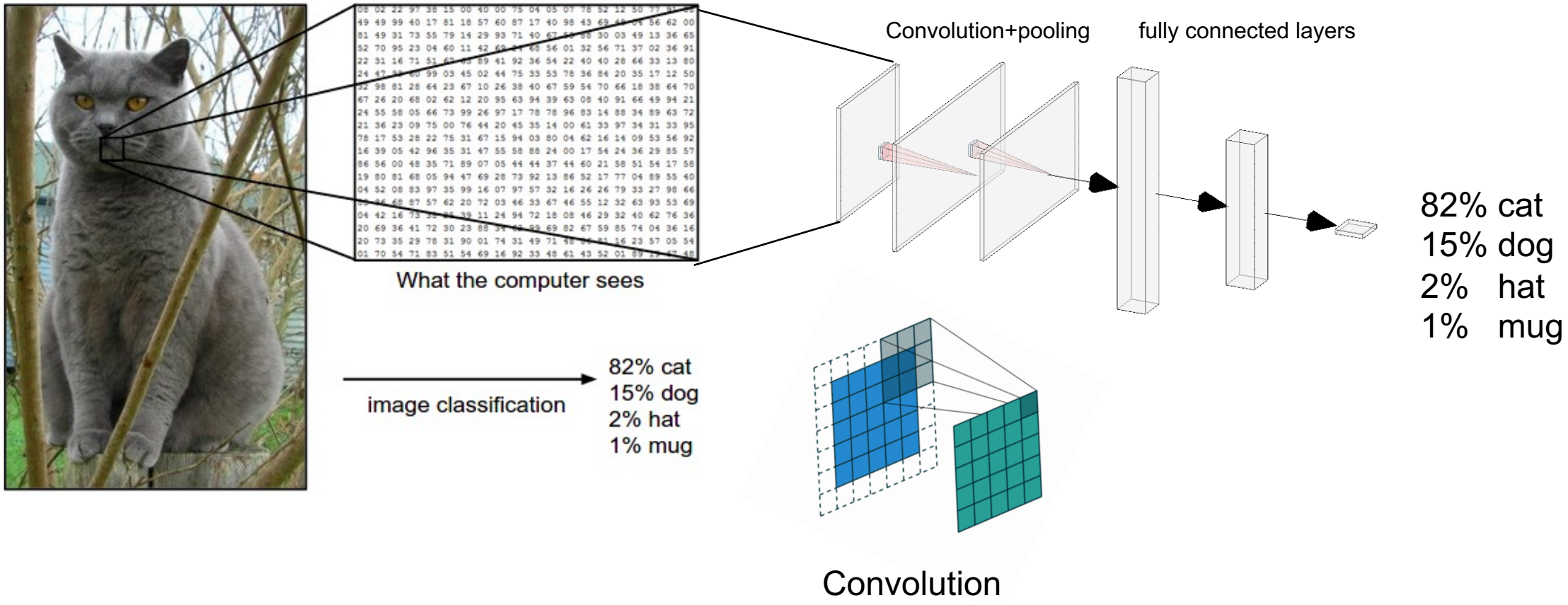


Best way to learn from spatial features?

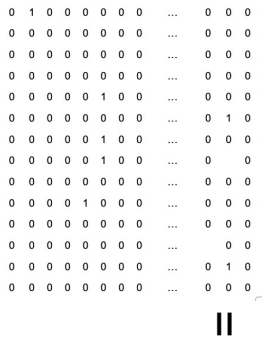
[1] A. Elnaggar *et al.*, "ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing," in *IEEE Transactions on Pattern Analysis and Machine Intelligence*, doi: 10.1109/TPAMI.2021.3095381.
[2] Mason, D.M., Friedensohn, S., Weber, C.R. *et al.* Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. *Nat Biomed Eng* **5,** 600–612 (2021).

Classification with Convolutional Neural Networks (CNNs)

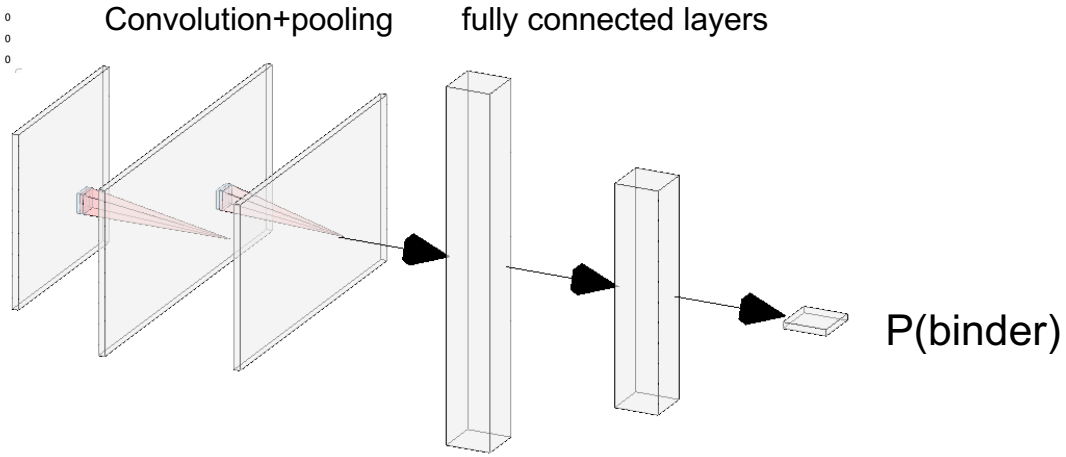
- CNNs is inspired from how brain perceives/recognize images



Classification with one-hot encoded representation and Convolutional Neural Networks (CNNs)



Vannilla CNN



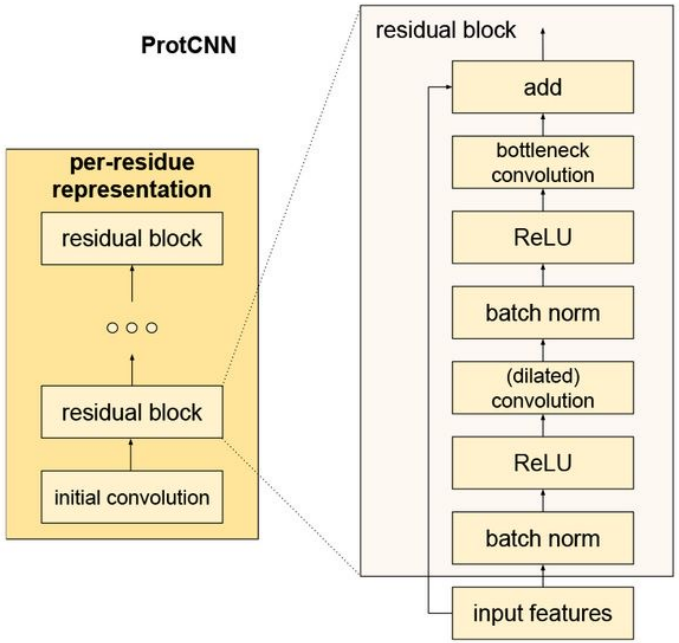
Simple architecture:

- Two convolution + max pooling layers
  - Extract spatial features and reserve spatial relationships (fits AA sequence structure)
- Two dense layers with batch normalization and dropouts to avoid overfitting

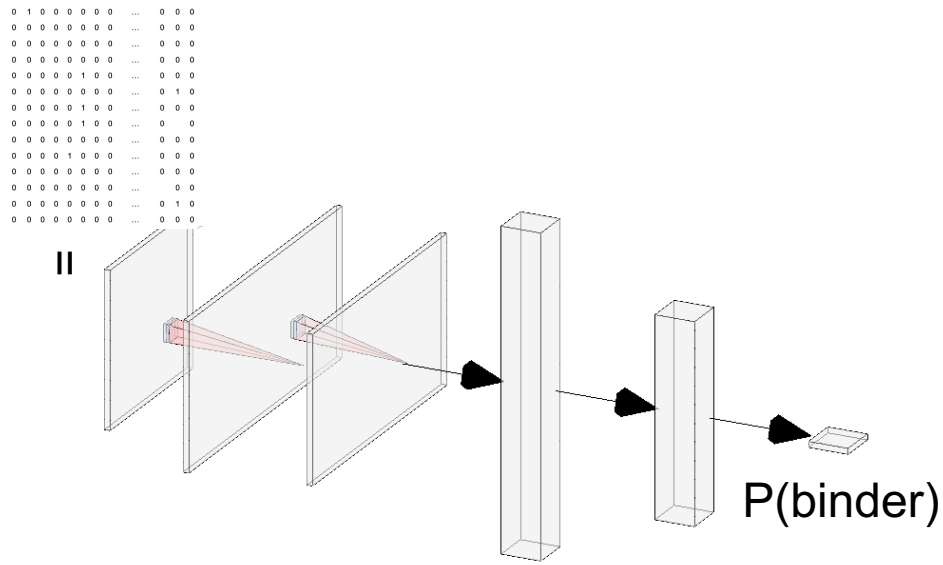
[1] Maxwell L. Bileschi, David Belanger, Drew Bryant, Theo Sanderson, Brandon Carter, D. Sculley, Mark A. DePristo, Lucy J. Colwell bioRxiv 626507; doi: https://doi.org/10.1101/626507
[2] arXiv:1512.03385

Classification with one-hot encoded representation and CNNs

Res-CNN[1]



Vannilla CNN



Key architecture: CNN + residual blocks + dilated convolution

- Residual blocks: avoid vanishing/exploding gradient problems[2]
- Dilated convolution : increase receptive field size, to include more spatial features with less computations

[1] Maxwell L. Bileschi, David Belanger, Drew Bryant, Theo Sanderson, Brandon Carter, D. Sculley, Mark A. DePristo, Lucy J. Colwell bioRxiv 626507; doi: https://doi.org/10.1101/626507
[2] arXiv:1512.03385

Classification with one-hot encoded representation:

Res-CNN slightly outperforms vanilla CNN architecture

Dataset:
- 3725 binding sequences
- 3620 non-binding sequences

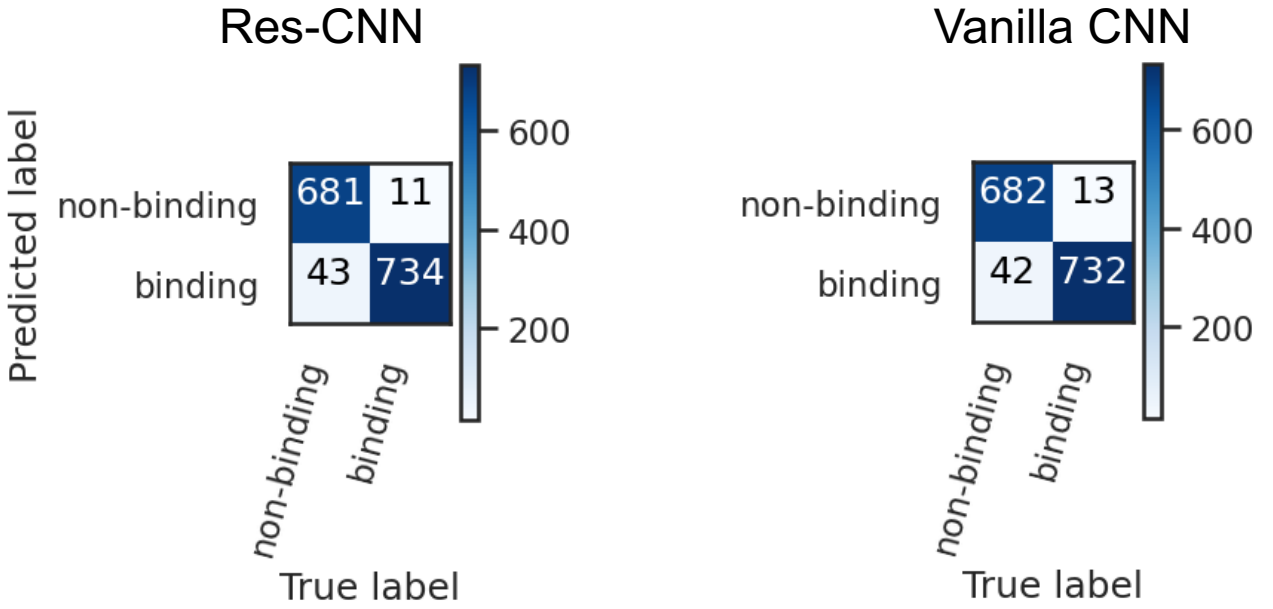
  Train : validation : test = 60:20:20

Input:
- 15 X 20 positional encoded matrix

Model:
- ResCNN & CNN
  - Trained for 200 epochs with early stopping and regularization

Performance evaluation
- Binary entropy loss
- F1, accuracy score

	Test - F1 score	Test - accuracy
Res-CNN	0.9634	0.9632
Vanilla CNN	0.9627	0.9626



Task 1: Predicting the binding status of novel Trastuzumab Variants

Conclusion:

Task 1 is a friendly start ☺, the key is to extract feature from the AA sequences

1. Highly accurate prediction of binding status of Trastuzumab can be achieved:
   - Highest: with random forest classifier (positional one-hot encoded) f1 score 0.97, accuracy 0.968
   - Res-CNN slightly outperforms vanilla CNN, slightly less performs the random forest classifier

2. CDR3 sequence embedding with positional one-hot encoding outperforms representations learned by pretraind BERT model

	Test - F1 score	Test - accuracy
Res-CNN with OHE	0.9634	0.9632
Vanilla CNN with OHE	0.9627	0.9626
Random forest with NLP encoding	0.669	0.503
Random forest with OHE	**0.970**	**0.968**

Task 1: Predicting the binding status of novel Trastuzumab Variants

Discussion & future perspective

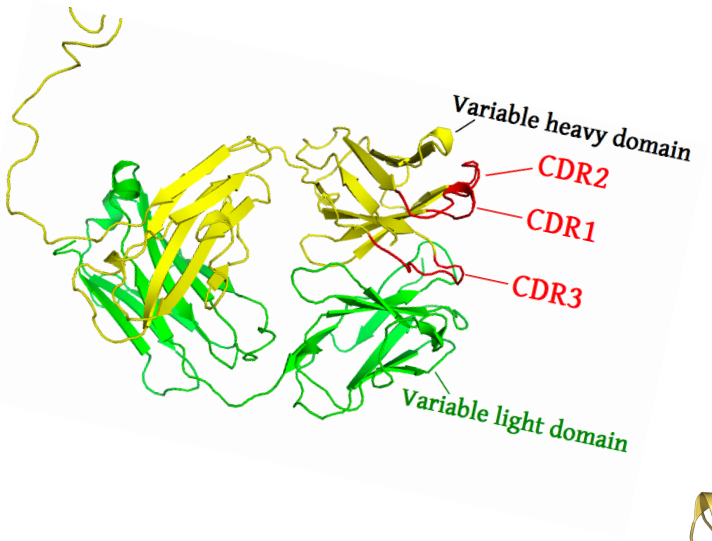
1. Positional one-hot encoding scheme retains positional relationships between AAs in a sequence
2. Learned sequence representations are 1024-dimensional numerical vector, might not reserve structural information
   ⇒ Averaging the AA representation might lead to loss of information
   ⇒ CDR3 Sequences are too short in comparison with protein that are used for pre-training
   ⇒ Needs for fine-tuning
   ⇒ Other representations can be experimented: such as protein descriptors
3. Skipping conventional tokenized & padding approach because:
   1. Sequence is too short (15 residues) to be padded
   2. Padding might lead to incomplete truncation on sequence, results in inaccurate feature extraction

*NLP-based approach was chosen out of personal interest/curiosity in experimenting new methods; positional OHE was chosen by its demonstrated good performance in literatures[1] and importance of AA and structural information to protein-antibody binding

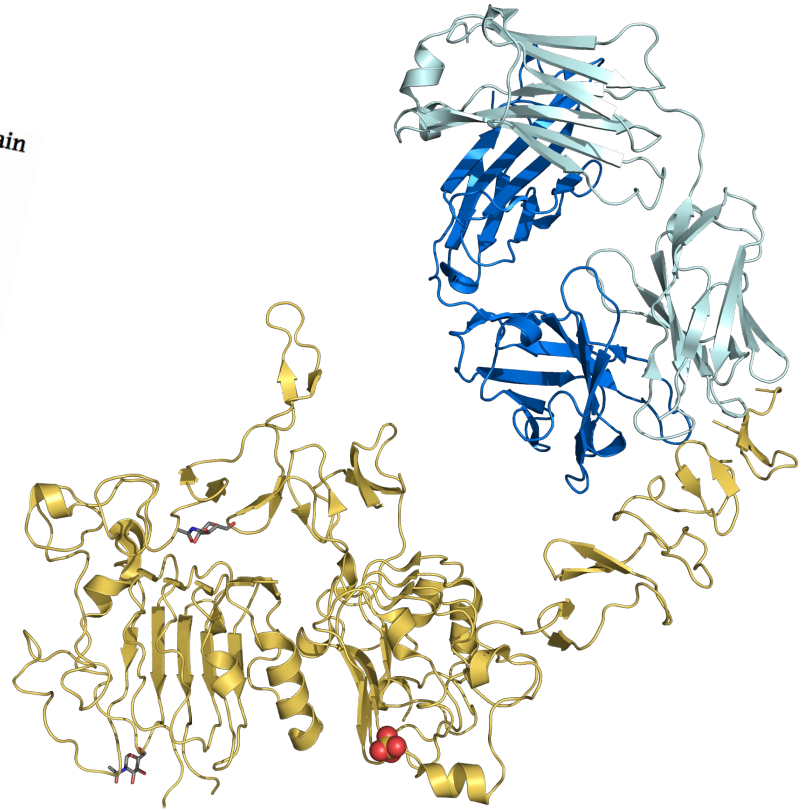
[1] Mason, D.M., Friedensohn, S., Weber, C.R. *et al.* Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. *Nat Biomed Eng* **5,** 600–612 (2021).

Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

- Very challenging problem due to small dataset (90 entries)



CDR3 sequences
of Trastuzumab variants



HER2

Regression

Binding affinities:
KD (nm)

https://en.wikipedia.org/wiki/HER2/neu

Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

Key outline:

1. Data quality check
2. Log normalization of KD(nm)
3. Sequence diverisity analysis

1. Data augmentation with AA replacement

1. CNN
2. K-nearest neighbor

Final prediction see: https://github.com/PinaColadast/CDR3_prediction_case/blob/main/task2_predicted.csv
Implementation detals see https://github.com/PinaColadast/CDR3_prediction_case/blob/main/deepCDR_task2.ipynb

Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

Data Augmentation

Data augmentation scheme

- Key challenges: 90 entry points (a few thousands is ideal for pattern prediction task)
  - predict numerical values with low data regime
    - Challenging to recover true distribution of datasets

Basic question: Can we collect more data?

If Yes

- Go and collect more data

=> No HER2 antibody binding affinities data available

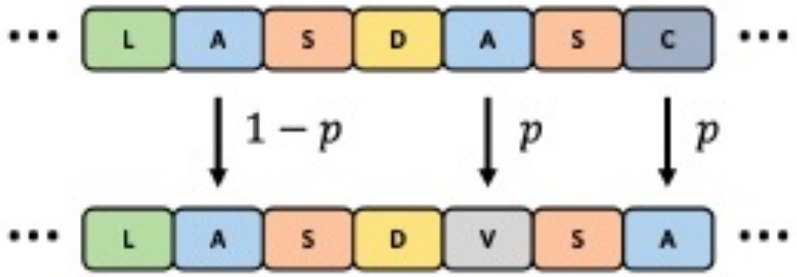
No

1. **Learning with original data**
2. **Learning with data augmentation**
3. Transfer learning with pretrained affinity model (no large-scale model founded)

Protein/AA sequence augmentation methods

Protein/AA sequence augmentation methods

- **Replacing AA residues (with probability *p*) with residues of most similar chemical properties[1,2]**
  - Retains AA properties that are crucial to bindng such as. Hydrogen donor/receptors



A:V, S:T, F:Y, K:R, C:M, D:E, N:Q, V:I, P:P, G:G, W:W, H:H, T:S, Y:F, M:C, E:D, Q:N, L:L, R:K, I:V

1. Pairing each naturally-occuring AA with a partner that belongs to the same class (aliphatic, hydroxyl, cyclic, aromatic, basic, or acidic)
2. Proline (only backbone cyclic), glycine (only an H side-chain), trytophan (indole side chain), histidine (basic side chain with no size or chemical reaction equivalent) remains same residue*

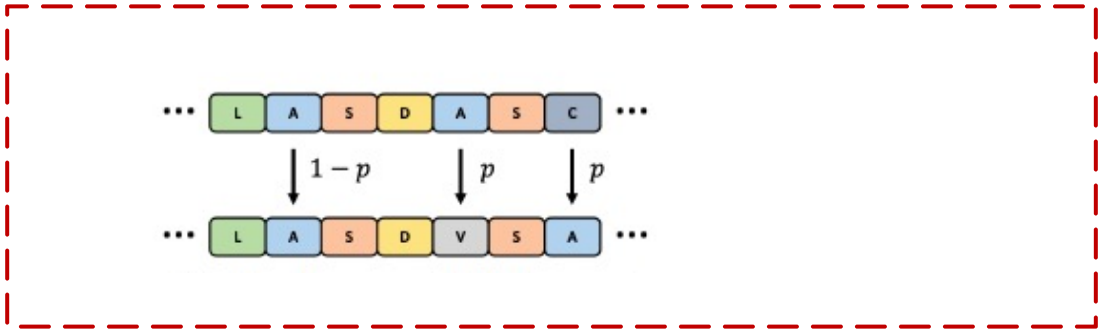
[1] Hongyu Shen, Layne C. Price, Taha Bahadori, Franziska Seeger **doi:** https://doi.org/10.1101/2021.02.18.431877
[2] French, S., Robson, B. What is a conservative substitution?. *J Mol Evol* **19,** 171–175 (1983). https://doi.org/10.1007/BF02300754

Protein/AA sequence augmentation methods with replacement with chemically similar residues

- The adaptation of replacement augmentation has following steps:
  1. Randomly select 1 position in each AA sequence (typically position 4-13 that are highly variable)
  2. With probability 0.1, the residue will be replaced, chance of 0.9 it won't be replaced
  3. Replacement follows pairwise rule:

```
A:V, S:T, F:Y, K:R, C:M, D:E, N:Q, V:I, P:P, G:G, W:W, H:H, T:S, Y:F, M:C, E:D, Q:N, L:L, R:K, I:V
```

  4. Repeat 1-3 steps 100 times for each sequence



Repeat 100 times for each sequence

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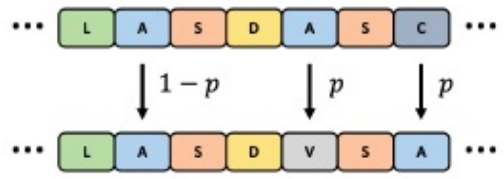
A:V, S:T, F:Y, K:R, C:M, D:E, N:Q, V:I, P:P, G:G, W:W, H:H, T:S, Y:F, M:C, E:D, Q:N, L:L, R:K, I:V
```

  4. Repeat 1-3 steps 100 times for each sequence

- Corresponding affinity value is simulated from parent log KD value plus 1% Gaussian noise ~N(0,1)



Repeat 100 times for each sequence

Affinity' = 99% * Affinity + 1% * Y~ N(0,1)

=> To simulate experiments measure variance

=> 5700 CDR3 sequences in total for training

[1] Hongyu Shen, Layne C. Price, Taha Bahadori, Franziska Seeger **doi:** https://doi.org/10.1101/2021.02.18.431877

Learning with positional one-hot encoding of CDR3 sequences

- Positional one-hot encoding deriving 15 X 20 matrix to represent each sequence:
  - Selected by task 1 benchmark result

Positional one-hot encoding

		A	C	D	E	F	G	B	J	MLQRS	T	V	W	G
C		0	1	0	0	0	0	0	0	…	0	0	0	0
S		0	0	0	0	0	0	0	0	…	0	0	0	0
R		0	0	0	0	0	0	0	0	…	0	0	0	0
Y		0	0	0	0	0	0	0	0	…	0	0	0	0
G		0	0	0	0	0	1	0	0	…	0	0	0	0
V		0	0	0	0	0	0	0	0	…	0	1	0	0
G	=>	0	0	0	0	0	1	0	0	…	0	0	0	0
G		0	0	0	0	0	1	0	0	…	0		0	0
M		0	0	0	0	0	0	0	0	…	0	0	0	0
F		0	0	0	0	1	0	0	0	…	0	0	0	0
P		0	0	0	0	0	0	0	0	…	0	0	0	0
Y		0	0	0	0	0	0	0	0	…		0	0	1
V		0	0	0	0	0	0	0	0	…	0	1	0	0
T		0	0	0	0	0	0	0	0	…	0	0	0	1
W		0	0	0	0	0	0	0	0	…	0	0	1	0

[1] Mason, D.M., Friedensohn, S., Weber, C.R. *et al.* Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. *Nat Biomed Eng* **5**, 600–612 (2021).

Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

Regression

Regression with original data and data augmentation:

With simplest K-nearest neighbor regressor (no restriction on data size)

Dataset:
- original data: 90
  - train:test = 60:20:20
- Augmented training set: 4000
  - Randomly selected from 5700 augmented training data

Input:
- 15 X 20 positional encoded matrix
  - Flattened to 300-d vector for algorithmic convenience

Model:
- KNN regressor: k=4
  - Hamming loss as distance metrics
    - Simiar to levenstein distance but normalized by length of vector
  - Parameters tuned

Performance evaluation
- Spearman rank correlation & mean squared error (MSE) (based on natural logarithm level)

Test set performance

	Rank correlation	MSE (log KD)
With augmentation	0.324**	1.200
Without augmentation	0.690**	0.760

KNN performs better without data augmentation
- **Rank correlation shows moderate to strong correlation**
- **KNN is not immune to noises in datasets**

**: statistically significant (p-value <0.01)

Regression with simple KNN exhibits comparable performance as Res-CNN

Dataset:
- Augmented training set: 4000
  - Randomly selected from 5700 augmented training data
  - Train: Val: Test = 60:20:20

Input:
- 15 X 20 positional encoded matrix

Model:
- Res-CNN
  - Similar model architecture to task1 adapted to regression

Performance evaluation
  - Spearman rank correlation & MSE (based on natural logarithm level)

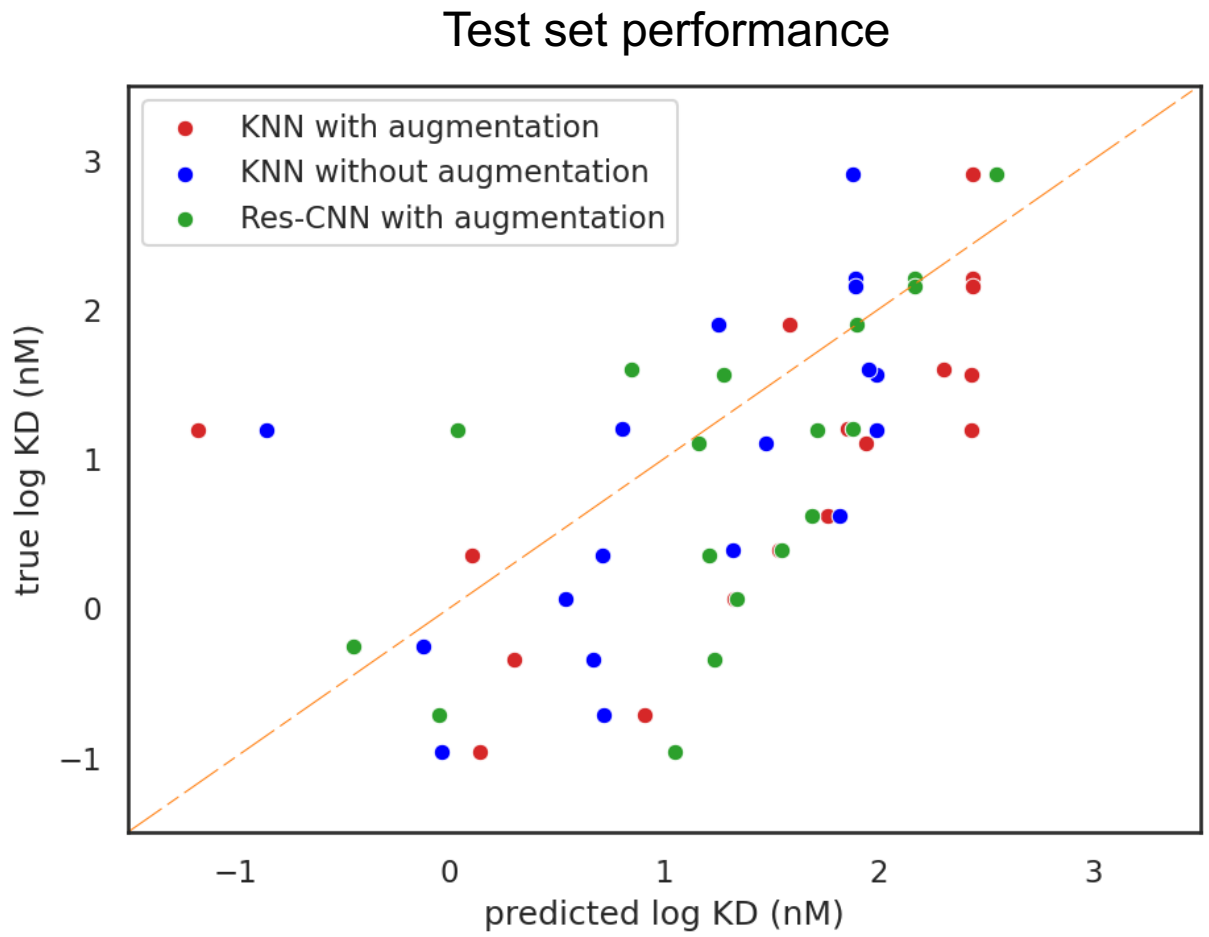
Test set performance comparison

	Rank correlation	MSE (log KD)
KNN With augmentation	0.324**	1.200
Res-CNN with augmentation	**0.702****	**0.82**
KNN without augmentation	**0.690****	**0.760**
Res-CNN Without augmentation	NA, model not converge due to too small size of data points	

❖ Res-CNN trained with data augmentation shows worse MSE but slightly better rank correlation

** statisitacally significant (p-value <0.01)

Regression with simple KNN slightly outperforms Res-CNN with data augmentation

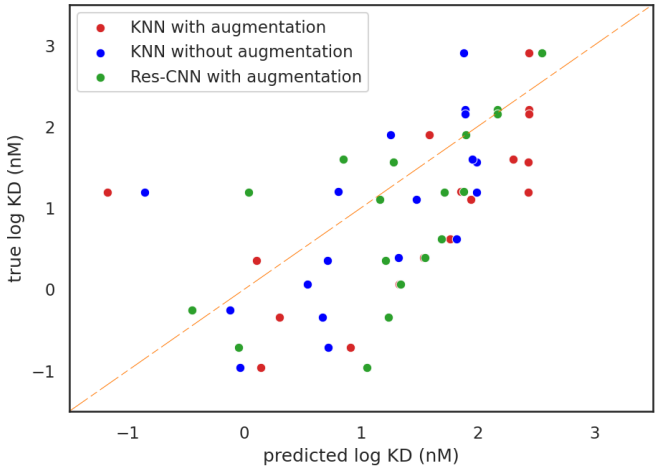


Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

Conclusion:

1. Challenging task to predict accurately with low data regime, lower accuracy than task 1
2. With data augmentation:
    i. KNN performs worse with data augmentation
        i. Pros: Might perform well under low data regime given selection of representative training data
        ii. Cons: KNN predicts by extropolating distance relationships between data points and performs weighted sum of neighbors, not robust against noise

    ii. Res-CNN performs better with augmentation
        i. Pros: Neural networks learns well with noisy and synthetic data
        ii. Cons: Deep learning requires larger dataset



Task 2: Predicting the Affinity of Novel Trastuzumab Variants Towards HER2

Future perspective:

1. More robust data augmentation techniques can be experimented
   - e.g. masking tokens in AA sequences
   - Literature[1] highlights other robust augmentation methods however without providing example code and algorithm, challenging to implement within limited timeframe

2. Transfer learning with pretrained protein properties model
   - Not so many large-scale affinity prediction data available
   - there might be other protein properties prediction dataset available, pretrained model can be used to initiate training => pretrained on task 1 dataset
3. more robust prediction algorithms (or postulate mathematical relationships between features and affinity)
4. Check prediction outliers to diagnose wrong prediction

5. **Best approach:**
   I. **Generate more data with experiment measurement ☺ (if possible)**
   II. Molecular simulation to mimic protein-antibody binding affnity as ground truth…(less recommended)

[1] Hongyu Shen, Layne C. Price, Taha Bahadori, Franziska Seeger **doi:** https://doi.org/10.1101/2021.02.18.431877

Take-away messages

- Data size/quality determines model performance
- Data-centric approach improves model performance more than model-centric approach
- **Protein/antibody engineering with machine learning is FUN ! ☺**

ETH zürich

Questions?

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Thank you!

For details of implementation (code) please check:
https://github.com/PinaColadast/CDR3_prediction_case

Back-up Slides

Machine/Deep learning optimizes antibody specificity by in-silico prediction

- Machine learning models trained from sequence and binding properties data can predict binding affinities
- Predicted performance serves as criterion to pre-select variants from a large candidates pool

=> Acclerate antiboody discovery and optimization

CDR3 sequences

CSRYGVGGMFPYVYW
CSRFGQAALYAYCYW

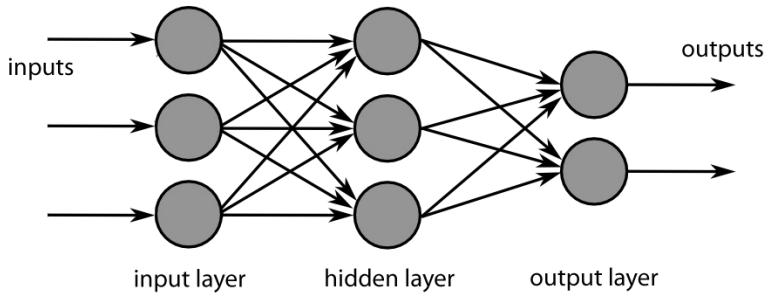
.....
CSRWVASGFFVYLYW
CSRYNNGGLYVYRYW

X = sequence, Y = affinity

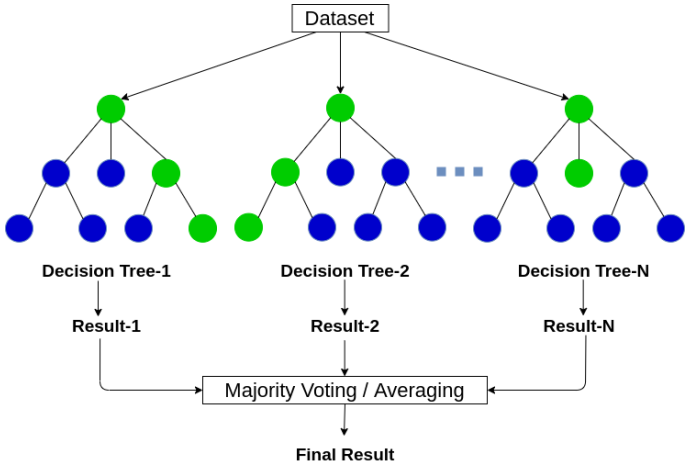
$$Y = f(X, \beta) + \varepsilon$$

Affinity

Strong binders?
Weak binders?
Non-binding?



Deep neural network



Random Forest

https://de.wikipedia.org/wiki/Deep_Learning

Feature engineering of CDR3 AA sequences representation

- Key challenges:
  1. To transform string-like AA sequences to machine readable numeric values

  ✓ Solutions:

  I. Natural language processing (NLP) approach to learn the data representation
  II. Binary encoding representing ab /presence of AAs
  III. Protein descriptors (protein fingerprints, electrostatic potential properties descriptors etc.)

  2. To reserve positional relationships of AA

  ✓ Solutions:

  1. Positional one-hot encoding of AAs
  2. Explicit protein descriptors

  3. To reserve functional properties of AA

  ✓ Solutions:

  I. Implicit categorical encoding of 20 natural AAs
  II. Explicit protein descriptors

❖ select AA sequences rather than nucleotide sequencs, as:

- AA impacts protein interaction directly
- More diverse categories (20) to encode

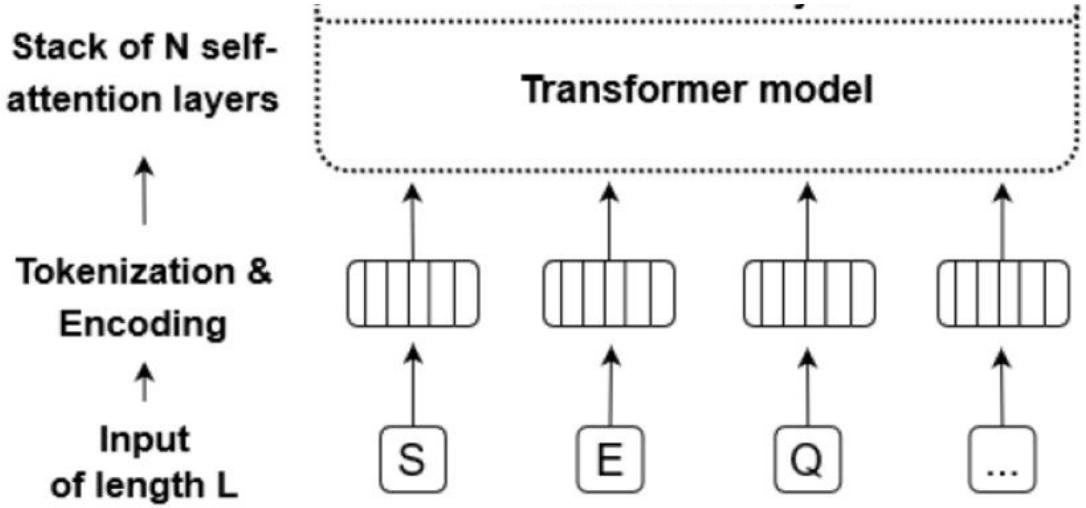
[1] Journal of Cheminformatics volume 5, Article number: 41 (2013)

Feature engineering of CDR3 AA sequences

I. Natural language processing (NLP) approach to learn 1024-vector numeric representation for each sequence

- AA sequence is a language with unique syntax and semantics

NLP-based representation learning[1]



Key ideas:
Leverage BERT (**B**idirectional **E**ncoder **R**epresentations from **T**ransformers) model pretrained with large-scale protein sequences to generate representation

Key steps:

1. Tokenize protein sequence and create positional encoding, feed forward to BERT

[1] A. Elnaggar *et al.*, "ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing," in *IEEE Transactions on Pattern Analysis and Machine Intelligence*, doi: 10.1109/TPAMI.2021.3095381.

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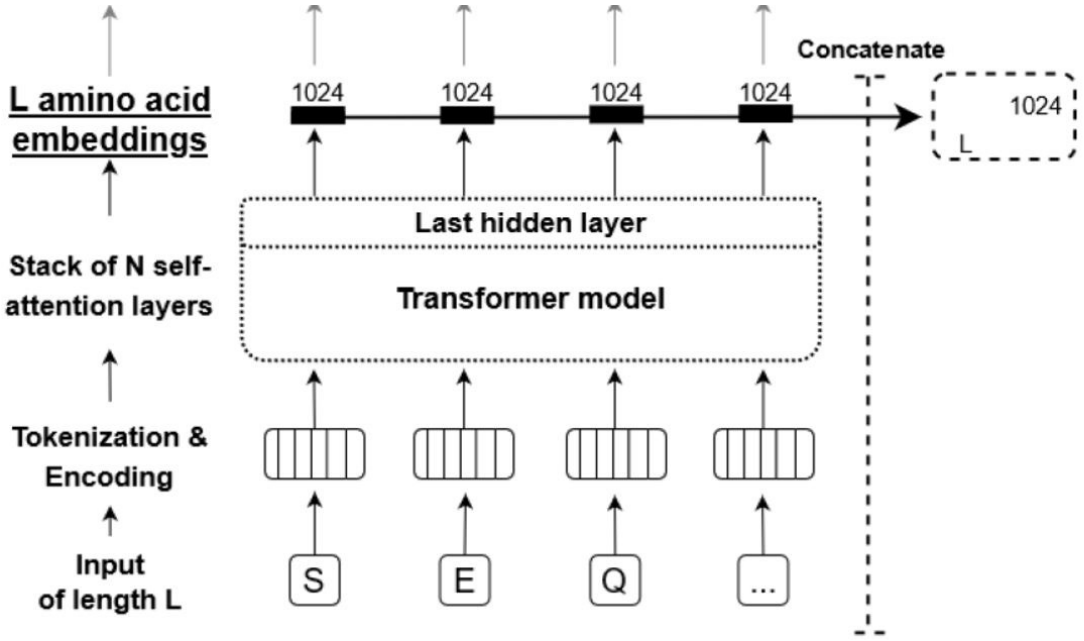

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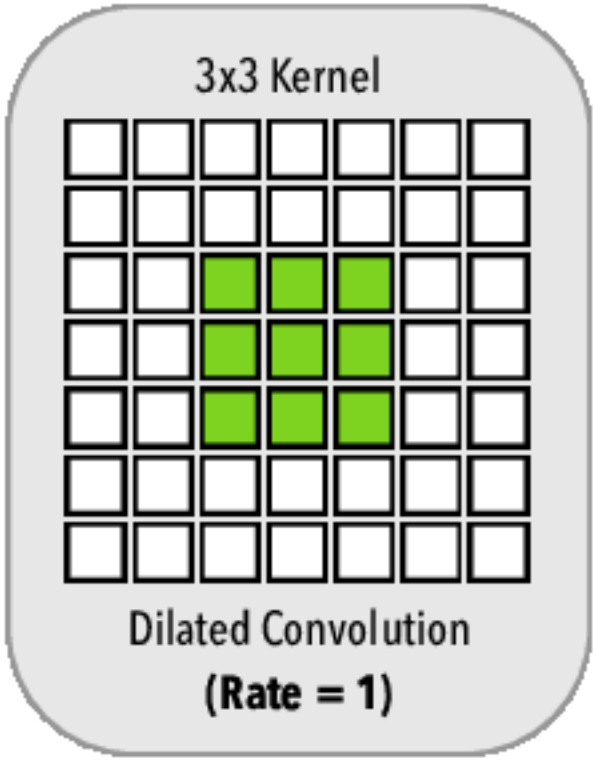
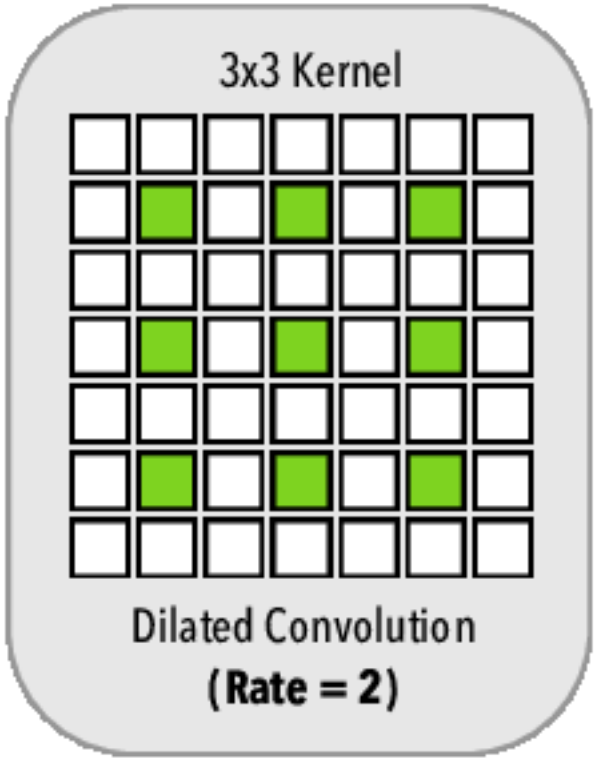
Key steps:

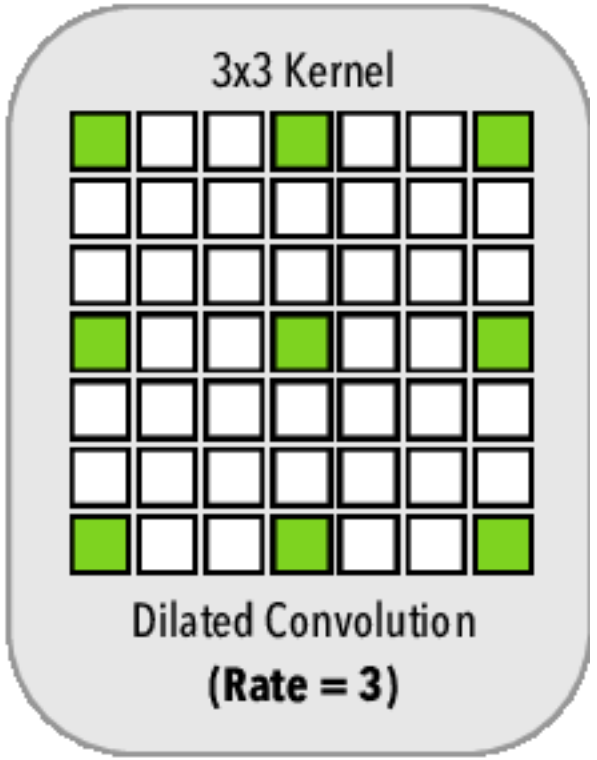
1. Tokenize protein sequence and create positional encoding, feed forward to BERT
2. Generate 1024- long vector embedding for each AA

[1] A. Elnaggar *et al.*, "ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing," in *IEEE Transactions on Pattern Analysis and Machine Intelligence*, doi: 10.1109/TPAMI.2021.3095381.

Dialated convolutions

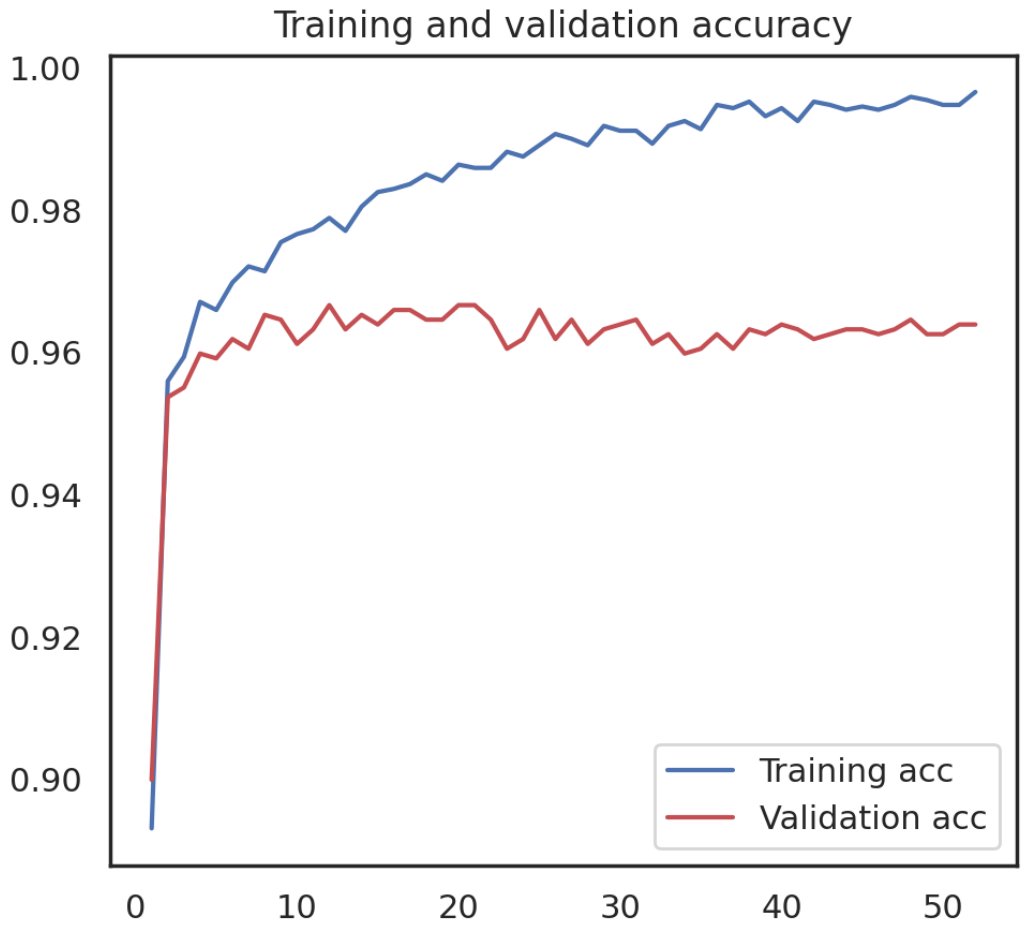


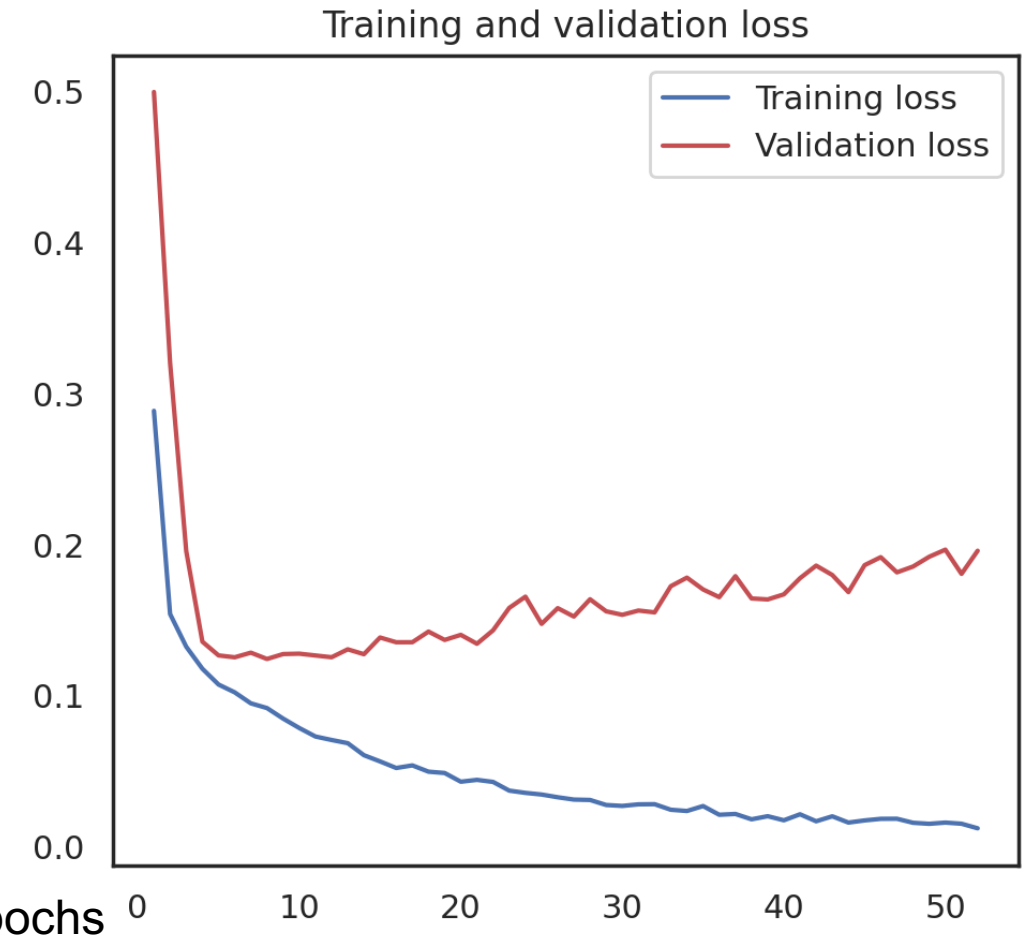




Classification with one-hot encoded representation:

Vanilla CNN architecture training history shows train loss approaches 0 with more epochs, validation loss oscillates after epoch 20

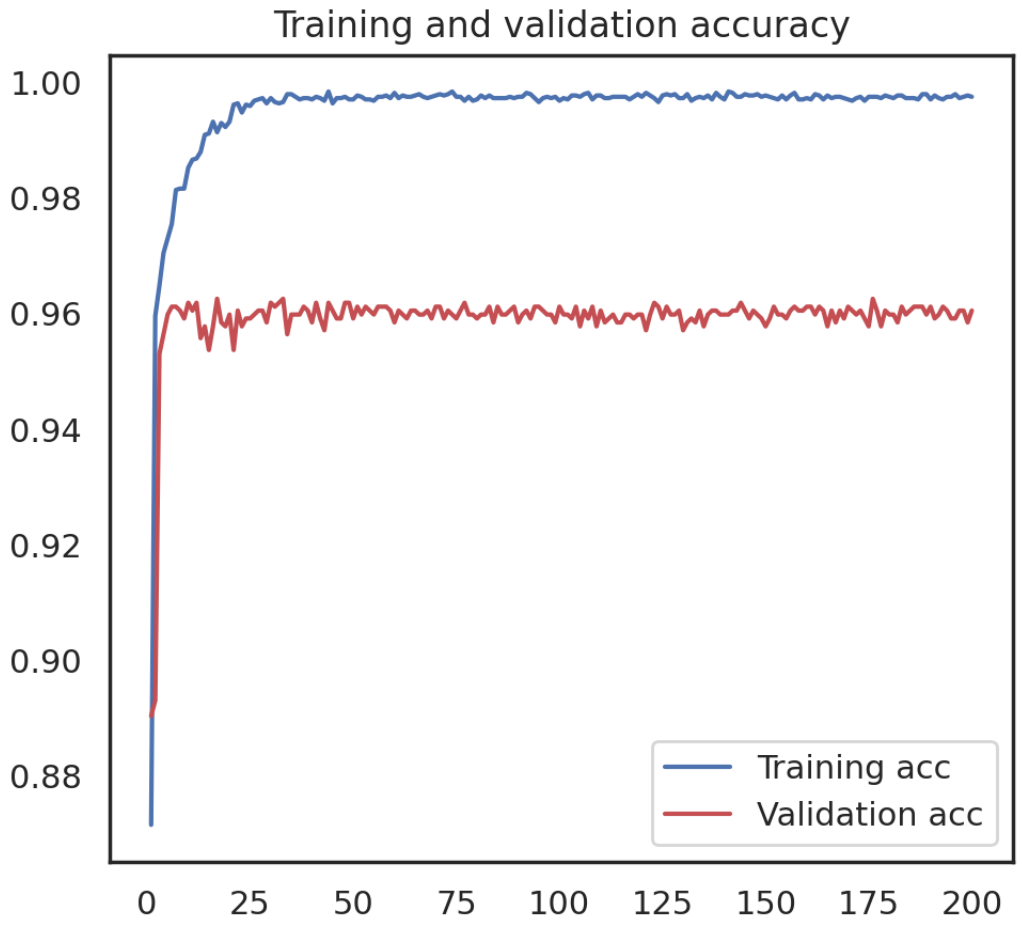


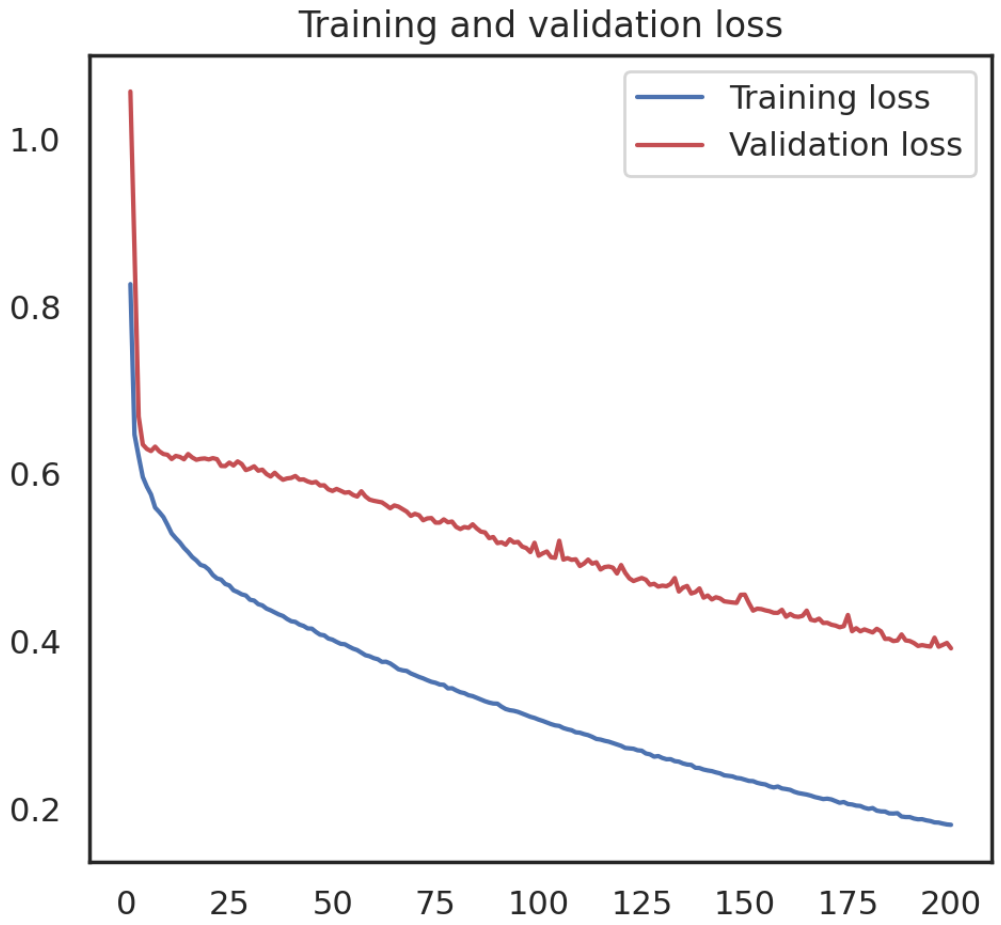


Epochs

Classification with one-hot encoded representation:

Res-CNN architecture training history shows train loss approaches 0 with more epochs, validation loss keep decreasing with accuracy reaching plateau after epoch 50





Epochs

Classification with NLP-learned & one-hot encoded representations: Positional one-hot encoding outperforms NLP-based representations

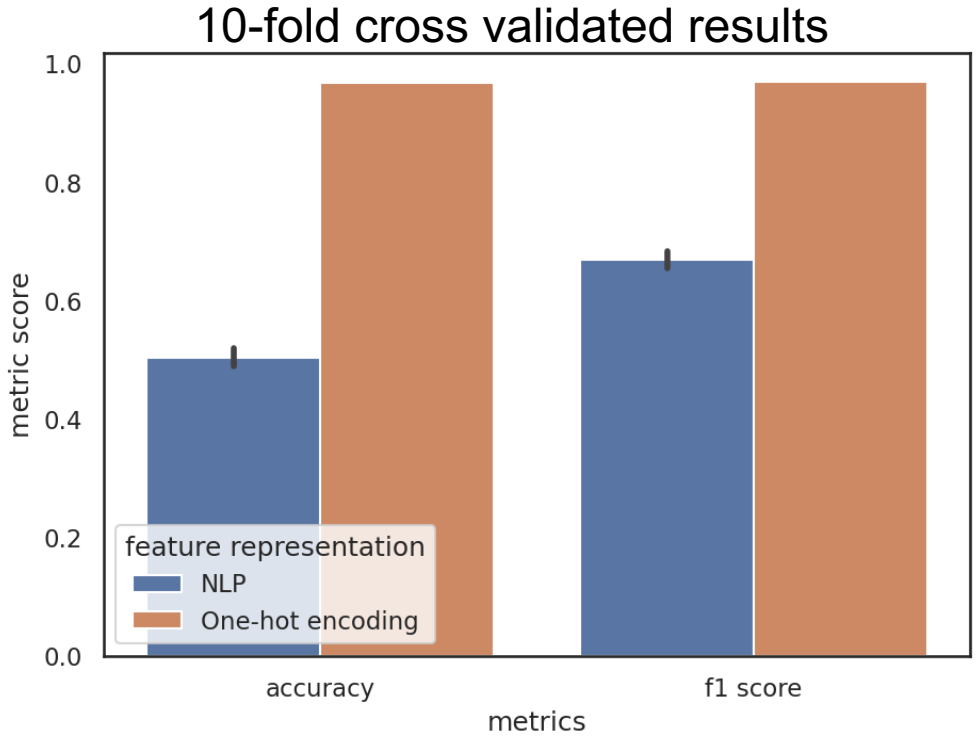
Dataset:
- 3725 binding sequences
- 3620 non-binding sequences

train:test = 80:20

Input:
- 1024-dimensional feature vector
- 15 X 20 positional encoded matrix
  - Flattened to 300-d vector for algorithmic convenience

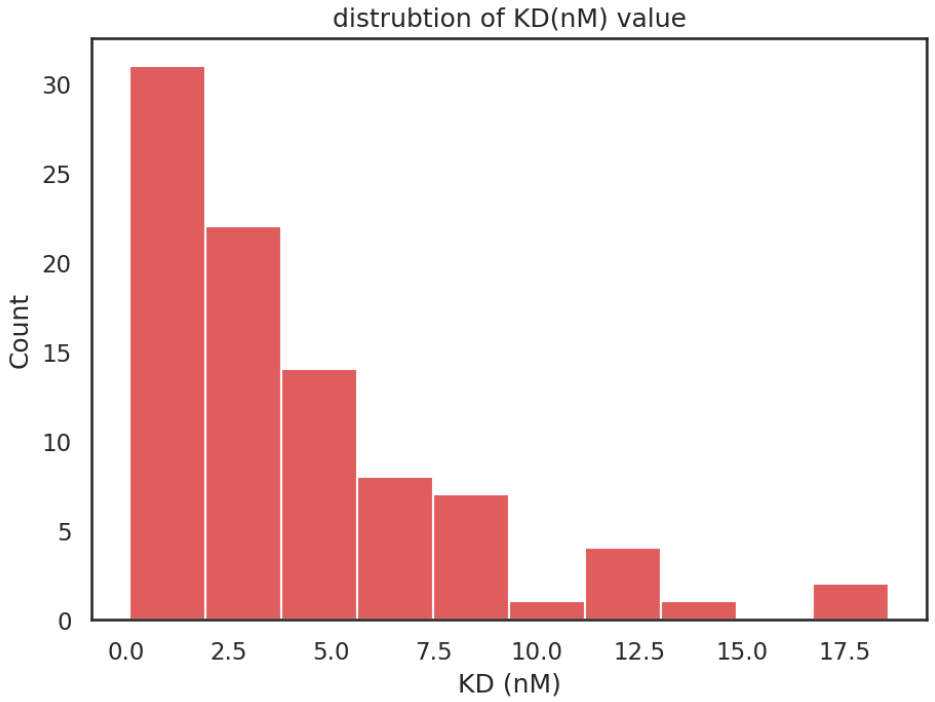
Model:
- random forest classifier
  - Parameters tuned by grid-search

Performance evaluation
- 10-fold cross-validated
- f1, accuracy score



Log(natural) normalization of KD values

- KD values exhibits right skewed distibution and with large fold gap between small and large values
- Log normalization of KD values gives less sample variance and smaller fold gap
  - Sequencing quality control was skipped due to absence of sequencing quality data (assuming high quality)
  - No repetitive data entries



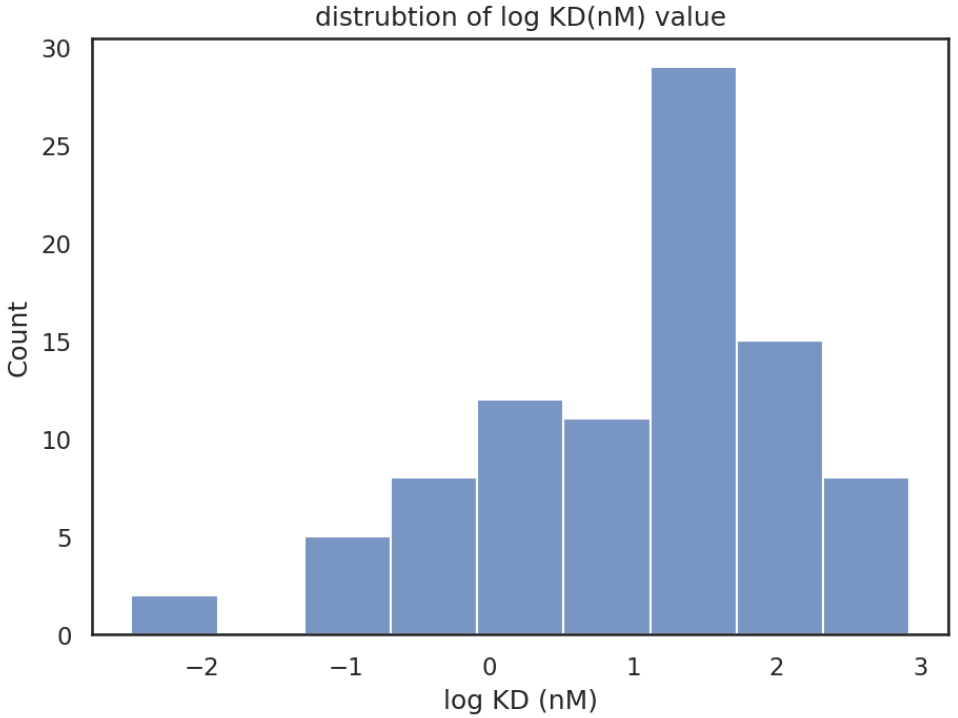
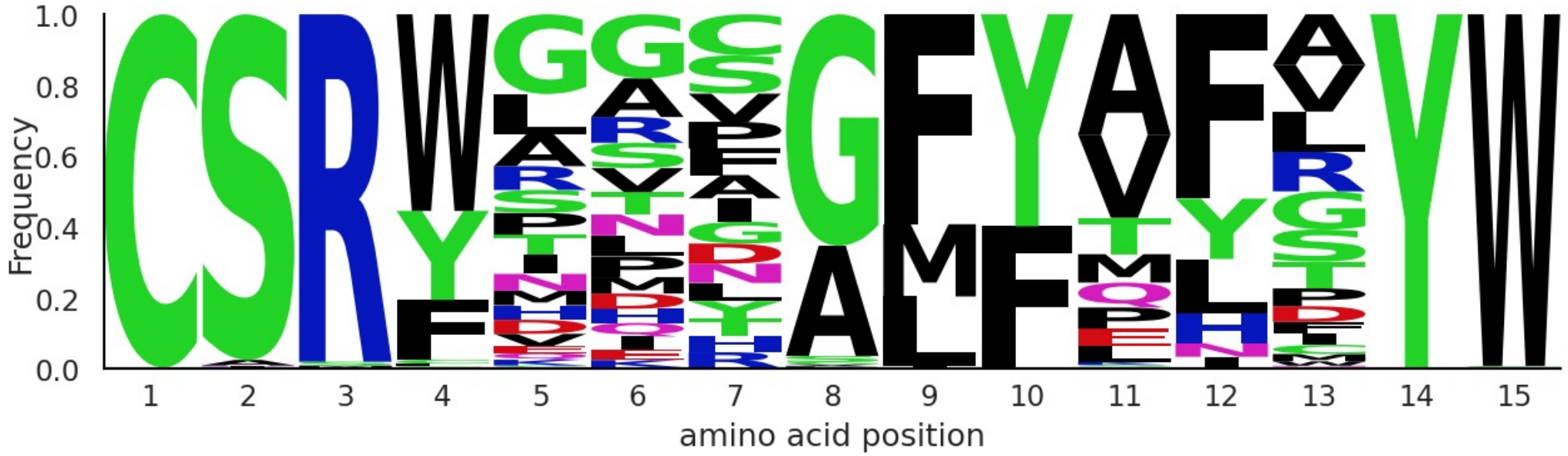


Figure: histgrams of binding affinity distributions

Sequence diversity analysis

- Length of AA sequences : 15 residues
- similar residue compositions and positions as data from task 1
  - Pos 1-3 : CSR mostly
  - Pos 14-15: YW



Protein/AA sequence augmentation methods

- Literature research has benchmarked AA sequence augmentation methods and highlighted its generailizibitliy
  - ❖ **Replacing residues (with probability p) with residues of most similar chemical properties[1,2]**
    - **Retains AA properties that are crucial to bindng such as. Hydrogen donor/receptors**

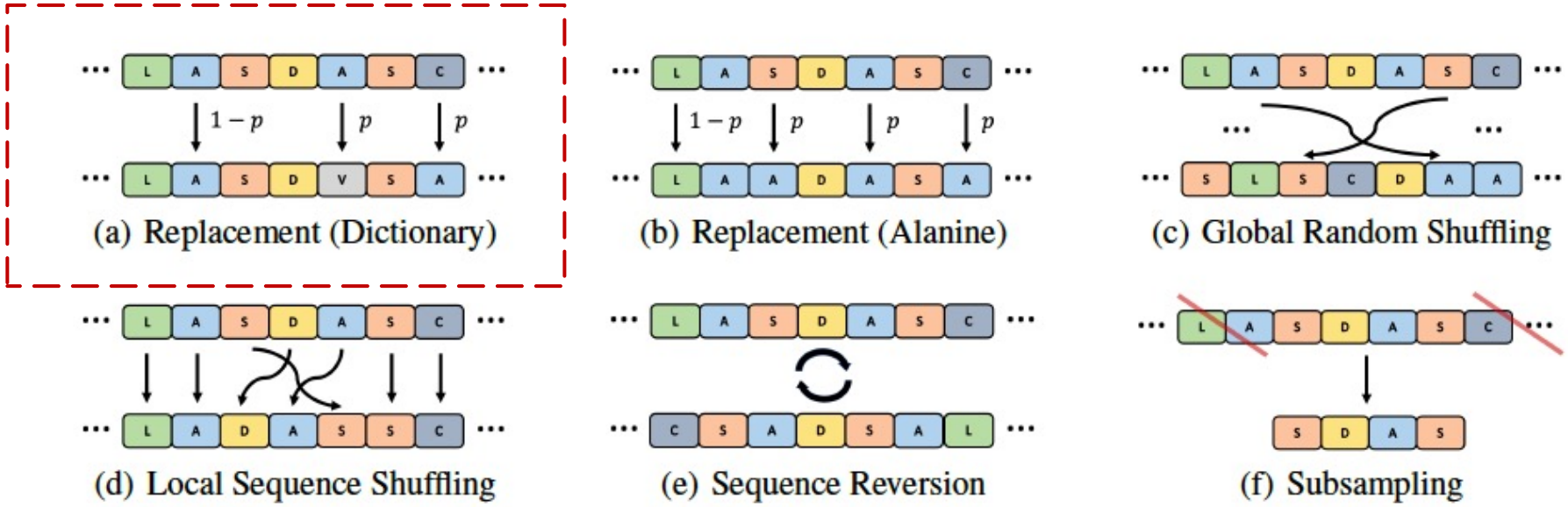


Figure 1: Diagram of data augmentations. We study randomly replacing residues (with probability p) with (a) a chemically-motivated dictionary replacement or (b) the single amino acid alanine. We also consider randomly shuffling either (c) the entire sequence or (d) a local region only. Finally, we look at (e) reversing the whole sequence and (f) subsampling to a subset of the original.

[1] Hongyu Shen, Layne C. Price, Taha Bahadori, Franziska Seeger **doi:** https://doi.org/10.1101/2021.02.18.431877
[2] French, S., Robson, B. What is a conservative substitution?. *J Mol Evol* **19,** 171–175 (1983). https://doi.org/10.1007/BF02300754

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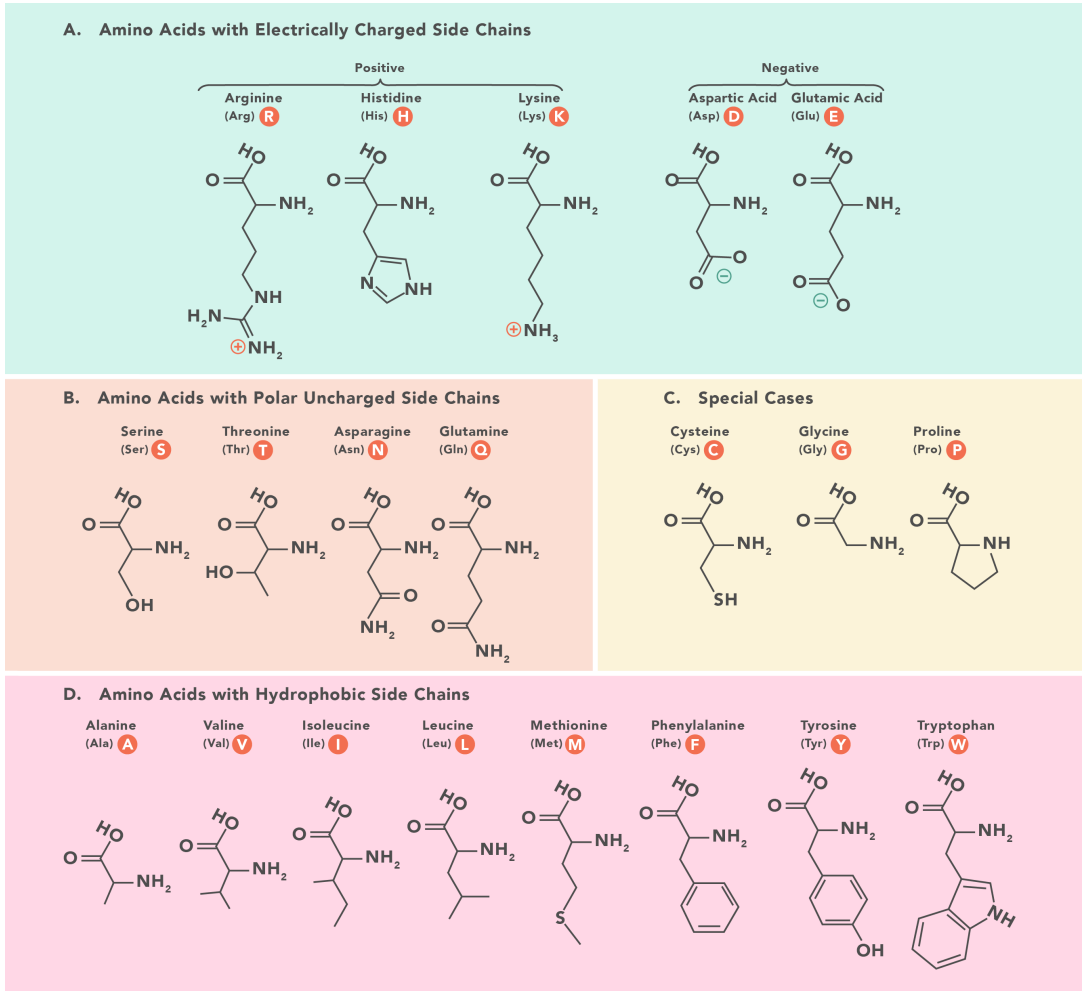
A:V, S:T, F:Y, K:R, C:M, D:E, N:Q, V:I, P:P, G:G, W:W, H:H, T:S, Y:F, M:C, E:D, Q:N, L:L, R:K, I:V



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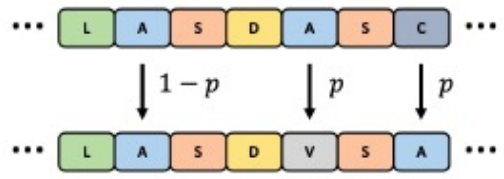
Protein/AA sequence augmentation methods with replacement with chemically similar residues

- The adaptation of replacement augmentation has following steps:
  1. Randomly select 1 position in each AA sequence (typically position 4-13, as 1-3, 14-15 are highly conserved)
  2. With probability 0.1, the residue will be replaced, chance of 0.9 it won't be replaced
  3. Replacement follows pairwise rule: *

     A:V, S:T, F:Y, K:R, C:M, D:E, N:Q, V:I, P:P, G:G, W:W, H:H, T:S, Y:F, M:C, E:D, Q:N, L:L, R:K, I:V

  4. Repeat 1-3 steps 100 times for each sequence

- Corresponding affinity value is simulated from parent log KD value plus 1% Gaussian noise ~N(0,1)



Repeat 100 times for each sequence

Affinity' = 99% * Affinity + 1% * N(0,1)

=> To simulate experiments measure variance

* note: proline (only backbone cyclic), glycine (only an H side-chain), tryptophan (indole side chain), or histidine (basic side chain with no size or chemical reaction equivalent) remains same residue

[1] Hongyu Shen, Layne C. Price, Taha Bahadori, Franziska Seeger **doi:** https://doi.org/10.1101/2021.02.18.431877

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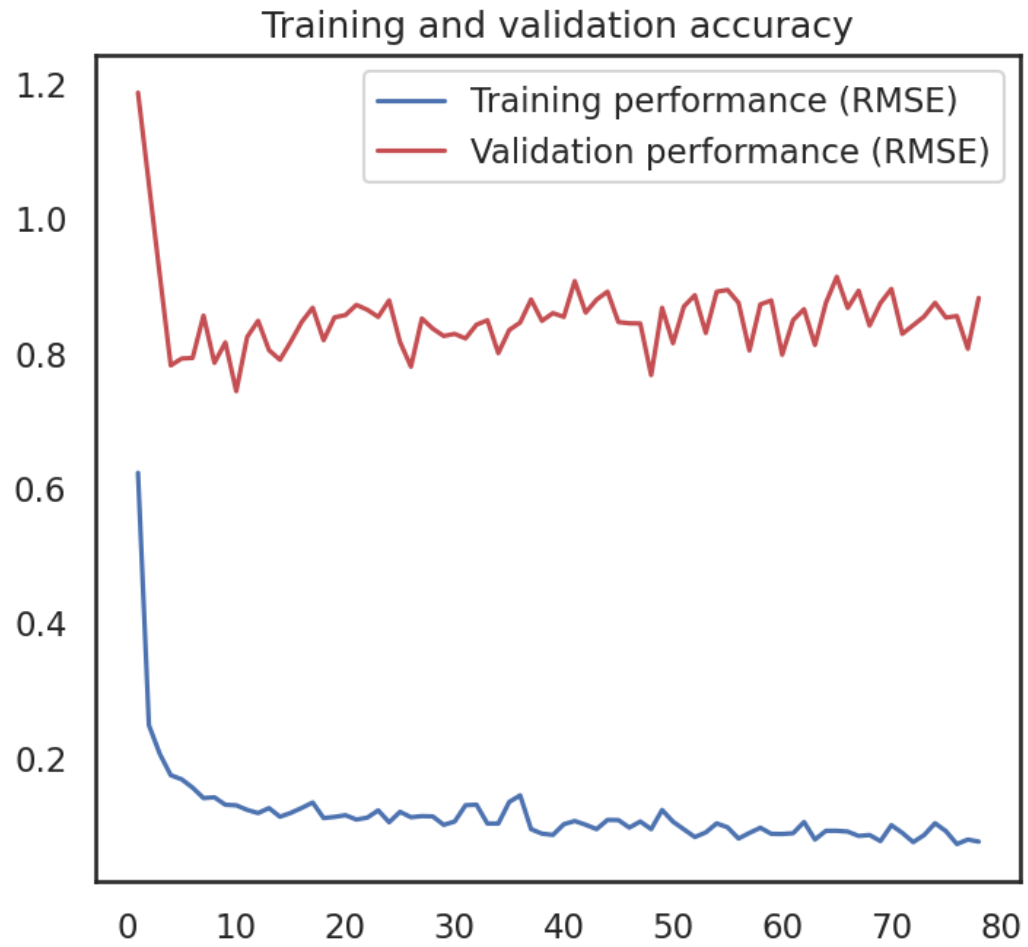
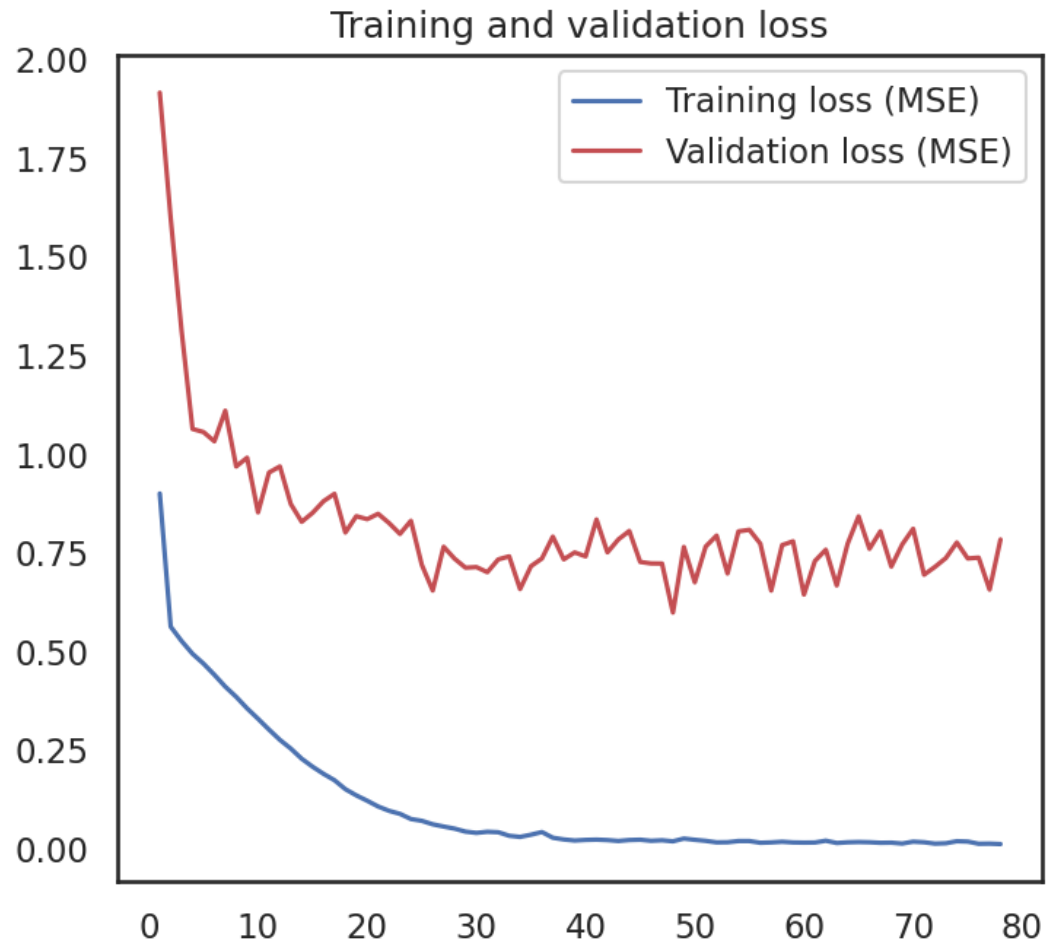
Regression with Res-CNN

Res-CNN architecture training history shows training loss approaches 0 with more epochs, validation loss oscillates, perhaps due to stuck in local minimum or overfits the training set





Epochs

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With simplest K nearest neighbor regressor (no restriction on data size)

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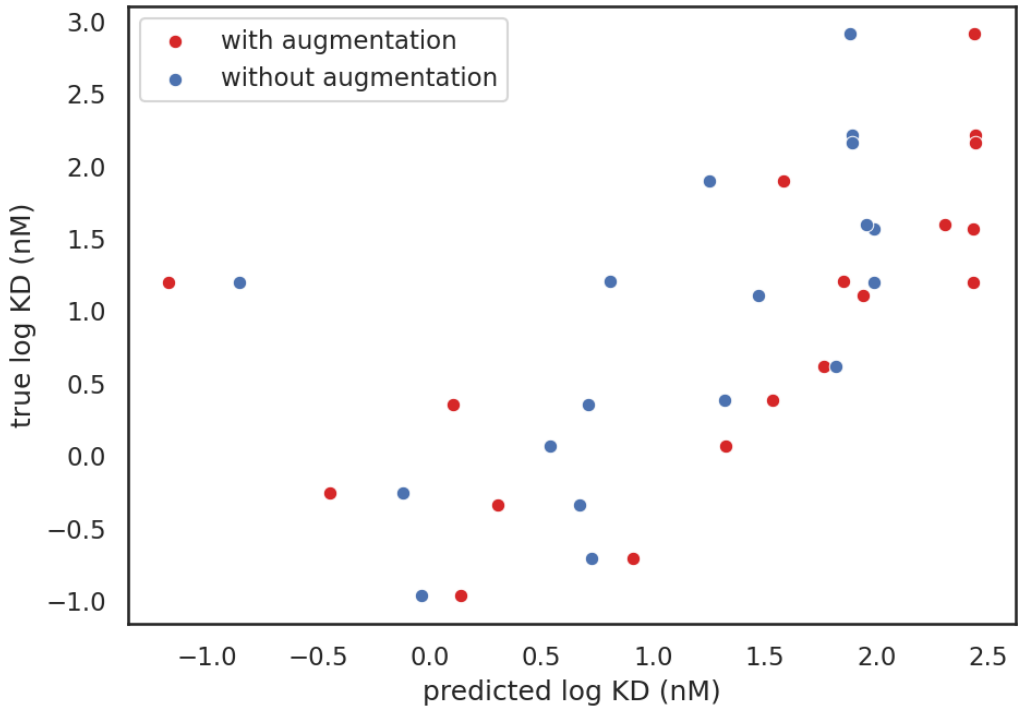

KNN performs better without data augmentation
- **predidcted value shifted by larger error with augmentation**
- **KNN can't handle noise very well, only extropolate values from distance relationships**