Finetuning ESM3 with Contrastive Preference Optimization for Antigen-Specific Antibody Design

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Abstract

Antibodies are therapeutic agents produced by our immune system that exhibit strong, highly-specific binding to potential disease-causing targets. In recent years, researchers have attempted to computationally design such antibodies, known as monoclonal antibodies, especially with the advent of machine learning methods. For instance, large, foundational protein language models such as ESM2 and ESM3 have shown great success in predicting protein properties and functions by unifying their features into a rich embedding space. However, due to the variability of antibody loop regions (CDRs), such foundation models have been less successful in antibody design and optimization tasks. Our work presents a framework to optimize existing foundation models, specifically ESM3, for antibody design from a purely sequence-based perspective. We perform a case study by redesigning the CDR3 sequence of Trastuzumab, a monoclonal antibody that binds to HER2, a protein regulating cell growth which is overexpressed in certain forms of cancer. To accomplish this, we compile a dataset of Trastuzumab CDR3 sequences, each classified as high, medium, or low binding, and perform Contrastive Preference Optimization (CPO) to finetune ESM3 to assign higher likelihoods for high affinity sequences. We observed that although the finetuning process does not enhance ESM3's embedding space by much, we find that sequences generated by the finetuned model greatly surpass those of existing protein language foundation models in terms of plausibility and edit distances from ground truth high affinity sequences. Furthermore, after folding and docking the generated antibody structures, we find that our finetuned model generates unique sequences with binding affinities comparable to those of the high affinity sequences from the training dataset. To this extent, we claim that CPO serves as an optimal framework for finetuning protein language models on highly-specific tasks such as antibody design. Our code is available at `https://github.com/anirudhvenk/antibody-dpo`.

1 Introduction

1.1 Background

Antibodies play an essential role in modern medicine, as they have high specificity and affinity for target molecules. These proteins are essential components of our immune system and have been used to treat cancer as well as manage autoimmune diseases. However, predicting antibody secondary structure and designing antibodies with improved biological characteristics remains a difficult and highly relevant problem in protein engineering.

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Engineering high-performance antibodies often requires improving their binding affinity and stability, which requires insight into their structure-function relationships. Conventional methods for antibody design have relied on tedious and resource-intensive experimental *in vitro* methodologies. However, recent advancements in computational techniques and machine learning models pose a tremendous opportunity to expedite and improve the antibody design process.

EvolutionaryScale's ESM3 (Hayes et al. 2024), a state-of-the-art protein language model, achieves groundbreaking performance in protein sequence generation and optimization, having been trained on evolutionary data, as well as other protein-specific modalities, namely secondary structure and solvent-accessible surface area. ESM3 is trained on billions of proteins, drawn from sources ranging from the Amazon Rainforest to the depths of the ocean, hydrothermal vents, and soil microbes. Due to its unique architecture, ESM3 can seamlessly bridge sequence, structure and function within a single language model framework. However, finetuning ESM3 for hyper-specific tasks such as antibody design remains a challenge that few have yet tackled.

The complementarity-determining regions (CDRs) of antibodies have high variability in order to recognize the many different antigens that they must bind to, and this variability presents a unique challenge for protein language models, which are usually excellent at learning conserved structures across protein families. In order to leverage the power of ESM3 for antibody design, we must design a specialized finetuning strategy that can transfer the model's general protein knowledge to the specificities of antibody antigen interactions.

1.2 Motivation

To tackle the problem of applying ESM3 to antibody design, we propose a method that performs Contrastive Preference Optimization, or CPO (Xu et al. 2024), for aligning ESM3 to generate high affinity CDRs. Originally developed for machine translation tasks, CPO is an offline reinforcement learning approach which trains models to avoid generating adequate but not perfect outputs. CPO learns from pairs of inputs where one specific example is preferred over the other. Through this approach, the base model learns to emphasize features associated with higher performance or utility. This approach is well suited to antibody design, as we often have datasets that classify antibody variants by their binding affinity to particular antigens.

Herein we report the results of finetuning ESM3 using CPO to produce high-affinity antibodies for HER2, a protein regulating cell growth and division. In particular, we focus on Trastuzumab, a targeted therapy drug used to treat certain types of cancer where HER2 is overexpressed and leads to rapid cell growth and metastasis.

We use a dataset with approximately 500,000 Trastuzumab CDR3 variants and their binding affinities for HER2, which are classified by high, medium, and low binding. The finetuning process involves three key steps:

1. Generating initial embeddings for the antibody CDR3 sequences using ESM3
2. Sampling pairs of embedded sequences from the dataset where one has a "high" binding affinity, and another has either a "medium" or "low" binding affinity, and the pair of embeddings exhibits high cosine similarity
3. Applying CPO on the paired dataset to steer ESM3 to generating sequences with high levels of binding

Through these steps, we can take advantage of the ESM3's foundational knowledge while refining its generative capabilities for developing HER2 binders.

1.3 Related Work

ESM2 (Lin et al. 2022), developed by FAIR at Meta, was a groundbreaking addition to the realm of protein language models. ESM2 is an encoder-only architecture that processes protein sequences as inputs, and provides rich embeddings for downstream tasks such as contact prediction and folding. As a result, it has become an important tool for computational protein engineering and prediction of protein function.

In parallel with the development of general protein language models, efforts have been made to build antibody-specific models, such as AntiBERTy (Ruffolo et al. 2021) and IgLM (Shuai et al. 2023).

These are models designed for analyzing antibody sequences, leveraging their specific structural and functional properties. For instance, AntiBERTy is pretrained on 558 million antibody sequences and captures antibody-specific patterns and relationships. The model is capable of performing such tasks as clustering antibodies into trajectories resembling affinity maturation, providing further insights for computational antibody engineering.

Although these antibody specific models are outstanding in their particular niche, they struggle with generalization across the wider structural and evolutionary protein space, in contrast to more universal models such as ESM3 that can leverage knowledge across protein families to guide antibody design.

2 Methods

Our methodology centers on leveraging contrastive learning within the embedding space of ESM3 to enhance its ability to discriminate and generate high-affinity antibody sequences. First, we constructed a specialized training set by identifying pairs of CDR3 variants that are similar in the embedding space yet functionally distinct in binding affinity. This process involved computing ESM3's embeddings for a large collection of Trastuzumab CDR3 sequences and then selecting high-affinity versus medium/low-affinity pairs based on cosine similarity measures. Next, we fine-tuned ESM3 using a hinge loss framework, freezing most structural parameters to preserve the model's underlying protein representations while optimizing sequence-specific parameters. By intensifying contrastive distinctions and preserving essential structural information, the model could more effectively identify, generate, and refine antibody sequences with improved binding characteristics.

2.1 Data Preparation

Our dataset included 500,000 Trastumuzab CDR3 variants, characterized by their binding affinity to HER2 (either high, medium, or low binding), derived from (Chinery et al. 2024). In order to construct the CPO dataset, we first computed ESM3's embedding for each sequence, then computed the cosine similarity between all high affinity sequences and all medium/low affinity sequences. This follows a similar methodology to CLEAN (Yu et al. 2023), where the goal is to obtain pairs of protein sequences that the model predicts are highly similar in the embedding space, yet are functionally vastly different, in order for the model to more effectively characterize positive and negative sequences in a contrastive loss framework. We then extract the top 10^6 pairs of sequences with the highest cosine similarity, with a 1:4 ratio of high-affinity to low-affinity sequences.

2.2 Training

Following the creation of the CPO dataset, we finetune ESM3 by first freezing all parameters that are not inherently related to the sequence generation task. This includes the structure/function embedding modules, all of the transformer blocks, and all decoder heads apart from the sequence head, resulting in around only 2.5 million trainable parameters out of the total 1.4 billion. We reasoned that finetuning structure/function specific modules with sequence data would lead to interference across tracks, thereby making the embedding space less informative.

Finetuning was performed by overriding HuggingFace's CPOTrainer class so that it was compatible with ESM3's model architecture. We trained ESM3 for around 45,000 steps with a learning rate of 0.005 before observing convergence. We used the hinge loss function, which penalizes predictions that are correct but too close to the decision boundary, and a β of 0.1, which indicates the deviation from the base model (lower beta means higher deviation). During the training process, we observed ESM3 attributing higher positive rewards to chosen sequences and lower negative rewards to rejected sequences, indicating the model's ability to differentiate between positive and negative binders, as shown in Figure 2. Furthermore, ESM3 became more confident in its generation of high-affinity binders compared to low-affinity binders during training, as shown by the log probabilities of each variant in Figure 3.

3 Experiments

We conducted a series of experiments to evaluate the finetuned model's ability to generate and discriminate high-affinity antibody sequences. First, we assessed the sequence embeddings, where

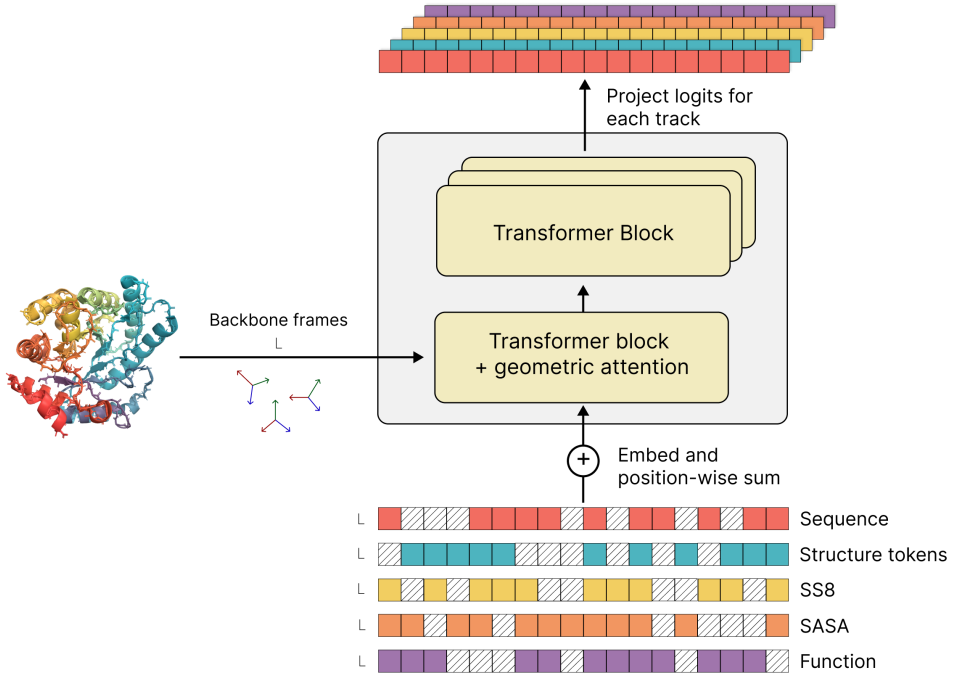

Figure 1: ESM3 model architecture. The only trainable parameters include the sequence embeddings and the output projection head for the sequence track.

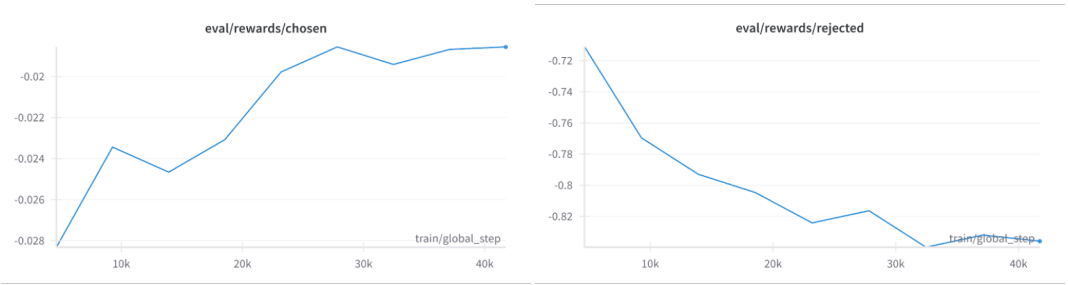

Figure 2: Rewards for preferred (chosen) and dispreferred (rejected) sequences during training.

the finetuned model demonstrated improved recall and marginal gains in distinguishing high and low affinity binders compared to the base model. Next, we evaluated the generated sequences using edit distance and AntiBERTy pseudo-log-likelihood as a plausibility score. The finetuned model consistently produced sequences that were closer to known high-affinity binders and exhibited higher plausibility than both the baseline ESM3 and IgLM models. Finally, structural and interaction quality analysis revealed that the finetuned model generated sequences with interaction energies comparable to those of high-affinity sequences from the training set, further demonstrating its ability to replicate functional properties critical for binding affinity. These results validate the finetuned model's effectiveness in antibody sequence design.

3.1 Sequence Embedding Evaluation

To evaluate the finetuned model's ability to distinguish between high and low-affinity binding sequences, we generated positive and negative embedding cluster centers. These centers were calculated by averaging the embeddings of high-affinity and low-affinity sequences, respectively. Each test set sequence embedding was classified based on its proximity to these centers, with closer proximity to the positive center indicating high binding affinity and vice versa. From the results shown in Figure 4, the finetuned model achieves a recall of 0.80 compared to the base model's recall of 0.75, indicating a slight improvement in its ability to correctly identify high-binding sequences.

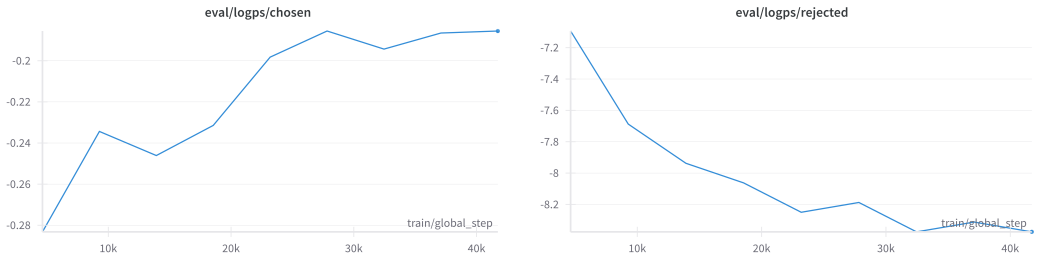

Figure 3: Log probabilities of chosen and rejected sequences during training.

However, the precision remains unchanged at 0.39 for both models, which indicates that while the model detects more true positives, it also generates a significant number of false positives. This imbalance is further reflected in the ROC curve presented in Figure 4, where the fine-tuned model shows marginal gains over the base model. The slight improvement in the ROC curve highlights the model's enhanced ability to separate positive and negative classes, though the separation is not yet robust.

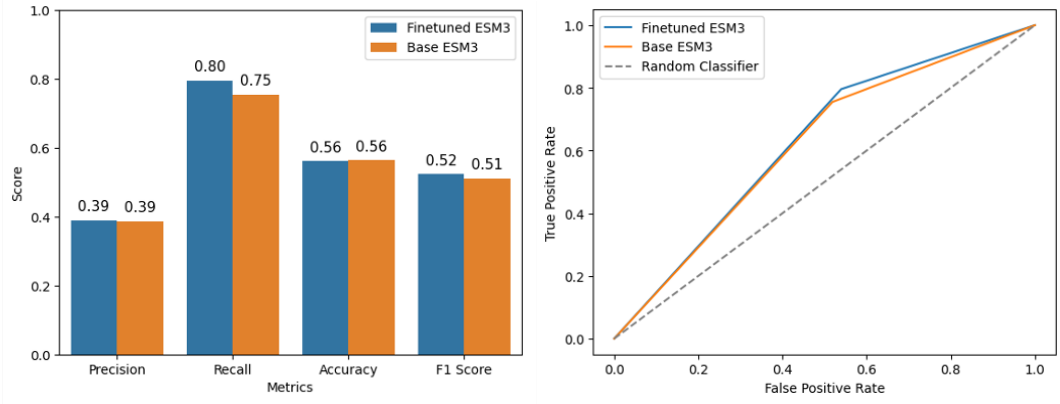

Figure 4: Classification metrics and ROC curve using base and finetuned ESM3 embeddings for classification.

3.1.1 Sequence Generation Evaluation

To evaluate the likelihood that the sequences generated by our model exhibit high binding affinity, we compare the finetuned ESM3 model against IgLM and the base (non-finetuned) ESM3 model. Specifically, we calculate the average edit distance of the CDR3 region for each generated sequence to all high-affinity sequences in the training set. This evaluation was performed for 1,000 sequences generated by each model. As shown in Figure 5, the fine-tuned model exhibits a lower average edit distance compared to both IgLM and the baseline ESM3 model, indicating that the sequences generated by the finetuned model are compositionally closer to high-affinity binders. Notably, the most frequent edit distance for both the fine-tuned model and IgLM is similar, around 8.0. However, the distribution of IgLM-generated sequences is more dispersed, with a higher proportion of sequences showing edit distances greater than 8.5. In contrast, the finetuned model displays a more concentrated distribution around the most frequent value, suggesting that it generates sequences that are consistently closer to the high-binding sequences. This dense distribution of entries around lower edit distances demonstrates the finetuned model's ability to learn and replicate sequence features that are characteristic of high-affinity binders. Compared to the baseline ESM3 model, which shows a broader distribution and higher average edit distances overall, the fine-tuned model clearly outperforms in generating sequences that are closer to the ground truth binders. These findings underscore the effectiveness of the finetuning process in guiding the model to produce biologically relevant CDR3 sequences with enhanced binding potential.

To assess the plausibility of sequences generated by the finetuned model compared to those generated by IgLM and the base ESM3 model, we used AntiBERTy to calculate the pseudo-log-likelihood (PLL) of the CDR3 region. Smaller negative PLL values indicate higher plausibility, as the sequence aligns more closely with patterns learned from the dataset. The distribution of PLL values across 1,000 generated sequences for each model is shown in Figure 6. The fine-tuned model outperforms both IgLM and the baseline ESM3 model, as evidenced by the shift in its PLL distribution toward higher plausibility. A significant proportion of sequences generated by the fine-tuned model fall within the range of -0.65 to -0.60, demonstrating consistently higher plausibility compared to the broader and more left-skewed distributions of IgLM and the baseline model. In contrast, the sequences generated by the baseline ESM3 model are heavily concentrated in lower plausibility regions around -0.75, while IgLM exhibits a slightly more favorable distribution but still fails to match the performance of the fine-tuned model. The improvement in plausibility for the finetuned model indicates that it has effectively learned the sequence-level patterns characteristic of biologically plausible CDR3 regions. This supports the hypothesis that finetuning enhances the model's ability to generate sequences that are not only syntactically valid but also closely aligned with real-world biological data.

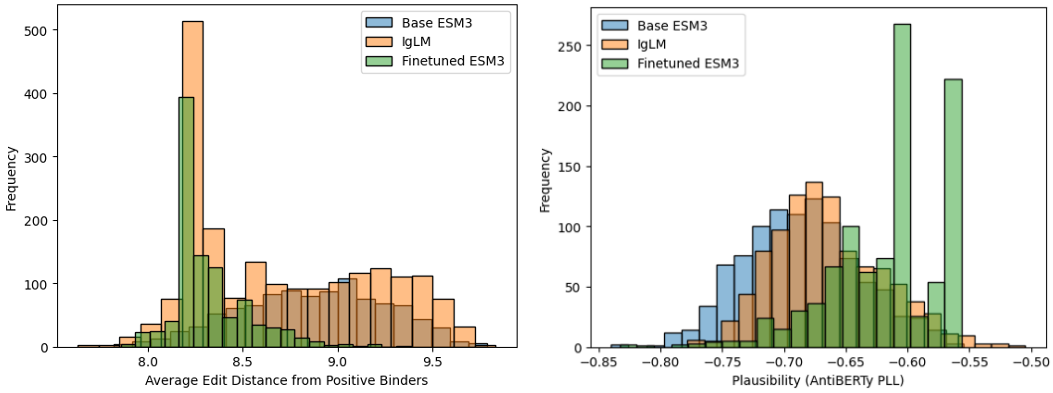

Figure 5: Average edit distance of each generated CDR3 sequence from the ground truth positive binder sequences and plausibility of each generated CDR3 sequence measured by AntiBERTy PLL.

3.1.2 Folding and Docking Evaluation

To evaluate the structural and interaction quality of the antibody sequences generated by the finetuned ESM3 model, we focused on the top five most plausible sequences. These were compared against five high-affinity sequences from the training set. The sequences were folded and docked using AlphaFold3 (Abramson et al. 2024) to predict the complexes' 3D structures, relaxed with OpenMM (Eastman et al. 2023) to optimize their energy configurations, and their interaction energies with HER2 were calculated using FoldX (Delgado et al. 2019). As shown in Figure 6, the top row displays the structures and interaction energies of the finetuned model's sequences, while the bottom row showcases the high-affinity sequences from the training set. The average interaction energy of the finetuned model's sequences was found to be -5.65 kcal/mol, which is comparable to the average interaction energy of -8.47 kcal/mol for the training set sequences. Notably, one of the sequences generated by the fine-tuned model, ARMDRYYFDY, achieved an interaction energy of -12.02 kcal/mol, which is highly competitive with the best-performing training set sequence (WHESDFYLFM) that exhibited a binding energy of -16.41 kcal/mol. These results demonstrate that the fine-tuned model has successfully learned structural patterns that contribute to high binding affinity. The comparable interaction energies between the generated and ground-truth sequences suggest that the finetuned model can produce sequences with functional properties that rival those of experimentally verified high-affinity binders. Furthermore, the structural similarities observed in the folded models further validate the ability of the finetuned ESM3 model to generate biologically relevant antibody sequences.

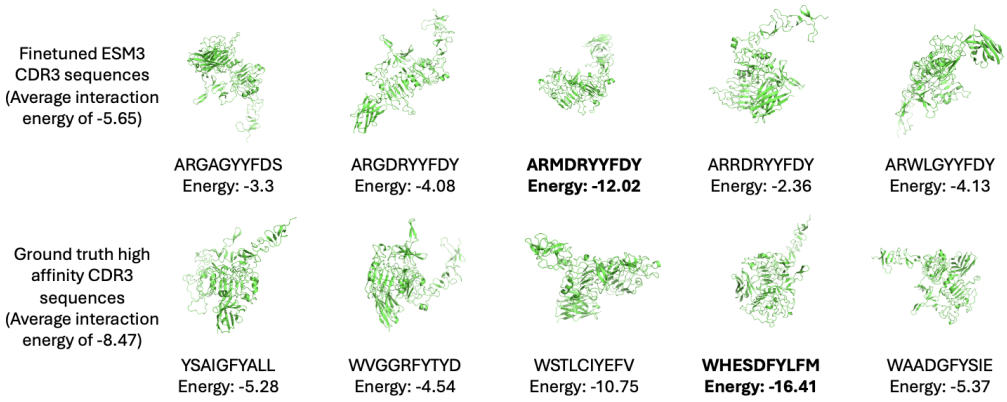

Figure 6: Interaction energy for designed CDR3s grafted into Trastuzumab docked against HER2.

4 Discussion and Future Work

Our work demonstrates the utility of finetuning ESM3 with contrastive preference optimization for antigen-specific antibody design. In particular, our results demonstrate how CPO-based finetuning enables ESM3 to generate potential CDR3 sequences that have high binding affinity, despite the fact that the base ESM3 model we finetuned was not trained on any antibody sequence data whatsoever. Furthermore, in comparison to existing baseline foundation models for both proteins and specifically antibodies, our finetuned ESM3 model outperforms such existing generations in terms of their plausibility, and edit distance from existing positive binders. In addition, after folding and docking the most plausible sequences generated by ESM3, we found that our complexes exhibited interaction energies comparable to those of the ground-truth high affinity CDR3 sequences from the training dataset, thereby further corroborating our claim in which our finetuned model can generate high-affinity binders.

Although we saw success in terms of ESM3's generative capabilities after finetuning, we failed to see similar improvement in terms of its representation learning capabilities. After finetuning, we saw only a very slight improvement in terms of ESM3's ability to classify high affinity and low affinity CDR3 sequences using the model's embedding space as a tool for discriminating betwen sequences. We can intuitively attribute this failure to the fact that ESM3's embedding space consists of a combination of sequential, structural, and functional data; since we are only finetuning ESM3 in the sequence space (and freezing all other parameters), there may not be enough information passed through these hidden layers to warrant improvements or separability within the embedding space. To combat this, one could perhaps implement a separate, trainable, self-attention-based track operating primarily on sequence representation learning.

There are numerous avenues for future work. First, we only trained on 10^6 pairs of sequences, when there are actually around $4 * 10^{10}$ possible pairs that exist in our training data. Although training on a dataset this large is intractable, we could perhaps find more nuanced relationships between pairs, after noticing that ESM3's embedding of the sequences may not have been as informative as we originally thought. Furthermore, the ESM3 model we finetuned had 1.4 billion parameters and is the smallest (but the only open-source) ESM3 variant. This model was not pretrained on any antibody sequential or structural data, making our results even more surprising given this context. However, we infer that if we were to finetune a larger ESM3 variant, perhaps one pretrained on antibody data, we could potentially see even better results. Finally, we could potentially integrate structural and functional information into the finetuning process, perhaps by providing information about antigen hotspots or refining the structure prediction pipeline by injecting structural data about the antibody/antigen itself. However, this would require an integration of only partial information into the model since we do not actually know the secondary structure of each of the potential CDR3 sequences, thereby complicating the task. However, we believe that adding such information would be critical in improving the effectiveness of our model and could potentially help improve the representation learning and generation capabilities.

5 Teamwork Division

The project was completed collaboratively, with specific responsibilities divided among team members as follows:

- **Anirudh Venkatraman:** Worked on data preparation, finetuning and evaluation.
- **Gopinath Balaji:** Worked on literature review and evaluation.
- **Veeresh Kande:** Worked on finetuning and evaluation.
- **Joint Efforts:** Report and slides preparation.

References

[1] Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A. J., Bambrick, J., Bodenstein, S. W., Evans, D. A., Hung, C.-C., O'Neill, M., Reiman, D., Tunyasuvunakool, K., Wu, Z., Žemgulytė, A., Arvaniti, E., … Jumper, J. M. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature, 630(8016), 493–500. https://doi.org/10.1038/s41586-024-07487-w

[2] Chinery, L., Hummer, A. M., Mehta, B. B., Akbar, R., Rawat, P., Slabodkin, A., Quy, K. L., Lund-Johansen, F., Greiff, V., Jeliazkov, J. R., & Deane, C. M. (2024). Baselining the buzz trastuzumab-her2 affinity, and beyond. https://doi.org/10.1101/2024.03.26.586756

[3] Delgado, J., Radusky, L. G., Cianferoni, D., & Serrano, L. (2019). FoldX 5.0: Working with RNA, small molecules and a new graphical interface. Bioinformatics, 35(20), 4168–4169. https://doi.org/10.1093/bioinformatics/btz184

[4] Hayes, T., Rao, R., Akin, H., Sofroniew, N. J., Oktay, D., Lin, Z., Verkuil, R., Tran, V. Q., Deaton, J., Wiggert, M., Badkundri, R., Shafkat, I., Gong, J., Derry, A., Molina, R. S., Thomas, N., Khan, Y., Mishra, C., Kim, C., … Rives, A. (2024). Simulating 500 million years of evolution with a language model. https://doi.org/10.1101/2024.07.01.600583

[6] Lin, Z., Akin, H., Rao, R., Hie, B., Zhu, Z., Lu, W., Smetanin, N., Verkuil, R., Kabeli, O., Shmueli, Y., Dos Santos Costa, A., Fazel-Zarandi, M., Sercu, T., Candido, S., & Rives, A. (2022). Evolutionary-scale prediction of atomic level protein structure with a language model. https://doi.org/10.1101/2022.07.20.500902

[7] Ruffolo, J. A., Gray, J. J., & Sulam, J. (2021). Deciphering antibody affinity maturation with language models and weakly supervised learning. arXiv. https://doi.org/10.48550/ARXIV.2112.07782

[8] Shuai, R. W., Ruffolo, J. A., & Gray, J. J. (2023). IgLM: Infilling language modeling for antibody sequence design. Cell Systems, 14(11), 979-989.e4. https://doi.org/10.1016/j.cels.2023.10.001

[9] Xu, H., Sharaf, A., Chen, Y., Tan, W., Shen, L., Van Durme, B., Murray, K., & Kim, Y. J. (2024). Contrastive preference optimization: Pushing the boundaries of llm performance in machine translation. arXiv. https://doi.org/10.48550/ARXIV.2401.08417

[10] Zhou, X., Xue, D., Chen, R., Zheng, Z., Wang, L., & Gu, Q. (2024). Antigen-specific antibody design via direct energy-based preference optimization. arXiv. https://doi.org/10.48550/ARXIV.2403.16576

[11] P. Eastman, R. Galvelis, R. P. Peláez, C. R. A. Abreu, S. E. Farr, E. Gallicchio, A. Gorenko, M. M. Henry, F. Hu, J. Huang, A. Krämer, J. Michel, J. A. Mitchell, V. S. Pande, J. PGLM Rodrigues, J. Rodriguez-Guerra, A. C. Simmonett, S. Singh, J. Swails, P. Turner, Y. Wang, I. Zhang, J. D. Chodera, G. De Fabritiis, and T. E. Markland. "OpenMM 8: Molecular Dynamics Simulation with Machine Learning Potentials." J. Phys. Chem. B 128(1), pp. 109-116 (2023).