Benchamaking deep generative models for diverse antibody sequence design

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Abstract

Computational protein design, i.e. inferring novel and diverse protein sequences consistent with a given structure, remains a major unsolved challenge. Recently, deep generative models that learn from sequences alone or from sequences and structures jointly have shown impressive performance on this task. However, those models appear limited in terms of modeling structural constraints, capturing enough sequence diversity, or both. Here we consider three recently proposed deep generative frameworks for protein design: (AR) the sequence-based autoregressive generative model, (GVP) the precise structure-based graph neural network, and Fold2Seq that leverages a fuzzy and scale-free representation of a three-dimensional fold, while enforcing structure-to-sequence (and vice versa) consistency. We benchmark these models on the task of computational design of antibody sequences, which demand designing sequences with high diversity for functional implication. The Fold2Seq framework outperforms the two other baselines in terms of diversity of the designed sequences, while maintaining the typical fold.

1 Introduction

Antibodies and their functional domains play key roles in research, diagnostics, and therapeutics. Among them, an attractive class is comprised of nanobodies, which are functional antibody domains with small size (∼15 kDa) and high stability (Tm up to 90 C), and therefore are of increasing therapeutic interest [3]. Designing functional sequences typically requires a combinatorial exploration of sequence space. To address this issue, one can impose sequence and/or structural constraints to narrow the search space.

Functionally diverse antibodies correspond to the highly stable immunoglobulin fold, a universal protein scaffold structure. Antigen binding specificity is largely determined by the sequence and structural diversity of the complementarity-determining regions (CDRs) that are displayed on canonical frameworks. Among the CDRs, CDR3 contributes most of the sequence and length diversity. As a result, a big focus in computational antibody design has been on sampling diverse CDR3s.

Along this direction, recently deep generative models have been used for sampling virtual sequences that were then prioritized for experimental validation. Most of those models are sequence-based that leveraged autoregressive neural nets such as LSTM. Such models have been successfully used for the entire functional antibody sequence design [13], as well as for designing only the CDRs given rest of the sequence as a context [14]. Nevertheless, such model does not explicitly account for the 3D
In the training stage, our model constructs a fold encoder: \( \text{Fold2Seq Encoder-Decoder Transformer} \) that adopts multi-layer \( \text{Transformer} \) and \( \text{GNN} \) as the encoder and decoder to implement the vanilla sequence embedding module (learnable model and a vanilla sequence embedding module (learnable). All training sequences are padded and \( \text{Transformer} \) is used to model the autoregressive likelihood. Determining regions (CDRs), which are complex and highly diverse parts of the sequence, determining antibody sequence. On the other hand, the work of [14] focused on the variable complementarity structure, represented as a graph over the residues. The key challenge was to account for long-range interactions with neighboring residues drive the folding event that defines its 1D sequence. Chemical composition and structural constraints associated with the characteristic immunoglobulin fold and may be limited in terms of capturing the broad landscape of CDRs.

In this paper, we benchmark three recently proposed state-of-the-art deep generative models – sequence-based, 3D structure-based, and 3D fold-based – by comparing the generated sequences of llama nanobodies. The structure-based and fold-based models generate full protein sequences by using a single representative 3D structure of the llama nanobody as an input, whereas the autoregressive sequence-based model designs CDR3 by conditioning on the preceding germline framework-CDR1-CDR2 nanobody sequence. The structure-based model leverages the precise backbone coordinates, whereas the fold-based model uses a fuzzy description of the secondary structures elements in 3D as the input. Machine learning, physicochemical, bioinformatics, and structural metrics of the generated sequences from a single llama nanobody sequence/structure and their comparison with the natural llama nanobody repertoire suggest that generated sequences strongly vary in term of novelty, diversity, consistency, and coverage.

## 2 Related Work

The protein/antibody design problem has been recently addressed using multiple machine learning approaches. For example, the sequence-based methods of [2] [13, 11] formulated the problem as an autoregressive process, leveraging deep recurrent networks such as an LSTM, to design the entire antibody sequence. On the other hand, the work of [14] focused on the variable complementarity determining regions (CDRs), which are complex and highly diverse parts of the sequence, determining the specificity of the antibody. Instead of recurrent networks, they applied causal dilated convolutions to model the autoregressive likelihood.

Alternatively, ML-physics hybrid methods have also been explored. For instance, [15], proposed a system, that starts from a random protein sequence, and the search process is guided by the physics-based constraints to satisfy the desired structural motifs. EVOdesign [11], on the other hand, is a structure-based approach that uses the evolutionary profiles to guide the sequence search. It first identifies the structural analogs from PDB and then constructs a structural sequence profile using MSA. The physics-based force fields are then used to search for the low free-energy sequence states.

Recently, a class of deep generative models that account for the 3D structural constraints, have been proposed. For example, [7] used a generative model for protein sequences design given a target structure, represented as a graph over the residues. The key challenge was to account for long-range dependencies in the protein sequence that are usually short-range in the 3D structure space.
Based on [7] the work of [9] then proposed Geometric Vector Perceptrons (GVPs) to allow for the embedding of geometric information at nodes and edges without reducing such information to scalars that may not fully capture complex geometry. The proposed graph network ensures that the vector and scalar outputs are equivariant and invariant with respect to rotations and reflections.

Designing proteins based on a rigid backbone structure is known to restrict the diversity and novelty of the sequences. For this reason, [4] proposed Fold2Seq, a transformer-based generative framework for designing protein sequences conditioned on a generic target fold, rather than the specific high resolution 3D structure. The fold here was defined as the spatial arrangement of the local secondary structure elements. The method also uses joint sequence–fold embedding to better capture the relationship between the two modalities.

Finally, a recent work of [8] proposed a generative model to specifically design only the CDRs of antibodies rather than the whole protein sequence. The approach is based on co-designing the CDR sequence and 3D structure of CDR as graphs. The output is built autoregressively while iteratively refining its predicted global structure, which in turn guides the next residue choice.

3 Method

Figure 1 shows the overview of our benchmarking study.

**Models** We examine three different models. AR [14]: the autoregressive approach that uses the causal dilated convolutions for the input prefix sequence to generate the CDR3 protein subsequence. GVP [9]: the encode-decoder GNN model that uses Geometric Vector Perceptrons to encode the scalar and vector structure information which is then decoded in the autoregressive manner to generate the entire protein sequence. Fold2seq [4]: another encode-decoder model based on transformer architecture that embeds the fuzzy 3D protein structure information (fold) in the joint sequence-fold embedding space and then decodes it autoregressively into the corresponding protein sequence. For the GVP and Fold2Seq outputs we run ANARCI [6] to extract the CDR regions.
Table 1: Sequence recovery rate (SRR) and NLL from the autoregressive sequence model [14] trained on natural llama nanobody repertoire for the sequences generated by the comparison methods and the sequences from the natural llama library, synthetic library, and next-generation sequencing library.

| Model     | Seq Recovery Rate (%) | NLL     |
|-----------|-----------------------|---------|
| Fold2Seq  | 30.711                | 2.572   |
| GVP       | 40.131                | 2.987   |
| AR        | 48.865                | 0.375   |
| Natural   | –                     | 0.371   |
| Synthetic | –                     | 4.912   |
| NGS       | –                     | 5.102   |

**Sequence Design** The sequence and structure corresponding to the Chain A of pdb id 3K3Q were used as inputs for this study. It is worth noting that this structure is included in the training of both GVP and Fold2Seq model, whereas a maximum of 58.94% sequence identity was found to be present between the input sequence and the AR training set.

We compare the full sequences as well as the CDRs across the generated ensembles. For this purpose, we extracted the CDRs from the generated sequences using the IMGT numbering scheme as returned by ANARCI software [6]. For the extracted CDRs, we estimate the percentage of unique sequences (uniqueness). Sequences that contain glycosylation sites, asparagine deamination motifs, or sulfur-containing amino acids (cysteine and methionine) were removed. For the AR model, we optionally considered an extra filter to exclude sequences that do not end with the final beta-strand of the nanobody template as in [14]. We denote the approach with final beta-strand filtering by AR filtered, while we call AR unfiltered the version without final beta-strand filtering.

**Evaluation Metrics** We define the set of the generated sequences (structures) conditioned on sequence/structure j as \( G_j \). In structure-based design, Sequence Recovery rate is defined as (SRR) for \( y_j \) as \( SRR_{structure}(j) = \frac{1}{|G_j|} \sum_{g \in G_j} SIM(x_g, x_j) \). A global alignment scheme and BLAST62 matrix, with a gap opening penalty of -10 and gap extending penalty of -1, were used for estimating pairwise sequence identity (SIM) and alignment score. Negative log likelihood (NLL) was estimated by using the autoregressive (AR) generative model trained on 1.2 million natural llama nanobody sequences [14], as following: \( NLL = - \sum_{k=1}^{K} \log(p(x_k | X_{<k})) \), which is sum of the cross-entropy between the true residue at each position and the predicted distribution over possible residues, conditioned on the preceding characters. Structural recovery of the three sequence design models by predicting the 3D structure of the top 100 generated sequences using pretrained models from AlphaFold2[1].

### 4 Results

Table 2: Uniqueness and novelty of the CDR3, CDR2, and CDR1 regions of the sequences generated by Fold2Seq, GVP, AR without final beta-stand filtering (AR unfiltered), AR with final beta-stand filtering (AR filtered), and the sequences from the natural llama library. Note that the AR approach has been trained to generate CDR3 only, given the preceding portion of a ground truth sequence.

| CDR   | Uniqueness | Novelty | Uniqueness | Novelty | Uniqueness | Novelty | Uniqueness | Novelty | Uniqueness | Novelty | Natural Llama |
|-------|------------|---------|------------|---------|------------|---------|------------|---------|------------|---------|---------------|
| CDR3  | Fold2Seq   | GVP     | AR unfiltered | AR filtered | Natural Llama |
|       | 100        | 88.33   | 87.57      | 13.85   | 100         |
|       | 43.36      | 32.71   | 11.92      | 8.97    | 32.64       |
| CDR2  | Uniqueness | Novelty | Uniqueness | Novelty | Uniqueness | Novelty | Natural Llama |
|       | 100        | 9.15    | –          | –       | 100         |
|       | 58.70      | 9.15    | –          | –       | 83.83       |
| CDR1  | Uniqueness | Novelty | Uniqueness | Novelty | Uniqueness | Novelty | Natural Llama |
|       | 92.49      | 56.20   | –          | –       | 100         |
|       | 60.75      | 51.99   | –          | –       | 83.37       |

Table[1] reports the average sequence recovery rate and average negative log likelihood from the trained autoregressive model in [14], estimated using 10k generated sequences. The autoregressive sequence model provides highest sequence recovery rate, followed by GVP and Fold2Seq. Both methods yield

[1]:https://github.com/kalininalab/alphafold_non_docker
sequences that share $\geq 30\%$ identity on average with the sequence of the input structure, implying fold-consistency of the generated sequences, as a $30\%$ sequence identity threshold typically suggests fold homology [12]. SRR of GVP is higher than Fold2Seq, consistent with earlier observation that a structure-based model yields higher sequence recovery than a fold-based model [4]. The negative log likelihood (NLL) from the trained autoregressive model was found to be consistent with the experimentally reported thermostability of unseen llama nanobody sequences, which is an important aspect of nanobody fitness [14]. Therefore, we also estimate the NLL of the generated sequences using Fold2Seq, GVP, AR, as well as a state-of-art synthetic library (Synthetic, constructed combinatorically using the position-specific amino acid frequencies of nanobody sequences with crystal structures in the PDB database) [10] and a large collection of nanobody sequences from next-generation sequencing repositories (ngs) [5]. As expected, AR-generated sequences show a NLL close to the natural nanobodies that were used for training of the model. Both synthetic and ngs libraries show very high NLL, indicating the bias of the trained AR toward natural llama nanobody repertoire. Among the two 3D-conditional generative models, interestingly, F2S sequences show lower NLL than GVP, implying F2S sequences are closer to the broader llama nanobody sequences.

As reported in Table 2, we estimate uniqueness and novelty of all three generative models with respect to the training set used for the AR model training. Fold2Seq outperforms both GVP and AR by a significant margin in term of uniqueness for all CDRs. Trend in novelty is not as clear though between GVP and Fold2Seq: while GVP produces more novel CDR3s, Fold2Seq performs better for CDR1s and CDR2s in term of novelty. For reference we also include in Table 2 uniqueness and novelty for 10,000 sequences selected at random from the natural llama library of [14]. This result implies that a fuzzy representation of a 3D fold allows generating sequences with novel and unique CDRs, when compared to a sequence-based and a backbone structure-based model.
This result is confirmed as we estimate the pairwise sequence similarity within the generated ensemble from a particular model (Figure 2). When compared to the database comprised of natural llama nanobody sequences, Fold2Seq sequences are more diverse (low similarity score within the ensemble) than GVP and AR sequences. GVP sequences show an interesting characteristic—majority of sequences are highly similar—indicating a mode collapse. In terms of similarity with the natural CDR3s, Fold2Seq sequences are more distant, followed by GVP and AR. The similarity distribution corresponding to GVP seem narrower, consistent with lack of diversity in generated samples.

Further, we analyze the physicochemical properties, such as isoelectric point (pI) and length of the CDR3 sequences. Figure 3 shows that the natural nanobody CDR3s populate broad ranges of isoelectric point and sequence length, with a preference toward pI around 6 and length around 14 and 18. Fold2Seq produces a significant coverage of the natural sequences, with a clustering around pI = 6 and length = 14, while still exhibiting non-zero density around the input. GVP produces sequences that are very close to the one corresponding to the input pdb only, while AR tends to generate short (around 6 amino acid) sequences with pI around 4.

Finally, we compare the structural recovery of the three generative frameworks by predicting the 3D structure of 100 random generated sequences using AlphaFold2. Figure 4(a) shows that, almost all of the sequences generated by the Fold2Seq and GVP share a sequence identity of ≥ 30% and TM-score of ≥ 0.5 with the groundtruth, indicating both methods perform similarly in terms of recovering correct fold topology [16]. However, GVP sequences exhibit higher TM-score than Fold2Seq ones, consistent with high consistency of the generated sequences with the input while lacking coverage and diversity (see Figure 4(a)). The AR sequences show high sequences identity as well as high TM-score, which is not surprising given that the model “grafts” a short (around 6 residue) sequences within the input sequence. In contrast, though Fold2Seq generates diverse and dissimilar sequences, corresponding structures turn out to be high-consistency, with a 1.8-3 Å backbone RMSD from the input structure (see Figure 4(b)).

In conclusion, this benchmarking study highlights key performance differences of three protein sequence design models on the nanobody sequence generation task. The backbone based GVP model provides high consistency with the input, while lacking the diversity present within the llama nanobody sequences. A sequence-based model, on the other hand, captures a limited spectrum of the llama nanobody repertoire. The sequence design model that employs a fuzzy representation of the
corresponding 3D fold as the input and generates sequences by leveraging representation learned by join training on 3D folds and 1D sequences provides better diversity, while still providing sufficient consistency with the input sequence/structure and coverage of the llama nanobody repertoire.

Ultimately, the choice of method should be guided by the task at hand. If one wishes to generate sequences very close to an input sequence/structure, GVP might be more appropriate. If one wishes to graft specific regions such as a short CDR3 within an input sequence, then the AR approach would be better suited. If one wishes to generate diverse sequences with broader coverage that “extrapolate” to a greater extent from a given input sequence, while still maintaining consistency with the input, then Fold2Seq would be a method of choice.

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