HOW TO HALLUCINATE FUNCTIONAL PROTEINS

A PREPRINT

Zak Costello
Agile Biofoundry
Joint BioEnergy Institute
Lawrence Berkeley National Lab
zak.costello@lbl.gov*

Hector Garcia Martin
Agile Biofoundry
Joint BioEnergy Institute
Lawrence Berkeley National Lab
hgmartin@lbl.gov

March 4, 2019

ABSTRACT

Here we present a novel approach to protein design and phenotypic inference using a generative model for protein sequences. BioSeqVAE, a variational autoencoder variant, can hallucinate syntactically valid protein sequences that are likely to fold and function. BioSeqVAE is trained on the entire known protein sequence space and learns to generate valid examples of protein sequences in an unsupervised manner. The model is validated by showing that its latent feature space is useful and that it accurately reconstructs sequences. Its usefulness is demonstrated with a selection of relevant downstream design tasks. This work is intended to serve as a computational first step towards a general purpose structure free protein design tool.

1 Introduction

Proteins are the main functional unit of life, performing a majority of tasks within the cell. These macromolecules perform a diverse set of functions including catalysis, structural support, mechanical transduction, molecular transport, and sensing. The ability to reliably engineer proteins with a specified function in a systematic way would be transformative for biology, allowing for the explicit design of molecular machines with a targeted function for a diverse array of applications.

However, proteins have proven difficult to engineer. Each protein is uniquely defined by a sequence of amino acids. This seemingly simple definition gives rise to dizzying complexity. While protein sequences can be trivially created by randomly choosing amino acids, not every protein sequence encodes a functional protein. In general functional protein sequences appear to be rare in the space of all possible sequences. For example it has been estimated that only 1 in 1 hundred billion random protein sequences has ATP binding activity. As such, there is an underlying syntax to these sequences that is required for function to be present. Syntactic correctness gives rise to recognized secondary (e.g. alpha helices) and tertiary structures (e.g. alpha/beta-barrel domains), which in aggregate lead to function. In part due to this complexity, no general method for reliably engineering proteins exists. Engineering proteins with a desired phenotype remains a piecemeal task that requires expert level skill to perform successfully.

Nonetheless, the discipline of protein engineering has enabled the creation of an array of novel and useful proteins. Sustainable and inexpensive biochemicals have been developed using modern metabolic engineering practices enabled by engineered proteins. Promising proteins for cancer therapeutics have been developed. Biosensors have been designed for rapid detection of various biomolecules for diagnostic and industrial purposes. In light of these successes, however, there is still no reliable general purpose engineering strategy.

A recently discovered class of machine learning models, known collectively as generative models, offer a way forward. Generative models are able to take an unlabeled data set, for example pictures of human faces, and learn how to create novel examples that are semantically accurate. In the case of human faces, generated images from a trained model would have noses, mouths, and eyes in the proper locations and proportions so that they would be human recognizable. Amazingly, this is done without ever having to tell the model what any of these features are or how they are related to the concept of a face. The models are simply trained on a collection of images. Generative models have been applied
successfully to many domains where unlabeled or sparsely labeled data is abundant. Impressive results have been published where this class of models has generated realistic examples of faces (8,9), audio (10), 3D objects (11), and RNA expression profiles (12).

To this end, we propose a general method for protein design and property inference which is uses only protein sequence and phenotype data. We developed a generative model variant trained on the full protein sequence space known as BioSeqVAE. BioSeqVAE can generate novel protein sequences that are syntactically correct and are likely to fold and function. This model is coupled with downstream supervised models that are used to search BioSeqVAE for valid protein sequences that impart a desired function. This approach, in theory, allows for the design of a protein with any desired function. We improve over previous work in the area of using generative models on protein sequences. Our model is general in the sense that it is trained on the entire known protein sequence space and therefore can produce a protein of any type. Additionally, our model has been engineered to generate proteins up to 1000 amino acids in length. To our knowledge, 1000 amino acids is longer than any machine learning model designed to generate proteins to date. In order to contextualize our model we review the protein engineering literature generally by contrasting our model with both de novo methods and directed evolution approaches.

Directed evolution approaches aim to iteratively enrich for a desired function through stages of mutation and selection of an initial protein sequence. It requires one or more starting proteins that can reasonably be evolved to have the desired function. This approach is advantageous because it does not require understanding of the relationship between sequence and function, and can still reach desired performance characteristics in a systematic way (13). These methods have resulted in substantial success in creating industrial catalysts (14,5). One important limitation of these methods is that they require a protein starting point that is able to be evolved to a desired function.

De novo methods use the principals of protein folding to design sequences with structure that results in a chosen function. First determining the structure of a protein with the function of interest is a more reasonable task to a human designer. Then de novo methods can find sequences that are likely to have the structure of interest. Like directed evolution, this approach has enjoyed wide success and has resulted in useful proteins designed from physical principals (15,6,3,16,17). It is distinguished from directed evolution by attempting to understand the relationship between sequence and function mediated through protein structure. Because of this, de novo techniques are not restricted to portions of the protein sequence space that has already been explored by nature.

There exists a small body of literature on the use generative models to infer protein properties or perform design. To our knowledge this has only been done on a limited scale. With protein sequence data, only sets of homologous sequences have been used for training (18,19,20). BioSeqVAE trains on the entire known proteome instead of specific families of proteins so that it can intuit the general syntax of a protein sequence. This more diverse training set introduces substantial challenges to constructing an well performing model. (18,19) used their models for downstream classification tasks and did not use them explicitly for design. Only (20) used their model explicitly for design. They used a novel generative modeling approach to generate small metaloproteins and peptides (20). This approach was limited in the size of proteins it could generate and did not use the entirety of the sequence space. We intend to use our model to design protein sequences with a collection of desireable protein phenotypes, instead of variants on a single family. In a recent review, (21) postulate the possibility of a model similar to BioSeqVAE. In the next section we describe several technical hurdles which had to be overcome in order to produce a functional model.

2 Model Development & Methods

Applying generative models to protein sequences presents unique challenges that require special modifications to the model architecture in order to perform well. BioSeqVAE is intended to be used for both phenotypic inference and design. In order to successfully perform both tasks an architechture was needed that could do all of the following:

(i) handle protein sequences with variable lengths
(ii) model interactions between distant amino acid residues
(iii) generate novel and realistic protein sequences
(iv) encode proteins into an informative feature vector

The first major design decision is to choose which type of generative model to use. Currently, generative models come in three flavors, variational autoencoders (22), generative adversarial networks (23), and normalizing flows (24,25,9). All of the first three requirements above are satisfied by any generative model. Only variational autoencoders and normalizing flow based models satisfy the fourth requirement. In this work, we choose to adopt a variational autoencoder model due to their relative maturity and ease of training, but note that normalizing flow based models are worth exploring in future work.
Generative models, such as the variational autoencoder, produce data with the same statistical properties that they were trained on. In the case of a generative model trained on pictures of human faces, novel but semantically valid faces can be efficiently generated. Images generated from the model have all of the characteristics required to be identified as a face including, eyes, ears, noses, and mouths. The same logic applies to BioSeqVAE, a generative model trained on functional protein sequences that fold in their native host. Therefore, the model should produce proteins that are likely to fold and function. We verify that the model is behaving in a way consistent with this assumption.

A variational autoencoder is a model that is trained to reconstruct its own input. In our case, it first encodes a protein sequence into a feature vector. The feature vector can be thought of as a summary of the important information in the protein sequence. The vector space where these feature vectors reside is generally known as the latent space. Then from that feature vector the variational autoencoder reconstructs the original protein sequence.

A fully trained variational autoencoder can be used in two modes, either as an encoder or decoder. The encoder can be used to take a protein sequence and find its associated feature vector. This feature vector can then be used for downstream classification or regression tasks. For example, we can determine where a given protein is likely to be localized given its sequence. The decoder can be used to generate arbitrary sequences that are likely to fold and function by sampling from the latent space. Further the latent space samples can be chosen so that sequences are likely to also have desired phenotypes. In the remainder of the section the model design and its uses are described detail.

2.1 Data Sources and Processing

The data to train BioSeqVAE was acquired from the UniProt database (26). We split the UniProt sequence database into two separate parts, SwissProt and TREMBL. The SwissProt Database is hand curated and contains about 550 thousand proteins. The TREMBL part of the Database is computationally predicted and contains approximately 140 million sequences. Sequences in the database were clustered into groups which shared over 80% homology. Then one sequence was chosen per cluster. This operation was performed using the Linclus command line tool (27). Since the goal of this model was to learn the general structures of protein sequences, we chose to include only representative sequences from clusters of proteins with similar homology. Sequences were further pruned by selecting sequences between 100 and 1000 amino acid residues in length. The data cleaning operation reduced the SwissProt and TREMBL datasets to 200 thousand and 45 million sequences respectively. For the experiments in this paper models were trained only on the SwissProt data set. The sequences were represented with one hot encoding with 21 categories, where 20 were amino acids and one represented sequence end.

2.2 Model Architecture

Here we briefly review the variational autoencoder and discuss the relevant modifications needed to make it work on protein sequence data. For complete coverage of variational autoencoding models see (22). We adapt a variational
autoencoding model to perform unsupervised learning on protein sequences (Figure 1). We start by constructing some data set, \( X = \{ x_i \}_{i=1}^N \), where \( X \subset \mathbb{R}^{21 \times 1000} \). In our case this is the set of all known protein sequences after the data cleaning protocol from above. Our objective is to maximize the likelihood of our generative model \( p_\theta(X) \) given our data. In general, this objective function,

\[
\max_\theta \log p_\theta(X),
\]

is intractable to evaluate. The main insight of the variational autoencoder is that one can introduce a set of latent variables \( z \in \mathbb{Z} \subset \mathbb{R}^m \) and break the model into an encoder and a decoder. In general \( m \) is a model hyperparameter that may be chosen. In this case we choose \( m = 250 \). The encoder estimates the distribution \( q_\phi(z|x) \) over latent variables in \( \mathbb{Z} \) given a particular datapoint \( x \). The decoder provides the distribution \( p_\theta(x|z) \) for output in data space \( \mathbb{X} \) given a particular point in the latent space \( z \in \mathbb{Z} \). Both the encoder and decoders are typically deep learning models parameterized by their weights \( \theta \) and \( \phi \) respectively. Starting from the objective function of the optimization problem in \( 1 \), we can use this insight to derive a computationally tractable lower bound on our objective using Jensen’s Inequality as below:

\[
\log p_\theta(x) = \log \int_\mathbb{Z} p_\theta(x, z) dz = \log \int_\mathbb{Z} p_\theta(x, z) \frac{q_\phi(z|x)}{q_\phi(z|x)} dz = \log \mathbb{E}_{q_\phi(z|x)} \left[ \frac{p_\theta(x, z)}{q_\phi(z|x)} \right] = \mathbb{E}_{q_\phi(z|x)} [\log p_\theta(x, z)] - \mathbb{E}_{q_\phi(z|x)} [\log q_\phi(z|x)]
\]

Now, instead of explicitly maximizing the intractable likelihood of the model, a tractable lower bound on that objective can be optimized. This lower bound is known as the evidence lower bound (ELBO). To gain a better intuition on this lower bound, the ELBO can be rewritten using the definition of the Kullback–Leibler (KL) divergence in an easier to interpret form,

\[
\mathcal{L}_{ELBO}(\theta, \phi, x) = \mathbb{E}_{q_\phi(z|x)} [\log p_\theta(x|z)] - D_{KL}(q_\phi(z|x)||p(z)),
\]

where \( p(z) \) is an easy to sample from distribution over \( Z \). For BioSeqVAE we choose this distribution to be the standard multivariate normal distribution, \( \mathcal{N}(0, I) \). The ELBO loss as expressed above has two terms with straightforward interpretations. The first term is the reconstruction loss, which measures how well a particular data point is reconstructed when run through both the encoder and decoder. The second term represents the closeness of the latent variable distribution to the chosen simple prior distribution, \( p(z) \). When this term in the loss is minimized, sampling points from the distribution of syntactically correct protein sequences efficient because valid points in \( Z \) are close to the distribution \( p(z) \).

For protein sequence design and phenotypic inference we need both an accurate reconstruction, and an informative latent space. To this end, we chose a high capacity decoder to encourage high reconstruction accuracy. However, this design choice can make the latent space encode uninformative features. Several modifications exist to help fix this problem by constraining the amount of mutual information between \( x \) and \( z \) in the encoding model \( 31 \). We use the result from \( 30 \) to augment the ELBO objective and force the model to encode informative features in the latent space. The resulting objective has the form

\[
\mathcal{L}_{INFOVAE}(\theta, \phi, x) = \mathbb{E}_{q_\phi(z|x)} [\log p_\theta(x|z)] - (1 - \alpha) D_{KL}(q_\phi(z|x)||p_\theta(z)) - (\alpha + \lambda - 1) D_{MMD}(q_\phi(z)||p_\theta(z))
\]

where \( \alpha \) and \( \lambda \) are hyperparameters weighting the mutual information and agreement with the chosen latent feature distribution respectively. The final term is the maximum-mean discrepancy divergence which is easily computed \( 30 \). Now that we have motivated the model structure and chosen an objective compatible with our goals, we move forward to specific encoder and decoder design considerations.
2.3 Design of the Encoder and Decoder

To implement a variational autoencoder a parameterized encoder, $q_\phi(z|x)$, and decoder, $p_\theta(x|z)$, must be designed. The encoder and decoder design of BioSeqVAE is inspired by PixelVAE (32) and includes modifications made to improve its function on protein sequence data. In the particular case of encoding protein sequences, we expect the data distribution to be highly complex in the sense of having many different interactions between amino acids. Whatever model is used to estimate the joint distribution over amino acids must be sufficiently comprehensive to express every proteomic device that is known to exist. Additionally, the model must be able to capture interactions between amino acids distant in sequence space. This requirement is due to a protein sequence representing a biomolecule that is embedded in three dimensions. Additionally, we require high fidelity reconstruction and an informative latent space. These specifications are addressed by the design considerations below.

Due to the complexity of the distribution we are trying to estimate, we start off with the assumption that the model will benefit from a very deep ResNet style convolutional network. The depth gives rise to an exponential increase in expressivity of the network (33). The ResNet architecture allows the network to be efficiently trained (34). The specific form of the residual layers used in both the encoder and decoder come from (28). This residual layer shows improved training times and overall performance compared to the original ResNet layer design.

In order to capture the distant interactions between residues dilated convolutions are used. Application of dilated convolutions allows for exponential instead of linear increase in the receptive field of the network as a function of depth (29; 35). The chosen network architecture has a receptive field large enough to capture dependencies between any pair of amino acids in the input sequence.

To free the variational autoencoder model from memorizing the fine details of protein sequences explicitly (e.g. the particular amino acid distribution of a beta sheet) we augment the decoder with an autoregressive module (32; 36; 37; 38; 39). The autoregressive module can learn the local structure of the amino acid sequence, leaving the latent space to encode the higher level details such as secondary structure into the feature space.

Combining all of the design considerations leads to the architecture visualized in Figure 1. Inside of each module the colored cubes each represents a layer type. Gray scale layers indicate a 1D dilated convolutional layer with skip connections in the style of resnet (28). The darkness of the grayscale layers indicates the magnitude of the dilation. Progressively darker gray indicates larger dilation. This is done in the pattern used in (29). Red layers indicate a 1D convolution where the length of the input is halved with a stride of two and the channels are doubled. Green layers indicate the reverse operation of red via a transposed 1D convolution. The encoder contains 25 convolutional resnet style blocks in total and two strided convolution layers for down scaling and channel doubling. The decoder reverses the encoder structure. In the auto regression module, gray scale blocks are causal (in the style of (40)) in addition to being 1D dilated convolution residual layers.

2.4 Training & Code Availability

To train this model the cleaned SwissProt database was used. The model was trained end to end using the ADAM optimizer (41). Complete code will be made available coinciding with final publication.

2.5 Protein Property Inference

Once an instance of BioSeqVAE is trained, the latent feature space can be used to predict the phenotype of a given protein. This task is performed using a supervised learning approach. A dataset relating sequence to function must be provided in order to learn which points in latent feature space relate to specific functions. It is important to note that this can be done for any imaginable protein property for which a dataset can be gathered. Some possible properties include Gene Ontology IDs, temperature stability, EC Number, or protein localization. In practice, much of the required data has already been gathered and is readily available across many bioinformatics databases. For this work we specifically used data present in the UniProt database.

Supervised models are created by first using BioSeqVAE to encode all protein sequences in the data set into a latent feature vector. Then that latent feature vector and the associated phenotype is used to train the model. In this work, we use a random forest model from scikit-learn without parameter tuning for training. When both the unsupervised variational autoencoding model and a set of supervised phenotype models are created targeted design of function becomes possible.
2.6 Protein Design

With BioSeqVAE the design problem is reduced to a search of the latent feature space, as every point in the space is associated with a protein sequence that is likely to fold and have some function. So the design task relies on downstream models to predict how points in the latent feature space relate to desirable phenotypes.

A set of models that relate points in the latent feature space to different phenotypes \{f_i\}_{i=1}^N, can be leveraged to generate enzymes with any combination desired properties. This allows design to be rephrased as an optimization problem in euclidean space as follows.

\[
\min_z \sum_{i=1}^m \alpha_i (f_i(z) - c_i)^2
\]

(2)

where \(f_i\) is the \(i\)th model \(c_i\) is the target (e.g. a specific sequence length) and \(\alpha_i\) is a weight. Once solved, the optimal point in latent feature space, \(\hat{z}\), is decoded to find a candidate protein to test in downstream experiments.

3 Results & Discussion

BioSeqVAE’s core capability is to encode protein sequences into an information rich latent feature space and generate protein sequences that are likely to fold and function. In this section we provide evidence for these claims that motivates future experimental validation. Analyses are first performed to validate the models core function (i.e. does the model perform like a valid variational autoencoder?). Once validated, we illustrate the usefulness of BioSeqVAE for design and phenotypic inference with two classification tasks. We demonstrate good performance on an enzyme classification task and a protein localization task while showing how the model can be used to design new sequences. The intent of these tasks is to demonstrate that the latent feature space encodes features that are useful for downstream learning rather than chasing state of the art performance. The ultimate objective is to develop models that allow the user to find points in the latent feature space that generate proteins with properties of interest. To emphasize this point, we generate representative sequences for each of the supervised models we present. The section is culminated by demonstrating how sequences that are likely to have a combination of desirable properties can be generated.

3.1 Model Validation

To validate the model is performing correctly, both qualitative and quantitative methods were employed. As an overall performance measure, the accuracy of encoding and then decoding the same protein was evaluated (i.e. reconstruction). Then, the distribution of the latent feature space was estimated to check that it was close a standard normal distribution. Finally, random samples were taken from the latent feature space and decoded to show qualitatively that the generated sequences look correct.

Figure 2: BioSeqVAE is able to reconstruct proteins in the test set with accuracy that is dependent on length. It is very effective at reconstructing short proteins and the accuracy trails off to around 50% at 1000 amino acids. On average reconstruction accuracy is 83.7% ± 12.1%.
BioSeqVAE accurately reconstructs proteins. To evaluate reconstruction accuracy, known proteins from the test set were embedded using the encoder, then decoded to predict the original sequence. 1000 random proteins from the test set were reconstructed. The percent agreement between the actual sequence and the predicted reconstruction was calculated. The results of this test are visualized in Figure 2. The average reconstruction accuracy is 83.7% ± 12.1%. The length of the protein is related to the reconstruction accuracy, with the algorithm performing better on shorter proteins. For proteins with less than 250 amino acids, we expect greater than 90% reconstruction accuracy. We expect with increasing latent space dimension, that reconstruction accuracy will be improved.

The latent feature space is can be sampled from easily and produces qualitatively valid random samples. To validate that the feature space can produce good protein sequence samples, 10,000 proteins from the test set were encoded into the feature space. The mean and covariance matrix for those encoded features was calculated. The KL divergence term in the loss encourages the latent feature space to have a standard multivariate normal distribution. In practice we do not reach that exact distribution but approximate it. In Figure 3, covariance matrix is plotted.

| Mean and Variance of Latent Space Components |
|---------------------------------------------|
| mean | variance |
| 0.04 | 0.02 |
| 0.00 | 0.02 |
| 0.06 |

Figure 3: The latent space can be well approximated as a multivariate gaussian with 250 dimensions. The dimensions of the Gaussian are close to independent. Using the mean and covariance matrix efficient samples representative of protein sequences can be synthesized. Samples of Random Protein sequences are obtained by sampling from the latent feature space then running them through the BioSeqVAE decoder. Qualitatively, we can see that these proteins do not have any obvious artifacts such as long amino acid repeats. When these sequences are BLASTed against UniProt database, they have small stretches of homology with sequences that were not in the training set demonstrating that they share qualities with known proteins.

Then, latent feature space samples were drawn from a multivariate normal with the estimated statistics shown in Figure 3. One thing to notice is that the diagonal components of the covariance matrix are largest, showing that the model disentangles features from the data set into a set of features that are closer to independent. In Figure 3, three samples from the latent feature space were reconstructed into protein sequences. Qualitatively, these sequences look good with
no long stretches of amino acid repeats or other visually obvious artifacts. Additionally, the random proteins have small stretches of homology with existing proteins when BLASTed against the UniProt database.

3.2 BioSeqVAE can be used to Generate Representative Examples of Proteins with a Desired Phenotype.

When sampling randomly from the latent feature space, the phenotype of the protein sequence that is generated is unknown. In order to discover the phenotype of that generated sequence, supervised learning methods are employed to learn the relationship between points in the latent space and a particular phenotype. This relationship will be easiest for the model to learn if BioSeqVAE encodes informative features. Specifically, any set of supervised phenotype models can be used to predict which points in the latent feature space correspond to proteins of interest. For example, the models can be used to find points in the latent space which correspond to proteins that are localized in the membrane and also catalyze a desired reaction. From those points in the latent space, BioSeqVAE can hallucinate syntactically valid proteins that are likely to have the desired phenotype. In this way we can pair the strengths of several models and use them for either design or phenotypic inference. We present two examples of this as a high level proof of concept. It is worth noting that this procedure can be done for arbitrarily complex phenotypes and with any number of models. The only requirement is that a sufficiently large supervised data set is available to train each phenotypic models.

3.2.1 Enzyme Type can be Accurately Predicted and Designed using BioSeqVAE

A powerful use case of our model is the capacity to create novel enzymes. To show an initial proof of principal, a data set of 60,000 enzyme sequences and their type were gathered. The enzyme classification scheme used was the Enzyme Commission [EC] number. The EC number is a hierarchical ontology for classifying enzymes. It has 4 levels, where the highest level is most coarse. This highest level is broken down into 6 general enzyme categories along with transporters as a 7th bin. We classified enzymes into each of the 6 highest level enzyme categories. Deep learning has been used to classify enzymes by EC number in the past and achieved high accuracy (42). Our objective was not to beat state of the art classification accuracy, but instead to show that we could generate novel examples of each of these enzyme types. Because these enzymes are hallucinated from BioSeqVAE they are likely to be syntactically valid.

A simple random forest classification model from scikit-learn (43) was applied to the collected enzyme sequence data set obtained from the UniProt database where both sequence and EC Number were known. The protein sequences were encoded into a 250 dimentional feature vector using BioSeqVAE. Then these features and the first element of the EC Number were used in a supervised learning setting to train a random forest classifier. The classifier achieved 70.6% cross validated error (Figure 4). Further, it was used to generate an enzyme likely to be of each type.

3.3 Protein Localization can be Predicted and Designed using BioSeqVAE

Proteins final destination within the cell is encoded in its sequence. As membrane proteins in particular can be difficult to work with in metabolic engineering applications, a method that can be used to convert a membrane protein into a cytosolic variant would be valuable. We demonstrate the generation of proteins that are likely to be secreted, localized to the membrane, the nucleus, and the cytosol. Again a phenotype model is trained to learn where a given protein is localized from its sequence.

The dataset was again gathered from the UniProt database. The resulting dataset contained 63,532 proteins with examples balanced evenly between each of the 4 predicted compartments. A random forest classifier with the same hyperparameters as the previous model was applied to this dataset. The results are visualized in Figure 5. Average classification accuracy of the model was 65.1% ± 0.5%. From this model proteins can be found which are most likely to belong to each of the tested compartments using the method from section 2.6.

3.4 BioSeqVAE Can be used to Design Proteins with Multiple Properties Simultaneously

In order to design proteins of interest, multiple phenotypes may have to be combined. This is possible by optimizing over both of the existing classifiers simultaneously and generating a candidate sequence that is likely to be localized to the membrane and also is an oxioreductase.

>Optimal Membrane Protein Oxioreductase
MLNSASTLYYPALIAALAYLL1LCIPKVSKFSGRSASIWAYASREGKGGRIWDRKASVRFSPQFRHYEYAAKRLP1VVRIFKNNQ
WQTVDLRKSPANGLRTCDRTFGPMHRDTITFVIDVLYLCQAMAAYDVYVQPLFLYSCRMRLKSVSLDGRLPASPRKRIFIGSA
ALAMRQDLTDGASIAIARTGVVWAA5RTPITVEGGHKLKHTQKCASLAGFAPQIELLLGLVQAQRPF1NFPFVRFGQSPYLDDXLLLP
WGLLAEMTHTDLALKIDONMNETENQYLMIL
Hallucinated Oxidoreductase, Confidence 35%

MIDPGEVTYRAGAQKEQFGGLIFFPKPVWLTSANQPKEDAVSVDSSGGRQTRLIGDKCRVDWKVRQVEAQLSDIYTDASLQAVLDEDEFRFYEAEKRNVRDLEDEPSVGKQFOEQDKINTGKHTMAGAATGFSDSCMAIGAEEMIAAQMPIGSARYKQRYQGFGIEANGNESQNLHILTSPVAMCCTPDMAFDDGGPDKIPFLGLRGKASLTAFAQRAARGAATTEGFHQVGD

Hallucinated Transferase, Confidence 31%

MSSSAGRKRSTKDVFYFLSLTCDTTTLTYCMALLFVIQRLDKHGRAAHDVVKARQLAQRQGILDERKSARDSPPIIIILLITLGPMKESLPLQILIDPLALPKEAHLVHPHLPREYPHYPVYPTEAAGLPALGSVTLKLRCPAATTVEKIIITQTFEVAQQLNANVSTSPGMIWARPASPMAMVQDQNLIPQCPGKSTALITQYDDSGWGDREPDFKVFHRAGAPNDPKLLASFalFLMLMLVLAVANDKSWNlMARGA
<Hallucinated Hydrolase, Confidence 37%

MTASPKNRQNVYLNQFNDIEIESPEVKSSHKBIGSNNAEVNFKNVRTDEAKQSFYIEIFENVSYEEDGSDAAFEYPIKHLODKPTSAARECGDQWLAKEEVLEPLSTRYIERTARDLIDQPLVPLPSVPFARQSPIEPNDFDELSFLGFLGQJKITKSMTHEKSNENVQANFFKSIYDRYRGYSYRHRDGSVPAYVIWILPSGARIISRTSAIGTVSFYSSMTYVSEITPGMADQAGFRPNVRPLLDIYCDARPIHKPDSQSLHFDAPYLEAQKITYVRPDQIGTVYEGLGKAIVAAITCFFKPFDLSSLNYDILKMATPVIALNDGGIGAFLLLDPHDKDDQHDLVLKGFATIQLAAAAEIEYWRIQNLHEHFEIL

Hallucinated Lyase, Confidence 26%

MVRSEVQFGDGGRSPRKLGRKGRAVILEVALCEVQAQAVAGPIARPCQKGLLHATAPATASAIAFVLSQFVPGHWTLSHHAQAFFAVADNADNFLQRVRYRTQLYMDIPRMMRMPNMACAGYLGETADSAFENRTQEGMACAFAVVIPISLGGRRSWPLSNVIGGTVAEVPALGLARANNWVIRASAADGLYPCYLDYTDDPPFDLYMLAMQILDIPQQAAVLAGAIRERLLLRLQRLTALKA

Hallucinated Isomerase, Confidence 27%

MQATLRQQKPSRCQEGALLMLRLAEFGCWGQRKTVQVDGRAVAGHVLARILISLEVPEVASEGWAMMLRPRLLYDLRKLDRSPTYVQPLRLACRSEQHQLAKSEDEAAKPDYKQFDLNDNIHSETLEASKEATGHSVHPGAPWTAADSPGANDRDKPAHEIMTHREDLATTAPATQFQRLEGALVLYLEAAALQGQQL

Hallucinated Ligase, Confidence 56%

MMEKLRYPVHMSDIQMDASAOQQRDTHMIHQGPFFARRIRQQVQPKYRSDDEASYAKSRLERPSQSQIRQGDYACEARPDSVFLSQAADDSTVCAKVASGKARNEMMLQFQVKELSNAPKAYQLGPVLRALERPSDFRQCDVALLPREDLIDRIGTVDDLRKQPVNIPIECKRERIDAIYQAGQDVWVGQLCMCGCQFYRVRKCTAEKRIYVDISLASSATIYMCEYAHRDGMANDTQTVSTVAIFRPLTRILYAAAMTRQLSQLSWPPAPVDRATVDTDDLEAIYLLYYYLNLNPYYTFPGWAVCMLWAGASTGQMILLQQAAATDLLQYYEGMTVLYKNNANVIIIFRKLCCMGHRRKLYLID

Figure 4: The latent feature space can be used to both determine which enzyme class that a sequence belongs to and create novel examples of that type of enzyme. A balanced dataset of 60000 examples of enzymes with known EC numbers were used to train this model. (left) The confusion matrix shows how well each class is predicted from a given point in the latent space. (right) The combined reciever operating characteristic curve for the classifier. (bottom) Using the supervised EC number classification model and the technique outlined in section 2.6, Representative sequences from each class of proteins were generated. When blasted against the UniProt database some show homology with proteins of their designated type.
Confusion Matrix for Compartment Classifier
(Overall Accuracy: 65.1% ± 0.5%)

| Predicted Compartment | Actual Compartment |
|------------------------|--------------------|
| cytoplasm              | 65                 |
| membrane               | 9.6                |
| nucleus                | 19                 |
| secreted               | 6.5                |
| cytoplasm              | 16                 |
| membrane               | 54                 |
| nucleus                | 20                 |
| secreted               | 10                 |
| cytoplasm              | 16                 |
| membrane               | 9.2                |
| nucleus                | 69                 |
| secreted               | 6.5                |
| cytoplasm              | 8.3                |
| membrane               | 8.7                |
| nucleus                | 12                 |
| secreted               | 71                 |

Receiver Operating Characteristic for Compartment Classifier (area = 0.91)

Figure 5: The latent feature space can be used to both determine protein localization and create novel examples of proteins with chosen localizations. (left) The confusion matrix shows how well each class is predicted from a given point in the latent space. (right) The combined reciever operating characteristic curve for the classifier. Using the supervised compartment prediction model and the technique outlined in section 2.6, Representative sequences localized to the following areas were generated: Nucleus, Membrane, Cytosol, Secreted.

This particular protein when blasted against the complete UniProt database has homology with a subunit of the ABC binding cassette transporter and a transmembrane protein of unknown function from Chara braunii. While this is suggestive that the designed protein has the specified phenotype, only actual experimentation will allow the design claims to be rigorously validated.

4 Conclusion

We have demonstrated that realistic protein sequences can be hallucinated from an unsupervised machine learning model, BioSeqVAE. The properties of sequences can be intuited from the latent feature space of BioSeqVAE. This paper is intended to demonstrate that protein sequences that are likely to fold and function are possible to create in the absence of structural information. This opens up the possibility to use much larger and easier to collect data sets and leverage those for the creation of novel proteins for an array of applications. This paper lays the groundwork for experimental testing of the hallucinated models in the lab and testing them for their
designed function. Additionally, it provides a novel way to tackle pathway completion when looking for proteins in pathways for orphaned metabolites. We fully intend to use this model to tackle these challenges.

5 Acknowledgements

This work was part of the DOE Agile BioFoundry (http://agilebiofoundry.org), supported by the U.S. Department of Energy, Energy Efficiency and Renewable Energy, Bioenergy Technologies Office, and the DOE Joint BioEnergy Institute (http://www.jbei.org), supported by the Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

References

[1] Pengfei Tian and Robert B Best. How many protein sequences fold to a given structure? a coevolutionary analysis. *Biophys J*, 113(8):1719–1730, oct 2017.

[2] Anthony D Keeffe and Jack W Szostak. Functional proteins from a random-sequence library. *Nature*, 410(6829):715, 2001.

[3] Justin B Siegel, Amanda Lee Smith, Sean Poust, Adam J Wargacki, Arren Bar-Even, Catherine Louw, Betty W Shen, Christopher B Eiben, Huu M Tran, Elad Noor, Jasmine L Gallerah, Jacob Bale, Yasuo Yoshikuni, Michael H Gelb, Jay D Keasling, Barry L Stoddard, Mary E Lidstrom, and David Baker. Computational protein design enables a novel one-carbon assimilation pathway. *Proc Natl Acad Sci USA*, 112(12):3704–3709, mar 2015.

[4] Hans Renata, Z Jane Wang, and Frances H Arnold. Expanding the enzyme universe: accessing non-natural reactions by mechanism-guided directed evolution. *Angew Chem Int Ed Engl*, 54(11):3351–3367, mar 2015.

[5] Devin L Trudeau, Toni M Lee, and Frances H Arnold. Engineered thermostable fungal cellulases exhibit efficient synergistic cellulose hydrolysis at elevated temperatures. *Biotechnol Bioeng*, 111(12):2390–2397, dec 2014.

[6] Daniel-Adriano Silva, Shawn Yu, Umut Y. Ulge, Jamie B. Spangler, Kevin M. Jude, Carlos Labão-Almeida, Lestat R. Ali, Alfredo Quijano-Rubio, Mikel Ruterbusch, Isabel Leung, Tamara Biary, Stephanie J. Crowley, Enrique Marcos, Carl D. Walkey, Brian D. Weitzner, Fátima Pardo-Avila, Javier Castellanos, Lauren Carter, Lance Stewart, Stanley R. Riddell, Marion Pepper, Gonçalo J. L. Bernardes, Michael Dougan, K. Christopher Garcia, and David Baker. De novo design of potent and selective mimics of IL-2 and IL-15. *Nature*, 565(7738):186–191, jan 2019.

[7] Viktor Stein and Kirill Alexandrov. Synthetic protein switches: design principles and applications. *Trends Biotechnol*, 33(2):101–110, feb 2015.

[8] Tero Karras, Timo Aila, Samuli Laine, and Jaakko Lehtinen. Progressive growing of GANs for improved quality, stability, and variation. *arXiv*, oct 2017.

[9] Diederik P. Kingma and Prafulla Dhariwal. Glow: Generative flow with invertible 1x1 convolutions. *arXiv*, jul 2018.

[10] Chin-Wei Huang, David Krueger, Alexandre Lacoste, and Aaron Courville. Neural autoregressive flows. *arXiv*, apr 2018.

[11] Jiajun Wu, Chengkai Zhang, Tianfan Xue, Bill Freeman, and Josh Tenenbaum. Learning a probabilistic latent space of object shapes via 3d generative-adversarial modeling. In D. D. Lee, M. Sugiyama, U. V. Luxburg, I. Guyon, and R. Garnett, editors, *Advances in Neural Information Processing Systems* 29, pages 82–90. Curran Associates, Inc., 2016.

[12] Romain Lopez, Jeffrey Regier, Michael B Cole, Michael I Jordan, and Nir Yosef. Deep generative modeling for single-cell transcriptomics. *Nat Methods*, 15(12):1053–1058, dec 2018.
[13] Frances H Arnold. Directed evolution: bringing new chemistry to life. *Angew Chem Int Ed Engl*, 57(16):4143–4148, apr 2018.

[14] Andrew R Buller, Paul van Roye, Jackson K B Cahn, Remkes A Scheele, Michael Herger, and Frances H Arnold. Directed evolution mimics allosteric activation by stepwise tuning of the conformational ensemble. *J Am Chem Soc*, 140(23):7256–7266, jun 2018.

[15] Justin B Siegel, Alexandre Zanghellini, Helena M Lovick, Gert Kiss, Abigail R Lambert, Jennifer L St Clair, Jasmine L Gallaher, Donald Hilvert, Michael H Gelb, Barry L Stoddard, Kendall N Houk, Forrest E Michael, and David Baker. Computational design of an enzyme catalyst for a stereoselective bimolecular diels-alder reaction. *Science*, 329(5989):309–313, jul 2010.

[16] Po-Ssu Huang, Scott E Boyken, and David Baker. The coming of age of de novo protein design. *Nature*, 537(7620):320–327, sep 2016.

[17] Ivan Coluzza. Computational protein design: a review. *J Phys Condens Matter*, 29(14):143001, apr 2017.

[18] Adam J Riesselman, John B Ingraham, and Debora S Marks. Deep generative models of genetic variation capture the effects of mutations. *Nat Methods*, 15(10):816–822, oct 2018.

[19] Jakob Nybo Nissen, Casper Kaae Sonderby, Jose Juan Almagro Armenteros, Christopher Heje Groenbech, Henrik Bjorn Nielsen, Thomas Nordahl Petersen, Ole Winther, and Simon Rasmussen. Binning microbial genomes using deep learning. *BioRxiv*, dec 2018.

[20] Anvita Gupta and James Zou. Feedback gan for dna optimizes protein functions. *Nature Machine Intelligence*, 1(2):105, 2019.

[21] Kevin K Yang, Zachary Wu, and Frances H Arnold. Machine learning in protein engineering. *arXiv preprint arXiv:1811.10775*, 2018.

[22] Diederik P Kingma and Max Welling. Auto-encoding variational bayes. *arXiv*, dec 2013.

[23] Ian Goodfellow, Jean Pouget-Abadie, Mehdi Mirza, Bing Xu, David Warde-Farley, Sherjil Ozair, Aaron Courville, and Yoshua Bengio. Generative adversarial nets. In Z. Ghahramani, M. Welling, C. Cortes, N. D. Lawrence, and K. Q. Weinberger, editors, *Advances in Neural Information Processing Systems 27*, pages 2672–2680. Curran Associates, Inc., 2014.

[24] Laurent Dinh, David Krueger, and Yoshua Bengio. NICE: Non-linear independent components estimation. *arXiv*, oct 2014.

[25] Laurent Dinh, Jascha Sohl-Dickstein, and Samy Bengio. Density estimation using real NVP. *arXiv*, may 2016.

[26] The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Res*, 46(5):2699, mar 2018.

[27] Martin Steinegger and Johannes Söding. Clustering huge protein sequence sets in linear time. *Nat Commun*, 9(1):2542, jun 2018.

[28] Kaiming He, Xiangyu Zhang, Shaoqing Ren, and Jian Sun. Deep residual learning for image recognition. In *The IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, June 2016.

[29] Fisher Yu, Vladlen Koltun, and Thomas Funkhouser. Dilated residual networks. In *2017 IEEE Conference on Computer Vision and Pattern Recognition (CVPR)*, pages 636–644. IEEE, jul 2017.

[30] Shengjia Zhao, Jiaming Song, and Stefano Ermon. InfoVAE: Information maximizing variational autoencoders. *arXiv*, jun 2017.

[31] Shengjia Zhao, Jiaming Song, and Stefano Ermon. The information autoencoding family: A lagrangian perspective on latent variable generative models. *arXiv preprint arXiv:1806.06514*, 2018.

[32] Ishaan Gulrajani, Kundan Kumar, Faruk Ahmed, Adrien Ali Taiga, Francesco Visin, David Vazquez, and Aaron Courville. PixelVAE: A latent variable model for natural images. *arXiv*, nov 2016.

[33] Maithra Raghu, Ben Poole, Jon Kleinberg, Surya Ganguli, and Jascha Sohl Dickstein. On the expressive power of deep neural networks. In *Proceedings of the 34th International Conference on Machine Learning-Volume 70*, pages 2847–2854. JMLR. org, 2017.
[34] Kaiming He, Xiangyu Zhang, Shaoqing Ren, and Jian Sun. Identity mappings in deep residual networks. In Bastian Leibe, Jiri Matas, Nicu Sebe, and Max Welling, editors, *Computer vision – ECCV 2016*, volume 9908 of *Lecture notes in computer science*, pages 630–645. Springer International Publishing, Cham, 2016.

[35] Fisher Yu and Vladlen Koltun. Multi-scale context aggregation by dilated convolutions. *arXiv*, nov 2015.

[36] Tim Salimans, Andrej Karpathy, Xi Chen, and Diederik P. Kingma. PixelCNN++: Improving the PixelCNN with discretized logistic mixture likelihood and other modifications. *arXiv*, jan 2017.

[37] Aaron van den Oord, Nal Kalchbrenner, Oriol Vinyals, Lasse Espeholt, Alex Graves, and Koray Kavukcuoglu. Conditional image generation with PixelCNN decoders. *arXiv*, jun 2016.

[38] Aaron van den Oord, Nal Kalchbrenner, and Koray Kavukcuoglu. Pixel recurrent neural networks. *arXiv*, jan 2016.

[39] Aaron van den Oord, Sander Dieleman, Heiga Zen, Karen Simonyan, Oriol Vinyals, Alex Graves, Nal Kalchbrenner, Andrew Senior, and Koray Kavukcuoglu. WaveNet: A generative model for raw audio. *arXiv*, sep 2016.

[40] Nal Kalchbrenner, Lasse Espeholt, Karen Simonyan, Aaron van den Oord, Alex Graves, and Koray Kavukcuoglu. Neural machine translation in linear time. *CoRR*, abs/1610.10099, 2016.

[41] Diederik P. Kingma and Jimmy Ba. Adam: A method for stochastic optimization. *CoRR*, abs/1412.6980, 2014.

[42] Yu Li, Sheng Wang, Ramzan Umarov, Bingqing Xie, Ming Fan, Lihua Li, and Xin Gao. Deepre: sequence-based enzyme ec number prediction by deep learning. *Bioinformatics*, 34(5):760–769, 2017.

[43] F. Pedregosa, G. Varoquaux, A. Gramfort, V. Michel, B. Thirion, O. Grisel, M. Blondel, P. Prettenhofer, R. Weiss, V. Dubourg, J. Vanderplas, A. Passos, D. Cournapeau, M. Brucher, M. Perrot, and E. Duchesnay. Scikit-learn: Machine learning in Python. *Journal of Machine Learning Research*, 12:2825–2830, 2011.