

# PepMLM: Target Sequence-Conditioned Generation of Peptide Binders via Masked Language Modeling

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## Abstract

Target proteins that lack accessible binding pockets and conformational stability have posed increasing challenges for drug development. Induced proximity strategies, such as PROTACs and molecular glues, have thus gained attention as pharmacological alternatives, but still require small molecule docking at binding pockets for targeted protein degradation (TPD). The computational design of protein-based binders presents unique opportunities to access “undruggable” targets, but have often relied on stable 3D structures or predictions for effective binder generation. Recently, we have leveraged the expressive latent spaces of protein language models (pLMs) for the prioritization of peptide binders from sequence alone, which we have then fused to E3 ubiquitin ligase domains, creating a CRISPR-analogous TPD system for target proteins. However, our methods rely on training discriminator models for ranking heuristically or unconditionally-derived “guide” peptides for their target binding capability. In this work, we introduce **PepMLM**, a purely target sequence-conditioned *de novo* generator of linear peptide binders. By employing a novel masking strategy that uniquely positions cognate peptide sequences at the terminus of target protein sequences, PepMLM tasks the state-of-the-art ESM-2 pLM to fully reconstruct the binder region, achieving low perplexities matching or improving upon previously-validated peptide-protein sequence pairs. After successful *in silico* benchmarking with AlphaFold-Multimer, we experimentally verify PepMLM’s efficacy via fusion of model-derived peptides to E3 ubiquitin ligase domains, demonstrating endogenous degradation of target substrates in cellular models. In total, PepMLM enables the generative design of candidate binders to any target protein, without the requirement of target structure, empowering downstream programmable proteome editing applications.

## Introduction

The development of therapeutics largely relies on the ability to design small molecule- or protein-based binders to pathogenic target proteins of interest.<sup>1</sup> These binders can either be used as inhibitors or as functional recruiters of effector enzymes.<sup>2</sup> For example, proteolysis targeting chimeras (PROTACs) or molecular glues are heterobifunctional small molecules that bind and recruit endogenous E3 ubiquitin ligases for targeted protein degradation (TPD).<sup>3,4</sup> Still, these small molecule-based methods rely on the existence of accessible cryptic or canonical binding sites, which are not present on classically “undruggable” intracellular proteins.<sup>5,6</sup> With the advent of deep learning-based structure prediction tools such as AlphaFold2,<sup>7</sup> combined with generative modeling,<sup>1</sup> algorithms such as RFDiffusion and MASIF-Seed enable researchers to conduct *de novo* protein binder design from target structure alone.<sup>8,9</sup> Nonetheless, much of the undruggable proteome, including dysregulated proteins such as transcription factors and fusion oncoproteins, are conformationally disordered, thus biasing design to a small subset of disease-related proteins.<sup>1,6</sup>

Over the past few years, deep learning has revolutionized natural language processing (NLP), particularly through the implementation of the attention mechanism.<sup>10</sup> This foundational advancement has transcended the boundaries of natural language analysis, finding pertinent applications in the modeling of other languages, such as proteins, which are fundamentally sequences of amino acids.<sup>11</sup> In recent times, several protein language models (pLMs), trained on distinct transformer architectures, such as ProtT5, ProGen2, ProtGPT2, and the ESM series, have accurately captured critical physicochemical properties of proteins.<sup>12–15</sup> Notably, ESM-2 currently stands as the state-of-the-art model in the realm of protein sequence encoding, essentially functioning as a BERT-style model that discerns co-evolutionary patterns among protein sequences via a masked language modeling (MLM) training task.<sup>16,17</sup> These models have been extended to powerful applications, including antibody design, the creation of novel proteins, and structure prediction, offering a streamlined approach to embedding useful protein information.<sup>13,14,16,17</sup> Recently, our lab has leveraged the expressivity of pLMs to both generate and prioritize effective peptidic binder motifs to targets of interest, enabling design of peptide-guided degraders, termed ubiquibodies (uAbs).<sup>18–20</sup> As such, uAbs represent a programmable, CRISPR-like approach for TPD. Our early models, Cut&CLIP and SaLT&PepPr, rely on the existence of interacting partner sequences as scaffolds for peptide design<sup>19,20</sup>. Most recently, our PepPrCLIP model generates *de novo* peptides by first sampling the ESM-2 latent space for naturalistic peptide candidates, and then screening these candidates through a contrastive model to determine target sequence specificity.<sup>21</sup> However, a purely *de novo*, target sequence-conditioned binder design algorithm has yet to be developed.

To achieve this goal, we introduce **PepMLM**, a novel Peptide binder design algorithm via Masked Language Modeling, built upon the foundations of ESM-2.<sup>16</sup> PepMLM innovates by employing a contiguous masking strategy that uniquely positions the entire peptide binder sequence at the terminus of target protein sequences, compelling ESM-2 to reconstruct the entire binding region (Figure 1A). PepMLM-derived linear peptides achieve low perplexities, matching or improving upon validated peptide-protein sequence pairs in the test dataset, and experimentally exhibit degradation capability of endogenous, disordered target substrates when incorporated into the uAb architecture. Overall, by focusing on the complete reconstruction of peptide regions, PepMLM represents the first example of target-conditioned *de novo* binder design from sequence alone, thus facilitating a deeper understanding of binding dynamics and paving the way for the development of more effective, targeted binders to unstructured proteins of interest.

## **Results**

We trained PepMLM using existing peptide-protein binding data sourced from the recent PepNN training set and the gold-standard Propedia dataset.<sup>22,23</sup> We subjected our curated dataset to a filtration process based on the lengths of the binder and target protein sequences, which were confined to 50 and 500 respectively. To remove redundancies, we applied a homology filter thresholded at 80%, resulting in a final training set of 10,000 samples and leaving 203 samples for testing.<sup>24</sup> Each entry in the dataset comprised a concatenated protein and binder sequence. During the training phase, we masked the entire peptide sequence, tasking the model to reconstruct them via the ESM-2-650M model. The discrepancy between the ground truth binder and the reconstructed binder induces a cross entropy loss, thereby forcing parameter updates via gradient descent. Post fine-tuning, we generate peptide binders of specific lengths by providing the model with a target protein sequence and a user-defined number of mask tokens, as illustrated in Figure 1A. Final settings and hyperparameters used to train our model are presented in Supplementary Table 1.

We considered two distinct decoding strategies during the generation phase. The default strategy, akin to ESM-2 or BERT-style models,<sup>16</sup> employs greedy decoding, wherein the token with the highest probability is selected at each site. Despite its efficacy, greedy decoding is limited to the generation of a single peptide binder. To augment the diversity of the peptides, we introduced top- $k$  sampling, allowing PepMLM to randomly select from the top  $k$  probable tokens at each site. In this decoding strategy, we evaluated perplexity alongside various  $k$  values, ranging from 2 to 10, on the test set of target proteins. For each target protein, we generated 10 binders of the same length as the ground truth binder. We observed that as  $k$  increased, perplexity also rose, indicating a decrease in model confidence (Figure 1B). While higher  $k$  values yielded more diverse binder sequences, they also corresponded with an increase in the number of outliers. To find a balance between sequence diversity and maintaining model confidence (as indicated by the lower perplexity), we settled on  $k = 3$  as our final selection.

To substantiate the efficacy of the generated peptides, we conducted a comprehensive series of computational benchmarks with test-set peptide-target pairs. The total 203 test-set target proteins were utilized to generate one peptide binder each, employing pre-trained ESM-2 embeddings and PepMLM (Supplementary Table 2). Subsequently, the pseudo-perplexity of the binder region was computed for four groups of target protein:binder pairs. For a significant portion of the test-set, known binders exhibited a reasonable perplexity range, with only a few outliers, validating the model's effective ability to model them accurately (Figure 1C). A comparative analysis revealed that the binders generated by PepMLM exhibited lower perplexity values, suggesting a higher likelihood of them making stable binding interactions with the target (Figure 1C). Moreover, our distribution analysis revealed that PepMLM closely mirrors the distribution peak of real binders, a deviation from the distribution shifts observed with the original ESM-2 model alone and with randomly generated binders (Figure 1D). Our observation underscores the model's ability to grasp the underlying conditional distribution of protein and peptide binding to a notable extent, enhancing the suitability of ESM-2 for peptide binder design through fine-tuning.

Next, to benchmark PepMLM's generation quality, we co-folded the test and generated binders with their respective target proteins utilizing AlphaFold-Multimer, which has been proven effective at predicting peptide-protein complexes.<sup>25,26</sup> The pLDDT and ipTM scores, verified metrics within AlphaFold2,<sup>7</sup> function as critical indicators of the structural integrity and the potential interface binding affinity of peptide-protein complex, respectively, providing a quantitative assessment of our generation. The extracted ipTM and pLDDT values from our benchmarking indicated a significant negative correlation ( $p < 0.01$ ) with PepMLM perplexity, affirming the model's reliability at prioritizing binders with stable binding capacity to the target (Supplementary Figure 1). Our further analysis, which involved sorting the test set based on their ipTM values and comparing them with the corresponding generated binders, revealed a hit rate of over 38%, with many showcasing promising ipTM scores exceeding 0.95 (Figures 2A and 2B). This data suggests that PepMLM can generate and prioritize effective binders, precluding the need for extensive downstream experimental screening.

To corroborate our *in silico* results on more unique targets, we sought to test PepMLM-generated binders in our uAb architecture to degrade pathogenic proteins in a cellular model of Ewing sarcoma, a pediatric bone malignancy with no approved targeted therapies.<sup>27</sup> As our targets, we chose two cancer-related proteins, 4E-BP2 and  $\beta$ -catenin, as well as the more structured histone H3 protein, a core epigenetic nuclear protein that comprises chromatin.<sup>28-30</sup> To design peptides, we first employed greedy decoding to determine the optimal binder length that yielded the lowest perplexity, followed by the generation of binders for each target sequence using top  $k$  sampling, where  $k$  was fixed at 3 as previously described (Supplementary Table 3). After cloning these peptides into our uAb backbone and transfecting into A673 Ewing sarcoma cells, we conducted Western blotting on whole-cell protein extracts with target-selective primary antibodies (Figure 2C). Our results demonstrate that select PepMLM-generated "guide" peptides induce binding and subsequent degradation of endogenous targets when fused to E3 ubiquitin ligase domains, demonstrating reduced protein levels relative to that of the non-targeting control uAb (Figure 2D), motivating further design and testing of effective degraders to diverse pathogenic targets.

To this point, we had utilized the lightweight ESM-2-650M model, enabling flexible fine-tuning and inference. To assess the performance of larger models, we additionally fine-tuned ESM-2-3B<sup>16</sup> for peptide generation (PepMLM-3B) and evaluated it using the same methodology as employed for the ESM-2-650M version of

PepMLM (PepMLM-650M). However, as illustrated in Supplementary Figure 2, we did not observe a substantial improvement in either perplexity or hit rate for PepMLM-3B (36.02%). Considering the associated resource and inference costs, we provide our PepMLM-650M model as a resource for effective linear peptide generation.

## **Discussion**

By simply redesigning a guide RNA, the CRISPR-Cas system enables targeting and modification almost any DNA sequence, a programmable process that has revolutionized biology.<sup>31</sup> Specifically, with the recent engineering of protospacer adjacent motif (PAM)-relaxed Cas variants, there is minimal restriction as to which user-defined DNA sequences can be bound and edited.<sup>32,33</sup> Unfortunately, an analogous, programmable system for “editing” proteins has yet to be developed, as the design of target-specific binders requires either accessible binding pockets for small molecules, which do not exist on “undruggable” targets such as those at flat protein-protein interaction interfaces, or conformationally-stable 3D structures, precluding the binding of disordered targets such as dysregulated transcription factors and fusion oncoproteins.<sup>1</sup> To mitigate these shortcomings, here, we introduce PepMLM, the first *de novo* binder design algorithm directly conditioned on the target sequence of a protein. By using generated peptides as guides for E3 ubiquitin ligase domains, and eventually other post-translational modification domains, this work serves as a step forward towards developing a fully modular proteome editing system.

We envision that further improvements can be made to PepMLM, enabling its adoption as a universal tool for peptide binder design. A first, simple step would be to integrate PepMLM generation with our recent PepPrCLIP contrastive discriminator to allow for ranking of peptides with high target specificity.<sup>21</sup> Moreover, we envision that PepMLM can be retrained with modification-aware and variant-aware pLM embeddings to enable specificity to post-translational isoforms over wild-type protein states. Finally, we are planning to integrate PepMLM generation with high-throughput lentiviral screening to both evaluate its hit rate experimentally and input experimental data back into the algorithm, creating an active learning-based optimization loop. As a note, we have not validated PepMLM’s ability to generate high affinity, standalone peptide binders, those that can be further stabilized via cyclization or stapling, though this may prove possible via the current algorithm.<sup>34,35</sup> Nonetheless, we envision that through additional development, our accessible peptide generator, coupled with variants of our uAb architecture, will enable a CRISPR-analogous system to bind and modulate any target protein, whether structured or not.

## **Methods**

### **Data Curation**

In the data curation phase, protein and peptide complexes were amalgamated from the PepNN and Propedia databases.<sup>22,23</sup> Initially, redundancy between the two datasets was eliminated, followed by the utilization of MMseqs2 to cluster the remaining protein sequences, setting a threshold of 0.8.<sup>24</sup> When protein sequences were identified within the same cluster and exhibited identical binder sequences, a single sequence was retained. This was followed by a manual filtering process, wherein protein sequences were sorted and those exhibiting high similarity (threshold of 80%) were removed to further mitigate homology issues. Consequently, a dataset comprising 10,203 entries was amassed, from which 10,000 were randomly allocated for training and 203 for testing. The maximum lengths for the binder and protein sequences were established at 50 and 500, respectively.

### **Conditional Peptide Modeling**

Peptide binders are modeled in a distinctive manner, wherein the peptides are modeled conditionally based on the full protein sequence. Let  $p = (p_1, p_2, p_3, \dots, p_n)$  represent the target protein sequence of length  $n$  and  $b = (b_1, b_2, b_3, \dots, b_m)$  denote the binder of length  $m$ . The protein and peptide sequences are concatenated, incorporating start, end, and padding tokens. Mask language modeling transforms this into a conditional modeling problem, where the objective is to reconstruct  $b$  given  $p$ , as the entire  $b$  region is masked during both training and generation phases. The entire model is updated with a Cross Entropy loss function, where

$p = (p_1, p_2, p_3, \dots, p_n)$  represents the target protein sequence of length  $n$  and  $b = (b_1, b_2, b_3, \dots, b_m)$  denote the binder of length  $m$ . The protein and peptide sequences are concatenated, incorporating start, end, and padding tokens. Mask language modeling transforms this into a conditional modeling problem, where the objective is to reconstruct  $b$  given  $p$ , as the entire  $b$  region is masked during both training and generation phases. The entire model is updated with Cross Entropy loss, which can be represented as:

$$\mathcal{L} = - \sum_{i=1}^m b_i \log(\hat{b}_i)$$

Through this methodology, the discrepancy between the generated binders and the ground truth is minimized, facilitating the learning of the conditional probability,  $\prod_{i=1}^m P(b_i|p)$ .

### PepMLM Training

The pre-trained protein language model, ESM-2, was utilized to facilitate full parameter fine-tuning. ESM-2, a transformer-based model, is adept at discerning coevolutionary patterns across protein sequences. The concatenated protein and peptide sequences were tokenized at the amino acid level and input into the model. Deviating from the original training strategy of ESM, the entire binder sequence was exclusively masked, compelling the model to learn the relationship between the peptide binder and the protein. The ESM-2-650M and ESM-2-3B models were both trained for PepMLM. Both versions were trained on an NVIDIA 8xA100 640 GB DGX GPU system with Pytorch 2.01 and Python 3.10.10. Specific parameters are shown in Supplementary Table 1.

### PepMLM Generation

During the generation phase, the target protein sequence, along with a designated number of mask tokens (at end), was input into the model. Subsequently, the model greedily decodes logits at each masked position to identify peptide binders. To infuse greater diversity into the generation process, top- $k$  sampling was implemented, wherein the model randomly selects the top  $k$  highest probability logits at each masked position.

### Pseudo-Perplexity of PepMLM

The pseudo-perplexity of ESM-2 was adapted to focus specifically on the evaluation of peptide binder generation. Notably, the perplexity calculation is confined to the binder region, or, in other words, the masked regions. Mathematically, the pseudo-perplexity is defined as:

$$\text{PseudoPerplexity}(b) = \exp \left\{ -\frac{1}{m} \sum_{i=1}^m \log p(b_i | b_{j \neq i}, p) \right\}$$

In this equation,  $b$  represents the binder sequence and  $m$  is the length of the binder sequence. This modification ensures a more focused evaluation of the generated peptide binders, aligning with the conditional modeling approach adopted in this study.

### Generated Peptide Benchmarking

To assess the efficacy of the generated peptide binders, two benchmarking studies were conducted: one on the test set and another on selected critical proteins. In the test set benchmarking, top  $k$  sampling ( $k=3$ ) was employed to generate a single peptide binder for each target protein. Additionally, the original ESM-2 model was utilized to generate peptides, and random peptides of equivalent length were created. For ESM-2 generation, specifically, mask tokens of the same length were added at the end of target protein sequences for analogous model prediction and decoding as for PepMLM. The perplexity of the PepMLM was compared across four groups. PepMLM-generated binders and test binders were folded using the AlphaFold2 ColabFold version 1.5.2, in conjunction with the protein sequences. Folding metrics including pLDDT and ipTM were

gathered, which were utilized to correlate perplexity findings. For each test target protein, the ipTM scores of the test and generated binders were compared to determine the overall hit rate. Notice, as top k sampling generates with randomness, the hit rate might vary or increase with different runs or k options.

For the selected critical proteins, greedy decoding generation was initially utilized to ascertain the optimal length, followed by the implementation of top-k sampling to generate several binders with a desired degree of diversity. Binders were generated to select target proteins for experimental testing.

## Generation of plasmids

All uAb plasmids were generated from the standard pcDNA3 vector, harboring a cytomegalovirus (CMV) promoter and a C-terminal IRES-mCherry cassette as a transfection control. An Esp3I restriction site was introduced immediately upstream of the CHIPΔTPR CDS and flexible GSGSG linker via the KLD Enzyme Mix (NEB) following PCR amplification with mutagenic primers (Genewiz). For uAb assembly, peptide sequences were human codon-optimized for complementary oligo generation (Genewiz). Oligos were annealed and ligated via T4 DNA Ligase into the Esp3I-digested uAb backbone. Assembled constructs were transformed into 50 μL NEB Turbo Competent *Escherichia coli* cells, and plated onto LB agar supplemented with the appropriate antibiotic for subsequent sequence verification of colonies and plasmid purification (Genewiz).

## Cell culture

A673 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, and 10% fetal bovine serum (FBS). For uAb testing, pcDNA3-uAb (500 ng) plasmids were transfected into cells as triplicates ( $4 \times 10^5$ /well in a 12-well plate) with Lipofectamine 2000 (Invitrogen) in Opti-MEM (Gibco).

## Cell fractionation and immunoblotting

On the day of harvest, cells were detached by addition of 0.05% trypsin-EDTA and cell pellets were washed twice with ice-cold 1X PBS. Cells were then lysed and subcellular fractions were isolated from lysates using a 1:100 dilution of protease inhibitor cocktail (Millipore Sigma) in Pierce RIPA buffer (ThermoFisher). Specifically, the protease inhibitor cocktail-RIPA buffer solution was added to the cell pellet, the mixture was placed at 4 °C for 30 min followed by centrifugation at 15,000 rpm for 10 min at 4 °C. The supernatant was collected immediately to a pre-chilled PCR tube, and after adding 4X Bolt™ LDS Sample Buffer (ThermoFisher) with 5% β-mercaptoethanol in a 3:1 ratio, the mixture was incubated at 95 °C for 10 min prior to immunoblotting. Immunoblotting was performed according to standard protocols. Briefly, samples were loaded at equal volumes into Bolt™ Bis-Tris Plus Mini Protein Gels (ThermoFisher) and separated by electrophoresis. iBlot™ 2 Transfer Stacks (Invitrogen) were used for membrane blot transfer, and following a 1 h room-temperature incubation in SuperBlock™ Blocking Buffer (ThermoFisher), proteins were probed with rabbit anti-4E-BP2 antibody (Cell Signaling, Cat # 2845; diluted 1:500), rabbit anti-Histone 3 antibody (Abcam, Cat # ab1791; diluted 1:500), rabbit anti-β-catenin antibody (Cell Signaling, Cat # 8480; diluted 1:500), or mouse anti-GAPDH antibody (Santa Cruz Biotechnology, Cat # sc-47724; diluted 1:500) for overnight incubation at 4°C. The blots were washed three times with 1X TBST for 5 min each and then probed with a secondary antibody, goat anti-rabbit IgG (H+L), horseradish peroxidase (HRP) (ThermoFisher, Cat # 31460, diluted 1:5000) or goat anti-mouse IgG (H+L) Poly-HRP (ThermoFisher, Cat # 32230, diluted 1:2000) for 1-2 h at room temperature. Following three washes with 1X TBST for 5 min each, blots were detected by chemiluminescence using an iBright 1500 Imaging System (ThermoFisher). Densitometry analysis of protein bands in immunoblots was performed using ImageJ software as described here: <https://imagej.nih.gov/ij/docs/examples/dot-blot/>. Briefly, bands in each lane were grouped as a row or a horizontal "lane" and quantified using FIJI's gel analysis function. Intensity data for the uAb bands was first normalized to band intensity of GAPDH in each lane then to the average band intensity for the uAb vector control cases across replicates.

## Statistical analysis and reproducibility

To ensure robust reproducibility of all results, experiments were performed with at least three biological replicates. Sample sizes were not predetermined based on statistical methods but were chosen according to the standards of the field (at least three independent biological replicates for each condition). All data were reported as average values with error bars representing standard deviation (SD). All graphs were generated using Prism 10 for MacOS. No data were excluded from the analyses. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

### **Supplementary Figures**

Supplementary Figure 1. Association between model perplexity and co-folding metrics.

Supplementary Figure 2. Evaluation of PepMLM-3B.

Supplementary Table 1. Settings and hyperparameters used to train PepMLM-650M.

Supplementary Table 2. Peptide sequences, PPL, and ipTM scores for AlphaFold-Multimer benchmarking.

Supplementary Table 3. Peptide sequences and PPL scores for uAb generation and experimental testing.

### **Author Contributions**

T.C. designed the PepMLM architecture, curated peptide-protein data, trained and evaluated trained models. T.C., S.V., V.S.K., R.P., A.H., and P.V. performed *in silico* benchmarking. S.P. conducted *in vitro* assays for uAb screening, with L.H., T.W., V.Y., E.H. and L.Z. providing technical assistance. P.C. conceived, designed, and directed the study.

### **Data and Materials Availability**

All data needed to evaluate the conclusions in the paper are present in the paper and supplementary tables and figures. Inference code and API to run PepMLM can be found at: <https://huggingface.co/TianlaiChen/PepMLM-650M>.

### **Competing Interests**

P.C. is a co-founder of UbiquiTx, Inc., and is the inventor of patents related to genetically-encoded proteome editing technologies. The remaining authors declare no competing interests.

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### **Declarations**

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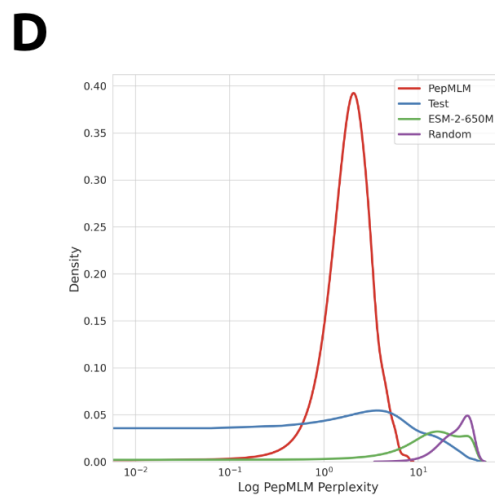
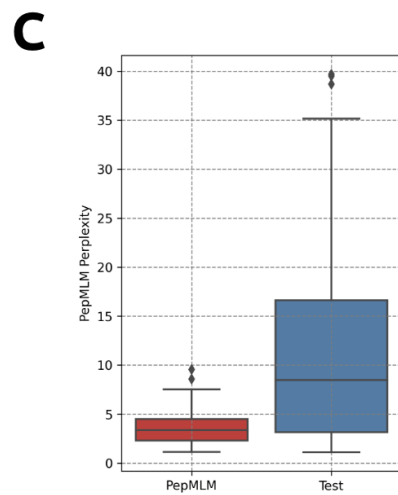
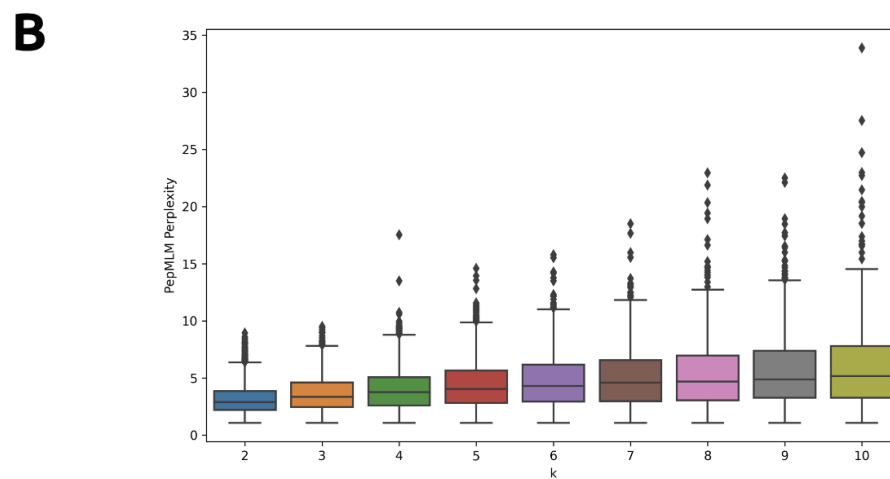
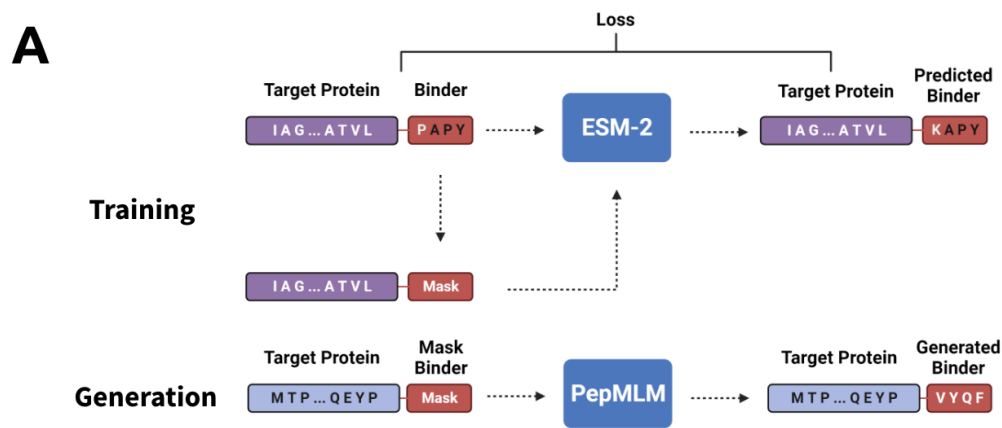
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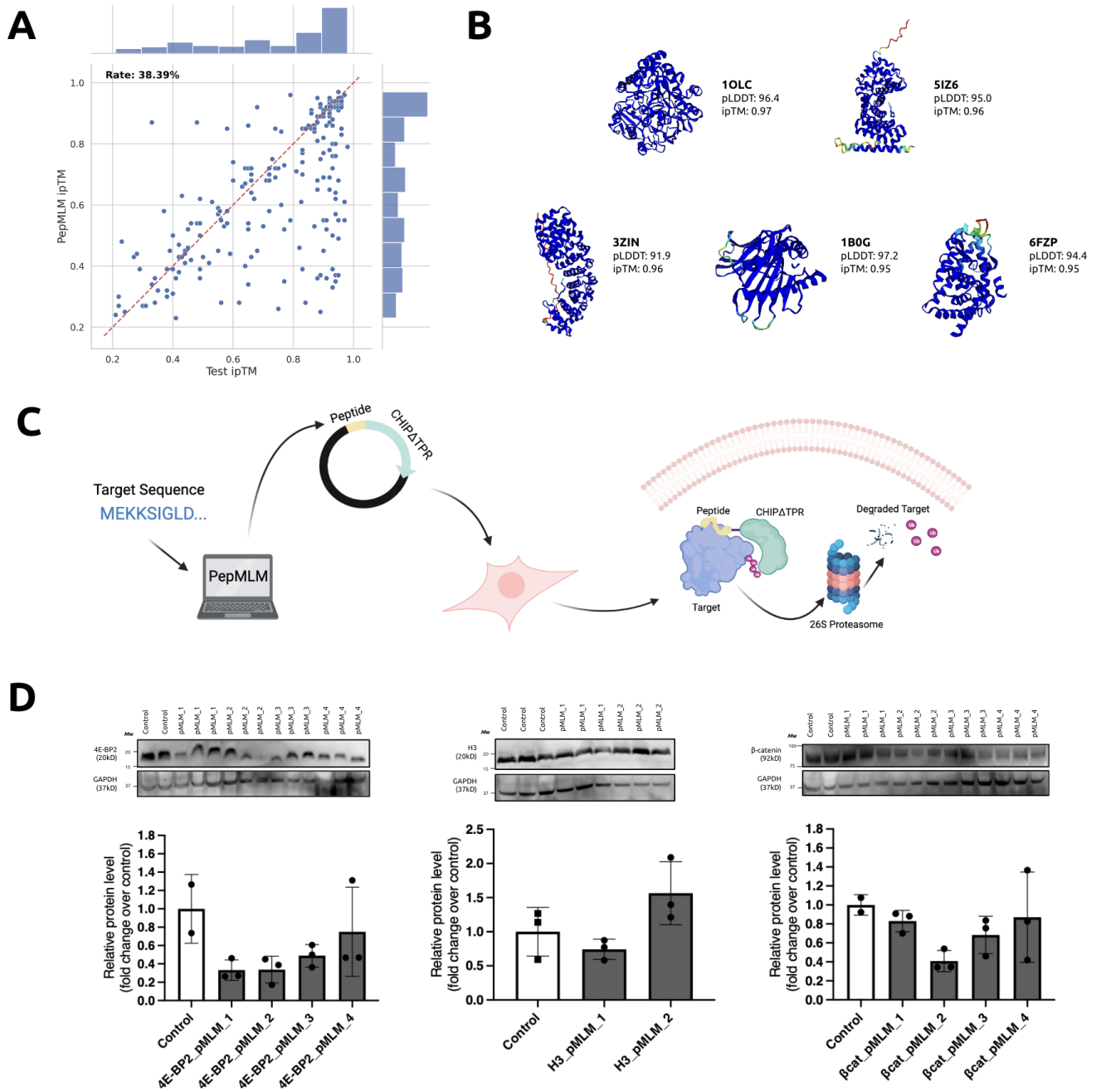
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**Figure 1. Overview and evaluation of the PepMLM model.** (A) The architecture of the PepMLM model. Based on the fine-tuning of ESM-2, the model incorporates the target protein sequence along with a masked binder region during the training phase. During the generation phase, the model can accept target protein sequences and mask tokens to facilitate the creation of peptides of specified lengths. (B) Top- $k$  sampling. Specified  $k$  values are plotted in relation to perplexities calculated for target proteins in the test set, via generation of 10 binders of the same length as the ground truth binder. (C) Perplexity distribution comparison. The perplexity values were calculated for test and generated peptides, encompassing the target proteins in the test set. (D) The density distribution visualization of the log perplexity values for target-peptide pairs, encompassing test peptides, PepMLM-650M-generated peptides, ESM-2-650M-generated peptides, and random peptides.



**Figure 2. Benchmarking of PepMLM-generated peptides.** (A) *In silico* hit-rate assessment. Utilizing AlphaFold-Multimer, ipTM scores were computed for both the generated and test peptides in conjunction with the target protein sequence. The entries are organized in accordance with the ipTM scores attributed to the test set peptides. The hit rate is characterized by the generated peptides exhibiting ipTM scores  $\geq$  those of the test peptides. (B) AlphaFold-Multimer co-folding of specified target proteins from the PDB and sampled peptide binders generated via PepMLM-650M, with the pLDDT values serving as the determinant for color coding. ipTM scores indicate binding stability. (C) Architecture and mechanism of uAb degradation system. CHIP $\Delta$ TPR is fused to the C-terminus of PepMLM-designed target-specific peptides, and can thus tag endogenous target proteins for ubiquitin-mediated degradation in the proteasome, post-plasmid transfection. (D) Degradation of endogenous targets in protein extracts of A673 Ewing sarcoma cells analyzed via immunoblotting. Blots are representative of independent transfection replicates ( $n = 3$ ). Relative degradation activity was determined by densitometry analysis of target protein signal normalized to sample-specific GAPDH signal.

## **Supplementary Information**

### **PepMLM: Target Sequence-Conditioned Generation of Peptide Binders via Masked Language Modeling**

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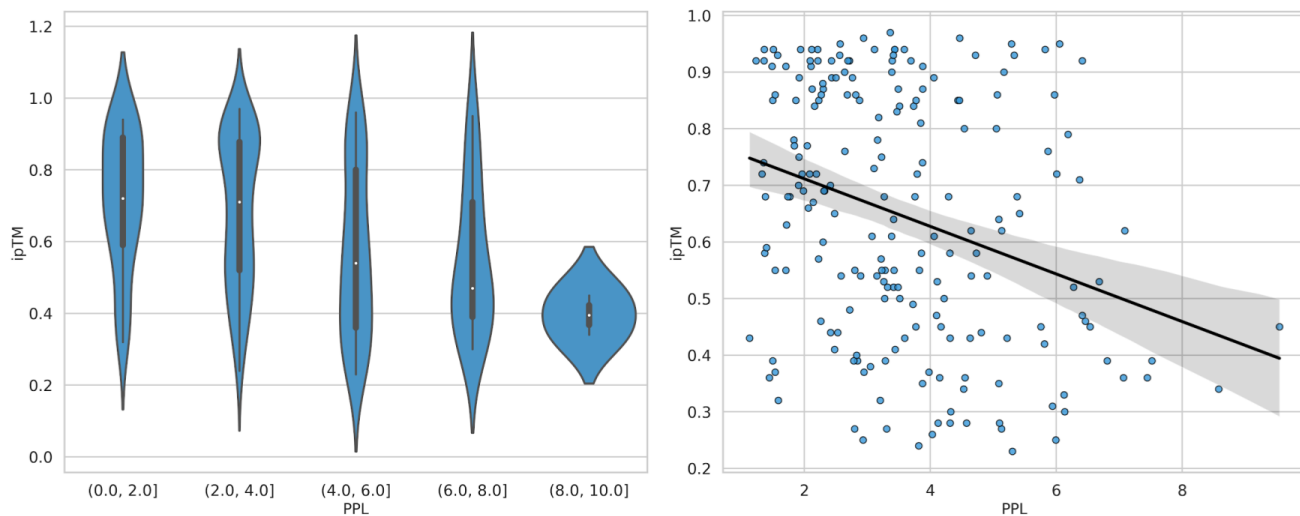
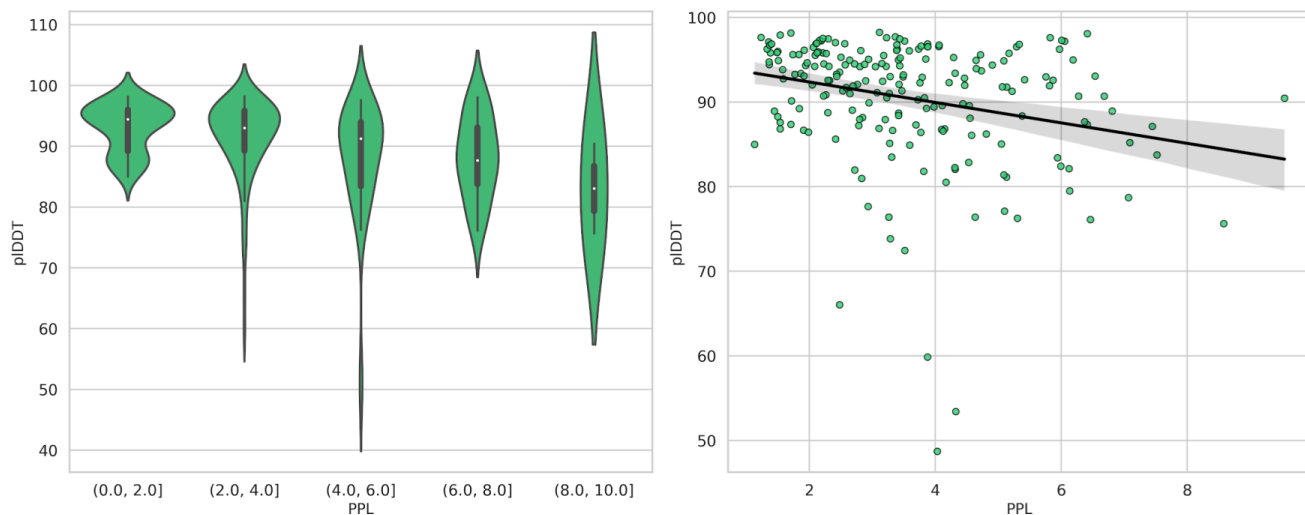
†Corresponding author: [pranam.chatterjee@duke.edu](mailto:pranam.chatterjee@duke.edu)

## **Supplementary Figures**

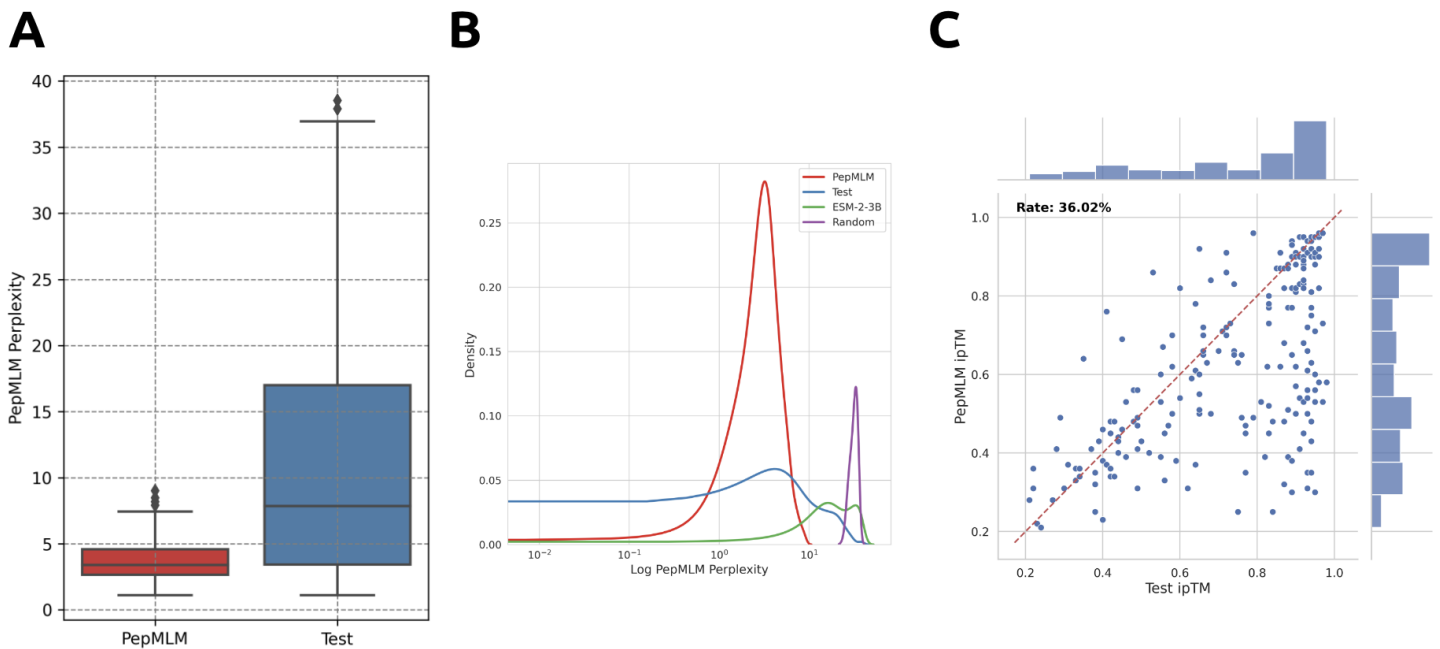
1. Association between model perplexity and co-folding metrics.
2. Evaluation of PepMLM-3B.

## **Supplementary Tables**

1. Settings and hyperparameters used to train PepMLM-650M.
2. Peptide sequences, PPL, and ipTM scores for AlphaFold-Multimer benchmarking.
3. Peptide sequences and PPL scores for uAb generation and experimental testing.

**A****B**

**Supplementary Figure 1. Association between model perplexity and co-folding metrics.** (A) Relationship between ipTM and perplexity (PPL). The initial segment of Figure A presents a violin plot, categorizing perplexity in 2-unit intervals. The subsequent segment delineates the raw data points, accompanied by a regression analysis, indicating a slope of  $-0.30$  ( $p < 0.001$ ). (B) Negative correlation between PPL and pLDDT, identified by a regression slope of  $-0.26$  ( $p < 0.001$ ). The violin plot underscores a marked decrement in specific folding metrics, most pronounced in ipTM, commensurate with elevated perplexity levels.



**Supplementary Figure 2. Evaluation of PepMLM-3B.** (A) Perplexity distribution comparison. The perplexity values were calculated for test and generated peptides, encompassing the target proteins in the test set. (B) The density distribution visualization of the log perplexity values for target-peptide pairs, encompassing test peptides, PepMLM-3B-generated peptides, ESM-2-3B-generated peptides, and random peptides. (C) *In silico* hit-rate assessment. Utilizing AlphaFold-Multimer, the ipTM scores were computed for both the generated and test peptides in conjunction with the target protein sequence. The entries are organized in accordance with the ipTM scores attributed to the test set peptides. The hit rate is characterized by the generated peptides exhibiting ipTM scores  $\geq$  those of the test set peptides.

**Supplementary Table 1.** Settings and hyperparameters used to train PepMLM-650M.

Name	Setting
Learning Rate	0.000798
Batch Size	16
Gradient Accumulation	2
Warm up steps	501
Number of training epochs	5
Optimizer	AdamW (default)
Trainer	Default HuggingFace Settings



**Supplementary Table 2.** Peptide sequences, PPL, and ipTM scores for AlphaFold-Multimer benchmarking.

Target	Peptide Name	Peptide Sequence	PPL	ipTM
4BPK-A	4BPK-C	XWAREIGAXARRMADDLNAQYX	N/A	0.93
4BPK-A	PpC_4BPK-A_1	GASMMRELAEWLRKG	0.9991984963	0.41
4BPK-A	PpC_4BPK-A_2	RSEIIQEMLGEAANEMF	0.9990072846	0.66
4BPK-A	PpC_4BPK-A_3	LALETLRVGDGVQRIHD	0.9968246818	0.78
4BPK-A	PpC_4BPK-A_4	GRYIHDGLIYEMGKRLIA	0.9967900515	0.36
4BPK-A	PpC_4BPK-A_5	LGEIDQELYKVVMYGEK	0.9960549474	0.36
4BPK-A	PpC_4BPK-A_6	DELERAIRELAAREKG	0.9960279465	0.75
4BPK-A	PpC_4BPK-A_7	VGLGVIDYLRRIGDQDK	0.9949992895	0.78
4BPK-A	PpC_4BPK-A_8	GITPIKEMGREIHEILR	0.99496907	0.86
4BPK-A	PpC_4BPK-A_9	TSPSYNPSCASYSSTSPR	-0.6418320537	0.43
4BPK-A	PpC_4BPK-A_10	THPTTTTATATTATSN	-0.6186317205	0.45
4BPK-A	PpC_4BPK-A_11	MHNTTTTTTTATTATSN	-0.6149728894	0.42
4BPK-A	PpC_4BPK-A_12	PHPSPEWCPCARSGAGDA	-0.6146063209	0.35
4BPK-A	PpC_4BPK-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.46
4BPK-A	PpC_4BPK-A_14	EHQGQLDEIEKNPAHLDI	N/A	0.41
4BPK-A	PpC_4BPK-A_15	PCYIPADPLRWYKPI	N/A	0.41
4BPK-A	PpC_4BPK-A_16	MRACPQKPCYRRRRGP	N/A	0.34
4BPK-A	PpC_4BPK-A_17	VVTAQGVYELQHDDTD	N/A	0.36
4BPK-A	PpC_4BPK-A_18	GPAGEEGKRGARGERGDP	N/A	0.39
4BPK-A	PpC_4BPK-A_19	MPVGMANFNHEPNTD	N/A	0.61
1LKL-A	1LKL-B	XYEEG	N/A	0.92
1LKL-A	PpC_1LKL-A_1	GGIPVALPNVNDNYA	0.978949368	0.66
1LKL-A	PpC_1LKL-A_2	PSVDESNEKELLYVK	0.976215899	0.71
1LKL-A	PpC_1LKL-A_3	VGVCYDKSQEQVPV	0.9750964642	0.83
1LKL-A	PpC_1LKL-A_4	LKEYTLVEEQSQLNK	0.9683961868	0.34
1LKL-A	PpC_1LKL-A_5	NPGIKYVENELESSM	0.967734158	0.82
1LKL-A	PpC_1LKL-A_6	GCMGPAVELLGAPPKD	0.9665395617	0.56
1LKL-A	PpC_1LKL-A_7	QEGCWPKEKEMLIAW	0.9599069357	0.33
1LKL-A	PpC_1LKL-A_8	HIMDMKNPVQGCELGDPQ	0.9566709995	0.41
1LKL-A	PpC_1LKL-A_9	MALSTLRRVLLGQQRNHR	-0.8509562016	0.47

1LKL-A	PpC_1LKL-A_10	KKDNPPRRPDMKPLKKDS	-0.8466350436	0.67
1LKL-A	PpC_1LKL-A_11	TKLNWTLRLYLKSKCRM	-0.842817843	0.69
1LKL-A	PpC_1LKL-A_12	MTYADHAASGRTSRRNAI	-0.8424198627	0.51
1LKL-A	PpC_1LKL-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.4
1LKL-A	PpC_1LKL-A_14	EHQQQLDEIEKNPAHLDI	N/A	0.63
1LKL-A	PpC_1LKL-A_15	PCYIPADPLRWYKPI	N/A	0.68
1LKL-A	PpC_1LKL-A_16	MRACPQKPCYRRRRGP	N/A	0.35
1LKL-A	PpC_1LKL-A_17	VVTAQGVYELQHDDTD	N/A	0.75
1LKL-A	PpC_1LKL-A_18	GPAGEEGKRGARGERGDP	N/A	0.67
1LKL-A	PpC_1LKL-A_19	MPVGMANFNHEPNTD	N/A	0.33
1BHF-A	1BHF-I	XFEEI	N/A	0.93
1BHF-A	PpC_1BHF-A_1	PSVDESNEKELLYVK	0.9817922711	0.66
1BHF-A	PpC_1BHF-A_2	VGVCYDKSQEQVPV	0.9799521565	0.85
1BHF-A	PpC_1BHF-A_3	GGIPVALPNVNDNYA	0.9796469808	0.37
1BHF-A	PpC_1BHF-A_4	NPGIKYVENELESSM	0.9780395627	0.7
1BHF-A	PpC_1BHF-A_5	LKEYTLVEEQSQLNK	0.9759145379	0.81
1BHF-A	PpC_1BHF-A_6	GCMGPAVELLGAPPKD	0.9717277288	0.53
1BHF-A	PpC_1BHF-A_7	QEGCWPKEKEMLIAW	0.9664136767	0.33
1BHF-A	PpC_1BHF-A_8	FVLMQKEAEDAEQLS	0.9625493884	0.33
1BHF-A	PpC_1BHF-A_9	KKDNPPRRPDMKPLKKDS	-0.8526343107	0.49
1BHF-A	PpC_1BHF-A_10	MALSTLRRVLLGQRNHR	-0.8471319675	0.39
1BHF-A	PpC_1BHF-A_11	MTYADHAASGRTSRRNAI	-0.84048599	0.45
1BHF-A	PpC_1BHF-A_12	TKLNWTLRLYLKSKCRM	-0.8372011781	0.26
1BHF-A	PpC_1BHF-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.39
1BHF-A	PpC_1BHF-A_14	EHQQQLDEIEKNPAHLDI	N/A	0.64
1BHF-A	PpC_1BHF-A_15	PCYIPADPLRWYKPI	N/A	0.69
1BHF-A	PpC_1BHF-A_16	MRACPQKPCYRRRRGP	N/A	0.3
1BHF-A	PpC_1BHF-A_17	VVTAQGVYELQHDDTD	N/A	0.69
1BHF-A	PpC_1BHF-A_18	GPAGEEGKRGARGERGDP	N/A	0.47
1BHF-A	PpC_1BHF-A_19	MPVGMANFNHEPNTD	N/A	0.5
4OAJ-A	4OAJ-B	NEKVSCV	N/A	0.87
4OAJ-A	PpC_4OAJ-A_1	MVHPFTEANSEYVFI	0.9954669476	0.82

4OAJ-A	PpC_4OAJ-A_2	KNQSEYSIYSECVLHLSL	0.9948153496	0.48
4OAJ-A	PpC_4OAJ-A_3	MAISEAKELEQVSAV	0.9942713976	00.86
4OAJ-A	PpC_4OAJ-A_4	MWITSQVTSQTTWRR	0.9942292571	0.77
4OAJ-A	PpC_4OAJ-A_5	MSKGLFMAAVPSLAS	0.9937303662	0.30
4OAJ-A	PpC_4OAJ-A_6	MKESDVVWPRLPLLH	0.9924523234	0.29
4OAJ-A	PpC_4OAJ-A_7	MLPAPQPPLPYGSDGS	0.992174983	0.28
4OAJ-A	PpC_4OAJ-A_8	MQPLPQPLLPYSSGGS	0.9920607805	0.30
4OAJ-A	PpC_4OAJ-A_9	MWEQAPPPPPWPPRP	-0.8082128167	0.20
4OAJ-A	PpC_4OAJ-A_10	AADEKEHKENKEEDHG	-0.7744987607	0.33
4OAJ-A	PpC_4OAJ-A_11	KAKEEKDEEQAKCQMEDA	-0.7654567361	0.40
4OAJ-A	PpC_4OAJ-A_12	MAAEEEKKDEKQLADSAA	-0.7598571777	0.58
4OAJ-A	PpC_4OAJ-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.61
4OAJ-A	PpC_4OAJ-A_14	EHQGQLDEIEKNPAHLDI	N/A	0.49
4OAJ-A	PpC_4OAJ-A_15	PCYIPADPLRWYKPI	N/A	0.31
4OAJ-A	PpC_4OAJ-A_16	MRACPQKPCYRRRRGP	N/A	0.27
4OAJ-A	PpC_4OAJ-A_17	VVTAQGVYELQHDDTD	N/A	0.31
4OAJ-A	PpC_4OAJ-A_18	GPAGEEGKRGARGERGDP	N/A	0.27
4OAJ-A	PpC_4OAJ-A_19	MPVGMANFNHEPNTD	N/A	0.72
4CIM-B	4CIM-Q	AADPLGQALRAIGDEFETRFR	N/A	0.94
4CIM-B	PpC_4CIM-B_1	QEMRKSECLRILDEHGR	0.983576715	0.41
4CIM-B	PpC_4CIM-B_2	LALETLRRVGDGVQRIHD	0.9821183085	0.81
4CIM-B	PpC_4CIM-B_3	LGEIDQELYKVVMYGEK	0.9809831977	0.35
4CIM-B	PpC_4CIM-B_4	GITPIKEMGREIHEILR	0.9802325964	0.86
4CIM-B	PpC_4CIM-B_5	GRYIHDGLIYEMGKRLIA	0.9797714353	0.37
4CIM-B	PpC_4CIM-B_6	VGLGVIDYLRRIGDQDK	0.9785536528	0.65
4CIM-B	PpC_4CIM-B_7	QIEYLAKQIVDNAIQQAK	0.9777933359	0.58
4CIM-B	PpC_4CIM-B_8	RIEQEYETQWDNIIDQAK	0.9769710302	0.39
4CIM-B	PpC_4CIM-B_9	LEGDHGSSGGNCSTPAI	-0.6792706251	0.33
4CIM-B	PpC_4CIM-B_10	MVTIMTVSNNASTTYKDK	-0.6757205129	0.38
4CIM-B	PpC_4CIM-B_11	TSPSYNPSCASYSSTSPR	-0.6677196622	0.53
4CIM-B	PpC_4CIM-B_12	MRYSAVYSSHPSSCGI	-0.6657535434	0.8
4CIM-B	PpC_4CIM-B_13	CCNPNPCPWRCSSAAEGL	N/A	0.51

4CIM-B	PpC_4CIM-B_14	EHQQQLDEIEKNPAHLDI	N/A	0.48
4CIM-B	PpC_4CIM-B_15	PCYIPADPLRWYKPI	N/A	0.46
4CIM-B	PpC_4CIM-B_16	MRACPQKPCYRRRRGP	N/A	0.33
4CIM-B	PpC_4CIM-B_17	VVTAQGVYELQHDDTD	N/A	0.35
4CIM-B	PpC_4CIM-B_18	GPAGEEGKRGARGERGDP	N/A	0.41
4CIM-B	PpC_4CIM-B_19	MPVGMANFNHEPNTD	N/A	0.55
3KF9-A	3KF9-B	KRRWKKNFIAVSAANRFKISS	N/A	0.91
3KF9-A	PpC_3KF9-A_1	KNRLQSLWMRLVKDFRL	0.9439585805	0.85
3KF9-A	PpC_3KF9-A_2	KQKLILAERKRKLNTWML	0.9421902895	0.73
3KF9-A	PpC_3KF9-A_3	MSKPKMPGHELIRRVIRR	0.9403369427	0.6
3KF9-A	PpC_3KF9-A_4	MTYADAIASGREDRRSAI	0.9389324784	0.37
3KF9-A	PpC_3KF9-A_5	MLEELREKQELIREKSNK	0.9374441504	0.32
3KF9-A	PpC_3KF9-A_6	MLKLLKEVRVLLGRGQ	0.9370013475	0.47
3KF9-A	PpC_3KF9-A_7	MRTRLKAAALERLAAR	0.9367336035	0.46
3KF9-A	PpC_3KF9-A_8	KLDKKLVEINLRKSPEE	0.9363824129	0.5
3KF9-A	PpC_3KF9-A_9	ADRCNNKQELKLVPA	-0.9044314623	0.37
3KF9-A	PpC_3KF9-A_10	HKQCAKLDPRPGYNRP	-0.896143198	0.42
3KF9-A	PpC_3KF9-A_11	MELNNLLDGGAVAAP	-0.8870944381	0.55
3KF9-A	PpC_3KF9-A_12	MEDIGILNGAAVPAK	-0.8820631504	0.42
3KF9-A	PpC_3KF9-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.46
3KF9-A	PpC_3KF9-A_14	EHQQQLDEIEKNPAHLDI	N/A	0.33
3KF9-A	PpC_3KF9-A_15	PCYIPADPLRWYKPI	N/A	0.28
3KF9-A	PpC_3KF9-A_16	MRACPQKPCYRRRRGP	N/A	0.28
3KF9-A	PpC_3KF9-A_17	VVTAQGVYELQHDDTD	N/A	0.35
3KF9-A	PpC_3KF9-A_18	GPAGEEGKRGARGERGDP	N/A	0.11
3KF9-A	PpC_3KF9-A_19	MPVGMANFNHEPNTD	N/A	0.4
6SEN-A	6SEN-L	XVPMRKRQLPASFWEEPX	N/A	0.94
6SEN-A	PpC_6SEN-A_1	SEKEYVEMLDFLESKLG	0.9829846621	0.87
6SEN-A	PpC_6SEN-A_2	EGDAMFRLGTDWPELHDR	0.9790226221	0.36
6SEN-A	PpC_6SEN-A_3	DLLDNMPNTVCPISKSI	0.9768525958	0.35
6SEN-A	PpC_6SEN-A_4	KRMRKTYNYVLEKLM	0.9756338	0.31
6SEN-A	PpC_6SEN-A_5	RYETKQIRRFLRIDL	0.9753730297	0.36

6SEN-A	PpC_6SEN-A_6	MSDKEYVEKLDRLYHKLQ	0.9751641154	0.34
6SEN-A	PpC_6SEN-A_7	MRLYKLVEMFMDPKGK	0.9750229716	0.65
6SEN-A	PpC_6SEN-A_8	MIEALRKQSLYLKFDPLL	0.9734063745	0.66
6SEN-A	PpC_6SEN-A_9	TDCALFIAWWNLVRQKLD	-0.8822892904	0.44
6SEN-A	PpC_6SEN-A_10	ETLWATREVDVLDISYER	-0.8517132401	0.35
6SEN-A	PpC_6SEN-A_11	KTDFKVRLEEYDIRFR	-0.851116538	0.29
6SEN-A	PpC_6SEN-A_12	ATFHDALPITLCDVQGLE	-0.823202908	0.33
6SEN-A	PpC_6SEN-A_13	CCNPNPCPWRCSSAAEGL	N/A	0.51
6SEN-A	PpC_6SEN-A_14	EHQQLDEIEKNPAHLDI	N/A	0.48
6SEN-A	PpC_6SEN-A_15	PCYIPADPLRWYKPI	N/A	0.34
6SEN-A	PpC_6SEN-A_16	MRACPQKPCYRRRRGP	N/A	0.31
6SEN-A	PpC_6SEN-A_17	VVTAQGVYELQHDDTD	N/A	0.29
6SEN-A	PpC_6SEN-A_18	GPAGEEGKRGARGERGDP	N/A	0.35
6SEN-A	PpC_6SEN-A_19	MPVGMANFNHEPNTD	N/A	0.32

**Supplementary Table 3.** Peptide sequences and PPL scores for uAb generation and experimental testing.

Target	UniProt ID	uAb Name	Peptide Sequence	PPL
$\beta$ -catenin	P35222	pMLM_beta-catenin_1	KKALQL	4.541961
$\beta$ -catenin	P35222	pMLM_beta-catenin_2	GKTFQV	5.699396
$\beta$ -catenin	P35222	pMLM_beta-catenin_3	GKALQV	4.793907
$\beta$ -catenin	P35222	pMLM_beta-catenin_4	KATLKL	5.595168
EIF4EBP2	Q13542	pMLM EIF4EBP2_1	DSTIVVQTK	5.365092
EIF4EBP2	Q13542	pMLM EIF4EBP2_2	DSSIVVQTP	6.203168
EIF4EBP2	Q13542	pMLM EIF4EBP2_3	ESTIVVTP	6.035627
EIF4EBP2	Q13542	pMLM EIF4EBP2_4	DDTLVVTP	6.283843
H3	P68431	pMLM_H3_1	DEDY	2.653573
H3	P68431	pMLM_H3_2	DEEY	3.240149