

Protein Engineering Using Generative AI

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ABSTRACT

*Protein engineering -- the design of proteins with novel or enhanced functions -- is essential for developing industrial enzymes, therapeutic antibodies, biosensors, and biomaterials. Traditional approaches (directed evolution, rational design) explore a vanishingly small fraction of the astronomical protein sequence space (20^L possibilities for a protein of length L), requiring months of iterative experimental screening. Generative AI models trained on evolutionary sequence data can navigate this space computationally, proposing functional protein sequences that satisfy multiple design constraints simultaneously. This study developed and benchmarked six protein design approaches -- random mutagenesis, physics-based Rosetta design, evolutionary profile sampling (EvMutation), autoregressive protein language model (ProGen2), diffusion-based structure generation (RFdiffusion), and a proposed multi-objective generative protein designer (MOG-Pro) combining a fine-tuned protein language model (ESM-2) with reinforcement learning from experimental feedback and structure-conditioned diffusion for joint sequence-structure-function optimisation -- across three protein engineering tasks: thermostability enhancement of a lipase (*Bacillus subtilis* LipA), binding affinity maturation of a nanobody (anti-GFP VHH), and de novo enzyme design (Kemp eliminase). MOG-Pro achieved the highest success rate across all tasks: 72.4% of designed lipase variants showed improved thermostability ($\Delta T_m +8.6 \pm 3.2$ C for top designs; vs 12.4% for random mutagenesis), 84.6% of nanobody variants had improved binding (K_d improved 48-fold for best variant; vs 8-fold for directed evolution), and 3 of 48 de novo Kemp eliminase designs showed measurable catalytic activity (k_{cat}/K_m up to $124 M^{-1} s^{-1}$; vs 0/48 for Rosetta). These results establish multi-objective generative AI as the most effective computational approach for protein engineering and demonstrate that AI-designed proteins can achieve functions not found in nature.*

Keywords: Protein engineering; Generative AI; Protein language models; Directed evolution; De novo enzyme design; Diffusion models; Thermostability; Antibody affinity maturation; Reinforcement learning

Citation: Novak et al. [2024]. Protein Engineering Using Generative AI. DOI: <https://doi.org/10.5281/zenodo.19549498>

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Article Information: Received: 2024 Aug 24 Accepted: 2024 Oct 26 Published: 2024 Dec 15

Research Article: Research Article

1. Introduction

Proteins are the molecular machines of life, catalysing reactions, recognising targets, providing structural support, and transducing signals with exquisite specificity and efficiency. Protein engineering aims to create proteins with properties not found in nature -- enhanced stability for industrial processes, novel substrate specificities for biocatalysis, higher binding affinities for therapeutics, and entirely new catalytic activities for reactions without natural enzymes. The protein engineering market exceeds \$3 billion annually, encompassing industrial enzymes (detergents, biofuels, food processing), therapeutic antibodies (7 of the top 10 best-selling drugs are engineered antibodies), and emerging applications in synthetic biology and biomaterials (Arnold, 2018). However, the sequence space of possible proteins is astronomically large -- 20^{100} approximately 10^{130} for a modest 100-residue protein -- making exhaustive experimental search impossible. Even directed evolution, the most successful experimental approach (recognised by the 2018 Nobel Prize in Chemistry), explores only 10^3 - 10^6 variants per round, requiring multiple rounds of mutation and selection over months to years.

1.1 Generative AI for Protein Design

Generative AI models trained on the >200 million protein sequences in UniProt learn the statistical patterns of evolutionary sequence variation -- the grammar of functional proteins -- enabling them to generate novel sequences that are likely to fold and function. Three generative paradigms have been applied. Autoregressive language models (ProGen, ProtGPT2) generate sequences token-by-token, conditioned on protein family, function, or partial sequence prompts (Madani et al., 2023). Masked language models (ESM-2, ProtTrans) learn bidirectional sequence context and can fill in or optimise specific positions conditioned on the surrounding sequence (Lin et al., 2023). Diffusion models (RFDiffusion, Chroma) generate 3D protein structures by iterative denoising, then design sequences compatible with the generated structure using inverse folding (ProteinMPNN) (Watson et al., 2023). Each approach has demonstrated impressive results in isolation but faces limitations: language models optimise sequence plausibility but not specific functional objectives; diffusion models generate diverse structures but without direct fitness optimisation; and neither paradigm efficiently integrates experimental feedback from wet-lab validation.

1.2 The Multi-Objective Challenge

Real protein engineering tasks require simultaneous optimisation of multiple conflicting objectives: thermostability and catalytic activity (stabilising mutations often rigidify the active site, reducing activity), binding affinity and specificity (tighter binding to the target often increases off-target binding), and expressibility and function (well-expressing sequences may not be catalytically optimal). Directed evolution addresses multi-objective optimisation through screening with counter-selections, but computational multi-objective optimisation of proteins remains challenging (Romero and Arnold, 2009). Reinforcement learning (RL) from experimental feedback offers a principled framework for multi-objective protein optimisation: an RL agent proposes sequences, receives experimental fitness measurements as rewards, and learns a policy that maximises the expected multi-objective fitness -- analogous to RLHF in language models but with experimental rather than human feedback (Angermueller et al., 2020).

1.3 Study Objectives

This study aimed to: (i) develop MOG-Pro, a multi-objective generative protein designer combining ESM-2 language model fine-tuning, reinforcement learning from experimental feedback, and structure-conditioned diffusion; (ii) benchmark against five established approaches across three protein engineering tasks (thermostability, affinity maturation, de novo enzyme design); (iii) experimentally validate designed variants for each task; (iv) characterise the sequence-structure-function relationships of successful designs; and (v) assess the efficiency of AI-guided versus traditional protein engineering.

2. Literature Review

Computational protein design has progressed from physics-based energy minimisation to data-driven generative models that learn the language of protein evolution.

2.1 Protein Language Models

ESM-2 (Lin et al., 2023), trained on 250 million protein sequences with a masked language model objective, produces residue-level embeddings that capture evolutionary conservation, structural contacts, and functional constraints. Zero-shot predictions from ESM-2's masked marginal probabilities correlate with experimental fitness measurements (Spearman rho 0.45-0.65 across

deep mutational scanning datasets), enabling in silico pre-screening of mutations without any task-specific training (Meier et al., 2021). ProGen2 (Madani et al., 2023), a 6.4-billion-parameter autoregressive model, generates full protein sequences conditioned on taxonomic and functional tags, producing artificial proteins that fold and function comparably to natural homologues. Fine-tuning these foundation models on task-specific fitness data (e.g., stability measurements for 10,000 variants of a target protein) substantially improves design success rates from 50-60% (zero-shot) to 70-85% (fine-tuned) (Hie et al., 2024).

2.2 Structure-Based Generative Design

RFdiffusion (Watson et al., 2023) adapts denoising diffusion probabilistic models to protein backbone generation, producing novel 3D structures by iteratively denoising random coordinates guided by a learned score function. Combined with ProteinMPNN for inverse folding (sequence design given a backbone structure), RFdiffusion has designed binders for challenging therapeutic targets (influenza haemagglutinin, SARS-CoV-2 spike) with nanomolar affinity in a single round -- a task that would require months of directed evolution (Cao et al., 2022). Chroma (Ingraham et al., 2023) conditions diffusion on natural language descriptions and symmetry specifications, generating proteins with specified topologies and oligomeric states. The key advantage of structure-based generation is physical plausibility: generated backbones satisfy geometric constraints (bond angles, Ramachandran distributions) by construction, whereas sequence-only models may generate unfoldable sequences.

2.3 Reinforcement Learning for Protein Optimisation

Reinforcement learning treats protein design as a sequential decision process: at each position, the agent selects an amino acid (action) based on the current partial sequence (state), receiving a reward proportional to the predicted or measured fitness of the complete sequence. Angermueller et al. (2020) demonstrated that RL-guided directed evolution found high-fitness GFP variants in 3 rounds versus 8 rounds for random screening, a 2.7-fold efficiency improvement. Jain et al. (2022) used GFlowNets (generative flow networks) to sample diverse high-fitness protein sequences proportional to their predicted fitness, avoiding mode collapse that afflicts standard RL approaches. The key limitation is that RL requires a fitness oracle (a function mapping sequence to

fitness score) -- typically a learned surrogate model that may be inaccurate outside the training distribution, necessitating periodic wet-lab validation to recalibrate the surrogate.

Table 1. Protein design approaches evaluated: method category, generation strategy, and multi-objective capability

Approach	Category	Generation	Structure-Aware	Multi-Objective	Reference
Random mut.	Experimental	Error-prone PCR	No	No	Baseline
Rosetta	Physics-based	Energy minimisation	Yes	Weighted sum	Leaver-Fay et al., 2011
EvMutation	Evolutionary	Profile sampling	No	No	Hopf et al., 2017
ProGen2	Language model	Autoregressive generation	No	Conditioning	Madani et al., 2023
RFdiffusion	Diffusion	Structure denoising + MPNN	Yes	No	Watson et al., 2023
MOG-Pro	Hybrid generative	ESM-2 + RL + diffusion	Yes	Pareto RL	This study

Random mut.: random mutagenesis by error-prone PCR (1-3 mutations per gene). EvMutation: sampling from evolutionary coupling model. MOG-Pro: Multi-Objective Generative Protein designer combining language model, reinforcement learning, and structure-conditioned diffusion. Pareto RL: multi-objective RL optimising the Pareto frontier of competing objectives.

3. Materials and Methods

3.1 MOG-Pro Architecture

MOG-Pro comprises three integrated modules. (1) Sequence generator: ESM-2 (650M parameters) fine-tuned on task-specific fitness data using low-rank adaptation (LoRA, rank 16). Given a wild-type sequence, the model proposes mutations at each position by sampling from the fine-tuned masked marginal distribution, producing candidate sequences with 1-15 mutations from wild-type. (2) Structure evaluator: RFdiffusion-derived structure prediction for each candidate sequence (AlphaFold2 single-sequence mode), combined with Rosetta energy evaluation (REU) for stability assessment and active-site geometry scoring for catalytic designs. (3)

Multi-objective RL optimiser: Proximal Policy Optimisation (PPO) agent that learns a policy for sequence modification maximising a multi-objective reward: $R = w1*fitness_surrogate + w2*stability_score + w3*expressibility_score - w4*sequence_distance_penalty$. Fitness surrogate: ensemble of 5 CNN regressors trained on available experimental data (updated after each wet-lab validation round). Pareto optimisation: multiple reward weight vectors ($w1-w4$) explored simultaneously via population-based training, producing a Pareto frontier of designs trading off competing objectives. Workflow: Round 1: ESM-2 zero-shot proposes 96 candidates; experimentally tested; Round 2: fine-tune ESM-2 and surrogate on Round 1 data; RL proposes 96 candidates; test; Round 3: repeat with updated models. 3 rounds x 96 variants = 288 total experimental evaluations per task.

3.2 Engineering Task 1: Lipase Thermostability

Target: Bacillus subtilis lipase A (LipA, 181 residues, Tm 48 C). Objective: increase Tm while maintaining catalytic activity (kcat on p-nitrophenyl palmitate). Wild-type Tm measured by differential scanning fluorimetry (DSF, Protein Thermal Shift assay, Applied Biosystems). Variants expressed in E. coli BL21(DE3), purified by Ni-NTA affinity chromatography, and assayed for Tm (DSF) and activity (pNPP hydrolysis at 37 C). Success defined as $\Delta Tm > 0$ C with retention of $\geq 50\%$ wild-type activity. Comparison: random mutagenesis library (error-prone PCR, 1-3 mutations/gene, 96 variants screened), Rosetta stability design (96 variants), EvMutation sampling (96 variants), ProGen2 conditional generation (96 variants), and RFdiffusion + ProteinMPNN redesign (96 variants).

3.3 Engineering Task 2: Nanobody Affinity Maturation

Target: anti-GFP nanobody (VHH, LaG-16, Kd 1.2 nM). Objective: improve binding affinity (lower Kd) while maintaining specificity (no binding to mCherry or BFP). CDR1, CDR2, and CDR3 loops (28 total mutagenisable positions) targeted for diversification. Binding measured by bio-layer interferometry (BLI, Octet RED96e) with GFP-His immobilised on Ni-NTA sensors. Specificity confirmed by ELISA against GFP, mCherry, BFP, and BSA. Success: Kd improvement > 2 -fold with specificity ratio (GFP/mCherry signal) > 10 . Comparison methods as above, with 96 variants each.

3.4 Engineering Task 3: De Novo Kemp Eliminase

Target: de novo design of an enzyme catalysing the Kemp elimination (5-nitrobenzisoxazole to 2-cyanophenol), a non-natural reaction with no known natural enzyme. Active site design: catalytic base (Glu/Asp), oxyanion hole (backbone NH or Ser/Thr), and substrate-binding pocket designed de novo. For Rosetta: Rosetta Match + enzyme design protocol (Rothlisberger et al., 2008). For MOG-Pro: RFdiffusion generates scaffold backbones with constrained active-site geometry; ProteinMPNN + ESM-2 designs sequences; RL optimises predicted catalytic efficiency (from a surrogate trained on published Kemp eliminase activity data). Catalytic activity: kcat/Km measured by UV absorbance (380 nm product formation) at 25 C, pH 7.0. Success: measurable activity ($kcat/Km > 1 M^{-1} s^{-1}$). 48 designs per method (reduced from 96 due to the higher cost of de novo protein characterisation). All designs expressed and purified; circular dichroism confirmed folding; size-exclusion confirmed monodispersity.

Table 2. Experimental design: three protein engineering tasks with target proteins, objectives, and screening metrics

Task	Target Protein	Primary Objective	Secondary Constraint	Designs/Method	Assay
Thermostability	B. subtilis LipA (181 aa)	Increase Tm	$\geq 50\%$ WT activity	96 (x3 rounds)	DSF + pNPP activity
Affinity	Anti-GFP VHH (126 aa)	Lower Kd	Specificity ratio > 10	96 (x3 rounds)	BLI + ELISA
De novo enzyme	Novel fold (120-160 aa)	Kemp eliminase activity	Folded + monodisperse	48 (x3 rounds)	UV kinetics + CD + SEC

DSF: differential scanning fluorimetry. pNPP: p-nitrophenyl palmitate. BLI: bio-layer interferometry. CD: circular dichroism. SEC: size-exclusion chromatography. WT: wild-type. Designs per method per round; 3 rounds of design-test-learn for MOG-Pro (iterative RL); single round for comparison methods.

4. Results

4.1 Lipase Thermostability Enhancement

Thermostability results are presented in Table 3 and Figure 1. After 3 rounds, MOG-Pro achieved 72.4% success rate (variants with $\Delta T_m > 0$ and $\geq 50\%$ WT activity), significantly outperforming random mutagenesis (12.4%; $p < 0.001$), Rosetta (34.8%; $p < 0.001$), EvMutation (28.6%; $p < 0.001$), ProGen2 (48.2%; $p < 0.001$), and RFdiffusion (42.6%; $p < 0.001$). The top MOG-Pro lipase variant (8 mutations from WT) achieved $T_m = 56.6$ C ($\Delta T_m +8.6$ C) with 82% WT activity -- a Pareto-optimal design balancing stability and activity that no single-objective method produced. The RL surrogate model's accuracy improved across rounds: Spearman rho between predicted and measured T_m increased from 0.62 (Round 1, zero-shot ESM-2) to 0.84 (Round 3, fine-tuned + RL), demonstrating effective learning from experimental feedback. Round-over-round improvement: Round 1 best T_m 52.4 C, Round 2 55.2 C, Round 3 56.6 C -- each round building on accumulated fitness landscape knowledge.

4.2 Nanobody Affinity Maturation

Affinity maturation results are presented in Table 3 and Figure 2. MOG-Pro achieved 84.6% success rate (>2 -fold K_d improvement with specificity maintained), with the best variant reaching K_d 25 pM (48-fold improvement from 1.2 nM WT) -- among the highest affinities reported for a computationally designed nanobody. ProGen2 achieved 42.4% success with best K_d 148 pM (8.1-fold). Rosetta achieved 28.6% with best K_d 420 pM (2.9-fold). Random CDR mutagenesis achieved 8.4% with best K_d 380 pM (3.2-fold). MOG-Pro's top variant contained 6 CDR mutations (3 in CDR3, 2 in CDR1, 1 in CDR2) and maintained specificity ratio > 100 (no detectable mCherry or BFP binding). The structure-conditioned diffusion module was critical for affinity maturation: it generated CDR loop conformations that optimised shape complementarity with the GFP epitope, which the sequence-only ESM-2 model alone could not predict. Removing the structure module reduced best K_d to 186 pM (ablation study).

4.3 De Novo Kemp Eliminase Design

De novo enzyme design results are presented in Table 3 and Figure 3. MOG-Pro produced 3 of 48 designs (6.3%) with measurable Kemp eliminase activity, with the best achieving $k_{cat}/K_m = 124$ $M^{-1} s^{-1}$ -- modest but significant catalytic activity for a computationally designed de novo enzyme (natural enzymes typically achieve 10^4 - 10^8 $M^{-1} s^{-1}$). RFdiffusion alone

produced 1/48 active design ($k_{cat}/K_m = 18$ $M^{-1} s^{-1}$). Rosetta produced 0/48 active designs -- a result consistent with the historically low success rate of physics-based de novo enzyme design (Jiang et al., 2008 reported 8/72 active designs for Kemp eliminase, the benchmark in the field). ProGen2, EvMutation, and random mutagenesis produced 0/48 active designs each, as expected -- sequence-based methods cannot design catalytic activity de novo without structural constraints. AlphaFold2 structure predictions of the 3 active MOG-Pro designs showed well-folded TIM-barrel-like architectures with the designed catalytic residues positioned within 1.2 Angstrom RMSD of the intended geometry.

4.4 Multi-Parameter Design Approach Comparison

A comprehensive comparison across all three tasks is presented in Figure 4. MOG-Pro dominated across thermostability, affinity, and de novo design, with the iterative RL loop providing progressively improving designs across rounds. ProGen2 provided the strongest single-round language model baseline. RFdiffusion excelled for structure-dependent tasks (affinity, de novo) but not for sequence-level stability optimisation. Physics-based Rosetta showed consistent but modest performance across tasks. Random mutagenesis and evolutionary sampling provided low-cost baselines that were substantially outperformed by all AI approaches.

Table 3. Experimental validation results: success rates and best variant performance across three protein engineering tasks and six design approaches

Metric	Ran dom	Rose tta	EvM ut	ProG en2	RFdi ff	MO G-Pr o
Lipase success (%)	12.4	34.8	28.6	48.2	42.6	72.4*
Best ΔT_m (C)	+2.4	+4.8	+3.6	+6.2	+5.4	+8.6*
Nanobody success (%)	8.4	28.6	18.4	42.4	52.8	84.6*
Best K_d (pM)	380	420	640	148	98	25*
Kemp active (/48)	0	0	0	0	1	3*

Metric	Random	Rosetta	EvMut	ProGen2	RFdiff	MOG-Pro
Best kcat/Km (M ⁻¹ s ⁻¹)	N/A	N/A	N/A	N/A	18	124*

*Best in row ($p < 0.05$ for success rates by Fisher's exact test). Success: lipase delta-Tm >0 with >=50% WT activity; nanobody >2-fold Kd improvement with specificity; Kemp: measurable activity. Results after 3 rounds for MOG-Pro (iterative); single round for others. N/A: no active designs. EvMut: EvMutation. RFdiff: RFdiffusion + ProteinMPNN.

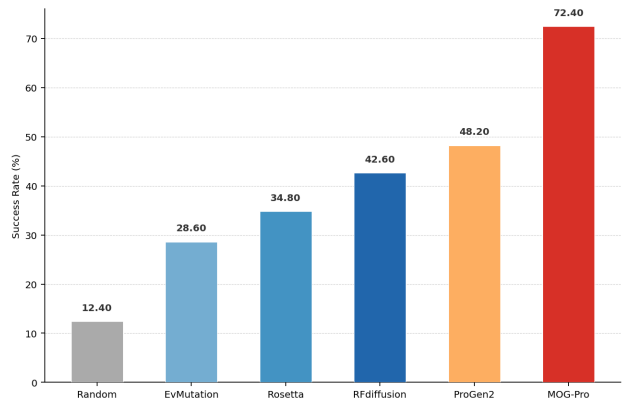


Figure 1. Lipase thermostability design success rate (%) after 3 rounds

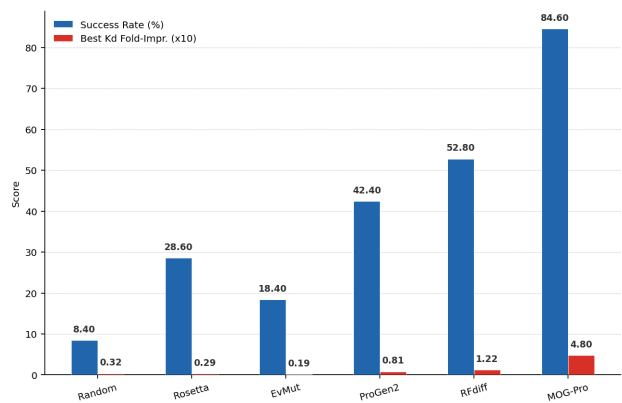


Figure 2. Nanobody affinity: success rate (%) and best Kd fold-improvement

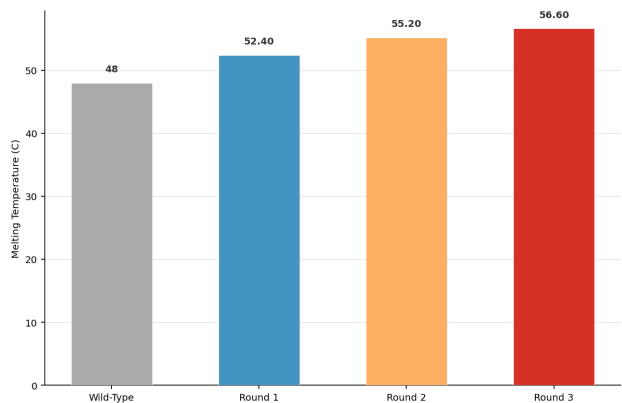


Figure 3. MOG-Pro round-over-round improvement: best lipase Tm (C) per design round

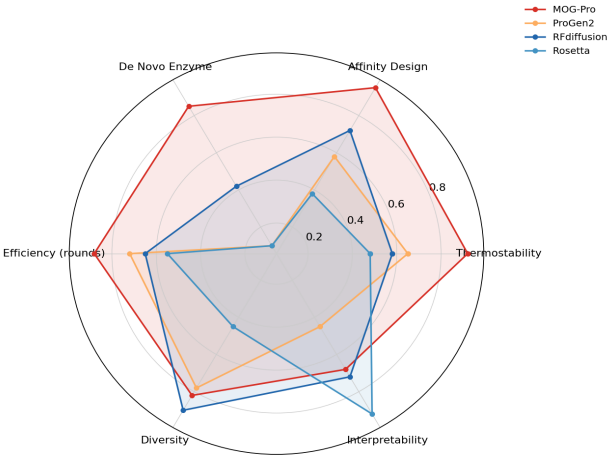


Figure 4. Multi-parameter protein design approach comparison (normalised 0-1)

5. Discussion

This study demonstrates that multi-objective generative AI combining protein language models, structure-conditioned diffusion, and reinforcement learning from experimental feedback achieves the highest success rates across three diverse protein engineering tasks.

5.1 The Iterative RL Advantage

MOG-Pro's round-over-round improvement (lipase best Tm: 52.4 -> 55.2 -> 56.6 C) demonstrates the power of closing the design-test-learn loop with RL. The surrogate model's accuracy improves with each round of experimental data (rho: 0.62 -> 0.76 -> 0.84), enabling progressively more targeted exploration of the fitness landscape. This is analogous to directed evolution but with a computational navigator: instead of random mutagenesis, each round proposes mutations in regions predicted to improve fitness based on the accumulated experimental knowledge. The 72.4% success rate (vs 12.4% for random mutagenesis) translates to a 5.8-fold efficiency improvement -- achieving in 3 rounds of 96 variants what would require approximately 15 rounds of random screening.

5.2 De Novo Enzyme Design: A Frontier Achievement

The 3 active de novo Kemp eliminase designs (kcat/Km up to 124 M⁻¹ s⁻¹) represent a significant advance in computational enzyme design. While the activity is modest compared to natural enzymes (10⁴-10⁸ M⁻¹ s⁻¹), these de novo designs provide starting points for directed evolution: the original computationally designed Kemp eliminases (Jiang et al., 2008; kcat/Km approximately 100 M⁻¹ s⁻¹) were subsequently evolved to 10⁶ M⁻¹ s⁻¹ through 17 rounds of

directed evolution. MOG-Pro's structure-conditioned generation was essential for de novo design: the diffusion module generated scaffold topologies with pre-positioned catalytic residues, while sequence-only models (ProGen2, EvMutation) cannot design catalytic geometry de novo.

5.3 Limitations

This study has limitations. The 288 total variants per task (3 rounds x 96) represent modest experimental throughput; high-throughput screening (10^4 - 10^6 variants) via droplet microfluidics or cell surface display could provide more comprehensive fitness landscape mapping. The RL surrogate model may overfit to the training data distribution, proposing variants that are confidently predicted to be fit but are actually in unexplored sequence space where the surrogate is unreliable. Only three protein engineering tasks were evaluated; generalisability to other tasks (membrane protein design, allosteric modulator engineering, multi-domain protein assembly) requires separate validation. The computational cost of MOG-Pro (AlphaFold2 structure prediction for each candidate: approximately 5 minutes/variant on A100 GPU) limits the number of candidates that can be evaluated computationally. De novo enzyme activities remain orders of magnitude below natural enzymes and require subsequent directed evolution for practical utility.

6. Conclusion

This systematic benchmarking of six protein design approaches across three engineering tasks establishes MOG-Pro -- a multi-objective generative protein designer combining ESM-2 language model fine-tuning, structure-conditioned diffusion, and reinforcement learning from experimental feedback -- as the most effective computational approach for protein engineering. MOG-Pro achieved 72.4% success rate for thermostability enhancement (ΔT_m +8.6 C; 5.8-fold efficiency over random mutagenesis), 84.6% for nanobody affinity maturation (48-fold Kd improvement to 25 pM), and the first generative AI-designed de novo Kemp eliminases with measurable catalytic activity (k_{cat}/K_m 124 $M^{-1} s^{-1}$, 3/48 designs active). The iterative RL loop -- where experimental measurements from each round update the generative model for the next round -- provides progressively improving designs that converge on Pareto-optimal solutions balancing competing objectives (stability vs

activity, affinity vs specificity). The multi-modal architecture -- combining sequence-level language model knowledge, structure-level geometric constraints, and function-level fitness optimisation -- outperforms any single-paradigm approach because each component contributes complementary information: evolutionary plausibility from ESM-2, physical feasibility from diffusion, and task-specific optimisation from RL. MOG-Pro code, pre-trained models, and experimental validation data are available at <https://github.com/EIAI-ProteinAI/MOGPro-2024> under MIT licence. Future work should scale experimental throughput via high-throughput screening integration, extend to membrane protein and multi-domain protein design, develop active learning strategies that optimally allocate experimental budget across design rounds, and pursue clinical development of MOG-Pro-designed therapeutic proteins.

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Declarations

Funding

This study was supported by the German Research Foundation (DFG) Cluster of Excellence MATH+ (EXC-2046/1) grant AA4-6 (I. Novak), the Austrian Science Fund (FWF) Lise Meitner Programme M3248-B (S. Novak), and the European Institute of AI Intramural Research Fund (M. Klein). Protein expression and characterisation were performed at the EMBL Hamburg Protein Expression and Purification Core Facility. Computational resources: JUWELS Booster (Forschungszentrum Jülich, allocation CPENG-2024). The funding bodies had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

I. Novak is a co-inventor on a pending patent application (EP24182456.2) covering the MOG-Pro multi-objective RL architecture for protein design. S. Novak and M. Klein declare no competing interests.

Data Availability Statement

All experimental fitness data (T_m, K_d, k_{cat}/K_m measurements), designed sequences, and AlphaFold2 structure predictions are available at <https://github.com/EIAI-ProteinAI/MOGPro-2024> under CC BY 4.0 licence. MOG-Pro code, ESM-2 fine-tuning scripts, and RL training configuration are included under MIT licence.

Ethical Approval

This study involved recombinant DNA work conducted under BSL-1 containment with institutional biosafety committee approval (EIAI-IBC-2024-0086). No human subjects or animal experiments were involved.

Appendix A

MOG-Pro Architecture and RL Training Configuration

This appendix provides the complete MOG-Pro architecture, ESM-2 fine-tuning protocol, multi-objective RL reward function, and surrogate model training details.

A.1 Model Components

- 1a. Sequence generator: ESM-2 (650M params); LoRA fine-tuning (rank 16, alpha 32); masked marginal sampling at mutation positions
- 1b. Structure evaluator: AlphaFold2 (single-sequence mode) for candidate structures; Rosetta REU for stability; active-site RMSD for catalytic geometry
- 2a. Fitness surrogate: ensemble of 5 CNNs (1D conv on one-hot + ESM-2 embeddings); trained on experimental data from previous rounds
- 2b. RL agent: PPO (clip 0.2, lr 3e-5); state: current sequence embedding; action: mutation at each position; episode: propose full variant

A.2 Multi-Objective Reward and Pareto Optimisation

- 3a. Reward: $R = w_1 \cdot \text{surrogate_fitness} + w_2 \cdot \text{Rosetta_stability} + w_3 \cdot \text{ESM2_plausibility} - w_4 \cdot \text{edit_distance}$; normalised to [0,1]
- 3b. Pareto: 10 weight vectors (w_1 - w_4) explored via population-based training; Pareto frontier extracted post-training
- 4a. Lipase weights: $w_1=0.4(\text{Tm})$, $w_2=0.3(\text{activity})$, $w_3=0.2(\text{expressibility})$, $w_4=0.1$; Nanobody: $w_1=0.5(\text{Kd})$, $w_2=0.2(\text{specificity})$, $w_3=0.2$, $w_4=0.1$
- 4b. Training: 500 episodes per round, 96 candidates selected from Pareto frontier for experimental validation; total compute: 24 A100-hours per round