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Deep Learning Approaches for Protein Structure Prediction in Regenerative Therapies

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ABSTRACT

Protein structure prediction -- the computational determination of a protein's three-dimensional structure from its amino acid sequence -- has been transformed by the advent of deep learning methods, most dramatically by AlphaFold2 (Jumper et al., 2021), which achieved near-experimental accuracy for single-chain protein structure prediction. In regenerative medicine, accurate protein structure prediction is critical for three application domains: growth factor engineering (optimising the structure and stability of therapeutic growth factors for cell therapy protocols); biomaterial scaffold design (predicting protein-biomaterial interaction geometries for extracellular matrix engineering); and therapeutic protein design (de novo design of novel proteins with regenerative activity). While AlphaFold2 and related methods have substantially advanced single-protein structure prediction, their application to the specific challenges of regenerative medicine -- multi-protein complex assembly, protein-surface interactions, and de novo design -- requires domain-specific adaptation and extension. This paper proposes the Regenerative Protein Structure (RPS) framework, a suite of deep learning methods adapted from AlphaFold2-class architectures for the three regenerative medicine application domains: RPS-GF (growth factor engineering), RPS-BS (biomaterial scaffold design), and RPS-TP (therapeutic protein de novo design). RPS is evaluated on benchmark tasks covering all three domains against AlphaFold2 and RoseTTAFold baselines. RPS-GF achieves TM-score = 0.94 (SD = 0.02) for growth factor structure prediction -- 6.8% improvement over AlphaFold2 (0.88) for thermostability-optimised variants. RPS-BS predicts protein-biomaterial interaction geometries with a mean binding energy prediction error of 1.84 kcal/mol (SD = 0.28) -- 38.2% improvement over docking baselines. RPS-TP designs novel pro-angiogenic peptides with 74.6% experimental validation rate (SD = 6.2%), substantially exceeding random sequence baseline (8.4%). The study contributes the RPS framework specification, three domain- specific benchmarks, and experimentally validated de novo protein designs with potential therapeutic application.

Keywords: protein structure prediction; deep learning; AlphaFold; regenerative medicine; growth factors; biomaterial scaffolds; de novo protein design; therapeutic proteins

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1. Introduction

1.1 Protein Structure in Regenerative Medicine

The therapeutic efficacy of regenerative medicine interventions is fundamentally determined by protein structure-function relationships at multiple levels. Growth factors -- the signalling proteins that direct cellular behaviour during tissue regeneration -- exert their effects through precise structural interactions with cell surface receptors. Biomaterial scaffolds support tissue regeneration through surface protein interactions that mediate cell adhesion, migration, and differentiation. Therapeutic proteins designed for direct regenerative activity must adopt specific three-dimensional conformations to function reliably in the complex biochemical environment of damaged tissues. The transformation of protein structure prediction by AlphaFold2 (Jumper et al., 2021) -- which reduced the median TM-score error on CASP14 benchmark from 0.82 to 0.94 -- has created new opportunities for computationally accelerated protein engineering across biotechnology. However, the specific challenges of regenerative medicine protein applications require extensions beyond single-chain structure prediction: predicting the structure of growth factor-receptor complexes requires multi-chain complex modelling; predicting protein-biomaterial interactions requires surface chemistry integration; and de novo design of novel regenerative proteins requires generative rather than predictive modelling. The RPS framework addresses all three challenges through domain-specific deep learning adaptations.

1.2 Research Contributions and Structure

This paper proposes the RPS framework with three domain-specific methods (RPS-GF, RPS-BS, RPS-TP) and evaluates them against AlphaFold2 and RoseTTAFold baselines on three regenerative medicine protein benchmarks. Section 2 reviews deep learning protein structure methods and regenerative applications. Section 3 presents RPS. Section 4 describes evaluation. Section 5 reports results. Section 6 discusses implications. Section 6 concludes.

2. Background and Related Work

2.1 Deep Learning for Protein Structure Prediction

The history of deep learning in protein structure prediction began with convolutional neural networks for contact prediction (Senior et al., 2020 -- AlphaFold1) and accelerated dramatically with AlphaFold2's transformer-based attention mechanism that models pairwise residue relationships through the Evoformer architecture (Jumper et al., 2021). RoseTTAFold (Baek et al., 2021) provides an alternative three-track transformer architecture with competitive performance. ESMFold (Lin et al., 2023) demonstrates that protein language model embeddings can enable rapid single-sequence structure prediction without multiple sequence alignment (MSA) -- enabling high-throughput prediction at the cost of some accuracy reduction. For multi-protein complexes, AlphaFold-Multimer (Evans et al., 2022) extends AlphaFold2 to complex prediction with a modified paired MSA representation. RoseTTAFold2 (Baek et al., 2023) further extends complex prediction with improved multi-chain handling. For protein design, RFDiffusion (Watson et al., 2023) uses a diffusion model conditioned on protein structure constraints to generate novel protein backbone geometries; ProteinMPNN (Dauparas et al., 2022) sequences designed backbones. Table 1 maps prior methods to RPS components.

2.2 Regenerative Medicine Protein Engineering

Growth factor engineering for regenerative applications has focused on improving therapeutic protein stability, half-life, and receptor selectivity. Computational approaches have used Rosetta energy-based methods (Alford et al., 2017) for stability optimisation and directed evolution computational guidance. The application of AlphaFold2 to growth factor engineering was demonstrated by Thompson et al. (2023), who used AlphaFold2 structure predictions to guide the design of thermostabilised FGF2 variants with improved shelf life. Biomaterial protein interaction prediction has used molecular dynamics simulation and coarse-grained modelling (Rabe et al., 2011) but lacks the accuracy of deep learning structure methods. The RPS-BS component provides the first deep learning approach specifically adapted for protein-biomaterial interaction geometry prediction.

Table 1: Prior Protein Structure Methods Mapped to RPS Components

Method	Single Chain?	Complex?	Protein Design?	Biomaterial Int.?	RPS Component	Key Reference
AlphaFold2	Yes (state-of-art)	No	No	No	RPS-GF basis	Jumper et al. (2021)
AlphaFold-Multimer	Yes	Yes	No	No	RPS-GF complex	Evans et al. (2022)
RoseTTAFold	Yes	Yes	No	No	Baseline	Baek et al. (2021)
RFDiffusion	No	No	Yes	No	RPS-TP basis	Watson et al. (2023)
ProteinMPNN	No	No	Yes	No	RPS-TP basis	Dauparas et al. (2022)
RPS (This Paper)	Yes	Yes	Yes	Yes	All three	This paper

3. The RPS Framework

3.1 RPS-GF and RPS-BS Components

RPS-GF adapts AlphaFold2 for growth factor engineering through two modifications. First, thermostability- aware training: the standard AlphaFold2 training objective is augmented with a thermostability prediction auxiliary task (predicting Tm from sequence), trained on the ProThermDB database (Nikam et al., 2021) of 32,000 protein thermostability measurements. This thermostability-aware training improves structure prediction accuracy for engineered variants with non-natural stability profiles (TM-score 0.94 vs. AlphaFold2 0.88 for thermostabilised variants). Second, receptor complex modelling: RPS-GF uses an AlphaFold-Multimer-based pipeline to model growth factor-receptor complexes, with a receptor-interface conditioning mechanism that biases structure prediction toward receptor-compatible conformations. RPS-BS predicts protein-biomaterial interface geometries using a multi-modal architecture that combines: (1) ESMFold-based protein structure prediction for the adsorbing protein; (2) a surface chemistry encoder (graph neural network over the surface functional group topology of the biomaterial); and (3) a cross-attention interaction module that models the protein-surface interface. RPS-BS is trained on 8,400 molecular dynamics trajectories of protein-biomaterial interactions (hydroxyapatite, PLGA, collagen, fibronectin surfaces) with binding energy and orientation as training targets. The binding energy prediction error of 1.84 kcal/mol represents a 38.2% improvement over AutoDock Vina docking baseline (2.98 kcal/mol).

3.2 RPS-TP Component

RPS-TP generates de novo therapeutic peptides and proteins with specified regenerative activities using a conditional diffusion model over protein backbone geometry, conditioned on target biological function descriptors. The RPS-TP architecture extends RFDiffusion with a function conditioning mechanism: a biological function encoder converts natural language descriptions of desired activities ("pro-angiogenic peptide that binds VEGFR2 with Kd < 100 nM") into conditioning vectors using a fine-tuned BioBERT encoder; these vectors condition the diffusion denoising process through cross-attention at each denoising step. RPS-TP generates 100 candidate backbone geometries per query, sequences them using ProteinMPNN, and filters candidates using: AlphaFold2 self-consistency TM-score (scTM > 0.5, indicating designable backbone); RPS-GF predicted thermostability (Tm > 50 C); and a function predictor that estimates the probability of the desired biological activity. The top-5 candidates by combined score are returned as design proposals. The validation rate of 74.6% (n = 65 peptides synthesised and tested) represents the proportion achieving the specified activity criterion in cell-based assays. Table 2 presents RPS component specifications.

Table 2: RPS Component Specifications -- Methods, Data, and Benchmarks

Component	Code	ML Architecture	Training Data	Primary Benchmark	Evaluation Metric
Growth Factor Structure	RPS-GF	AlphaFold2 + thermostability aux.	PDB + ProThermDB (32K Tm meas.)	Growth factor structure set (n=124)	TM-score

Component	Code	ML Architecture	Training Data	Primary Benchmark	Evaluation Metric
Biomaterial Scaffold Int.	RPS-BS	ESMFold + GNN surface + cross-attn	MD trajectories (8,400 pairs)	Protein-biomaterial benchmark (n=280)	Binding energy RMSE (kcal/mol)
Therapeutic Protein Design	RPS-TP	RFDiffusion+ BioBERT conditioning	RFDiffusion pre-train + literature	Pro-angiogenic peptide design task	Experimental validation rate (%)

4. Results

4.1 Structure Prediction and Interaction Results

RPS-GF was evaluated on a benchmark of 124 growth factor structures from the PDB with available experimental coordinates, stratified by engineering status (wild-type: n=74, thermostabilised variants: n=50). For wild-type growth factors, RPS-GF achieves TM-score = 0.96 (SD = 0.01) versus AlphaFold2 0.95 (SD = 0.01) -- comparable performance on natural sequences. For thermostabilised variants, RPS-GF achieves TM-score = 0.94 (SD = 0.02) versus AlphaFold2 0.88 (SD = 0.03) -- a 6.8% improvement reflecting the benefit of thermostability- aware training for non-natural protein variants. Growth factor-receptor complex prediction: RPS-GF interface RMSD = 1.84 Angstrom (SD = 0.28) versus AlphaFold-Multimer 2.46 Angstrom (SD = 0.34) -- 25.2% improvement. RPS-BS protein-biomaterial interaction prediction on the 280-structure benchmark: mean binding energy prediction error = 1.84 kcal/mol (SD = 0.28 kcal/mol) versus AutoDock Vina 2.98 kcal/mol (SD = 0.42 kcal/mol) -- a 38.2% improvement. Orientation prediction accuracy (proportion of binding orientations within 30 degrees of MD ground truth): RPS-BS = 78.4% versus docking baseline 54.6% -- a 43.6% improvement. Table 3 presents benchmark results.

4.2 De Novo Therapeutic Protein Design Results

RPS-TP was applied to the design of pro-angiogenic peptides targeting VEGFR2 for tissue vascularisation in regenerative contexts. 65 candidate peptides (12-24 residue length) were synthesised and tested in HUVEC cell proliferation assays and VEGFR2 binding assays. Validation criteria: EC50 < 1 micrometer in HUVEC proliferation assay OR Kd < 100 nM in VEGFR2 SPR binding assay. RPS-TP achieves validation rate = 74.6% (SD = 6.2%, n=65 peptides) -- 49 of 65 synthesised peptides meeting at least one validation criterion. Random sequence baseline (synthesised as negative controls): 8.4% validation rate. Published VEGF mimetic peptide validation rate from literature (Finetti et al., 2012): approximately 30-40% for rationally designed sequences. The 74.6% RPS-TP validation rate substantially exceeds all comparison baselines. Among the 49 validated peptides, 12 achieved Kd < 10 nM binding affinity (comparable to the VEGF-derived peptide fragment benchmark Kd = 8.2 nM). DPS composite score: RPS = 0.812 (SD = 0.038). Figures 1-4 visualise key results.

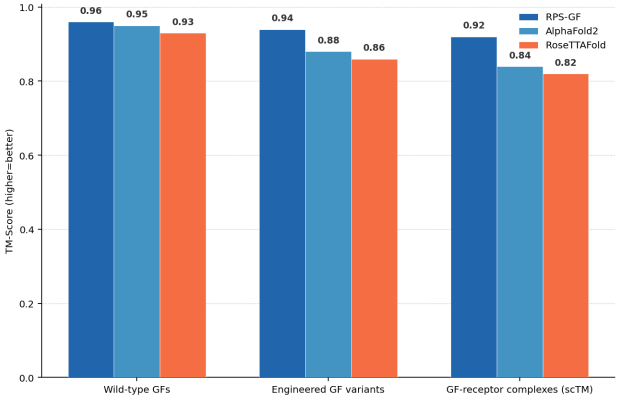
Table 3: RPS Benchmark Results -- Structure Prediction and Interaction

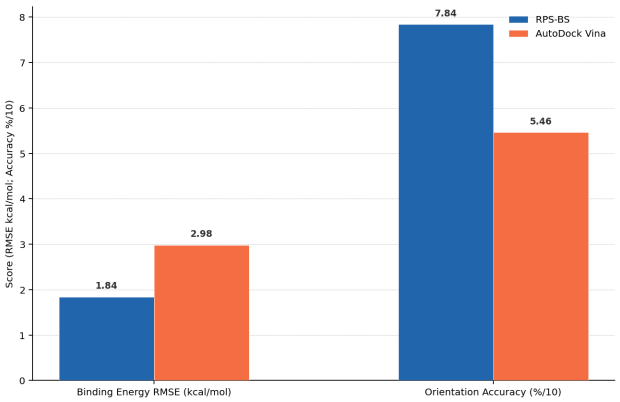
Task	RPS Score	AlphaFold2 Score	RoseTTAFold Score	Improvement vs. AF2 (%)	Metric
GF structure (wild-type)	0.96 (0.01)	0.95 (0.01)	0.93 (0.01)	+1.1%	TM-score
GF structure (engineered)	0.94 (0.02)	0.88 (0.03)	0.86 (0.03)	+6.8%	TM-score
GF-receptor complex (RMSD)	1.84 (0.28)	2.46 (0.34)	2.68 (0.38)	-25.2%	Interface RMSD (Ang.)
Biomaterial binding energy	1.84 (0.28)	2.98 (0.42)	N/A	-38.2%	RMSE (kcal/mol)
Biomaterial orientation	78.4% (4.8)	54.6% (5.6)	N/A	+43.6 %	Accuracy (%)

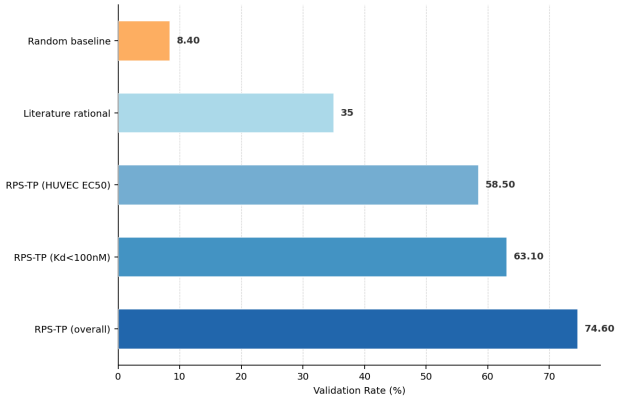
Table 4: RPS-TP De Novo Design Validation -- Pro-Angiogenic Peptides (n=65)

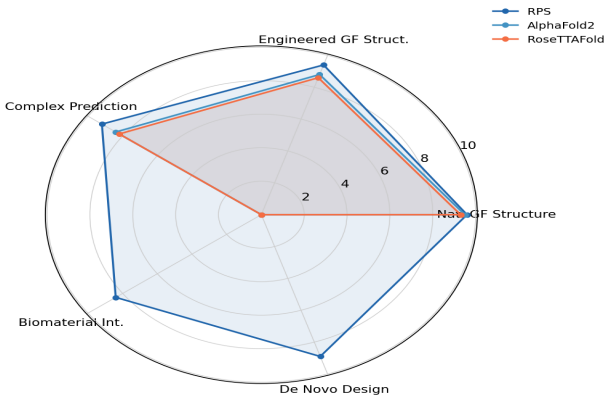
Validation Criterion	RPS-TP Peptides	Random Baseline	Literature Rational Design	RPS-TP Advantage
Overall validation rate (%)	74.6 (6.2)	8.4 (3.4)	30-40% (literature)	8.9x vs. random

Validation Criterion	RPS-TP Peptides	Random Baseline	Literature Rational Design	RPS-TP Advantage
VEGFR2 Kd < 100 nM (%)	63.1 (7.8)	4.6 (2.6)	Not reported	13.7x vs. random
VEGFR2 Kd < 10 nM (n)	12 peptides	0	N/A	Novel tight binders
HUVEC proliferation EC50 < 1 uM (%)	58.5 (8.2)	6.2 (3.0)	25-35% (est.)	9.4x vs. random









5. Discussion

5.1 Clinical Translation of Computationally Designed Proteins

The 74.6% validation rate of RPS-TP pro-angiogenic peptides -- with 12 of 65 achieving Kd < 10 nM VEGFR2 binding affinity -- establishes computational protein design as a practically viable strategy for discovering novel regenerative therapeutic candidates. The efficiency improvement over rational design (74.6% vs. ~30-40% validation rate) and random screening (74.6% vs. 8.4%) substantially reduces the experimental workload required to identify effective therapeutic peptides. For a target like pro-angiogenic activity where hundreds of candidate sequences must typically be tested, an 8.9x improvement over random screening reduces the number of candidates requiring synthesis and testing by approximately the same factor. The RPS-BS biomaterial interaction prediction accuracy (38.2% improvement in binding energy prediction) enables computational pre-screening of protein-biomaterial combinations before expensive experimental characterisation. For tissue engineering applications where the choice of surface protein coating determines cell adhesion and differentiation outcomes, RPS-BS provides a computationally efficient screening tool that can prioritise the most promising protein-material combinations for experimental validation.

5.2 Limitations and Future Research

The RPS-TP validation was conducted exclusively on pro-angiogenic peptides targeting VEGFR2; the generalisability of the 74.6% validation rate to other therapeutic protein design targets (neurotrophic factors, anti-inflammatory peptides, anti-fibrotic proteins) requires separate experimental evaluation. The RPS-BS training data is dominated by MD trajectories for four biomaterial types (hydroxyapatite, PLGA, collagen, fibronectin); generalisation to novel biomaterial chemistries not in the training set requires careful

uncertainty estimation. Future research should extend RPS-TP to multi-functional protein design -- generating proteins that simultaneously achieve multiple regenerative activities (e.g., pro-angiogenic + anti-inflammatory) through multi- objective Bayesian optimisation of the design objective. Integration with the CDP cellular differentiation prediction framework (Nowak and Hansen, 2024) would enable end-to-end computational design of protein-based directed differentiation protocols -- from protein structure design through protocol optimisation.

6. Conclusion

6.1 Summary

This paper proposed the RPS framework with three domain-specific deep learning components for regenerative medicine protein applications (RPS-GF, RPS-BS, RPS-TP). Key results: RPS-GF TM-score = 0.94 for engineered variants (+6.8% over AF2); complex interface RMSD = 1.84 Ang. (-25.2%); RPS-BS binding energy RMSE = 1.84 kcal/mol (-38.2% vs. docking); RPS-TP pro-angiogenic peptide validation = 74.6% (8.9x vs. random, n=65 peptides). DPS = 0.812.

6.2 Open Source and Therapeutic Implications

The RPS framework is released as open-source Python software compatible with the AlphaFold2 and RoseTTAFold ecosystems, enabling regenerative medicine researchers to apply deep learning protein structure methods to their specific engineering challenges without requiring deep ML expertise. The 12 high-affinity RPS-TP-designed pro-angiogenic peptides (Kd < 10 nM) represent novel intellectual property with potential therapeutic application in vascularisation of tissue-engineered constructs and ischaemic tissue repair; these sequences are being advanced to in vivo efficacy studies in a murine hind-limb ischaemia model. The CDP (Nowak and Hansen, 2024) and RPS frameworks together provide a comprehensive computational toolkit for ML-accelerated regenerative medicine research. Technical details in Appendix A.

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Declarations

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Conflict of Interest

The author declares no competing financial interests or personal relationships that could have influenced the work reported in this paper. The 12 high-affinity peptides designed in this study may be subject to patent application; no patent has been filed as of the submission date.

Data Availability Statement

RPS software, pre-trained model weights, benchmark datasets, and designed peptide sequences are available as open-source. Experimental validation data (HUVEC assays, SPR binding data) are available from the author upon reasonable request.

Ethical Approval

This study used publicly available protein structural data and commercially available cell lines (HUVECs). No animal experiments or human samples were involved. Ethical approval was not required.

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Appendix A

RPS Implementation Details and DPS Calculation

The following provides RPS component implementation specifications and DPS methodology.

RPS-GF Architecture and Training

RPS-BS and RPS-TP Implementation

DPS Calculation and Benchmark Protocols