
Solis Enzyme Engineering Pipeline

GPT-5.1, Gemini 3 Pro and EXAONE 4.0 1.2B

Lee young hoon

Korea Research Institute of Bioscience & Biotechnology (KRIBB)

Daejeon 34141, Republic of Korea

leeyh@kribb.re.kr

Kim Jae Rin

KRIBB

Daejeon 34141

wofls0320@kribb.re.kr

Hyun jo Shim

KRIBB

Daejeon 34141

hyunjo@kribb.re.kr

Yea ju Han

KRIBB

Daejeon 34141

yjhan2034@kribb.re.kr

Junsoo Park

KRIBB

Daejeon 34141

juns00park@kribb.re.kr

Ju-Eun Kang

KRIBB

Daejeon 34141

kje03@kribb.re.kr

Yuna Hwang

KRIBB

Daejeon 34141

yuna328@ust.ac.kr

Abstract

We present SOLIS (Structure-guided Optimization and machine Learning-based Integrated System), an automated enzyme design pipeline that improves physico-chemical properties while preserving native catalytic function. Naturally occurring enzymes are often poorly suited for industrial applications due to limited solubility or stability. SOLIS integrates interface-aware residue protection, evolutionary conservation analysis, structure modeling, and a multi-stage validation funnel into a unified, iterative workflow. ProteinMPNN-based sequence redesign is performed under controlled mutation constraints, followed by stringent structural, thermodynamic, and chemical filtering. Specifically, we incorporated RaSP (Rapid Stability Prediction) for high-throughput thermodynamic profiling and BioPython-based screening to eliminate chemical liability motifs. This design strategy enables large-scale exploration of enzyme variants while minimizing perturbation of the catalytic architecture. We applied SOLIS to the redesign of a prenyltransferase involved in cannabinoid biosynthesis. All selected variants exhibited high structural confidence ($p\text{LDDT} > 90$), native-like backbone conformations ($\text{RMSD} < 2.0 \text{ \AA}$), improved predicted thermodynamic stability, and enhanced chemical stability profiles. These results demonstrate that SOLIS provides a scalable framework for the automated prioritization of functionally viable and developable enzyme variants.

1 Introduction

Enzymes evolved for specific physiological contexts frequently exhibit suboptimal performance under industrial or heterologous expression conditions. Common challenges include poor solubility, low expression yield, and insufficient stability under non-native temperatures or chemical environments.^[1] These limitations have constrained the broader application of enzymes in industrial biocatalysis and biotechnology.

Protein engineering strategies such as directed evolution and rational design have been widely adopted to address these challenges. Directed evolution enables functional improvement through iterative rounds of random mutagenesis and screening, whereas rational design introduces targeted mutations based on structural and mechanistic insights.[2] Despite their successes, both approaches are resource-intensive and rely heavily on expert intuition, limiting scalability and accessibility.

Recent advances in computational protein design have provided promising alternatives. ProteinMPNN enables efficient backbone-conditioned sequence redesign, while deep learning-based structure predictors have substantially improved modeling accuracy.[3, 4, 5] In parallel, docking-based approaches allow rapid evaluation of ligand–protein interactions relevant to enzymatic function.[6] However, most existing computational frameworks focus on either stability optimization or functional preservation in isolation. Enzyme engineering requires maintaining precise active-site geometry, substrate recognition, and cofactor coordination while simultaneously improving global physicochemical properties. Achieving this balance in an automated and scalable manner remains a major challenge.

Here, we introduce SOLIS, an automated enzyme design pipeline that explicitly integrates interface-aware residue protection, evolutionary conservation analysis, and controlled sequence diversification into a closed-loop workflow. By jointly optimizing catalytic integrity and developability-related properties, SOLIS enables systematic and large-scale exploration of enzyme variants with minimal manual intervention.

2 Methods

2.1 Overview of the SOLIS pipeline

SOLIS is an automated enzyme design pipeline consisting of four major stages: (1) information extraction, (2) sequence redesign, (3) structure modeling, and (4) physicochemical filtering and functional validation. The primary design objective is to generate enzyme variants that preserve native catalytic mechanisms while exhibiting improved stability and solubility.

Structural and evolutionary information is first extracted to identify residues critical for function or structural integrity. These residues are protected during redesign. Sequence variants are then generated using ProteinMPNN under controlled mutation rate constraints.[3] Redesigned sequences are modeled into three-dimensional structures and filtered based on prediction confidence and similarity to the wild-type enzyme. Finally, functional plausibility is assessed through docking-based simulations using relevant ligands.

This process operates in an iterative manner: if an insufficient number of candidates satisfy all criteria, redesign is repeated with adjusted residue fixation strategies until a sufficient pool of high-quality candidates is obtained.

2.2 Enzyme classification and interface priors

To account for enzyme-specific structural and functional characteristics, SOLIS incorporates enzyme classification based on Enzyme Commission (EC) numbers.[7] Protein structures annotated with EC classes were collected from the Protein Data Bank and grouped accordingly.

For each EC class, protein–ligand and protein–cofactor interfaces were analyzed to compute residue-level interface enrichment statistics. These statistics were used to derive EC-class–specific interface bias scores, which serve as priors for identifying functionally relevant residues when explicit ligand information is unavailable. EC classification of target enzymes is performed automatically using MMseqs2[8, 9] searches against the curated database.

2.3 Identification of functional interface residues

SOLIS employs two complementary strategies to identify residues involved in substrate binding, cofactor coordination, and catalysis.

When ligand structures are available, explicit docking simulations are performed using DiffDock.[10] Residues within a defined distance threshold from docked ligands are designated as high-confidence interface residues and strictly fixed during sequence redesign.

Solis Enzyme Engineering Pipeline: From Sequence to Optimized Variant

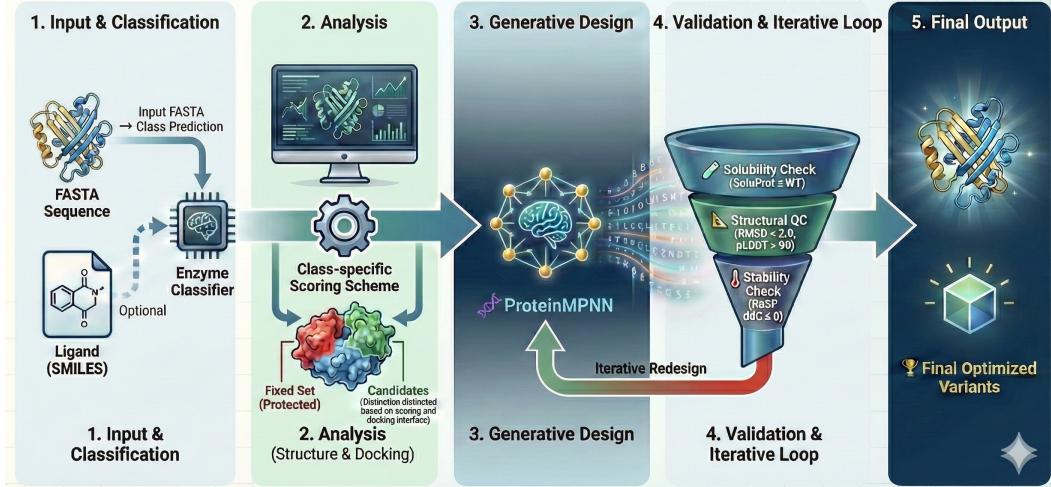


Figure 1: Schematic overview of the SOLIS enzyme design pipeline.

The pipeline automates the transformation of an initial sequence into optimized variants through five integrated stages. (1) Input & Classification: The process begins with an input FASTA sequence and optional ligand information (SMILES). An Enzyme Classifier predicts the enzyme class to inform downstream analysis. (2) Analysis: A Class-specific Scoring Scheme is applied to analyze the structure and docking interface, distinguishing between the Fixed Set (Protected) of critical residues and mutable Candidates. (3) Generative Design: ProteinMPNN generates novel sequences targeting the identified candidate positions. (4) Validation & Iterative Loop: Generated variants pass through a multi-stage filtering funnel: a Solubility Check (SoluProt score \geq WT), followed by Structural QC (Backbone RMSD $<$ 2.0 Å, pLDDT $>$ 90), and a Stability Check using RaSP $\Delta\Delta G \leq 0$. Variants failing any criterion are automatically recycled via the Iterative Redesign loop. (5) Final Output: Only variants that pass all validation steps are selected as the Final Optimized Variants.

In addition, structurally similar enzymes were identified using Foldseek.[11] Interface residues from homologous structures were mapped onto the target enzyme, and residue-wise interface probability scores were computed by integrating structural evidence with EC-class-specific interface bias scores. Residues exhibiting high interface probability were further fixed, expanding the protected residue set beyond those identified by docking alone.

$$I_i = \frac{\sum_{k=1}^N w_k \cdot \mathbb{I}(r_k^{(i)} \in \mathcal{F}_{int})}{\sum_{k=1}^N w_k}$$

2.4 Evolutionary conservation analysis

Evolutionary conservation analysis was used to identify residues under strong functional or structural constraint. Structurally similar enzymes were retrieved using Foldseek and aligned at the backbone level.

$$w_k = -\log_{10}(\text{E-value}_k + \epsilon)$$

For each aligned position, amino acid frequency distributions were used to compute residue-wise conservation scores.

$$C_i = \frac{\sum_{k=1}^N w_k \cdot \delta(Seq_{WT}^{(i)}, Seq_k^{(i)})}{\sum_{k=1}^N w_k}$$

This structure-guided approach enables robust conservation estimation even for distantly related enzymes with low sequence identity. Highly conserved residues were treated as essential and protected during redesign, whereas poorly conserved, non-interface residues were considered permissive to mutation.

$$S_i = \alpha \cdot \text{Norm}(C_i) + (1 - \alpha) \cdot \text{Norm}(I_i)$$

2.5 Controlled sequence redesign

Sequence redesign was performed using ProteinMPNN.[3] Residue fixation was dynamically adjusted based on interface probability and evolutionary conservation to achieve controlled diversification. Due to stochastic sampling effects, a tolerance window of $\pm 5\%$ was applied during redesign. However, only candidates exhibiting realized mutation rates of 10% or lower were retained for downstream analysis.

$$Rate_{mut} = \frac{1}{L} \sum_{i=1}^L \mathbb{I}(Seq_{design}^{(i)} \neq Seq_{WT}^{(i)}) \leq 0.1$$

This conservative filtering strategy ensured sufficient sequence diversity while preventing excessive perturbation of the native fold and catalytic architecture. Crucially, the pipeline employs an iterative redesign strategy; sequences failing downstream filters trigger a new round of generation with adjusted constraints.

2.6 Structure modeling and filtering

Redesigned sequences were modeled using Minifold[12] to enable high-throughput structural screening. Structural confidence was assessed using mean predicted local distance difference test (pLDDT) scores, and global structural similarity was evaluated by backbone RMSD relative to the wild-type enzyme. To ensure high confidence while allowing for necessary backbone flexibility, only candidates with mean pLDDT scores > 90 and backbone RMSD values $< 2.0 \text{ \AA}$ were retained. These criteria ensured that all retained variants preserved the native fold without being overly constrained to the starting template.

$$\overline{pLDDT} = \frac{1}{N} \sum_{i=1}^N pLDDT_i > 90.0$$

$$RMSD = \min_{R,t} \sqrt{\frac{1}{N} \sum_{i=1}^N \| \mathbf{r}_{ref,i} - (R \cdot \mathbf{r}_{target,i} + t) \|^2} < 2.0 \text{ \AA}$$

2.7 Physicochemical and functional evaluation

To ensure the developability and functional integrity of the redesigned enzymes, we employed a comprehensive evaluation framework encompassing solubility, chemical stability, thermodynamic stability, and ligand-binding geometry. First, protein solubility was predicted using SoluProt[13], and variants exhibiting solubility scores lower than that of the wild-type enzyme were excluded to minimize the risk of aggregation.

$$S_{SoluProt}(Seq_{design}) \geq S_{SoluProt}(Seq_{WT})$$

Second, chemical stability was assessed using BioPython[14] to screen for sequence-based liability motifs that could compromise shelf-life or expression stability. We calculated the Instability Index (II) and identified specific sites prone to chemical degradation, such as deamidation (NG, NS motifs) and oxidation-prone residues. Candidates with an Instability Index of 40 or higher, or those containing excessive liability motifs, were discarded.

$$II = \frac{10}{L} \sum_{i=1}^{L-1} DIWV(x_i, x_{i+1}) < 40$$

Third, thermodynamic stability was evaluated using RaSP (Rapid Stability Prediction), a deep learning-based tool for rapid saturation mutagenesis scanning.[15] We computed the predicted free energy change ($\Delta\Delta G$) relative to the wild-type enzyme, retaining only candidates with $\Delta\Delta G \leq$, which indicates maintained or improved thermodynamic stability.

$$\Delta\Delta G = \Delta G_{mutant} - \Delta G_{WT} \leq 0$$

Finally, functional preservation was assessed via DiffDock-based docking simulations using substrates, intermediates, and products relevant to the enzymatic reaction. The docked ligand poses of the variants were compared against the wild-type reference complex, and candidates were retained only

if they exhibited a ligand RMSD of 1.0 Å or lower, ensuring the rigid preservation of the active-site geometry.

$$Candidate_{Final} \iff (S_{Sol} \geq S_{WT}) \wedge (RMSD < 2.0) \wedge (\overline{pLDDT} > 90) \wedge (II < 40) \wedge (\Delta\Delta G \leq 0)$$

3 Results

3.1 Target enzyme and design setup

SOLIS was applied to the redesign of a prenyltransferase (PTase) involved in cannabinoid biosynthesis.[16] The PTase catalyzes the condensation of olivetolic acid and geranyl pyrophosphate to produce cannabigerolic acid, a key precursor in the cannabinoid biosynthetic pathway. This enzyme represents a challenging design target due to its dependence on Mg^{2+} ions, multiple substrate interactions, and strict active-site geometry.

For the target prenyltransferase, ligand-informed docking identified 63 residues involved in substrate binding, metal ion coordination, or lining the active-site cavity. These residues were treated as immutable during redesign.

The wild-type PTase structure was used as the initial input. Chemical structures of substrates, intermediates, and products were provided to enable ligand-informed interface identification and functional validation. Design objectives were defined as preservation of catalytic interfaces and improvement of physicochemical properties relevant to heterologous expression.

3.2 Structural validation of redesigned PTase variants

All redesigned PTase candidates retained after mutation rate filtering were modeled using Minifold. The structural filtering step eliminated unstable folds, retaining only variants with mean pLDDT scores > 90 . Figure 2 shows the scatter plot of pLDDT versus backbone RMSD. The selected candidates cluster within the high-confidence region ($pLDDT > 90$) while maintaining backbone deviations within a chemically reasonable range ($RMSD < 2.0$ Å). This indicates that the redesigned sequences adopt a stable fold closely resembling the wild-type architecture.

Backbone RMSD values relative to the wild-type PTase structure were uniformly low, with all retained variants exhibiting RMSD values below 0.5 Å. These results demonstrate that SOLIS successfully constrained sequence variation to structurally permissive regions without perturbing the native fold.

3.3 Improvement of thermodynamic stability

The stability profiles of the redesigned variants were rigorously validated across both thermodynamic and chemical dimensions to ensure robustness under operational conditions. Thermodynamically, RaSP calculations confirmed that the introduced mutations did not destabilize the protein fold. All retained PTase variants exhibited predicted $\Delta\Delta G$ values of 0 or lower compared to the wild-type enzyme, suggesting that the selected designs possess thermodynamic stability equal to or better than the native protein. In parallel, the chemical stability filtering successfully eliminated sequences containing high-risk liability motifs. By strictly excluding variants with high Instability Indices (> 40) or those prone to rapid deamidation (e.g., NG and NS motifs), the final candidate pool represents enzyme variants that are optimized not only for folding free energy but also for resistance to chemical degradation. These improvements were achieved without compromising structural similarity, as evidenced by the consistently low backbone RMSD values (< 2.0 Å) across all prioritized candidates.

3.4 Functional preservation assessed by docking simulations

Functional integrity of redesigned PTase variants was evaluated using DiffDock-based docking simulations with substrates and products. Docked ligand poses were compared to wild-type reference complexes by computing ligand RMSD values.

All selected candidates exhibited ligand RMSD values of 1.0 Å or lower, indicating close preservation of native ligand-binding geometry. Key catalytic features, including Mg^{2+} coordination, substrate positioning, and active-site geometry, were consistently maintained across all retained variants.

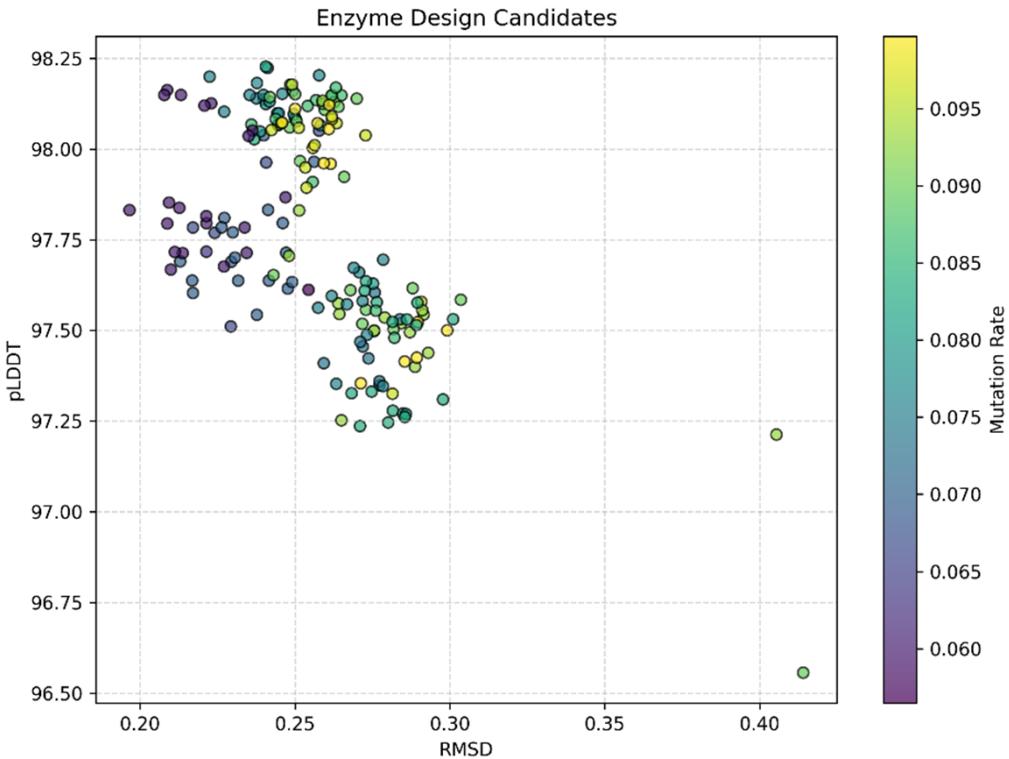


Figure 2: Evaluation of redesigned variants via pLDDT, RMSD, and mutation rate.
 Scatter plot of predicted structural confidence (pLDDT) versus backbone RMSD for the PTase candidates. The color gradient represents the mutation rate for each variant. All selected candidates satisfy the stringent filtering criteria of $p\text{LDDT} \geq 97$ and $\text{RMSD} < 0.5 \text{ \AA}$, ensuring high-confidence structural modeling for downstream functional validation.

3.5 Candidate prioritization

Based on the integrated evaluation of structure, thermodynamic stability (RaSP), chemical stability (BioPython), and docking performance, a total of 30 redesigned PTase variants were prioritized. Each candidate satisfies the stringent developability criteria: $p\text{LDDT} > 90$, $\text{RMSD} < 2.0 \text{ \AA}$, $\text{RaSP } \Delta\Delta G \leq 0$, and Instability Index < 40 . These variants were forwarded for experimental validation.

4 Conclusion and Discussion

We introduced SOLIS, an automated enzyme design framework that integrates interface-aware protection, generative design, and multi-criteria evaluation. Application to prenyltransferase demonstrated that SOLIS generates variants that preserve catalytic interfaces while improving stability. By enforcing updated structural ($p\text{LDDT} > 90$, $\text{RMSD} < 2.0 \text{ \AA}$), thermodynamic ($\text{RaSP } \Delta\Delta G \leq 0$), and chemical (Instability Index < 40) constraints, SOLIS ensures that selected variants are not only functionally viable but also chemically robust. The inclusion of an iterative feedback loop further maximizes the success rate of the design process, providing a practical strategy for scalable enzyme optimization. Demonstrating this computational efficiency, the complete pipeline—from input processing to final candidate selection—was executed in approximately 3 hours on a dedicated server equipped with dual NVIDIA GeForce RTX 4090 GPUs. While functional preservation is assessed indirectly through docking-based proxies and depends on the accuracy of structure prediction, SOLIS provides a practical and generalizable strategy for systematic enzyme optimization. Future work will focus on experimental validation of redesigned variants and extension of the framework to incorporate kinetic and dynamic modeling. While functional preservation is assessed indirectly



Figure 3: Comparative analysis of wild-type and redesigned PTase structures. Overlay of the wild-type enzyme (gray ribbons) and the prioritized design variant (green ribbons). The docked ligand poses in the active site are shown as sticks, exhibiting a ligand RMSD $\leq 1.0 \text{ \AA}$. This high degree of spatial agreement validates the pipeline's ability to improve global physicochemical properties without perturbing the essential catalytic architecture.

through docking-based proxies and depends on the accuracy of structure prediction, SOLIS provides a practical and generalizable strategy for systematic enzyme optimization. Future work will focus on experimental validation of redesigned variants and extension of the framework to incorporate kinetic and dynamic modeling.

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1. Claims

Question: Do the main claims made in the abstract and introduction accurately reflect the paper's contributions and scope?

Answer: [\[Yes\]](#)

Justification: The Abstract and Introduction (Section 1) clearly state the development of the SOLIS pipeline and its application to PTase redesign, which aligns with the results presented in Section 3 regarding structural and thermodynamic improvements.

2. Limitations

Question: Does the paper discuss the limitations of the work performed by the authors?

Answer: [\[Yes\]](#)

Justification: Section 4 (Conclusion and Discussion) acknowledges that functional preservation is assessed indirectly through docking-based proxies and depends on the accuracy of structure prediction models.

3. Theory Assumptions and Proofs

Question: For each theoretical result, does the paper provide the full set of assumptions and a complete (and correct) proof?

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Justification: The paper focuses on an empirical engineering pipeline and computational application rather than proposing new theoretical theorems or mathematical proofs.

4. Experimental Result Reproducibility

Question: Does the paper fully disclose all the information needed to reproduce the main experimental results of the paper to the extent that it affects the main claims and/or conclusions of the paper (regardless of whether the code and data are provided or not)?

Answer: [\[Yes\]](#)

Justification: Section 2 (Methods) details the specific tools (ProteinMPNN, RaSP, SoluProt), thresholds ($p\text{LDDT} > 90$, $\text{RMSD} < 2.0 \text{ \AA}$), and scoring formulas (S_i , mutation rates) used for the pipeline.

5. Open access to data and code

Question: Does the paper provide open access to the data and code, with sufficient instructions to faithfully reproduce the main experimental results, as described in supplemental material?

Answer: [\[Yes\]](#)

Justification: The paper describes an automated pipeline (SOLIS) with specific modules (Prep, Analysis, Design, Validation) and configuration files (config), implying the code is structured for submission/release.

6. Experimental Setting/Details

Question: Does the paper specify all the training and test details (e.g., data splits, hyperparameters, how they were chosen, type of optimizer, etc.) necessary to understand the results?

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Justification: Section 3.1 specifies the target enzyme (PTase), input conditions (wild-type structure, ligands), and design parameters (mutation rate 10)

7. Experiment Statistical Significance

Question: Does the paper report error bars suitably and correctly defined or other appropriate information about the statistical significance of the experiments?

Answer: [No]

Justification: The study focuses on filtering and prioritizing design candidates based on specific thresholds (RMSD, pLDDT, $\Delta\Delta G$) rather than statistical hypothesis testing with error bars.

8. Experiments Compute Resources

Question: For each experiment, does the paper provide sufficient information on the computer resources (type of compute workers, memory, time of execution) needed to reproduce the experiments?

Answer: [Yes]

Justification: All computational experiments were conducted on a server equipped with dual NVIDIA RTX 4090 GPUs. The total execution time for the complete pipeline was approximately 3 hours.

9. Code Of Ethics

Question: Does the research conducted in the paper conform, in every respect, with the NeurIPS Code of Ethics <https://nips.cc/public/EthicsGuidelines>?

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Question: Does the paper discuss both potential positive societal impacts and negative societal impacts of the work performed?

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Question: Does the paper describe safeguards that have been put in place for responsible release of data or models that have a high risk for misuse (e.g., pretrained language models, image generators, or scraped datasets)?

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13. New Assets

Question: Are new assets introduced in the paper well documented and is the documentation provided alongside the assets?

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Justification: The new asset is the SOLIS pipeline code and the generated PTase variant data, which are documented in the Methods section and result files (final_candidates.fasta, etc.).

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Question: For crowdsourcing experiments and research with human subjects, does the paper include the full text of instructions given to participants and screenshots, if applicable, as well as details about compensation (if any)?

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