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A Computational Evaluation of IsPETase Variants: Thermodynamic Stability vs. PET Substrate Binding

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Abstract

PET plastic is widely used in various applications, including packaging, textiles, and consumer goods, despite its slow natural degradation. A breakthrough discovery by Yoshida et al. identified *Ideonella sakaiensis*, a bacterium that breaks down PET via IsPETase. However, the wild-type IsPETase has slow degradation rates and is thermally unstable for PET degradation, leading researchers to engineer thermostable variants. Whether these stability-enhancing mutations cause unintended effects on ΔG binding, especially with smaller oligomer sizes comparable to microplastics, remains unexplored. Here, 13 IsPETase variants and 1-4mer PET oligomers were compiled for molecular docking (AutoDock Vina) to calculate binding free energy. ChimeraX extracted structural metrics for the catalytic gap, hydrogen-bond count, and interface surface area. ΔG binding progressively became more negative as the oligomer length increased (r= -0.9837, p=0.016), indicating stronger overall binding to longer oligomers. Among engineered mutants, ΔTm positively correlated with ΔG binding for the 2-mer and 3-mer lengths (2-mer: r=0.7592, p=0.018; 3-mer: r=0.7310, p=0.025), suggesting that more thermostable variants bound these intermediate lengths less tightly. This weakened binding coincided with a widened catalytic gap, consistent with the rigidification of the binding cleft as a possible effect of stabilizing mutations, which potentially limited the accommodation of intermediate PET ligands. Importantly, none of the correlations survived Benjamini-Hochberg correction for multiple comparisons, so this study's findings should be interpreted as exploratory with trends rather than established correlations. These findings nonetheless highlight a previously overlooked stability-binding trade-off, offering future research possibilities with implications for engineering PETase variants geared for microplastic and PET degradation.

Introduction

- ★ Polyethylene terephthalate (PET) is among the most widely produced plastics (~31 million tonnes in 2025) and resists natural degradation due to its aromatic backbone and semi-crystalline structure.
- ★ *Ideonella sakaiensis* uses PET as its primary carbon source via IsPETase, but the wild-type enzyme degrades PET slowly and is unstable above 40°C, prompting engineering of thermostable variants.
- ★ How stability-enhancing mutations affect substrate binding (ΔG binding) to short 1- 4mer PET oligomers remains poorly understood.
- ★ This study examines these trade-offs using computationally predicted free energy changes (ΔΔG) for single-point mutants and measured melting temperature shifts (ΔTm) for extensively engineered variants.

Question/Hypothesis

- ★ Does an increase in predicted thermodynamic stability (ΔΔG stability/ΔTm) among engineered IsPETase variants correlate with an altered binding free energy (ΔG binding) for shorter PET substrate units (1-4mer)?

Methods

1. **Dataset Curation & Structural Preparation**
   a. The wild-type IsPETase (PDB ID: 6EQE) and 13 literature-validated variants spanning a thermodynamic stability gradient were selected.
   b. Ligand preparation: Structures of the 1-4 short PET oligomers were obtained via PubChem SMILES strings.
2. **Thermodynamic Stratification & Metrics**
   a. Since ΔΔG predictors are unreliable for multi-point mutants, a dual-metric approach was used:

Stratum:	Variant type:	Metric used:	Stability range:
Destabilized control	Single-point	ΔΔG: (FireProt v 2.1)	+ shift in free energy
Native baseline	Wild-type	Reference point	ΔΔG = 0.00 kcal/mol
Stabilized variants	Multi-point	Experimental ΔTm	Moderate and drastic melting point increases

3. **Molecular Docking & Binding Simulations**
   a. 52 docking runs were performed (13 variants x 4 oligomer lengths) in AutoDock Vina via the PyRx interface to extract the ΔG binding of each pair.
4. **Structural Metrics Analysis (UCSF ChimeraX): 3 key binding properties extraction**
   i. Catalytic Gap (Å): The distance between the nucleophilic oxygen of Ser160 and the PET ester bond carbonyl carbon.
   ii. Hydrogen Bond Count: The total H-bonds between the enzyme binding cleft and the oligomer oxygen atoms.
   iii. Interface Contact Area (Å^2): The total enzyme-substrate contact surface area to quantify the binding "grip".

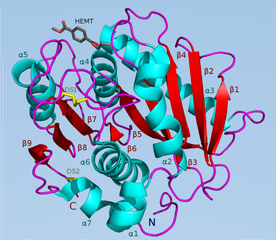


Figure 1: Depiction of the wild-type IsPETase enzyme and its regions

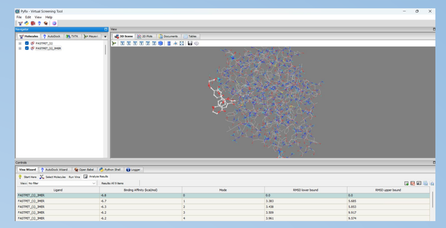


Figure 2: Example of the PyRx interface when docking enzymes-FASTPETase with 3-mer PET oligomer

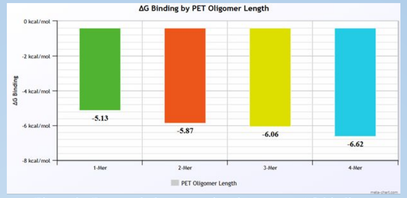


Figure 3: Bar graph demonstrating the average ΔG binding for each PET oligomer length (1-4mer).

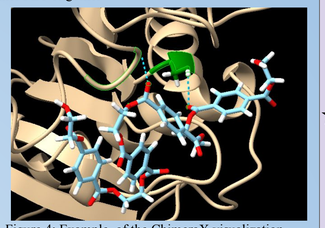


Figure 4: Example of the ChimeraX visualization-FASTPETase with 4-mer PET oligomer

Results

- ★ **Docking trends: binding affinity vs. oligomer lengths:**
  - Across 52 docking runs, ΔG binding became more negative with oligomer length, indicating tighter binding. Mean ΔG binding values for all 13 variants were calculated as -5.13 ± 0.11 (1-mer), -5.87 ± 0.47 (2-mer), -6.06 ± 0.86 (3-mer), and -6.62 ± 0.58 kcal/mol (4-mer).
  - A Pearson correlation confirmed a significant negative correlation between oligomer length and mean ΔG binding, indicating the active site accommodates longer PET chains more tightly.
- ★ **Engineering trade-offs between thermostability and binding affinity:**
  - Single-point mutants showed no significant correlation between the ΔΔG and ΔG binding at any oligomer length, though the sample size was small.
  - Among multi-point engineered mutants, higher ΔTm values correlated significantly with less negative ΔG binding for the 2-mer and 3-mer, meaning highly engineered variants bound these intermediates less tightly. The 1-mer and 4-mer trended similarly, but did not reach significance.
- ★ **ChimeraX Analysis:**
  - Higher thermostability was associated with a larger catalytic gap (1-mer, 3-mer), fewer hydrogen bonds (4-mer), and reduced interface contact area (2-mer), suggesting stabilization rigidifies the cleft and loosens enzyme-substrate engagement.
- ★ **Analysis and limitations**:
  - Trends were held when recomputed using the highest and lowest ΔTm values in the range, but none survived the Benjamini-Hochberg correction, despite being very near, so results should be read as highly plausible trends that indicate an underlying trade-off rather than a confirmed effect.

Discussion

- ★ **Main findings:** Highly thermostabilized variants bound to the 2-mer and 3-mer oligomers less tightly. Since MHET is known to cause product inhibition, this suggests stability-driven binding changes act at intermediate oligomer lengths rather than MHET itself, revealing a continued product inhibition issue.
- ★ **Stability-flexibility trade-off:** A higher ΔTm rise was associated with a wider catalytic gap (1-mer, 3-mer), consistent with the stability-flexibility tradeoff, where enhanced stability limits the enzyme's ability to engage with oligomers and slows catalysis.
- ★ **Magnitude of effect:** The ΔG is associated with the equilibrium association constant K_a, as ΔG°= -RT ln ($C° K_{a)}$; small ΔG shifts produce large changes in affinity. E.g.: for the 2-mer, the 1.3 kcal/mol difference between PETase V22 (-6.6 kcal/mol) and HotPETase (-5.3 kcal/mol) corresponds to roughly 9-fold weaker binding for HotPETase.
- ★ **Limitations**: Small single-point sample size (n=4), no single ΔΔG predictor covering both variant groups, non-equivalent stability metrics (ΔΔG/ΔTm), and Vina's lack of full protein flexibility. Since no results survived BH correction, findings are exploratory and require wet-lab verification.

Conclusion

- ★ Longer PET oligomers bound the IsPETase active site more tightly, while higher thermostability (ΔTm) trended with weaker binding to intermediate oligomers: 2-mer and 3-mer, suggesting a possible stability-binding trade-off in engineered variants. Because no results survived the BH correction, these findings are exploratory and should be validated with larger samples, molecular dynamics, and wet-lab experiments.

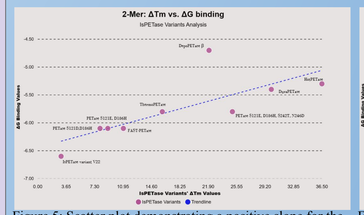


Figure 5: Scatter plot demonstrating a positive slope for the trendline between ΔTm and ΔG binding: 2-mer

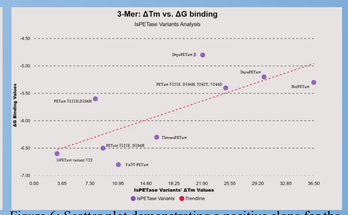


Figure 6: Scatter plot demonstrating a positive slope for the trendline between ΔTm and ΔG binding: 3-mer

Key Citations:

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