

Structural Analysis and Strategic Truncation of PBLP, a Malarial Hydrolase

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TBIOMD 499



INTRO

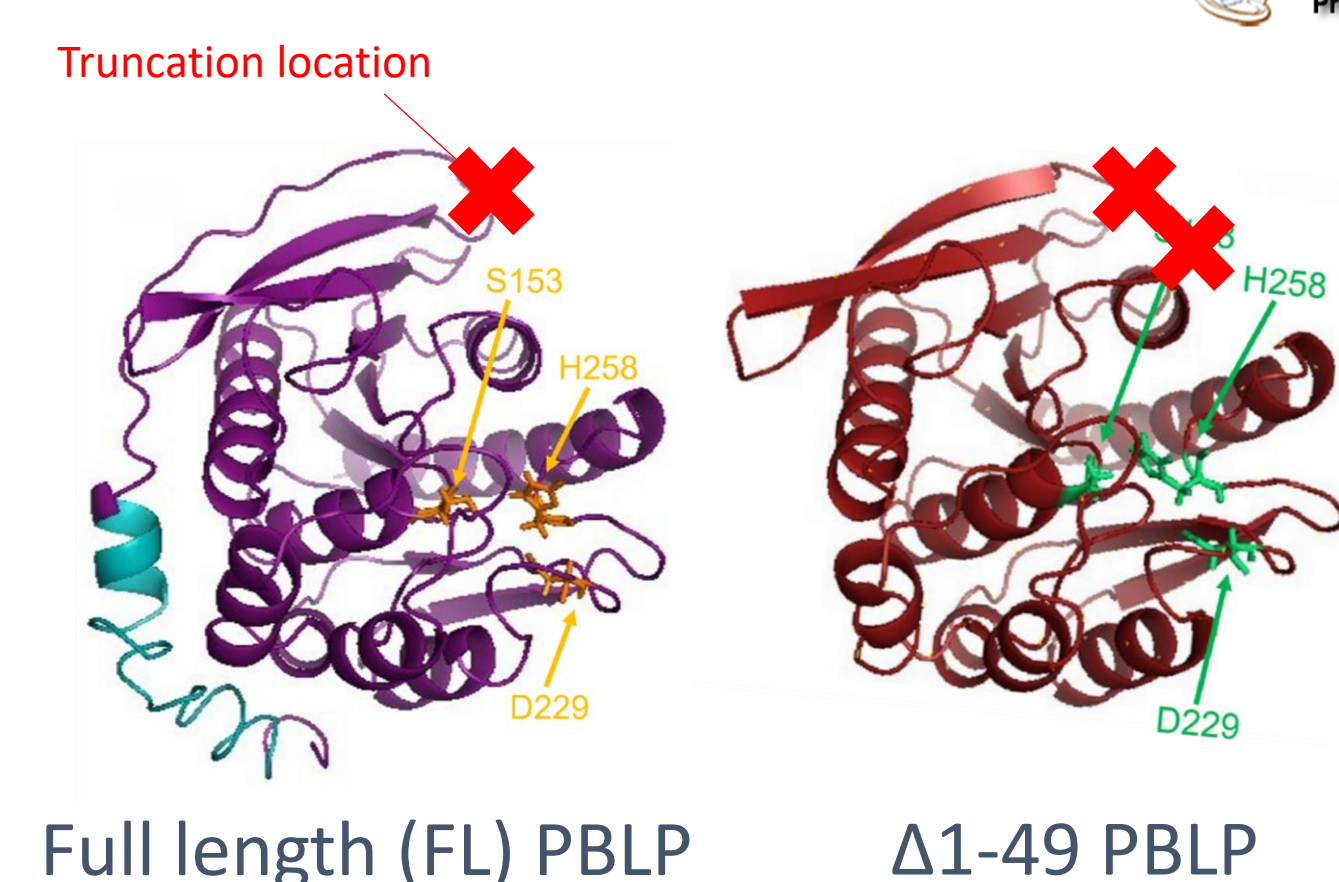
- PBLP stands for "*Plasmodium* BEM46-like protein" Discovered by Dr. Groat Carmona (2015).
- Expressed throughout all life malarial parasite life stages and modulates the replication of invasive forms.

PROJECT OVERVIEW

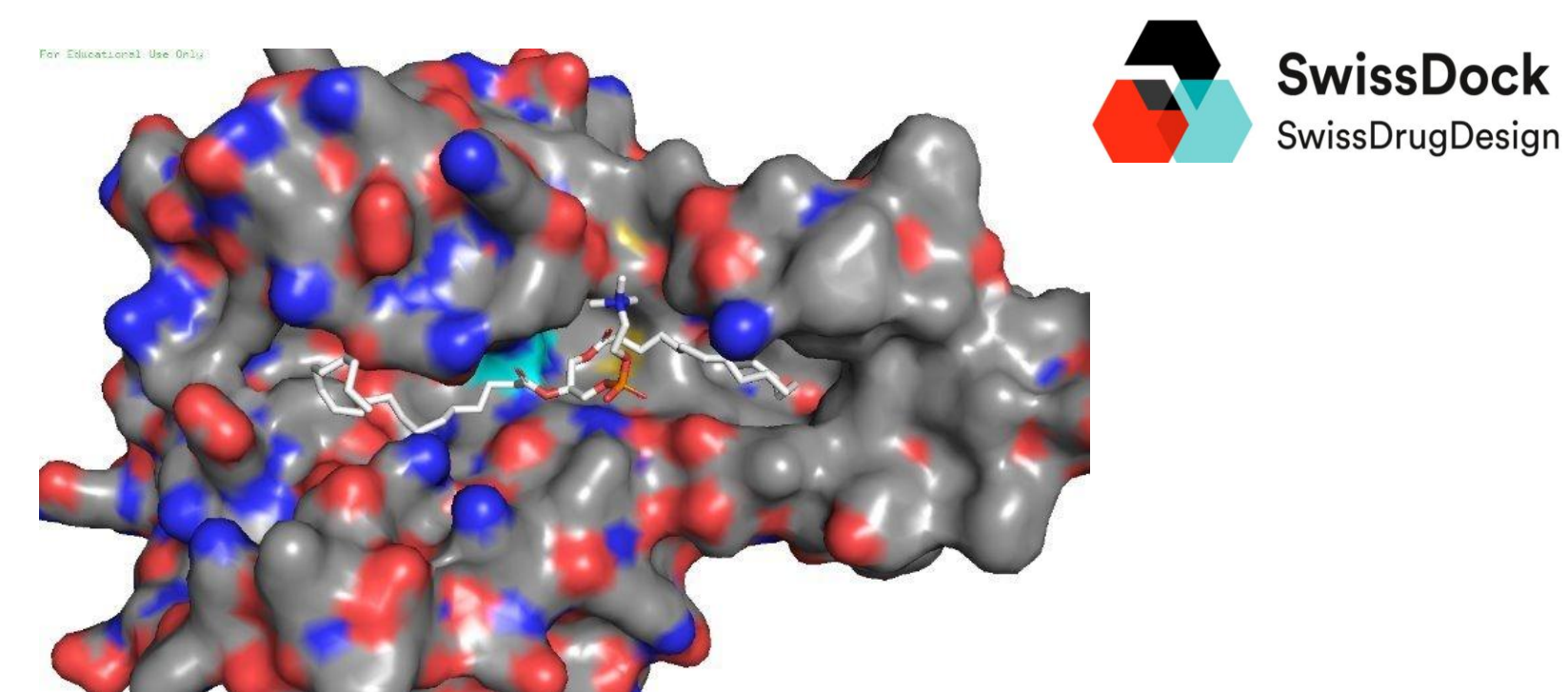
- **The big question:** what does PBLP catalyze?
- **The problem:** PBLP is hard to purify.

BACKGROUND

I-TASSER program used to generate preliminary models of PBLP. 1-49 region is mostly hydrophobic and didn't appear to interact with catalytic domain.

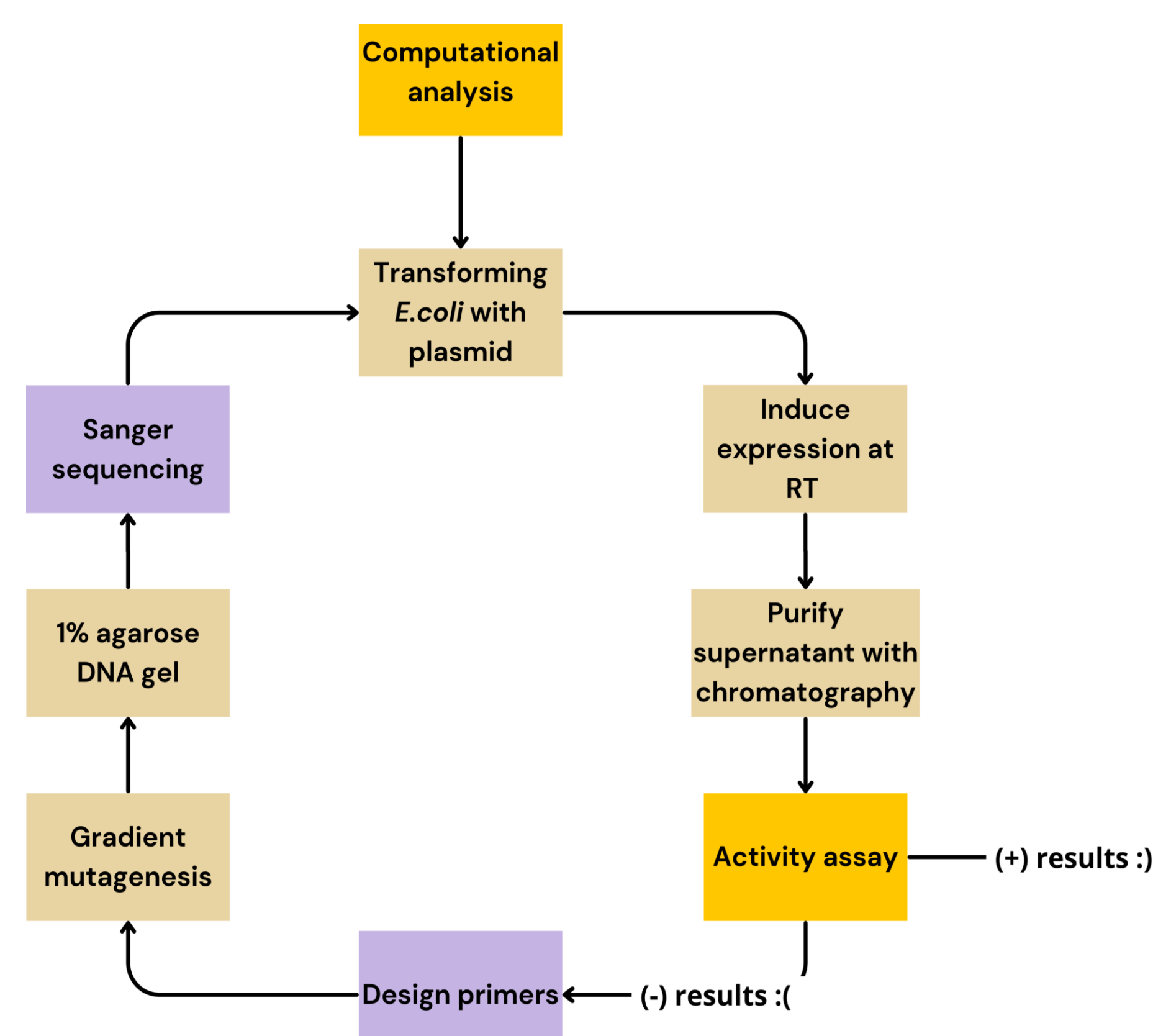


Best SwissDock results: phosphatidylcholine docked to FL PBLP, with a binding affinity of -4.017 kcal/mol.



PREVIOUS WORK

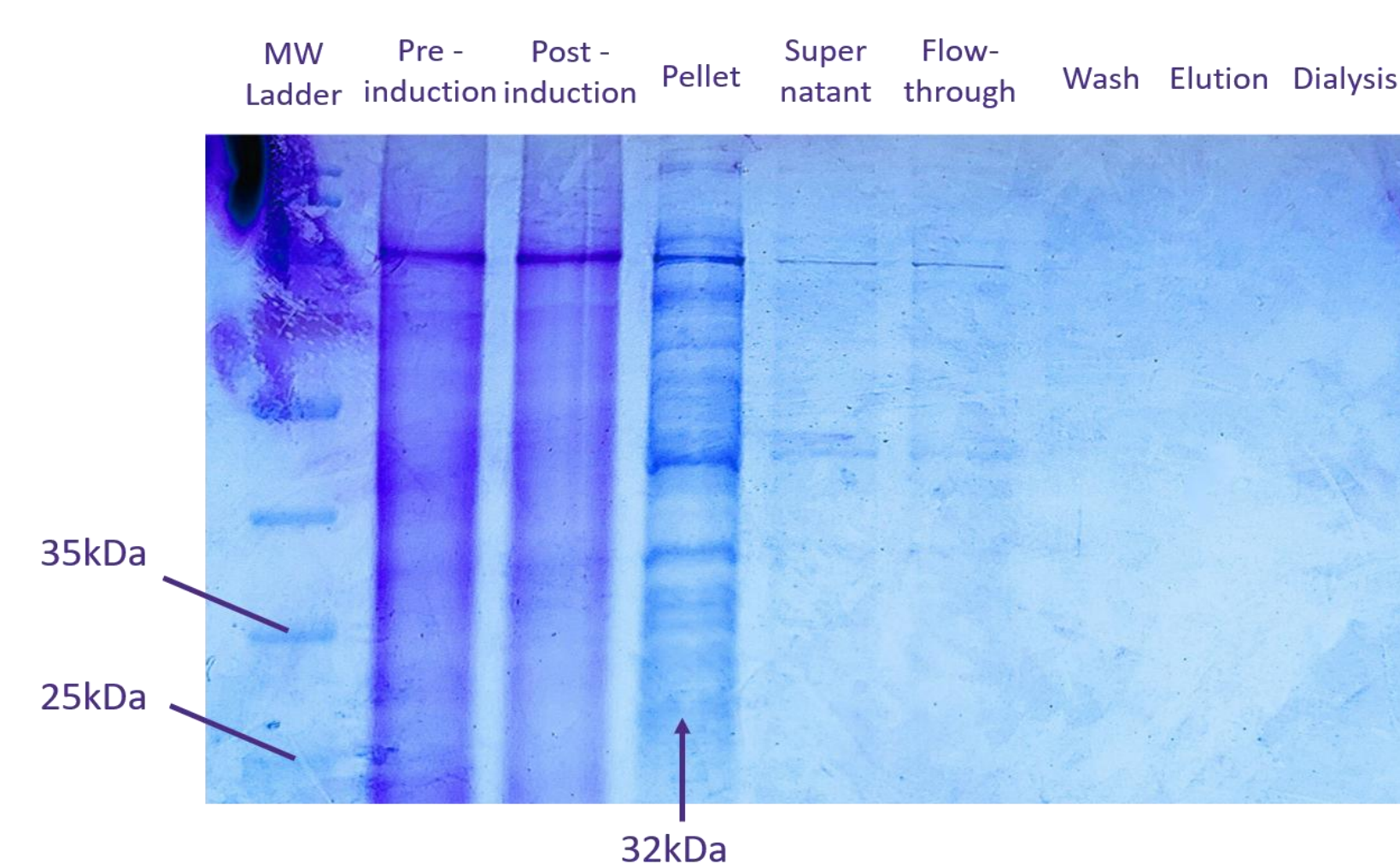
- **Steps we took:** Used truncated construct and optimized purification.
- **What went wrong?:** Low protein concentration.



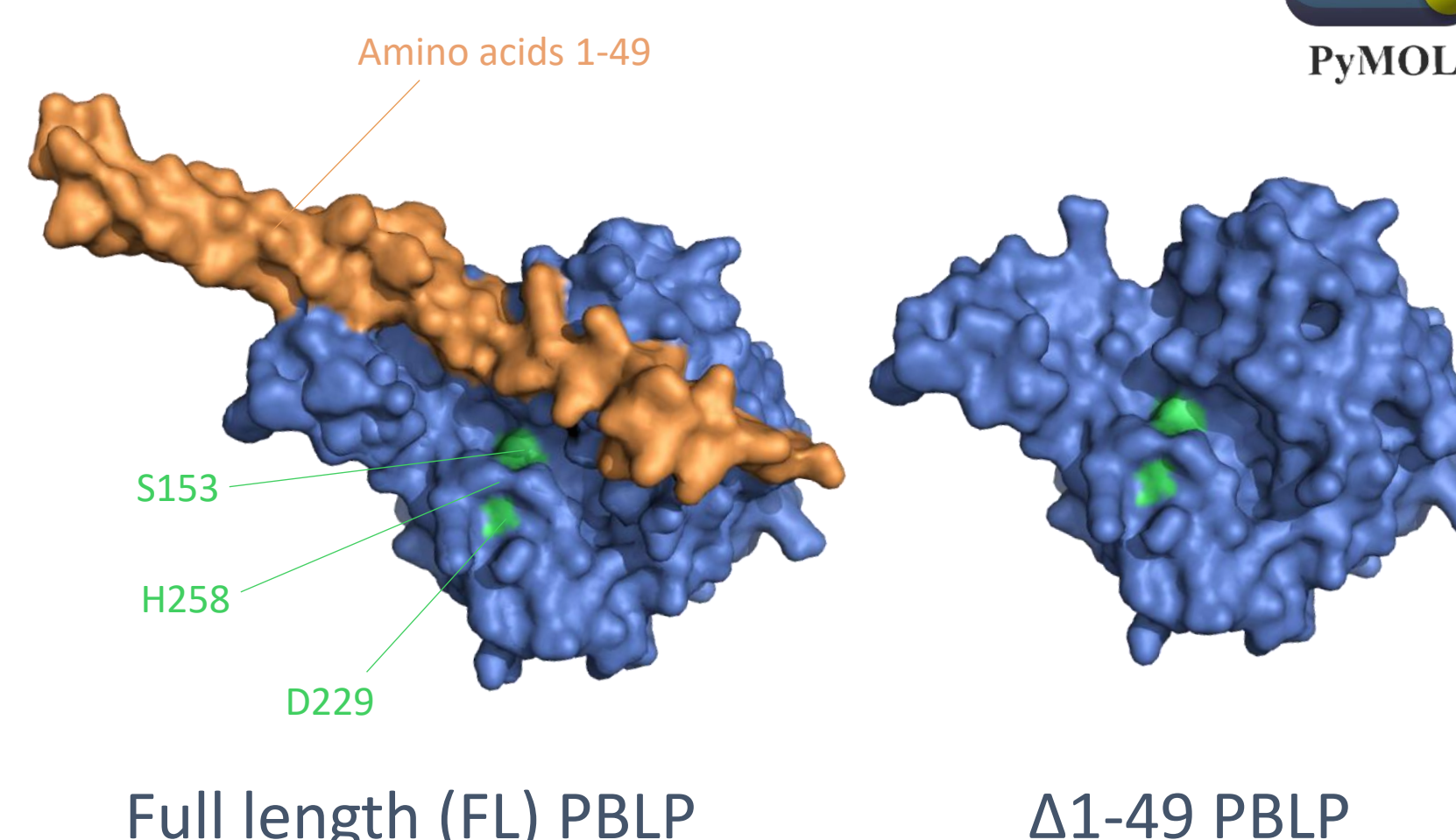
HYPOTHESIS

Earlier assay attempts to determine catalytic activity inconclusive, could not produce enough protein to respond to substrate panel.

SDS-PAGE GEL

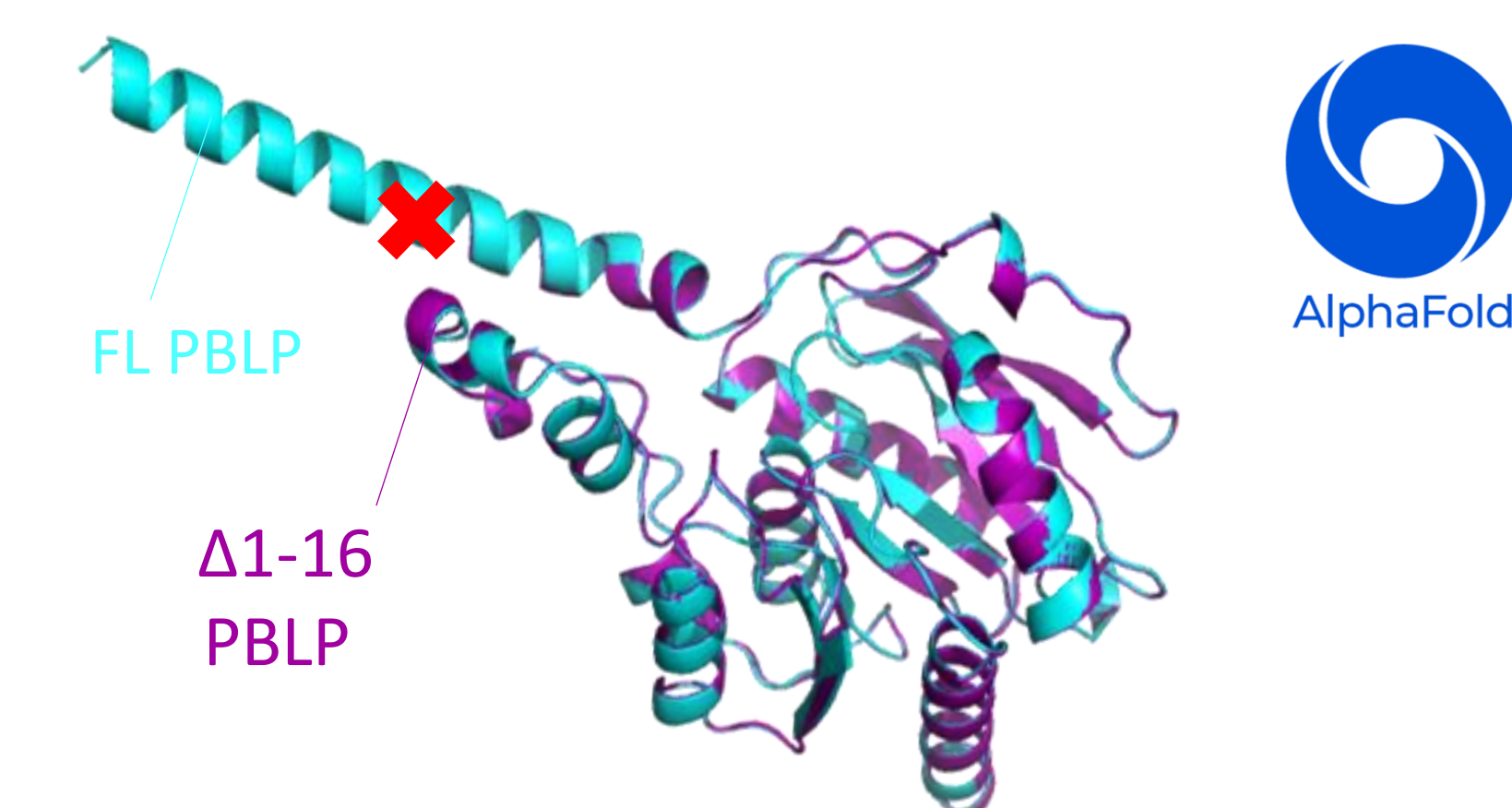


The 1-49 truncation results in loss of binding pocket, causing poor folding and inaccurate specificity.

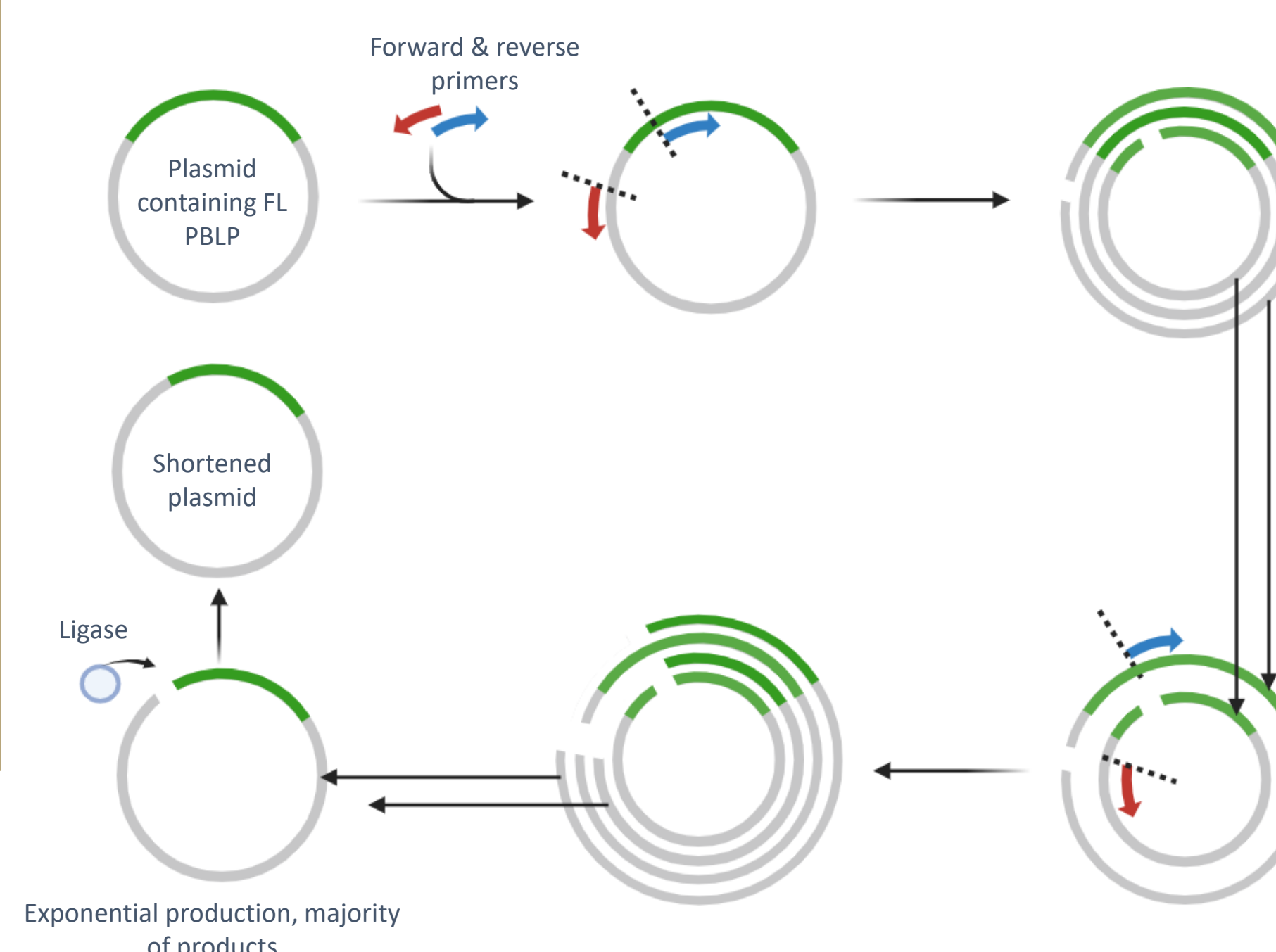


RESULTS

- Reduce the truncation.
- Out of 18 possibilities ran.
 - Removing 16 amino acids produced ribbon diagram most similar to FL.

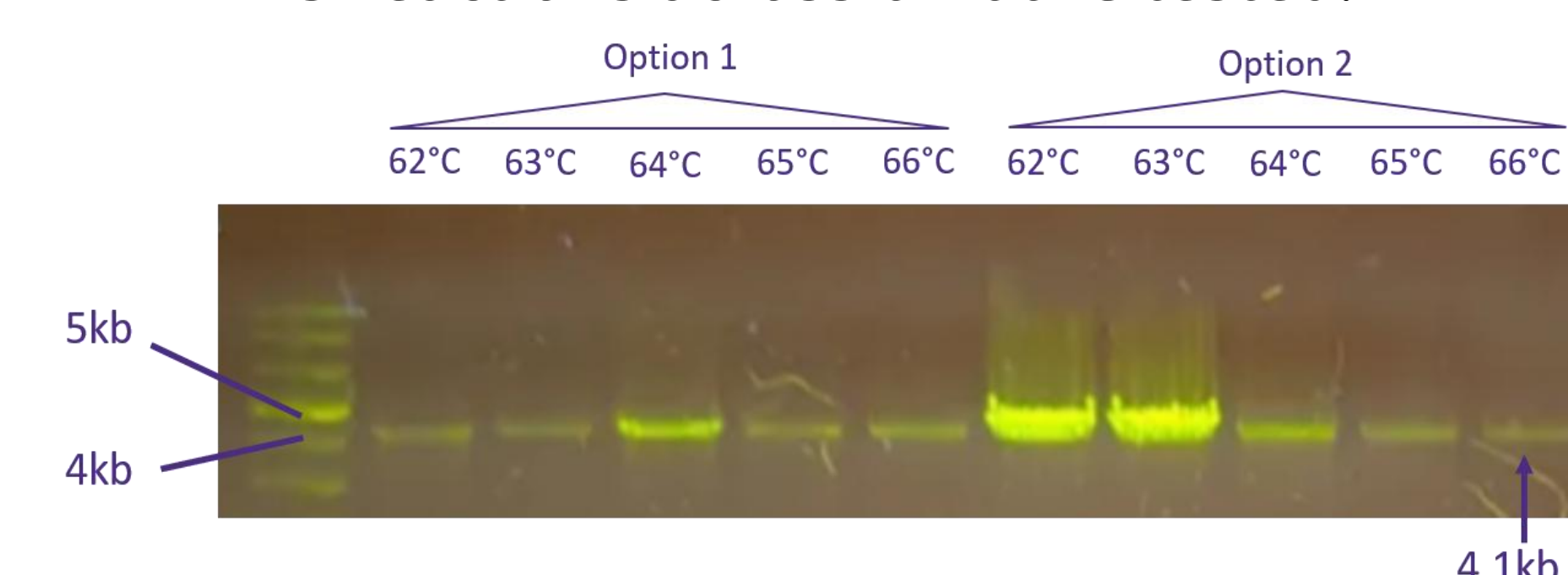


MUTAGENESIS PCR



1% AGAROSE GEL

- 2 different reverse primers explored.
- x5 reactions across 62-66°C tested.



SANGER SEQUENCING

Expected amino acids were removed from plasmid ORF for both reverse primer constructs.

Option 1

Score	Expect	Method	Identities	Positives	Gaps
548 bits(1411)	0.0	Compositional matrix adjust.	272/298(91%)	273/298(91%)	25/298(8%)
Query 1	MKKTATAIAVALAGFATVAQA	-----	-----	SLVFINTYIYFAQD	35
	MKKTATAIAVALAGFATVAQA			SLVFINTYIYFAQD	
Sbjct 1	MKKTATAIAVALAGFATVAQAQDGHGPEFELMLKVIISIIIIASLVFINTYIYFAQD			SLVFINTYIYFAQD	60

Option 2

Score	Expect	Method	Identities	Positives	Gaps
533 bits(1372)	0.0	Compositional matrix adjust.	263/287(92%)	264/287(91%)	22/287(7%)
Query 1	MKKTATAIAVALAGFATVAQAQDGH	-----	-----	SLVFINTYIYFAQD	38
	MKKTATAIAVALAGFATVAQAQDGH			SLVFINTYIYFAQD	
Sbjct 1	MKKTATAIAVALAGFATVAQAQDGHGPEFELMLKVIISIIIIASLVFINTYIYFAQD			SLVFINTYIYFAQD	60

FUTURE DIRECTIONS

- Induce BL-21 *E. coli* with mutated plasmids.
- Optimize purification protocol.
- Run activity assays on truncated products.

ACKNOWLEDGEMENTS

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Lab Mates: Gio, José
Discovered PBLP: Dr. Groat Carmona