Experimental Log (29 June – 28 August 2020)

Week 1 : 29/6 – 3/7

Date	Milestone	Remarks
29 June	Introduction	by instructors
	Finalized project directions	team's decision
30 June	Literature review on bacterial degradation of plastics	
1 July		
2 July	Decision of the types of plastic polymers to work on	Final decision : PET and PUR
	Selected suitable plastic-degrading enzymes from natural sources	PETase, Papain, PueA
3 July	Obtained full sequences of enzymes	Sequence verified by alignment tools for uniqueness

Week 2 : 6/7 – 10/7

Date	Milestone	Remarks
6 July	Searched for 3D structures of target enzymes in Protein Data Bank	pdb file of PETase and papain are obtained
7 July	Constructed the structures of polymers	
	PET and PUR by Chewdraw, from monomer to hexamer	

Week 3 : 13/7 - 17/7

Date	PulA	PueA	PudA	Remarks
13 July	Structure homology modelling by MODELLER			
14 July	Structure energy minimization via YASARA			High energy in PulA/PudA conformation
15 July	Enzyme structure verified by PROCHECK			*Only PueA was selected
				Simulations of PulA / PudA conformation are highly unstable

Week 4 : 20/7 - 24/7

Date	On-screen Tool				
	Autodock Vina	**DockThor**	**EDock**	**SwissDock**	**PatchDock**
3 August	Performed molecular docking between PET and PETase	Performed molecular docking between PET and PETase	Performed molecular docking between PET and PETase	Performed molecular docking between PET and PETase	Performed molecular docking between PET and PETase
4 August	Obtained LIGPLOTdiagram	Obtained LIGPLOTdiagram	Obtained LIGPLOTdiagram	Obtained LIGPLOTdiagram	Obtained LIGPLOTdiagram
5 August	Identified binding residues	Identified binding residues	Identified binding residues	Identified binding residues	Identified binding residues
6 August	PETase active site residue of Joo et al. (2008) positioned				
7 August	Result matched with Joo et al. (2008). Autodock Vina is selected as the project docking server				

Week 5 : 27/7 - 31/7

Date	Milestone	Remarks
27-28 July	Determined the binding affinity between PET-PETase (Monomer-Pentamer)	Stable binding (ΔG<-7): Tetramer
29 July	Determined the binding affinity between PUR-papain (Monomer-Pentamer)	Stable binding (ΔG<-7): Tetramer
30 July	Determined the binding affinity between PUR-papain (Monomer-Pentamer)	Stable binding (ΔG<-7): Trimer & Tetramer
31 July	Binding complex stability determination	
	The structures of PET (tetramer)-PETase, PUR (tetramer)-papain and PUR (tetramer)-pueA complexes were stabilized and ready for mutagenesis	

Week 6 : 3/8 - 7/8

Date	Milestone
3-5 August	The amino acid residues of PUR (tetramer)-papain and PUR (tetramer)-pueA were identified
	Conserved regions were recognised
6-7 August	Polarity of the amino acid residues was identified and calculated, with polar amino residues listed out

Week 7 : 10/8 - 15/8

Date	Milestone
10 August	Mutagenesis induction through PyMOL and structure regenerated
11-13 August	Mutant enzymes generated
	Complex structures validated for stability check
14-15 August	PUR docking with mutated enzymes

Week 8 : 17/8 - 21/8

Date	Milestone	Remarks
17 August	Mutant complex-PUR affinity verification	Papain G23W and PueA R392F gained enhanced affinity with PUR
18 August	Papain G23W and PueA R239F amino acid sequence obtained and back translated to nucleotide sequence	
19-21 August	Biobricks construction designed	Components : Promoter, PETase, Papain G23W, pueA R392F, linkers, RBS

Week 9 : 24/8 - 28/8

Date	Milestone	Remarks
24 August	T7 mutant, RBS and linker sequence obtained	
25 August	DNA constructed	Biobricks sequence : Promoter- > RBS- > Papain- > linker- > pueA- > linker- > PETase
26 August	Biobrick sequence checked	