Development of a One-pot Reaction for Drop-in Biofuel Production Using Solvent-free Liquid enzymes

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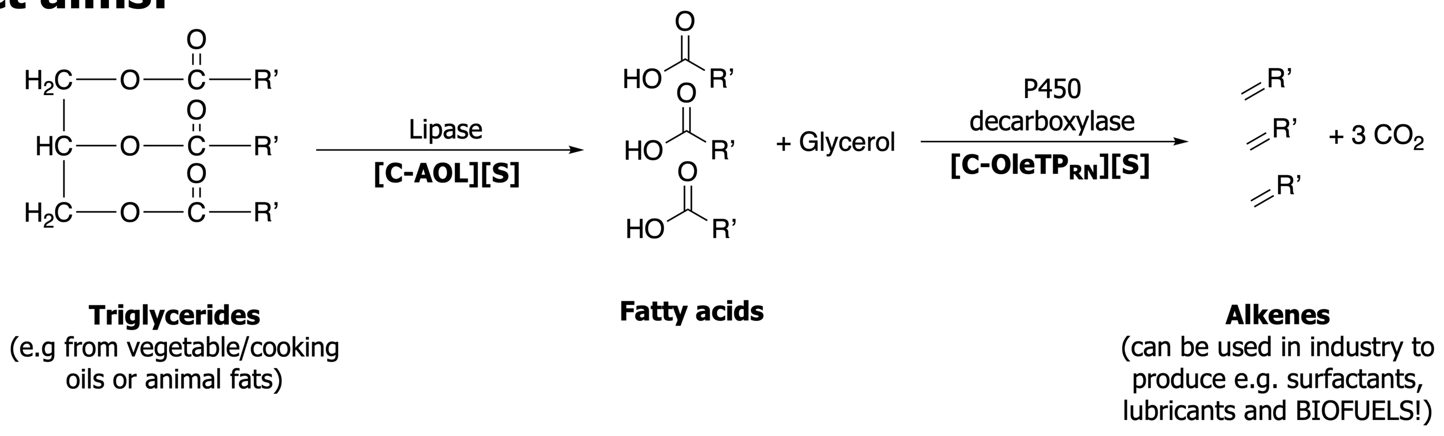
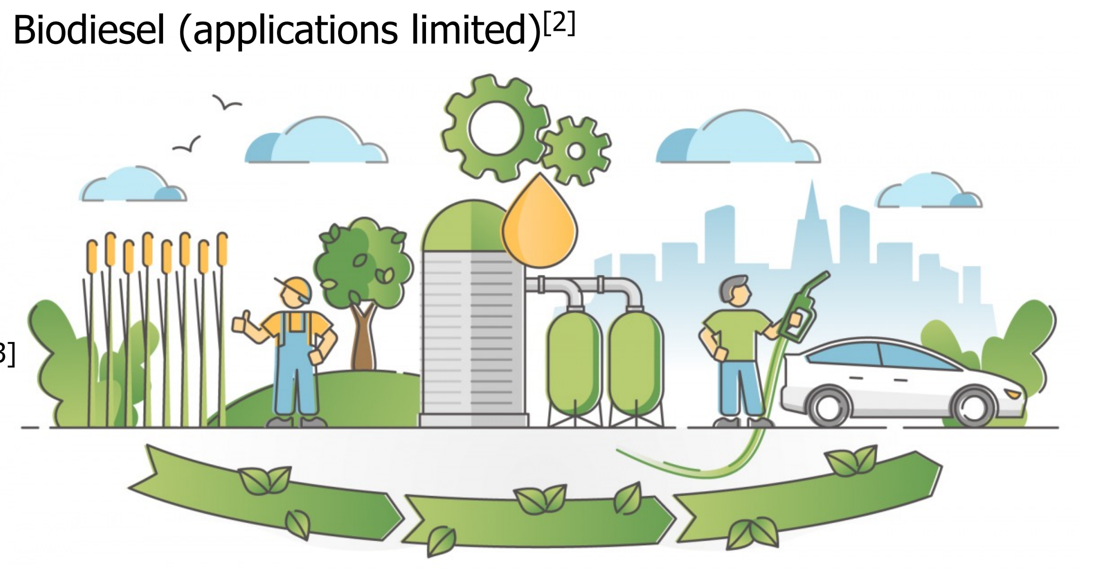
Project aims:



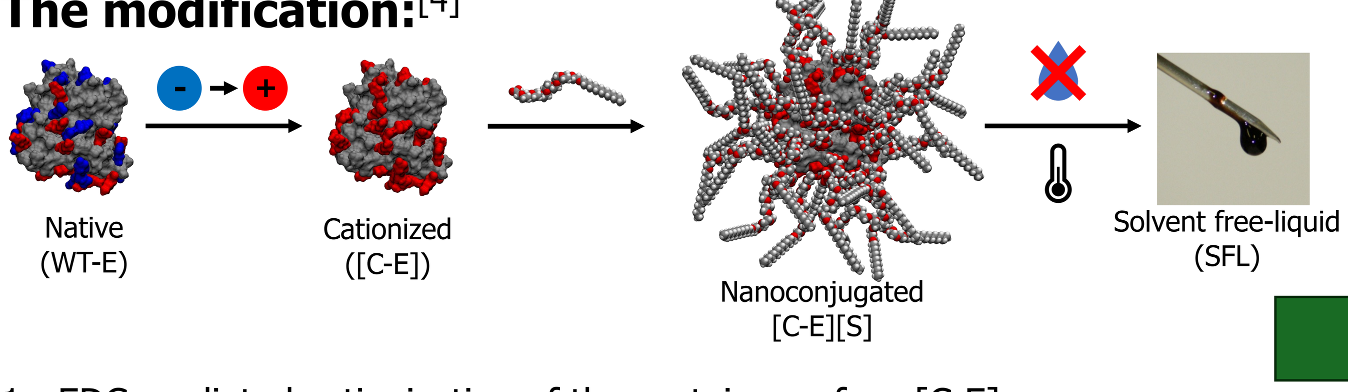
1. Modify enzymes relevant to the conversion of triglycerides to alkenes
2. Test effects of modification on enzyme properties and their solubility in organic solvents (e.g. ionic liquids, deep eutectic solvents)
3. Couple enzymes to produce an efficient and scalable one pot reaction with potential for further downstream applications.

Biofuels:

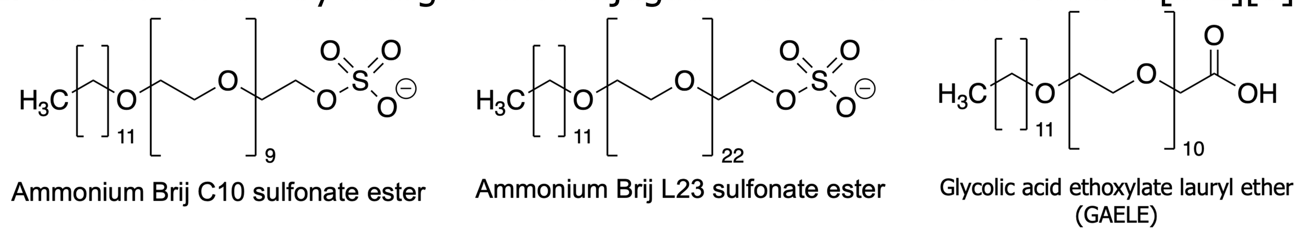
- Renewable energy source[1]
- Most common = Bioethanol and Biodiesel (applications limited)[2]
- Drop in biofuels:[3]
  - Structurally similar to fossil fuels
  - Compatible with existing infrastructure
- Can be produced using various methods e.g. **BIOCATALYSIS**[3]



The modification:[4]



1. EDC mediated cationization of the proteins surface [C-E]
2. Stoichiometric by charge nanoconjugation of surfactant molecules [C-E][S]



3. Lyophilization and thermal annealing of the sample

Produce a **solventless nanoconstruct, increasing the compatibility of biocatalysis with industrial processes:**[4]

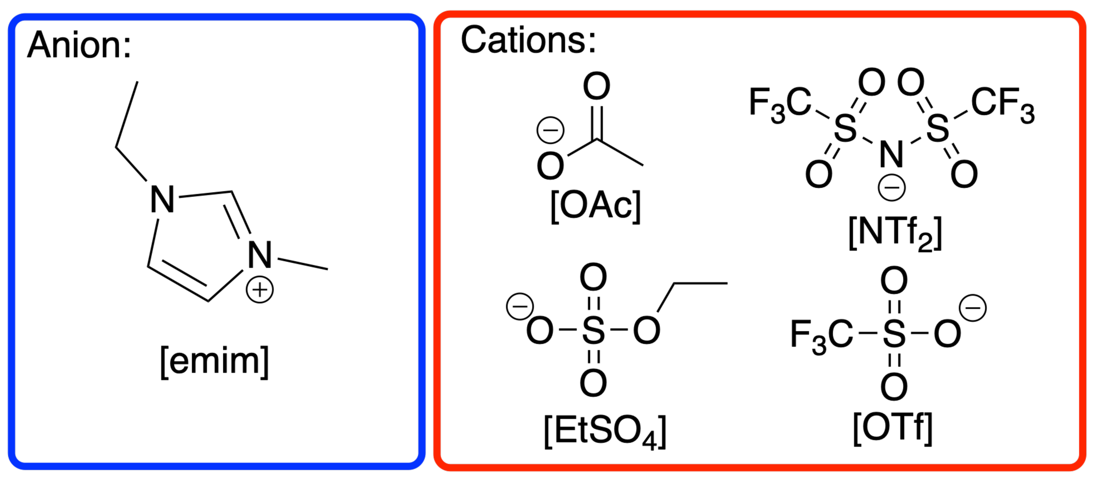
↑ Thermal stability of enzymes
↑ Solubility in organic solvents

+ Retain biological structure and activity

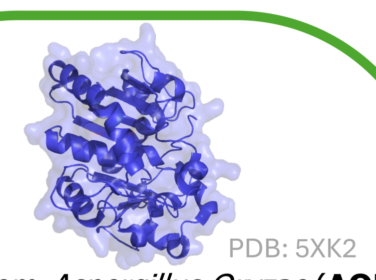
BUT: SFL too viscous → require a carrier solvent [5]

Ionic Liquids & Deep eutectic solvent:[6]

- Tunable properties
- Stable & non-flammable
- Good for biocatalysis



Lipase Modification:



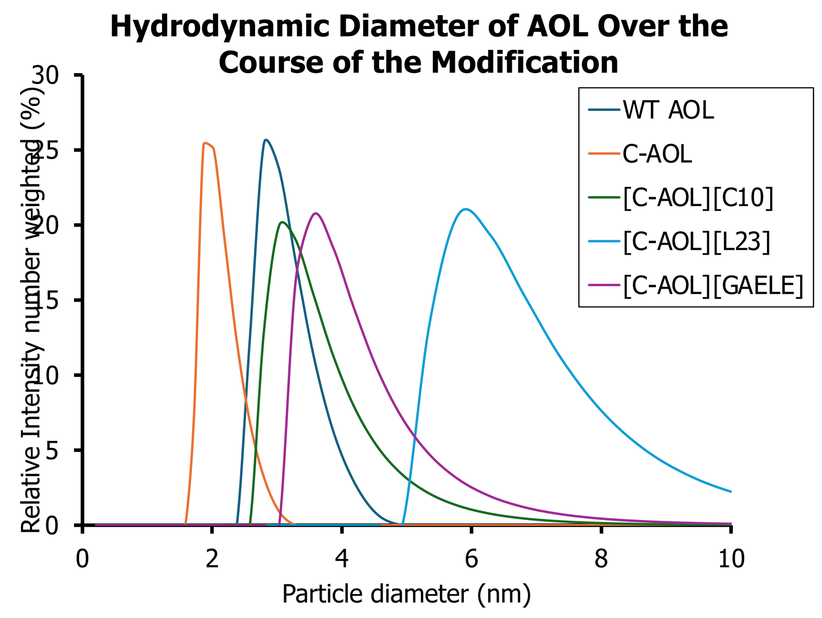
Lipase from *Aspergillus Oryzae* (**AOL**).
- Purchased (Sigma Aldrich) and purified.
- Contains 6 central β-sheet surrounded by 5 long and 6 short α-helices [7]
- Contains characteristic **lipase catalytic triad** (S, D, H) [7]

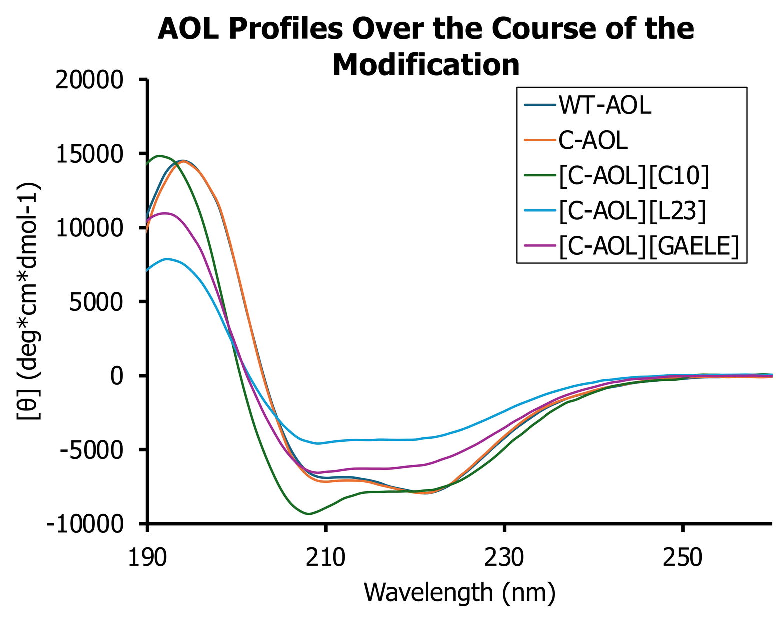
→ Effects of the modification on enzyme structure:

- Secondary structure = retained over the course of the modification.
- Hydrodynamic diameter = increases accordingly, with the addition of larger surfactants.
- Increase in half denaturation temperature.

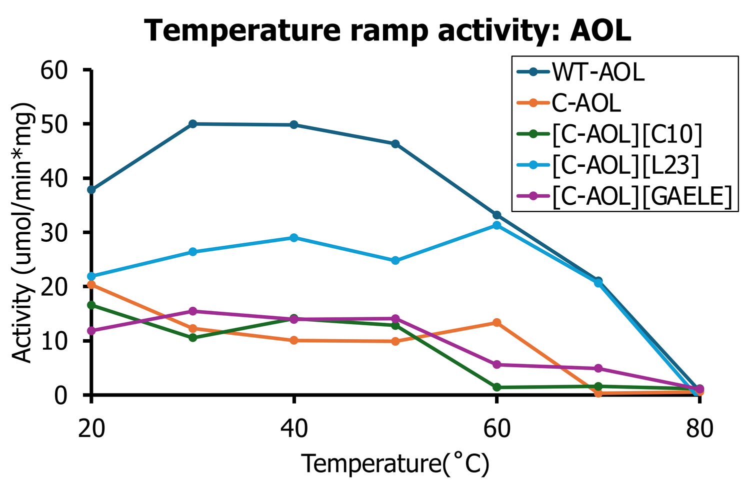
Secondary Structure of AOL Throughout the Modification (BestCell)

Secondary Structure(%)	WT AOL	C-AOL	[C-AOL] [C10]	[C-AOL] [L23]	[C-AOL] [GAELE]
α-Helix	15.2	18.0	20.2	7.6	12.9
β-Sheet	27.4	27.2	27.5	29.4	33.8
Turns	14.7	12.6	7.5	28.9	7.5
Unordered	42.7	42.2	44.8	34.0	45.8





→ Effects of the modification on enzyme temperature resistance:

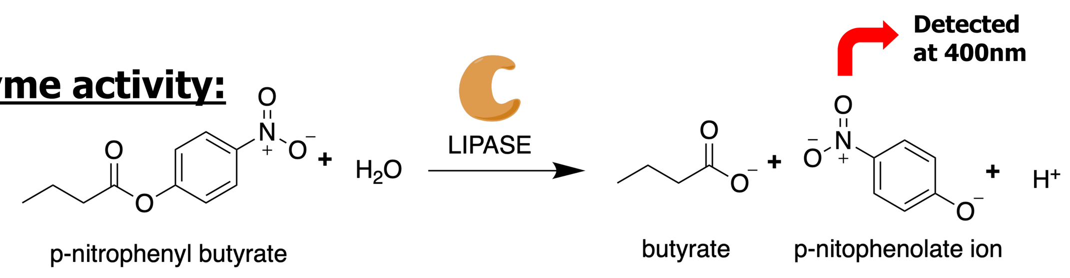


Temperature Ramp – Values Obtained from Data Analysis

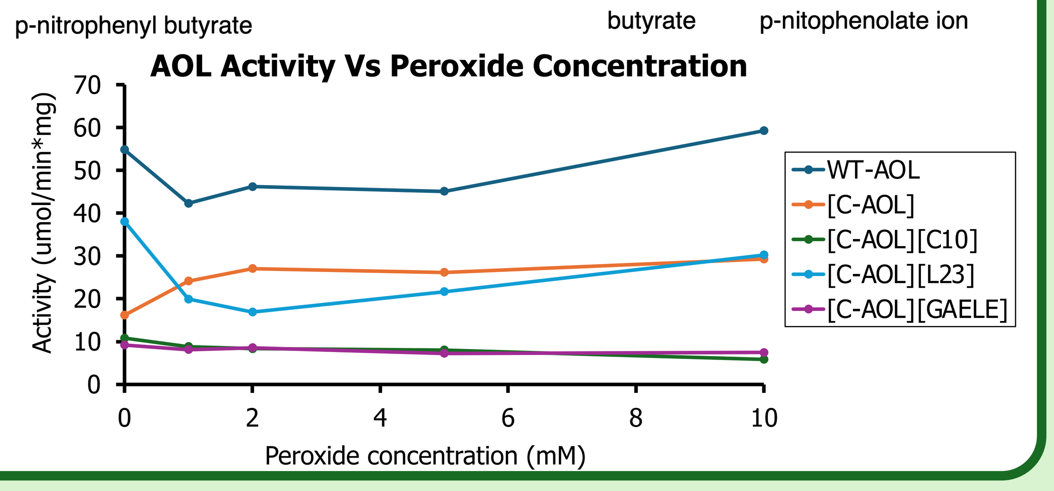
	WT AOL	C-AOL	[C-AOL] [C10]	[C-AOL] [L23]	[C-AOL] [GAELE]
T_m (°C)	70.98	78.60	Not Determinable (ND): Raw data suggests that enzyme has not fully denatured by 95°C		
ΔS_m (J K^{-1} mol^{-1})	1138.4	509.25			
ΔH_m (kJ mol^{-1})	80.81	40.03			

→ Effects of the modification on enzyme activity:

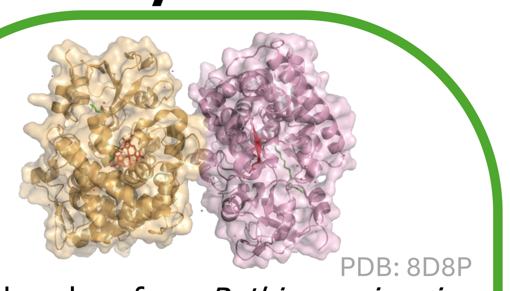
Observed using 4-NPB activity assay:
- Generally, highly active enzyme.
- Activity maintained throughout the modification.
- Enzymes resistance to peroxide concentration assessed.



Activity of AOL (umol*min^{-1}*mg^{-1})	
WT AOL	64.65
C-AOL	25.42
[C-AOL][C10]	18.56
[C-AOL][L23]	28.35
[C-AOL][GAELE]	11.85



Decarboxylase Modification:



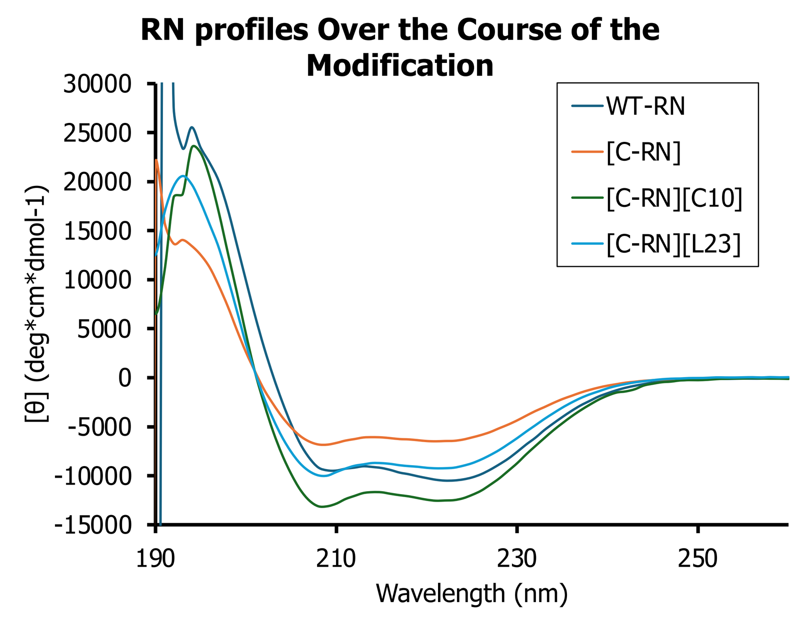
Decarboxylase from *Rothia nassimurium* (OleTP$_{RN}$ **/RN**).
- Provided by a collaborator in Universidade Estadual de Campinas.
- P450, CYP 152 **peroxygenase.** [8]
- Contains thiolate ligated **heme** iron complex. [8]

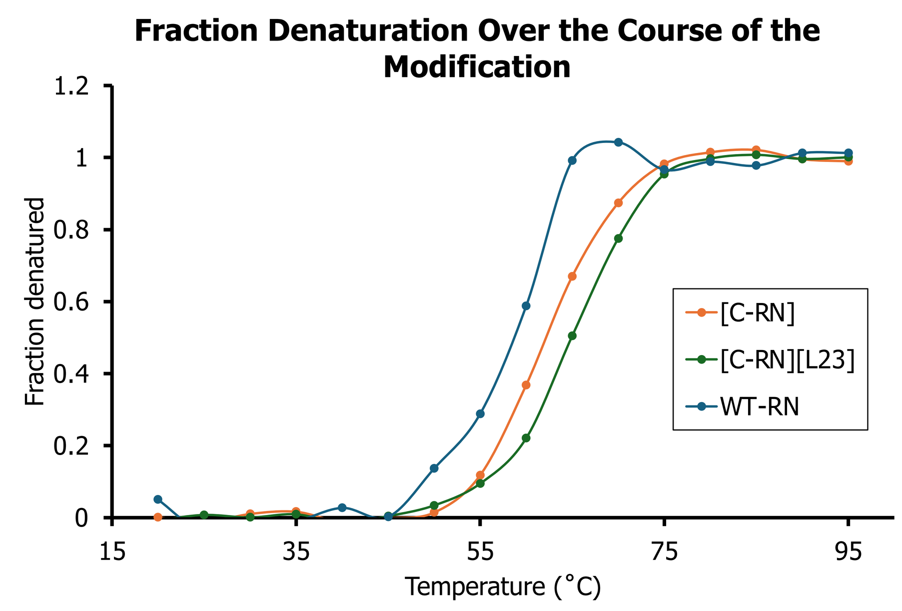
Effects of the modification on enzyme structure:

- Secondary structure = retained over the course of the modification.
- Hydrodynamic diameter = increases accordingly, with the addition of larger surfactants.
- Increase in half denaturation temperature inferred.

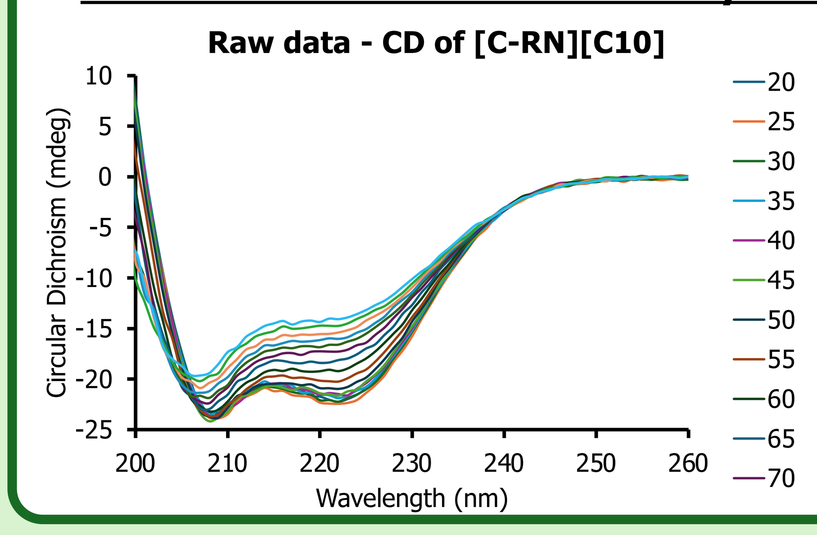
Secondary Structure of RN Throughout the Modification (BestCell)

Secondary Structure (%)	WT RN	C-RN	[C-RN] [C10]	[C-RN] [L23]
α-Helix	27.3	15.7	30.5	25.2
β-Sheet	20.7	25.2	13.8	19.6
Turns	12.2	14.1	12.3	13.4
Unordered	39.7	45.0	43.3	41.9





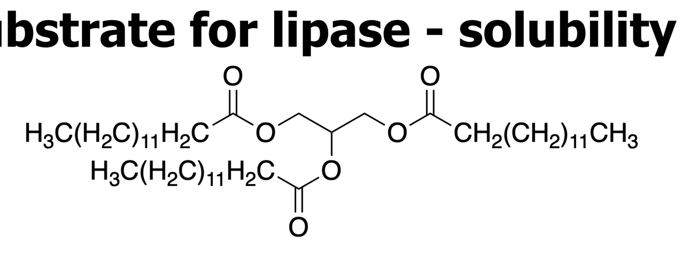
→ Effects of the modification on enzyme temperature resistance:



Temperature Ramp – Values Obtained from Data Analysis

	WT RN	C-RN	[C-RN] [C10]	[C-RN] [L23]
T_m (°C)	55.67	62.35	ND	63.08
ΔS_m (J K^{-1} mol^{-1})	956	865.4		810.31
ΔH_m (kJ mol^{-1})	53.22	53.96		51.11

Substrate for lipase - solubility assay:



Test solubility of substrate in ionic liquids:
- Assess feasibility of reaction in differed solvents.
- When heated to 60°C homogeneous mixture (up to 40mg/ml) achieved.
- At room temperature:

Maximum Concentration of Glyceryl Trimyristate in Ionic Liquids

	[emim][OAC]	[emim][EtSO$_4$]	[emim][OTf]	[emim][NTf$_2$]
Mass (mg) of trimyristate per mL of solvent	0.556	0.714	0.833	1.25

*Solubility increases with hydrophobicity

Conclusions:

- Successfully modified and characterized the lipase and decarboxylase, retaining biological structure and activity.
- Assessed the effects of the modification on the enzymes structure and activity.
- Assessed the solubility of the substrate in a range of ionic liquids and deep eutectic solvents.

Going Forward/ Future work in the field:

- Perform & optimize coupled reaction for the one-pot conversion of triglycerides to alkenes analyzing the data obtained using GC-MS and comparing against standards.
- Assess recyclability of enzymes and perform a Life Cycle Assessment to confirm the benefits of downstream applications
- Consider the modification and integration into the one pot reaction of the previously stipulated Oxidase – Decarboxylase chimera to avoid issues related to peroxide addition.[9]

References:

[1] N. S. Mat Aron *et al*, Sustainability of the four generations of biofuels – A review, *Int J Energy Res*, 2020, **44**, 9266–9282.
[2] J. Keasling *et al*, Microbial production of advanced biofuels, *Nat Rev Microbiol*, 2021, **19**, 701–715.
[3] P. Intasian *et al*, Biocatalysis, and Metabolic Engineering for Enabling a Circular Economy and Sustainability, *Chem Rev*, 2021, **121**, 10367–10451.
[4] A. P. S. Brogan, Preparation and application of solvent-free liquid proteins with enhanced thermal and anhydrous stabilities, New J. Chem, 2021, 45, 6577.
[5] A. P. S. Brogan and J. P. Hallett, Solubilizing and Stabilizing Proteins in Anhydrous Ionic Liquids through Formation of Protein–Polymer Surfactant Nanoconstructs, J Am Chem Soc, 2016, **138**, 4494–4501.
[6] A. Kumar *et al*, Lipase catalysis in organic solvents: advantages and applications, Biol Proced Online, 2016, **18**, 2.
[7] D. Lan *et al* , Structure-Guided Rational Design of a Mono- and Diacylglycerol Lipase from Aspergillus oryzae: A Single Residue Mutant Increases the Hydrolysis Ability, *J Agric Food Chem*, 2021, **69**, 5344–5352.
[8] L. L. Rade *et al*, Dimer-assisted mechanism of (un)saturated fatty acid decarboxylation for alkene production, Proceedings of the National Academy of Sciences, 2023, **120**, e2221483120.
[9] S. Matthews *et al*, Production of alkenes and novel secondary products by P450 OleTJE using novel H2O2-generating fusion protein systems, FEBS Lett, 2017, **591**, 737–750.
[Image] Queensland Government. What are biofuels? How we make and use biofuels in Queensland. [internet]. 2023 [cited 7/3/2024]. Available from: https://www.statedevelopment.qld.gov.au/news/what-are-biofuels-how-we-make-and-use-biofuels-in-queensland