IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

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| **Project Title:** | **Interaction of digestive enzymes to food macro-micro nutrients** | |
| **Project Number** | **IMURA1053** | |
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| **IITB Department**: | Department of Chemistry | |

**Research Clusters:** **Research Themes:**

| **Highlight which of the Academy's CLUSTERS this project will** | **Highlight which of the Academy's Theme(s) this project will address?** | |
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| address? (*Please nominate JUST **one.** For more information, see www.iitbmonash.org*) | | (*Feel free to nominate more than one. For more information, see www.iitbmonash.org*) | |
|---|---|---|---|
| 1 | *Material Science/Engineering (including Nano, Metallurgy)* | ***1*** | *Artificial Intelligence and Advanced Computational Modelling* |
| 2 | *Energy, Green Chem, Chemistry, Catalysis, Reaction Eng* | 2 | *Circular Economy* |
| 3 | *Math, CFD, Modelling, Manufacturing* | 3 | *Clean Energy* |
| 4 | *CSE, IT, Optimisation, Data,* | 4 | *Health Sciences* |
| 5 | *Sensors, Systems, Signal Processing, Control* | 5 | *Smart Materials* |
| 6 | *Earth Sciences and Civil Engineering (Geo, Water,* | 6 | *Sustainable Societies* |
| 7 | *Climate)* | 7 | *Infrastructure* |
| 8 | *Bio, Stem Cells, Bio Chem, Pharma, Food* | | |
| | *Semi-Conductors, Optics, Photonics, Networks, Telecomm, Power Eng* | | |
| | *HSS, Design, Management* | | |

## The research problem

Enzymes are indispensable bio-catalysts in a significant number of metabolic reactions in humans. In the context of food digestion, a suite of digestive enzymes (i.e., amylases, proteases, and lipases) act on carbohydrate, protein, and lipid macronutrients present within ingested food to convert them into low molecular weight absorbable compounds. The rate and extent of these conversions are increasingly seen as important aspects of human metabolic health and the nutritional value of foods and diets. It is also well-known that enzymes interact non-specifically to non-catalysed substrates.[1,2,3]. Based on binding energy arguments, it is typically assumed that interactions with non-catalysed substrates are minimal compared with those of specific substrates through the enzyme active site(s). In the last decade, considerable research effort has been directed at the structure of enzymes, mode of catalysis and factors affecting macronutrient catalysis (e.g., starch, protein, and lipids) by digestive enzymes (amylase, protease and lipase). Surprisingly, despite the fact of its importance, there has been effectively no systematic study on how digestive enzymes interact with non-catalysed substrates ( dietary fibre and phenolics). This shows that interactions in the digestive tract between key digestive enzymes and non-substrates could be a new paradigm for understanding how to control the rate and extent of food digestion. This presents opportunities to address current non-communicable diseases (e.g., obesity, metabolic syndrome, diabetes, etc.) as well as a lack of understanding in the food processing sector in tailoring food products that generate desirable nutrient digestion profiles and are also improving human health.

[1]Dunaif, G., & Schneeman, B. O. (1981). The American journal of clinical nutrition, 34(6), 1034-1035.
[2] Dhital, S., Gidley, M.J. and Warren, F.J., 2015. Carbohydrate Polymers, 123, pp.305-312.
[3]Li, H., & Dhital, S. (2022). Bioactive Carbohydrates and Dietary Fibre, 28, 100319.

## Project aims

1. **Elucidation of the kinetics of association and dissociation of enzymes with fibres and phenolics**. The kinetics of binding of enzymes on dietary fibre will be studied by a solution depletion method[2] where the activity of unbound enzymes from the solution is measured using specific assays for each enzyme (i.e., amylase, lipase and protease). By varying the inhibitor concentration (i.e., dietary fibre) and controlling reaction time, a range of parameters will be determined, including binding capacity, equilibrium binding extent, and binding selectivity.
2. **Defining the mode of interaction of enzymes with dietary fibre and phenolics**. The quantitative description of the forces that govern molecular associations requires the determination of changes in thermodynamic parameters, including free energy of binding, enthalpy and entropy of binding, and heat capacity changes. The thermal stability of the bound enzyme will be investigated using Nano-Differential Scanning Calorimetry (Nano-DSC). Isothermal Titration Microcalorimetry (ITC) will be used to understand thermodynamic parameters for interactions between enzymes and dietary fibres. Structural changes in enzymes after binding with non-catalytic substrates will be studied using Fluorescence Quenching (FQ) and Circular Dichroism (CD) Spectroscopy. We will also focus on developing a new method to study the binding of enzymes on dietary fibres and the interaction of digestive enzymes with phenolics by using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) analysis.
3. **Investigate the effect of the interaction of enzyme-fibre and enzyme-phenolics on in vitro hydrolysis of starch, protein and lipids.** The effect will be monitored in the different food systems, and design rules for lowering the metabolic response from the ingested foods will be achieved.
4. ***In silico* simulations of enzyme- fibre/phenolics interactions.** Computational studies e.g. molecular docking, will be carried out to identify the thermodynamic characteristics of enzyme – non-catalysed substrate interactions, as well as determine the position and conformation of the ligand on the enzyme of interest. Furthermore, the binding affinity between enzymes and substrates can be assessed, in the presence of bound non-catalysed substrates, to determine any retardation of enzymatic activity for comparison with experimentally determined results. Finally, possible binding locations determined via docking studies will give some indication of the binding stoichiometry of enzyme–non-catalysed substrate interactions, which can then be validated experimentally with the method of continuous variations.

## How skills/experience of the IITB and the Monash supervisor(s) support the proposed project

Monash supervisor Dr Sushil Dhital has wider expertise in the interaction of digestive enzymes with fibre ( soluble and insoluble) and phenolics, as well as the in-vitro hydrolysis of starch, protein, and lipids, which are relevant to objectives 1 and 3. On the other hand, IITB supervisor Prof Nand Kishore is renowned for his work in Chemical and Biological Thermodynamics and In silico simulations of enzyme- fibre/phenolics interactions. Thus the overall studies require the complementary expertise of both supervisors, Dr Sushil Dhital and Prof Nand Kishore.

## What is expected of the student when at IITB and when at Monash?

At IITB, the student will work with Prof Nand Kishore to achieve objectives 2 and 4. At Monash,

the student will work on objectives 1 and 3.

### Expected outcomes

Using the complementary expertise of Dr Sushil Dhital and Prof Nand Kishore, the project, for the first time, will investigate the kinetics, mode of interaction and thermodynamics of association and dissociation of enzymes with fibres and phenolics, followed by the advanced computer simulation to model the interaction.

### How will the project address the Goals of the above Themes?

The outputs of the project will be the first of their kind for the mechanistic understanding of the relations between dietary fibre/phenolics structures and their interactions with enzymes in relation to catalytic conversion of substrate to products. The techniques and knowledge developed in this project will be important to understanding the human and industrial processes that use enzymic catalysis. The data obtained will also allow the design of food formulations, which attenuate the rate of digestion, particularly of starch and lipids, linked to nutritional and health outcomes through a reduction in risks of non-communicable diseases. The project will form the basis for processors to engineer high-value foods that improve human health through digestion control, thus giving a slow and steady release of nutrients that aligns with Research Clusters (6)- Bio-Chemistry and Food; and the Research Theme (4) – health sciences

### Potential RPCs from IITB and Monash

Dr Lavaraj Devkota will be involved in the project at Monash. Lavaraj has wider expertise in phenolics

## Capabilities and Degrees Required

Good understanding on enzymes
Knowldge in dietary fibre
Hands on experience in instrumental techniques
Experience in molecular docking is plus