Supplementary

Mathematical Modelling

Theoretical Framework of Binding and Dissociation:

The simplest receptor–ligand interaction can be represented as:

$$P + R \Leftrightarrow PR$$

where R is the free receptor, L the ligand, RL the receptor–ligand complex, k_{on} the association rate constant, and k_{off} the dissociation rate constant.

The differential equations can describe the rate of formation and breakdown of complexes:

$$\frac{d[RL]}{dt} = k_{on} *[R] * [L] - k_{off} * [RL]$$

$$\frac{d[R]}{dt} = -k_{on} *[R] * [L] + k_{off} * [RL]$$

$$\frac{d[L]}{dt} = -k_{on} *[R] * [L] + k_{off} * [RL]$$

At equilibrium, the forward and reverse rates are equal, giving rise to the dissociation constant:

$$k_D = \frac{k_{on}}{k_{off}}$$

where a lower k_D value indicates stronger binding affinity.

The half-life of a receptor–ligand complex, representing the time required for half of the complexes to dissociate, can be calculated as:

$$t_{\frac{1}{2}} = \frac{lnln\,(2)}{k_{off}}$$

This parameter provides valuable insights into the stability of receptor–ligand interactions and is widely used in drug design and molecular recognition studies.

Diffusion:

Molecules in solutions move randomly and spread out over time.

This process follows Fick's law of diffusion, which we implemented numerically using finite-difference methods.

We modelled peptide motion using Fick's First Law:

$$J = D\frac{\partial C}{\partial x}$$

which relates flux J to the concentration gradient $\frac{\partial C}{\partial x}$ with the diffusion coefficient D.

The time evolution of concentration is described by Fick's Second Law.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

These equations are derived from first principles and are widely used to describe how small molecules spread in liquids, gels, and tissues.

Calculation of Diffusion Coefficient (D) and Damköhler Number (Da):

Diffusion coefficient D(Stokes–Einstein equation):

$$D = \frac{k_B T}{6\pi n r_h}$$

Where,

$$k_{B\,=1.380649\times10^{-23}JK^{-1}}$$
$$T = 298K$$
$$n = 0.89 \times 10^{-3} Pas$$
$$r_{h=0.68\ nm}$$
$$D = \frac{(1.380649 \times 10^{-23}(298)}{6\pi(0.89 \times 10^{-3})(0.68 \times 10^{-9})}$$

$$= \frac{4.11 \times 10^{-21} J}{1.14 \times 10^{-11}}$$
$$D = 3.61 \times 10^{-10}\frac{m^2}{s}$$
$$D = 361\mu\frac{m^2}{s}$$

Timescales :

Diffusion timescale:

$$\tau_{diff} = \frac{L^2}{D}$$
$$= \frac{(1.0 \times 10^{-5})^2}{3.61 \times 10^{-10}}$$
$$= 0.28\ s$$

Reaction timescale:

$$\tau_{react=\frac{1}{k_{on}R_o}}$$
$$= \frac{1}{1.35}$$

$$0.74\ s$$

Damköhler number:

$$D_{a=\frac{\tau_{diff}}{\tau_{react}}}$$

$$= \frac{0.28}{0.74}$$

$$= 0.37$$

Since D_a<1, the system is in a reaction-limited regim. Peptides diffuse across the domain in ~0.28 s, while binding occurs on a slower ~0.74 s timescale.

An ordinary differential equation (ODE) model was constructed describing the reversible binding of ligand (L) and receptor (R):

$$d[RL]/dt \ = \ k_{off} \ *[R] * [L] - \ k_{off} \ * [RL]$$

where k_on ($M^{-1}s^{-1}$) is the association rate constant, k_off(s^{-1}) is the dissociation rate constant, and $[RL]$ is the ligand–receptor complex concentration. The dissociation constant k_d was calculated from the Gibbs free energy change:

$$k_d = \exp\ (\Delta G/((R * T)\ )\ )$$

$$k_{off} = k_{on} \ * k_d$$

CALCULATIONS:

$$\Delta G = RTlnk_d$$

$$k_{d= e^{\frac{\Delta G}{(RT)}}}$$

Given:

$$\Delta G = \ -7.77 \frac{kcal}{mol}$$

$$T = 298\ K$$

$$R = 0.0019872041\ \frac{kcal}{mol}$$

$$RT = 0.0019872041 \times 298$$

$$= 0.5921868218 \frac{kcal}{mol}$$

$$\frac{\Delta G}{RT} = \frac{-7.77}{0.5921868218}$$

$$= 13.12085935$$

$$k_{d} = e^{-13.12085935}$$

$$k_{d} \approx 2.0030103 \times 10^{-6}\ M$$

$$k_{off} = o.67669 s^{-1}$$

$$k_{on} = \frac{k_{off}}{k_d}$$

$$= \frac{0.67669}{2.0030103 \times 10^{-6} M}$$

$$\approx 337812.8$$

$$k_{on} \approx 3.38 \times 10^{5}\ M^{-1} s^{-1}$$

$$t_{\frac{1}{2}} = \frac{ln2}{k_{off}}$$

$$= \frac{0.69314718056}{0.67669}$$

$$t_{\frac{1}{2}} = 1.02\ s$$

Sustainability

SDG 8 – Decent Work and Economic Growth

Excessive dietary salt intake is a major contributor to hypertension and cardiovascular diseases (CVDs), which impose a significant economic burden through rising healthcare costs, productivity loss, and premature mortality, particularly in India, where average salt consumption far exceeds WHO recommendations. These health impacts reduce workforce efficiency and result in long-term income losses and GDP decline. SaltEnPep addresses this challenge by producing saltiness-enhancing peptides using synthetic biology, allowing reduced sodium intake without compromising taste. By preventing salt-induced CVDs at the source, SaltEnPep supports a healthier workforce, lowers healthcare expenditure, and contributes to sustained economic productivity while also promoting innovation-driven employment in biotechnology and bioengineering sectors.

SDG 3 – Good Health and Well-Being

High salt exposure affects health both directly through excessive consumption and indirectly through occupational exposure during salt mining and processing, leading to hypertension, dehydration,

respiratory issues, and long-term systemic disorders, while complete salt restriction risks hyponatremia. SaltEnPep provides a balanced, preventive solution by enabling optimal sodium intake with enhanced salt perception, thereby reducing risks associated with overconsumption without compromising physiological sodium needs. Additionally, by reducing dependence on large-scale salt mining and processing, SaltEnPep minimises occupational exposure and associated health risks, contributing to the reduction of non-communicable diseases and environmentally induced illnesses in line with targets 3.4 and 3.9 also.

SDG 11 – Sustainable Cities and Communities

Conventional biochemical purification and salt production processes generate chemical waste, contribute to environmental toxicity, and strain municipal waste management systems. SaltEnPep adopts a sustainable purification strategy using chitin affinity chromatography, an eco-friendly, biodegradable material derived from seafood industry waste, eliminating the need for toxic chemicals. The reuse of chitin beads and reliance on food-grade, non-hazardous materials significantly reduce laboratory waste generation. This circular-economy approach transforms biological waste into a valuable resource, minimises urban environmental impact, and aligns with sustainable waste management practices essential for resilient cities and communities.

SDG 12 – Responsible Consumption and Production

Salt extraction and mining are resource-intensive processes that contribute to rising material and carbon footprints through energy consumption, chemical usage, and environmental degradation. SaltEnPep promotes responsible production by reducing reliance on traditional salt manufacturing while introducing a low-waste, enzyme-based peptide production system rooted in synthetic biology. Complementing this technological approach, SaltEnPep also emphasises public awareness through educational outreach, enabling informed dietary choices and encouraging sustainable consumption behaviours. By combining innovation with community engagement, the project addresses both material footprint reduction and responsible lifestyle adoption.

SDG 17 – Partnerships for the Goals

Achieving sustainable health and environmental outcomes requires collaboration across disciplines and sectors, yet gaps often exist between academia, industry, healthcare, and policy frameworks. SaltEnPep actively bridges this gap by engaging with health professionals, biotechnologists, educators, and food-sector experts through Integrated Human Practices (IHPs) and outreach initiatives. These partnerships facilitated knowledge exchange, ethical evaluation, and design refinement, ensuring that the solution is scientifically robust, socially acceptable, and scalable. By fostering multi-stakeholder collaboration, SaltEnPep strengthens collective action toward sustainable development and responsible innovation.

Entrepreneurship

At the core of the venture is a proprietary platform of saltiness-enhancing peptides developed through synthetic biology. These short amino acid sequences, identified from natural proteins such as soybean

and egg albumin, are engineered to interact with human salt taste receptors, increasing the perception of saltiness even at reduced sodium concentrations. Produced using engineered microbial fermentation systems, the peptides can be purified efficiently and formulated as food-compatible powders or sprays that integrate seamlessly into existing manufacturing lines. Because this solution works within current recipes and processing infrastructure, SaltEnPep avoids costly reformulations for manufacturers, a critical adoption factor. This forms the foundation of the company's value proposition: enabling sodium reduction without sensory compromise, operational disruption, or negative label perception. For food companies, the "job to be done" is regulatory compliance and healthier positioning while retaining consumer loyalty; SaltEnPep removes the pain of taste loss and reformulation risk while delivering the gain of product differentiation and health alignment.

The venture operates on a B2B ingredient supply and licensing model, embedding itself within food manufacturing value chains rather than competing at the consumer brand level. Revenue is generated through bulk ingredient sales, long-term supply agreements, licensing of peptide technology to flavour houses, and co-branding collaborations that allow manufacturers to market reduced-sodium products supported by SaltEnPep technology. Once integrated into a product formulation, switching costs for customers become high, strengthening retention and recurring demand. Economies of scale in microbial fermentation further improve margins over time. Financially, early development is supported through research grants, biotech incubators, and seed investment, transitioning toward strategic partnerships with large food companies for pilot adoption, and later scaling through revenue-backed growth and potential Series A/B funding. Over a ten-year horizon, the plan progresses from R&D validation and regulatory approval to regional manufacturing partnerships, international market entry, platform expansion into other taste-modulating peptides, and eventual positioning as either a major acquisition target for a global ingredient corporation or a licensing-driven profitability model.

Market analysis indicates strong growth in sodium-reduction technologies, driven by regulatory frameworks, increasing processed food consumption, and rising non-communicable disease awareness. The initial geographic focus is India and the Asia-Pacific region, where sodium intake levels, hypertension prevalence, and processed food market growth converge, creating both public health urgency and commercial demand. Expansion into North America and Europe follows, supported by mature regulatory systems and clean-label consumer preferences. Market demography spans large-scale food manufacturers, institutional food suppliers, and flavour companies serving mass-market packaged foods — sectors characterised by high production volumes and a strong need for scalable ingredient solutions.

The competitive landscape includes mineral salt substitutes, flavour masking agents, and emerging biotech taste-modulation approaches. SaltEnPep differentiates itself through minimal flavour alteration, clean-label alignment, and compatibility with existing production systems. Porter's Five Forces analysis suggests moderate supplier power due to reliance on biotech production inputs, but strong buyer retention once integration occurs; barriers to entry increase with proprietary peptide IP and regulatory approvals, reducing long-term competitive threats. Broader PESTLE factors favour growth: political and regulatory pressure to reduce sodium, economic expansion of processed food sectors, social health awareness, technological advances in fermentation, legal pathways for novel food ingredients, and environmental benefits from efficient microbial production compared to traditional chemical synthesis.

Risks include regulatory approval timelines, scale-up complexities, cost competitiveness, and industry adoption rates. These are mitigated through early regulatory engagement, use of well-characterised microbial hosts, scalable fermentation design, and pilot collaborations with manufacturers to demonstrate sensory equivalence. Intellectual property protection through patents covering peptide sequences, production methods, and applications strengthens defensibility, while trade secrets protect formulation

know-how. Stakeholders include food manufacturers, regulatory bodies, healthcare systems, investors, and consumers; alignment is achieved through shared benefits of health improvement, regulatory compliance, and economic opportunity.

Strategically, SaltEnPep leverages its strengths in scientific innovation, clean-label positioning, and scalable B2B integration while navigating weaknesses such as capital-intensive scale-up and early-stage awareness. Opportunities arise from expanding health regulations and functional ingredient markets, while threats include evolving competitor technologies and regulatory shifts. Ultimately, SaltEnPep transforms a global dietary health issue into a sustainable enterprise by aligning technological innovation, market demand, and regulatory momentum. Its long-term trajectory supports both measurable public health impact and strong commercial returns, making it a compelling venture in the future of preventive nutrition and food biotechnology.

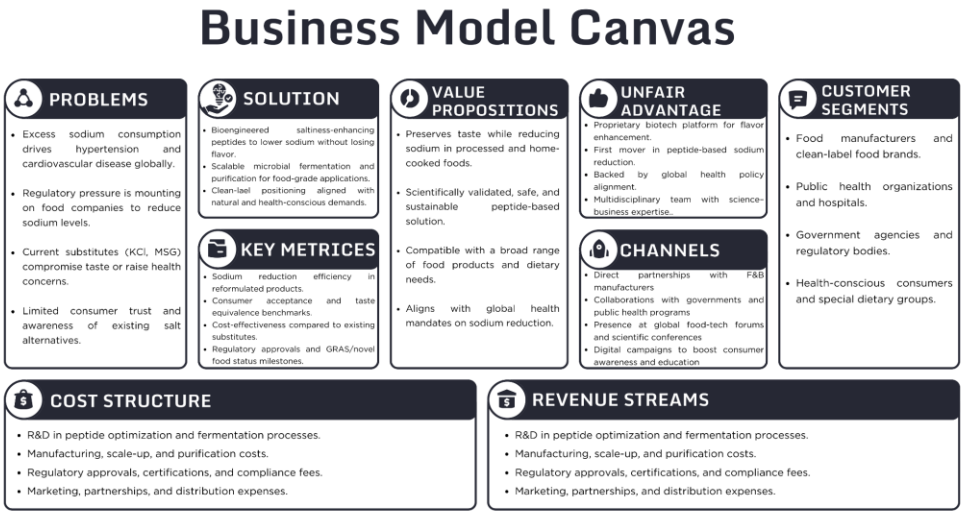

Fig 1: Business Model Canvas

Fig 2: PESTEL Analysis

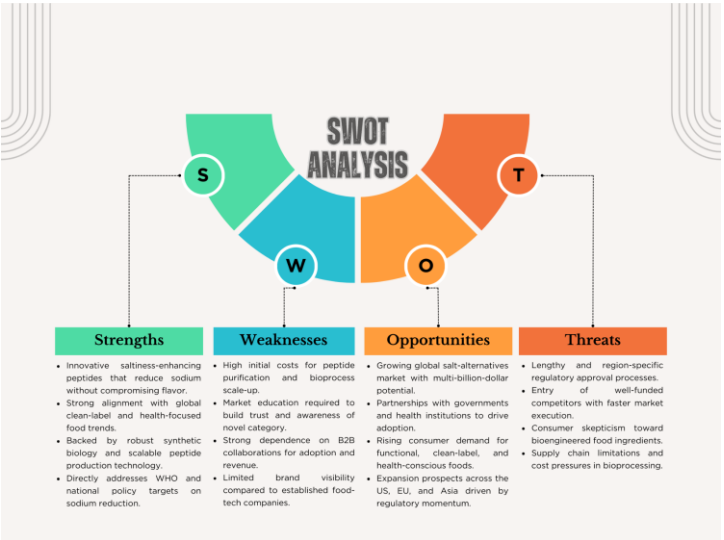

Fig 3: SWOT Analysis

Biosafety and Biosecurity

Our team at Rajalakshmi Engineering College upholds biosafety as a core pillar of responsible synthetic biology. Before beginning any experimental work, all members completed certified training in Biosafety and Microbiology in collaboration with Balaji Medical College. This program reinforced containment strategies, emergency response, aseptic techniques, and best laboratory practices, ensuring every procedure was guided by a strong safety mindset.

We conduct our work using Escherichia coli BL21 (DE3), a Risk Group 1 organism suitable for Biosafety Level 1 (BSL-1) conditions. Our laboratories are equipped with laminar airflow units, HEPA-filtered biosafety cabinets, and secure access systems that maintain controlled, sterile environments.

Standard use of Personal Protective Equipment (PPE), adherence to Good Microbiological Techniques (GMT), and rigorous sterilisation and waste management protocols ensure contamination-free and environmentally responsible research. All biological and chemical wastes are segregated, sterilised, and disposed of in accordance with national biomedical waste management guidelines.

Genetic constructs were designed with safety in mind, screened for allergenicity and toxicity, and restricted from secretion or environmental release. Our team also follows strict chemical safety measures and maintains updated infrastructure with emergency preparedness systems.

Collectively, these frameworks transform biosafety from a checklist into an integral research culture—ensuring every step of our work upholds ethical responsibility, technical precision, and environmental responsibility.

Education

At SaltEnPep, we view education as a means to enable responsible innovation, critical thinking, and curiosity rather than the mere transfer of knowledge. Through a seven-event outreach series conducted between July and September 2025, we engaged diverse audiences ranging from high school students to undergraduate biotechnology students through three school outreach programs, one hands-on summer camp, and three college-level sessions. Using storytelling, games, surveys, ethical debates, hands-on experiments, and real-world case studies, we introduced synthetic biology as a solution-oriented, interdisciplinary field addressing challenges in health, agriculture, food, sustainability, and the environment. Pre- and post-outreach surveys conducted during school events revealed a clear shift in student thinking—from associating biotechnology primarily with GM crops and cloning to proposing innovative, application-based ideas such as plastic-degrading microbes, edible packaging, vaccine development, fortified foods, and biosensors—demonstrating increased contextual and application-focused understanding.

School outreach events at Maharishi International Residential School (123 students), Kendriya Vidyalaya Anna Nagar (100+ students), and Immaculate Hearts Girls Higher Secondary School (90+ students) combined foundational SynBio concepts, recombinant DNA technology, iGEM project examples across villages, ethics and biosafety discussions, and interactive activities such as DNA puzzles, debates, and design challenges. A one-day summer camp introduced younger students to laboratory environments through FIST lab tours, DNA isolation, and mini SDS-PAGE, emphasising safety, responsibility, and scientific curiosity. College-level outreach at Rajalakshmi Engineering College reached over 700 biotechnology students through sessions that connected academic theory to industrial applications, research pathways, wet-lab and dry-lab integration, and the iGEM competition. Across all events, SaltEnPep was presented as a real-world case study demonstrating socially responsible innovation through saltiness-enhancing peptides. Collectively, these initiatives bridged classroom learning with real-life science, inspired career exploration, strengthened ethical awareness, and reinforced synthetic biology as a powerful tool for designing solutions to global and local challenges.

Integrated Human Practices

Our Human-Centred Design lies in its vast interdependence with expert validation at each stage of carrying out the project. We conducted these analyses critically at each step into progression of our project, which ensures that only what was accepted was continued.

Phase 1: Ideation

The ideation phase focused on understanding the real-world relevance of sodium reduction and defining a clinically meaningful problem statement. Early consultations with clinicians and dieticians highlighted excessive sodium intake as a major contributor to hypertension, cardiovascular disease, renal strain, and poor treatment compliance. These discussions revealed that existing salt substitutes—particularly potassium-based alternatives—are unsuitable for many high-risk populations, including cardiac patients, the elderly, and salt-sensitive individuals.

Senior cardiologists Dr. Kamalanathan and Dr. Shree Datta played a significant role in validating the medical necessity of a non-potassium salt alternative and emphasised that taste retention is essential for patient adherence. Dietetic perspectives reinforced that sodium reduction strategies must align with cultural dietary habits and sensory expectations. Public perception surveys conducted in parallel revealed uneven awareness of hidden salt consumption, especially among younger populations, underscoring the need for solutions that combine taste preservation with public education. These insights collectively shaped the core concept of a safe, peptide-based saltiness enhancer.

Phase 2: Technical and Experimental Advancement & Conceptualisation

The second phase translated clinical insights into a technically robust and experimentally feasible design. Expert mentorship guided the refinement of molecular strategy, computational validation, and bioprocess workflows. Dr V. Aravindhan provided critical direction by recommending a shift from simple peptide repeats toward engineered fusion protein formats to improve molecular stability, expression efficiency, and reproducibility. He also emphasised a phased workflow beginning with dry-lab validation before wet-lab experimentation.

Computational validation was strengthened through sustained mentorship from Mr Nandha Kumar and scientific validation from Dr Venkatesh Chellappa, who confirmed the reliability of peptide–protein docking and molecular dynamics simulations and advised on scalability and parameter optimisation. This ensured informed candidate selection before laboratory validation.

Bioprocess and purification strategies were streamlined following technical consultation with Dr. Ganesh, who guided enzyme selection, tag minimisation, intein-based purification design, and scalability considerations. Advanced analytical validation using UHPLC and mass spectrometry further strengthened confidence in peptide identity and purity. Sensory evaluation was supported through electronic tongue training and validation at CSIR-CFTRI, enabling objective assessment of saltiness enhancement and reinforcing the importance of instrument-driven taste profiling.

Phase 3: Validation and Regulatory Compliances

The final phase focused on translational feasibility, regulatory alignment, and real-world deployment strategies. Regulatory and food technology consultations clarified that the peptide-based product would likely fall under the FSSAI “Novel Food” or “Food Additive” category, requiring rigorous safety, stability, allergenicity, and toxicity assessments. Emphasis was placed on transparent labelling, matrix studies across food systems, shelf-life testing, and alignment with Codex and international regulatory frameworks.

Clinical experts stressed the importance of long-term safety data, affordability, and physician engagement to build trust and enable prescription-level credibility. Strategic guidance supported a Business-to-Business (B2B) entry model, prioritising hospitals, institutional kitchens, and healthcare-linked food services as controlled adoption environments before broader consumer rollout. Together, these insights established a clear regulatory and validation roadmap, positioning the project as a clinically responsible, regulatory-ready, and scalable public health intervention.

Fig 4: Integrated Human Practices - The Three Phases