Advanced Drug Delivery Reviews 222 (2025) 115603

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

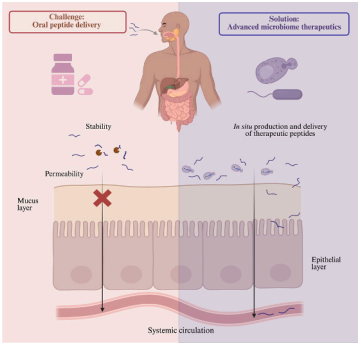
journal homepage: www.elsevier.com/locate/adr

Advanced microbiome therapeutics for oral delivery of peptides and proteins: Advances, challenges, and opportunities☆

Hitesh P. Gelli [a], Ruben Vazquez-Uribe [b], Stephen T. Buckley [c], Jan Terje Andersen [d,e,f], Morten Otto Alexander Sommer [a,*]

[a] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby, Denmark
[b] Center for Microbiology, VIB, Leuven, Belgium
[c] Novo Nordisk, Research and Early Development, Måløv, Denmark
[d] Department of Pharmacology, University of Oslo, Oslo, Norway
[e] Department of Immunology, Oslo University Hospital Rikshospitalet, Oslo, Norway
[f] Precision Immunotherapy Alliance, University of Oslo, Oslo, Norway

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Drug delivery
Peptides and proteins
Therapeutics
Oral delivery
Engineered microbes
eLBPs
Absorption
Stability

ABSTRACT

Peptide and protein medicines have changed the therapeutic landscape for many diseases, yet oral delivery remains a significant challenge due to enzymatic degradation, instability, and poor permeability in the gastrointestinal tract. Advanced Microbiome Therapeutics (AMTs) could overcome some of these barriers by producing and releasing therapeutic peptides directly in the gastrointestinal tract. AMTs can localize peptide production at the site of absorption, providing either sustained or controlled release while potentially reducing side effects associated with systemic administration. Here, this review assesses the status of AMTs for oral peptide delivery and discusses the potential integration of permeation enhancers, mucoadhesive systems, and receptor-mediated transport strategies to improve oral bioavailability further. Combining these approaches could pave the way for more widespread oral delivery strategies for peptide and protein medicines.

☆ This article is part of a special issue entitled: 'Live Biotherapeutics' published in Advanced Drug Delivery Reviews.

* Corresponding author.
E-mail address: msom@bio.dtu.dk (M.O. Alexander Sommer).

https://doi.org/10.1016/j.addr.2025.115603
Received 3 April 2025; Received in revised form 1 May 2025; Accepted 6 May 2025
Available online 9 May 2025

1. Introduction

Robust oral administration of therapeutic peptides and proteins is a long-standing goal within the pharmaceutical industry to improve patient compliance and therapeutic outcomes [1]. However, oral delivery of large and structurally complex biomolecules is challenged by poor intestinal stability and limited absorption [2]. Their considerable molecular size and susceptibility to enzymatic degradation in the harsh conditions of the gastrointestinal (GI) tract severely limit their oral bioavailability and therapeutic efficacy [3,4]. To address these limitations, researchers have aimed to develop delivery systems based on nanoparticles, such as polymeric nanoparticles [5], and chemical modification methods, including PEGylation [6], have been established to improve both the stability and permeability of therapeutic molecules. Recently, oral microdevices have also emerged as an innovative approach, capable of shielding therapeutic peptides from degradation and enabling their precise release and absorption within the GI tract [7]. Despite these promising advancements, each strategy is accompanied by specific drawbacks, including the potential modification of the biological activity of therapeutic agents and concerns regarding their long-term safety and possible toxicity [8–10].

The approach of employing engineered microbes as carriers for the oral delivery of therapeutic payloads (small molecules, peptides, and proteins), commonly known as Advanced Microbiome Therapeutics (AMTs) or Engineered Live Biotherapeutic Products (eLBPs), represents a potentially transformative method to overcome existing challenges associated with oral drug administration [11]. Specific microbes, including several probiotic strains, have been deployed as chassis for therapeutic purposes due to their beneficial probiotic attributes, established safety profiles, inherent robustness, and ability to temporarily colonize various regions of the GI tract [12,13]. As such, AMTs are increasingly recognized as a promising means for enabling oral delivery of therapeutics aimed at managing inflammatory disorders [14–16] and cardiometabolic diseases [17–19]. Still, the promising outcomes of these studies have predominantly resulted from local action within the GI tract rather than systemic exposure. Indeed, few studies have demonstrated significant elevation of the therapeutic agents in systemic circulation outside of the GI region.

In this review, we first outline the significant challenges associated with the oral delivery of therapeutic peptides, such as peptide stability in the GI tract and limited intestinal permeability. We then discuss why AMTs represent a promising platform for oral peptide delivery, highlighting their advantages over traditional delivery methods, including their ability for sustained or controlled release. Subsequently, we delve into key therapeutic areas where AMTs have demonstrated effectiveness in delivering peptide-based therapeutics, including inflammatory disorders and cardiometabolic diseases, and further we pinpoint current limitations that hinder successful clinical translation. Finally, we elaborate on the integration of pharmaceutical strategies, such as permeation enhancers, mucoadhesive systems, and receptor-mediated transport strategies that could further enhance AMTs as oral peptide drug delivery systems.

2. Barriers to successful oral peptide delivery

Oral delivery of peptide- based therapeutics is challenged by many factors, including lack of stability towards GI pH, digestive enzymes as well as limited absorption across the mucus layer and the intestinal epithelium (Fig. 1), restricting the overall bioavailability to less than 1–2 % [20].

The acidic gastric environment denatures most peptides and proteins, causing degradation before absorption can occur [22,23]. The pH varies widely across the GI tract, influenced by factors including diet, health, age, and sex. In healthy individuals, the pH is highly acidic in the stomach (pH 1.0–2.5), neutralizes in the duodenum (pH 6.0–6.5), and rises to pH 7.0–7.5 in the distal ileum, while the colonic pH ranges from pH 5.0–7.0 [24,25]. Gastric pH can influence the ionization state of the peptide-based drugs, potentially leading to alterations in their structure or biological activity [26].

Digestive enzymes, primarily found in the stomach and small intestine, are essential for breaking down dietary proteins into absorbable units like short peptides and amino acids. Yet, this activity limits oral delivery of peptide drugs [27]. In the stomach, Pepsin is a key proteolytic enzyme that operates optimally at acidic pH, effectively degrading proteins. Indeed, the half-life of native GLP-1 is less than 2 min at pH 2.6, and approximately 5.5 min at pH 5 when exposed to pepsin. Similarly, Semaglutide (with SNAC) has shown a half-life of 16 min at pH 2.6 and 34 min at pH 5 [28]. In the small intestine, additional enzymes, including trypsin, chymotrypsin, and carboxypeptidase, play critical roles in protein digestion. Trypsin cleaves peptide bonds specifically after the basic amino acids Lysine and Arginine. Chymotrypsin, on the other hand, primarily targets aromatic and large hydrophobic amino acids, including Phenylalanine, Tyrosine, and Tryptophan, and less efficiently Leucine and Methionine. Carboxypeptidase complements these enzymes by sequentially removing amino acids from the carboxyl-terminal end of peptide chains. In human gastric fluid (HGF) and human

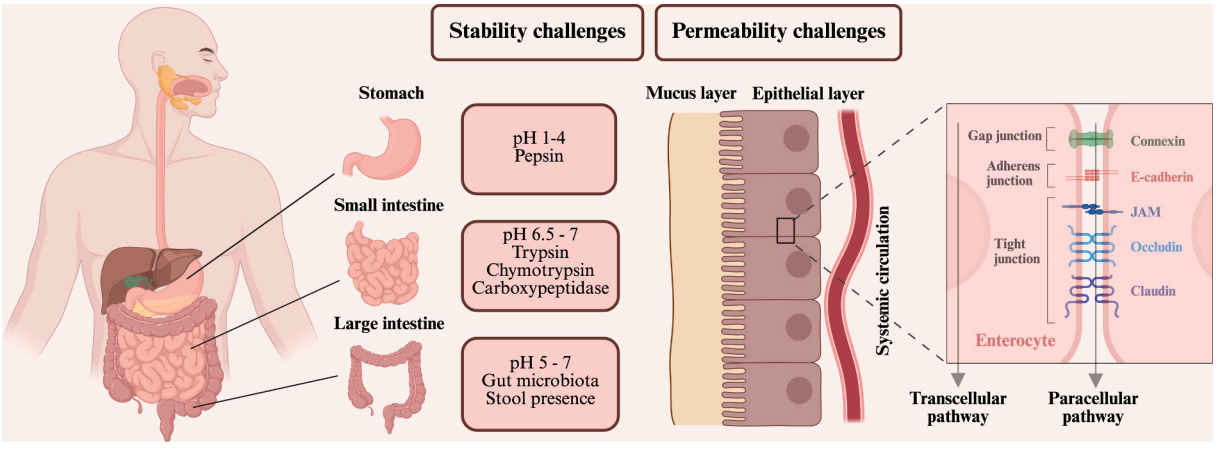


Fig. 1. Key challenges in oral peptide and protein delivery. Stability challenges arise from varying pH conditions and enzymatic degradation in different GI anatomic regions, including the presence of pepsin in the stomach (pH 1–4), trypsin and chymotrypsin in the small intestine (pH 6.5–7), and microbial activity in the large intestine (pH 5–7). Permeability challenges involve crossing the mucus layer and selective epithelial cell barrier, where tight junction proteins (e.g., occludin, claudin) inhibit and regulate paracellular transport. Adapted from Miao et al. (2023) [21], Journal of Nanobiotechnology. https://doi.org/10.1186/s12951-023-01991-3. Adapted under Creative Commons License (CC BY).

intestinal fluid (HIF), peptide drugs exhibit rapid degradation. Calcitonin, glucagon, secretin, and insulin were completely degraded within 2 min in both fluids, highlighting their extreme susceptibility to enzymatic degradation [29]. Similarly, in guinea pigs, insulin degradation in the small intestine was rapid, with a half-life of 2–4 min, primarily driven by trypsin-like, chymotrypsin-like, and aminopeptidase enzymes [30]. These findings emphasize the critical enzymatic barriers to oral peptide drug stability.

The mucus barrier significantly impacts the oral delivery of peptide therapeutics due to its protective role in the GI tract. Composed primarily of water and mucins it forms a complex network with two layers: a dense inner layer tightly attached to the epithelium, protecting against pathogens, and a loosely attached outer layer colonized by commensal microbiota [31,32]. The diffusion coefficient of peptides and proteins decreases with increasing molecular weight in intestinal mucus, where steric hindrance becomes the predominant barrier to macromolecular transport [33]. Additionally, charge distribution strongly influences peptide diffusion, as seen in the case of a highly anionic synthetic peptide (−12 charge), which diffused more freely in reconstituted gastric mucin gels than a cationic peptide (+8 charge). Peptides with near-zero charge (+2) showed no restriction, and in some cases, their diffusion was enhanced in the presence of mucins [34]. Furthermore, an in vivo study in rats has demonstrated that the duodenal mucus restricts the diffusion of macromolecules ranging from 3.5 to 89 kDa, highlighting its role in limiting peptide bioavailability [35].

The GI epithelium presents a major barrier to the effective absorption of peptide- and protein-based therapeutics, but they may cross the cell epithelial layer via transcellular or paracellular routes. The transcellular route involves either passive diffusion across the apical and basolateral membranes or via active transport mechanisms such as membrane fusion, transcytosis, and intracellular absorption followed by systemic secretion [36,37]. However, due to their large size, peptides and proteins rarely permeate cell membranes via transcellular routes [38,39]. Additionally, intracellular degradation by cytosolic enzymes may further limit their absorption through this route [40]. The paracellular pathway involves drug transport through water-filled pores, or paracellular spaces, between adjacent cells. The intestinal epithelium has a surface area of $\sim 2 \times 10^6$ cm^2, with only 0.01–0.1 % ($\sim$200–2000 cm^2) consisting of paracellular space. In theory, this may allow systemic absorption of peptides and proteins at picomolar to nanomolar levels, which are sufficient to elicit biological effects [41]. However, tight junctions within the paracellular space limit macromolecule absorption. These intercellular structures, regulated by proteins like claudins, occludins, junctional adhesion molecules (JAMs), and zonula occludens (ZO-1, ZO-2, and ZO-3), act as structural barriers to the passage of macromolecules [42,43]. Accordingly, drug bioavailability decreases sharply as molecular weight exceeds 700 Da [44], resulting in poor absorption of larger therapeutic peptides and proteins.

Furthermore, the gut microbiota can influence the bioavailability of peptides through the action of microbial enzymes, regulating host gene expression, and competing for substrates. Strong evidence links gut microbiota to the efficacy and safety of various drugs. For instance, it was discovered that tyrosine decarboxylases from *Enterococcus faecalis* metabolize the Parkinson's drug Levodopa (L-DOPA), reducing its efficacy while also increasing m-tyramine production from dopamine, which may lead to severe adverse effects [45,46]. A systematic study assessed the metabolic capabilities of 76 gut microbial strains to metabolize 271 oral drugs, revealing that 176 drugs (66 %) underwent bacterial metabolism. Among the most extensively metabolized compounds were proton pump inhibitors (PPIs) (pantoprazole, omeprazole, tenatoprazole), the chemotherapeutic agent melphalan, the antimalarial artemisinin, and the Parkinson's drug mesylate, all of which were degraded by nearly all strains tested. Among the gut bacteria, *Bacteroides dorei* and Clostridium sp. exhibited broad-spectrum metabolic activity, metabolizing 164 and 154 drugs, respectively [47]. While substantial research has focused on small-molecule drugs, studies on the microbiota-mediated metabolism of peptide-based therapeutics remain limited. One study in 2020 demonstrated that a specific metalloprotease (GelE) from *E. faecalis* could degrade GLP-1, an incretin hormone used to treat type 2 diabetes. However, this study was conducted *in vitro*, and its findings have not yet been validated in preclinical animal models or human studies. The scarcity of such studies may be attributed to the limited number of orally available peptide drugs and the fact that their primary absorption occurs in the stomach [28] or upper GI tract [48], where microbial interactions are less pronounced compared to the lower regions of the GI tract. An alternative approach being explored is colon-targeted drug delivery, which offers lower protease activity and prolonged transit time, potentially enhancing stability and absorption of peptide and protein therapeutics [49]. In this context, gut microbiota could play a crucial role, as microbial metabolism may significantly influence the efficacy, bioavailability, and safety of peptide drugs.

3. AMTs in oral delivery of peptides and proteins

AMTs are microbes, such as bacteria and yeast, engineered to deliver therapeutic payloads in a targeted manner to prevent, treat, or cure a disease. AMTs provide a biological solution by leveraging microbial systems naturally suited for the gut environment. Unlike traditional drug formulations, which often face significant barriers in the GI tract, AMTs utilize engineered microbes to protect, produce, and release therapeutic peptides *in situ*, overcoming many of the limitations associated with oral peptide delivery, especially in the stomach and upper intestines. Additionally, they can be engineered to respond to specific stimuli, such as pH or metabolite levels, to ensure precise control over drug release [50], in addition to their ability to offer sustained release of biopharmaceuticals, particularly peptides and proteins (Fig. 2).

3.1. Sustained release of therapeutic peptides

Sustained-release platforms offer multiple benefits such as reduced dosing frequency, decrease in adverse side effects and improve efficacy-dose relationship for a drug [51]. Traditional sustained-release platforms like synthetic formulations [52], polymer-based systems [53], or surface-modified materials [54] encapsulate the drugs within a matrix that degrades over time. Developing sustained-release formulations is challenging for proteins due to their fragility and structural complexity [51]. Additionally, the proteins are prone to denaturation [55] or aggregation [56], which can enhance their immunogenicity [57,58]. Unlike traditional sustained-release systems, which rely on controlled degradation of synthetic matrices, AMTs achieve sustained release by continuously synthesizing and secreting peptides or proteins *in situ* over extended periods. This can be achieved through constitutive expression systems, which use promoters that drive continuous transcription of the target gene regardless of the environmental conditions. The level of therapeutic production from constitutive systems is largely determined by transcriptional and translational control elements. At the transcriptional level, promoter strength refers to how effectively the promoter can recruit the cell's transcription machinery. Strong promoters are recognized more efficiently by RNA polymerase, leading to more frequent initiation of mRNA synthesis, while weaker promoters result in less frequent transcription. The DNA sequence of the promoter itself determines this strength by influencing how tightly and how often RNA polymerase binds and initiates transcription. This directly affects how much mRNA is available for translation into protein. Thus, selecting an appropriate promoter is key to achieving the desired level of protein expression [59,60]. Such systems have been validated in bacteria- and yeast-based AMTs *in vivo* in rodents [61,62]. Numerous constitutive promoters have been studied for protein expression in common AMT chassis like *Lactococcus lactis* [63], *Escherichia coli* Nissle [61], and *Saccharomyces bouldardii* [62,64]. At the translational level, ribosome binding site (RBS) sequences control the efficiency of ribosome recruitment to the mRNA, affecting protein synthesis rates [65]. The

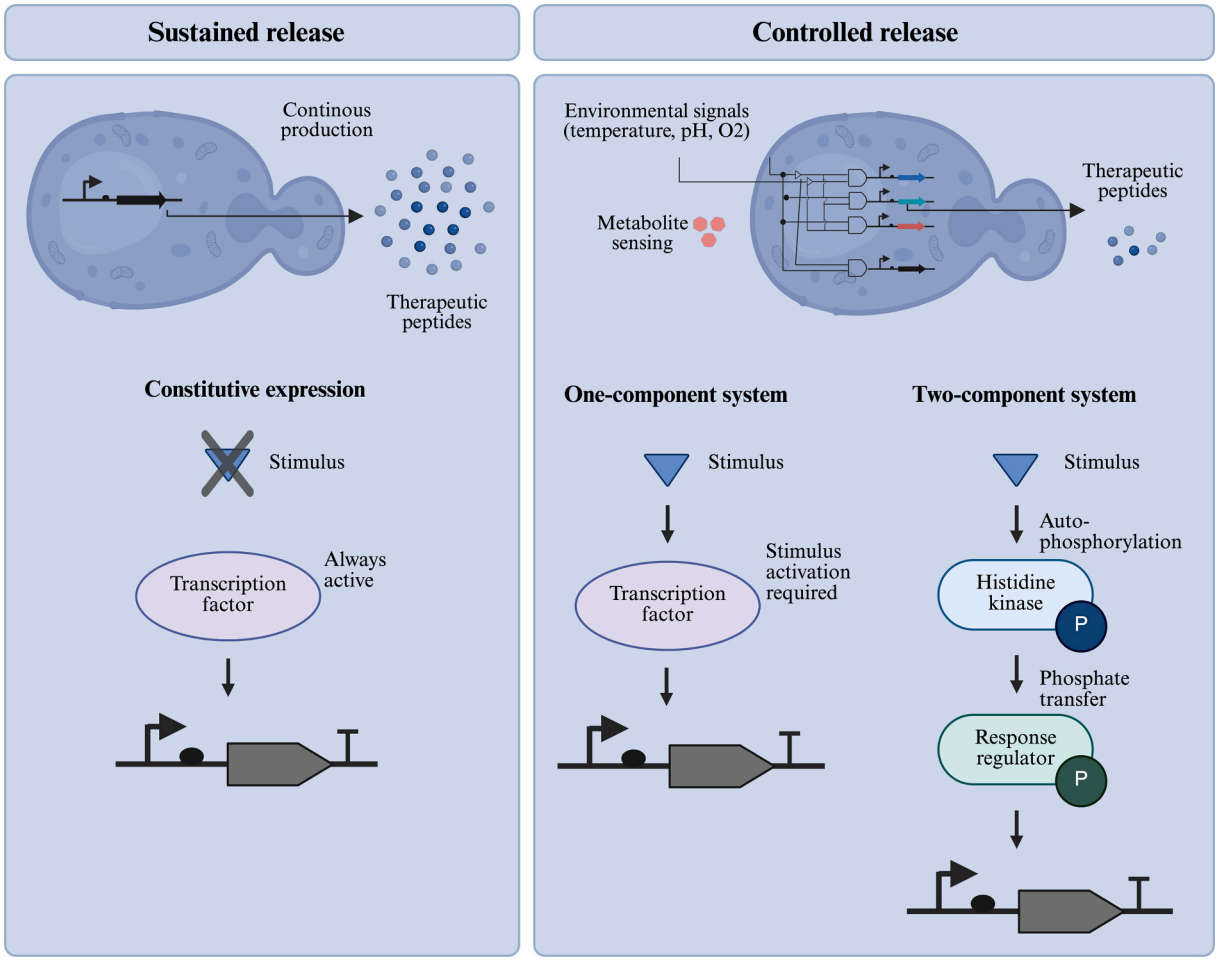


Fig. 2. Schematic of sustained vs. controlled therapeutic peptide release in AMTs. ***Left:*** *Constitutive expression enables continuous peptide production without external stimuli.* ***Right:*** *Controlled systems respond to environmental stimuli (oxygen, temperature, and pH) or metabolites, using either one-component (direct sensing by transcription factor) or two-component systems (stimulus detection via histidine kinase and response regulator signaling).*

RBS is a short sequence located just before the start codon, and it often includes a Shine-Dalgarno (SD) sequence that helps guide the ribosome to the correct position on the mRNA. This sequence pairs with a complementary region on the 16S rRNA of the ribosome, allowing the ribosome to align correctly and begin translation. Variations in the RBS sequence and its spacing from the start codon influence the strength and stability of ribosome binding, thereby modulating translation initiation efficiency. Thus, selecting an appropriate RBS sequence is key to achieving the desired level of protein expression [66–68]. Finally, engineered transcriptional terminators are used to stop transcription at the correct location, preventing unintended read-through into downstream genes and ensuring precise control over gene expression [69]. These molecular tools collectively enhance the ability of AMTs to act as reliable and sustained sources of therapeutic peptides.

3.2. *Controlled release of therapeutic peptides*

While sustained release ensures continuous therapeutic peptide production, there are cases where an unregulated, constant drug release may not be optimal, and controlled release is necessary. Controlled release refers to the ability to regulate the timing, location, and amount of a therapeutic peptide being delivered. It offers several advantages, including the prevention of excessive drug accumulation leading to toxicity, mimicking physiological secretion patterns for improved therapeutic efficacy, and enhancing patient safety by reducing the risk of overexposure [70]. Traditionally, the controlled release of peptide drugs has been achieved through formulation-based approaches, such as encapsulation in polymer-coated tablets, hydrogels, and microspheres, where drug release occurs through diffusion, polymer degradation, or pH-sensitive mechanisms [71]. However, these methods present significant challenges including the possible toxicity of materials used, the need for invasive procedures, and high manufacturing cost [71].

AMTs offer an alternative approach to controlled release by leveraging inducible genetic tools and regulatory networks, rather than relying on physical or chemical formulations. Unlike constitutive systems that drive continuous gene expression, inducible systems are only activated under defined conditions, allowing for precise and adjustable peptide production [72]. These systems typically fall into two categories: one-component systems, such as ligand-responsive transcription factors, and two-component systems, which use separate sensor and response proteins to regulate transcription in response to external cues.

One-component systems involve a single cytoplasmic protein typically a transcription factor that both detects a specific input and modulates transcription. These transcription factors are often engineered to bind ligands such as small molecules, host metabolites, or microbial signals. Upon ligand binding, the transcription factor undergoes a conformational change that alters its affinity for DNA. This change can enable the factor to either recruit RNA polymerase and initiate transcription, or block polymerase access to repress gene expression [73]. Such systems are widely used in synthetic biosensors, where inducible promoters are coupled to ligand-responsive regulators to achieve tight, signal-dependent control of therapeutic peptide secretion.

Two-component systems offer more modularity and sensitivity. They consist of a membrane-bound histidine kinase sensor and a cytoplasmic

response regulator. The kinase detects an external stimulus such as pH, bile acids, or inflammation-associated signals and autophosphorylates on a conserved histidine residue. This phosphate is then transferred to an aspartate residue on the response regulator, activating it. The activated regulator binds to promoter regions of target genes to initiate or repress transcription [74]. These systems enable AMTs to respond to extracellular signals that do not easily enter the cell, offering an additional layer of environmental responsiveness. The AMTs could be engineered to respond dynamically to external cues such as gut inflammation, quorum sensing, or specific biomolecules.

Several studies from the literature highlight how AMTs have been designed to leverage environmental stimuli for targeted therapeutic production [75]. In one study, researchers developed a self-tunable engineered probiotic yeast designed to treat inflammatory bowel disease (IBD). The yeast was genetically engineered to sense extracellular ATP, a key mediator of intestinal inflammation, and respond by producing apyrase, an enzyme that degrades ATP. This responsive yeast system effectively suppressed intestinal inflammation, demonstrating efficacy comparable to, or exceeding that of standard-of-care therapies [76]. In addition, inducible biosensing systems have been developed to sense compounds including nitric oxide [77,78], pH [50,79], and bile acids [50,79].

Another approach involves using quorum sensing signals, which allow engineered microbes to synchronize their behavior based on their population density. This method can be used to ensure that therapeutic production is triggered only when the microbial population reaches a certain threshold, enhancing collective efficacy and reducing unnecessary resource use. In a study, a genetically modified probiotic *E. coli* strain was developed to sense and eliminate the pathogen *Pseudomonas aeruginosa*. The engineered bacteria used quorum sensing to detect *P. aeruginosa*'s signaling molecules, triggering the production of antimicrobial peptides and enzymes that disrupt the pathogen biofilms [80]. By combining environmental sensing with precise therapeutic responses, these engineered microbes may pave the way for a significant step forward in the development of personalized medicine. They offer the ability to treat challenging diseases by responding to the specific markers within the GI tract of the patients, paving the way for safer, more effective, and controlled therapies.

4. Pre-clinical studies using AMTs for oral peptide delivery

Several studies have deployed engineered bacteria and yeast for delivery of therapeutic peptides (Table 1**)**. The first organism employed as an AMT was *Lactococcus lactis*, engineered to deliver interleukin-10 (IL-10) for the treatment of IBD [15]. Since then, various bacterial chassis, including *Bifidobacterium longum* [81] and *Lactobacillus casei* [82], and notably, *E. coli* Nissle [83], have been employed. Additionally, the probiotic yeast *S. boulardii* [84] has been used as an AMT chassis. *L. lactis* and *E. coli* Nissle are among the most researched bacteria in AMTs, with *S. boulardii* being the primary yeast chassis. These strains offer advantages such as good probiotic abilities, safety profiles, availability of well-established tools for genetic engineering, and their ability to survive in the GI tract, making them ideal for AMT applications.

4.1. Local applications of AMTs for gastrointestinal diseases

Intestinal health has gained significant attention in recent years due to its crucial role in overall well-being, with conditions such as IBD comprising of Crohn's disease and ulcerative colitis, posing major clinical challenges. Management of IBD requires prolonged immunosuppressive therapies that can pose risks of systemic side effects [98]. *L. lactis* has been widely investigated in IBD treatment, with several engineered strains undergoing both preclinical and clinical evaluation. Among these, strains engineered to express trefoil factors (TFFs) and anti-tumor necrosis factor-α (TNF-α) specific nanobodies have been tested in dextran sulfate sodium (DSS)-induced colitis models [90,99]. TFFs are protective peptides naturally expressed in specific regions of the GI tract, playing a crucial role in maintaining epithelial integrity and promoting mucosal healing. Notably, *L. lactis*-mediated in situ production of TFF-3, a goblet cell-derived peptide present in both the small and large intestines, demonstrated superior efficacy in colitis healing compared to the direct administration of purified TFF peptides [90]. Another bioengineered *L. lactis* strain was designed to counteract excessive TNF-α release, a key driver of colonic inflammation. This strain secreted anti-TNF-α nanobodies, which provided the therapeutic benefits of TNF inhibition without the adverse effects typically associated with systemic anti-TNF therapies. Due to their smaller molecular size, these nanobodies exhibited greater stability and a reduced likelihood of triggering systemic immune responses, making them a promising localized treatment for colitis [99]. *L. lactis* strains have also been engineered to express key anti-inflammatory cytokines such as IL-10 and IL-27. In a preclinical colitis model, IL-27-secreting *L. lactis* demonstrated superior efficacy in resolving inflammation than strains expressing IL-10 alone. This effect was attributed to an IL-27-mediated increase in intestinal epithelial IL-10 production, which enhanced mucosal immune regulation [100]. Currently, *L. lactis* remains the only engineered bacterial strain to have reached phase II clinical trials for IBD [16]. This study assessed a strain engineered to secrete human IL-10 (hIL-10) for its therapeutic potential in moderately active ulcerative colitis. While the strain was well-tolerated, the clinical outcomes did not show significant superiority over placebo or injected IL-10 [101]. Beyond *L. lactis*, engineered yeast probiotics have also been explored for IBD treatment. Researchers in one study developed a self-tunable *S. cerevisiae* strain capable of sensing extracellular ATP (eATP), a pro-inflammatory signal, and responding by secreting an ATP-degrading enzyme (apyrase) [76]. In a mouse model of colitis, these engineered yeasts significantly reduced intestinal inflammation, fibrosis, and dysbiosis, achieving therapeutic efficacy comparable to standard anti-TNF therapies. This study highlights the potential of engineered yeast as an adaptive and localized therapeutic strategy for IBD [76].

4.2. Systemic applications of AMTs for metabolic disorders

The increasing demand for GLP-1 receptor agonists in the management of type 2 diabetes mellitus (T2DM) and obesity has led to extensive research efforts focused on developing effective oral formulations. While injectable GLP-1 analogs such as exenatide, liraglutide, and semaglutide have shown substantial clinical benefits, the need for more patient-friendly alternatives has driven interest in oral delivery approaches. Among these, nanoparticle-based delivery systems have been widely investigated as potential carriers for GLP-1 and its analogs. Several studies have demonstrated that loading of GLP-1 receptor agonists into nanosystems can enhance their stability, improve intestinal absorption, and extend the hypoglycemic effects in diabetic animal models [102–106]. However, despite these promising results, nanoparticle-based GLP-1 delivery remains far from clinical translation due to significant challenges, including poor bioavailability, rapid degradation, and high production costs. To date, no such formulation has reached clinical trials, and the only available polymeric microparticle system, Byetta®, still relies on subcutaneous administration. Currently, the only approved oral formulation of a GLP-1 receptor agonist is Rybelsus® (oral semaglutide), which has demonstrated efficacy in managing blood glucose levels. However, its overall bioavailability is only about 0.8 % [107], highlighting the need for improved drug delivery strategies to enhance absorption and therapeutic outcomes. This major limitation presents an opportunity to explore alternative delivery approaches that can maximize the benefits of GLP-1 therapy while minimizing systemic losses.

AMTs have been widely studied for *in situ* delivery of GLP-1 and its agonists. Duan et al. were the first to demonstrate that genetically engineered bacteria could secrete GLP-1(1–37), laying the foundation for microbial-based peptide delivery [108]. Nearly a decade later, they

Table 1
Overview of AMTs used in pre-clinical studies to deliver therapeutic peptides and proteins orally.

Strain	Oral dosing (CFU)	Treatment duration	Drug	Target disease	Quantification of protein/peptide	Protein modification	Therapeutic effect	Reference
Lactococcus lactis	$2X10^9$ CFU/dose	Dosed five times per week for 6 weeks	Proinsulin and IL-10	Type I diabetes (T1D)	No quantification	None, but co-administered with low-dose anti-CD3 therapy.	• 48 % of the treated mice remained normoglycemic for 6 weeks post-treatment. • Reduced beta-cell destruction.	[85]
L. lactis	$1 X 10^9$ CFU/mL	Single oral dose	GLP-1	Type II diabetes mellitus (T2DM)	GLP-1 secretion measured to be 60 pM in 12 h from 10^4 CFU/mL culture supernatants.	None	• Reduction in blood glucose by 10–20 %.	[18]
L. lactis	$1X10^{10}$ CFU/dose	Dosed daily for 9–21 days (study-dependent)	GLP-1 (1–37)	T2DM	• GLP-1 secretion measured to be 130 pg/mL in culture supernatants. • Elevated GLP-1 levels measured in the portal vein.	None	• Improved glucose tolerance. • Elevated fasting and glucose-stimulated insulin levels.	[19]
L. lactis	$2 X 10^7$ CFU/dose	Dosed daily for 14 days	IL-10	IBD	• IL-10 secretion measured to be 3 μg/mL in culture supernatants. • IL-10 was measured to be 7 ng in the colon.	None	• Reduction in colitis severity by 50 %. • Prevention of colitis onset in IL-10 knockout mice.	[15]
Saccharomyces cerevisiae	$2 X 10^8$ CFU/dose	Dosed daily for 7 days, 2 cycles with a one-week interval	Apyrase	IBD	ATPase activity measured to be the equivalent of 280 pM apyrase per μL.	None	• Reduced intestinal inflammation and fibrosis. expression of IFN-γ and IL-17. • Suppressed the • Increase in the level of IL-10. • Limited gut dysbiosis by restoring microbiome diversity.	[76]
Saccharomyces boulardii	$1 X 10^9$ CFU/dose	Dosed daily for 5 days	Atrial Natriuretic Peptide (ANP)	IBD	No quantification	Hexamutant version designed to enhance therapeutic effects	• Improved body weight, DAI and survival rate. • Reduced the levels of TNF- α, IL-1β in the colon. • Increased the level of IL-6 in the colon.	[86]
Escherichia coli Nissle	300 μL of 10^8, 10^9, or 10^{10} CFU/ml	Dosed daily for 8 weeks	GLP-1 (7–37)	Obesity	No quantification	Modifications were made to the GLP-1 (7–37) sequence to protect from DPP-IV to improve stability.	• Reduced body weight and food intake. • Lower hepatic fat accumulation and triglyceride levels. • Improved glucose tolerance and insulin sensitivity.	[87]
E. coli Nissle	$1 X 10^8$ CFU/dose	Daily dosing for 13 days	Trefoil factor 3 TFF3 fused to CsgA	IBD	No quantification	TFF3 genetically fused to csgA curli fibres	• Reduction in weight loss and DAI. • Decrease in IL-6, IL-17A, and TNF-α. • Enhanced mucosal healing and improved intestinal barrier integrity.	[83]

(*continued on next page*)

Table 1 (*continued*)

E. coli Nissle	1 X 10^8 CFU/dose	Single dose	Anti-TNF nanobody (NbTNF)	IBD	Below levels of detection in serum and no detection in colon homogenates	Secreted as SSOspC2-NbTNF homodimer for improved secretion.	• Reduction in TNF −α • Prevented injury and inflammation in TNBS induced colitis model.	[88]
S. boulardii	10^8 CFU/dose	Daily dosing for 29 days	Exendin-4	Obesity	• Exendin-4 levels measured to be 15 nM/OD_{600} (aerobic) and 11 nM/OD_{600} (anaerobic) in culture supernatants. • Exendin-4 was detected in the plasma of few mice only. Values were close to detection limit (0.5 pmol/L).	None	• Reducing food intake by 25% under cold exposure. • 4-fold higher weight loss in treatment group under cold exposure. • Improved glucose metabolism and lipid homeostasis under cold exposure.	[84]
L. lactis	5 X 10^9 CFU/dose	Daily dosing for 7 days	Bovine lactoferricin-lactoferrampin fusion peptide	Acute colitis	Protein levels were measured to be 40—–60 ng/mL in culture supernatants and 50–100 ng/mL in cell lysates.	Fusion protein contains a flexible linker $(GGGS)_2$	• Reduced DAI. • Attenuated weight loss. • Reduced colon shortening. • Restored intestinal barrier integrity. • Suppressed the production of pro-inflammatory cytokines. • Improved intestinal gut microbiota population.	[89]
L. lactis	2 X 10^9 CFU/dose	Dosed daily for 5 days (therapeutic) and for 7 days (prophylactic)	Murine trefoil factors (mTFF1, mTFF2, and mTFF3)	Acute colitis	TFF secretions measured to be 300–500 ng/mL depending on the variant	None	• Reduced mortality. • Reduced weight loss. • Lower inflammatory scores. • Significant prevention and healing of acute colitis.	[90]
L. lactis	2 X 10^9 CFU/dose	Dosed daily for 4 days	Anti-TNF alpha scFv	Ulcerative colitis	Protein expression by western blot (31 kDa). No quantification.	None	• Significant reduction in DAI. • Restoration of colon length. • Decreased C-reactive protein levels (CRP).	[91]
Lactococcus casei	5 X 10^9 CFU/dose	Daily dosing for 9 days	Manganese superoxide dismutase (MnSOD)	Ulcerative colitis	MnSOD activity was measured to be 325 μmol/min/mg of protein	None	• Significant reduction in colonic histological damage score. • Reduction of ROS in vitro and ex vivo. • Decreased infiltration of inflammatory cells.	[82]
Bifidobacterium longum	6X10^8 CFU/dose	Daily dosing for 28 days post L-arabinose induction	Oxyntomodulin	Obesity	• Peptide level was measured to be approx. 2000 pg/mL in culture supernatants. • Peptide level was measured to be approx. 50 pg/mL in the intestinal content.	None	• Significant decrease in body weight. • Reduced consumption of food. • Reduced. triglyceride levels in plasma. • Decreased plasma ghrelin levels.	[92]

(*continued on next page*)

Table 1 (*continued*)

S. boulardii	$_{1\ X\ 10}9$ CFU/dose	Daily dosing for 7 days (prophylactic), for 4 days (therapeutic), for 13 days (recurrent prevention).	Tetra-specific antitoxins against TcdA and TcdB toxins	Clostridioides difficile infection (CDI)	Presence of the proteins was confirmed by western blot and ELISA. No quantification.	None	• Significantly reduced mortality, weight loss, and diarrhea in CDI recurrent model. • Decreased histopathology and inflammation. • Significantly reduced toxin levels. • Demonstrated both prophylactic and therapeutic effects.	[93]
B. longum	$2X10^9$ CFU/dose	Daily dosing for 7 days	human MnSOD (manganese superoxide dismutase)	Ulcerative colitis	Enzyme concentration was measured to be approx. 200 – 300 pg/mL after 30h in the culture supernatants. Enzyme concentration was measured to be approx. 20 pg/mL in the intestinal tissues.	hMnSOD was fused to PEP-1 cell-penetrating peptide	• Reduction in pro-inflammatory cytokines and tissue damage in colon. • Decreased neutrophil infiltration and inflammation.	[81]
E. coli Nissle	$3\ X\ 10^9$ CFU to $7.2\ X\ 10^{11}$ (dose response)	Single dose	Phenylalanine degrading enzyme: Phenylalanine ammonia lyase (PAL)	Phenylketonuria	PAL activity was measured in vitro and in vivo in mice and cynomolgus monkeys	None	• Reduced blood phenylalanine concentration by 38% in mice. • Inhibited increase in serum phenylalanine after oral phenylalanine challenge in cynomolgus monkeys.	[94]
E. coli Nissle	$1\ X\ 10^9$ CFU per cube.	Administered through gelatin cubes.	Aldafermin	Metabolic dysfunction-associated steatotic liver disease (MASLD)	Aldafermin was measured to be 10–15 ng/mL/OD_{600} in culture supernatants.	None	• Reduced body weight • Reduced hepatic steatosis • Decreased MASLD plasma biomarkers in mice.	[95]
Lactobacillus reuteri	$1\ X\ 10^9$ CFU/dose	Single dose (delayed-type hypersensitivity) Daily dosing for 21 days (collagen-induced arthritis)	ShK-235 (kv1.3 channel blocker)	Rheumatoid arthritis	Peptide was measured to be approx. 450 pM in culture supernatants. Peptides was measured to be apprx. 7 nM in serum.	None	• Reduced collagen-induced arthritis severity by 84%. • Lowered delayed-type hypersensitivity (DTH) by 30%. • Resulted in less bone and join damage.	[96]
Lactobacillus paracasei	$1\ X\ 10^{10}$ CFU/dose	Twice daily dosing for 7 or 14 days.	GLP-1	T2DM	GLP-1 level was measured to be 300 ng/dose.	Pentameric GLP-1	• No significant glucose lowering effect.	[97]

Abbreviations: CFU, colony-forming unit; IBD, inflammatory bowel disease; IL, interleukin; TNBS, 2,4,6-trinitobenzene sulfonic acid; DSS, dextran sodium sulfate; IFN, interferon; ROS, reactive oxygen species; TNF, tumor necrosis factor; DAI, disease activity index.

advanced this strategy by converting intestinal cells to function as glucose-responsive insulin-secreting cells, highlighting the relevance of GLP-1 over its active form in enterocyte reprogramming [17]. Later, another study engineered *L. lactis* to produce GLP-1, showing its potential in mitigating systemic inflammation-induced memory impairment and amyloidogenesis [109]. Their findings also suggested a novel therapeutic role for this strain in neuropsychiatric disorders by reducing neuroinflammation [110,111]. The antidiabetic effects of engineered probiotics were further confirmed in both obese mouse [112] and monkey [113] models, emphasizing their role in the gut-pancreas-liver axis [114]. In 2016, Arora et al. utilized *L. lactis* to deliver GLP-1 in mice, leading to an increase in GLP-1 levels within the portal vein. However, there was no conclusive evidence that the elevated GLP-1 originated from the absorption of the microbially produced peptide [19]. Another promising study involved genetically modified *E. coli Nissle* for GLP-1 delivery for treating Parkinson's disease and exhibited neuroprotective effects [115]. Additionally, *Clostridium butyricum* engineered to produce GLP-1 demonstrated beneficial effects on blood pressure and cardiac hypertrophy in rat models [116]. In another study, *L. lactis* was engineered to act as a light-responsive probiotic, secreting GLP-1 under optogenetic control, showing adequate glucose and weight regulation in rodent models [117]. Efforts to enhance oral GLP-1 delivery have also involved stabilizing the peptide against enzymatic degradation. For instance, GLP-1(7–36)-Gly8 and its modified variants have been successfully delivered via recombinant Lactobacillus strains in diabetic rat models [97]. *Lactobacillus paracasei* has been explored as an oral vector for delivering GLP-1 [97]. Furthermore, probiotic yeast like *S. boulardii* have been engineered to deliver Exendin-4, a GLP-1

agonist, in mice and have shown promising anti-obesity effects [84]. Using a strain like *S. boulardii* could provide additional benefits as studies have proven its natural metabolic-modulating and anti-obesity effects [118], suggesting an added therapeutic dimension beyond peptide delivery. Overall, advances in microbiome-based GLP-1 RA delivery systems highlight their potential as innovative therapeutic platforms for metabolic disorders.

Overall, AMTs are a promising drug delivery platform for treating metabolic diseases and IBD due to targeted and site-specific delivery of therapeutic peptides directly in the GI tract, which can enhance therapeutic outcomes and improve patient compliance by providing non-invasive oral treatment. Additionally, these microbes could help restore gut microbial balance, restore intestinal barrier function, and modulate local immune responses, addressing key underlying factors in the pathophysiology of metabolic diseases and IBD as evidenced from the studies.

4.3. Shortcomings of the pre-clinical studies

Although preclinical studies have demonstrated promising therapeutic potential for AMTs, some shortcomings need to be addressed. AMT colonization and persistence in the GI tract are rarely quantified, with few studies examining how long AMTs remain in the host and their CFU levels in different regions of the GI tract. This information is critical as AMT efficacy depends on sustained colonization and therapeutic production at the target site. Without these data it is unclear if the strains reside in the gut transiently or are cleared rapidly. In contrast, traditional oral drugs are evaluated using pharmacokinetic parameters like retention, half-life, and clearance [119]. Another limitation is the lack of appropriate positive controls. While negative controls (strains containing empty vectors) are common, only one study [120] included a positive control, such as subcutaneous Exenatide injection. Including such benchmarks is essential to assess whether AMTs offer real advantages over conventional routes. Without them, efficacy comparisons remain incomplete.

Another important limitation is the considerable variability in experimental conditions across studies. Differences in AMT dosing regimens (ranging from 10^8 to 10^{10} per dose), dosing frequency, and treatment duration likely contribute to the differences in the therapeutic outcomes. Furthermore, the lack of quantification of the therapeutic production, either in vitro or within the GI tract remains a concern. Precise dosing is central to drug development, ensuring optimal efficacy and minimizing side effects [121], yet few AMT studies report how much therapeutic is produced in situ, making it difficult to establish dose–response relationships. For strains intended to deliver systemically absorbed therapeutics, direct measurements of circulating drug levels should be assessed. Quantifying local and systemic levels are essential for understanding pharmacokinetic parameters such as drug stability, Cmax, and Tmax.

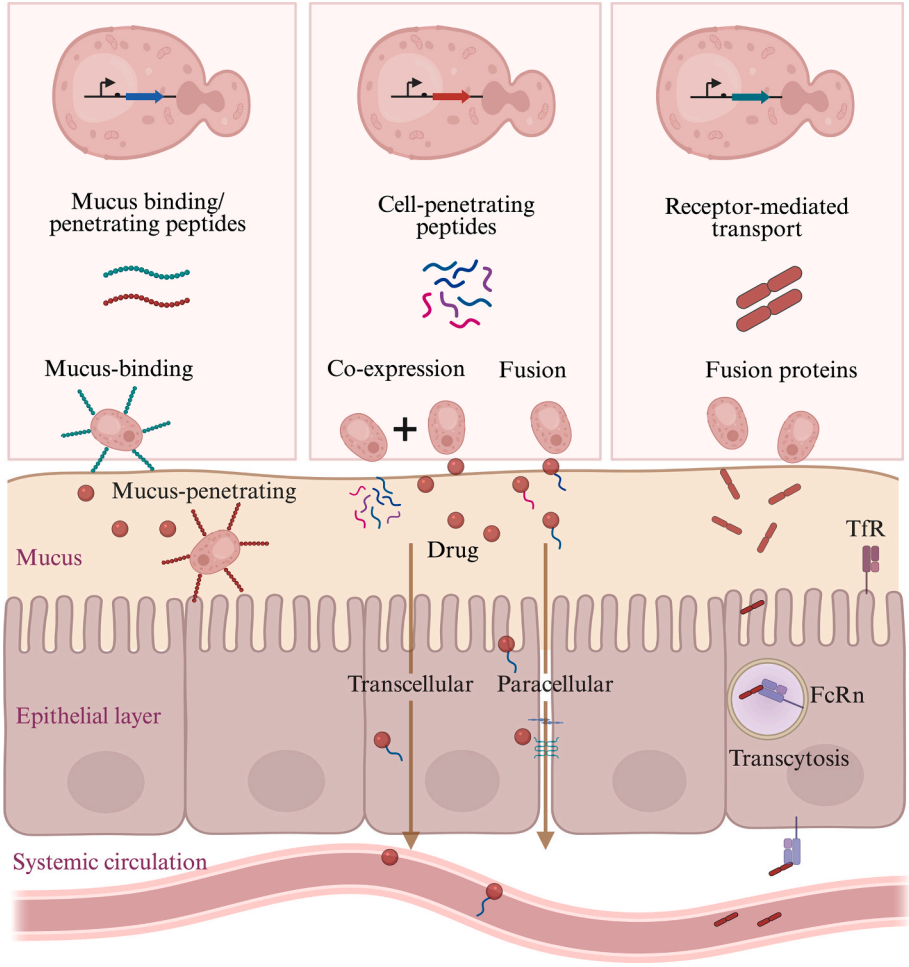


Fig. 3. Strategies for enhancing oral peptide delivery using AMTs. *This figure illustrates strategies for enhancing oral peptide drug delivery using AMTs by integrating key concepts from traditional drug delivery systems. By incorporating established drug delivery tools such as cell-penetrating peptides (CPPs), mucus-binding peptides, and fusion proteins, AMTs can improve peptide stability, enhance intestinal absorption, and increase systemic bioavailability.*

5. Strategies for enhancing systemic peptide delivery using AMTs

The therapeutic efficacy of peptide and protein drugs often depends on their entry into systemic circulation. While AMTs may pave the way for efficient oral delivery of therapeutic peptides locally in the GI tract, the entry of these drugs into the systemic circulation remains a challenge. Integrating strategies from conventional drug delivery platforms could further enhance AMTs by addressing the challenges of intestinal absorption and bioavailability. This section discusses how established drug delivery approaches can be leveraged to optimize AMTs for improved peptide delivery (Fig. 3).

5.1. Mucoadhesive and mucus-penetrating strategies for improved residence time

Mucoadhesive and mucus-penetrating systems are two major strategies used to enhance the retention time of therapeutics in the GI tract by interacting with the mucosal layer. Mucoadhesive systems anchor the drug delivery system to the mucus through strong interactions with GI mucins, prolonging residence time and making them attractive candidates for long-term drug delivery. However, their effectiveness is limited by mucus turnover, which typically occurs within 1–7 h [122–124]. In contrast, mucus-penetrating systems are designed to bypass the mucus layer and interact directly with the epithelium, allowing for potentially prolonged drug delivery. Since their clearance depends on epithelial cell turnover, which occurs approximately every 3–5 days [125], these systems may offer a significant residence time compared to mucoadhesive systems. By incorporating either of these modalities, the drug delivery systems may interact with the mucins or the epithelium and extend the residence time and thereby improving the bioavailability of orally administered therapeutics. Mucoadhesive strength can be evaluated *in vitro* using assays that measure bacterial binding to purified mucins, as well as *ex vivo* retention tests on intestinal segments [126]. *In vivo* colonization and persistence can be assessed by quantifying CFUs from fecal samples of the animal over time or by tracking fluorescently labeled or bioluminescent strains through non-invasive imaging [127]. These approaches help determine the residence time of engineered microbes and provide functional evidence of enhanced mucoadhesion in the GI tract.

In the case of AMTs, some probiotic bacterial strains naturally exhibit mucoadhesive ability [128]. For instance, *Lactobacillus rhamnosus*, one of the well-studied lactic acid bacteria, has been shown to have unique pili that produce mucus-binding proteins, thereby enhancing its mucoadhesive function [129,130]. Mucoadhesive strategies can be implemented in AMTs to further improve their colonization and retention in the GI tract. Microbial cell-surface display systems, which allow for peptides and proteins to be displayed on the cell surface by fusing them with an anchoring motif, have been used for various biotechnological applications including vaccine development [131], antibody production [132], peptide library construction for screening [133], and biosensor development [134]. This has further been used in AMT applications to enable precise targeting to certain cells or tissues in situ in the context of IBD [135] and cancer [136]. Similarly, this concept can also be implemented in AMTs for improved mucoadhesion in the GI tract by displaying mucoadhesive, mucus-penetrating, or epithelial receptor-binding modalities on the surface of a bacterial or yeast cell. For instance, in one study [135], *S. boulardii* was engineered to bind to abundant extracellular matrix proteins in the GI tract through tunable antibody display. This design enabled an additional gut residence time and 100-fold increased CFUs of the engineered strains in the colon.

5.2. Cell-penetrating peptides for improved transepithelial transport

Cell-penetrating peptides (CPPs) are short peptides, typically 5–30 amino acids long, that enhance the delivery of therapeutic molecules by crossing epithelial cell barriers. These peptides can be derived from natural proteins, synthesized de novo, or engineered as chimeric constructs to optimize their properties [137]. Over 1,500 CPPs have been identified, showcasing a broad spectrum of sequences and structures facilitating their interaction with cell membranes. CPPs have been shown to mediate the uptake of diverse cargo including small molecules, nucleic acids, and proteins, and allow for either intracellular or transepithelial delivery [138,139]. Traditionally, CPPs have been studied for their ability to facilitate intracellular delivery by enhancing permeation across cell membranes. This mechanism often involves a combination of endocytosis and direct translocation, with pathways influenced by the peptide sequence, cell type, and environmental factors [140]. However, CPPs also exhibit the capacity to enhance transport across epithelial cell barriers [141–144], which is relevant for oral administration followed by delivery to the systemic circulation. In fact, CPPs have been shown to facilitate transport of peptides such as Exendin-4 [145], insulin [146,147], and parathyroid hormone, across the epithelium *in vitro* or *in vivo* in rodents. However, as discussed above, oral delivery of such peptides is limited due to degradation in the GI tract, but AMTs may offer an ideal platform for enhanced local in situ peptide production.

AMTs can be engineered to express CPPs and facilitate the transport of therapeutic peptides across the intestinal epithelium. In a recent study, *S. boulardii* was engineered to deliver CPPs in the GI tract of mice [148]. To assess its effect on macromolecule absorption, FITC-dextran (4 kDa), a model compound similar in size to GLP-1 agonists was administered orally due to its inert nature and measurable fluorescence. Increased systemic levels of FITC-dextran indicated enhanced intestinal absorption, thereby providing proof-of-concept for potentially using AMTs to enhance drug bioavailability. Nevertheless, while FITC-dextran is a useful model to illustrate the permeability enhancement, its representativeness is limited as it does not fully replicate the structure or biological activity of therapeutic peptides. Therefore, translating these findings to clinical applications necessitates caution and further studies should involve therapeutic peptides of interest to verify the absorption enhancement effects. As such, it may be attractive to explore the engineering of microbial strains that simultaneously express the CPPs and the therapeutic molecules or combine microbial strains expressing the two modalities. Another possibility would be to tailor-design fusions consisting of a combination of CPPs with therapeutic peptides or proteins without affecting their functional properties. Such strategies may offer new opportunities for design of AMTs. However, as the CPPs are non-specific to cell types and higher concentrations could lead to negative impact on host and gut microbiome [144].

5.3. Receptor-mediated transport

Receptor-mediated endocytosis is a crucial cellular process that enables the internalization of extracellular substances through specific receptor-ligand interactions, playing a key role in uptake of vitamins, hormones as well as transferrin [149]. Such natural transport pathways may be leveraged for cellular uptake following oral delivery of therapeutic peptides and proteins, facilitating transcellular transport across selective polarized intestinal epithelium and release into the systemic circulation [150]. Various nutritional receptors, particularly vitamin receptors, have been recognized for their potential in enhancing therapeutic delivery, as they actively facilitate the transport of essential vitamins across the GI tract [151]. For instance, targeting the receptors of vitamins B12 [152,153], folate [154], and biotin [155,156] have resulted in the improved oral delivery of therapeutic peptides including GLP-1 and insulin. Additionally, saccharide receptors, such as those for mannose [157], galactose [158], and hyaluronic acid [159], are present on intestinal epithelial cells and may facilitate mucoadhesion or the transport of the peptides if they are made to target these receptors [151]. One particularly promising target for receptor-mediated transcellular transport is the transferrin receptor (TfR), which is extensively expressed across the small intestine. TfR is responsible for the

internalization and transcytosis of transferrin-bound iron in a pH dependent manner, and this pathway may be explored to enhance transport of therapeutic peptides across the intestinal barrier [160]. For instance, the oral bioavailability of insulin was increased by 29.6 % in mice through the use of transferrin-coated nanoparticles [161].

Another attractive receptor is the neonatal Fc receptor (FcRn), which is broadly expressed by polarized epithelial cell layers where it mediates transcytosis of immunoglobulin G (IgG) and albumin across the mucosal barriers in a pH dependent manner [162,163]. Specifically, upon cellular uptake, FcRn engages the ligands simultaneously via non-overlapping binding sites within mildly acidic pH of endosomes that follows by transport to the opposite side of the cells where exposure to the neutral pH of the extracellular space triggers release of the ligands [162,164,165]. This pathway is an attractive gateway for mucosal delivery of intact IgG Fc and albumin fused therapeutic modalities upon intranasal or pulmonary administration [166–168]. As such, it may also be explored for transmucosal delivery of proteins engineered to engage FcRn in the context of AMTs. In addition, as FcRn acts as a homeostatic regulator of both IgG and albumin via a similar pH dependent cellular recycling mechanism [164,169], which results in a plasma half-life of 3 weeks at average in humans, proteins delivered to the circulatory system will have increased exposure, which will improve bioavailability. For instance, long-acting albumin (albiglutide) [170] and IgG1 Fc (dulaglutide) [171] fused GLP-1 have been reported. While oral delivery of such drugs is challenging due to degradation in the GI tract, AMTs could address stability concerns through *in situ* production.

To enable receptor targeting in AMTs, suitable receptor-ligand interactions can be identified through methods such as phage display [172], yeast surface display [173], or computational modeling to discover peptides with high affinity for intestinal receptors like FcRn or TfR. AMTs may be engineered to express peptides that mimic the ligands for the receptors as a strategy to enhance the intestinal absorption of the fused therapeutic peptides. Once a high-affinity peptide is identified it can be genetically fused to the therapeutic protein using linkers to preserve function and improve stability, followed by codon optimization for expression in microbial hosts. These gene constructs can then be introduced into plasmid vectors or the genome of the microbes. However, no study has so far investigated design of bacteria or yeast strains expressing peptides with such properties for mucosal delivery. However, it is well established that bacteria can express peptides and antibody-derived fragments, and when combined with FcRn-binding peptides or an albumin-derived peptide, which were recycled and transcytosed in an *in vitro* cellular system [174]. Similarly, yeast strains can be engineered to secrete albumin and fusions, which also show extended plasma half-life in human FcRn transgenic mice upon intravenous administration [175]. These strategies offer a modular framework for engineering AMTs that facilitate systemic entry of therapeutic peptides via receptor-mediated transport, thereby improving their bioavailability.

6. Clinical translational barriers and opportunities

Despite their promise, the clinical translation of orally administered AMTs/ eLBPs is limited by regulatory, manufacturing, and biological challenges. Regulatory uncertainty arises because AMTs represent a novel therapeutic class with minimal precedent. Manufacturing also presents major hurdles as AMTs must be produced at scale with consistent quality, formulated for long-term stability, and delivered orally without loss of viability. Developing cGMP-compliant production and formulation methods that ensure shelf-stable, room-temperature products without relying on cold storage is essential [176,177]. Furthermore, the biological complexity of host–microbiome interactions and disease pathology complicates AMT design and evaluation [178]. They face the highly variable GI environment and inter-patient differences in microbiota. A clear mechanistic understanding of disease and robust biomarkers are often lacking, making it difficult to predict and quantify therapeutic effects or define dose–response relationships [176,179]. Indeed, many AMT candidates that showed efficacy *in vitro* or in animal models have failed to translate similarly in humans, with clinical trials frequently being terminated for lack of efficacy [180]. This translational gap underscores the challenge of designing clinical trials for AMTs that must account for complex endpoints like microbial engraftment, host immunological changes, etc., and deal with high variability, all while maintaining rigorous controls and blinding.

Encouragingly, several strategies are being developed to overcome current barriers and accelerate the clinical translation of AMTs. Researchers are designing genetic circuits that enable precise, disease-responsive therapeutic production using validated biosensors and logic gates [181,182]. Biocontainment remains a major focus in the AMT design. Strategies such as kill switches and auxotrophic dependencies are being used to restrict survival outside the intended environment [179,183–185]. More advanced multilayered containment systems are being explored, including gene circuits that trigger self-destruction upon therapy completion or environmental escape [186]. Efforts are also underway to engineer obligate anaerobes and commensal strains better suited to colonize the human gut [187,188]. Physical containment through advanced delivery technologies such as pH-responsive capsules and magnet-guided systems adds another layer of targeting and safety, improving colonization and limiting off-target effects [189,190]. Complementing these experimental approaches are computational tools that can model gut physiology, microbial dynamics, and therapeutic kinetics. Integrating with multi-omics data and machine learning, these models could potentially enable *in silico* optimization of dosing, colonization, and efficacy, potentially making preclinical testing more predictive of human outcomes [191,192]. Finally, interdisciplinary collaboration is needed to address regulatory, clinical, and societal challenges. Continuous dialogue with regulators, more clinical successes, and transparent engagement with healthcare providers and patients are crucial to building trust in AMTs [193,194].

7. Conclusion and perspectives

AMTs represent a promising platform for oral delivery of therapeutic peptides and proteins and have the potential to be tailored for targeted delivery and enhanced bioavailability. By leveraging their ability to colonize, synthesize, and secrete the biologics in the GI tract, AMTs overcome some of the key challenges such as enzymatic degradation and limited stability. To date, AMTs have demonstrated encouraging preclinical success, particularly in the context of local inflammation such as IBD. AMTs have shown significant promise by enabling site-specific delivery of anti-inflammatory agents, resulting in improved therapeutic efficacy and reduced systemic side effects. However, their application in treating chronic metabolic disorders has been limited by the poor absorption of therapeutics into the systemic circulation. These challenges could potentially be addressed by integrating pharmaceutical strategies like the use of mucoadhesive strategies, CPPs, and receptor-mediated transport. However, the clinical translation of AMTs remains largely untapped with most of the work confined to early development and their broader therapeutic potential not yet fully realized. Key challenges to improving the safety and efficacy of AMTs include limited understanding and control over their localization, residence time, and dosing dynamics within the GI tract. Addressing these challenges will require more rigorous and standardized study designs, aimed at characterizing the *in situ* behaviour of AMTs such as their colonization, persistence, and clearance alongside the pharmacokinetics of the therapeutic payload, including its absorption, distribution, metabolism, and excretion, where relevant. To accelerate this process, predictive modeling approaches that integrate gut physiology with microbial growth and metabolism could enable rapid *in silico* testing of genetic and delivery strategies prior to experimental validation. These computational tools may guide the rational design of AMTs with improved performance characteristics. Building on these insights, optimization of microbial chassis for enhanced therapeutic expression, secretion, and

regional targeting within the GI tract will be essential for increasing the precision and applicability of AMTs. Finally, patient and environmental safety considerations must be addressed, particularly about long-term microbial stability, immunogenicity, and unintended off-target effects. Robust biocontainment strategies such as kill-switch mechanisms and tightly regulated expression systems will be vital to minimize biosafety risks. Overall, with continued advances in microbial engineering and integration with pharmaceutical delivery strategies, AMTs can evolve into a clinically viable platform for both local and systemic delivery of peptide and protein therapeutics. Further research will be essential to translate these promising concepts into safe and effective therapies.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT-4.5 to improve the readability and language of the manuscript for some parts. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

S.T.B is an employee at Novo Nordisk and holds shares in the company. J.T.A has ownership interests in Authera AS and is an inventor of the inventions claimed in patent families arising from WO2017158426, WO2019057564, and WO2015063611A2. M.O.A.S is a co-founder and board member of Clinical-Microbiomics and SNIPR Biome, and a board member of Novonesis. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funded by The Novo Nordisk Foundation under NNF grant number: NNF20CC0035580 NNF Challenge program CAMiT under Grant agreement: NNF17C0028232.

Data availability

No data was used for the research described in the article.

References

[1] A.L. Smart, S. Gaisford, A.W. Basit, Oral peptide and protein delivery: intestinal obstacles and commercial prospects, *Expert Opin. Drug Deliv.* 11 (2014) 1323–1335.
[2] M. Goldberg, I. Gomez-Orellana, Challenges for the oral delivery of macromolecules, *Nat. Rev. Drug Discov.* 2 (2003) 289–295.
[3] J.M. Harris, R.B. Chess, Effect of pegylation on pharmaceuticals, *Nat. Rev. Drug Discov.* 2 (2003) 214–221.
[4] D.J. Drucker, Advances in oral peptide therapeutics, *Nat. Rev. Drug Discov.* 19 (2020) 277–289.
[5] L.M. Ensign, R. Cone, J. Hanes, Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers, *Adv. Drug Deliv. Rev.* 64 (2012) 557–570.
[6] Y. Yang, et al., Recent advances in oral and transdermal protein delivery systems, *Angew. Chem.* 135 (2023).
[7] J. Byrne, et al., Devices for drug delivery in the gastrointestinal tract: a review of systems physically interacting with the mucosa for enhanced delivery, *Adv. Drug Deliv. Rev.* 177 (2021) 113926.
[8] V. Truong-Le, P.M. Lovalenti, A.M. Abdul-Fattah, Stabilization challenges and formulation strategies associated with oral biologic drug delivery systems, *Adv. Drug Deliv. Rev.* 93 (2015) 95–108.
[9] T.A. Al-Hilal, F. Alam, Y. Byun, Oral drug delivery systems using chemical conjugates or physical complexes, *Adv. Drug Deliv. Rev.* 65 (2013) 845–864.
[10] B. Homayun, X. Lin, H.-J. Choi, Challenges and recent progress in oral drug delivery systems for biopharmaceuticals, *Pharmaceutics* 11 (2019) 129.
[11] R. Vazquez-Uribe, K.A. Hedin, T.R. Licht, M. Nieuwdorp, M.O.A. Sommer, Advanced microbiome therapeutics as a novel modality for oral delivery of peptides to manage metabolic diseases, *Trends Endocrinol. Metab.* (2024), https://doi.org/10.1016/j.tem.2024.04.021.
[12] G. Samonis, et al., *Saccharomyces boulardii* and *Candida albicans* experimental colonization of the murine gut, *Med. Mycol.* 49 (2011) 395–399.
[13] K.A. Hedin, et al., Effects of broad-spectrum antibiotics on the colonisation of probiotic yeast Saccharomyces boulardii in the murine gastrointestinal tract, *Sci. Rep.* 12 (2022) 8862.
[14] N.S. Dosoky, L.S. May-Zhang, S.S. Davies, Engineering the gut microbiota to treat chronic diseases, *Appl. Microbiol. Biotechnol.* 104 (2020) 7657–7671.
[15] L. Steidler, et al., Treatment of Murine Colitis by *Lactococcus lactis* Secreting Interleukin-10, *Science* 1979 (289) (2000) 1352–1355.
[16] H. Braat, et al., A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease, *Clin. Gastroenterol. Hepatol.* 4 (2006) 754–759.
[17] F.F. Duan, J.H. Liu, J.C. March, Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes, *Diabetes* 64 (2015) 1794–1803.
[18] P. Agarwal, P. Khatri, B. Billack, W.-K. Low, J. Shao, Oral delivery of glucagon like peptide-1 by a recombinant lactococcus lactis, *Pharm. Res.* 31 (2014) 3404–3414.
[19] T. Arora, et al., Microbially produced glucagon-like peptide 1 improves glucose tolerance in mice, *Mol. Metab.* 5 (2016) 725–730.
[20] Y. Han, et al., Multifunctional oral delivery systems for enhanced bioavailability of therapeutic peptides/proteins, *Acta Pharm. Sin. B* 9 (2019) 902–922.
[21] Y.-B. Miao, et al., Cracking the intestinal lymphatic system window utilizing oral delivery vehicles for precise therapy, *J. Nanobiotechnol.* 21 (2023) 263.
[22] S.K. Dubey, et al., Oral peptide delivery: challenges and the way ahead, *Drug Discov. Today* 26 (2021) 931–950.
[23] P. Tyagi, S. Pechenov, J. Anand Subramony, Oral peptide delivery: Translational challenges due to physiological effects, *J. Control. Rel.* 287 (2018) 167–176.
[24] D.F. Evans, et al., Measurement of gastrointestinal pH profiles in normal ambulant human subjects, *Gut* 29 (1988) 1035–1041.
[25] J. Fallingborg, Intraluminal pH of the human gastrointestinal tract, *Dan. Med. Bull.* 46 (1999) 183–196.
[26] G. Chen, W. Kang, W. Li, S. Chen, Y. Gao, Oral delivery of protein and peptide drugs: from non-specific formulation approaches to intestinal cell targeting strategies, *Theranostics* 12 (2022) 1419–1439.
[27] P. Langguth, et al., The challenge of proteolytic enzymes in intestinal peptide delivery, *J. Control. Release* 46 (1997) 39–57.
[28] S.T. Buckley, et al., Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist, *Sci. Transl. Med.* 10 (2018).
[29] J. Wang, V. Yadav, A.L. Smart, S. Tajiri, A.W. Basit, Toward oral delivery of biopharmaceuticals: an assessment of the gastrointestinal stability of 17 peptide drugs, *Mol. Pharm.* 12 (2015) 966–973.
[30] K. Ikesue, P. Kopečkovà, J. Kopeček, Degradation of proteins by guinea pig intestinal enzymes, *Int. J. Pharm.* 95 (1993) 171–179.
[31] M. Boegh, H.M. Nielsen, Mucus as a barrier to drug delivery – understanding and mimicking the barrier properties, *Basic Clin. Paharmacol. Toxicol.* 116 (2015) 179–186.
[32] M.E.V. Johansson, K.A. Thomsson, G.C. Hansson, Proteomic analyses of the two mucus layers of the colon barrier reveal that Their main component, the muc2 mucin, is strongly bound to the Fcgbp protein, *J. Proteome Res.* 8 (2009) 3549–3557.
[33] A. Bernkop-Schnürch, F. R., Investigations into the diffusion behaviour of polypeptides in native intestinal mucus with regard to their peroral administration, *Pharm. Pharmacol. Commun.* 2 (1996) 361–363.
[34] L.D. Li, et al., Spatial configuration and composition of charge modulates transport into a mucin hydrogel barrier, *Biophys. J.* 105 (2013) 1357–1365.
[35] G. Flemström, et al., Adherent surface mucus gel restricts diffusion of macromolecules in rat duodenum in vivo, *Am. J. Physiology-Gastrointestinal and Liver Physiol.* 277 (1999) G375–G382.
[36] M. Laksitorini, V.D. Prasasty, P.K. Kiptoo, T.J. Siahaan, Pathways and progress in improving drug delivery through the intestinal mucosa and blood–brain barriers, *Ther. Deliv.* 5 (2014) 1143–1163.
[37] J. Brunner, S. Ragupathy, G. Borchard, Target specific tight junction modulators, *Adv. Drug Deliv. Rev.* 171 (2021) 266–288.
[38] P. Lundquist, P. Artursson, Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations and studies in human tissues, *Adv. Drug Deliv. Rev.* 106 (2016) 256–276.
[39] Yang, N. J. & Hinner, M. J. Getting Across the Cell Membrane: An Overview for Small Molecules, Peptides, and Proteins. in 29–53 (2015). doi:10.1007/978-1-4939-2272-7_3.
[40] M. Kristensen, H.M. Nielsen, Cell-penetrating peptides as carriers for oral delivery of biopharmaceuticals, *Basic Clin. Paharmacol. Toxicol.* 118 (2016) 99–106.
[41] N. Salamat-Miller, T.P. Johnston, Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium, *Int. J. Pharm.* 294 (2005) 201–216.
[42] B. Lee, K.M. Moon, C.Y. Kim, Tight junction in the intestinal epithelium: its association with diseases and regulation by phytochemicals, *J. Immunol. Res.* 2018 (2018) 1–11.
[43] J. Renukuntla, A.D. Vadlapudi, A. Patel, S.H.S. Boddu, A.K. Mitra, Approaches for enhancing oral bioavailability of peptides and proteins, *Int. J. Pharm.* 447 (2013) 75–93.
[44] Z. Antosova, M. Mackova, V. Kral, T. Macek, Therapeutic application of peptides and proteins: parenteral forever? *Trends Biotechnol.* 27 (2009) 628–635.
[45] V. Maini Rekdal, E.N. Bess, J.E. Bisanz, P.J. Turnbaugh, E.P. Balskus, Discovery and inhibition of an interspecies gut bacterial pathway for Levodopa metabolism, *Science* 1979 (364) (2019).

[46] K.G. Jameson, E.Y. Hsiao, A novel pathway for microbial metabolism of levodopa, *Nat. Med.* 25 (2019) 1195–1197.
[47] M. Zimmermann, M. Zimmermann-Kogadeeva, R. Wegmann, A.L. Goodman, Mapping human microbiome drug metabolism by gut bacteria and their genes, *Nature* 570 (2019) 462–467.
[48] S. Tuvia, et al., Oral octreotide absorption in human subjects: comparable pharmacokinetics to parenteral octreotide and effective growth hormone suppression, *J. Clin. Endocrinol. Metab.* 97 (2012) 2362–2369.
[49] L.E. McCoubrey, et al., Colonic drug delivery: formulating the next generation of colon-targeted therapeutics, *J. Control. Release* 353 (2023) 1107–1126.
[50] T.H. Vaaben, R. Vazquez-Uribe, M.O.A. Sommer, Characterization of eight bacterial biosensors for microbial diagnostic and therapeutic applications, *ACS Synth. Biol.* 11 (2022) 4184–4192.
[51] F. Wu, T. Jin, Polymer-based sustained-release dosage forms for protein drugs, challenges, and recent advances, *AAPS Pharm. Sci. Tech.* 9 (2008) 1218–1229.
[52] K. Wadher, M. Umekar, R. Kakde, Formulation and evaluation of a sustained-release tablets of metformin hydrochloride using hydrophilic synthetic and hydrophobic natural polymers, *Indian J. Pharm. Sci.* 73 (2011) 208.
[53] A. Bose, T.W. Wong, N. Singh, Formulation development and optimization of sustained release matrix tablet of Itopride HCl by response surface methodology and its evaluation of release kinetics, *Saudi Pharma. J.* 21 (2013) 201–213.
[54] N. Reddy, R. Reddy, Q. Jiang, Crosslinking biopolymers for biomedical applications, *Trends Biotechnol.* 33 (2015) 362–369.
[55] G. Jiang, Assessment of protein release kinetics, stability and protein polymer interaction of lysozyme encapsulated poly(?,?-lactide-co-glycolide) microspheres, *J. Control. Release* 79 (2002) 137–145.
[56] T.G. Park, W. Lu, G. Crotts, Importance of in vitro experimental conditions on protein release kinetics, stability and polymer degradation in protein encapsulated poly (d,l-lactic acid-co-glycolic acid) microspheres, *J. Control. Release* 33 (1995) 211–222.
[57] A. Braun, L. Kwee, M.A. Labow, J. Alsenz, Protein aggregates seem to play a key role among the parameters influencing the antigenicity of interferon alpha (IFN-alpha) in normal and transgenic mice, *Pharm. Res.* 14 (1997) 1472–1478.
[58] H. Schellekens, N. Casadevall, Immunogenicity of recombinant human proteins: causes and consequences, *J. Neurol.* 251 (2004).
[59] M.S. Bienick, et al., The interrelationship between promoter strength, gene expression, and growth rate, *PLoS One* 9 (2014) e109105.
[60] H. Redden, N. Morse, H.S. Alper, The synthetic biology toolbox for tuning gene expression in yeast, *FEMS Yeast Res.* (2014), https://doi.org/10.1111/1567-1364.12188.
[61] J. Armetta, et al., *Escherichia coli* promoters with consistent expression throughout the murine gut, *ACS Synth. Biol.* 10 (2021) 3359–3368.
[62] C. Sands, K.A. Hedin, R. Vazquez-Uribe, M.O.A. Sommer, Saccharomyces boulardii promoters for control of gene expression in vivo, *Microb. Cell Fact.* 23 (2024) 16.
[63] P.R. Jensen, K. Hammer, The sequence of spacers between the consensus sequences modulates the strength of prokaryotic promoters, *Appl. Environ. Microbiol.* 64 (1998) 82–87.
[64] D. Durmusoglu, et al., Improving therapeutic protein secretion in the probiotic yeast Saccharomyces boulardii using a multifactorial engineering approach, *Microb. Cell Fact.* 22 (2023) 109.
[65] F.N. Santos-Navarro, A. Vignoni, Y. Boada, J. Picó, RBS and promoter strengths determine the cell-growth-dependent protein mass fractions and their optimal synthesis rates, *ACS Synth. Biol.* 10 (2021) 3290–3303.
[66] S. Oesterle, D. Gerngross, S. Schmitt, T.M. Roberts, S. Panke, Efficient engineering of chromosomal ribosome binding site libraries in mismatch repair proficient Escherichia coli, *Sci. Rep.* 7 (2017) 12327.
[67] I. Farasat, et al., Efficient search, mapping, and optimization of multi-protein genetic systems in diverse bacteria, *Mol. Syst. Biol.* 10 (2014).
[68] H.M. Salis, E.A. Mirsky, C.A. Voigt, Automated design of synthetic ribosome binding sites to control protein expression, *Nat. Biotechnol.* 27 (2009) 946–950.
[69] F. de Felippes, et al., The key role of terminators on the expression and post-transcriptional gene silencing of transgenes, *Plant J.* 104 (2020) 96–112.
[70] G. Kaur, et al., Oral controlled and sustained drug delivery systems, in: *Drug Targeting and Stimuli Sensitive Drug Delivery Systems*, Elsevier, 2018, pp. 567–626, https://doi.org/10.1016/B978-0-12-813689-8.00015-X.
[71] S. Adepu, S. Ramakrishna, Controlled drug delivery systems: current status and future directions, *Molecules* 26 (2021) 5905.
[72] E. Pedone, et al., A tunable dual-input system for on-demand dynamic gene expression regulation, *Nat. Commun.* 10 (2019) 4481.
[73] L.E. Ulrich, E.V. Koonin, I.B. Zhulin, One-component systems dominate signal transduction in prokaryotes, *Trends Microbiol.* 13 (2005) 52–56.
[74] F. Jacob-Dubuisson, A. Mechaly, J.-M. Betton, R. Antoine, Structural insights into the signalling mechanisms of two-component systems, *Nat. Rev. Microbiol.* 16 (2018) 585–593.
[75] Z.-P. Zou, et al., Genetically engineered bacteria as inflammatory bowel disease therapeutics, *Eng. Microbiol.* 4 (2024) 100167.
[76] B.M. Scott, et al., Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease, *Nat. Med.* 27 (2021) 1212–1222.
[77] R. McKay, et al., A platform of genetically engineered bacteria as vehicles for localized delivery of therapeutics: toward applications for Crohn's disease, *Bioeng. Transl. Med.* 3 (2018) 209–221.
[78] K.J. Chua, et al., An engineered probiotic produces a type III interferon IFNL1 and reduces inflammations in *in vitro* inflammatory bowel disease models, *ACS Biomater. Sci. Eng.* 9 (2023) 5123–5135.
[79] B. Benbouziane, et al., Development of a Stress-Inducible Controlled Expression (SICE) system in Lactococcus lactis for the production and delivery of therapeutic molecules at mucosal surfaces, *J. Biotechnol.* 168 (2013) 120–129.
[80] N. Saeidi, et al., Engineering microbes to sense and eradicate *Pseudomonas aeruginosa* , a human pathogen, *Mol. Syst. Biol.* 7 (2011).
[81] M. Liu, et al., Oral engineered Bifidobacterium longum expressing rhMnSOD to suppress experimental colitis, *Int. Immunopharmacol.* 57 (2018) 25–32.
[82] L. Watterlot, et al., Intragastric administration of a superoxide dismutase-producing recombinant Lactobacillus casei BL23 strain attenuates DSS colitis in mice, *Int. J. Food Microbiol.* 144 (2010) 35–41.
[83] P. Praveschotinunt, et al., Engineered E. coli Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut, *Nat. Commun.* 10 (2019) 5580.
[84] K.A. Hedin, et al., Cold exposure and oral delivery of GLP-1R agonists by an engineered probiotic yeast strain have antiobesity effects in mice, *ACS Synth. Biol.* 12 (2023) 3433–3442.
[85] D.P. Cook, et al., Intestinal delivery of proinsulin and IL-10 via lactococcus lactis combined with low-dose anti-CD3 restores tolerance outside the window of acute type 1 diabetes diagnosis, *Front. Immunol.* 11 (2020).
[86] C.-H. Liu, J.-H. Chang, Y.-C. Chang, K.Y. Mou, Treatment of murine colitis by Saccharomyces boulardii secreting atrial natriuretic peptide, *J. Mol. Med.* 98 (2020) 1675–1687.
[87] J. Ma, C. Li, J. Wang, J. Gu, Genetically engineered *escherichia coli* nissle 1917 secreting GLP-1 analog exhibits potential antiobesity effect in high-fat diet-induced obesity mice, *Obesity* 28 (2020) 315–322.
[88] J.P. Lynch, et al., Engineered Escherichia coli for the in situ secretion of therapeutic nanobodies in the gut, *Cell Host Microbe* 31 (2023) 634–649.e8.
[89] L. Song, et al., Oral delivery of a Lactococcus lactis strain secreting bovine lactoferricin–lactoferrampin alleviates the development of acute colitis in mice, *Appl. Microbiol. Biotechnol.* 103 (2019) 6169–6186.
[90] K. Vandenbroucke, et al., Active delivery of trefoil factors by genetically modified Lactococcus lactis prevents and heals acute colitis in mice, *Gastroenterology* 127 (2004) 502–513.
[91] M.J. Chiabai, et al., Mucosal delivery of Lactococcus lactis carrying an anti-TNF scFv expression vector ameliorates experimental colitis in mice, *BMC Biotech.* 19 (2019) 38.
[92] R.T. Long, et al., Bifidobacterium as an oral delivery carrier of oxyntomodulin for obesity therapy: inhibitory effects on food intake and body weight in overweight mice, *Int. J. Obes.* 34 (2010) 712–719.
[93] K. Chen, et al., A probiotic yeast-based immunotherapy against *Clostridioides difficile* infection, *Sci. Transl. Med.* 12 (2020).
[94] V.M. Isabella, et al., Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria, *Nat. Biotechnol.* 36 (2018) 857–864.
[95] V. Iannone, et al., Changes in liver metabolic pathways demonstrate efficacy of the combined dietary and microbial therapeutic intervention in MASLD mouse model, *Mol. Metab.* 78 (2023) 101823.
[96] Y. Wang, et al., A bioengineered probiotic for the oral delivery of a peptide Kv1.3 channel blocker to treat rheumatoid arthritis. *Proceedings of the National Academy of Sciences*, 2023.
[97] Y. Lin, et al., Oral delivery of pentameric glucagon-like peptide-1 by recombinant lactobacillus in diabetic rats, *PLoS One* 11 (2016) e0162733.
[98] T. Zenlea, M.A. Peppercorn, Immunosuppressive therapies for inflammatory bowel disease, *World J. Gastroenterol.* 20 (2014) 3146–3152.
[99] K. Vandenbroucke, et al., Orally administered L. lactis secreting an anti-TNF Nanobody demonstrate efficacy in chronic colitis, *Mucosal Immunol.* 3 (2010) 49–56.
[100] M.L. Hanson, et al., Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice, *Gastroenterology* 146 (2014) 210–221.e13.
[101] M. Pesce, et al., Next-generation probiotics for inflammatory bowel disease, *Int. J. Mol. Sci.* 23 (2022) 5466.
[102] L. Zhang, et al., Tf ligand-receptor-mediated exenatide-Zn $^{2+}$ complex oral-delivery system for penetration enhancement of exenatide, *J. Drug Target.* 26 (2018) 931–940.
[103] Y. Song, et al., Oral delivery system for low molecular weight protamine-dextran-poly(lactic-co-glycolic acid) carrying exenatide to overcome the mucus barrier and improve intestinal targeting efficiency, *Nanomedicine* 14 (2019) 989–1009.
[104] S. Pinto, et al., Nanoparticles targeting the intestinal Fc receptor enhance intestinal cellular trafficking of semaglutide, *J. Control. Release* 366 (2024) 621–636.
[105] N. Shrestha, et al., Oral hypoglycaemic effect of GLP-1 and DPP4 inhibitor based nanocomposites in a diabetic animal model, *J. Control. Release* 232 (2016) 113–119.
[106] Q. Zhang, et al., A silica-based pH-sensitive nanomatrix system improves the oral absorption and efficacy of incretin hormone glucagon-like peptide-1, *Int. J. Nanomed.* 4983 (2012), https://doi.org/10.2147/IJN.S30013.
[107] R.V. Overgaard, A. Navarria, S.H. Ingwersen, T.A. Bækdal, R.J. Kildemoes, Clinical pharmacokinetics of oral semaglutide: analyses of data from clinical pharmacology trials, *Clin. Pharmacokinet.* 60 (2021) 1335–1348.
[108] F. Duan, K.L. Curtis, J.C. March, Secretion of insulinotropic proteins by commensal bacteria: rewiring the gut to treat diabetes, *Appl. Environ. Microbiol.* 74 (2008) 7437–7438.
[109] T. Chen, et al., Engineered commensal bacteria prevent systemic inflammation-induced memory impairment and amyloidogenesis via producing GLP-1, *Appl. Microbiol. Biotechnol.* 102 (2018) 7565–7575.
[110] X. Fang, et al., Therapeutic effect of GLP-1 engineered strain on mice model of Alzheimer's disease and Parkinson's disease, *AMB Express* 10 (2020) 80.

[111] X. Fang, P. Tian, X. Zhao, C. Jiang, T. Chen, Neuroprotective effects of an engineered commensal bacterium in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine Parkinson disease mouse model via producing glucagon-like peptide-1, *J. Neurochem.* 150 (2019) 441–452.

[112] L. Wang, et al., Engineered bacteria of MG1363-pMG36e-GLP-1 attenuated obesity-induced by high fat diet in mice, *Front. Cell. Infect. Microbiol.* 11 (2021).

[113] J. Luo, H. Zhang, J. Lu, C. Ma, T. Chen, Antidiabetic effect of an engineered bacterium Lactobacillus plantarum-pMG36e -GLP-1 in monkey model, *Synth. Syst. Biotechnol.* 6 (2021) 272–282.

[114] H. Hu, et al., Improvement effect of a next-generation probiotic *L. plantarum* -pMG36e-GLP-1 on type 2 diabetes mellitus *via* the gut–pancreas–liver axis, *Food Funct.* 14 (2023) 3179–3195.

[115] H. Wu, et al., Neuroprotective effects of an engineered *Escherichia coli* Nissle 1917 on Parkinson's disease in mice by delivering <scp>GLP</scp> -1 and modulating gut microbiota, *Bioeng. Transl. Med.* 8 (2023).

[116] X. Wang, et al., Engineered probiotics Clostridium butyricum <scp>-pMTL007-GLP</scp> -1 improves blood pressure via producing <scp>GLP</scp> -1 and modulating gut microbiota in spontaneous hypertension rat models, *J. Microbial. Biotechnol.* 16 (2023) 799–812.

[117] X. Zhang, et al., A micro-nano optogenetic system based on probiotics for in situ host metabolism regulation, *Nano Res.* 16 (2023) 2829–2839.

[118] Everard, A., Matamoros, S., Geurts, L., Delzenne, N. M. & Cani, P. D. *Saccharomyces boulardii* Administration Changes Gut Microbiota and Reduces Hepatic Steatosis, Low - Grade Inflammation, and Fat Mass in Obese and Type 2 Diabetic *db / db* Mice. *mBio* **5**, (2014).

[119] L. Diao, B. Meibohm, Pharmacokinetics and pharmacokinetic–pharmacodynamic correlations of therapeutic peptides, *Clin. Pharmacokinet.* 52 (2013) 855–868.

[120] J. Luo, et al., Administration of a next-generation probiotic *escherichia coli* nissle 1917-GLP-1 alleviates diabetes in mice with type 1 and type 2 diabetes, *Can. J. Infect. Dis. Med. Microbiol.* 2025 (2025).

[121] R. Maheshwari, et al., Drug Disposition Considerations in Pharmaceutical Product, in: *Dosage Form Design Considerations*, Elsevier, 2018, pp. 337–369, https://doi.org/10.1016/B978-0-12-814423-7.00010-1.

[122] M.E.V. Johansson, Fast renewal of the distal colonic mucus layers by the surface goblet cells as measured by in vivo labeling of mucin glycoproteins, *PLoS One* 7 (2012) e41009.

[123] N. Navabi, M.E.V. Johansson, S. Raghavan, S.K. Lindén, Helicobacter pylori infection impairs the mucin production rate and turnover in the murine gastric mucosa, *Infect. Immun.* 81 (2013) 829–837.

[124] H. Schneider, T. Pelaseyed, F. Svensson, M.E.V. Johansson, Study of mucin turnover in the small intestine by in vivo labeling, *Sci. Rep.* 8 (2018) 5760.

[125] G.L. Eastwood, Gastrointestinal epithelial renewal, *Gastroenterology* (1977) 962–975.

[126] L. Etienne-Mesmin, et al., Experimental models to study intestinal microbes–mucus interactions in health and disease, *FEMS Microbiol. Rev.* 43 (2019) 457–489.

[127] M.-L. Foucault, L. Thomas, S. Goussard, B.R. Branchini, C. Grillot-Courvalin, In vivo bioluminescence imaging for the study of intestinal colonization by *escherichia coli* in mice, *Appl. Environ. Microbiol.* 76 (2010) 264–274.

[128] M.L. Van Tassell, M.J. Miller, Lactobacillus adhesion to mucus, *Nutrients* 3 (2011) 613–636.

[129] I. von Ossowski, et al., Mucosal adhesion properties of the probiotic *lactobacillus rhamnosus* GG SpaCBA and SpaFED Pilin subunits, *Appl. Environ. Microbiol.* 76 (2010) 2049–2057.

[130] M. Kankainen, et al., Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human- mucus binding protein, *Proc. Natl. Acad. Sci.* 106 (2009) 17193–17198.

[131] J.-S. Lee, K.-S. Shin, J.-G. Pan, C.-J. Kim, Surface-displayed viral antigens on Salmonella carrier vaccine, *Nat. Biotechnol.* 18 (2000) 645–648.

[132] P. Martineau, et al., A genetic system to elicit and monitor anti-peptide antibodies without peptide synthesis, *Nat. Biotechnol.* 9 (1991) 170–172.

[133] B. Westerlund-Wikstrom, et al., Functional expression of adhesive peptides as fusions to Escherichia coli flagellin, *Protein Eng. Des. Sel.* 10 (1997) 1319–1326.

[134] J.K. Dhillon, P.D. Drew, A.J.R. Porter, Bacterial surface display of an anti-pollutant antibody fragment, *Lett. Appl. Microbiol.* 28 (1999) 350–354.

[135] M.K. Heavey, et al., Targeted delivery of the probiotic Saccharomyces boulardii to the extracellular matrix enhances gut residence time and recovery in murine colitis, *Nat. Commun.* 15 (2024) 3784.

[136] S.-H. Park, et al., RGD peptide cell-surface display enhances the targeting and therapeutic efficacy of attenuated *salmonella* -mediated cancer therapy, *Theranostics* 6 (2016) 1672–1682.

[137] S.G. Patel, et al., Cell-penetrating peptide sequence and modification dependent uptake and subcellular distribution of green florescent protein in different cell lines, *Sci. Rep.* 9 (2019) 6298.

[138] P. Agrawal, et al., CPPsite 2.0: a repository of experimentally validated cell-penetrating peptides, *Nucleic Acids Res.* 44 (2016) D1098–D1103.

[139] B.R. Liu, S.-H. Chiou, Y.-W. Huang, H.-J. Lee, Bio-membrane internalization mechanisms of arginine-rich cell-penetrating peptides in various species, *Membranes (Basel)* 12 (2022) 88.

[140] S. El-Andaloussi, T. Holm, U. Langel, Cell-penetrating peptides: mechanisms and applications, *Curr. Pharm. Des.* 11 (2005) 3597–3611.

[141] R.G. Diedrichsen, et al., Revealing the importance of carrier-cargo association in delivery of insulin and lipidated insulin, *J. Control. Release* 338 (2021) 8–21.

[142] M. Kristensen, R. Guldsmed Diedrichsen, V. Vetri, V. Foderà, H. Mørck Nielsen, Increased carrier peptide stability through ph adjustment improves insulin and PTH(1-34) delivery in vitro and in vivo rather than by enforced carrier peptide-cargo complexation, *Pharmaceutics* 12 (2020) 993.

[143] K. Ohtake, H. Natsume, H. Ueda, Y. Morimoto, Analysis of transient and reversible effects of poly-l-arginine on the in vivo nasal absorption of FITC-dextran in rats, *J. Control. Release* 82 (2002) 263–275.

[144] H.P. Gelli, R. Vazquez-Uribe, M.O.A. Sommer, Screening for effective cell-penetrating peptides with minimal impact on epithelial cells and gut commensals in vitro, *Front. Pharmacol.* 13 (2022).

[145] N. Kamei, et al., Effective nose-to-brain delivery of exendin-4 via coadministration with cell-penetrating peptides for improving progressive cognitive dysfunction, *Sci. Rep.* 8 (2018) 17641.

[146] E.J.B. Nielsen, et al., In vivo proof of concept of oral insulin delivery based on a co-administration strategy with the cell-penetrating peptide penetratin, *J. Control. Release* 189 (2014) 19–24.

[147] N. Kamei, et al., Usefulness of cell-penetrating peptides to improve intestinal insulin absorption, *J. Control. Release* 132 (2008) 21–25.

[148] H.P. Gelli, K.A. Hedin, M.F. Laursen, R.-V. Uribe, M.O.A. Sommer, Enhancing intestinal absorption of a macromolecule through engineered probiotic yeast in the murine gastrointestinal tract, *Trends Biotechnol.* (2024), https://doi.org/10.1016/j.tibtech.2024.10.019.

[149] Mani, I. & Singh, V. An overview of receptor endocytosis and signaling. in 1–18 (2023). doi:10.1016/bs.pmbts.2022.06.018.

[150] M.M. Attwood, J. Jonsson, M. Rask-Andersen, H.B. Schiöth, Soluble ligands as drug targets, *Nat. Rev. Drug Discov.* 19 (2020) 695–710.

[151] Q. Zhu, et al., Oral delivery of proteins and peptides: challenges, status quo and future perspectives, *Acta Pharm. Sin. B* 11 (2021) 2416–2448.

[152] K.B. Chalasani, G.J. Russell-Jones, A.K. Jain, P.V. Diwan, S.K. Jain, Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles, *J. Control. Release* 122 (2007) 141–150.

[153] J. Alsenz, G.J. Russell-Jones, S. Westwood, B. Levet-Trafit, P.C. de Smidt, Oral absorption of peptides through the cobalamin (vitamin B12) pathway in the rat intestine, *Pharm. Res.* 17 (2000) 825–832.

[154] J. Li, et al., An efficient controlled release strategy for hypertension therapy: folate-mediated lipid nanoparticles for oral peptide delivery, *Pharmacol. Res.* 157 (2020) 104796.

[155] Y. Youn, Improved peroral delivery of glucagon-like peptide-1 by site-specific biotin modification: design, preparation, and biological evaluation, *Eur. J. Pharm. Biopharm.* 68 (2008) 667–675.

[156] X. Zhang, et al., Biotinylated liposomes as potential carriers for the oral delivery of insulin, *Nanomedicine* 10 (2014) 167–176.

[157] H. Poursadegh, et al., Incorporating mannose-functionalized hydroxyapatite/metal-organic framework into the hyaluronic acid hydrogel film: a potential dual-targeted oral anticancer delivery system, *Int. J. Biol. Macromol.* 274 (2024) 133516.

[158] A. Jain, et al., Galactose engineered solid lipid nanoparticles for targeted delivery of doxorubicin, *Colloids Surf. B Biointerf.* 134 (2015) 47–58.

[159] Y. Wu, et al., Hyaluronic acid nanoparticles for targeted oral delivery of doxorubicin: lymphatic transport and CD44 engagement, *Int. J. Biol. Macromol.* 273 (2024) 133063.

[160] N. Amet, W. Wang, W.-C. Shen, Human growth hormone–transferrin fusion protein for oral delivery in hypophysectomized rats, *J. Control. Release* 141 (2010) 177–182.

[161] J.-J. Zou, et al., Efficient oral insulin delivery enabled by transferrin-coated acid-resistant metal-organic framework nanoparticles, *Sci. Adv.* 8 (2022).

[162] M. Bern, et al., An engineered human albumin enhances half-life and transmucosal delivery when fused to protein-based biologics, *Sci. Transl. Med.* 12 (2020).

[163] G.M. Spiekermann, et al., Receptor-mediated immunoglobulin g transport across mucosal barriers in adult life, *J. Exp. Med.* 196 (2002) 303–310.

[164] M. Pyzik, L.K. Kozicky, A.K. Gandhi, R.S. Blumberg, The therapeutic age of the neonatal Fc receptor, *Nat. Rev. Immunol.* 23 (2023) 415–432.

[165] E.S. Ward, R.J. Ober, Targeting FcRn to generate antibody-based therapeutics, *Trends Pharmacol. Sci.* 39 (2018) 892–904.

[166] W. Li, et al., An FcRn-targeted mucosal vaccine against SARS-CoV-2 infection and transmission, *Nat. Commun.* 14 (2023) 7114.

[167] J.A. Dumont, et al., Delivery of an erythropoietin-fc fusion protein by inhalation in humans through an immunoglobulin transport pathway, *J. Aerosol Med.* 18 (2005) 294–303.

[168] A.J. Bitonti, et al., Pulmonary delivery of an erythropoietin Fc fusion protein in non-human primates through an immunoglobulin transport pathway, *Proc. Natl. Acad. Sci.* 101 (2004) 9763–9768.

[169] C. Chaudhury, et al., The major histocompatibility complex–related Fc Receptor for IgG (FcRn) binds albumin and prolongs its lifespan, *J. Exp. Med.* 197 (2003) 315–322.

[170] E.L. St Onge, S.A. Miller, Albiglutide: a new GLP-1 analog for the treatment of type 2 diabetes, *Expert Opin. Biol. Ther.* 10 (2010) 801–806.

[171] E. Jimenez-Solem, M.H. Rasmussen, M. Christensen, F.K. Knop, Dulaglutide, a long-acting GLP-1 analog fused with an Fc antibody fragment for the potential treatment of type 2 diabetes, *Curr. Opin. Mol. Ther.* 12 (2010) 790–797.

[172] W.A.D.R.J.L. Erkki Koivunen, R. P, Identification of receptor ligands with phage display peptide libraries, *J. Nucl. Med.* 40 (1999) 883–888.

[173] Meanor, J. N., Keung, A. J., Rao, B. M. & Gera, N. Yeast Display Guided Selection of pH-Dependent Binders. in 293–311 (2022). doi:10.1007/978-1-0716-2285-8_16.

[174] V.W. Kelly, S.J. Sirk, Short FcRn-binding peptides enable salvage and transcytosis of scFv antibody fragments, *ACS Chem. Biol.* 17 (2022) 404–413.

[175] J.T. Andersen, et al., Single-chain variable fragment albumin fusions bind the neonatal Fc Receptor (FcRn) in a species-dependent manner, *J. Biol. Chem.* 288 (2013) 24277–24285.
[176] M.R. Charbonneau, V.M. Isabella, N. Li, C.B. Kurtz, Developing a new class of engineered live bacterial therapeutics to treat human diseases, *Nat. Commun.* 11 (2020) 1738.
[177] A.M. Brennan, Development of synthetic biotics as treatment for human diseases, *Synth. Biol.* 7 (2022).
[178] T. Ozdemir, A.J.H. Fedorec, T. Danino, C.P. Barnes, Synthetic biology and engineered live biotherapeutics: toward increasing system complexity, *Cell Syst.* 7 (2018) 5–16.
[179] M.K. Heavey, D. Durmusoglu, N. Crook, A.C. Anselmo, Discovery and delivery strategies for engineered live biotherapeutic products, *Trends Biotechnol.* 40 (2022) 354–369.
[180] J.W. Rutter, L. Dekker, K.A. Owen, C.P. Barnes, Microbiome engineering: engineered live biotherapeutic products for treating human disease, *Front. Bioeng. Biotechnol.* 10 (2022).
[181] T. Chien, et al., Enhancing the tropism of bacteria via genetically programmed biosensors, *Nat. Biomed. Eng.* 6 (2021) 94–104.
[182] A. Armstrong, M. Isalan, Engineering bacterial theranostics: from logic gates to in vivo applications, *Front. Bioeng. Biotechnol.* 12 (2024).
[183] A.M. Kunjapur, et al., Synthetic auxotrophy remains stable after continuous evolution and in coculture with mammalian cells, *Sci. Adv.* 7 (2021).
[184] K.A. Hedin, V. Kruse, R. Vazquez-Uribe, M.O.A. Sommer, Biocontainment strategies for in vivo applications of Saccharomyces boulardii, *Front. Bioeng. Biotechnol.* 11 (2023).
[185] A.G. Rottinghaus, A. Ferreiro, S.R.S. Fishbein, G. Dantas, T.S. Moon, Genetically stable CRISPR-based kill switches for engineered microbes, *Nat. Commun.* 13 (2022) 672.
[186] Y. Ma, A. Manna, T.S. Moon, Advances in engineering genetic circuits for microbial biocontainment, *Curr. Opin. Syst. Biol.* 36 (2023) 100483.
[187] J. Arnold, J. Glazier, M. Mimee, Genetic engineering of resident bacteria in the gut microbiome, *J. Bacteriol.* 205 (2023).
[188] N. Hayashi, Y. Lai, J. Fuerte-Stone, M. Mimee, T.K. Lu, Cas9-assisted biological containment of a genetically engineered human commensal bacterium and genetic elements, *Nat. Commun.* 15 (2024) 2096.
[189] S. Aghlara-Fotovat, et al., Hydrogel-encapsulation to enhance bacterial diagnosis of colon inflammation, *Biomaterials* 301 (2023) 122246.
[190] H. Xing, et al., Magnetically targeted delivery of probiotics for controlled residence and accumulation in the intestine, *Nanoscale* 17 (2025) 8588–8598.
[191] M.R. Charbonneau, et al., Development of a mechanistic model to predict synthetic biotic activity in healthy volunteers and patients with phenylketonuria, *Commun. Biol.* 4 (2021) 898.
[192] J.D. Brunner, N. Chia, Metabolic model-based ecological modeling for probiotic design, *Elife* 13 (2024).
[193] J.J. Luke, et al., Phase I Study of SYNB1891, an engineered *E. coli* nissle strain expressing STING agonist, with and without atezolizumab in advanced malignancies, *Clin. Cancer Res.* 29 (2023) 2435–2444.
[194] D. Barberio, Navigating regulatory and analytical challenges in live biotherapeutic product development and manufacturing, *Front. Microb.* 3 (2024).