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A Highly Potent Nanomedicine for Precision Pancreatic Enzyme Replacement Therapy

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Abstract

Oral enzyme replacement therapy benefits from a luminal site of action that is readily accessible without requiring transport across physiological barriers. Yet the use of native or engineered enzymes as precision treatments to restore digestive functions is constrained by biochemical barriers that rapidly compromise enzyme activity, localization and efficacy. Here, we report PER301, a precision multienzyme nanomedicine designed to overcome these barriers by combining enzyme shielding with mucoadhesive intestinal retention in a single nanoparticle architecture. By co-localizing lipolytic, proteolytic, and amylolytic enzymes within a precisely engineered organosilica framework, PER301 creates a nano-confined environment to sterically exclude endogenous proteases while allowing target substrates to access the immobilized enzymes. Because pancreatic lipases depend on interfacial activation, a conformational transition that may be restricted by nanoscale confinement, we developed a biomimetic templating strategy to favor its active state and introduced hydrophobic domains to promote lipid partitioning into the catalytic environment. To support translation, we developed a scalable manufacturing process for this multifunctional nano-architecture, yielding reproducible liter-scale batches and a dry-powder formulation with long-term stability (16 months) under ambient conditions. In canine and porcine models of exocrine pancreatic insufficiency, PER301 demonstrated up to a 69-fold increase in digestive potency compared to the standard-of-care pancreatic enzyme replacement therapy (PERT). Notably, chitosan-mediated mucoadhesion extended intestinal residence time, fully restoring lipid absorption to healthy levels and maintaining digestive activity for 17 hours post-administration. Biodistribution studies demonstrated that the nanoparticle system is non-absorbable, with no evidence of systemic translocation, organ accumulation, or toxicity, supporting a strictly localized safety profile. Together, these findings position PER301 as a translatable

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nanomedicine platform for localized, long-acting enzyme replacement therapy, offering a route to precision oral biologics that overcome the stability, localization and dosing limitations of conventional treatments.

Introduction

Therapeutic enzymes represent one of the most potent classes of biologics,¹⁻³ yet their clinical translation is fundamentally constrained by the difficulty of maintaining stability, localization, and activity *in vivo*. These challenges are particularly acute at mucosal interfaces, where enzymes are exposed to rapid dilution, proteolytic degradation, and poorly controlled spatial distribution.^{4,5} Although mucoadhesive materials based on electrostatic, hydrogen-bonding, covalent, or bio-inspired catechol interactions have been explored to prolong gastrointestinal residence time,^{6,7} their application as a precision strategy to spatially confine enzymatic activity remains largely underdeveloped. Addressing this gap is essential for the development of oral biotherapeutics that can deliver sustained, site-specific catalytic activity under physiologically hostile conditions.

Exocrine pancreatic insufficiency (EPI) exemplifies this challenge. Characterized by insufficient pancreatic enzyme secretion and severe nutrient malabsorption,⁸ EPI commonly arises from cystic fibrosis, chronic pancreatitis, and pancreatic adenocarcinoma. Patients with EPI experience chronic diarrhea, weight loss, and nutritional deficiencies that substantially impair quality of life. In healthy physiology, efficient digestion relies on the orchestrated activity of lipases, amylases, and proteases, a concerted process maintained by synchronized pancreatic secretion. By contrast, pancreatic enzyme replacement therapy (PERT) delivers exogenous enzymes as a concentrated bolus that lacks the spatiotemporal regulation of endogenous secretion, leaving them immediately vulnerable to autolysis and rapid dilution upon entering the duodenal lumen. Although enteric coatings facilitate gastric transit, they provide no protection once the enzymes are liberated into the hostile intestinal environment, resulting in a rapid loss of catalytic potency.^{9,10} These limitations drive a high pill burden and often fail to fully resolve malabsorption, underscoring the need for a more durable, site-specific therapeutic system.¹¹

Here, we report a precision multienzyme nanomedicine designed to maintain high catalytic activity regardless of the degradative conditions in the intestinal tract. We engineered a nanoparticle architecture that creates a privileged catalytic space by co-localizing a synergistic cocktail of proteolytic, amylolytic, and lipolytic enzymes within a porous organosilica framework. This design utilizes nano-scale confinement to shield the enzymes from endogenous proteases while preserving substrate accessibility. To promote localized efficacy,

the surface is functionalized with chitosan, leveraging mucoadhesive interactions to confine enzymatic activity at the mucosal interface.

Importantly, this design is implemented through a robust and scalable manufacturing process that enables reproducible, liter-scale production of multienzyme nanomedicines while maintaining consistent enzyme loading and activity; an aspect that remains largely unexplored in nanomedicine-based enzyme delivery. Our results show that this engineered system restores digestive function in established *in vivo* models of EPI while maintaining a strictly localized and non-absorbable profile, ensuring that therapeutic efficacy is achieved without systemic exposure. Collectively, these findings establish a translatable and safe strategy for high-potency, localized enzyme replacement therapy.

Results and Discussion

To implement the concept of a shielded, multi-catalytic environment within a translatable system, we engineered a nanoparticle architecture featuring a co-localized multienzyme cocktail (Figure 1) sourced from pancreatin (a clinically established porcine-derived mixture).¹² We selected spherical silica nanoparticles (SNPs)¹³ as the core scaffold due to their high structural uniformity and capacity for precise surface functionalization. Following amino-modification of the core SNPs with (3-aminopropyl)triethoxysilane (APTES), pancreatin was covalently tethered via glutaraldehyde-mediated multipoint attachment.^{14,15} This covalent immobilization strategy was chosen to ensure structural stability and prevent the dissociation of the enzymes upon exposure to the dynamic intestinal environment.

A central challenge in this design was the engineering of a protective barrier that excludes degradative proteases while remaining permeable to macromolecular nutrients. To achieve this, a soft, porous organosilica shell was grown around the protein–silica conjugate.¹⁶ Based on the crystallographic dimensions of the key enzymes involved, lipase, amylase, and trypsin (PDB codes: 1LPB,¹⁷ 1BVN,¹⁸ 1MCT¹⁹), we optimized the shell thickness to approximately 7 nm. This dimension serves as a selective molecular gate: it is sufficiently dense to sterically exclude bulky endogenous proteases, yet porous enough to allow the rapid diffusion of substrate molecules into the catalytic core. To further optimize the interface for lipid digestion, benzyltriethoxysilane (BTES) was integrated into the silica matrix, increasing the lipophilicity of the porous framework. We hypothesized that this hydrophobic modification would facilitate the transport of triglyceride substrates to the immobilized lipase by increasing their partitioning into the shell. This mechanism was confirmed using a fluorescent artificial lipase substrate, 1,2-Di-O-lauryl-rac-glycerol-3-(glutaric acid 6-methylresorufin ester). The BTES-modified nanoparticles (Layer ATB, Figure 2a) exhibited a 3-fold increase in specific activity compared

to the purely hydrophilic variant (Layer AT, Figure 2a). This result demonstrates that the engineered lipophilic shell effectively concentrates hydrophobic substrates within the catalytic core, overcoming diffusion limitations and significantly enhancing reaction kinetics at the nano-interface.

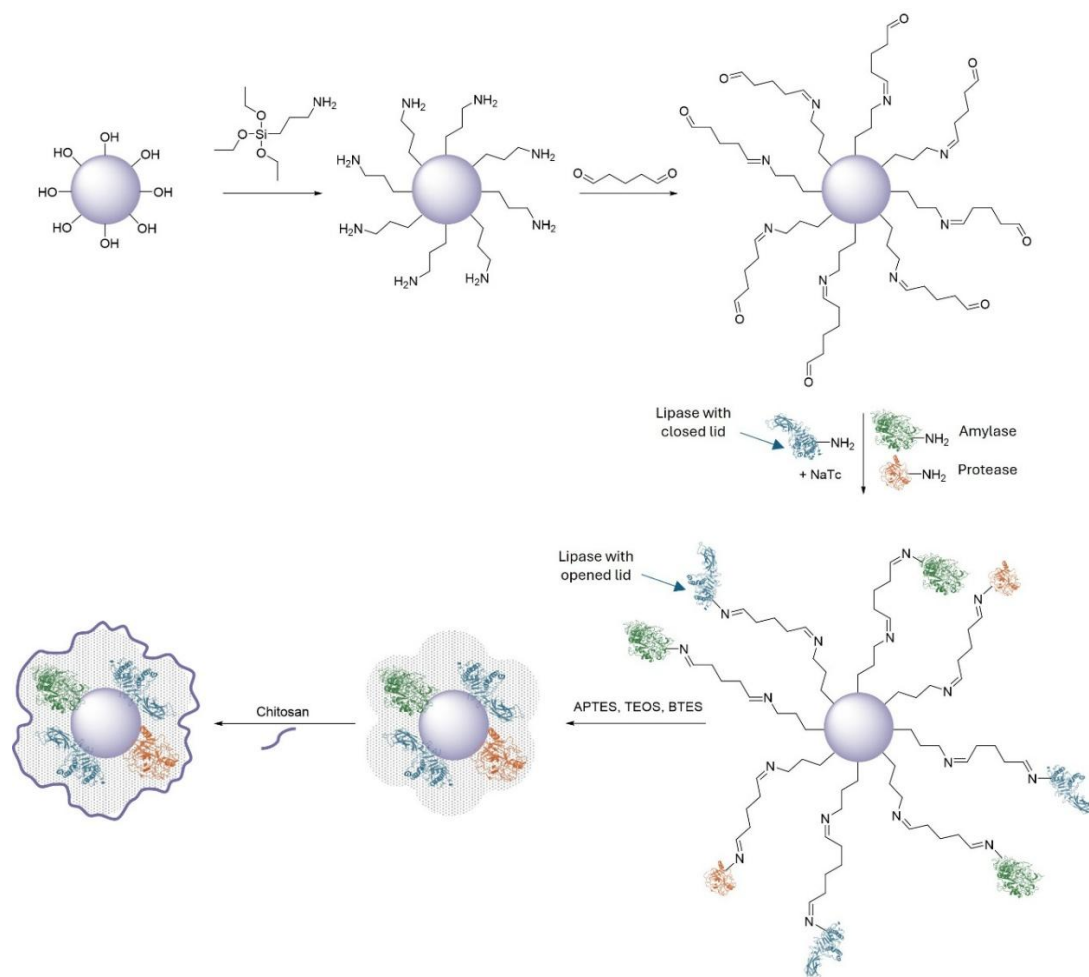


Figure 1. Schematic illustration of multienzyme nanomedicine synthesis (PER301). Silica nanoparticles are first functionalized with amine groups using APTES, followed by glutaraldehyde-mediated activation to introduce aldehyde-bearing linkers for enzyme conjugation. A synergistic enzyme cocktail comprising lipase, amylase and protease is then covalently immobilized onto the nanoparticle surface in the presence of sodium taurocholate (NaTc), which promotes opening of the lipase lid and preserves access to the active site. Subsequent organosilica condensation using APTES, TEOS and BTES generates a porous protective framework around the immobilized enzymes, enabling substrate accessibility while shielding enzymes from proteolytic degradation. Finally, chitosan coating confers mucoadhesive functionality, yielding a localized multienzyme nanomedicine designed for sustained catalytic activity at the intestinal mucosal interface.

However, nano-confinement introduces a fundamental steric trade-off: while the organosilica shell protects the immobilized enzymes from proteolytic degradation, it can also restrict access of colipase, a 10 kDa cofactor that supports the interfacial activation of pancreatic lipase through lid opening.²⁰ To address this, we employed a biomimetic templating strategy using sodium taurocholate (NaTc) as a sacrificial molecular chaperone.^{21,22} NaTc promotes the active, interface-stabilized conformation of pancreatic lipase, allowing this high-activity state to

be imprinted into the rigidifying silica framework. Following shell formation, NaTc was quantitatively recovered in the supernatant, confirming its removal from the nanoparticles and its role as a sacrificial template (Figure S1). This conformational imprinting effect was validated via pH-stat titration (Figure 2b), where NaTc-templated nanoparticles (SNPs-PCN(NaTc)-ATB) exhibited a twofold increase in specific activity compared with non-templated variants (SNPs-PCN-ATB).

To maximize the enzyme content within the architecture, we systematically varied the pancreatin concentration during immobilization (1X to 10X, corresponding to 1- to 10-fold). This resulted in a correlated increase in immobilized protein content from 1.1 mg.mL⁻¹ to 4.6 mg.mL⁻¹ (Figure 2c). Lipase activity analysis by pH-stat titration revealed that the 10X loading condition (PER301) yielded a fivefold increase in specific activity compared to the 1X baseline (SNPs-PCN(NaTc)-ATB, Figure 2b), achieving a high catalytic potency of 1536 U g⁻¹ of nanoparticle (Figure 2d). This optimized loading was selected for all subsequent studies, as it achieved superior catalytic performance.

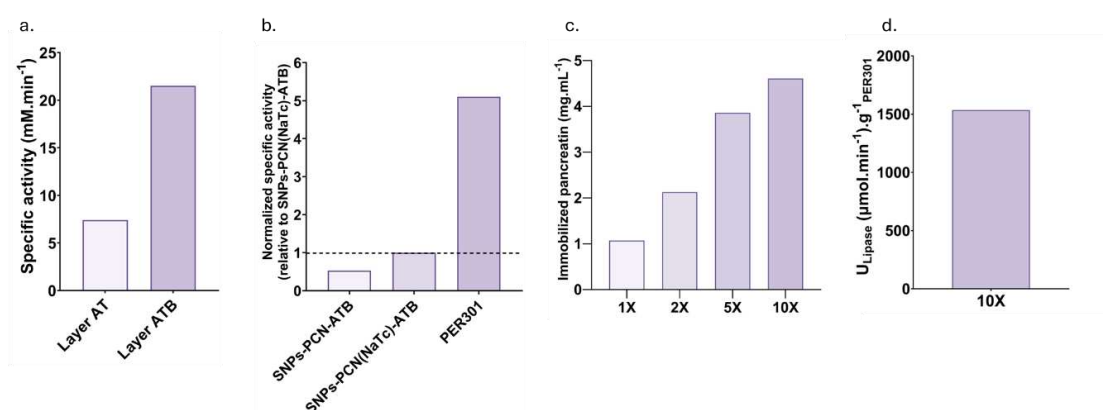


Figure 2. Multi-parametric optimization of PER301 catalytic potency. a) Lipase activity comparison between hydrophilic (Layer AT) and hydrophobic (Layer ATB) nanoparticles. b) Normalized specific activity of non-templated nanoparticles (SNPs-PCN-ATB) compared to the fully optimized, NaTc-templated drug substance (PER301). c) Protein quantification of pancreatin immobilized on the SNP surface across a loading gradient, with 10X loading defined as the PER301 formulation. d) Specific lipase activity of the optimized PER301 formulation as determined by pH-stat titration.

With the catalytic core thus optimized, the final modification involved functionalizing the outer surface with chitosan to impart mucoadhesive properties.²³ This was achieved by leveraging the high cationic charge density of chitosan to drive robust electrostatic interactions with the negatively charged silanol groups on the organosilica surface. Quantification via colorimetric assay revealed a chitosan loading of $293 \pm 8 \mu\text{g mL}^{-1}$ (Figure S2), which corresponds to a 20% fractional coverage of the nanoparticle surface. This self-assembly process ensures a stable, polycationic corona that facilitates interaction with the negatively charged mucin glycoproteins of the intestinal lining. This final modification yielded the PER301 drug substance (PER301-DS). Scanning Electron Microscopy (SEM) comparison of the final PER301 nanoparticles

(Figure S3b) with the core SNPs (Figure S3a) further confirmed a uniform shell thickness of approximately 7 nm surrounding the immobilized pancreatin, in close agreement with the design specifications derived from enzyme structural dimensions. This iterative refinement, from conformational imprinting to surface functionalization, yielded a streamlined, single-component system achieving superior catalytic performance without the need for exogenous cofactors.

Having established the nanoparticle architecture, we next evaluated whether this design could be implemented as a robust and scalable manufacturing process. We assessed the system's reproducibility through a series of characterization studies on independent production batches synthesized at a liter scale, yielding approximately 50 g of nanoparticles per batch. Protein quantification assays of reaction supernatants collected after organosilica layer growth confirmed efficient enzyme immobilization and demonstrated high batch-to-batch consistency ($n=3$), with an average loading of $4.6 \pm 1.2 \text{ mg mL}^{-1}$ (Figure 3a), a result in close agreement with our small-scale pilot studies. Catalytic performance remained equally robust across scales; pH-stat titration revealed a mean lipase activity of $1553 \pm 178 \text{ U g}^{-1}$ of nanoparticles (Figure 3b), confirming that enzymatic potency is preserved during scale-up. Morphological characterization via scanning electron microscopy (SEM) revealed a monodisperse particle size distribution with an average diameter of $65 \pm 8 \text{ nm}$ (Figure 3c). This uniformity across multiple liter-scale batches demonstrates that the PER301 synthetic framework is amenable to standardized manufacturing, a critical prerequisite for clinical translation.

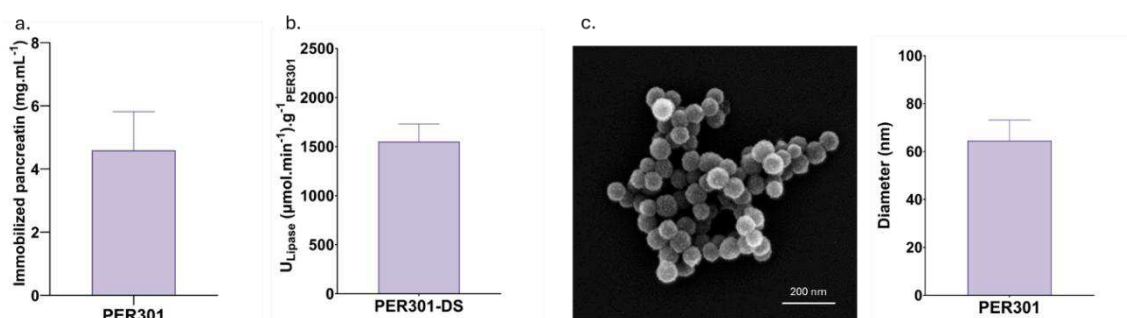


Figure 3. Batch-to-batch reproducibility and structural characterization of the PER301 drug substance (PER301-DS). a) Consistency of enzyme loading density across multiple production batches, as determined by protein quantification (Lowry assay) of reaction supernatants. b) Inter-batch reproducibility of specific lipase activity as determined by pH-stat titration. c) Particle size distribution and morphological uniformity of PER301, based on statistical analysis of representative scanning electron micrographs across independent synthesis lots.

However, beyond the reproducible synthesis of the drug substance, the development of a translatable nanoparticle system necessitates a dry-state formulation compatible with industrial downstream processing and oral administration. Lyophilization is a widely established pharmaceutical strategy for stabilizing biologics.²⁴ However, initial characterization of the PER301 drug substance (PER301-DS) revealed a low collapse temperature of $-55 \text{ }^{\circ}\text{C}$ (Figure

S4a–c). This presented a significant manufacturing bottleneck as such low temperatures require excessively prolonged and energy-intensive freeze-drying cycles.^{24,25} To overcome this limitation, a comprehensive formulation screening was performed to evaluate the effects of freezing rates and cryoprotectant compositions. The dual objective was to increase the collapse temperature while preserving the delicate enzymatic activity of the immobilized and shielded pancreatin. This screening identified three formulations that fully retained enzyme activity over lyophilization (Figure S5). From these candidates, a lead formulation was selected to maximize drug loading, achieving 64 wt% PER301 within a stabilizing excipient matrix of histidine, trehalose, and cysteine. This optimized matrix yielded the final PER301 drug product (PER301-DP) and successfully increased the collapse temperature to $-33\text{ }^{\circ}\text{C}$ (Figure S4d–f), enabling a markedly more efficient and industrially viable freeze-drying process.

To validate the robustness of the process, enzymatic activities were quantified across independent 1-liter batches, yielding approximately 78 g of PER301-DP post-lyophilization. The resulting drug product exhibited highly consistent multi-enzymatic performance. Protease and amylase activities were evaluated using the biorelevant INFOGEST protocols²⁶ to more accurately reflect the physiological conditions of the small intestine compared to standard pharmacopeial assays. Average activities were recorded at $1629 \pm 118\text{ U}_{(\mu\text{mol min}^{-1})}\text{ g}^{-1}$ PER301-DP for lipase (Figure 4a),²⁷ $796 \pm 17\text{ U}_{(\mu\text{mol min}^{-1})}\text{ g}^{-1}$ PER301-DP for protease (Figure 4b), and $79 \pm 2\text{ U}_{(\text{mg}^{-1}\text{ min}^{-1})}\text{ g}^{-1}$ PER301-DP for amylase (Figure 4c). Batch-to-batch reproducibility was further supported by a consistent residual moisture content²⁴ of $3.1 \pm 0.4\%$ (Figure 4d), a critical parameter for long-term biologic stability.

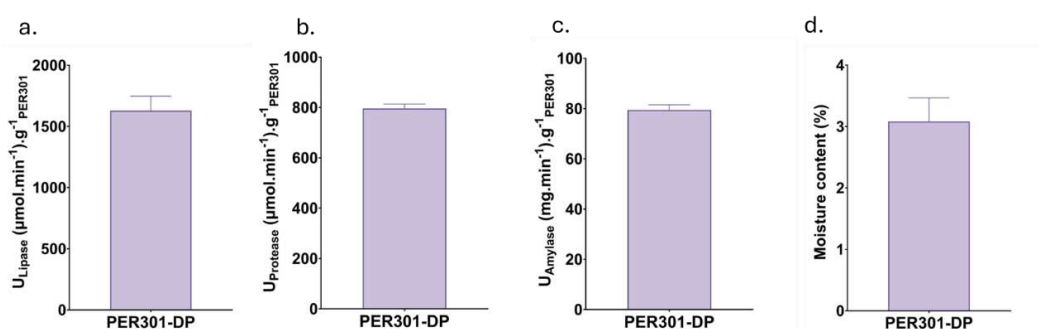


Figure 4. Multi-enzymatic robustness and pharmaceutical characterization of the PER301 drug product (PER301-DP). a–c) Inter-batch reproducibility of specific (a) lipase, (b) protease, and (c) amylase activities, validated using biorelevant protocols mimicking small intestinal physiological conditions. d) Batch-to-batch uniformity of moisture content as determined by Karl Fischer titration.

The long-term stability of PER301-DP was evaluated under ambient, non-light-protected conditions to simulate non-ideal storage (Figure S6). The formulation maintained full enzymatic activity for 10 months, and while activity was measured at 87% and 73% after 16 and 22 months respectively, these fluctuations were not statistically significant ($P > 0.05$). This

exceptional ambient shelf-life suggests a robust product profile that simplifies storage and eliminates the logistical constraints of cold-chain management. Morphologically, PER301-DP presented as a fine, low-density powder (Figure S7a,b). Laser diffraction analysis revealed a primary particle size distribution centered at approximately 6 μm (Figure S7c).

Given the high catalytic potency of the mucoadhesive PER301-DP formulation, which prolongs residence time in the small intestine and sustains enzymatic activity at the mucosal interface, we estimate that a standard therapeutic dose would require significantly less material than conventional PERT. Nevertheless, because mucoadhesion is designed to concentrate enzymatic activity at the intestinal mucosal interface, we assessed whether this localized payload could compromise epithelial integrity using a human intestinal barrier model.²⁸ Preliminary *in vitro* cytotoxicity assessments (CCK-8 assay)²⁹ showed that PER301 did not reduce metabolic activity at doses up to 1,000 capsules (Figure S8). Consistent with these findings, ATP-based measurements³⁰ showed that cellular energy levels remained comparable to untreated controls across the tested dose range, with no evidence of cytotoxicity. A marked loss of ATP signal was observed only at an extreme exposure equivalent to 50,000 capsules (Figure S9), far exceeding any physiologically relevant or therapeutically achievable dose. Importantly, the absence of dose-dependent decreases in viability across the relevant concentration range indicates that PER301 does not impair epithelial integrity under conditions representative of clinical use, supporting a robust safety profile for the nanomedicine.

To isolate the contribution of surface chemistry from enzymatic activity, we prepared two reference systems incorporating a catalytically inactive protein (e.g., bovine serum albumin) and a ¹¹¹In-radiotracer:³¹ PER300F, which features the same surface functionalization as the therapeutic particle, and PER300N, a non-functionalized control. The radiotracer was integrated by covalently conjugating a DOTA-chelator to the SNP core prior to surface functionalization (Figure 6), enabling precise *in vivo* tracking and quantification of residence time while excluding catalytic contributions. We evaluated these systems in a minipig model, administering the nanoparticles directly into the duodenum via endoscopy to isolate their behavior at the clinical site of action. SPECT/CT imaging performed at multiple time points (15 min to 72 h) revealed that chitosan functionalization achieved a threefold increase in small-intestinal residence time compared to the non-functionalized counterparts (Figure 5). Consistently, non-functionalized PER300N particles transited more rapidly to the colon, where they showed higher radioactivity at matched time points.

To assess the systemic pharmacokinetic profile, radioactivity was quantified in blood, urine, and feces. Following intraduodenal administration, both ¹¹¹In-PER300F and ¹¹¹In-PER300N exhibited negligible blood concentrations (0.00002%ID/g, Figure S10). This trace signal was

transient, appearing at 3 hours post-administration, synchronous with the peak absorption observed in the free ^{111}In control group (0.0002%ID/g, Figure S11). Because the nanoparticle-associated signal was tenfold lower than that of free ^{111}In and occurred at the same time point, it is most consistent with minor transient isotope de-labelling rather than systemic translocation of intact nanoparticles.

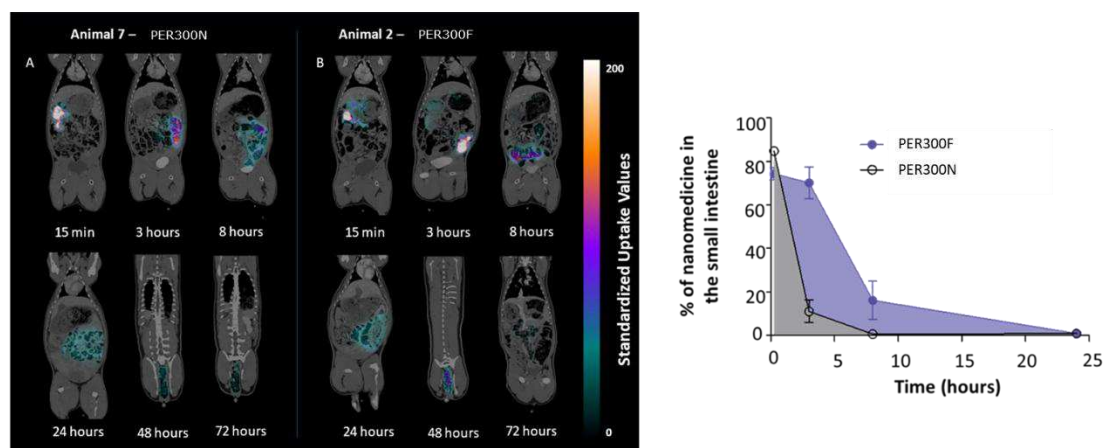


Figure 5. Mucoadhesive functionalization extends small-intestinal residence of PER301 analogues in minipigs. Quantitative SPECT/CT-based analysis of the longitudinal distribution of radiolabeled ^{111}In -nanoparticles following direct duodenal administration. Chitosan-functionalized analogues (PER300F) exhibit an approximately threefold increase in small-intestinal residence time compared with non-functionalized nanoparticles (PER300N), as quantified by area under the curve (AUC) analysis. Representative SPECT/CT images acquired at indicated time points demonstrate that while non-functionalized nanoparticles rapidly transit to the colon, PER300F remains localized within the small-intestinal lumen for an extended duration. Color scale represents standardized uptake values (SUV). Data are expressed as mean $\pm$ s.d. (n = 5 animals).

Excretion was restricted to the fecal route, peaking at 48 hours (Figure S12). The slightly higher fecal recovery observed for ^{111}In -PER300N correlates with the more rapid transit observed via SPECT/CT. Minimal radioactivity was detected in urine at 72 hours (Figure S13), further corroborating the absence of systemic circulation. Collectively, these biodistribution data validate that chitosan functionalization significantly enhances mucoadhesion and prolongs intestinal retention without compromising the non-systemic safety profile of the nanoparticle system.

To translate these findings into a deliverable therapeutic, the PER301-DP powder was filled into enteric-coated capsules (Eudracap).³² These capsules are designed to dissolve selectively in the proximal small intestine at pH values above 5.5, ensuring targeted release in the duodenum while protecting the enzymatic payload from gastric acidity. This delivery strategy is further optimized by administration under fasted conditions, promoting the robust adhesion of the released nanomedicine to the intestinal mucosa prior to the arrival of dense luminal contents.

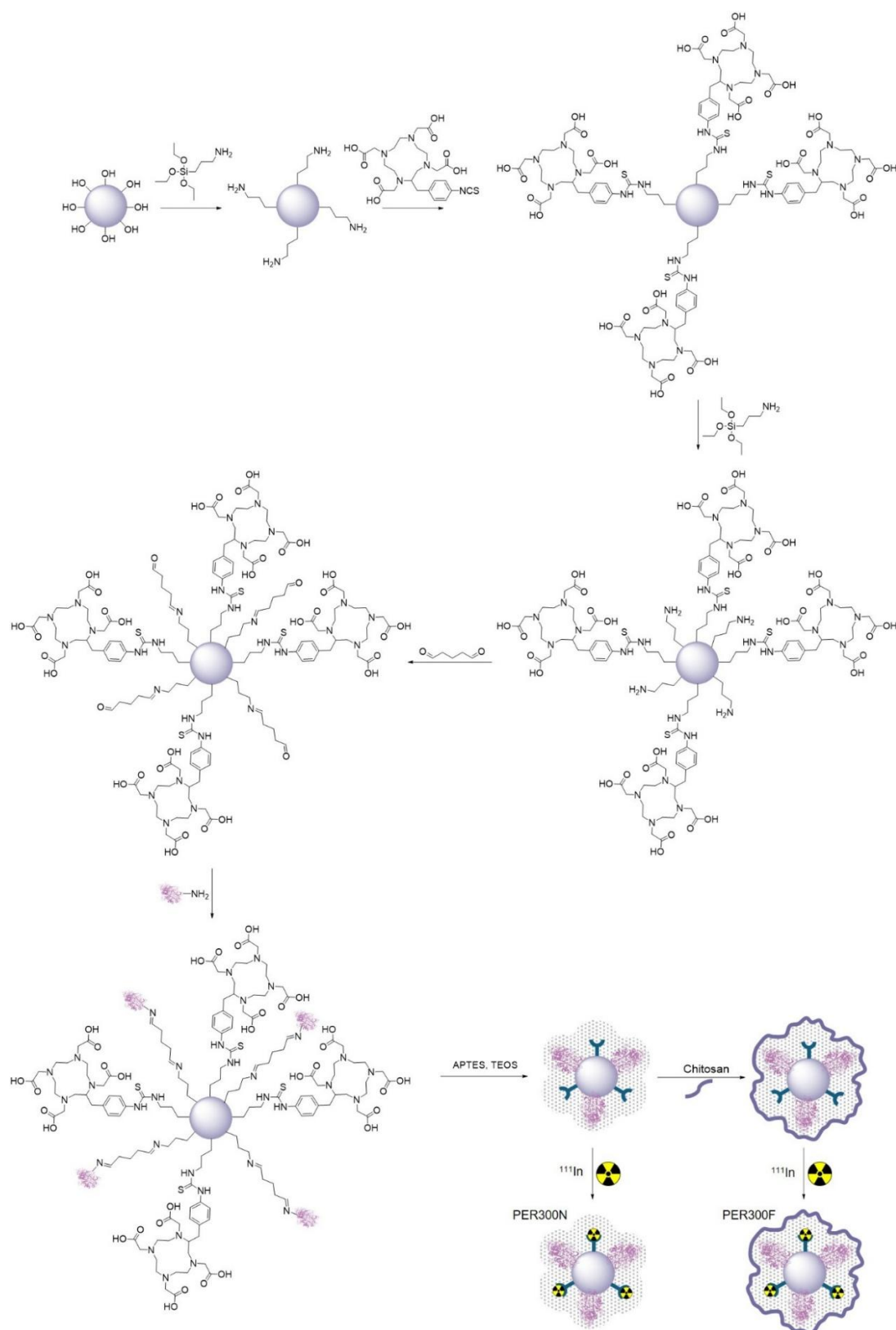


Figure 6. Design and radiolabeling of catalytically inactive PER301 analogues for *in vivo* biodistribution studies. Bovine serum albumin was immobilized on silica nanoparticle cores in place of pancreatin to generate catalytically inactive reference particles. A DOTA chelator was covalently coupled to the SNP core before organosilica shell growth and surface functionalization, enabling stable incorporation of ^{111}In for quantitative SPECT/CT tracking. Chitosan-functionalized particles (PER300F) and non-functionalized controls (PER300N) were used to distinguish the contribution of mucoadhesive surface chemistry to intestinal retention while excluding effects arising from enzymatic activity.

The *in vivo* therapeutic efficacy of PER301-DP was first evaluated in a 3-day study using a pancreatic duct–ligated (PDL) dog model. Following confirmation of EPI (Table S8),³³ animals were evaluated in a within-subject design to compare PER301-DP against a standard veterinary PERT. The PER301-DP dose was precisely calculated based on the total lipid content of the provided meal and a target digestion window of 2.5 hours, reflecting the expected intestinal transit time. This resulted in an administered lipase activity of only 859 U twice daily, whereas the standard veterinary PERT required 32,790 U, representing a nearly 40-fold reduction in administered activity. Despite this drastically lower dose, PER301-DP treatment successfully reversed malabsorption, as evidenced by a rapid stabilization in body weight (Figure 7c). This clinical recovery was underpinned by a marked reduction in steatorrhea, with fecal fat content significantly decreasing compared to untreated controls (Figure 7a). While the veterinary PERT failed to achieve a statistically significant reduction in fecal fat compared to untreated controls, PER301-DP significantly reduced fecal fat to 3.9%, approaching the healthy baseline of 2.4%. When normalized to the total units administered, PER301-DP exhibited a 69-fold higher digestive potency than the standard of care (Figure 7b).

A subsequent 7-day exploratory study was conducted in PDL dogs eight weeks post-surgery to assess sustained *in vivo* efficacy. PER301-DP was administered twice daily at a total activity of 792 U. Fat digestion served as the primary endpoint and was evaluated using both a substrate absorption challenge test (SACT), based on post-prandial plasma triglyceride (TG) levels, and the monitoring of fasted TG concentrations. Following seven days of treatment, PER301-DP markedly improved lipid absorption, as evidenced by the SACT, which revealed sustained TG uptake up to six hours after administration (Figure 7d). Notably, after seven days of treatment, lipid absorption was fully restored to levels comparable to healthy animals, as evidenced by the AUC analysis of the SACT (Figure 7e). Consistent with prolonged digestive activity, blood samples collected from fasted animals 17 hours after the last meal exhibited significantly elevated TG concentrations throughout the treatment period (Figure 7f), indicating continuous lipid digestion well beyond the dosing interval.

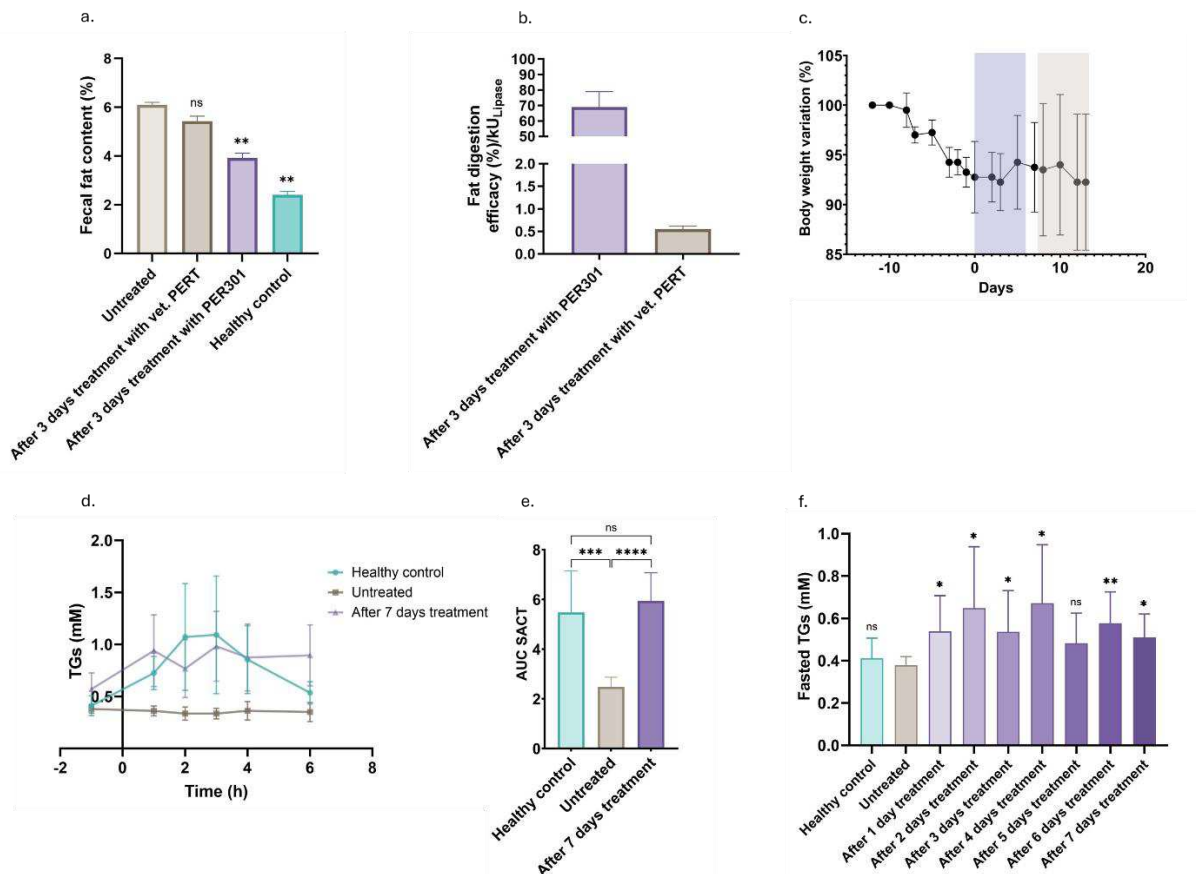


Figure 7. Evaluation of PER301-DP therapeutic efficacy in a canine PDL model. a–c) Assessment of fat malabsorption and physical recovery over a 3-day treatment period (n= 4 animals). a) Fecal fat content (%) compared to untreated controls. b) Lipolytic efficacy of PER301-DP compared to standard veterinary PERT, expressed as the percentage of fat digestion per kilounit (kU) of lipase administered. c) Body weight variations following pancreatic duct ligation (PDL) surgery; the post-surgical weight decline was stabilized following administration of PER301-DP (purple) or standard veterinary PERT (grey). d–f) Plasma triglyceride profiles during a 7-day efficacy study. d) Temporal triglyceride excursions following oral administration of PER301-DP and a high-fat diet. e) Calculated Area Under the Curve (AUC) for postprandial triglycerides. f) Fasting triglyceride levels. Statistical analysis was performed using Student's t-test. Data are compared to untreated controls: ns, $P > 0.05$, $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), and $P \leq 0.0001$ (****).

A confirmatory study was performed in the PDL minipig model, which closely recapitulates human intestinal physiology but constitutes a stringent system due to species-specific gastric features (e.g., diverticulum ventriculi) that cause erratic and prolonged capsule retention (45–1440 min).³⁴ This anatomy creates a challenging delivery environment for enteric-coated formulations, as asynchronous gastric emptying can lead to sub-therapeutic dosing. The study employed a within-subject design, with each animal serving as its own control. As with the canine study, the PER301-DP dose (1,043 U administered twice daily) was calculated based on the dietary fat load and a theoretical 2.5-hour digestion window. Even under these challenging conditions, this minimal dose achieved fat digestion results comparable to the standard of care (SOC) administered twice daily at 40,000 U. Both treatments significantly reduced fecal fat relative to the untreated EPI state (Figure 8d), with PER301-DP demonstrating a 33-fold higher digestive potency per unit of lipase activity (Figure 8e). The immediate weight gain (Figure 8a) and improved stool quality (Figure 8c)³⁵ reversed upon

cessation of treatment, confirming a direct dose-response. Consistent with the dog model, elevated fasted TG levels (Figure 8b) corroborated a prolonged therapeutic window of at least 17 hours, underscoring the functional advantage of intestinal mucoadhesion and suggesting that the architecture remains active even during inter-prandial periods.

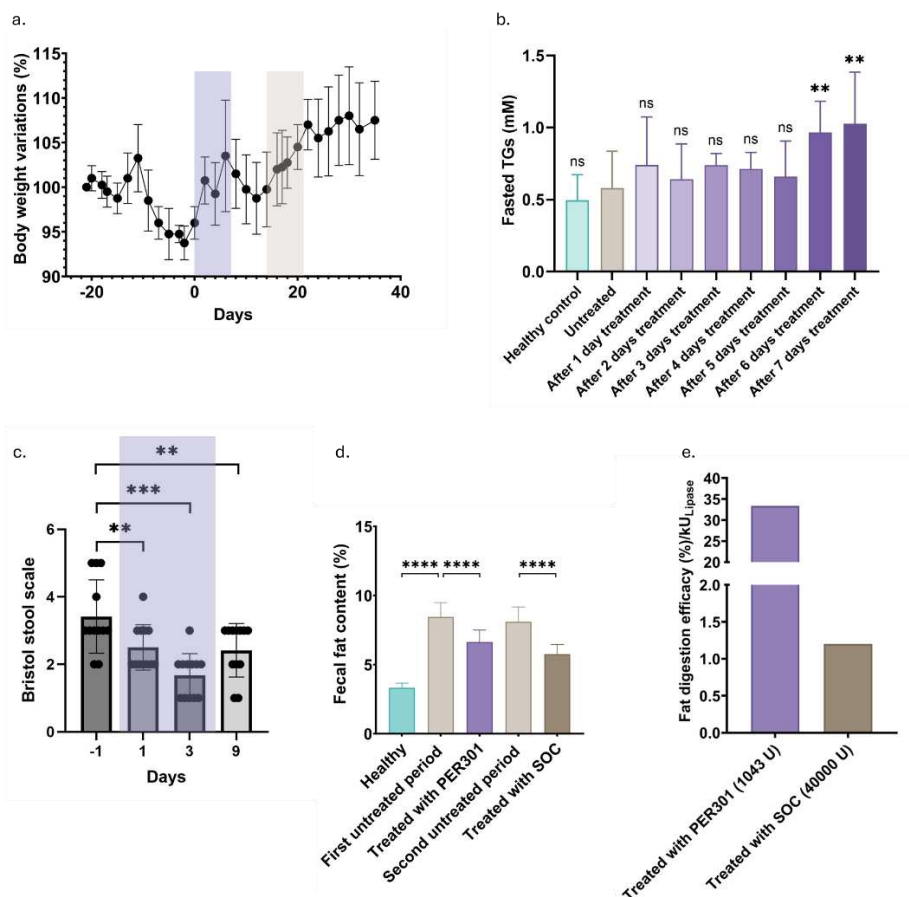


Figure 8. Evaluation of PER301-DP therapeutic efficacy in a porcine PDL model over a 7-day treatment period. a) Body weight variations following pancreatic duct ligation (PDL); the post-surgical weight decline was reversed, with body weight increasing following administration of PER301-DP (purple) or standard of care (SOC, grey). b) Fasting plasma triglyceride levels. c) Bristol Stool Scale consistency following PER301-DP administration. Purple indicates the PER301-DP treatment group. d) Fecal fat content (%) compared to untreated controls. e) Comparative lipolytic potency of PER301-DP versus SOC, normalized per unit of lipase. Statistical analysis was performed using Student's t-test. All data are presented as mean ± s.d. (n = 4 animals): ns, $P > 0.05$, $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), and $P \leq 0.0001$ (****).

Conclusion

In summary, we have developed a robust nanoparticle system that fundamentally redefines the delivery of therapeutic enzymes for exocrine pancreatic insufficiency. By engineering a privileged catalytic space that protects enzymes from both autolysis and degradation by endogenous proteases while significantly increasing their intestinal residence time, we achieved a transformative leap in catalytic efficiency and therapeutic duration.

Our findings demonstrate that PER301 can restore physiological digestion in large animal models using only a small fraction of the enzymatic activity required by conventional therapies.

Beyond potency, the integration of chitosan-mediated mucoadhesion addresses the bolus-effect of traditional PERT, creating a persistent catalytic interface that remains active for at least 17 hours after administration. This prolonged retention, coupled with enhanced stability, suggests the potential for once-daily dosing, representing a substantial reduction in pill burden and a significant improvement in patient quality of life. Furthermore, the successful transition from liquid-phase drug substance to a stable, industrially viable drug product (PER301-DP) with 16-month ambient stability demonstrates that this architecture is ready for clinical translation. This strategy of shielded, site-specific, and prolonged enzymatic activity provides a versatile blueprint for the oral delivery of other labile biologics, potentially expanding the therapeutic landscape for diverse mucosal and metabolic disorders.

References

- 1 Michailidou, F. Engineering of therapeutic and detoxifying enzymes. *Angew. Chem. Int. Ed.* **62**, e202308814 (2023).
- 2 van der Beek, N. A. M. E. *et al.* Clinical insights in enzyme replacement therapy for metabolic storage disorders: Lessons from pompe disease. *Lancet Neurol.* **24**, 230-245 (2025).
- 3 Stern, S. *et al.* Clinical pharmacology considerations for first-in-human clinical trials for enzyme replacement therapy. *J. Inherit. Metab. Dis.* **47**, 1096-1106 (2024).
- 4 Ensign, L. M., Cone, R. & Hanes, J. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Adv. Drug Deliv. Rev.* **64**, 557-570 (2012).
- 5 Chu, J. N. & Traverso, G. Foundations of gastrointestinal-based drug delivery and future developments. *Nat. Rev. Gastroenterol. Hepatol.* **19**, 219-238 (2022).
- 6 Kumar, R., Islam, T. & Nurunnabi, M. Mucoadhesive carriers for oral drug delivery. *J. Controlled Rel.* **351**, 504-559 (2022).
- 7 Li, M. *et al.* Advanced bioinspired multifunctional platforms focusing on gut microbiota regulation. *ACS Nano* **18**, 20886-20933 (2024).
- 8 Iglesia, D. d. I., Vujasinovic, M., Löhr, J. M. & Dominguez-Muñoz, J. E. Pancreatic exocrine insufficiency. *eClinicalMedicine* **94** (2026).
- 9 Layer, P. & Gröger, G. Fate of pancreatic enzymes in the human intestinal lumen in health and pancreatic insufficiency. *Digestion* **54**, 10-14 (2009).
- 10 Guarner, L., Rodríguez, R., Guarner, F. & Malagelada, J. R. Fate of oral enzymes in pancreatic insufficiency. *Gut* **34**, 708-712 (1993).
- 11 Lowe, M. E. & Whitcomb, D. C. Next generation of pancreatic enzyme replacement therapy: Recombinant microbial enzymes and finding the perfect lipase. *Gastroenterol.* **149**, 1678-1681 (2015).
- 12 Lieb, J. G., Patel, D., Karnik, N. & Toskes, P. P. Study of the gastrointestinal bioavailability of a pancreatic extract product (Zenpep) in chronic pancreatitis patients with exocrine pancreatic insufficiency. *Pancreatology* **20**, 1092-1102 (2020).
- 13 Stöber, W., Fink, A. & Bohn, E. Controlled growth of monodisperse silica spheres in the micron size range. *J. Colloid Interface Sci.* **26**, 62-69 (1968).
- 14 Briand, M. L. *et al.* Partially shielded enzymes capable of processing large protein substrates. *Chem. Commun.* **56**, 5170-5173 (2020).
- 15 Giunta, C. I. *et al.* Tuning the properties of natural promiscuous enzymes by engineering their nano-environment. *ACS Nano* **14**, 17652-17664 (2020).
- 16 Corroero, M. R. *et al.* Enzyme shielding in an enzyme-thin and soft organosilica layer. *Angew. Chem. Int. Ed.* **55**, 6285-6289 (2016).
- 17 Egloff, M.-P. *et al.* The 2.46 Å resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. *Biochemistry* **34**, 2751-2762 (1995).
- 18 Wiegand, G., Epp, O. & Huber, R. The crystal structure of porcine pancreatic α -amylase in complex with the microbial inhibitor tendamistat. *J. Mol. Biol.* **247**, 99-110 (1995).
- 19 Huang, Q., Liu, S. & Tang, Y. Refined 1.6 Å resolution crystal structure of the complex formed between porcine β -trypsin and MCTI-A, a trypsin inhibitor of the squash family: Detailed comparison with bovine β -trypsin and its complex. *J. Mol. Biol.* **229**, 1022-1036 (1993).
- 20 van Tilbeurgh, H., Sarda, L., Verger, R. & Cambillau, C. Structure of the pancreatic lipase-procolipase complex. *Nature* **359**, 159-162 (1992).
- 21 Haque, N. & Prabhu, N. P. Lid closure dynamics of porcine pancreatic lipase in aqueous solution. *Biochim. Biophys. Acta* **1860**, 2313-2325 (2016).
- 22 Haque, N. & Prakash Prabhu, N. Binding orientation and interaction of bile salt in its ternary complex with pancreatic lipase-colipase system. *Biochem. Biophys. Res. Commun.* **499**, 907-912 (2018).

- 23 Bernkop-Schnürch, A. & Dünnhaupt, S. Chitosan-based drug delivery systems. *Eur. J. Pharm. Biopharm.* **81**, 463-469 (2012).
- 24 Wang, W. Lyophilization and development of solid protein pharmaceuticals. *Int. J. Pharm.* **203**, 1-60 (2000).
- 25 Tang, X. & Pikal, M. J. Design of freeze-drying processes for pharmaceuticals: Practical advice. *Pharm. Res.* **21**, 191-200 (2004).
- 26 Brodkorb, A. *et al.* Infogest static in vitro simulation of gastrointestinal food digestion. *Nat. Protocols* **14**, 991-1014 (2019).
- 27 United States Pharmacopeial Convention. *The United States Pharmacopeia 43rd Revision and The National Formulary 38th Edition*, 3382 (2019).
- 28 Mahler, G. J., Shuler, M. L. & Glahn, R. P. Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. *J. Nutr. Biochem.* **20**, 494-502 (2009).
- 29 Tominaga, H. *et al.* A water-soluble tetrazolium salt useful for colorimetric cell viability assay. *Anal. Commun.* **36**, 47-50 (1999).
- 30 Crouch, S. P., Kozlowski, R., Slater, K. J. & Fletcher, J. The use of atp bioluminescence as a measure of cell proliferation and cytotoxicity. *J. Immunol. Methods* **160**, 81-88 (1993).
- 31 Price, E. W. & Orvig, C. Matching chelators to radiometals for radiopharmaceuticals. *Chem. Soc. Rev.* **43**, 260-290 (2014).
- 32 Cole, E. T. *et al.* Enteric coated HPMC capsules designed to achieve intestinal targeting. *Int. J. Pharm.* **231**, 83-95 (2002).
- 33 Jacobson, D. G., Curington, C., Connery, K. & Toskes, P. P. Trypsin-like immunoreactivity as a test for pancreatic insufficiency. *N. Engl. J. Med.* **310**, 1307-1309 (1984).
- 34 Suenderhauf, C. & Parrott, N. A physiologically based pharmacokinetic model of the minipig: Data compilation and model implementation. *Pharm. Res.* **30**, 1-15 (2013).
- 35 Lewis, S. J. & Heaton, K. W. Stool form scale as a useful guide to intestinal transit time. *Scand. J. Gastroenterol* **32**, 920-924 (1997).

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