Oral Delivery of Macromolecules

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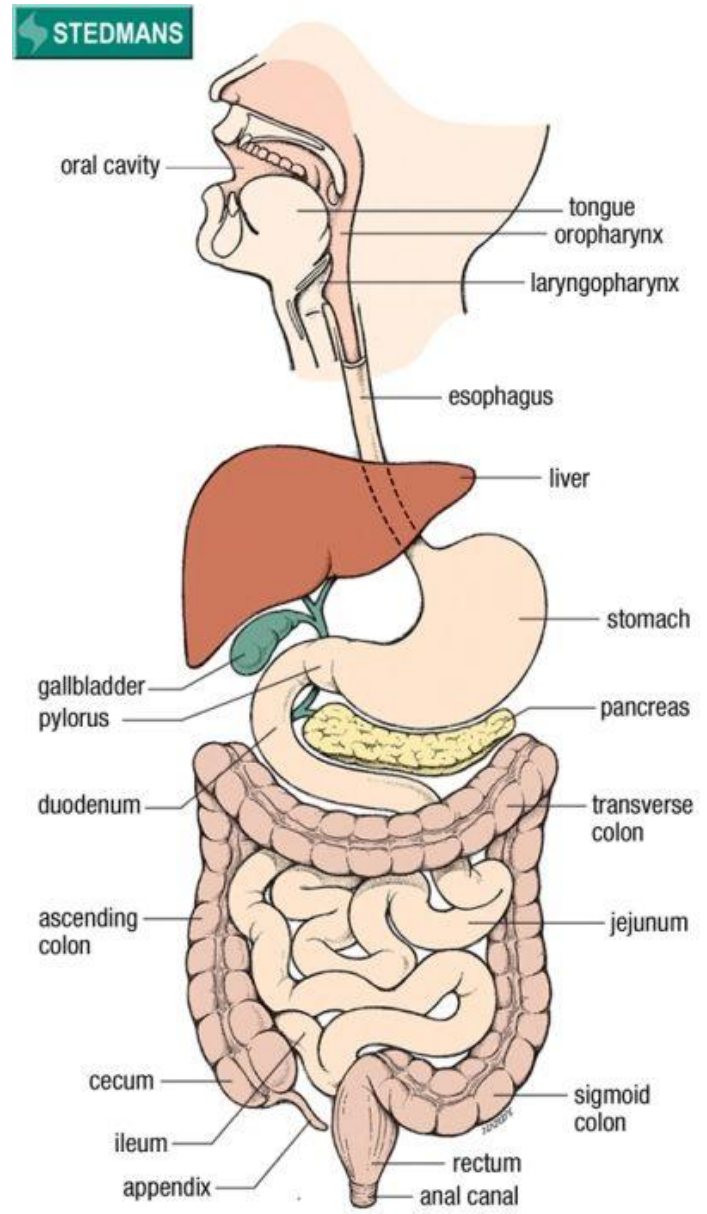
Basic Issues

- The gastrointestinal (GI) tract is exposed to (bombarded by?) an incredibly diverse range of materials and particulates
- There are multiple physical, physiological, and biological barriers in place to effectively restrict non-specific uptake from the GI tract
- Durable damage to that restricted uptake barrier can result in the loss of homeostasis that is incompatible with life
- The GI tract of organized to hydrolyze polymers that compromise most macromolecular drugs
- Location and actions of these hydrolysis events are choreographed to optimize nutrient availability at specific regions of the GI tract
- Hydrolyzed subunits of these foodstuff polymers are then selectively absorbed for utilization as energy sources or for biosynthesis

Organization and Functions of GI Tract

- Propulsion
  - Swallowing (oropharynx)
  - Peristalsis (esophagus, stomach, small intestine, large intestine)
- Chemical digestion
  - Enzymes designed to function in specific regions due to pH optima and activation or pro-forms
- Mechanical digestion
  - Chewing (mouth)
  - Churning (stomach)
  - Segmentation (small intestine)
- Absorption
  - Nutrients, ions, and water to blood and lymph (small intestine)
  - Bacterial-products (phylloquinone, cyanocobalamin, thiamine, riboflavin) and water to blood (large intestine)



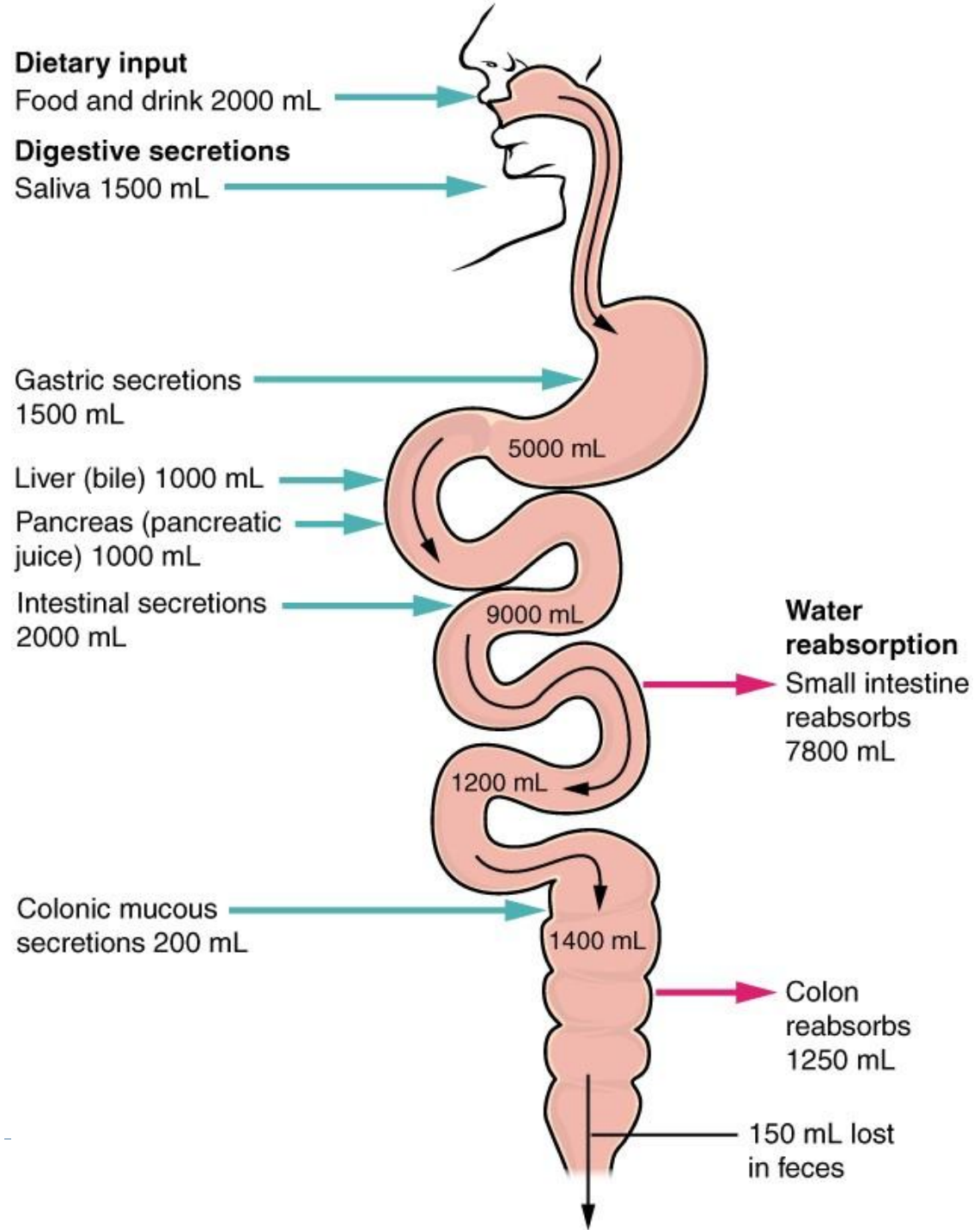
digestive system and associated structures

Enzymatic Hydrolysis in the Human GI Tract

	Carbohydrates		Proteins		Nucleic acids		Fats		Solubilizers
Oral cavity, oesophagus	**Salivary amylase**	**Poly- › Di-saccharides**							**Water from saliva (1.5 L/day)**
Stomach (fundus, body, pylorus)	**Amylase from saliva**		**Pepsin**	**Low pH denaturation Proteins › large peptides**			**Gastric lipase**	**Tri-glycerides**	**Water from gastric juice (2 L/day)**
Small intestine lumen (duodenum, jejunum, ileum)	**Pancreatic amylases**		**Pancreatic enterokinase, chymotrypsin, trypsin, carboxy-peptidases**	**Larger peptides › smaller peptides**	**Pancreatic nucleases**	**DNA, RNA › nucleotides**	**Pancreatic lipases**	**Fat globules › doplets**	**Water (2 L/day) and bile salts from gall bladder and pancreas**
Small intestine brush border (duodenum, jejunum, ileum)	**Disaccharidasee nzymes, sucrase, maltase, lactase**		**Aminopeptidases, carboxypeptidases, dipeptidases**	**Small peptides › amino acids**	**Nucleotidases Nucleosidases Phosphatases**		**Nucleosides Nitrogenous bases, sugars, phosphates**	**Glycerol, fatty acids, glycerides**	**Water (3 L/day) Bile salt uptake in distal ileum**
Colon (ascending, transverse, descending, sigmoid									**Water absorption (7-9 L/day)**

Follow the Water

- All digestive enzymes are hydrolases
- Lumen of esophagus and small intestine are potential spaces
  - Space is expanded to accommodate digesting packets of foodstuff: chyme to chyle
- Organizations to maintain cavities
  - Mouth – skeletal
  - Stomach – complex muscle organization
  - Large intestine – taenia coli (haustra)
- Management of water inflex and efflux are paramount to the digestive process, occurring constantly but also regulated locally in association with a meal

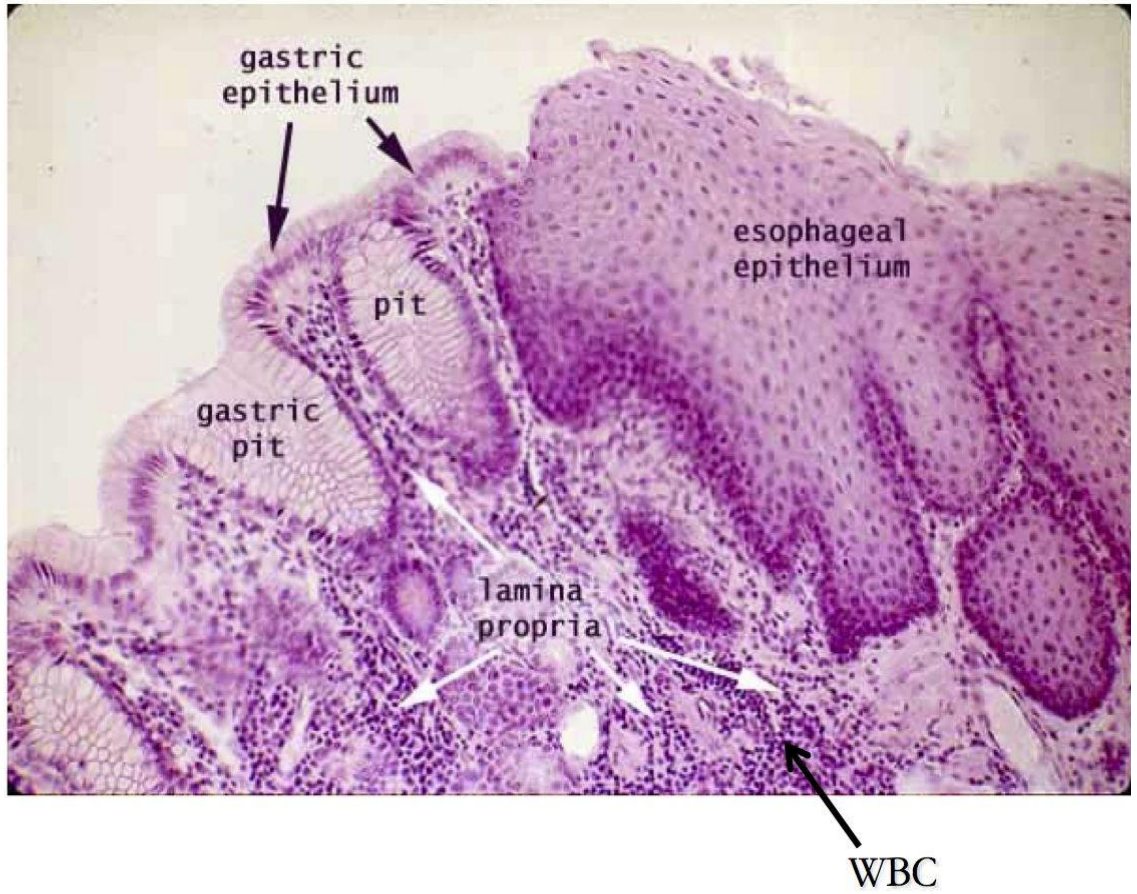


Holy Grail of Biopharmaceuticals

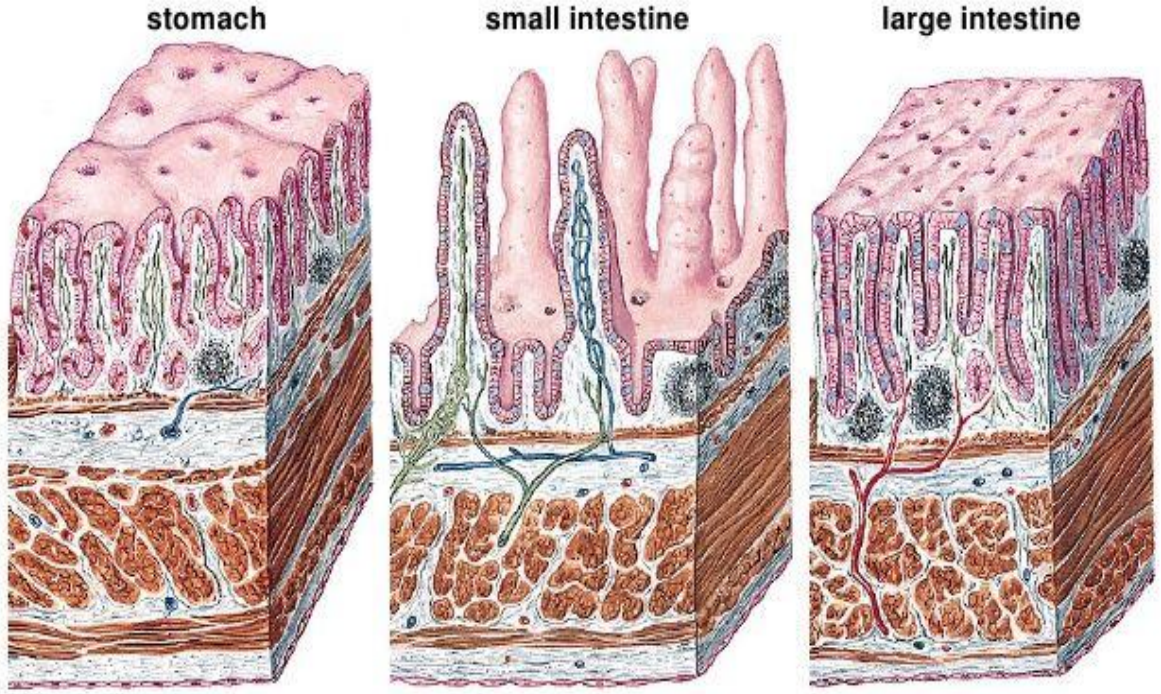
- Oral delivery of macromolecules (protein, peptide, nucleic acid-based therapeutics) are currently only efficiently administered only by subcutaneous (SC) injection or intravenous (IV) infusion.
- There are several issues limit making oral delivery of biopharmaceuticals a clinical/pharmaceutical reality.
  - Instability in the digestive tract – looks like food
    - Acidic stomach
    - Pancreatic enzymes
    - Glycocalyx enzymes
  - Inability to cross the intestinal mucosa – protection
    - Mucus as binding agent for viruses, etc.
    - Epithelium can restrict water and ion flux
- Overcoming these barriers has been the focus of research for 100 years

Epithelia – the Ultimate Barrier to Macromolecules

Esophagus - Stomach Junction

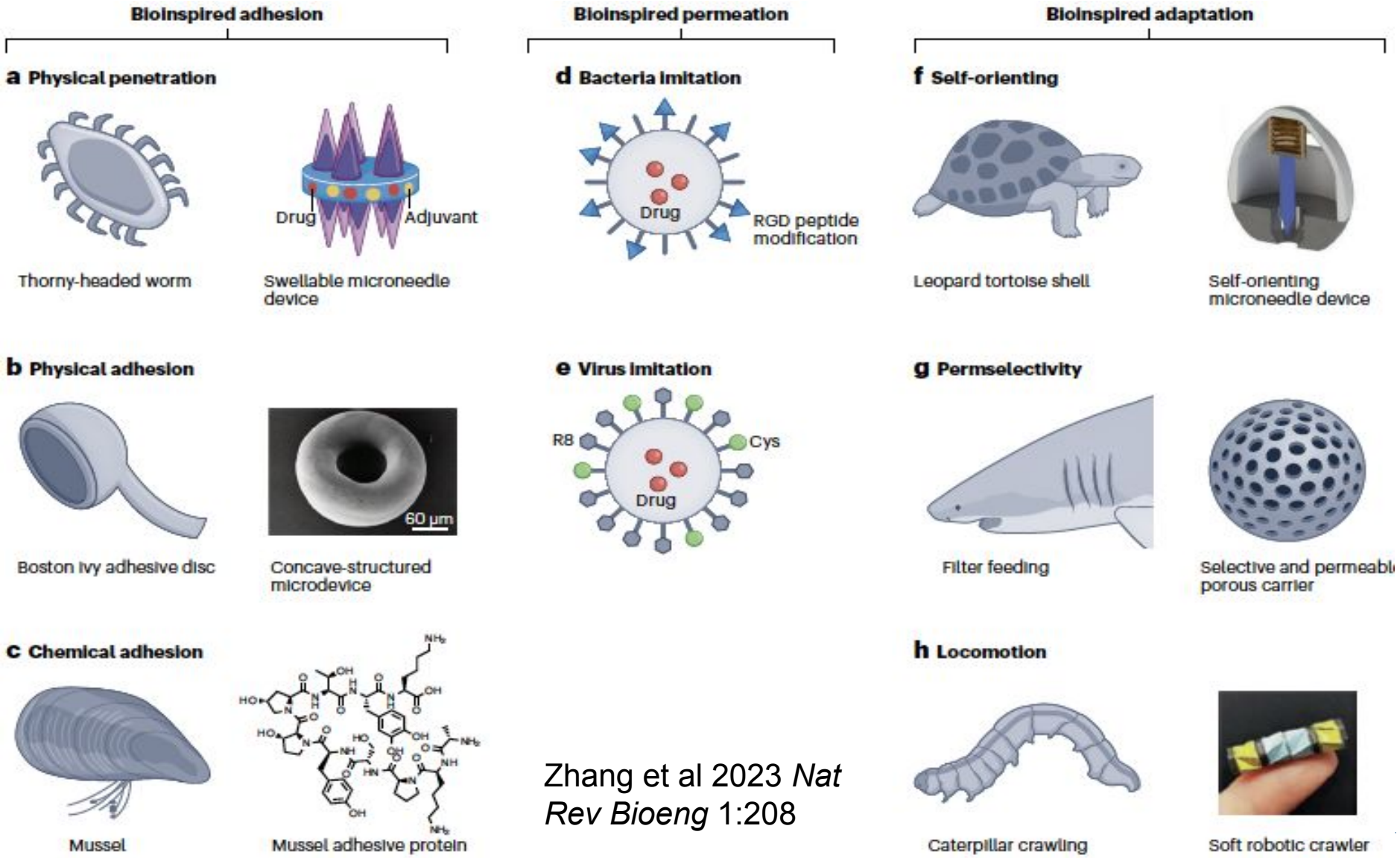


Comparison of histology of stomach, small- and large intestines



Note differences in surface area, innervation, and blood/lymph vessels

Physical Approaches



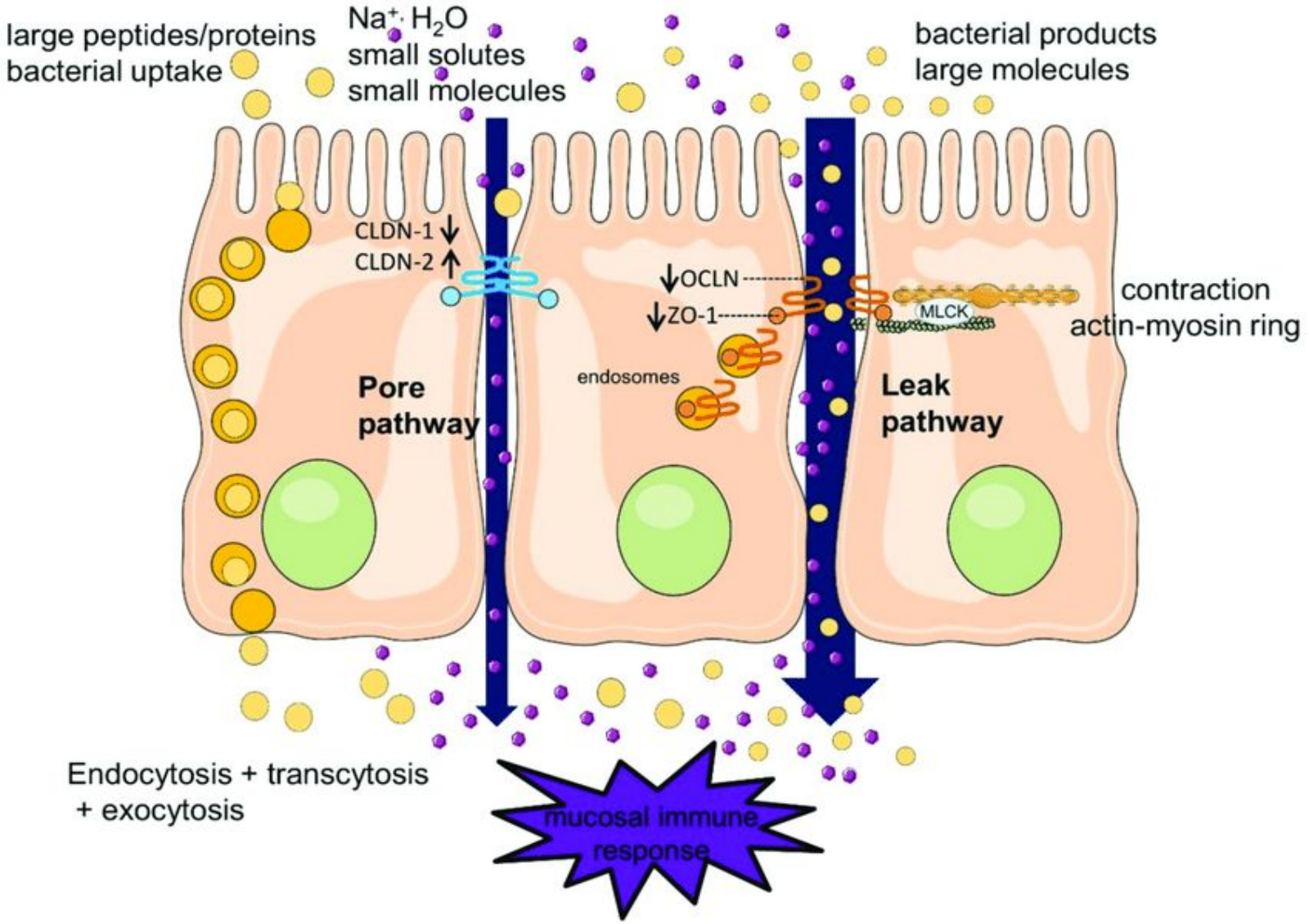
Zhang et al 2023 *Nat Rev Bioeng* 1:208

Physicochemical Approaches

- **Make the biopharmaceutical more stable** – You ingest all kinds of very stable materials the size of biopharmaceuticals. These end up in your poop, not in your blood. Enzyme inhibitors can block normal biological processes.
- **Incorporate the biopharmaceutical into nanoparticles** – There are many nanoparticles that enter your GI tract, the only ones that get into epithelial cells are viruses, and they are typically not that efficient. Viruses rely on replication to be injective.
- **Increase membrane solubility** – Biologically relevant biopharmaceuticals typically are designed to function either a cell membrane surface or inside of a cell. Making them able to pass through membranes will likely make them inactive or incapable to being where they need to be to be active.
- **Improve mucus penetration capacity** – This issue occurs with artificial systems. Viruses and biopharmaceuticals have no problem with mucus.

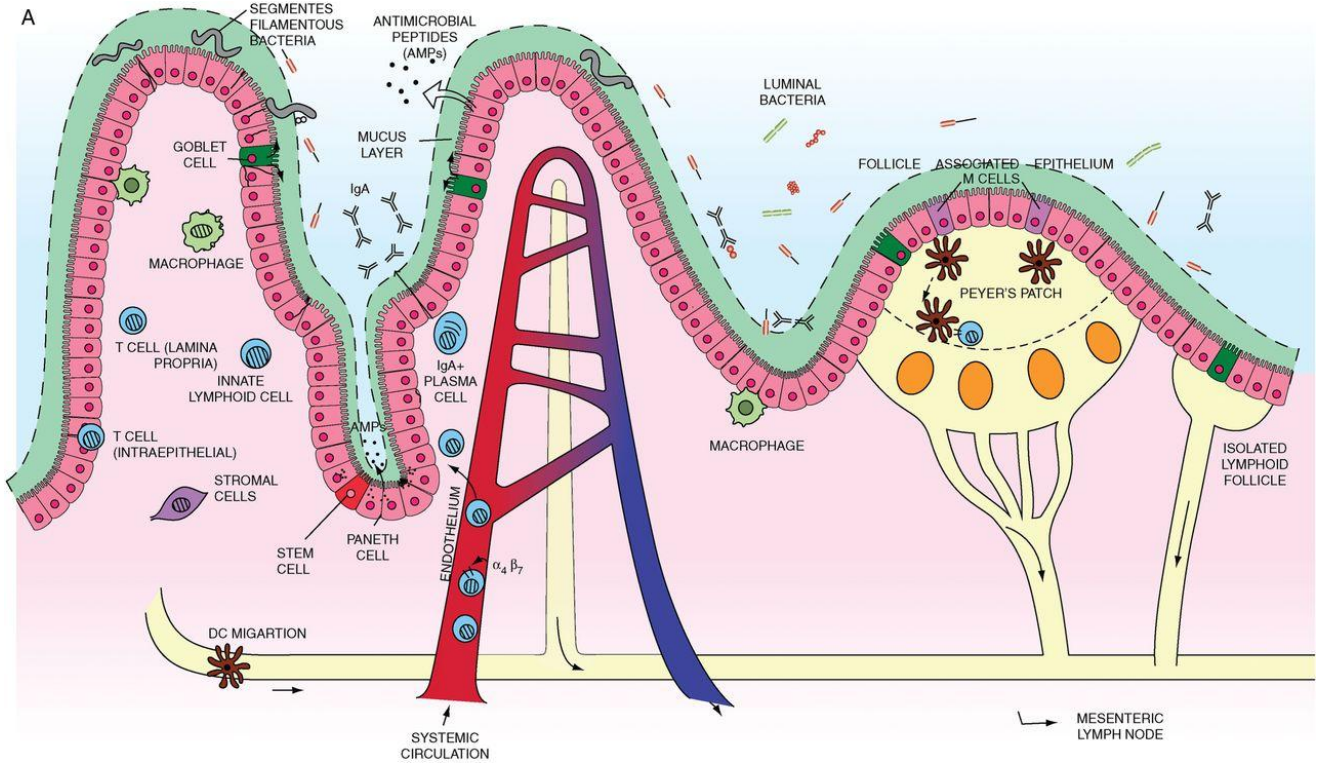
Paracellular vs Transcellular

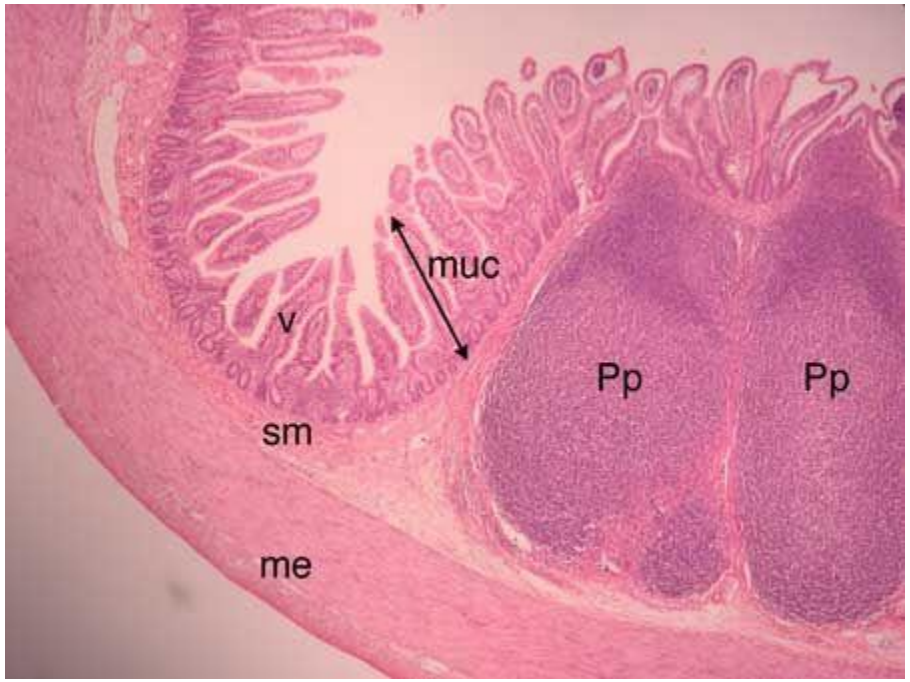
- Physicochemical properties of relevant macromolecules (protein, peptide, nucleic acid-based therapeutics) limit their transport to leak pathway paracellular and vesicular transcytosis.
- Multiple methods are used by epithelial cells to block non-specific transcytosis.
- Activation of leak pathway is associated with tight junction dysfunction and inflammatory pathologies.



Note, small molecules are naturally absorbed though the pore paracellular route.

The Reality of Microfold (M) Cells





In humans, Peyer's patches (PPs) consist of hundreds lymphoid follicles aggregated into an oval shape in the terminal ileum.

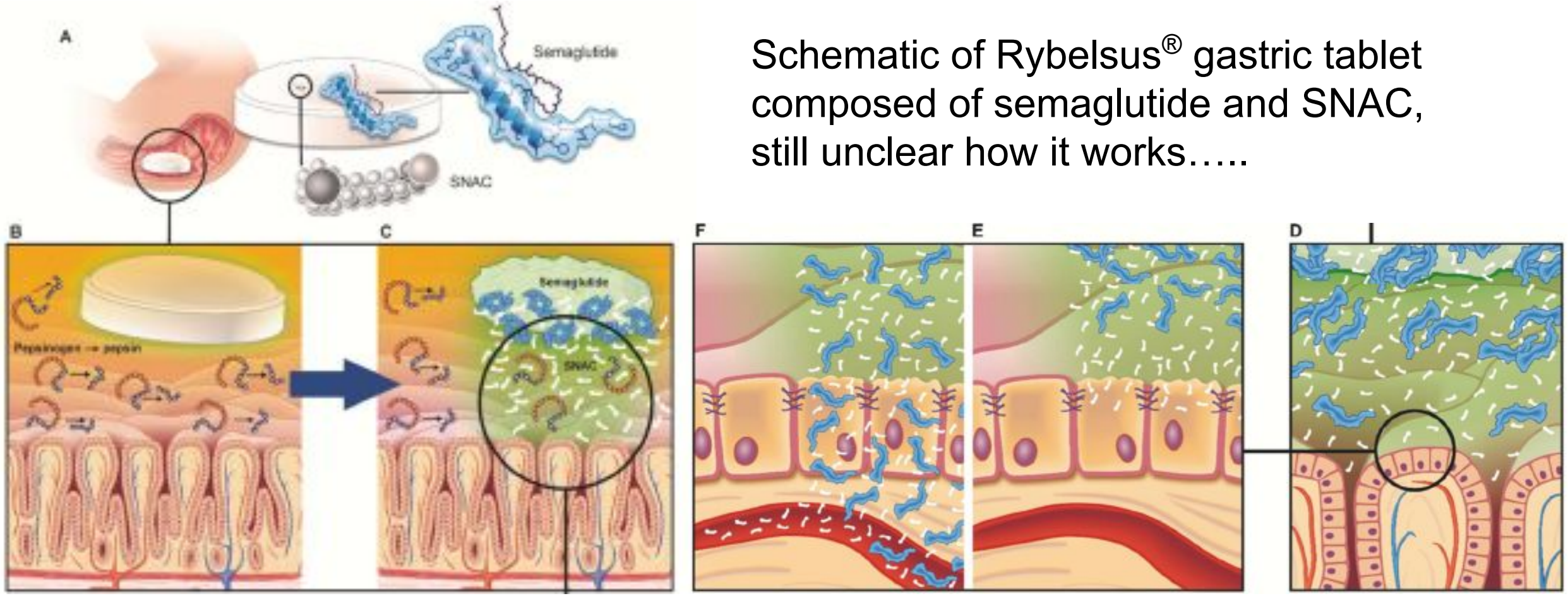
In mice, there is a different organization and distribution with 6–8 PPs with 4 or 5 lymphoid follicles dispersed at equal intervals along the entire length of the small intestine.

Cader and Kaser 2013 *Gut* 62:1653; Ohno 2016 *J Biochem* 159:151

Current State of Affairs for Peptide Delivery

- **Permeability enhancers (PEs) identified through empirical testing**
  - Over 250 paracellular and transcellular PEs have been described
  - Transiently alter permeability properties pf the epithelium and/or the nature of the drug to enhance its transcellular movement
  - Without a Mechanism of Action (MoA), clinical translation is challenging
  - Generally safe in nature, these PEs must be co-delivered at high levels
  - At such high levels, a PE could also act to block peptidase activities
  - Provide low single digit increase in bioavailability (BA)
- **Polymeric (nano/micro)particles**
  - Believed to improve oral delivery outcome, no translation yet
    - Drug stabilization
    - Improved access to apical cell surface of epithelium

A Clinically Validated Peptide Delivery Approach



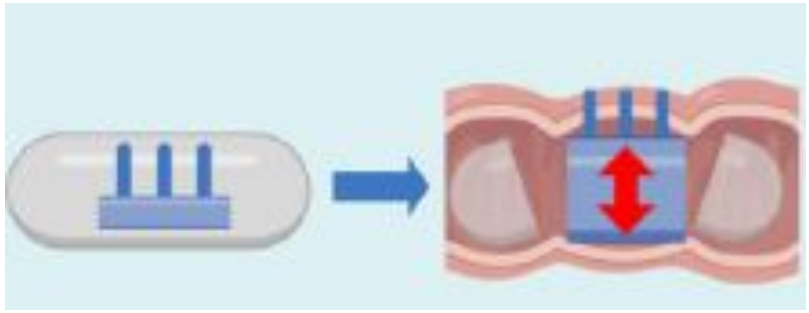
Schematic of Rybelsus® gastric tablet composed of semaglutide and SNAC, still unclear how it works…..

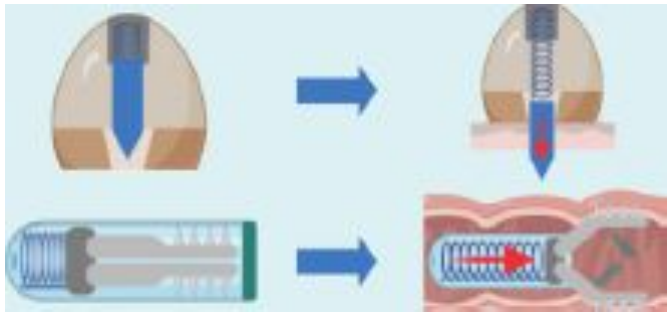
Kim *et al* 2022 *Pharmaceutics 15*:1585

Current State of Affairs for Protein Delivery

- **Specific cell surface receptors**
  - Cyanocobalamin/intrinsic factor/cubulin receptor – Challenged by limited receptor-mediated pathway availability
  - Transferrin/transferrin receptor – Uncertain how this receptor that is typically targeted to the basolateral surface can work for apical uptake
  - Immunoglobulin G/Neonatal Fc receptor (FcRn)
  - Toxin-based transcytosis pathway - validated in Phase 2
- **Physical delivery using microneedles** – Some validated in Phase 2

RaniPill™





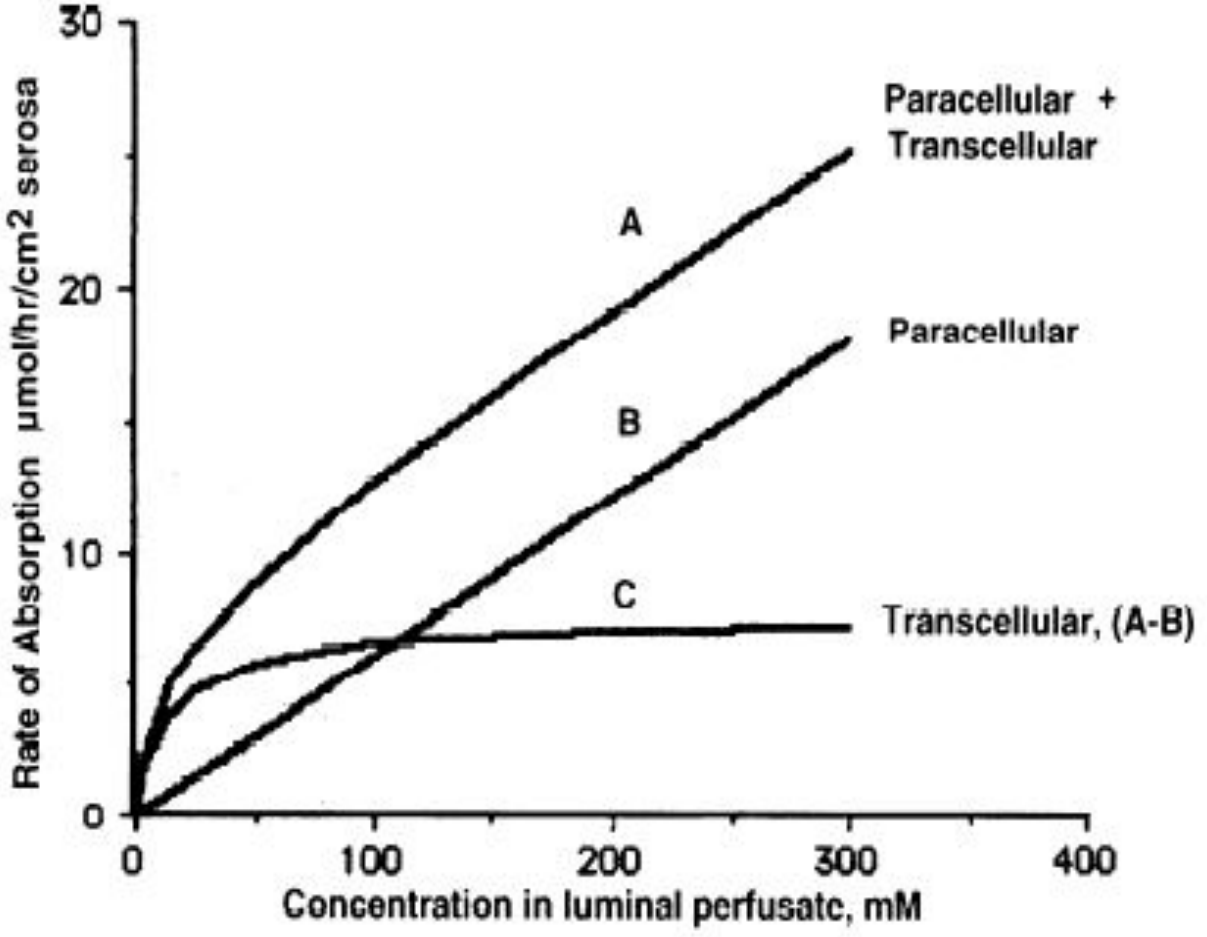
SOMA/LUMI

Zizzari *et al* 2021 *Drug Discov Today 26*:1097

What Makes Sense?

- **Use endogenous mechanisms that occur in man**
  - Great outcomes in rodents usually does not translate to the clinic
- **Approaches for repeated use without epithelial damage**
  - Commercial viability requires repeat customers
- **Non-immunogenic materials**
  - Even PEGs can show immunological activity
  - GI tract, however, has the benefit of immunosuppressive properties
- **Consider GI tract physiology**
  - Replacing a shot with a pill is not always practical
  - Intestinal activity and extraction
  - Hepatic-portal vasculature

Nutrients Modulation Paracellular Uptake



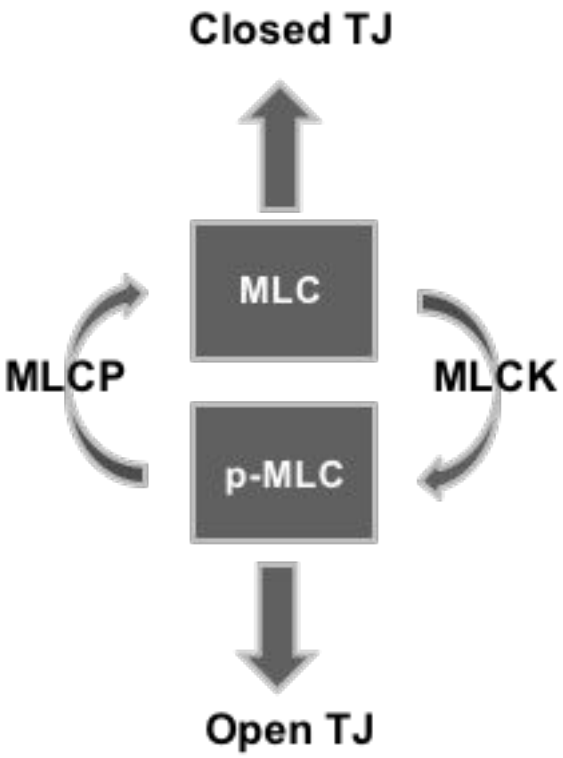
Specific for apical Na^+-dependent transporters for essential amino acids and glucose as secondary dietary uptake mechanism.

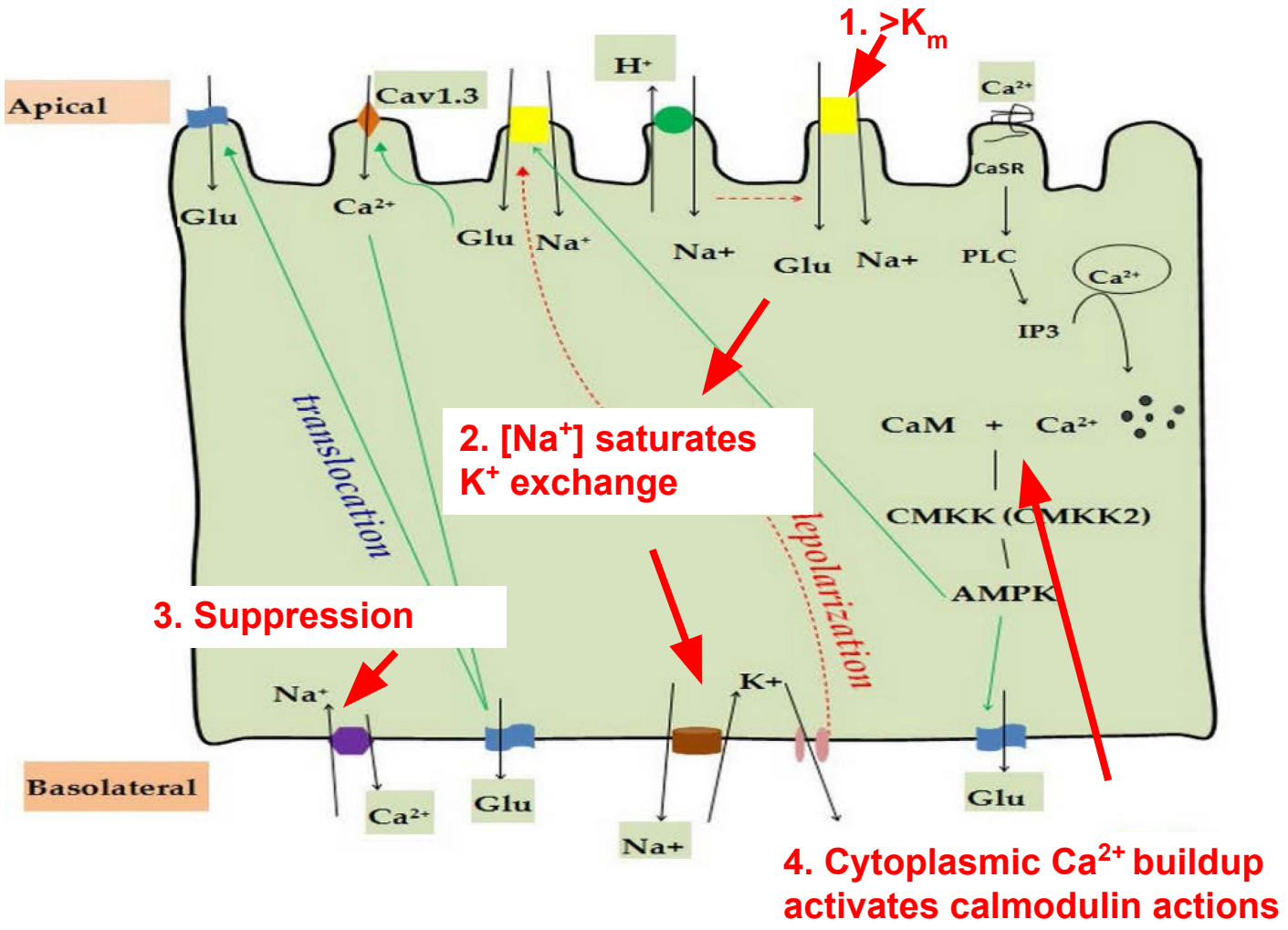
Free glucose is usually <25 mM in the blood and body fluids, but digestion can produce levels of 300 mM in the microenvironment of intestinal TJs.

Uptake of tryptophan, the limiting essential amino acid in the human diet, is associated with enterocyte secretion of antimicrobial peptides.

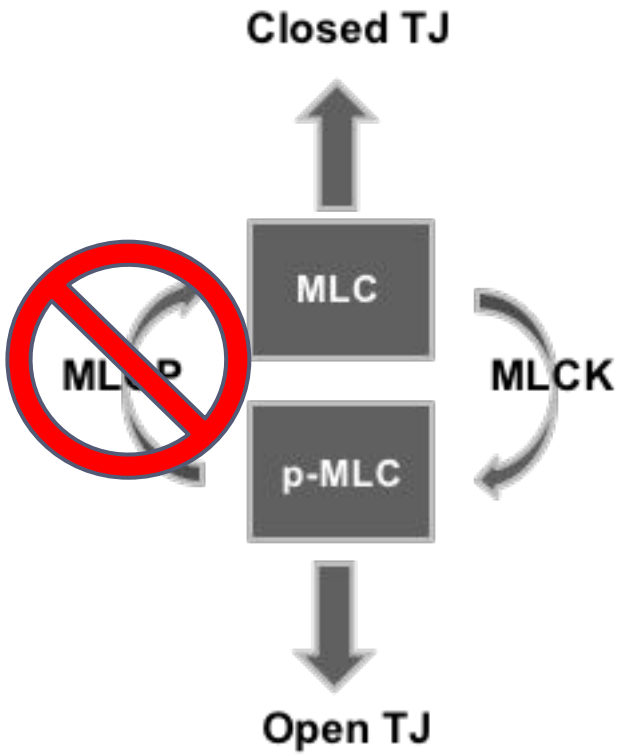
Pappenheimer 1993 *AJP Gastro* 265: G409; Bröer 2023 *Ann Rev Nutr* 43:12.1

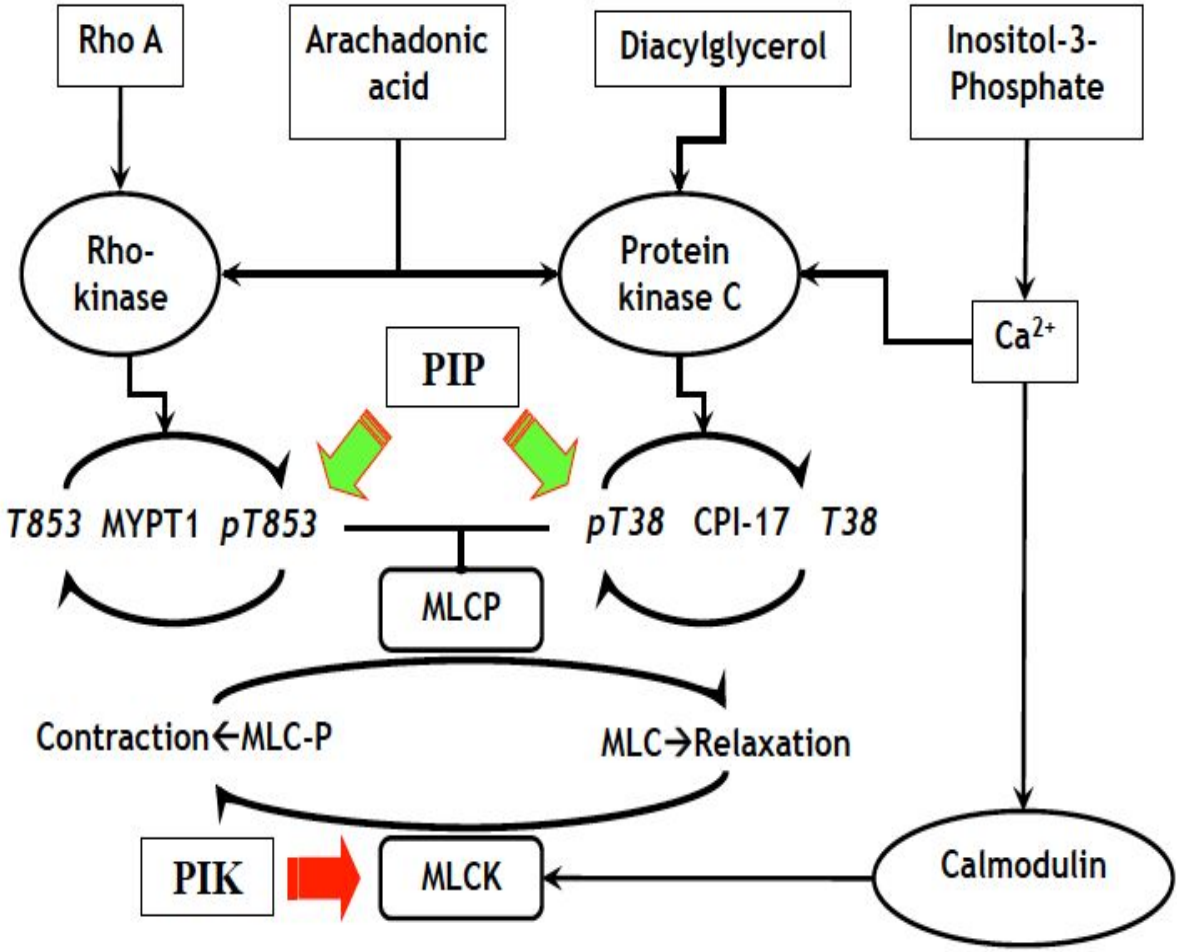
Nutrient Uptake Regulation of $[Ca^{2+}]_i$



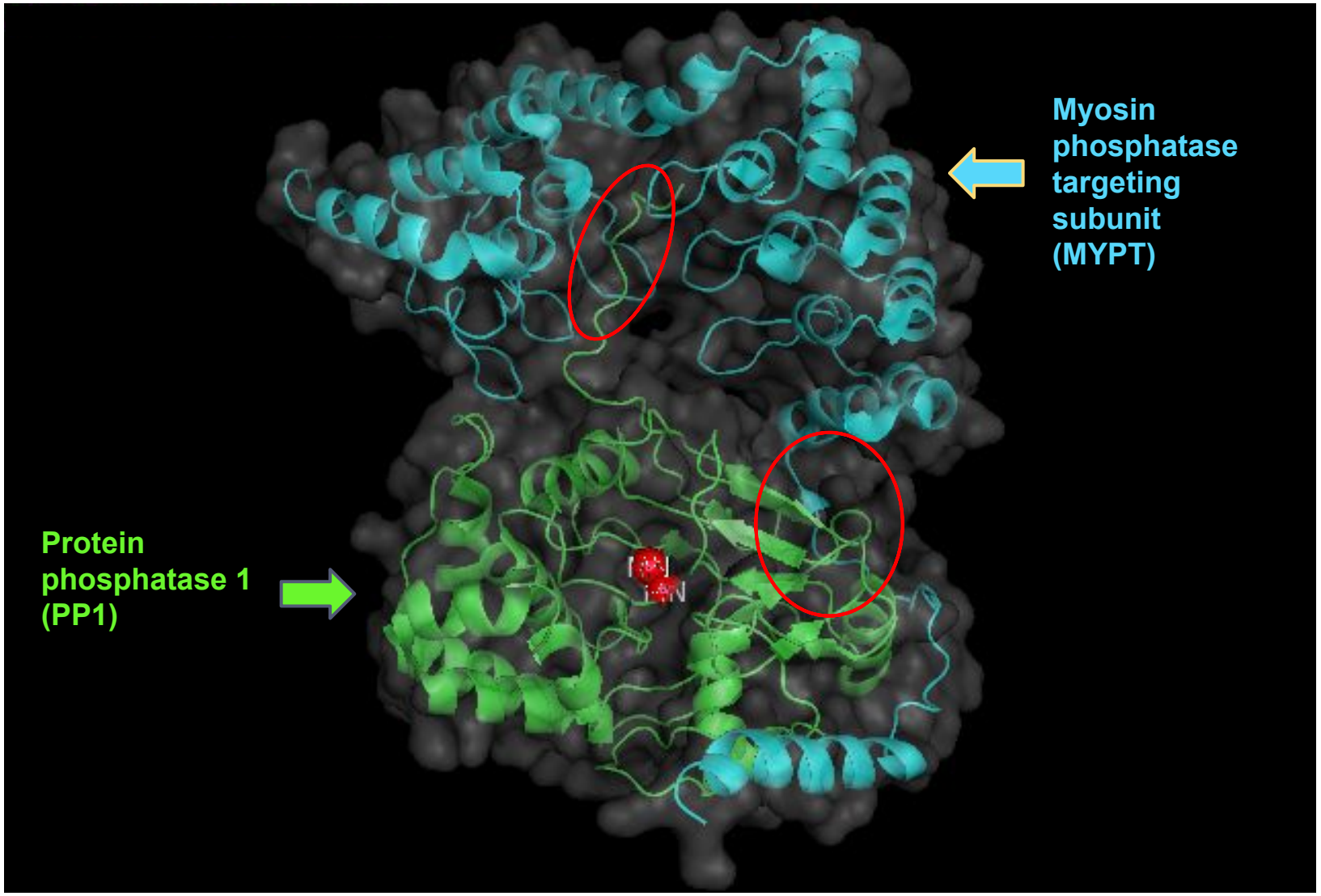


Regulation of Myosin Light Chain Phosphatase





MYPT1-PP1 Protein Interactions

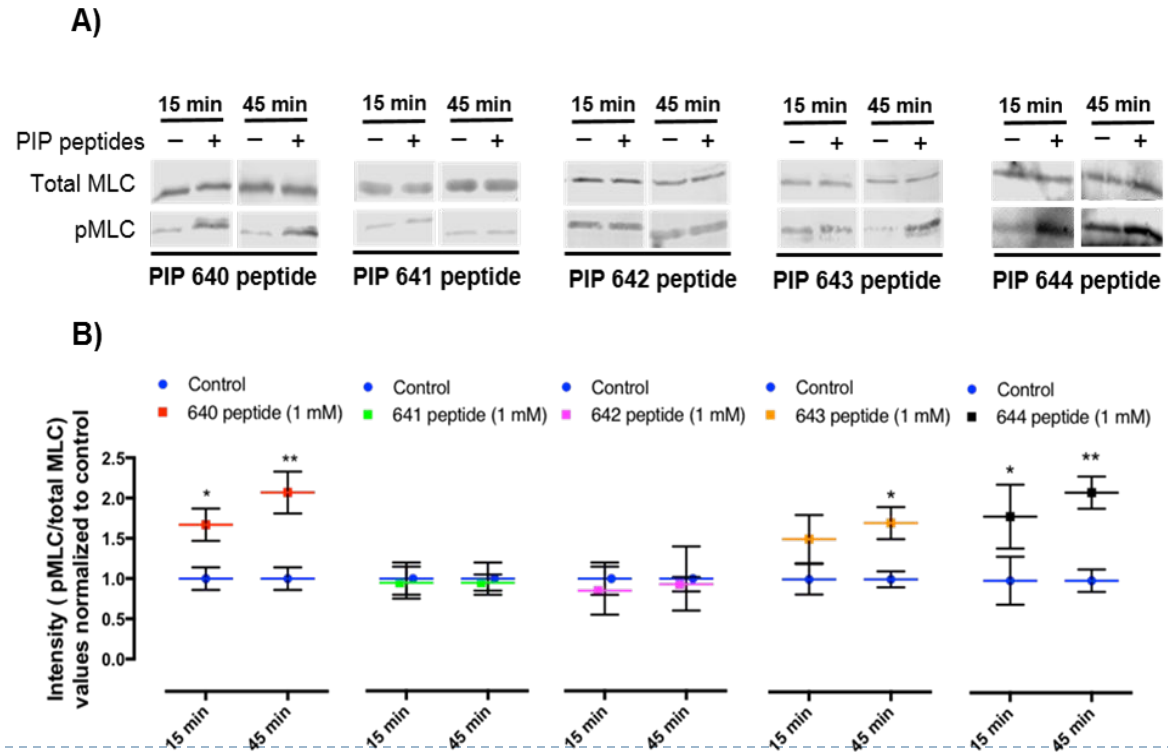


Terrak *et al* 2004 *Nature 429:780*

Site Specific Requirements of PIP 640

- Anticipated inter-facial contact sites involving glutamic acid and tyrosine are essential for full function of PIP 640
- Aspartic acid was not critical for pMLC induction. Changing to either a neutral (alanine) or positive (arginine) amino acid did not block pMLC induction.

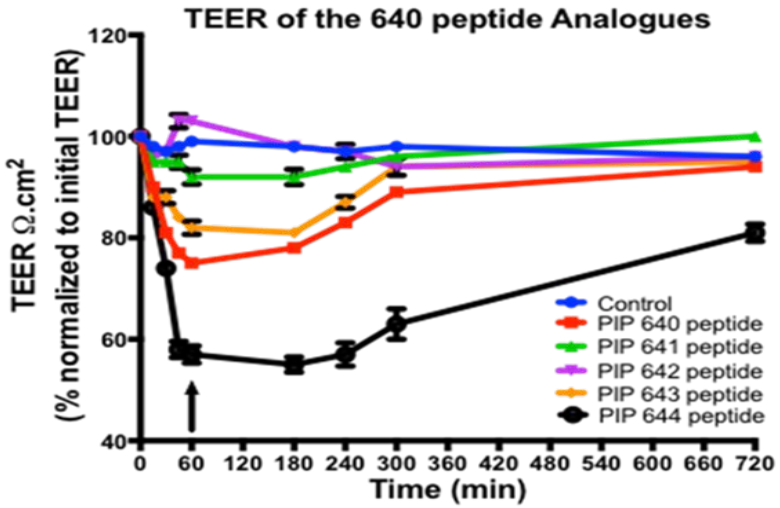
Peptides	Sequence	Aspect tested
PIP 640	rrdykvevrr-NH_2	--------
PIP 641	rrdykvavrr-NH_2	Target binding
PIP 642	rrdakvevrr-NH_2	Target binding
PIP 643	rraykvevrr- NH_2	Specificity
PIP 644	rrrykvevrr- NH_2	Specificity



Almansour *et al* 2018 *J Controlled Rel* 279:208

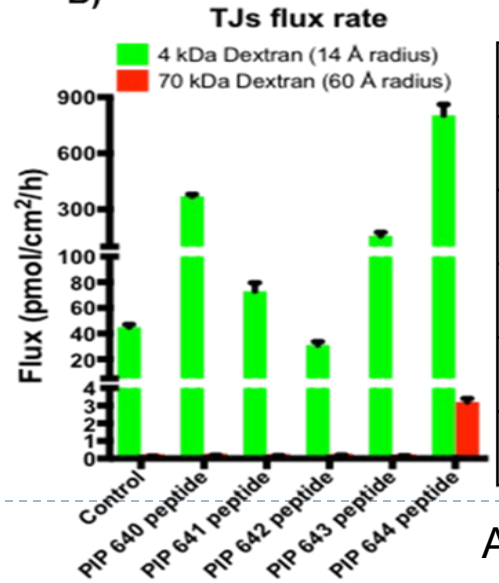
Altered Efficacy of PIP 640 Peptides

A)



TEER contrast	*Sig.*	TEER contrast	*Sig.*
Control- PIP 640 peptide	*	PIP 640 peptide- PIP 641 peptide	**
Control- PIP 641 peptide	n.s	PIP 640 peptide- PIP 642 peptide	*
Control- PIP 642 peptide	n.s	PIP 640peptide- PIP 643 peptide	n.s
Control- PIP 643 peptide	*	PIP 640 peptide- PIP 644 peptide	*
Control- PIP 644 peptide	**	----------	----

B)

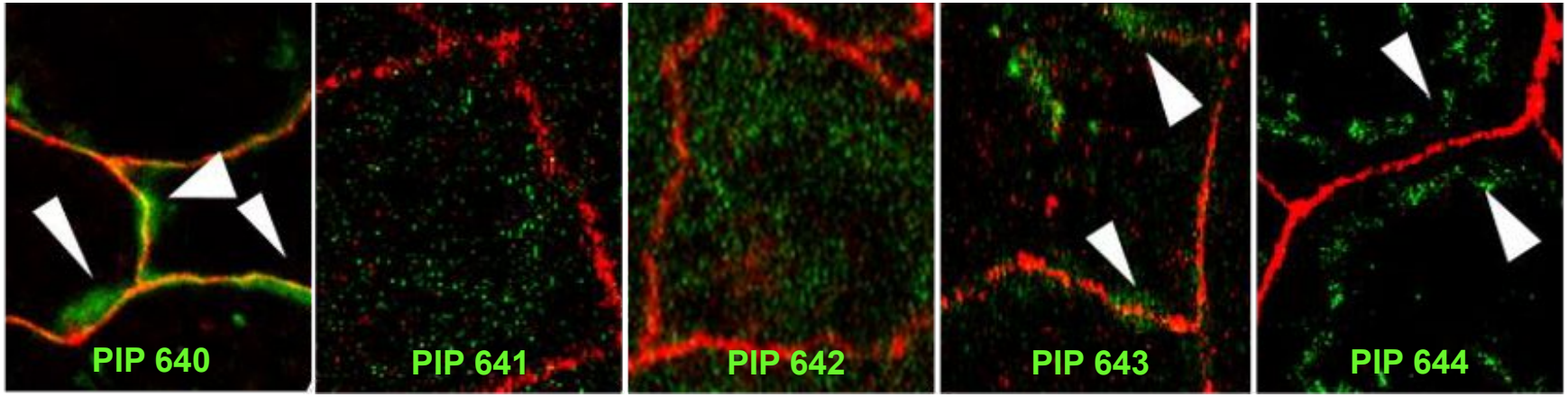


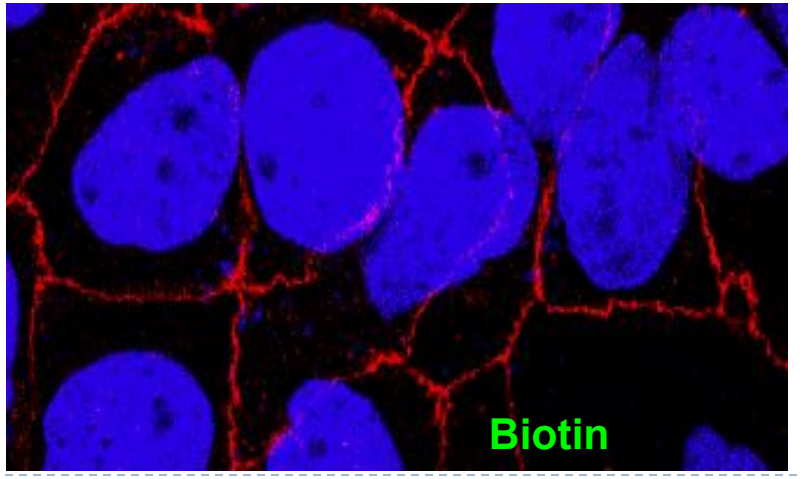
Flux contrast	*Sig.* 4 kDa	*Sig.* 70 kDa	Flux contrast	*Sig.* 4 kDa	*Sig.* 70 kDa
Control- PIP 640 peptide	****	n.s	PIP 640 peptide- PIP 641 peptide	****	n.s
Control- PIP 641 peptide	n.s	n.s	PIP 640 peptide- PIP 642 peptide	****	n.s
Control- PIP 642 peptide	n.s	n.s	PIP 640peptide- PIP 643 peptide	****	n.s
Control- PIP 643 peptide	***	n.s	PIP 640 peptide- PIP 644 peptide	****	****
Control- PIP 644 peptide	****	****	----------	----	----

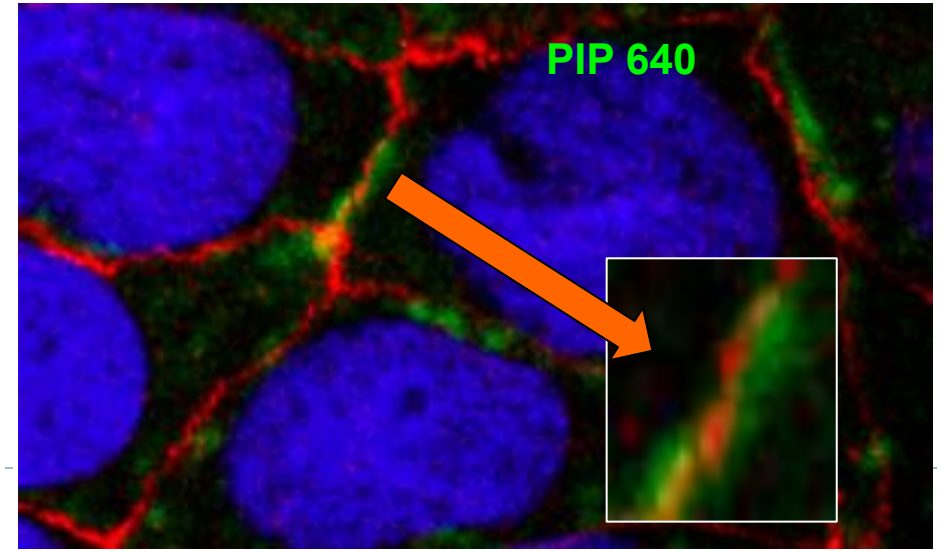
Almansour, *et al.* 2018 *J Controlled Rel* 279:208

640 Peptide Localizes to TJs

Caco-2 cells were labeled *in vitro* for Occludin, Nuclei and biotin-PIP 640 peptides using streptavidin-Alexa 488







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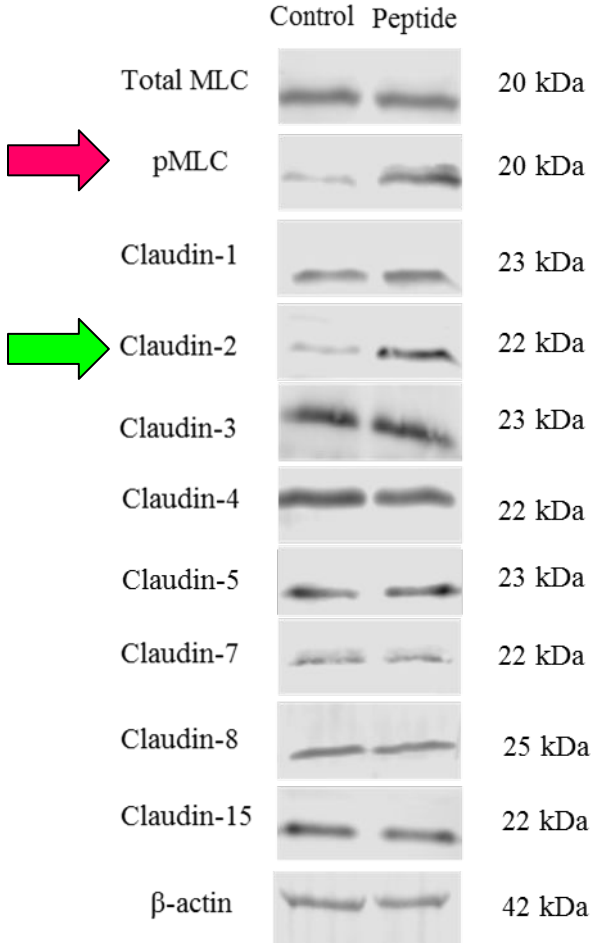
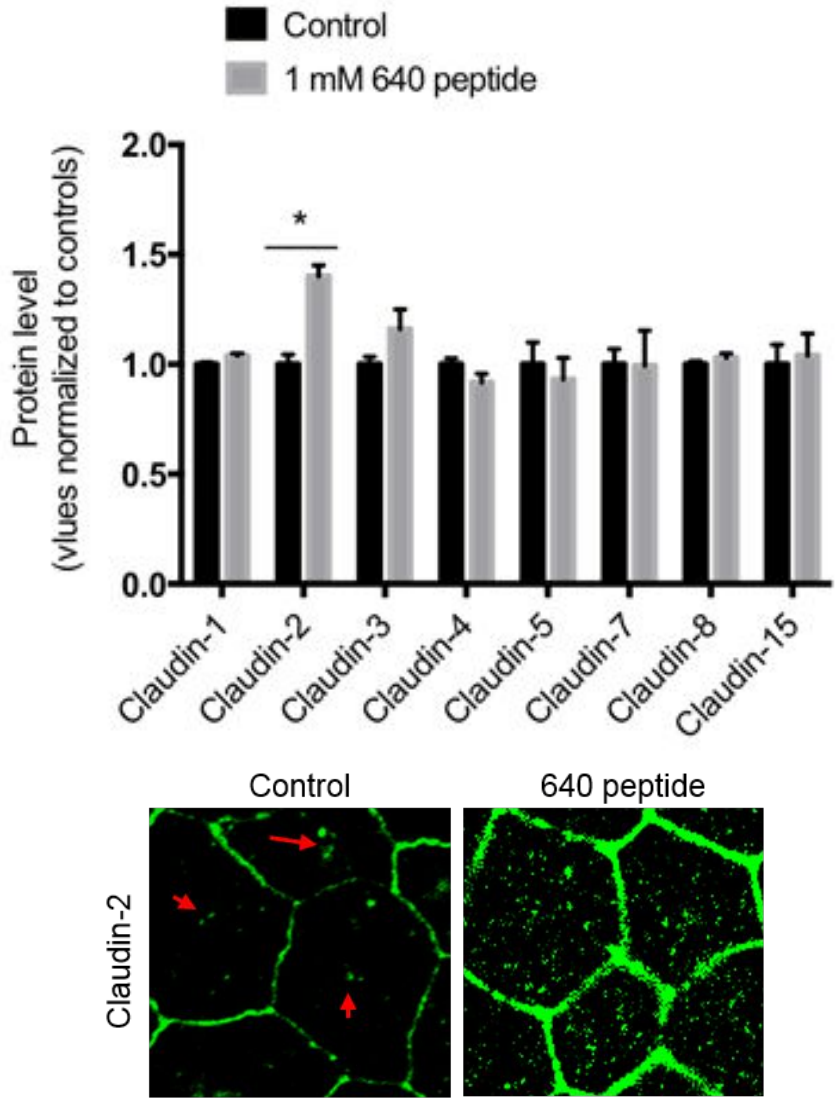
PIP 640 Increases Claudin-2 Protein Level

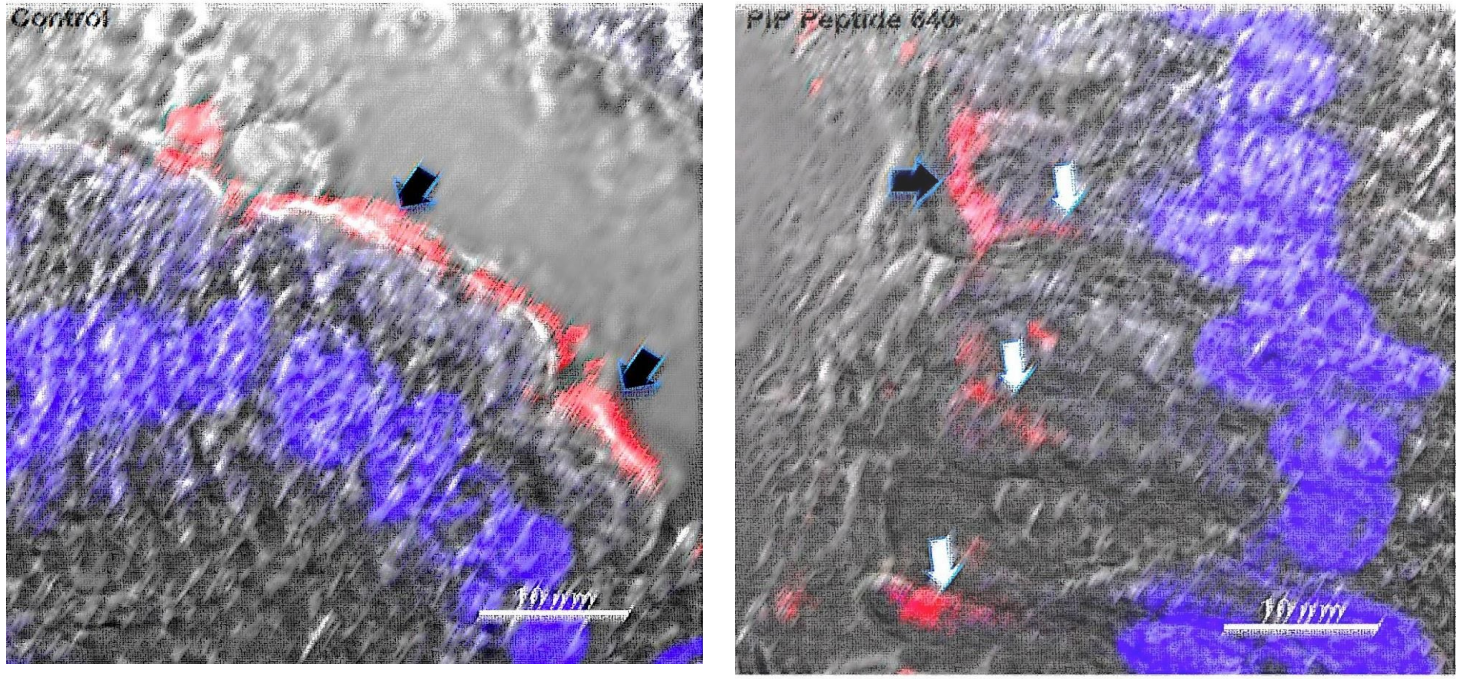


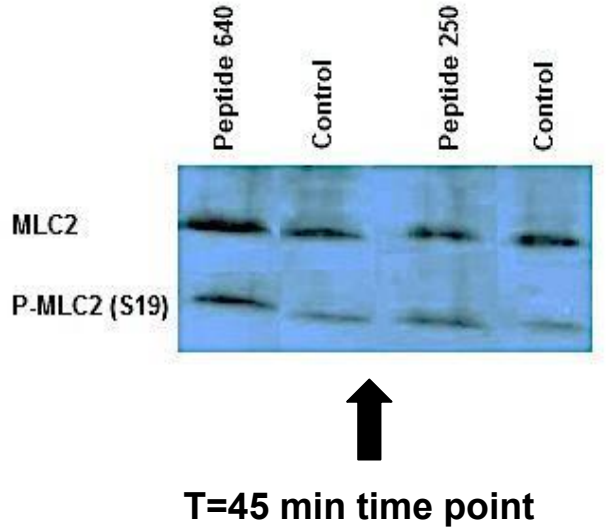


PIPs enhances paracellular (TJ) solute uptake and permselective properties to supress endotoxin absorption. For comparison, sodium caprate (C_{10}) transiently removes claudin-5 and tricellullin from TJs as part of its actions to increase permeability (Krug *et al* 2012 *Biomaterials* *34*:275) as well as altered intracellular Ca^{2+} and membrane fluidity (Twarog *et al* 2019 *Pharmaceutics* 11:78) which are non-specific effects.

Almansour, *et al.* 2018 *J Controlled Rel* 279:208

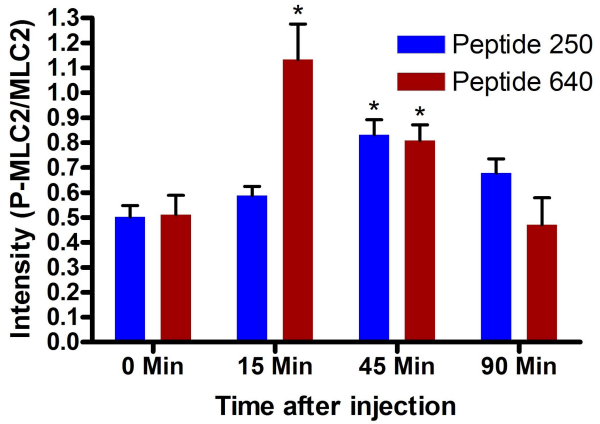
In Vivo Mechanism of Action





- Cy3-Insulin remains at luminal surface after intra-luminal intestinal injection
- Co-administration with peptide 640 enhances the presence of Cy3 label to sites consistent with paracellular transport

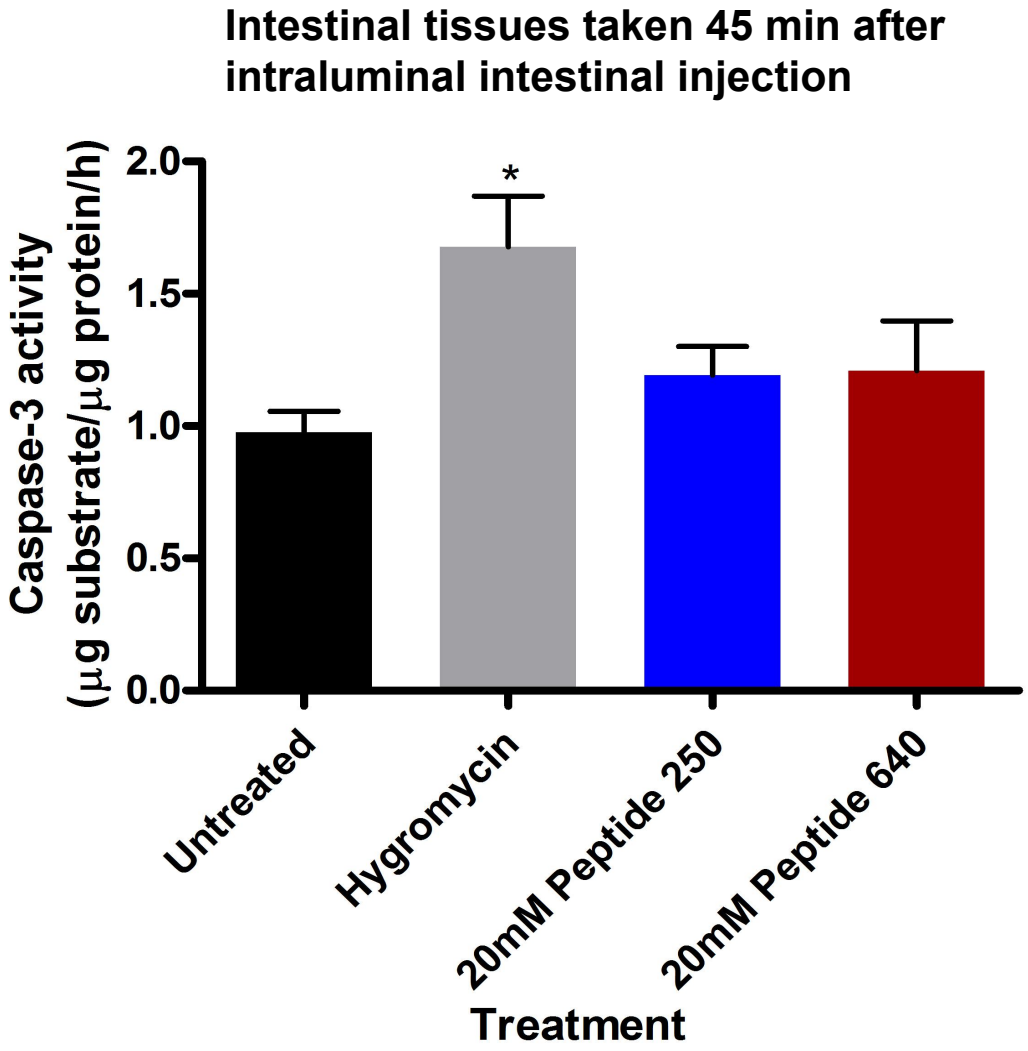
B



Taverner *et al* 2015 *J Controlled Rel* 210:189

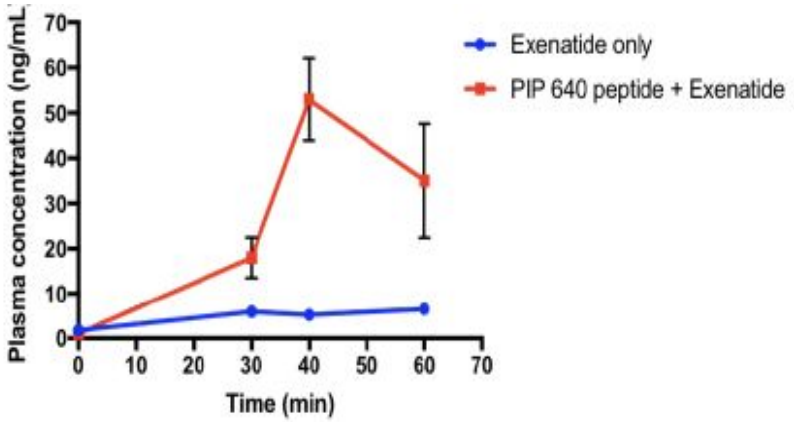
PIP Peptide Toxicity *In Vitro* and *In Vivo*

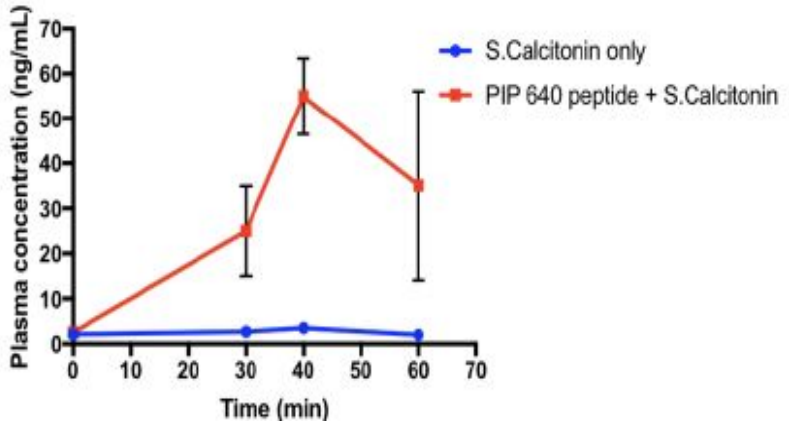
- For all *in vitro* conditions tested, complete TEER recovery was achieved by 24 h following PIP peptide removal.
- Initial *in vitro* studies using Caco-2 cells suggested that peptide 250 and 640, tested at concentrations that modulated TEER and paracellular permeability, did not affect cell viability as assessed by the mitochondrial membrane polarity marker MTS.

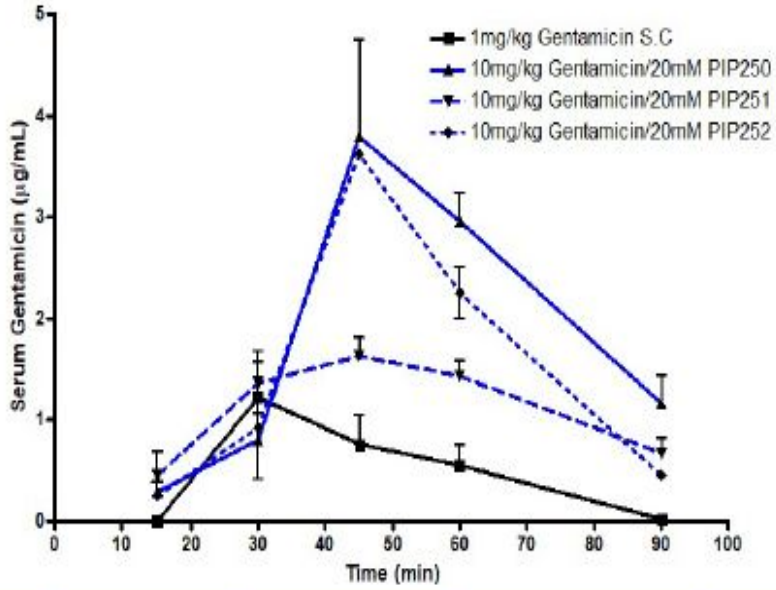


Taverner *et al* 2015 *J Controlled Rel* 210:189

In Vivo Uptake of Clinical Candidates



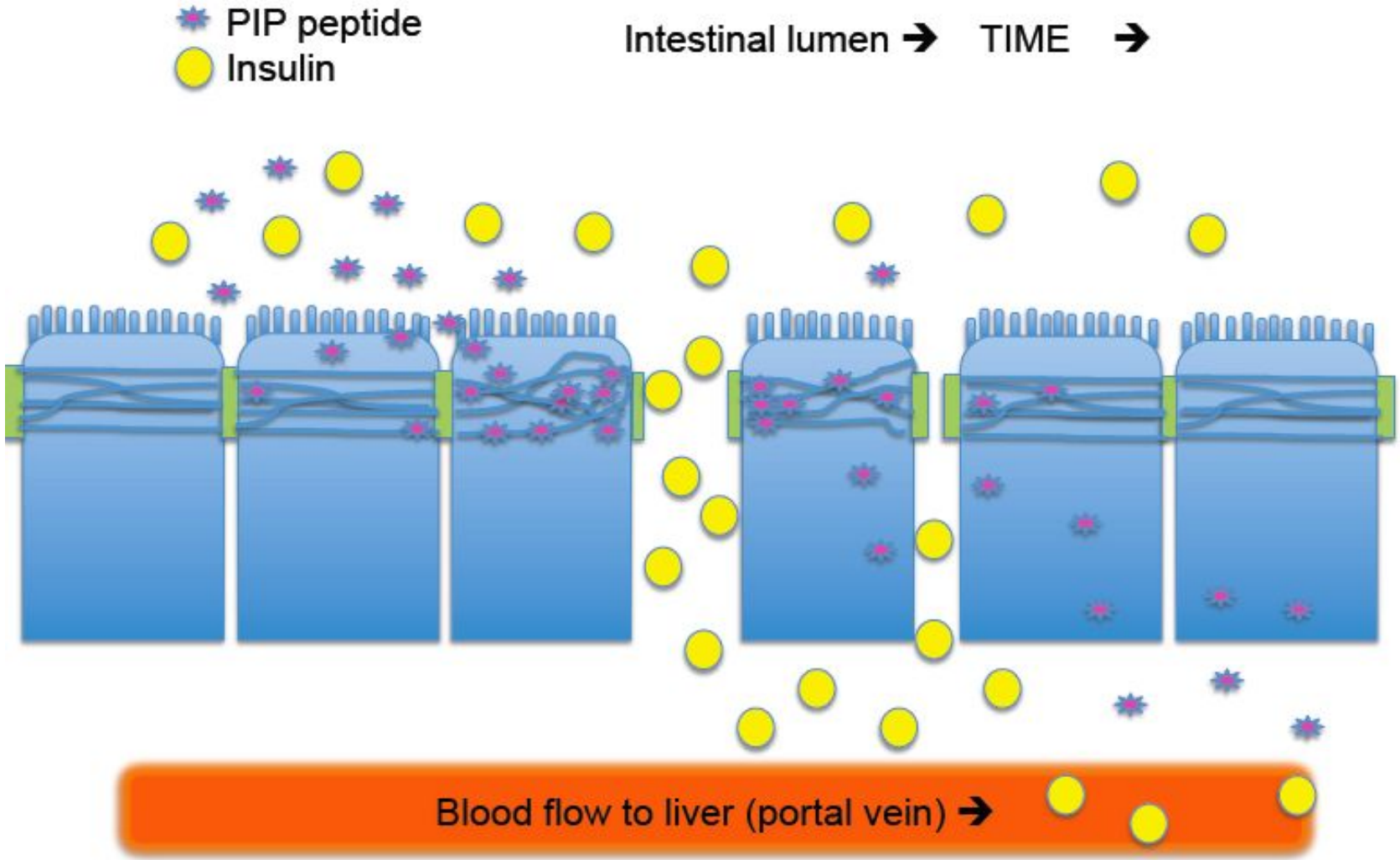




Peptide	T_{max} (min)	C_{max} (µg/mL)	AUC (µg/mL·min)	F_{REL}
PIP250	45	3.79 ± 1.67	155 ± 30.4	36.5%
PIP251	45	1.63 ± 0.31	90.5 ± 2.9	21.3%
PIP252	45	3.62 ± 0.02	127.4 ± 2.1	31.0%

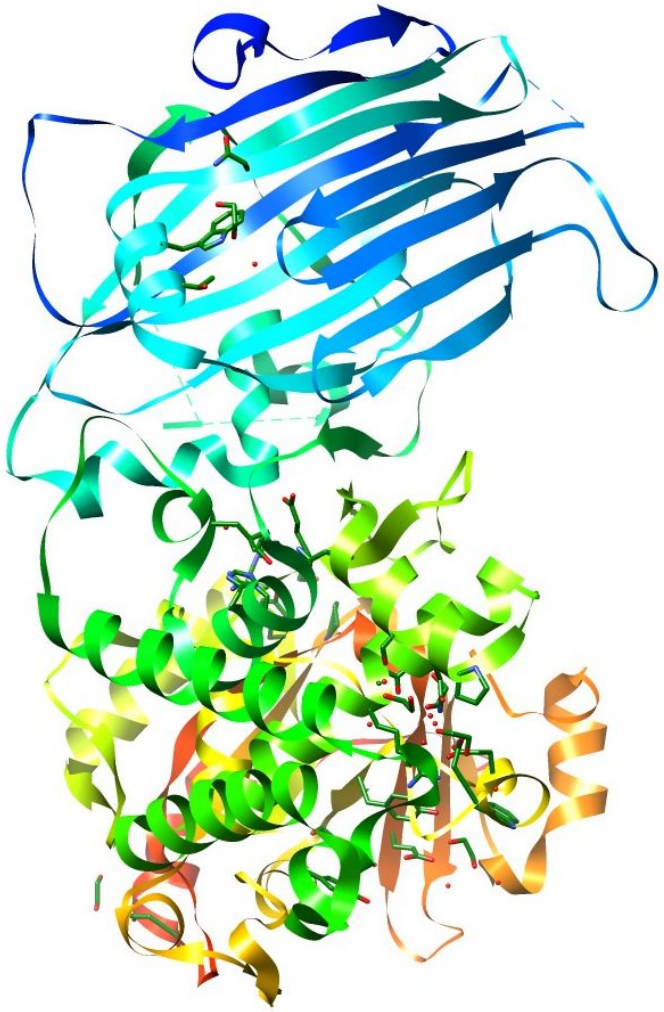
Almansour, *et al.,* 2018 *J Controlled Rel* 279:208; Taverner, et al., unpublished data

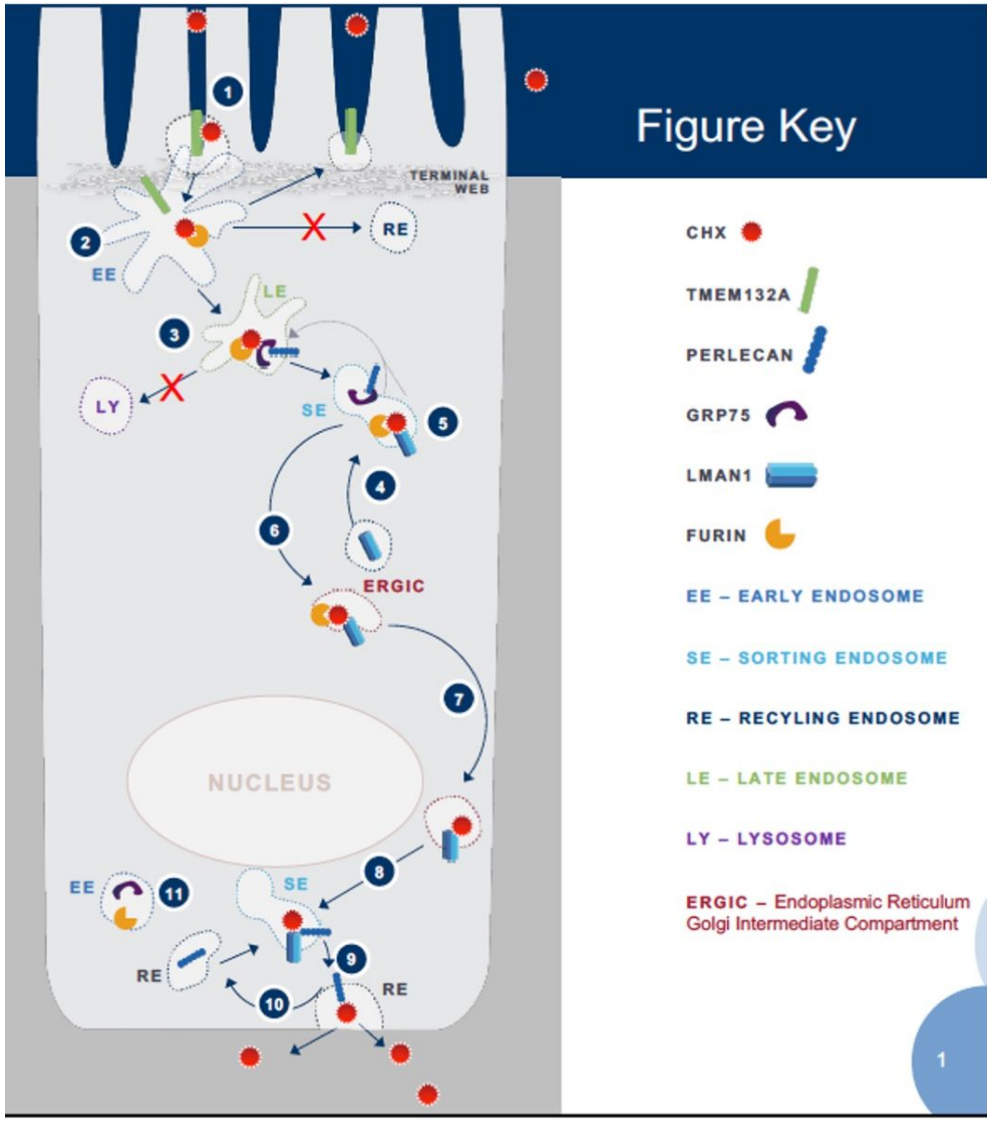
The Concept of PIP Peptide Actions



Taverner et al 2015 *J Controlled Rel* 210:189

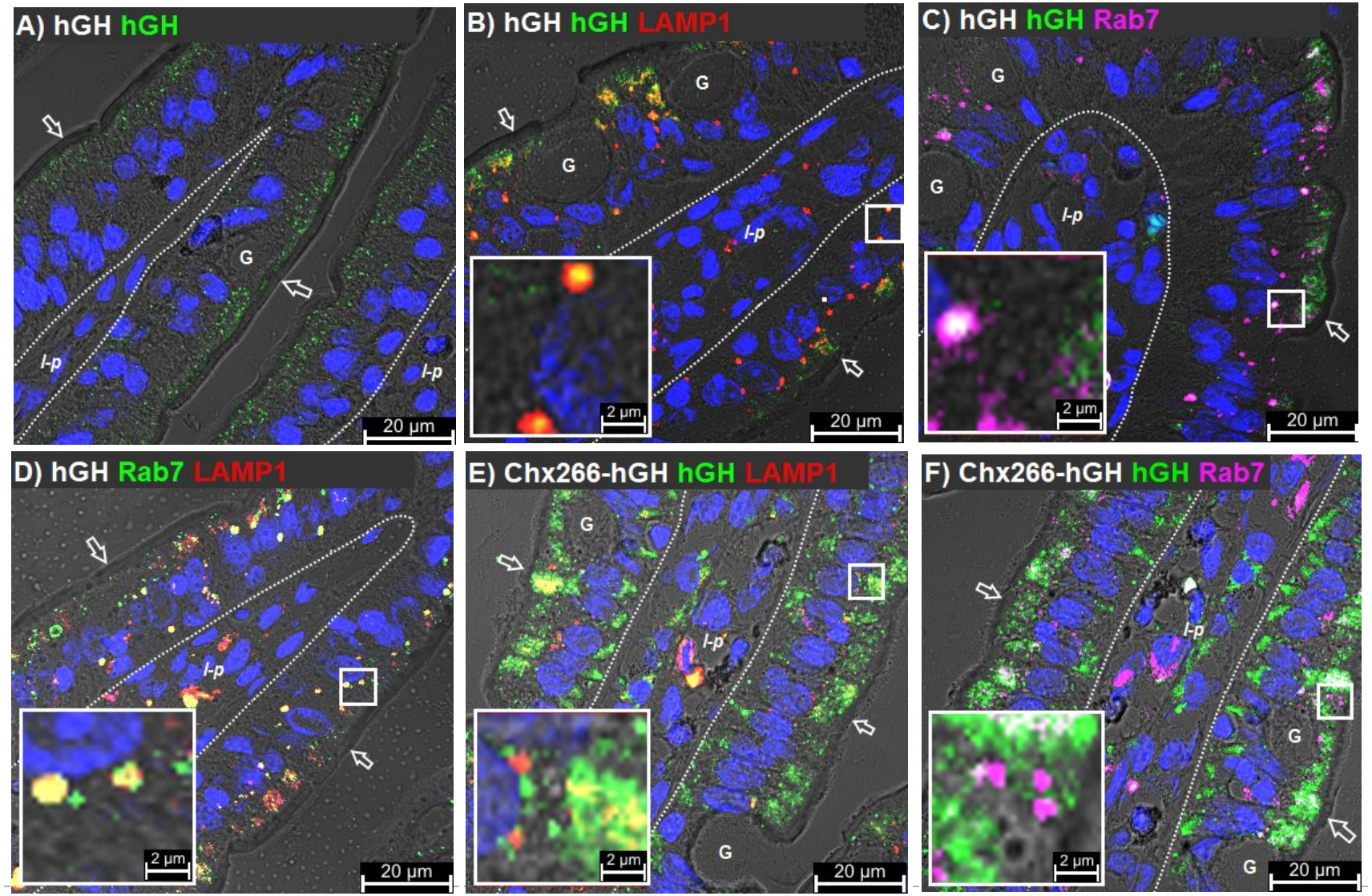
Cholix Undergoes Transcytosis





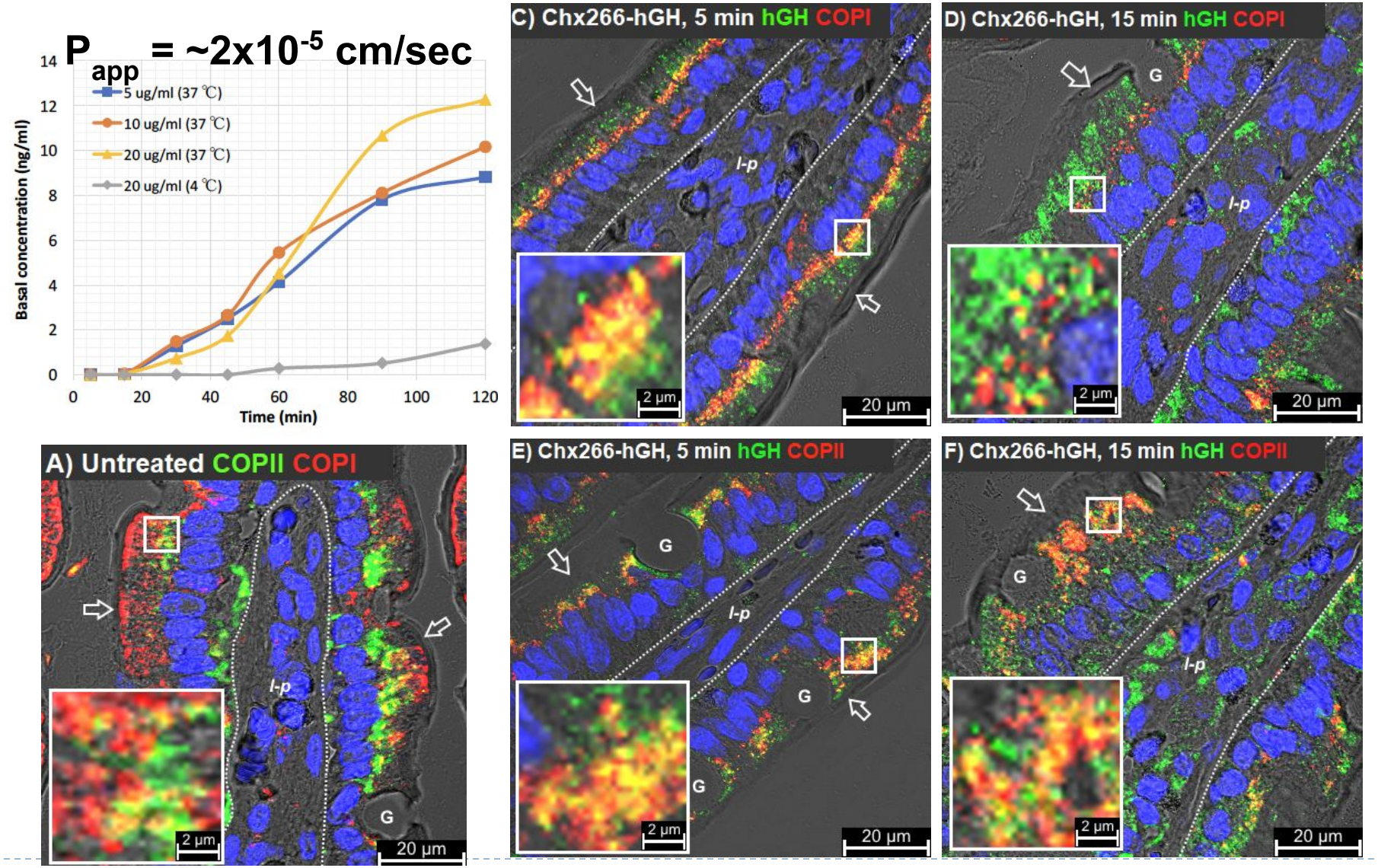
Liu, et al., 2022 *Tissue Barriers, Mar 9:2039003.*

Cholix Avoid Lysosomes



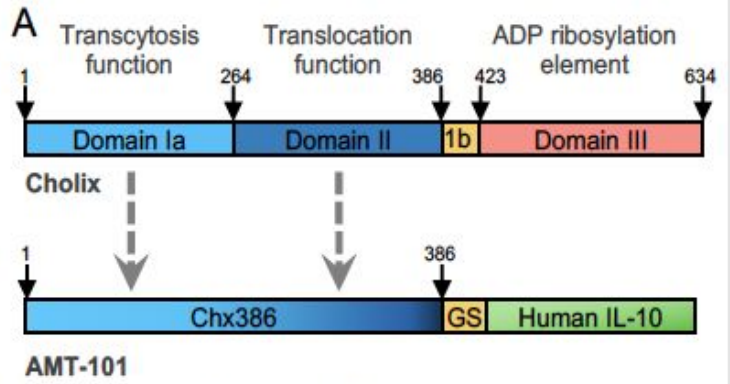
Taverner, et al., 2020 *Tissue Barriers, 8(1):1710429.*

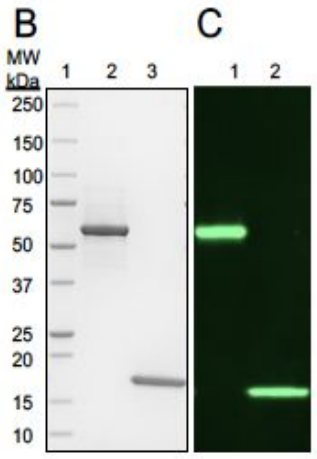
Cholix Transcytosis is Efficient and Rapid

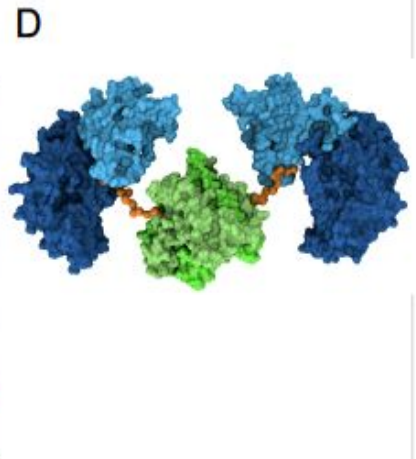


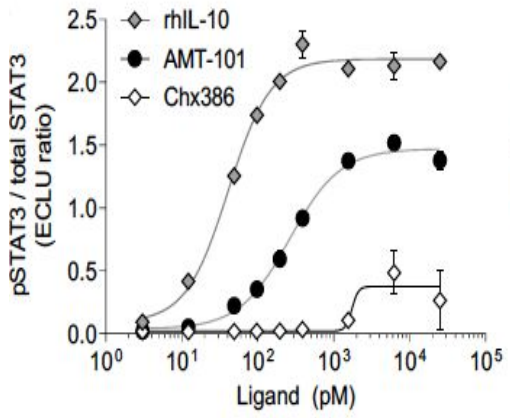
Taverner, et al., 2020 *Tissue Barriers, 8(1):1710429.*

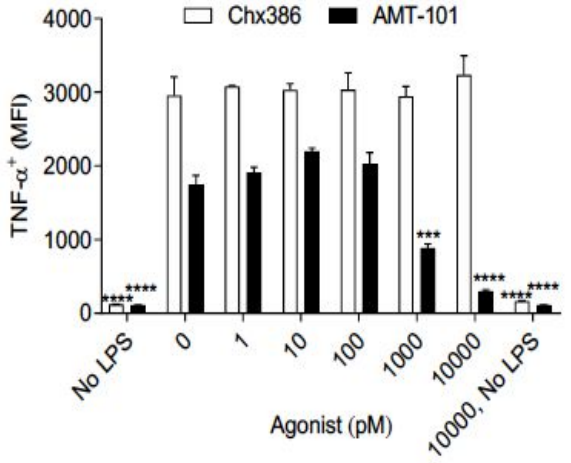
AMT-101: Cholix-IL10 dimer

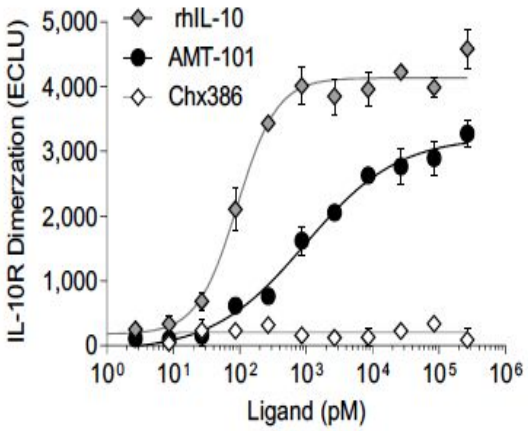


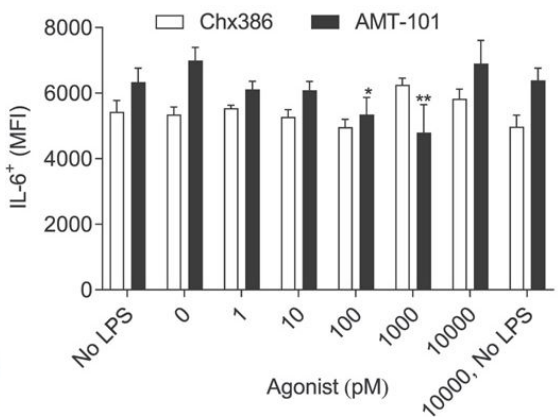






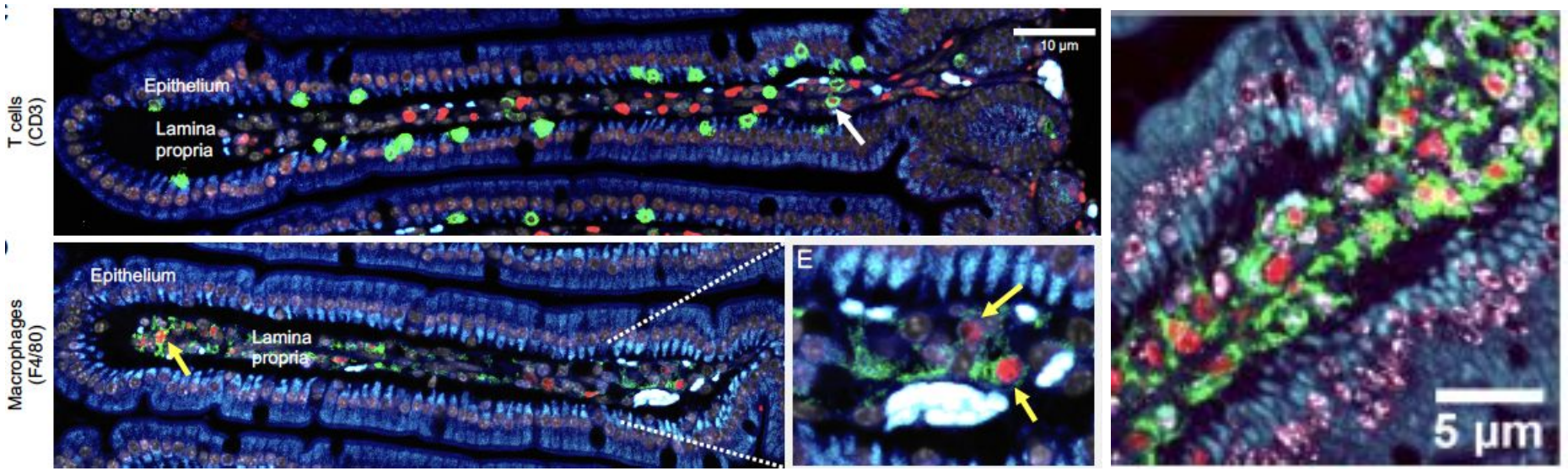


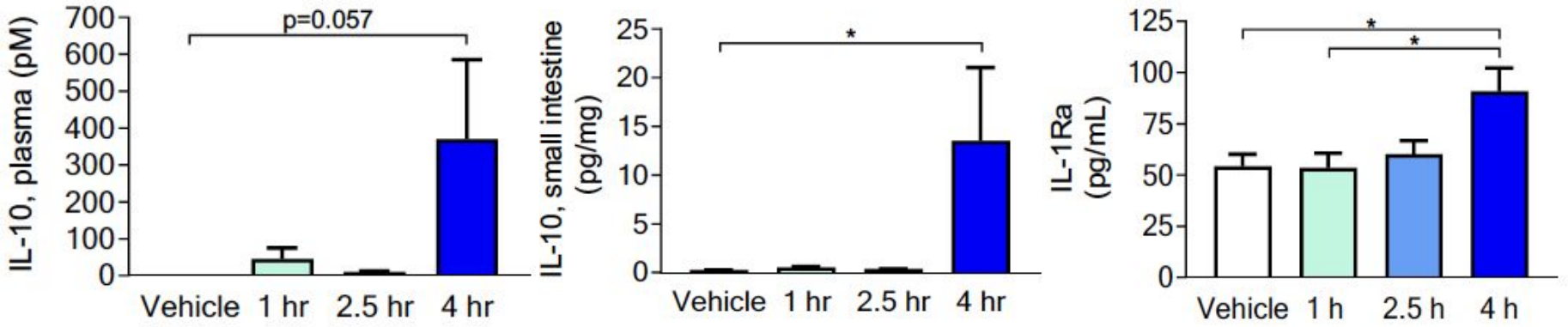




Fay et al., 2020 *J Immunol, 205(11):3191-3204.*

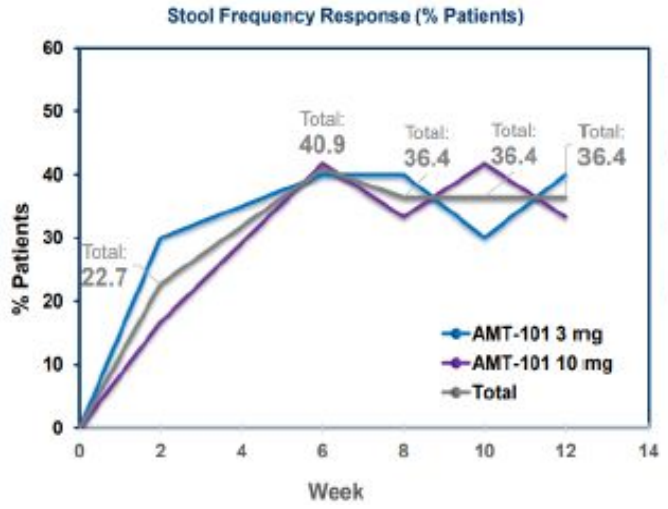
AMT-101 Targets Macrophage



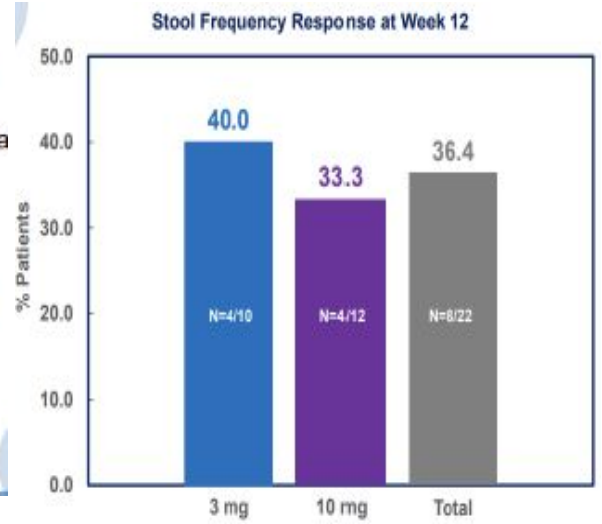


Fay et al., 2020 *J Immunol, 205(11):3191-3204.*

First Phase 2 Readout of AMT-101



- AMT-101 demonstrated a rapid onset of stool frequency response as early as week 2
- Rapid response is consistent with Phase 1b data in UC patients
- Clinical response was maintained through duration of treatment in both dose arms
- Top-line interim data demonstrated additional symptomatic improvements in fecal urgency, incontinence and abdominal cramps



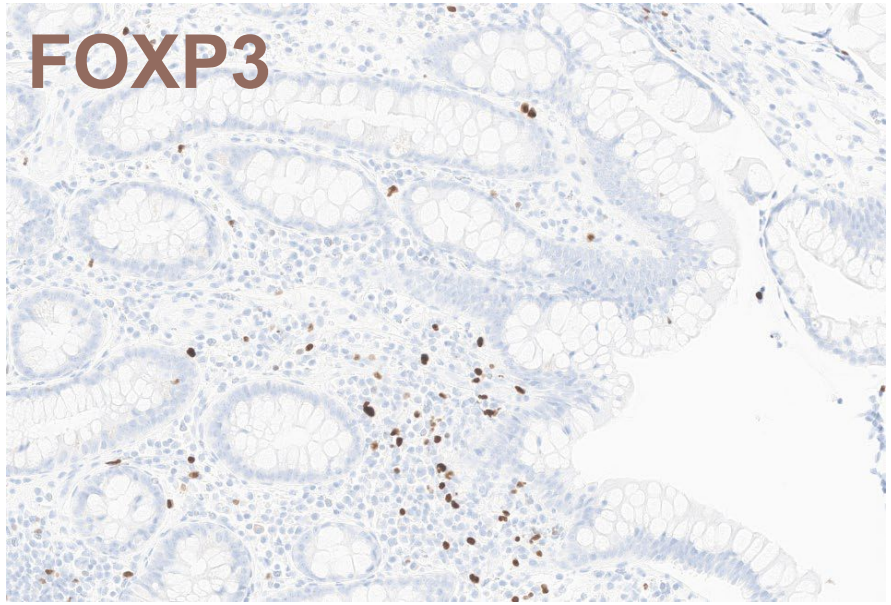
Pre-specified Efficacy Endpoint	Endpoint Definition	Patients Achieving Response, n(%) AMT-101 3mg (n=10)	AMT-101 10mg (n=12)	Total (n=22)
Stool Frequency Response (%) at Week 12	Reduction of = 3 stools and = 30% from baseline, OR = post-colectomy normal	4 (40.0%)	4 (33.3%)	8 (36.4%)
Histologic Healing Response (%) at Week 12	Geboes score = 3.1	2 (20.0%)	3 (25.0%)	5 (22.7%)

- Pre-specified, stringent efficacy endpoint selected to demonstrate stool frequency response
- 36.4% (8/22) patients achieved a stool frequency response of ≥ 3 stools and ≥ 30% from baseline, OR ≤ post-colectomy normal
- Pre-specified protocol criteria (% of patients achieving stool frequency response)
  - ≥ 30%: Program may proceed to Phase 3
  - 15% to 30%: Additional evaluation
  - < 15%: Program will not advance to Phase 3
- DMC recommends advancing to Phase 3 with 3 mg dose

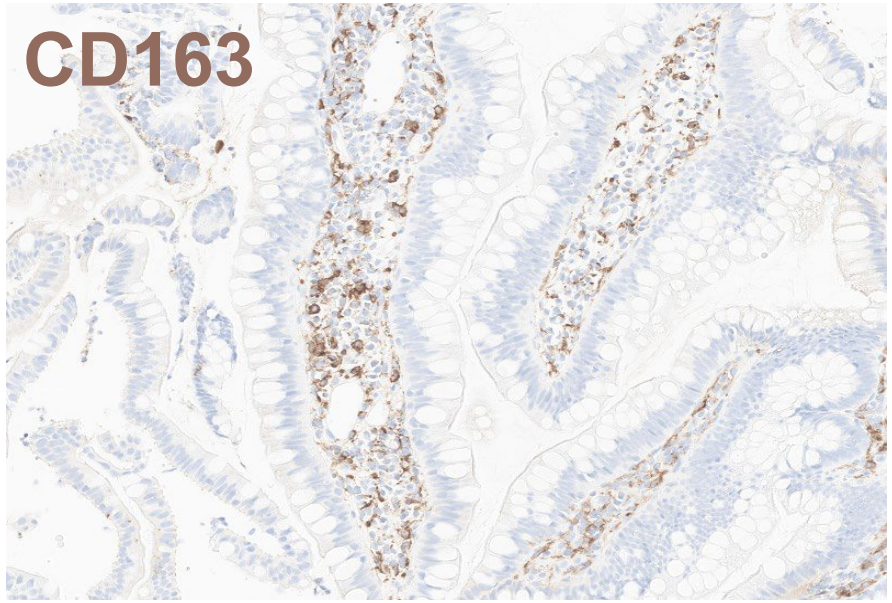
Company Reports

Evidence of AMT-101 Target Engagement

AMT-101 is designed to be restricted to the intestine following its uptake to maximize local immuno-modulation actions while minimizing the ability of systemic IL-10 to induce anemia and thrombocytopenia.



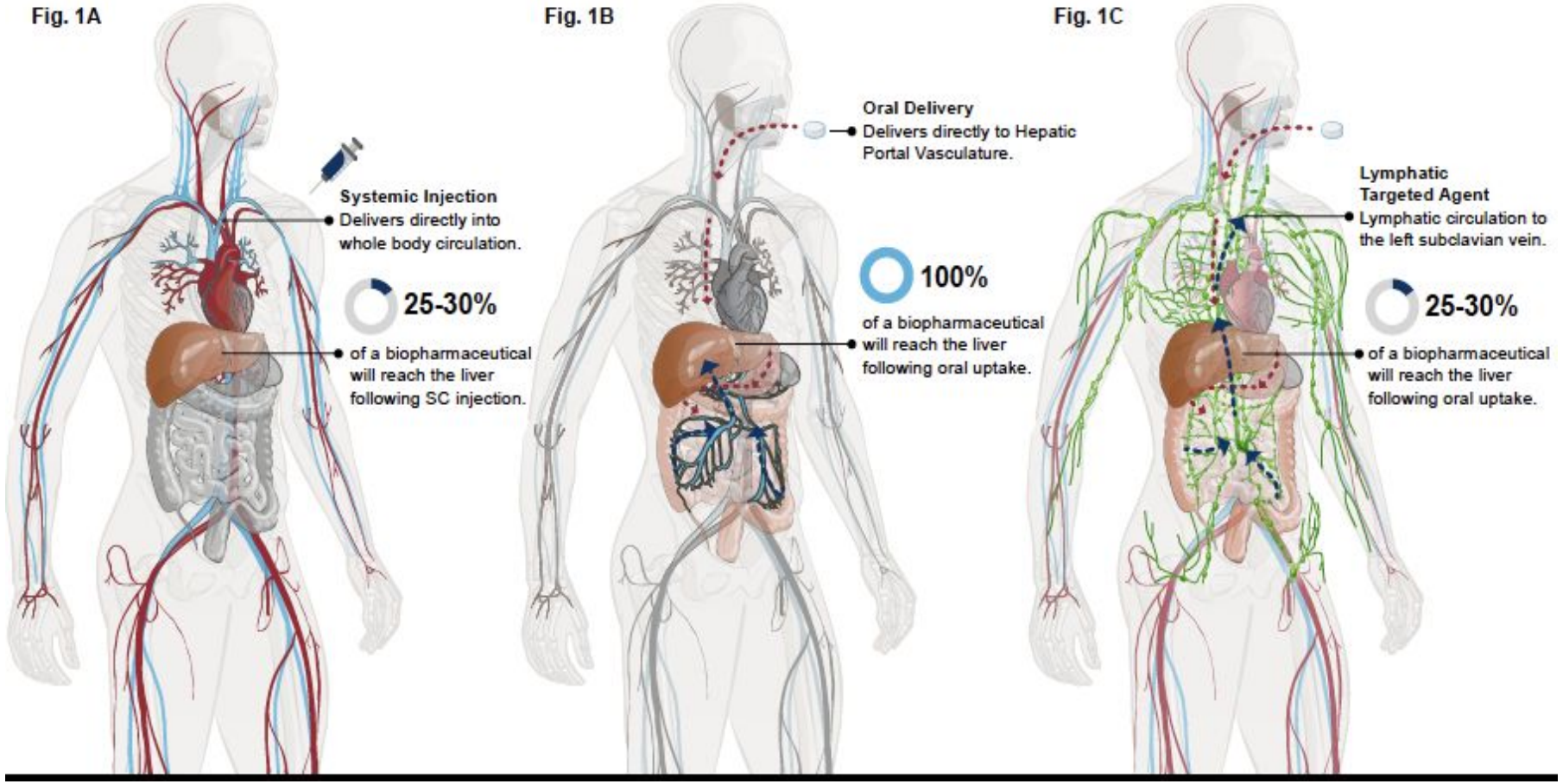
FOXP3 - Regulatory T cell marker
Increases observed in both dosage groups
Results consistent with IL-10 target engagement

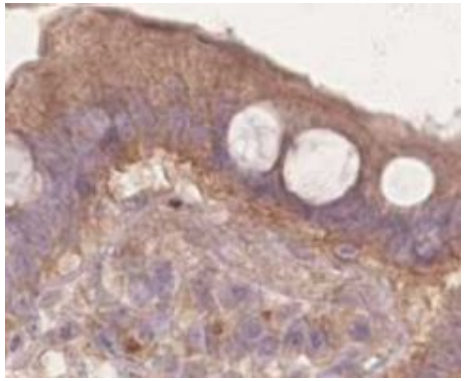


CD163 - M2-macrophage marker
Increases primarily observed in 3mg-dosed subjects
Positive correlation observed in patient's FOXP3^{+}

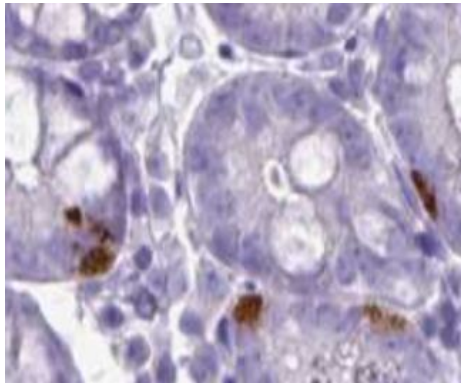
Tissue levels of IL-10 were also increased with both the 3mg and 10mg doses providing evidence of active transport.

Biological Considerations of Oral Delivery





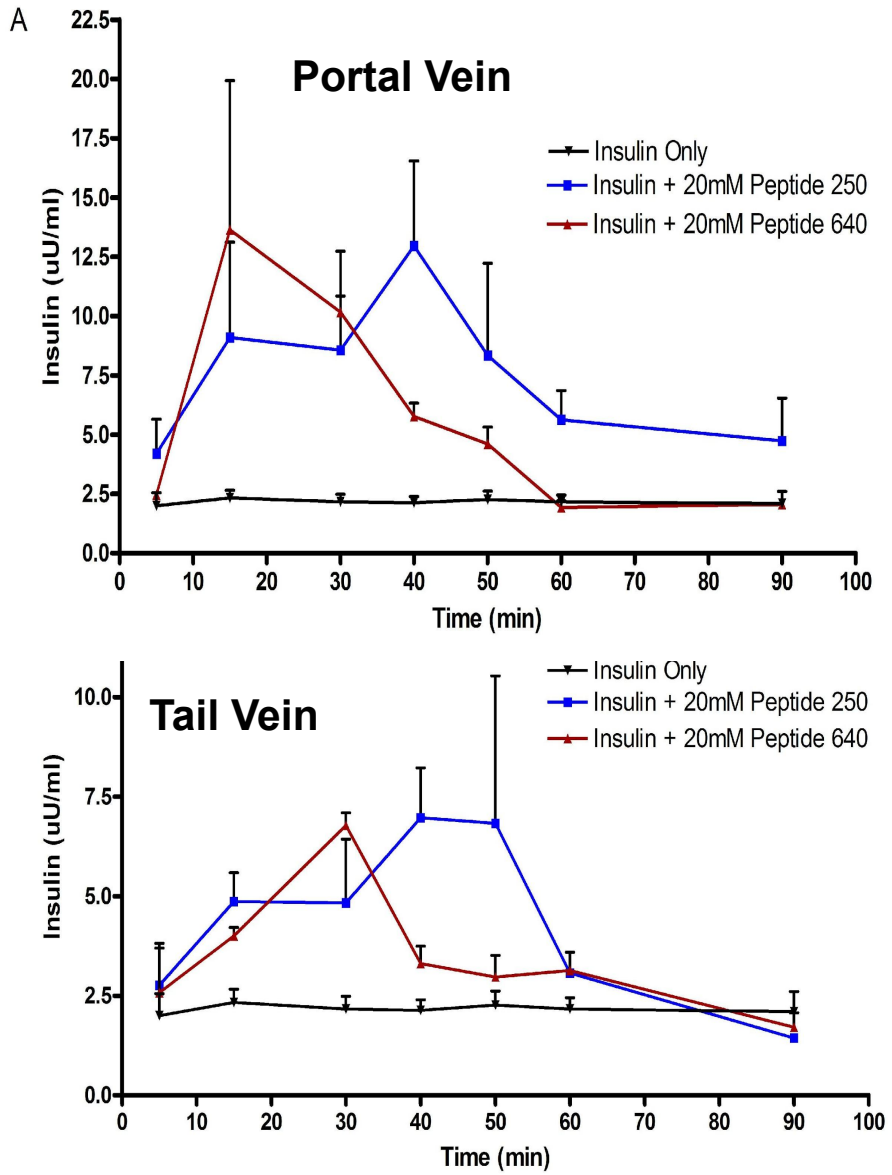
Insulin receptor



GLP-2 receptor

When considering the oral uptake of a biopharmaceutical there can be issues of local actions/ impact/ extraction prior to systemic access.

Insulin Levels *In Vivo*



- Portal vein insulin detected within 10-15 min
- Peptide 640 showed slightly faster onset and recovery for enhanced insulin levels compared to peptide 250
- Relative to SC, insulin %BA in the portal vein was ~4% for peptide 250 and ~3% for peptide 640
- Following hepatic extraction, systemic insulin %BA was ~1.6% for peptide 250 and ~1.4% for peptide 640

Taverner *et al* 2015 *J Controlled Rel* 210:189

Summary

An improved understanding of endogenous pathways and properties now provide novel opportunities for the oral delivery of peptide and protein therapeutics.

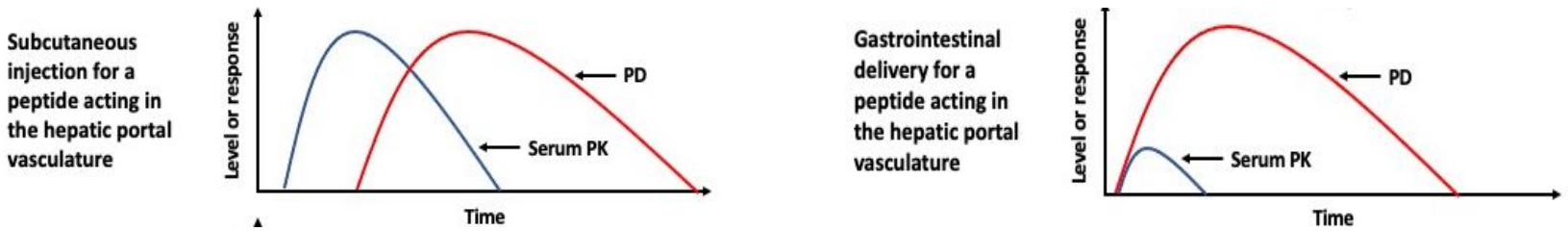
Anticipated human outcomes can be achieved from the start.

It is important to align therapeutic goals with the approach.

Drugs optimized for and validated through systemic delivery may not be the optimal agent for oral delivery approaches.

Drug properties used to select lead candidates may need to be re-envisioned to maximize technology-specific outcomes.

Oral delivery outcome assessments for many peptide and protein therapeutics may need to shift from PK to PD.



Tools that provide efficient and consistent oral peptide and protein uptake can be used to interrogate new biology and thus, novel therapies.

Those who made it happen

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