Design of prodrugs targeting the intestinal di/tripeptide transporter hPEPT1 (SLC15A1

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Faculty of Health and Medical Sciences

Design of prodrugs targeting the intestinal di/tri-peptide transporter PEPT1 (SLC15A1)

Birger Brodin

Drug Transporters in ADME

Pharmaceutics and Drug Delivery Section

Department of Pharmacy

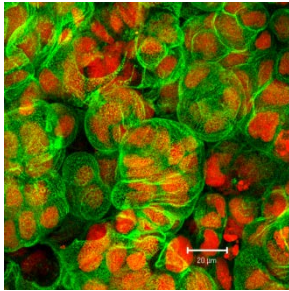Carsten Uhd Nielsen   Bente Steffansen

Membrane transporters may determine drug ADME properties
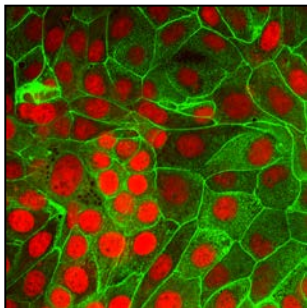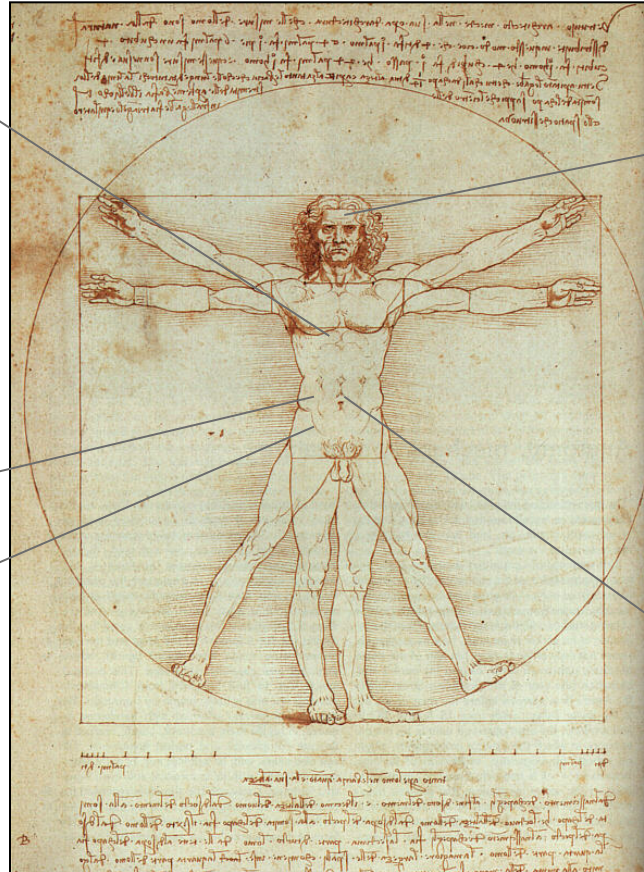
Lung:
Absorption, metabolism.
Calu-3 cells.

Brain
Influx/Efflux across the BBB
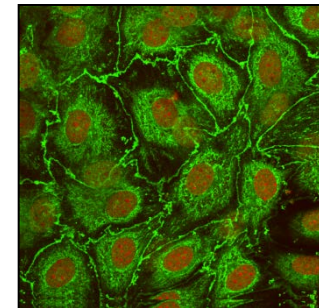
Bovine endothelial cells

Liver:
Metabolism, distribution

Kidney;
Reabsorption and efflux

GI;
Absorption and efflux

**Kidney tubule cells
LLC-PK1, SKPT, MDCK**
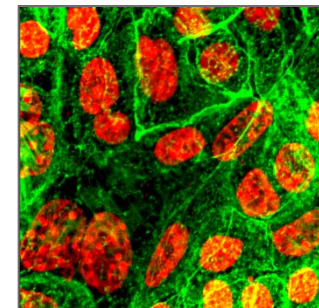
Intestinal cells, Caco-2

Localisation of PEPT1
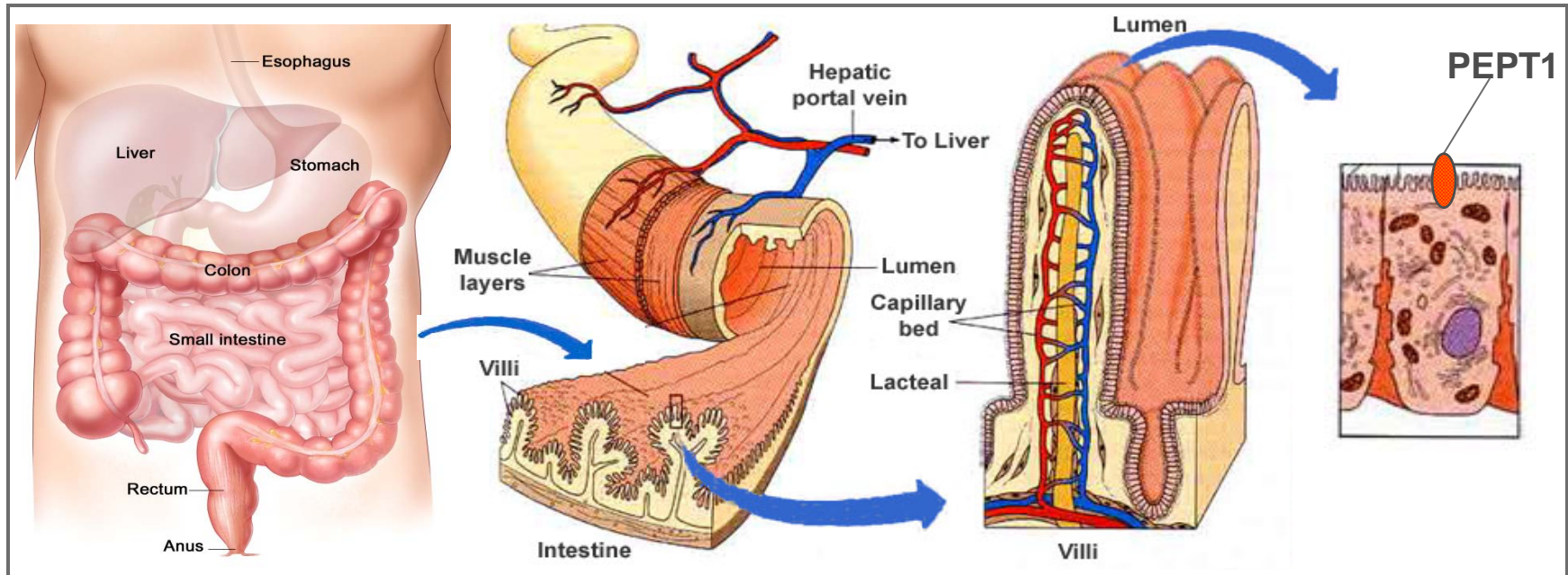
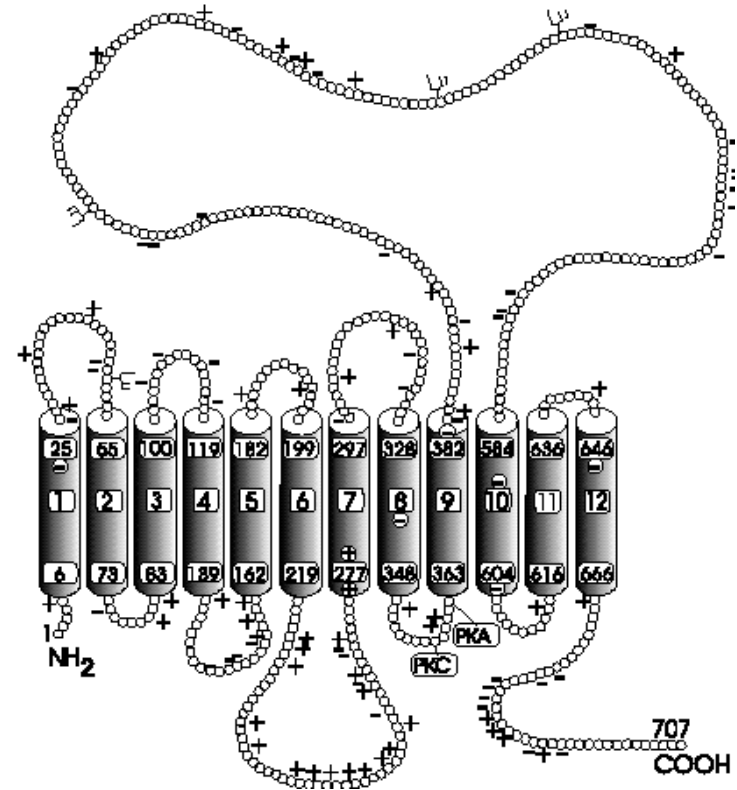Adapted from http://www.colorado.edu and www.meb.uni-bonn.de/cancer.gov

The PEPT1 protein

- hPEPT1 is expressed in the small intestine, pancreas, kidney and liver

- Consist of 708 amino acids, ~79 kDa

- Predicted to contain 12 transmembrane domains

- Proton coupled and electrogenic

- Ancestral forms dates back to prokaryotes

Fei et al. 1994

The di/tri-peptide transport pathway
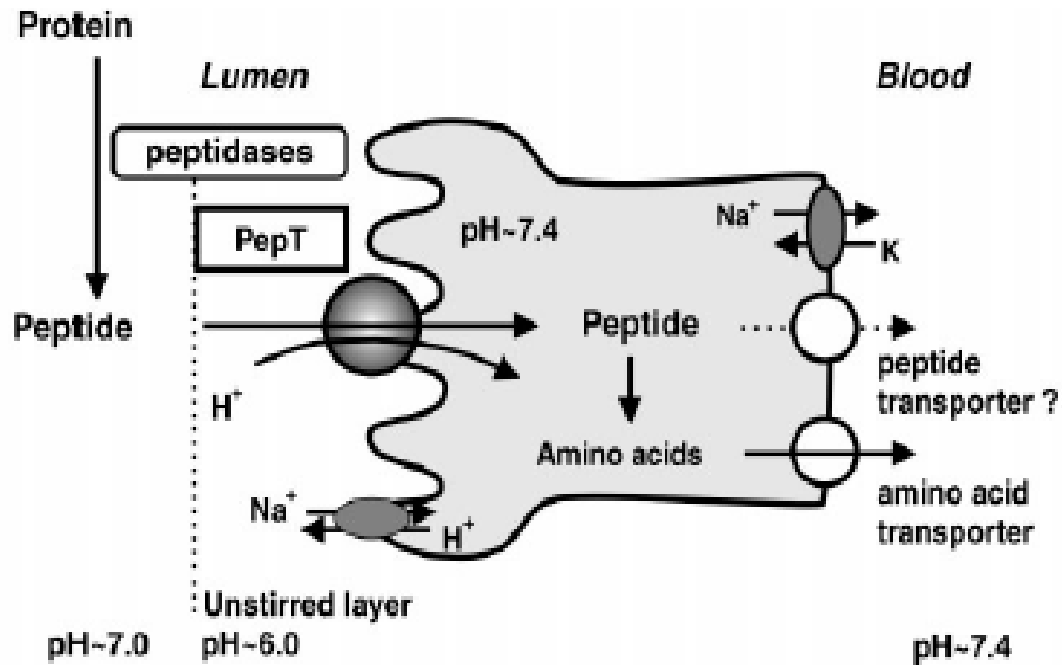
Adapted from Nielsen,M.J. 2006

Human peptide transporter 1 (hPEPT1) substrates

Di-and tripeptides (~8400 combinations)

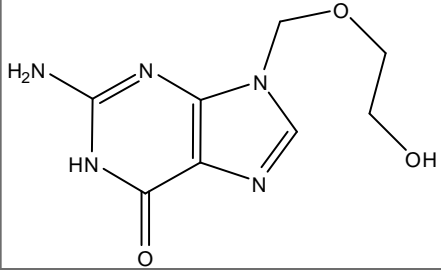β-lactam antibiotics (aminopenicillins and cephalosporins)

Bestatin (anticancer drug)
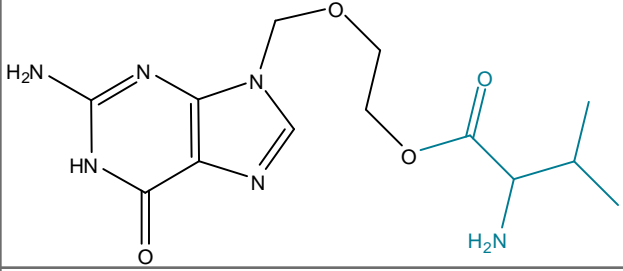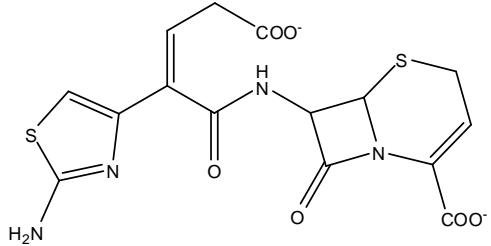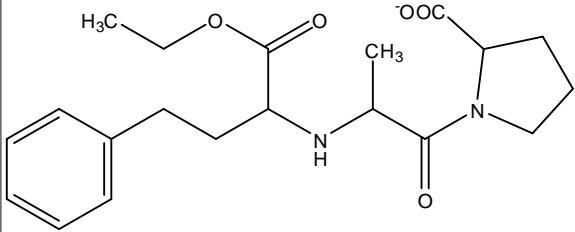
Valaciclovir (transport form of Aciclovir)

Both acidic, neutral and basic peptides are transported

The transport system has a preference for L-amino acids

Examples of Drugs taken up via hPEPT1

Substance	Structure	Ki (mM)	Bioavailability
Acyclovir		Not a substrate	15 %
Valacyclovir		0,7	54 %
Ceftibuten		0,3	80 %
Enalapril		4,6	60 %

Ref.: Bretschneider et al. 1999, Ganapathy et al. 1998, Herrera-Ruiz et al. 2004 and Micromedex

hPepT1 is a high capacity "promiscous" transporter

The hPEPT1 drug delivery project

Aim: To investigate the potential of hPEPT1-mediated transport as a drug delivery route for peptidomimetics or peptidomimetic prodrugs.

Initial strategy: To make a structure-affinity relationship of ligands binding to hPEPT1, in order to be able to identify molecular features in the substrate, necessary for binding.

The hPEPT1 prodrug concept

Drug

R1

Initial studies:
Backbone configurations
Linker stability
Model drug size
Focus on R1

Linker/bond

NH_3+

N

O

O⁻

O

C-terminal

**Stable backbone
(pro-moiety)**

R2
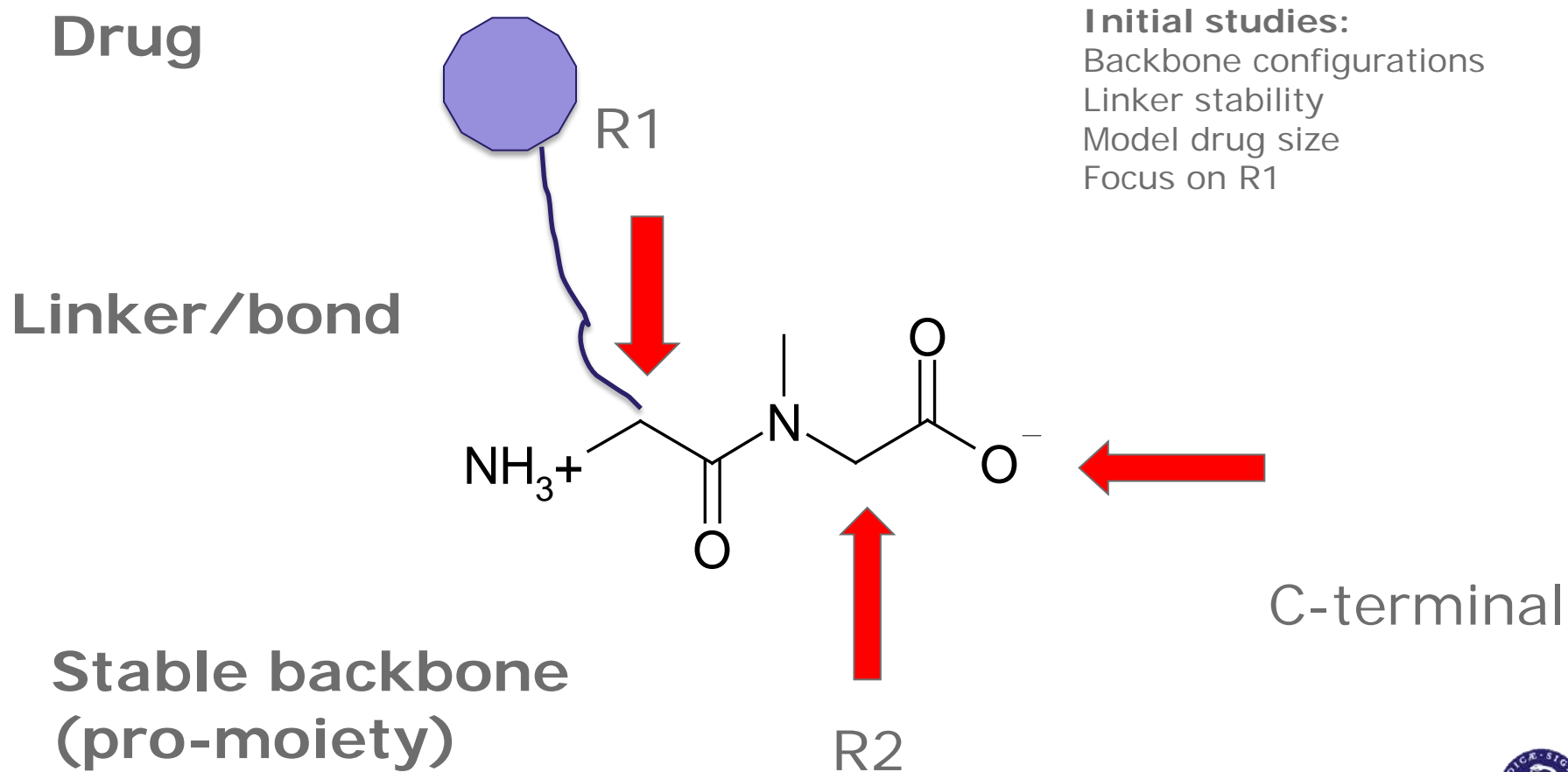
The assay system, the Caco-2 intestinal cell culture model

Grows on permeable membranes for 21 days in 95%O2/5%CO2 .

Media contains salts, serum, L-glutamine , NEEA & Pen/Strep.

Growth is dependent on passage number, serum batches and technician.

Monolayer transport characteristics must be characterized routinely by measuring TEER, mannitol fluxes and standard substrates, along with CLSM imaging of morphology.

Caco-2 cells express hPEPT1 n the apical membrane after 21 days of culture

http://inn.ingrm.it/Ricerca/transport/Heavymetal.htm

hPEPT1 localization
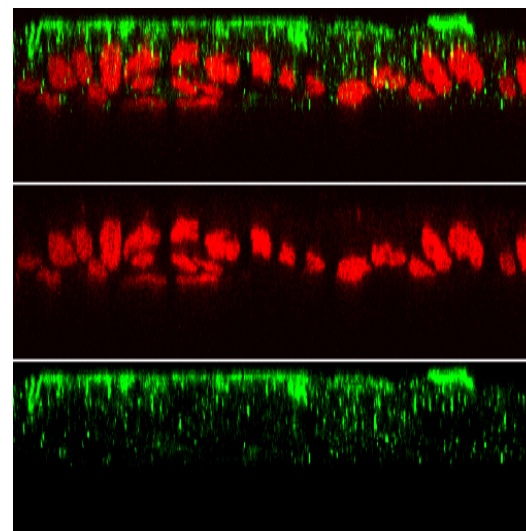
Nielsen et al, 2001

The assay system, hPepT1 activity and substrate affinity is measured using radiolabelled stable dipeptide

Structure-activity relationships were made by competition assays estimating inhibition constants (Ki) against a known stable radiolabelled substrate, glycylsarcosine (14C-Gly-Sar)

competing substrate

Radiolabelled stable peptide i.e. Gly-Sar
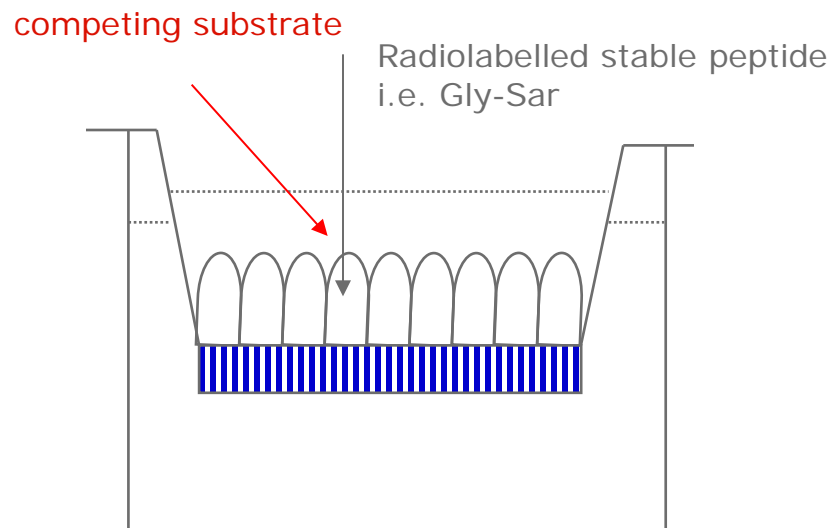
Glu(Acyclothymidine)-Sar, a promising model pro-drug?.
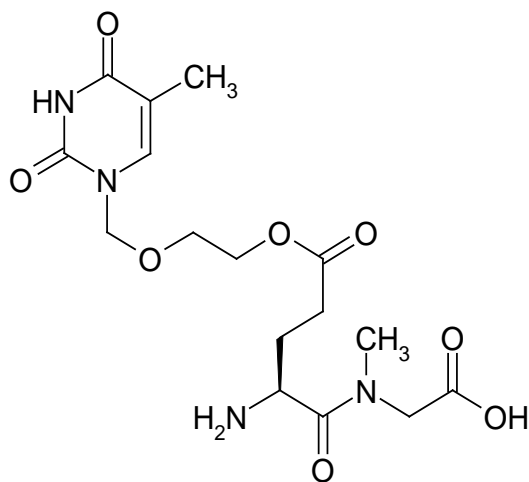
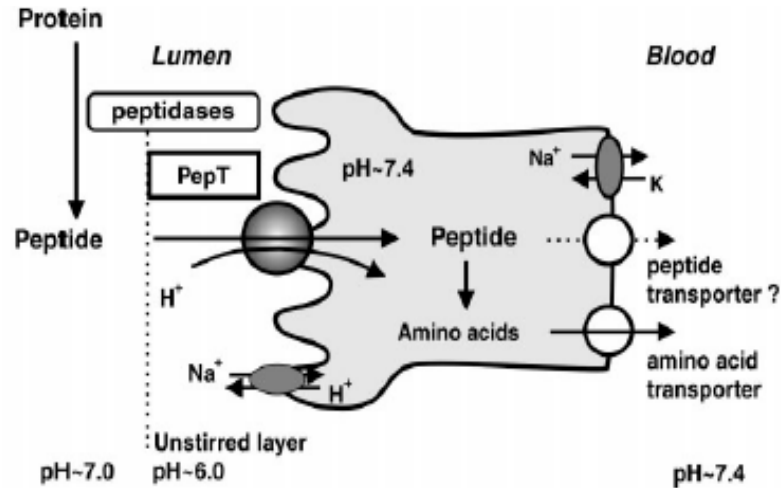Ki 0.072±0.002mM (natural substrates have Ki's below 2mM)

Stable backbone (methylated)

t½ at pH 7.4 is 3.7 hours (drug release)

Translocation ?

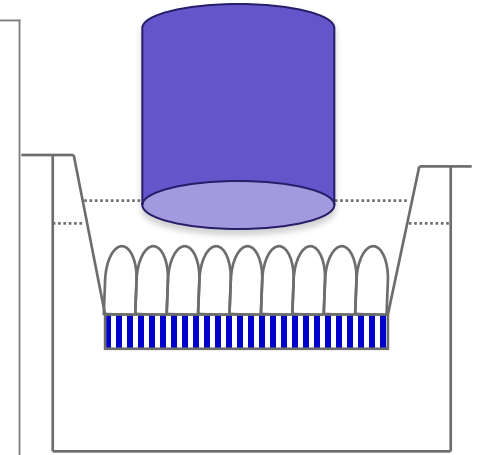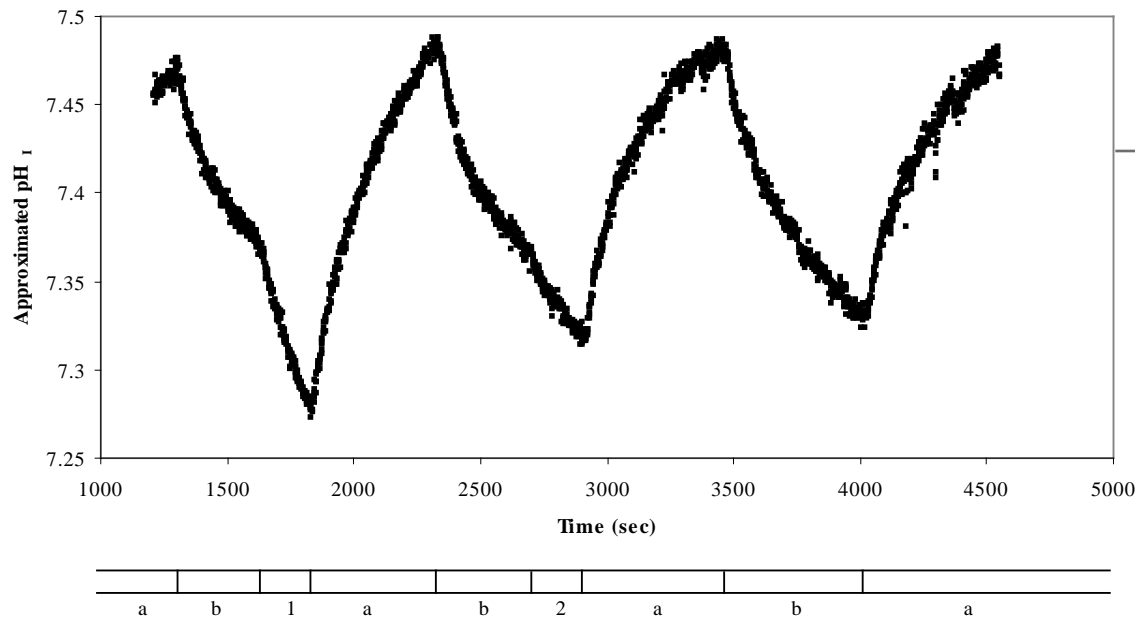Erikson et al, Eur J Pharm Sci 2005 25, 145-154

Translocation can be tested by measuring pH_i

Adapted from Nielsen,M.J. 2006

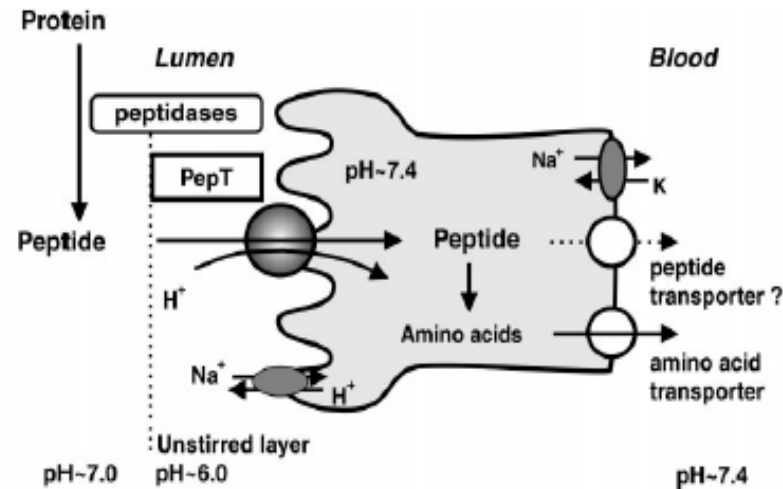# Addition of substrate causes intracellular acidification

Ussing-chamber on microscope stage BCECF for pHi measurements

a)   buffer pH = 7.4,
b)   buffer pH 6.0,
1)   2 mM Gly-Sar in buffer pH 6.0
2)   0.23 mM III (Glu(Acyclothymidine)-Sar).

Erikson et al, Eur J Pharm Sci 2005 25, 145-154

Failure; Affinity does not necessarily imply translocation

Derivatisation in the R1 position led to a number of **inhibitors** of PEPT1-mediated substrate uptake

In order to predict uptake via PEPT1, translocation must be measured (in-silico models has previously primarily been based on competition studies)

New strategy in the hPEPT1 drug delivery project

Aim: To investigate the potential of hPEPT1-mediated transport as a drug delivery route for peptidomimetics or peptidomimetic prodrugs.

Revised strategy: To make a structure-translocation affinity relationsship of lsubstrates transported by hPEPT1, in order to be able to identify molecular features in the substrate, necessary for binding and subsequent translocation.

Revised goal: To obtain a predictive computational model for hPEPT1-substrates.

PEPT1 substrate translocation estimated via intracellular acidification or changes in membrane potential

H$^+$

δ$^+$
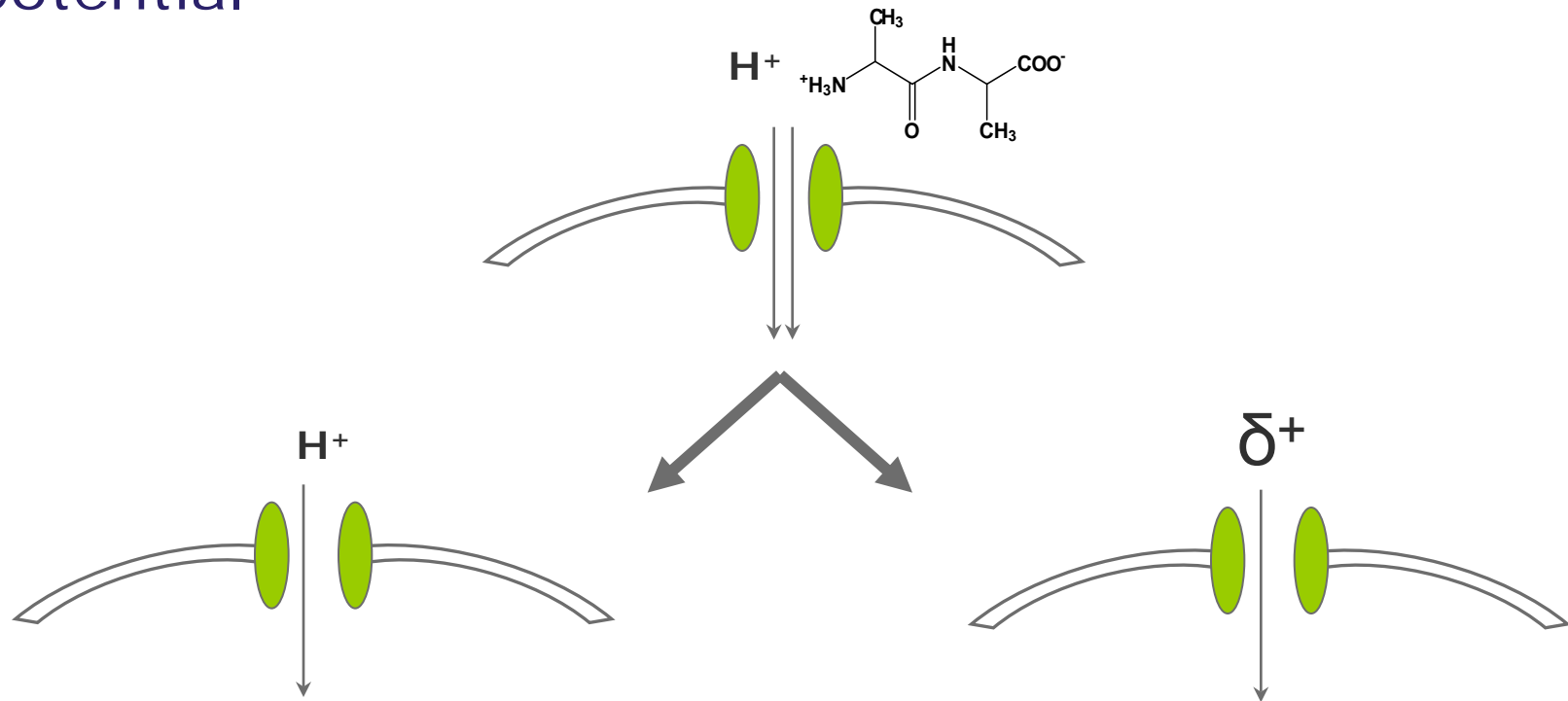
Translocation is accompanied by acidification

Translocation is accompanied by depolarisation

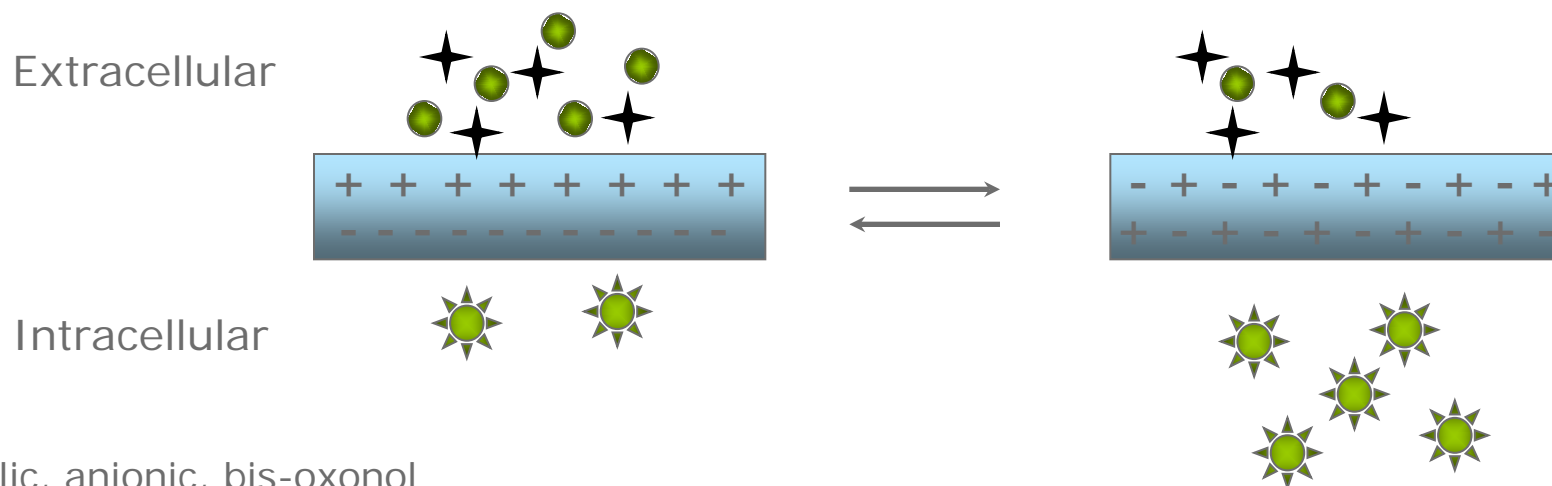- coupling ratio varies with charge of substrate

- one charge per substrate

Assay: A membrane potential assay and plate reader setup was employed

Ex: 544 nm

Em: 590 nm
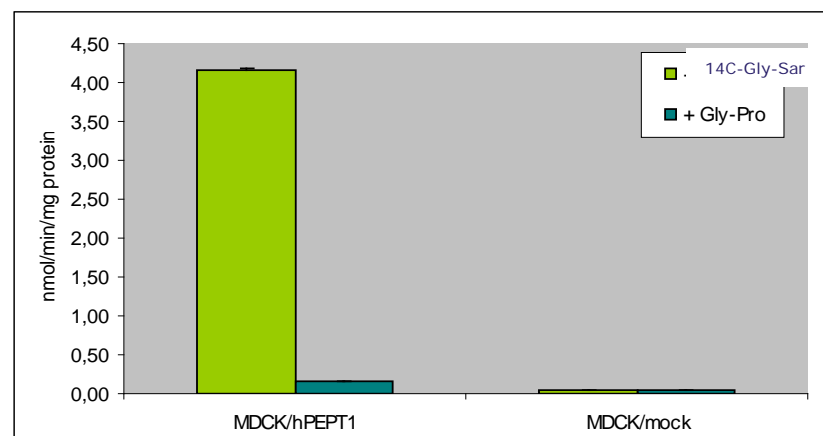
Extracellular

Intracellular

Lipofilic, anionic, bis-oxonol dye.
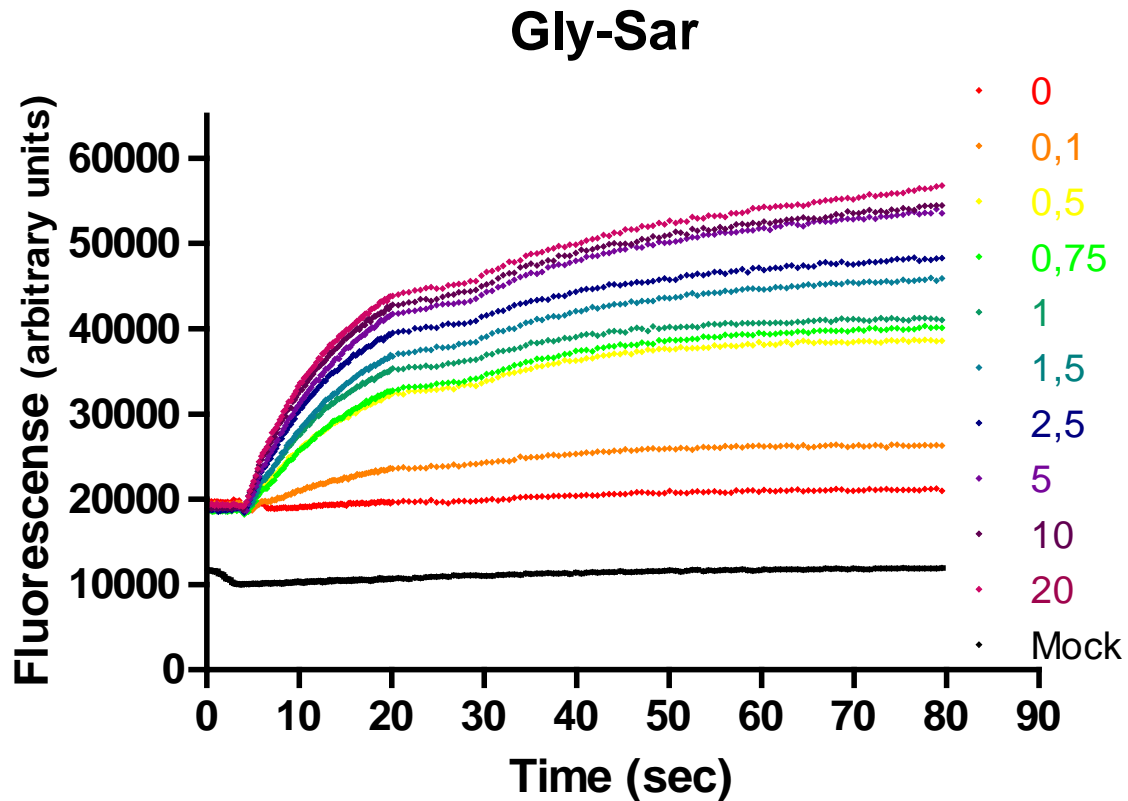FLIPR membrane potential assay
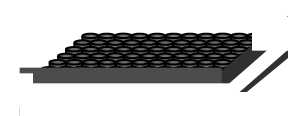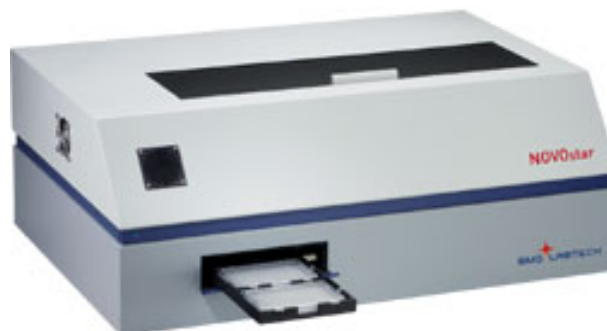
Assay: MDCK-hPEPT1 cells

MDCK-hPEPT1 cells were grown under standard conditions for 3 days

Kindly donated from Bristol-Meyers Squibb Company

Translocation of substrates can be measured directly

Test set

- 55 di- and tripeptides was selected, using computational methods (PCA, VolSurf descriptors).

- The compounds were been tested for their *translocation* properties (Km,Vmax).

- A structure-translocation relationship was constructed (QSAR, tested against a randomly choosen test set).

Hydrophilic interactions in the side chains lowered lumen-cell translocation rate
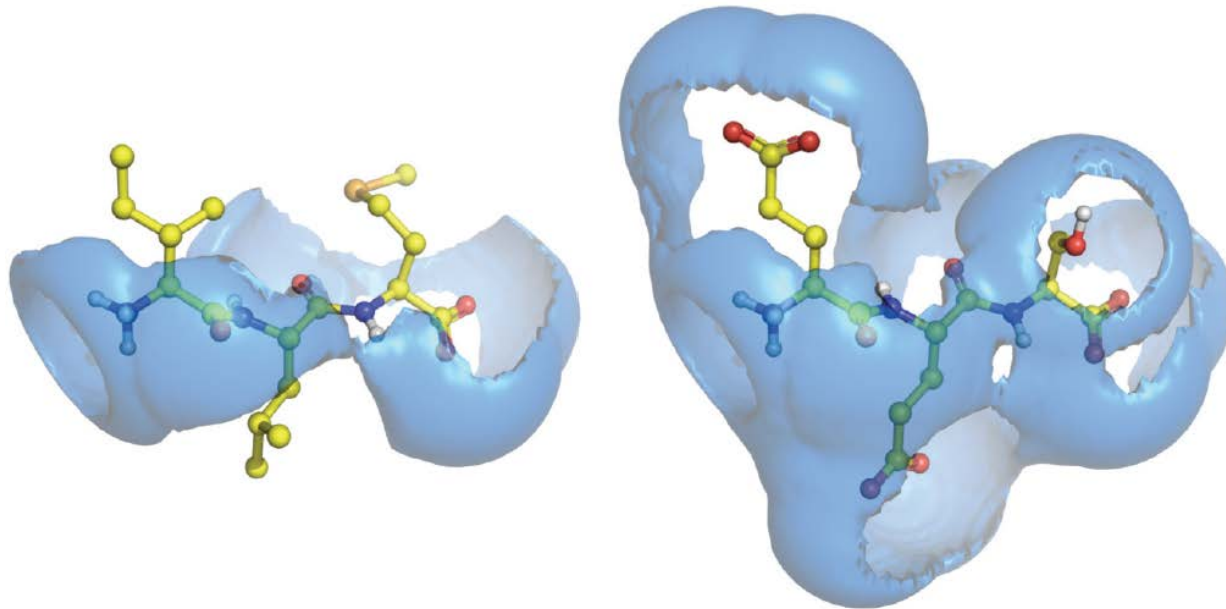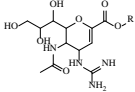

Figure 8: Two tripeptides, Ile-Leu-Met (good substrate, K_m^{app} = 0.43 mM; left) and Glu-Glu-Ser (poor substrate, K_m^{app} = 28 mM; right), displayed with their hydrophilic molecular interaction field at -4.0 kcal/mol. Adapted from (Omkvist *et al.* 2010c).

Met-Pro-Pro inhibitor: K_i=20 um

Omkvist et al, 2010.

Recent examples of prodrugs designed for carrier-mediated transport via hPEPT1.

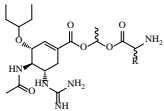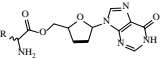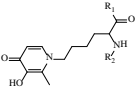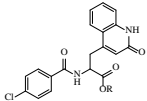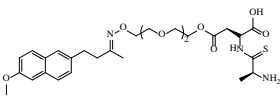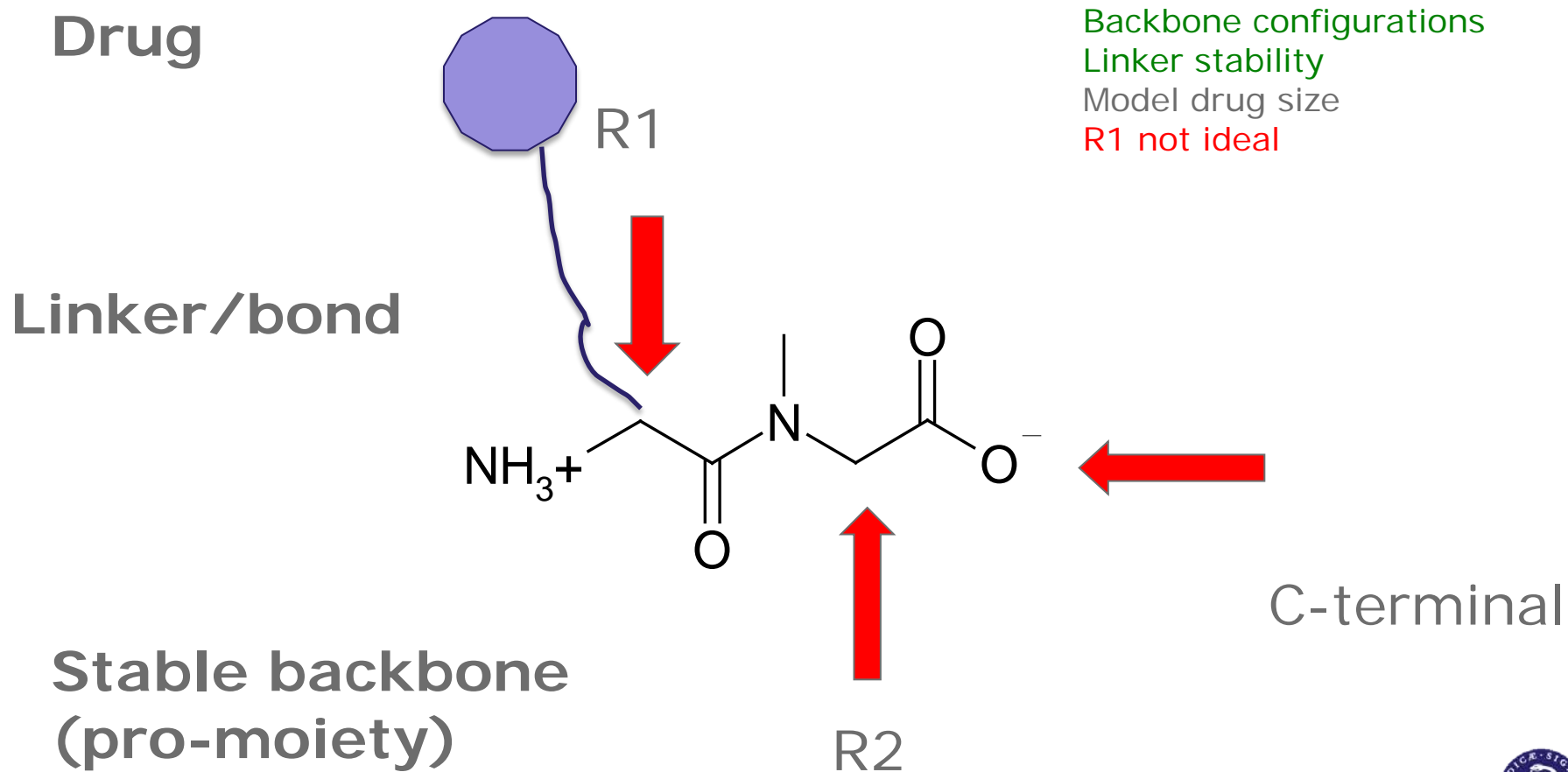| Parent drug	Structure	Promoiety (R)	hPEPT1 affinity	Comparison of prodrug versus parent drug	Reference
[3-(hydroxymethyl)-phenyl]guanidine (3-HPG)		Val Ile	0.65 (IC_{50}, mM)[a] 0.63 (IC_{50}, mM)[a]	Both prodrugs showed increased permeability as compared to the parent drug in an in situ rat perfusion study	Sun et al., 2010 [81]
Zanamivir		Val	1.19 (IC_{50}, mM)[a]	The valyl ester prodrug showed increased permeability in both cellular transport studies in Caco-2 cells and in in situ rat perfusion studies as compared to the parent drug.	Gupta et al., 2011 [82]
Guanidin oseltamivir carboxylate (GOC)		Val	0.19 (IC_{50}, mM)[a]	The ester prodrug showed increased cellular uptake, rat intestinal permeability and bioavailability in mice when compared to the parent drug	Gupta et al., 2013 [83]
Didanosine		Val	0.27 (IC_{50}, mM)[a]	Oral bioavailability in rats was increased from 8 % for the parent drug to 47 % for the valyl ester prodrug	Yan et al., 2011 [84]
Maltosine		Ala-R R-Ala	0.33 (K_i, mM)[a] 0.16 (K_i, mM)[a]	Electrophysiological measurements in hPEPT1-expressing oocytes showed significant increase in transport of the prodrugs as compared to maltosine	Geissler et al., 2011 [89]
Rebamipide		Ser-Gly	ND	Electrophysiological measurements in hPEPT1-expressing oocytes, cellular transport studies in Caco-2 cells and intestinal in situ rat perfusion experiments showed increased transport of the dipeptide ester prodrug as compared to rebamipide.	Kikuchi et al., 2009 [91]
Thiodipeptide conjugate of nabumetone		-	0.46 (K_i, mM)[b]	Cellular transport studies in Caco-2 cells showed increased permeability of the prodrug when compared to the known hPEPT1 substrate PheΨ[CS-NH]-Ala	Foley et al., 2009 [92]

a) In competition with Gly-Sar

b) In competition with D-Phe-Gln

Saaby et al, 2013.

Dias 24

The hPEPT1 prodrug concept

Drug

R1

Linker/bond

Backbone configurations
Linker stability
Model drug size
R1 not ideal

NH₃+

C-terminal

Stable backbone (pro-moiety)

R2

Challenges and future directions

Expansion of substrate sets to include more diverse compounds

Crystal structure of hPEPT1 for understanding of binding and translocation pocket

True "rational" design not possible yet

Characterisation of the basolateral peptide transport exit step remains.

Acknowledgements

From the group

Carsten Uhd Nielsen, Bente Steffansen, Diana Omkvist, Andre Huss Eriksson, Rikke Bjerring Andersen, Anne Engelbrecht Thomsen

From the Faculty

Lars Olsen, Flemming Steen Jørgensen, Simon Birksø Jensen

Funding

PDA (Predictive Drug Absorption consortium), Novo Nordic foundation, Carlsberg foundation.

BioSim Consortium.

Thank you for your attention.
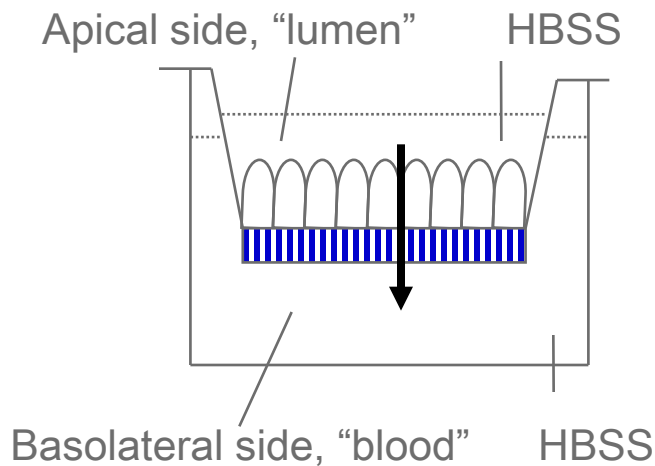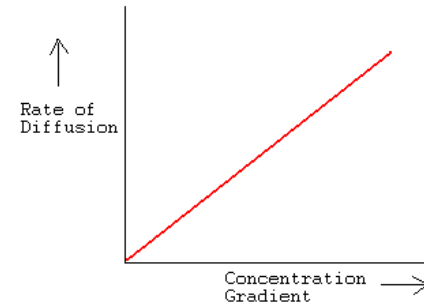
Supplementary slides

We measure fluxes and kinetics of drug transport in barrier tissues

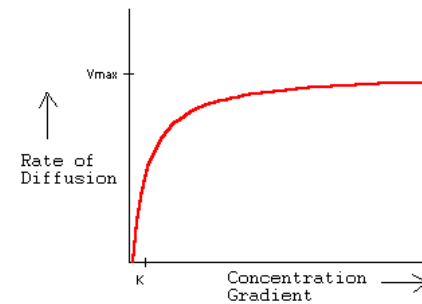Intestinal cells, Caco-2

Flux $\quad J = \dfrac{m}{A * t} \quad \left(\dfrac{mol}{cm^2 * s}\right)$

Rate of Diffusion / Concentration Gradient

Apical side, "lumen"   HBSS

Basolateral side, "blood"   HBSS

Kinetics $\quad J_o = \dfrac{J_{max}[S]}{K_m + [S]}$

Vmax

Rate of Diffusion / Concentration Gradient

Absorption of peptides from the small intestine
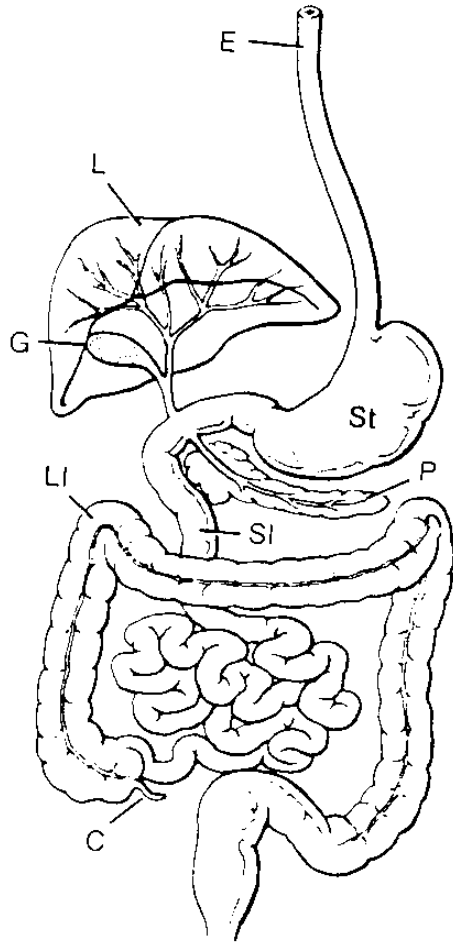
Proteins are broken down to peptides and amino acids in the stomach and in the small intestine.

Three hours after a protein meal (50 g bovine serum albumine), 120 mM of amino acids in the small intestine are in the form of peptides (with the majority consisting of 2-4 peptides), 30 mM are in the form of free amino acids

Peptide transport probably accounts for ~50 % of total amino acid uptake

# Molecular characteristics of human di/tri-peptide transporters

Transporter	hPepT1	hPepT1-RF*	hPepT2	
Family	POT	POT	POT	
Accession No.	U13173	AB001328	PS01023 g2833279	
Chromos. loc.	13q.34-q.35		3q13.3-q21	
Protein length	708 a.a.	208 a.a.	729 a.a.	
TMD's	12		12	
Substrate	oligopeptides peptoid drugs	-	oligopeptides peptoid drugs	
Tissue distrib.	intestines	intestines	Kidney, brain(?)	
Function	H^+-dependent transport	pH-sensing regulatory factor	H^+-dependent transport	

*Possible alternative splicing product of hPepT1

Expression is however questionable (Sondergaard et al, 2013)

Proposed model for substrate binding to hPEPT1
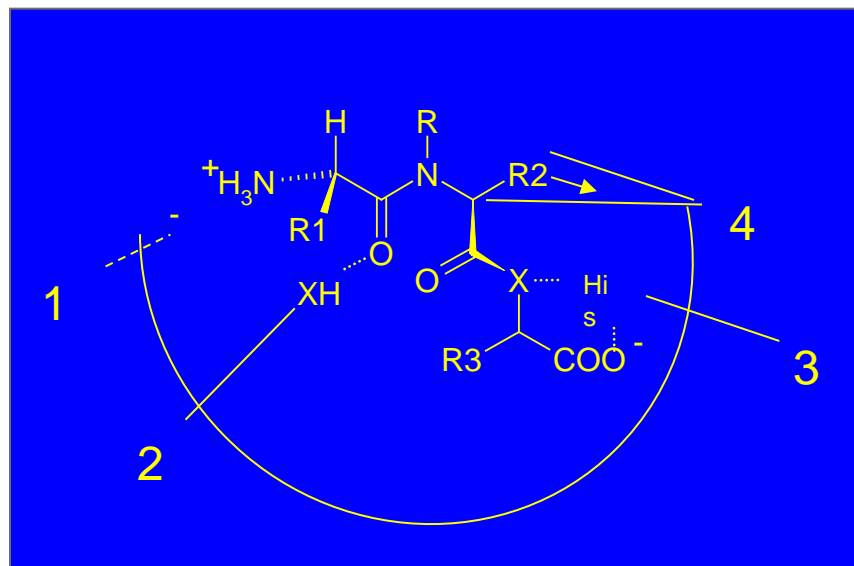
1) Ionic interaction

2) Hydrogen bonding

3) Interaction with histidine residue

4) Hydrophobic pocket

Bailey et al. Angew. Chem. Int. Ed. 39:505-508, 2000

The backbone of a substrate must mimick a di- or a tripeptide.

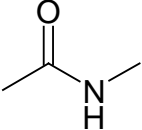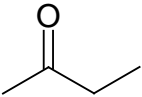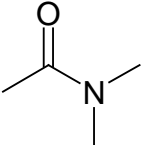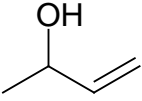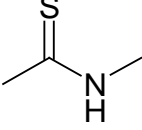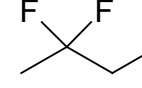Properties of the side chains (R1 and R2) influences binding

Di-and tripeptides as substrates

- The peptide transporter accepts di- and tripeptides (400 + 8000 possible combinations)

- Both acidic, neutral and basic peptides are transported

- The transport system has a preference for L-amino acids
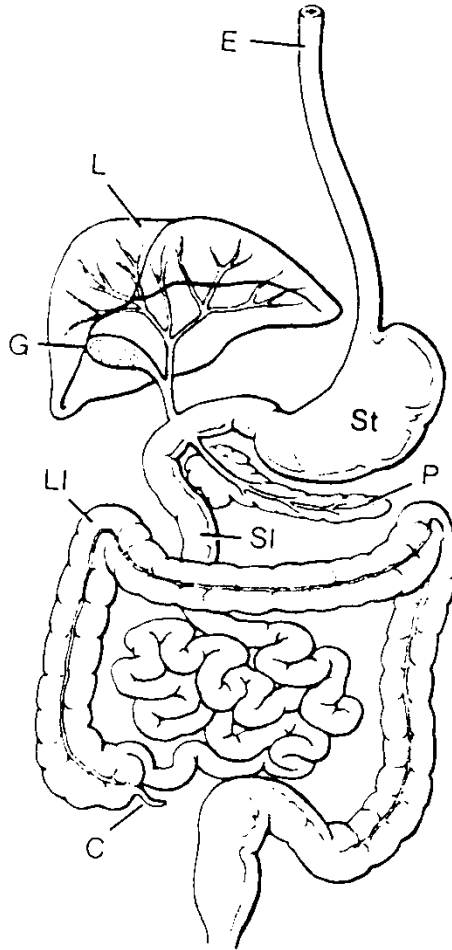
Allowed backbone conformations

	Regular peptide bond L/D peptide	+ +
	Ketomethylene	+
	N-methyl amide	+
	Methylene amino	-
	Hydroxyethylidene	-
	Thio amide	+
	Fluoroalkane	+

+ Affinity to PEPT
- No affinity to PEPT

Steffansen et al., 2005 EJPB
60, 241-245 Andersen 2006
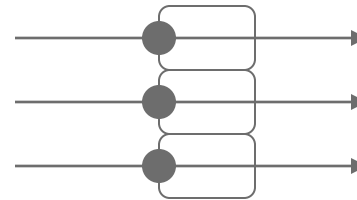
Transporters in GI drug absorption

Absorptive nutrient transporters

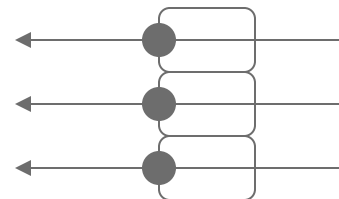*Amino acids*

Glucose

Di/tri-peptides

Organic ions

Efflux transporters

Organic ions

Hydrophobic xenobiotics

Adapted from "Animal Physiology"
Eckert,Randall & Augustine 1988