Biopharmaceuticals

GLUCOSE HOMEOSTASIS

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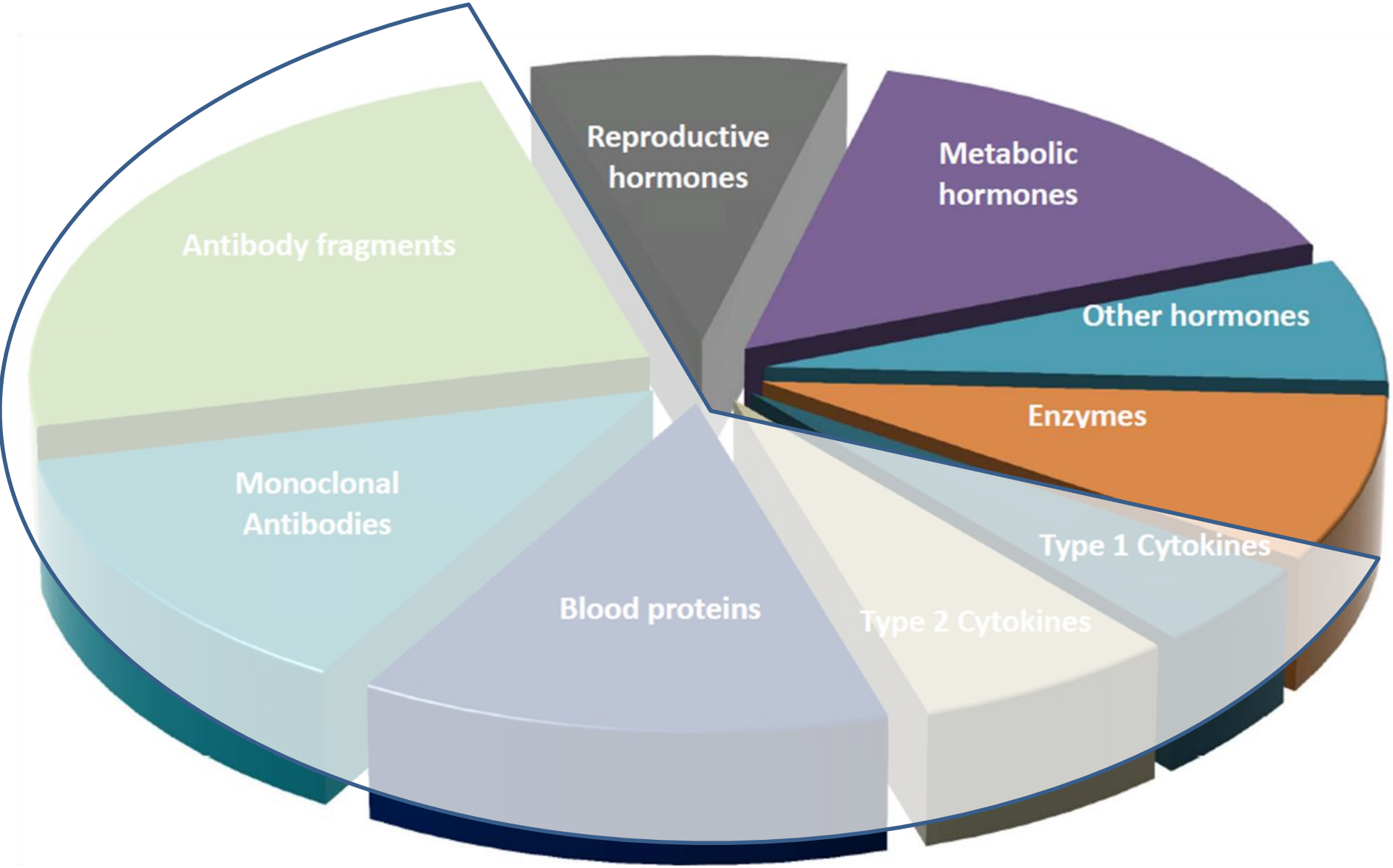
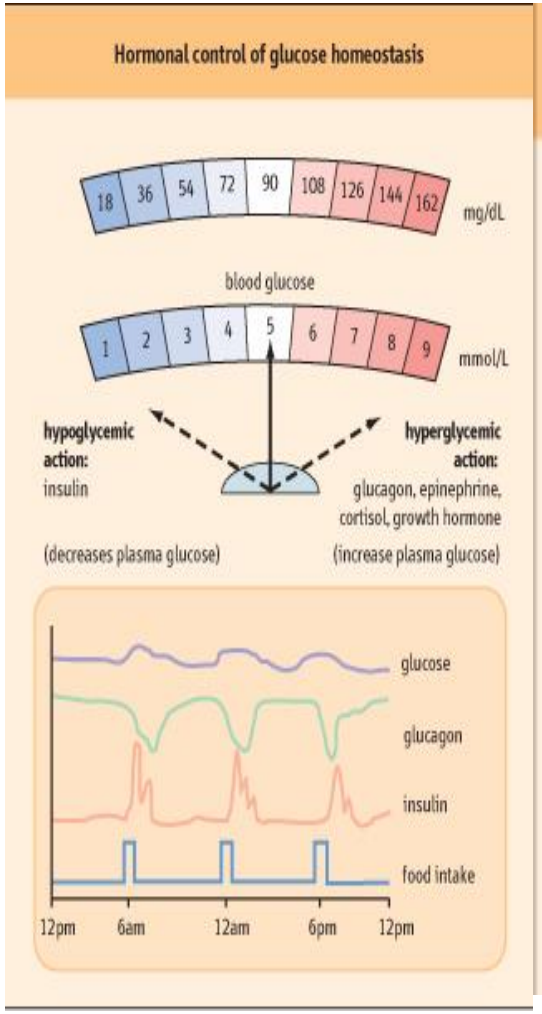
Hormones

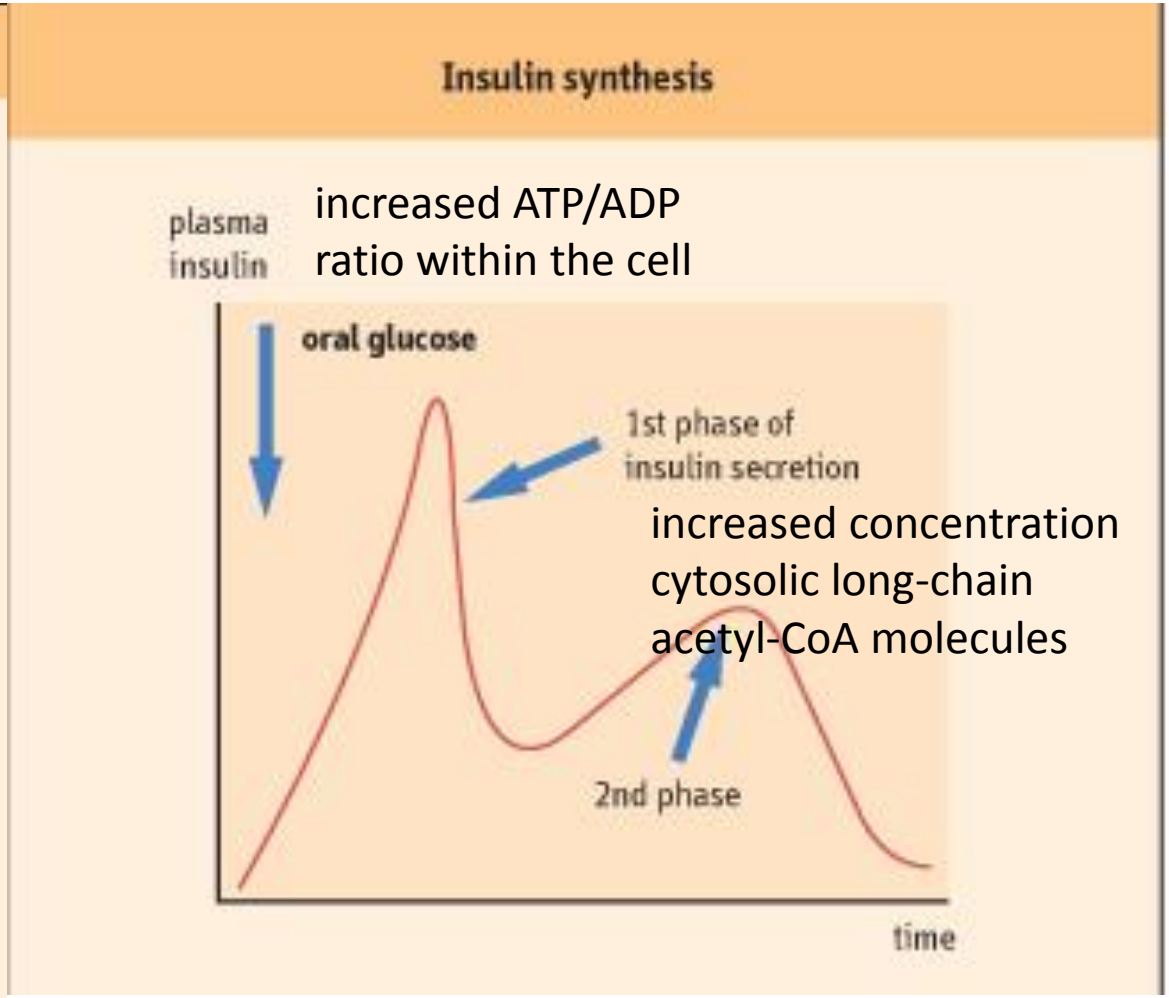

Reproductive hormones
Metabolic hormones
Other hormones
Enzymes
Type 1 Cytokines
Type 2 Cytokines
Blood proteins
Monoclonal Antibodies
Antibody fragments

Therapeutic Hormones

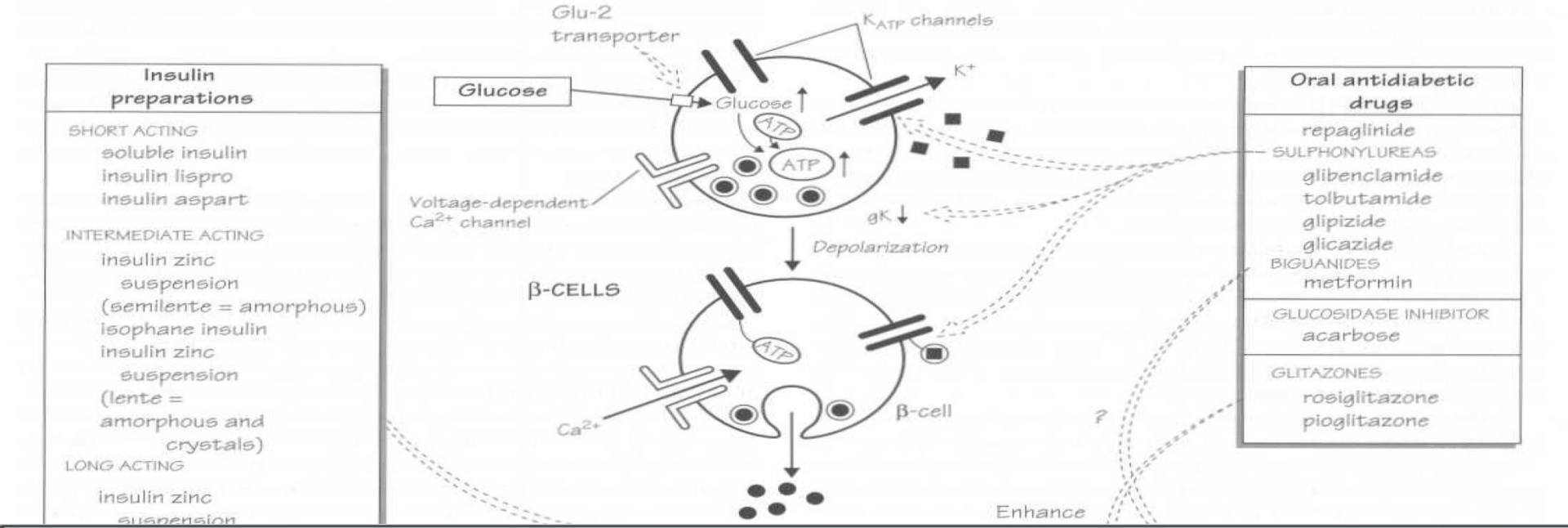
- **Hormonal control of Glucose homeostasis:** Insulin & Glucagon
- **Hypothalamo pituitary regulatory Hormones:**
- Human growth hormone
- Gonadotrophins

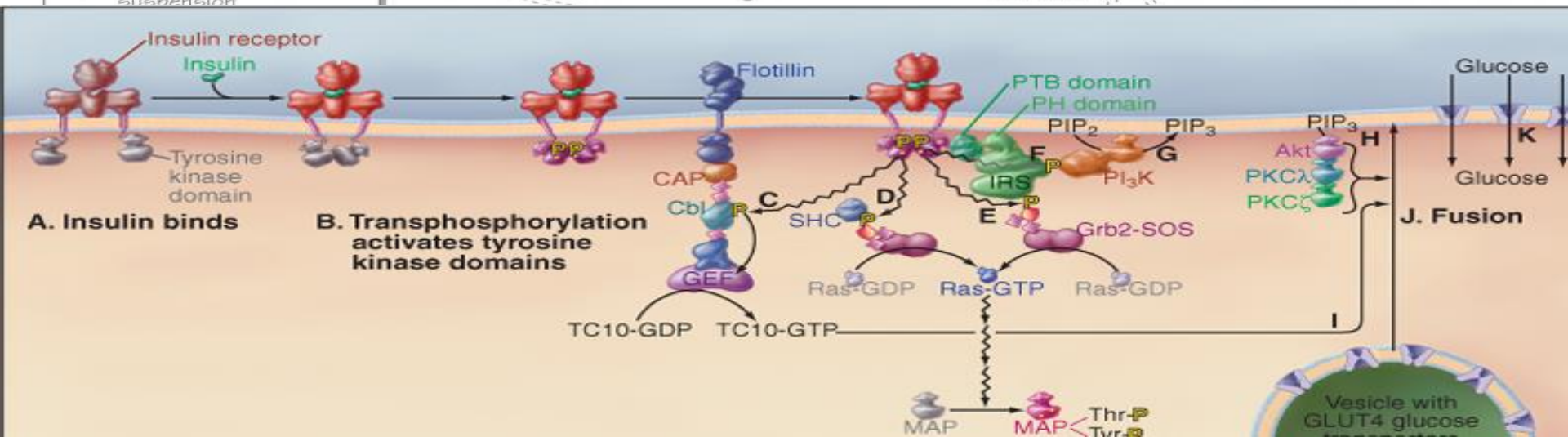
Hormonal control of glucose homeostasis



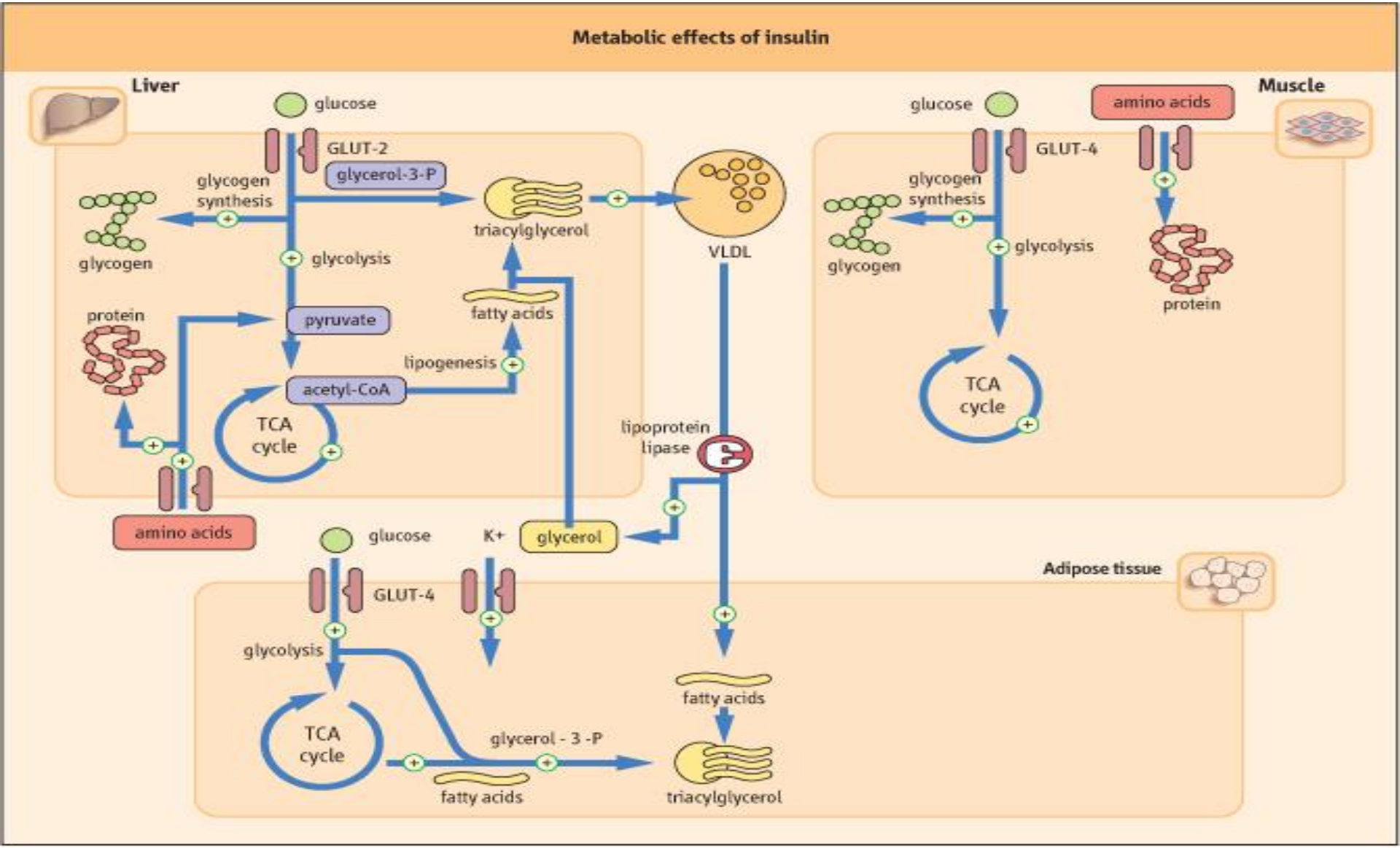


Insulin Pharmacology at Glance

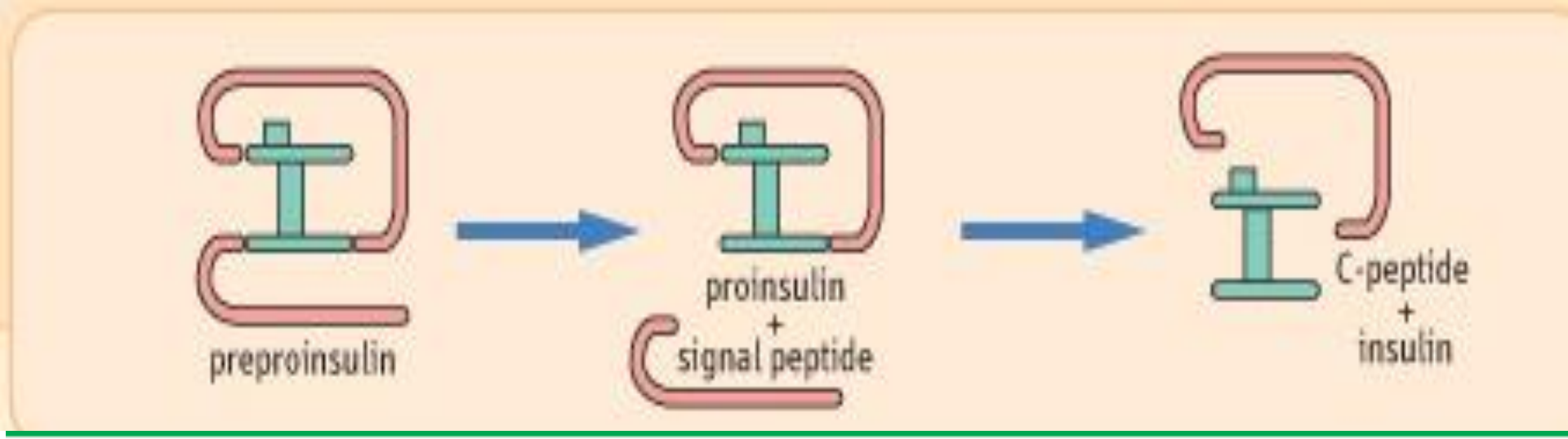


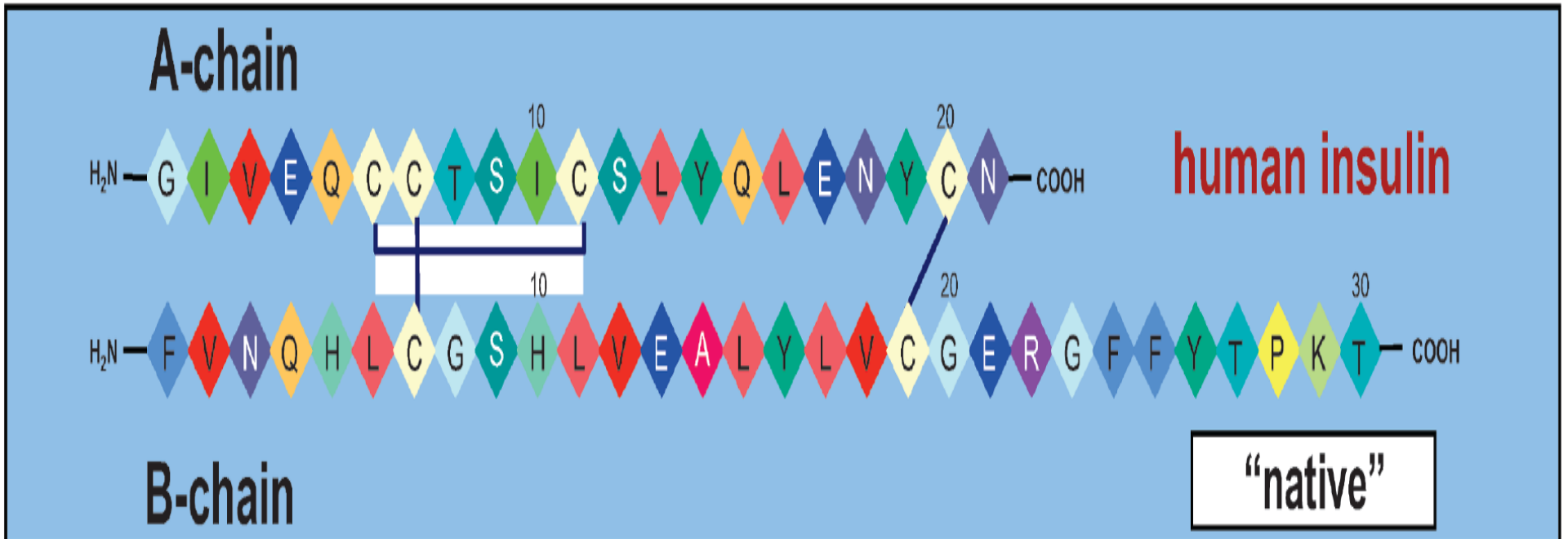


Insulin



The insulin molecule





The insulin molecule

The insulin protein production

```
P01317 INS_BOVIN        1   MALWTRLRPLLALLALWPPPPARAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREVEG   60
P01315 INS_PIG          1   MALWTRLLPLLALLALWAPAPAQAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREAEN   60
P01308 INS_HUMAN        1   MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTREAED   60
P01322 INS1_RAT         1   MALWMRFLPLLALLVLWEPKPAQAFVKQHLCGPHLVEALYLVCGERGFFYTPKSRREVED   60
P01323 INS2_RAT         1   MALWIRFLPLLALLILWEPRPAQAFVKQHLCGSHLVEALYLVCGERGFFYTPMSRREVED   60
P01326 INS2_MOUSE       1   MALWMRFLPLLALLFLWESHPTQAFVKQHLCGSHLVEALYLVCGERGFFYTPMSRREVED   60
P01329 INS_CAVPO        1   MALWMHLLTVLALLALWGPNTGQAFVSRHLCGSNLVETLYSVCQDDGFFYIPKDRRELED   60
P01325 INS1_MOUSE       1   MALLVHFLPLLALLALWEPKPTQAFVKQHLCGPHLVEALYLVCGERGFFYTPKSRREVED   60
                            ***  ::  :**** **     ***.:**** .***:** ** : **** *  *** *

P01317 INS_BOVIN       61   PQVGALELAGGPGAGGL-----EGPPQKRGIVEQCCASVCSLYQLENYCN          105
P01315 INS_PIG         61   PQAGAVELGGGL--GGLQALALEGPPQKRGIVEQCCTSICSLYQLENYCN          108
P01308 INS_HUMAN       61   LQVGQVELGGGPGAGSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN          110
P01322 INS1_RAT        61   PQVPQLELGGGPEAGDLQTLALEVARQKRGIVDQCCTSICSLYQLENYCN          110
P01323 INS2_RAT        61   PQVAQLELGGGPGAGDLQTLALEVARQKRGIVDQCCTSICSLYQLENYCN          110
P01326 INS2_MOUSE      61   PQVAQLELGGGPGAGDLQTLALEVAQQKRGIVDQCCTSICSLYQLENYCN          110
P01329 INS_CAVPO       61   PQVEQTELGMGLGAGGLQPLALEMALQKRGIVDQCCTGTCTRHQLQSYCN          110
P01325 INS1_MOUSE      61   PQVEQLELGGSP--GDLQTLALEVARQKRGIVDQCCTSICSLYQLENYCN          108
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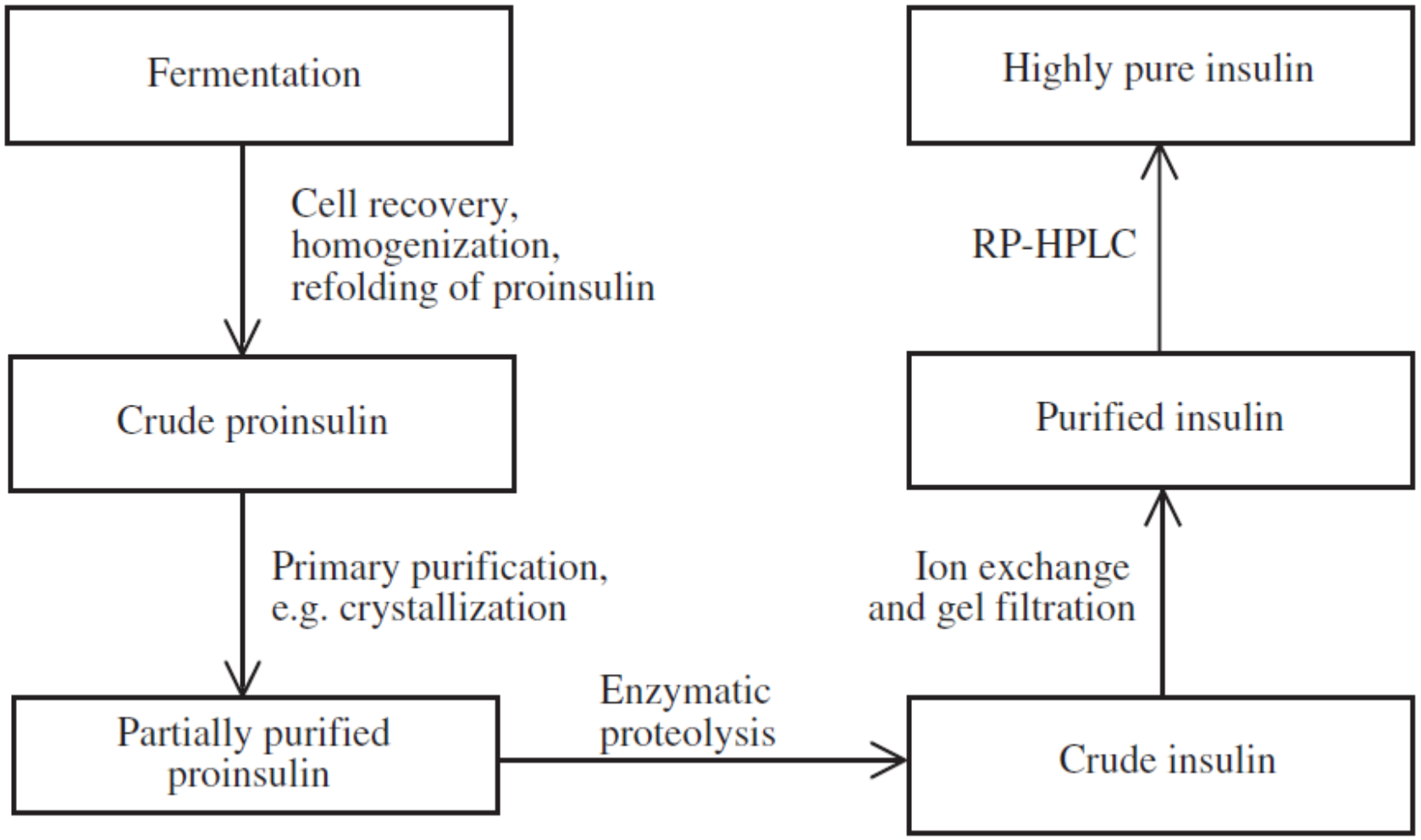
The insulin protein production



The Recombinant insulin Soluble Formulation

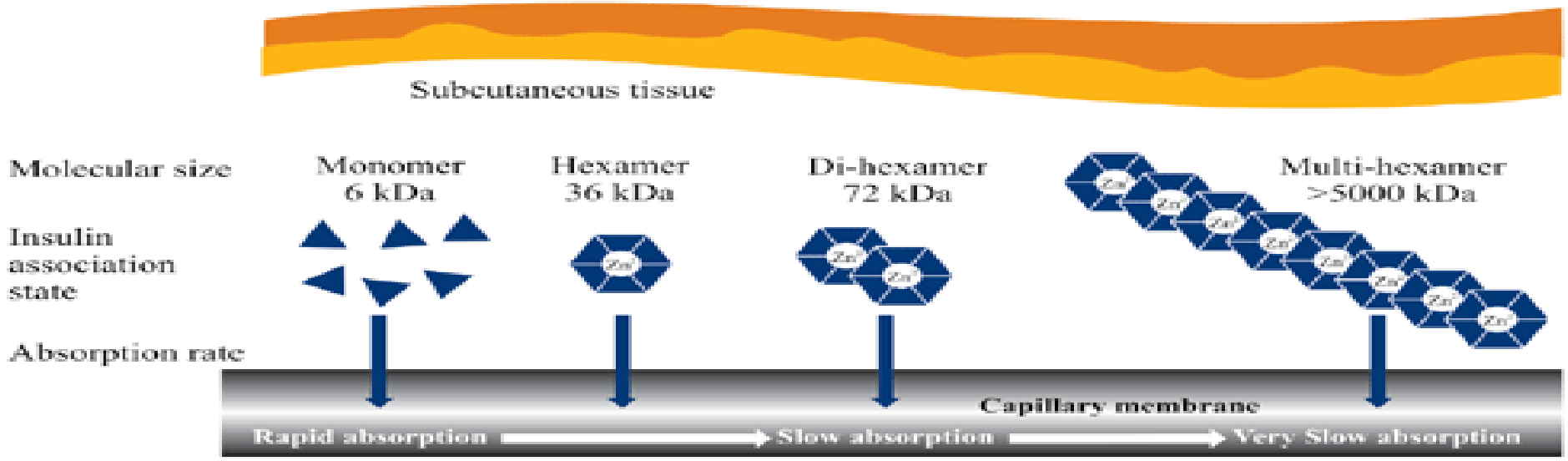
- Soluble formulations are sometimes referred to as '**regular insulin**' or '**unmodified insulin**'; or '**neutral insulin**' is a short-acting preparation that can be given intravenously.
- Making a solution (acidic or neutral pH)

Insulin Regular

Bill of Materials (Batch Size 2500 L to give 241545 Vials)					
Scale/mL		Item	Material	Qty	UOM
100.00	U	1	Insulin human, USP, 2% excess, 26.5 U/mg	9.519	g
2.50	mg	2	Metacresol, USP	6.25	g
16.00	mg	3	Glycerin, USP	40.00	kg
1.00	mL	4	Water for injection, USP	QS	kg
QS	mL	5	Hydrochloric acid, 10% solution, for pH adjustment	2.215	mL
QS	mL	6	Sodium hydroxide, 10% solution, for pH adjustment	3.30	mL

Note: Adjust the quantity of insulin based on activity.

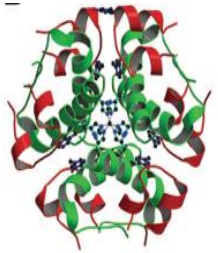
The Recombinant insulin Prolonged Formulation



“long-acting” insulins are usually administered in order to mimic low baseline endogenous insulin levels.

The Recombinant insulin Prolonged Formulation

- **protamine zinc insulin**, contains an excess amount of protamine



Insulin Human 70/30

Bill of Materials (Batch Size 1 L)					
Scale/mL		Item	Material	Qty	UOM
1000	U	1	Insulin human, USP, approximately 2% excess	1000,000	U
0.011	mg	2	Zinc oxide, USP; to give 0.025 mg/100 U	0.011	g
0.73	mg	3	Liquefied phenol, USP, equivalent to 0.65 mg/mL, calculated at 89% phenol	0.73	g
1.60	mg	4	Metacresol, USP	1.60	g
16.00	mg	5	Glycerin, USP (parenteral)	16.00	g
0.241	mg	6	Protamine sulfate, USP (purified) to provide 0.270 mg base/100 U in NPH crystallization part	0.241	g
3.78	mg	7	Sodium phosphate dibasic, USP	3.78	g
QS	mL	8	Water for injection, USP	QS	
QS	mL	9	Hydrochloric acid, 10% solution, for pH adjustment	QS	
QS	mL	10	Sodium hydroxide, 10% solution, for pH adjustment	QS	

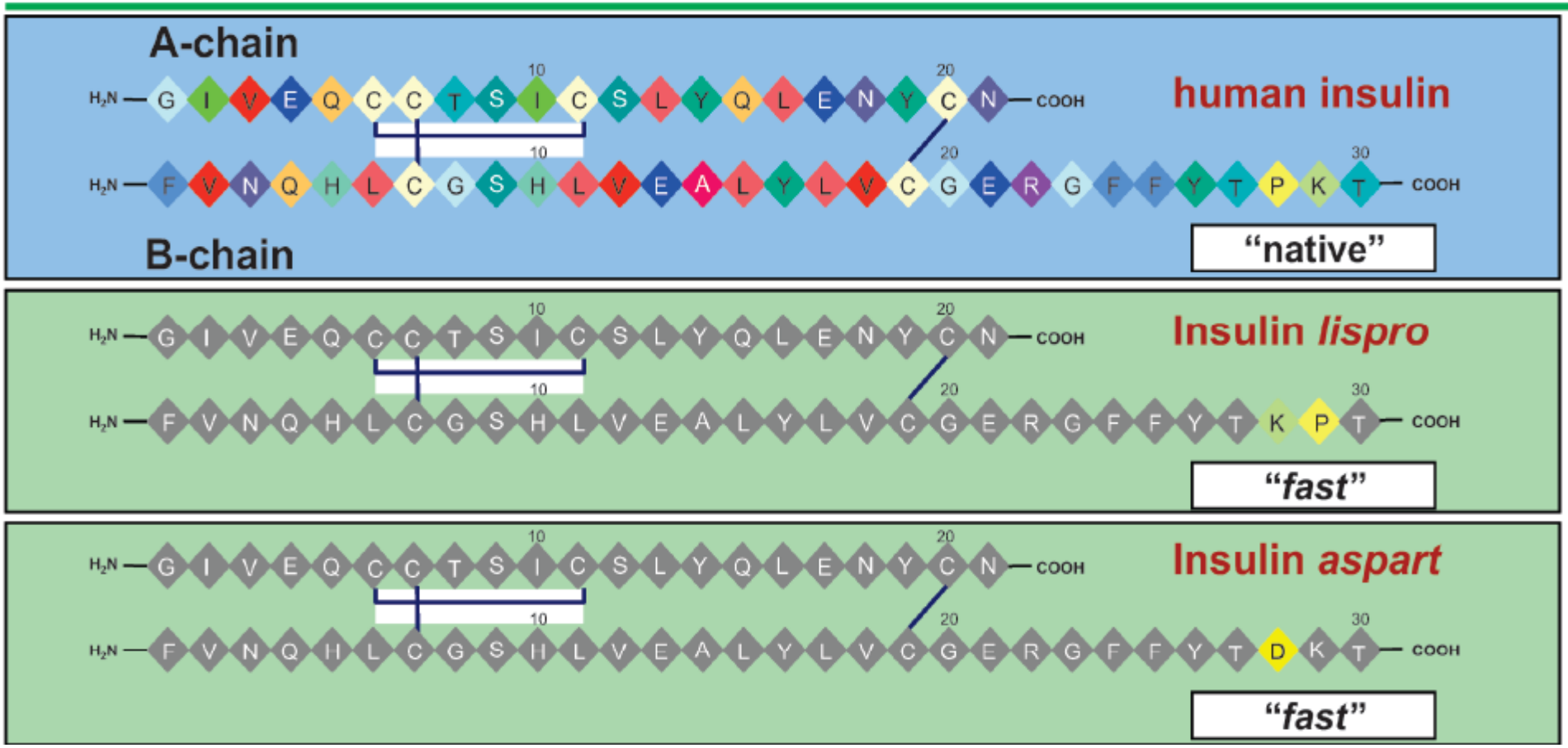
The Recombinant insulin molecule

analogues

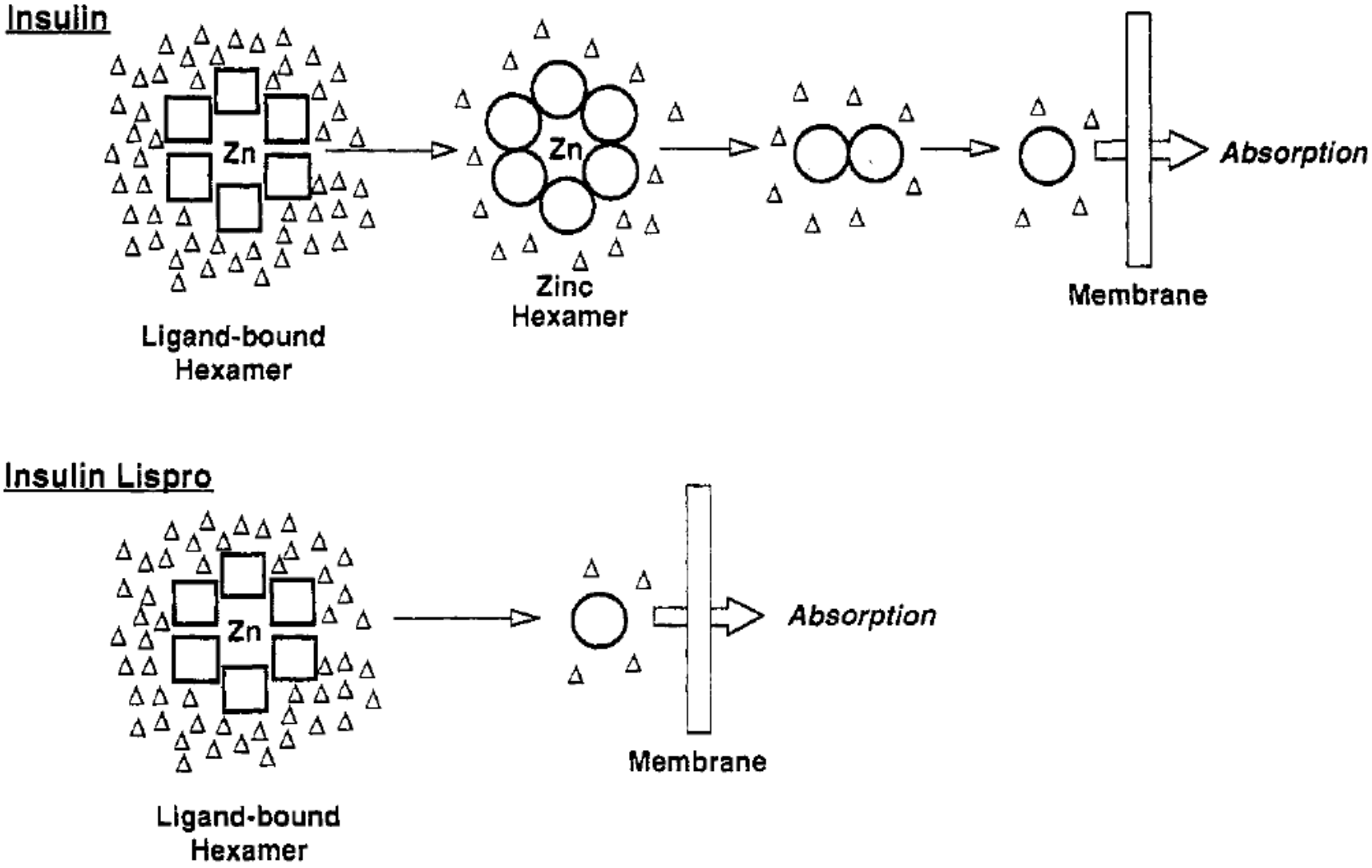
- **With altered pharmacokinetic profiles:**
- Rapidly acting: before meals (5 to 15 min)
  - **Insulin lispro** (LysB28, ProB29)
  - **Insulin aspart** (AspB28)
  - **Insulin glulisine** (LysB3, GluB29)
- long-acting: (basal insulin analogue)
  - **NPH** (intermediate)
  - **Insulin glargine:**
  - **Insulin detemir** (NN-304); **Levemir**

The Recombinant insulin molecule

analogues fast acting

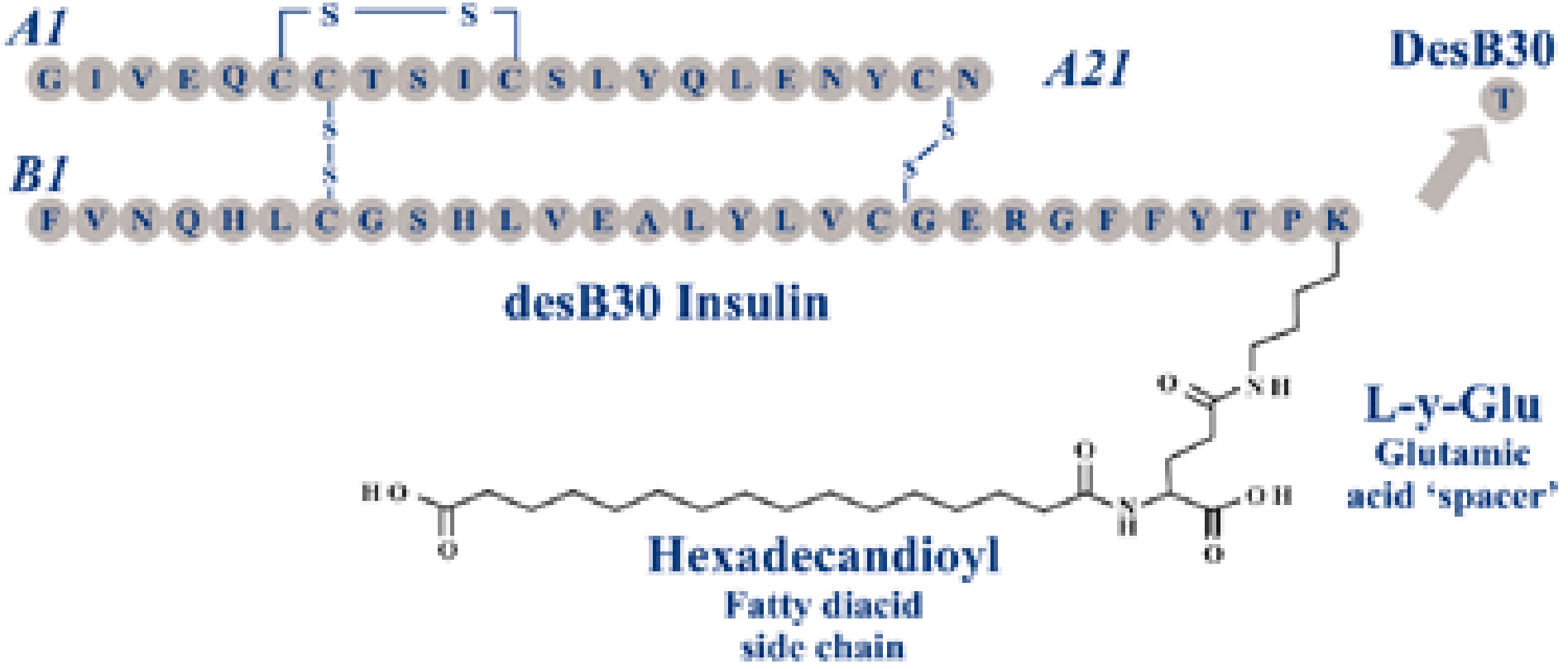


The Recombinant insulin molecule



The Recombinant insulin molecule

analogues slow acting



The Recombinant insulin molecule

analogues

- **With altered pharmacokinetic profiles:**
- Rapidly acting: before meals (5 to 15 min)
  - **Insulin lispro** (LysB28, ProB29)
  - **Insulin aspart** (AspB28)
  - **Insulin glulisine** (LysB3, GluB29)
- long-acting: (basal insulin analogue)
  - **NPH** (intermediate)
  - **Insulin glargine:**
  - **Insulin detemir** (NN-304); **Levemir**

Pharmacokinetic of insulin analogues

Category	Onset (hours after administration)	Peak activity (hours after administration)	Duration (h)
Short-acting	0.5–1	2–5	6–8
Intermediate-action	2	4–12	up to 24
Long-acting	4	10–20	up to 36

	Human Insulin Chain[a]					
	A	B				
Amino Acid position	21	28	29	30	Insertion at 31 and 32	Pharmacological Attributes[b]
Insulin human (native form)	Asn	Pro	Lys	Thr	None	Peak at 4–5 hr and dissipated by 14–15 hr; $t_{1/2}$ = 1.5 hr
Insulin lispro (*Humalog*)	Asn	***Lys***	***Pro***	Thr	None	Rapid acting and peak at 1 hr, instead of 3 hr; effect dissipated by 6–8 hr; $t_{1/2}$=1 hr
Insulin aspart (*NovoLog*)	Asn	***Asp***	Lys	Thr	None	Rapid acting and peak at 1 hr and sustained for another 2 hr; $t_{1/2}$=1.3 hr
Insulin glargine (*Lantus*)	***Gly***	Pro	Lys	Thr	***Arg*** and ***Arg***	Sustained release over 24 hr; onset 4–6 hr
Insulin detemir (*Levemir*)	***Gly***	Pro	***Lys-Myr***[c]	None	None	Sustained response over 16–18 hr; on set ~4–6 hr
Insulin degludec[d] (*Degludec*)	***Gly***	Pro	***Lys-Hdg***[e]	None	None	Sustained response over 24 hr; onset ~4–6 hr

The Recombinant insulin molecule

Product	Company	Therapeutic indication	Approved
Humulin (rhInsulin produced in *E. coli*)	Eli Lilly	diabetes mellitus	1982 (US)
Novolin (rhInsulin produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	1991 (US)
Humalog (Insulin lispro, an insulin analog produced in *E. coli*)	Eli Lilly	diabetes mellitus	1996 (US and EU)
Insuman (rhInsulin produced in *E. coli*)	Hoechst	diabetes mellitus	1997 (EU)
Liprolog (Bio Lysprol, short-acting insulin analog produced in *E. coli*)	Eli Lilly	diabetes mellitus	1997 (EU)
NovoRapid (Insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	1999 (EU)
Novomix 30 [contains insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae* (see *NovoRapid*) as one ingredient]	Novo Nordisk	diabetes mellitus	2000 (EU)
Novolog (Insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae*; see also *NovoRapid*)	Novo Nordisk	diabetes mellitus	2001 (US)

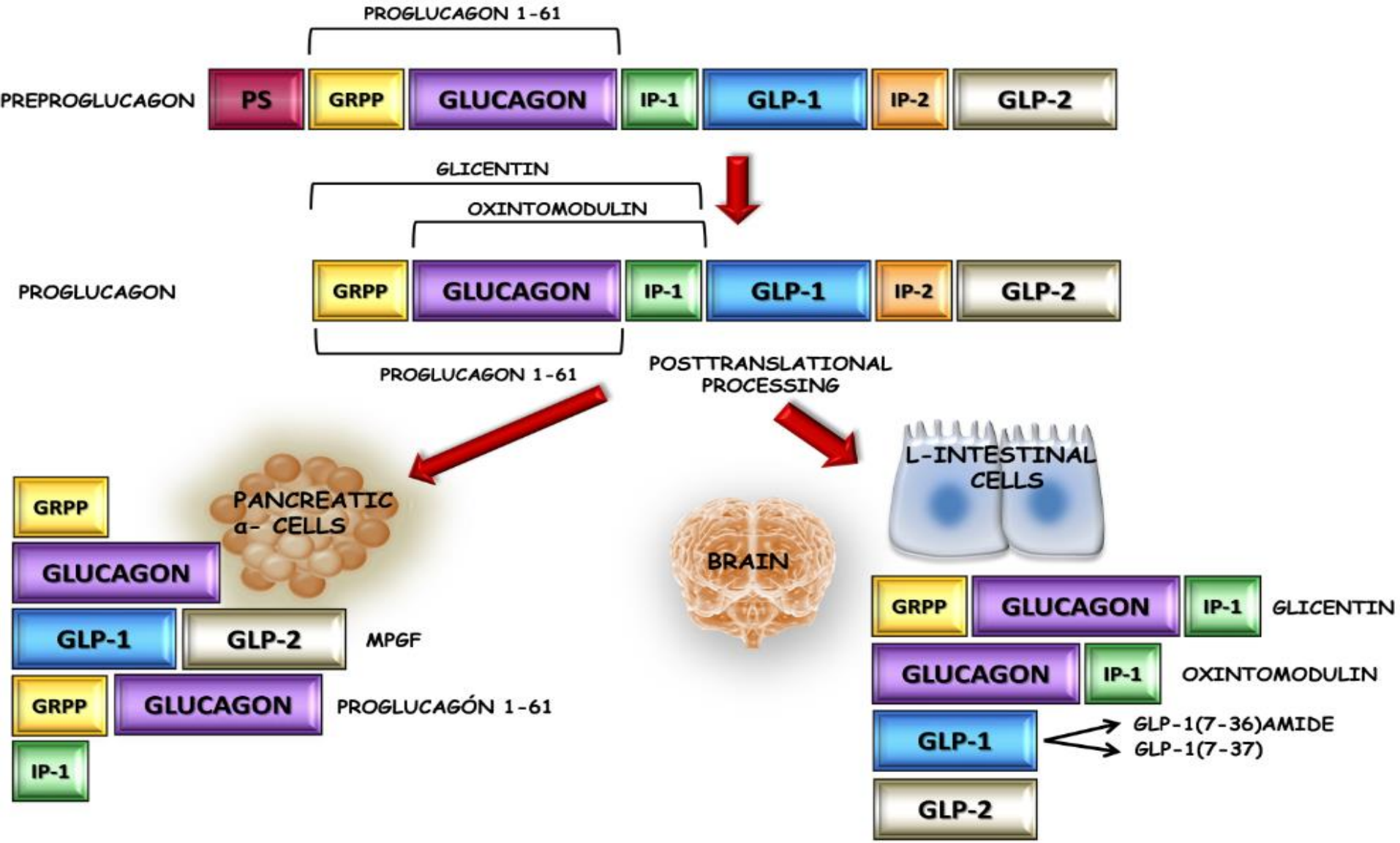
The Recombinant insulin molecule

Product	Company	Therapeutic indication	Approved
Novolog mix 70/30 (contains insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae* as one ingredient; see also *Novomix 30*)	Novo Nordisk	diabetes mellitus	2001 (US)
Actrapid/Velosulin/Monotard/Insulatard/Protaphane/Mixtard/Actraphane/Ultratard (all contain rhInsulin produced in *S. cerevisiae* formulated as short/intermediate/long-acting product)	Novo Nordisk	diabetes mellitus	2002 (EU)
Lantus (Insulin glargine, long-acting rhInsulin analog produced in *E. coli*)	Aventis	diabetes mellitus	2000 (US and EU)
Optisulin (Insulin glargine, long-acting rhInsulin analog produced in *E. coli*, see *Lantus*)	Aventis	diabetes mellitus	2000 (EU)
Levemir (Insulin detemir, long-acting rhInsulin analog produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	2004 (EU)
Apidra (Insulin Glulisine, rapid-acting insulin analog produced in *E. coli*)	Aventis	diabetes mellitus	2004 (US)

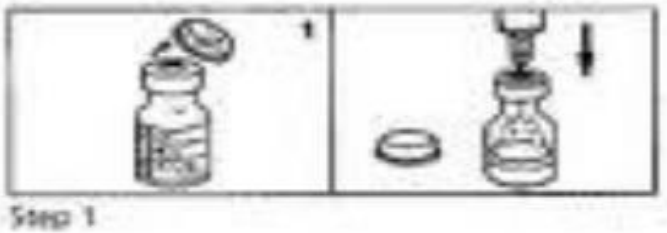
Insulin Delivery

Formulation	Administration route	Company	Clinical trial
Spray (Oral-lyn™)	Oral	Generex	Phase III
Capsule (ORMD 0801)	Oral	Oramed	Phase II
Tablet (IN-105)	Oral	Biocon	Phase II
Tablet (EMISPHERE™)	Oral	Emisphere Technologies	Phase II
Bioadhesive NP (Nodlin)	Oral	Shanghai Biolaxy	Phase I
Spray	Intranasal	Nastech	Phase II
Spray	Intranasal	MDRNA	Phase II
Dry powder (Technosphere®, Afrezza)	Pulmonary	Mannkind	Resubmitted new drug application
Dry powder (AIR®)	Pulmonary	Eli Lilly	Phase III
Liquid insulin (AERx®)	Pulmonary	Novo Nordisk	Phase III
PassPort™ patch	Transdermal	Altea Therapeutics	Phase II
TPM-02/insulin gel	Transdermal	Phosphagenics	Phase II

Glucagon



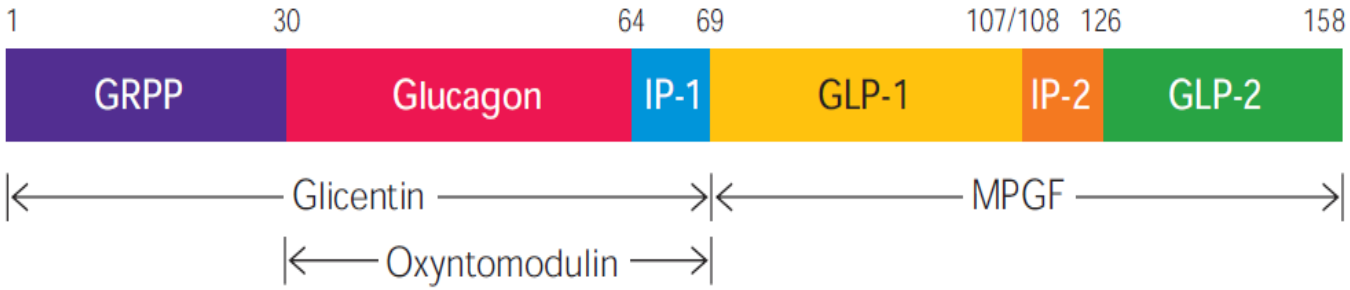
Glucagon Production



Glucagon Administration

- Glucagon, 0.5–1.0 units (0.5–1.0 mg freeze-dried), is administered by **s.c.** , **i.m**. or **i.v.** injection.
- In the cases of insulin-induced **hypoglycaemia**: treated by oral or i.v. administration of glucose, and sometimes treated by administration of glucagon.
- Glucagon recombinant is also used during radiologic examinations to **temporarily inhibit movement of the gastrointestinal tract**. It and anticholinergic drugs are equally effective for this use.
- be effective in **anaphylaxis**, biliary tract pain, beta-adrenergic blocker overdose, and esophageal obstruction and as premedication in endoscopic procedures**.**

New Diabetes therapeutics



Pancreas
MPGF

Glucagon

HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
Glucagon receptor antagonism target
for treatment of type 2 diabetes

Intestine
Glicentin
Oxyntmodulin

GLP-1

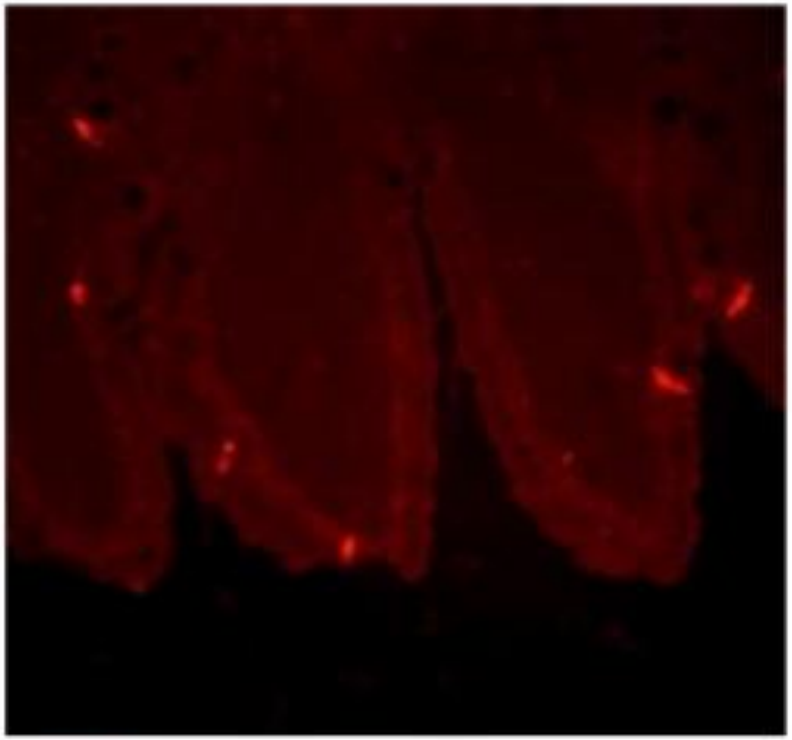
HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG
Major new drug class for
type 2 diabetes

GLP-2

HADGSFSDEMNTILDNLAARDFINWLIQTKITD
Clinical trials for the treatment of Crohn's
disease and short-bowel syndrome

GLP-1 and GIP are the 2 major incretins in human

- Both are peptide **hormones** (30 and 42 amino acids)
- Secreted from endocrine cells in the small intestinal mucosa
- **GLP-1**: distal, L-cells (mainly ileum, colon)
- **GIP**: proximal, K-cells (mainly duodenum)
- Released in response to meal ingestion

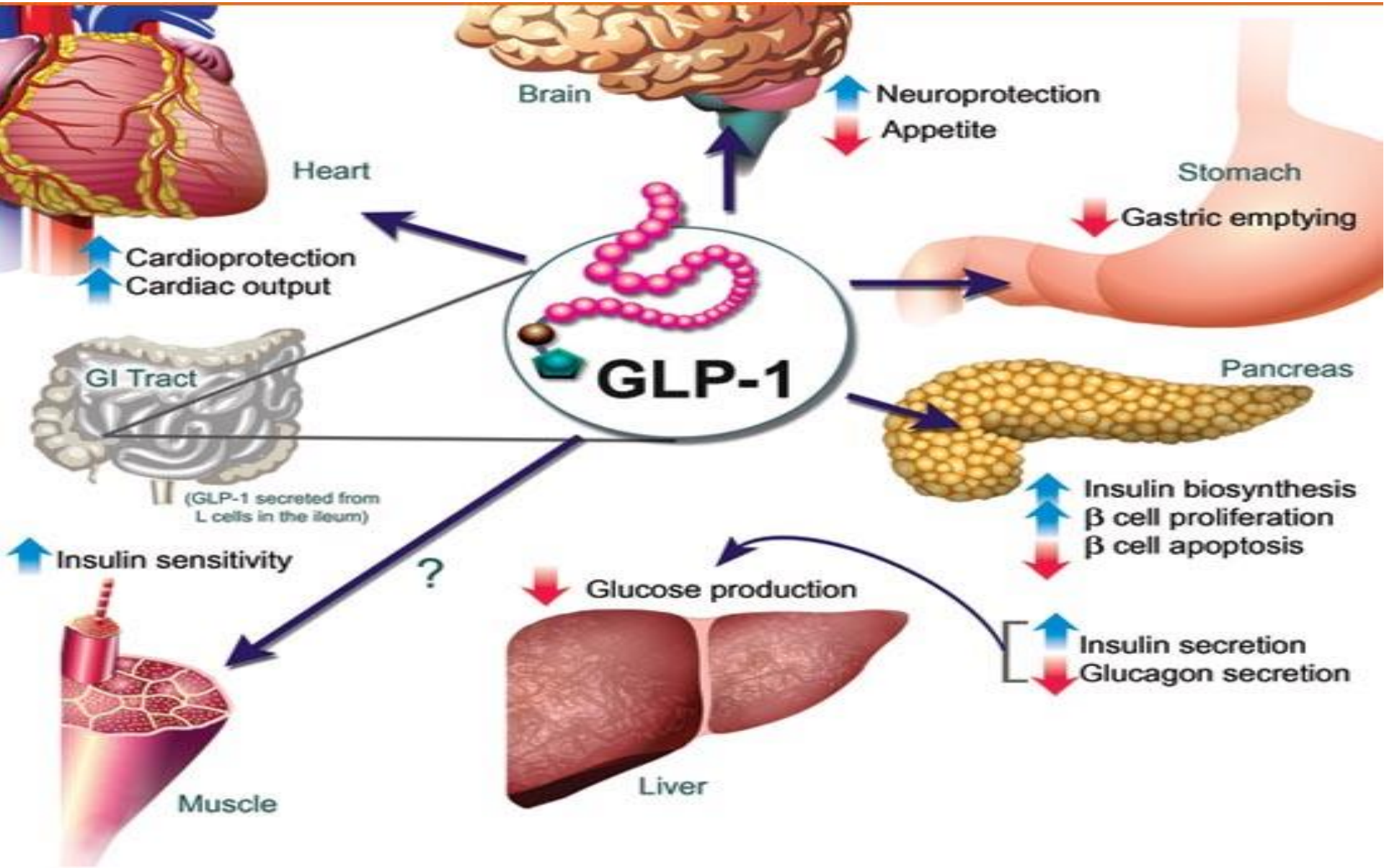


GLP-1 positive endocrine L-cells in human small intestine

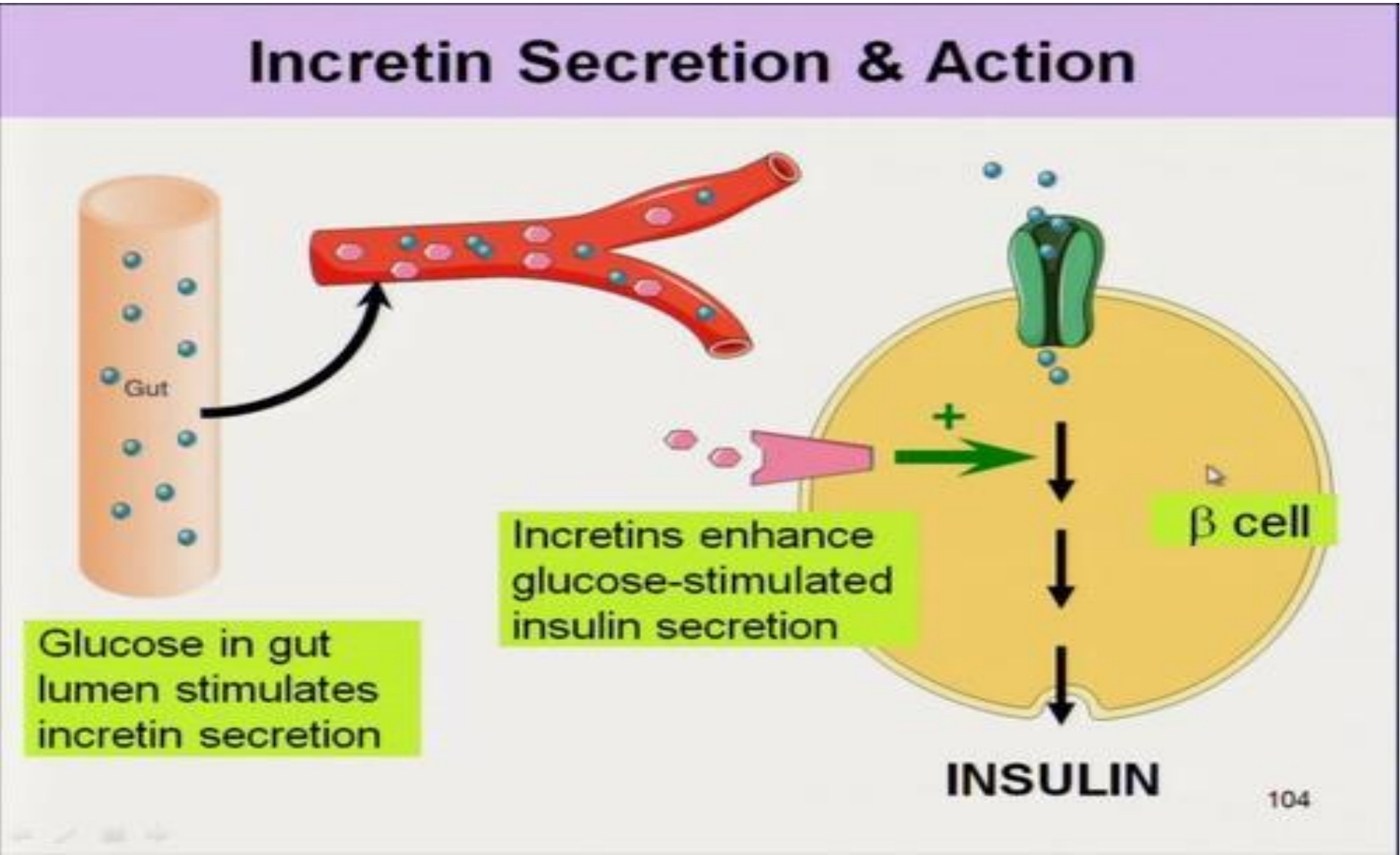
Incretins

- Incretins are **a group of metabolic hormones that stimulate a decrease in blood glucose levels.**
- Incretins do so by
  - **increasing insulin released**
  - **reducing gastric emptying and may directly reduce food intake**
  - **inhibiting glucagon release**
- The two main incretins are
  - **Glucagon-like peptide-1 (GLP-1)**
  - **Gastric Inhibitory Peptide (GIP)**

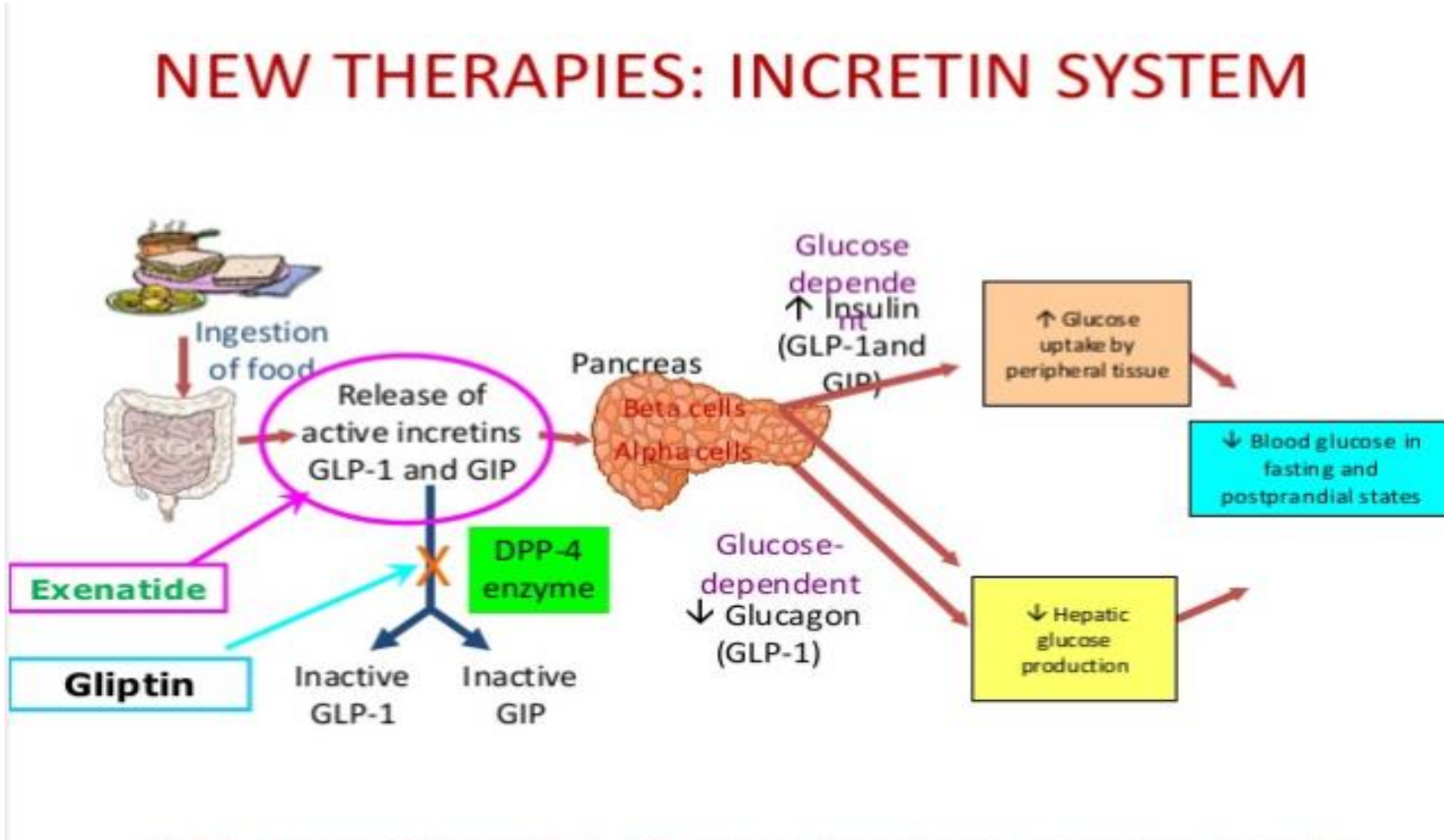
Incretins


Brain
Neuroprotection
Appetite
Heart
Cardioprotection
Cardiac output
Stomach
Gastric emptying
GI Tract
GLP-1
Pancreas
(GLP-1 secreted from
L cells in the ileum)
Insulin biosynthesis
β cell proliferation
β cell apoptosis
Insulin sensitivity
?
Glucose production
Insulin secretion
Glucagon secretion
Muscle
Liver

Incretins


Incretin Secretion & Action
Gut
Glucose in gut lumen stimulates incretin secretion
Incretins enhance glucose-stimulated insulin secretion
+
β cell
INSULIN
104

Incretins



GLP-1=glucagon-like peptide-1; GIP=glucose-dependent insulinotropic polypeptide.

Incretins

DIPEPTIDYL PEPTIDASE - 4 INHIBITORS

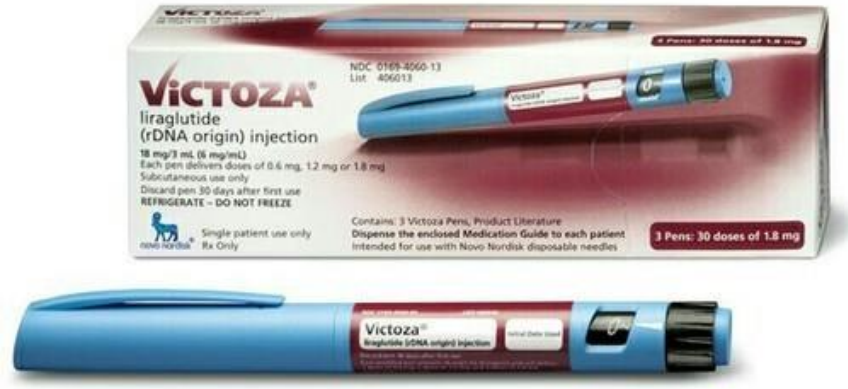
Drugs belonging to this class:

- Sitagliptin (FDA approved 2006)
- Vildagliptin (EU approved 2008)
- Saxagliptin (FDA approved 2009)
- Linagliptin (FDA approved 2011)

Exenatide

- Trade name: ***Byetta***
- Exenatide is a synthetic 39-amino acid peptide amide. It has a molecular weight of 4200 Da.
- It is an **incretin** mimic that acts as an **agonist** at the **"Glucagon-like peptide 1 receptor"** to enhance insulin secretion in the presence of raised glucose concentrations;
- It also suppresses inappropriate glucagon secretion and slows gastric emptying. → lower BLOOD glucose, and reduce food intake.

Liraglutide



- Trade name: ***Victoza***
- **Liraglutide** is a recombinant 31-amino acid peptide. It has a molecular weight of 3700 Da.
- It is an **incretin** mimic that acts as an **agonist** at the **"Glucagon-like peptide 1 receptor"** to enhance insulin secretion in the presence of raised glucose concentrations;

The pancreas

Endocrine gland	Hormone	Function	Secretion control is made by
Pancreas	Insulin	Increase uptake of glucose into the cell; promotes glycogenesis; lowers blood sugar levels	Raised blood glucose levels
	Glucagon	Promotes glycogenolysis; Increases blood sugar levels	Low blood glucose levels
	Somatostatin	Mild inhibition of insulin and glucagon preventing fluctuations in blood glucose levels. Decreases gut motility and secretion of digestive juices	Blood glucose levels