Lysosomal Storage Diseases and Glycan Degradation

Learning Objectives

- ***What is the location and mechanism of glycoproteins, glycolipids, and glycosaminoglycans degradation?***
- ***How are acid hydrolases delivered to lysosomes?***
- ***Describe the general features and symptoms of patients with lysosomal storage diseases.***
- ***How are lysosomal storage diseases treated?***

Outline

- ***Catabolism of Glycoproteins, Glycolipids, Glycosaminoglycans***
  - ✓ ***Lysosome***
    - ✓ ***Function***
    - ✓ ***Biogenesis and targeting of acid hydrolases***
      - ✓ ***Mannose 6-phosphate tag***
      - ✓ ***P-type lectins***
      - ✓ ***Disease: Mucolipidosis II (MLII)***

- ***Lysosomal Storage Diseases (LSDs)***
  - ✓ ***Classes***
  - ✓ ***Clinical manifestations, inheritance and prevalence***
  - ✓ ***Major treatment options***
  - ✓ ***Examples***
    - ✓ ***Fabry disease***
    - ✓ ***Mucopolysaccharidoses (MPS)***
    - ✓ ***Pompe disease***

Lysosomes are the Recycling Centers of the Cell

- ***Lysosomes are membrane bound organelles found in almost all cell types***
- ***Lysosome has ~60 different hydrolytic enzymes used for the controlled intracellular digestion of macromolecules.***
- ***Degraded macromolecules are recycled and used in pathways for energy production (TCA cycle) or in biosynthetic pathways (protein synthesis).***
- ***All hydrolytic enzymes are acid hydrolases that are optimally active near the pH 5 maintained within lysosomes.***
- ***V-type ATPases are responsible for acidifying intracellular compartments; lysosomes are the most acidified compartment in mammalian cells with a pH of 5.0 or less.***

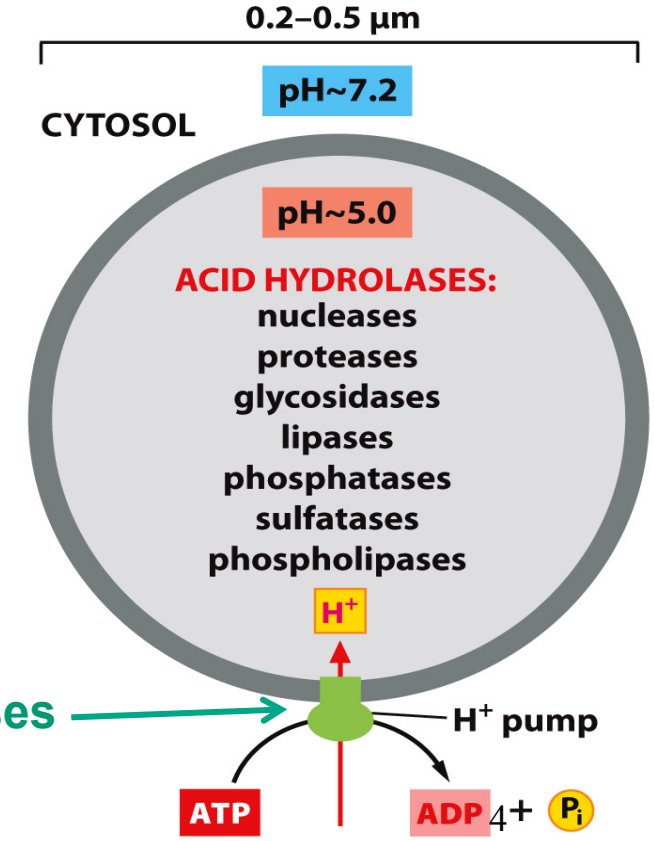


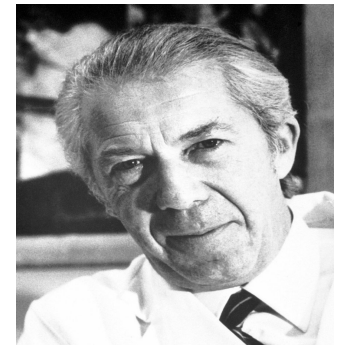
Figure 13-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

Functions of Lysosomes

Numerous functions are carried out by lysosomes including:

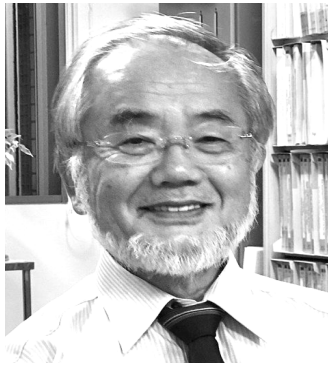
- ✓ ***Disposal of abnormal/aggregated proteins & defective organelles***
- ✓ ***Downregulation of cell surface signaling receptors (e.g., EGF receptor)***
- ✓ ***Release of endocytosed nutrients (e.g., cholesterol from LDL)***
- ✓ ***Degradation of pathogenic organisms (phagocytosis)***
- ✓ ***Cellular survival (autophagy)***

Nobel Prize

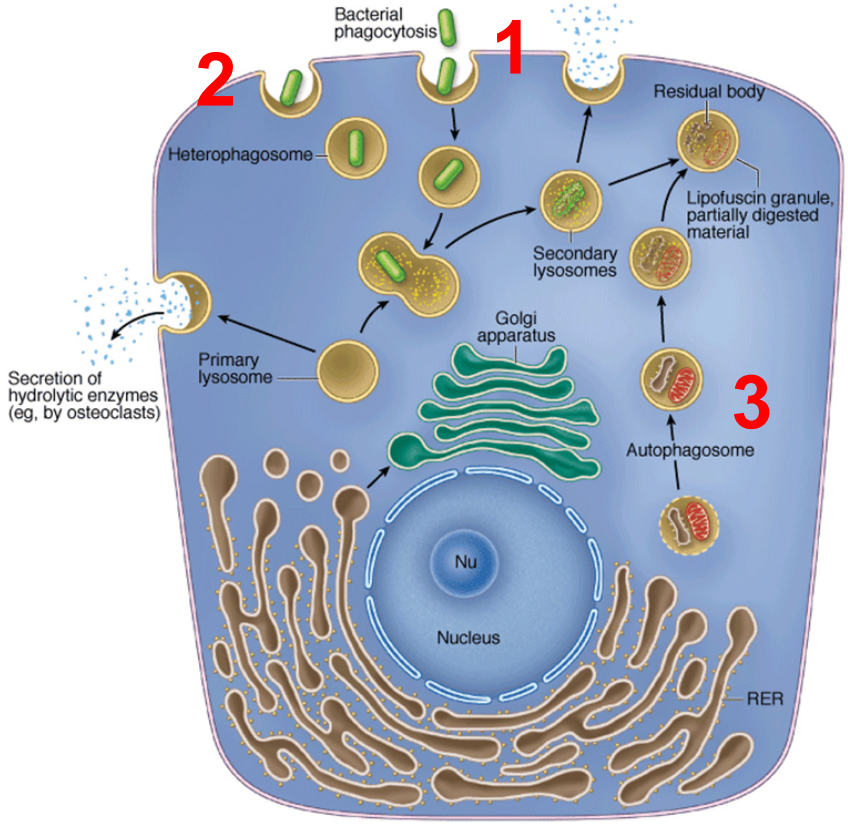


Christian de Duve, 1974
discovered lysosome

Nobel Prize



Yoshinori Ohsumi, 2016
Autophagy



Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition:* http://www.accessmedicine.com

Three pathways to the lysosome:

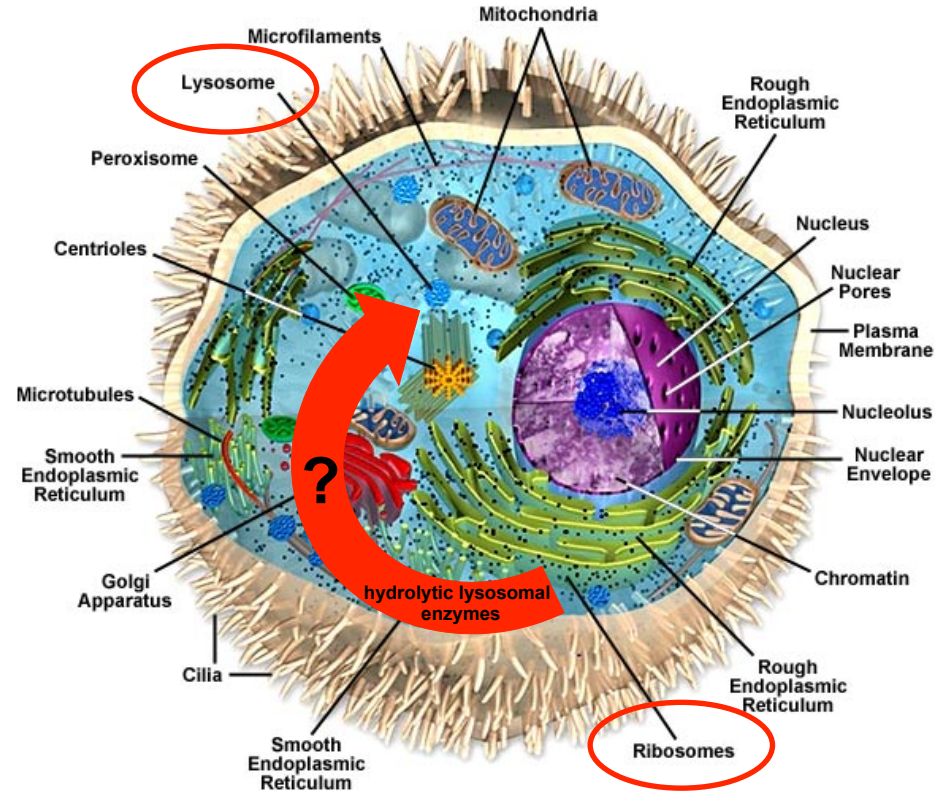
1) Phagocytosis
2) Endocytosis
3) Autophagy

Autophagy (**"self-eating"**) **is a major catabolic, energy-producing pathway that involves the lysosomal degradation of cytoplasmic proteins and organelles.**

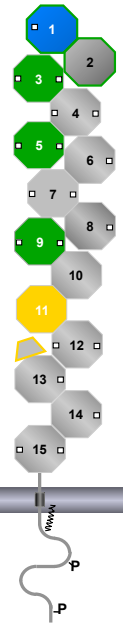
- ✓ **Housekeeping function (quality control mechanism) to eliminate damaged organelles and protein aggregates.**
- ✓ **During starvation, autophagy increases and provides an internal source of nutrients for energy generation and thus survival.**

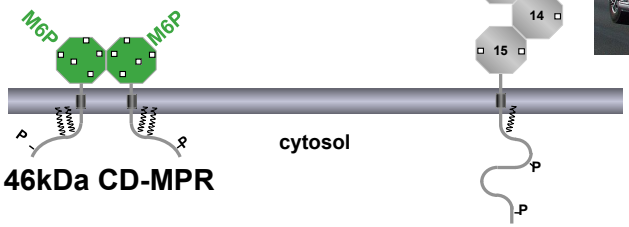
How to Build a Lysosome?

Mannose 6-phosphate



P-type Lectins/Receptors





46kDa CD-MPR

300kDa CI-MPR

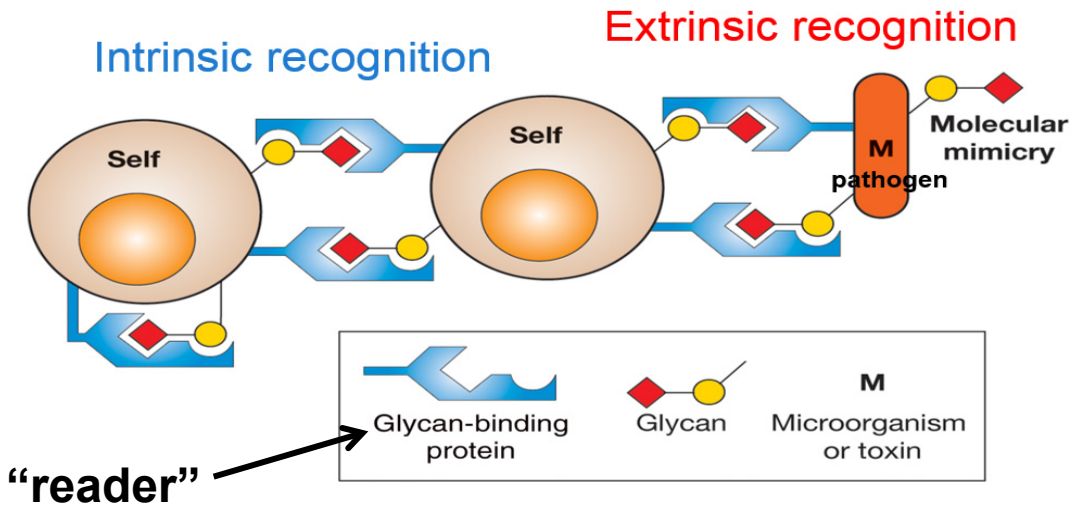
Mannose 6-phosphate receptors are the delivery trucks that recognize the "zip code" and carry the ~60 different, newly synthesized mannose 6-phosphate-tagged hydrolytic lysosomal enzymes (packages) from the Golgi to pre-lysosomal compartments.

"Write / Read / Erase"

- ***Writers*** *enzymes that add a mark*
  (glycosyltransferases)

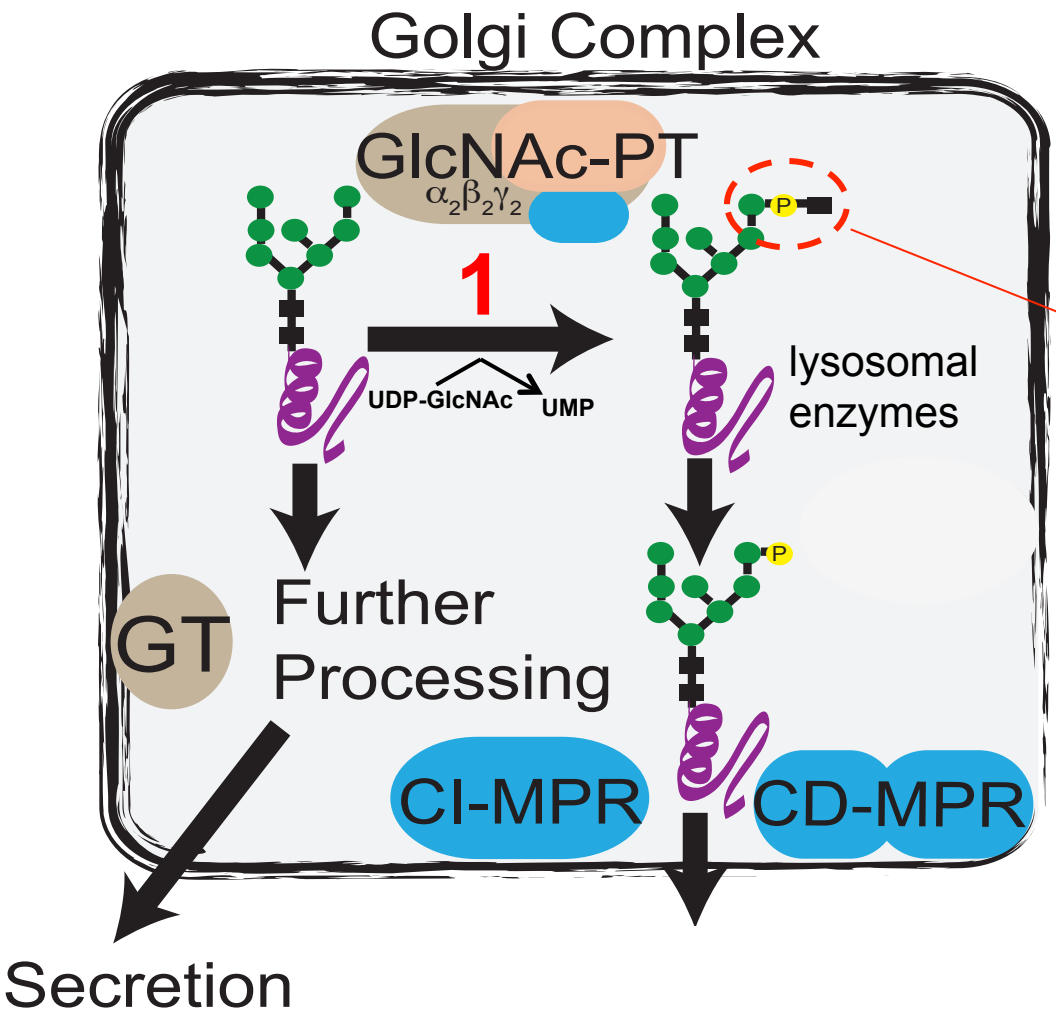
- ***Readers*** *proteins that bind to and interpret the mark*
  (lectins: glycan-binding proteins)

- ***Erasers*** *enzymes that remove the mark*
  (glycosidases)

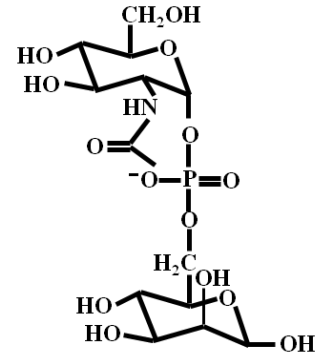


Modified from Glycobiology. 2016;27(1):3-49

Delivery of lysosomal enzymes to lysosomes by Mannose 6-Phosphate Receptors (MPRs) – Man-6-P tag (*N*-glycans)

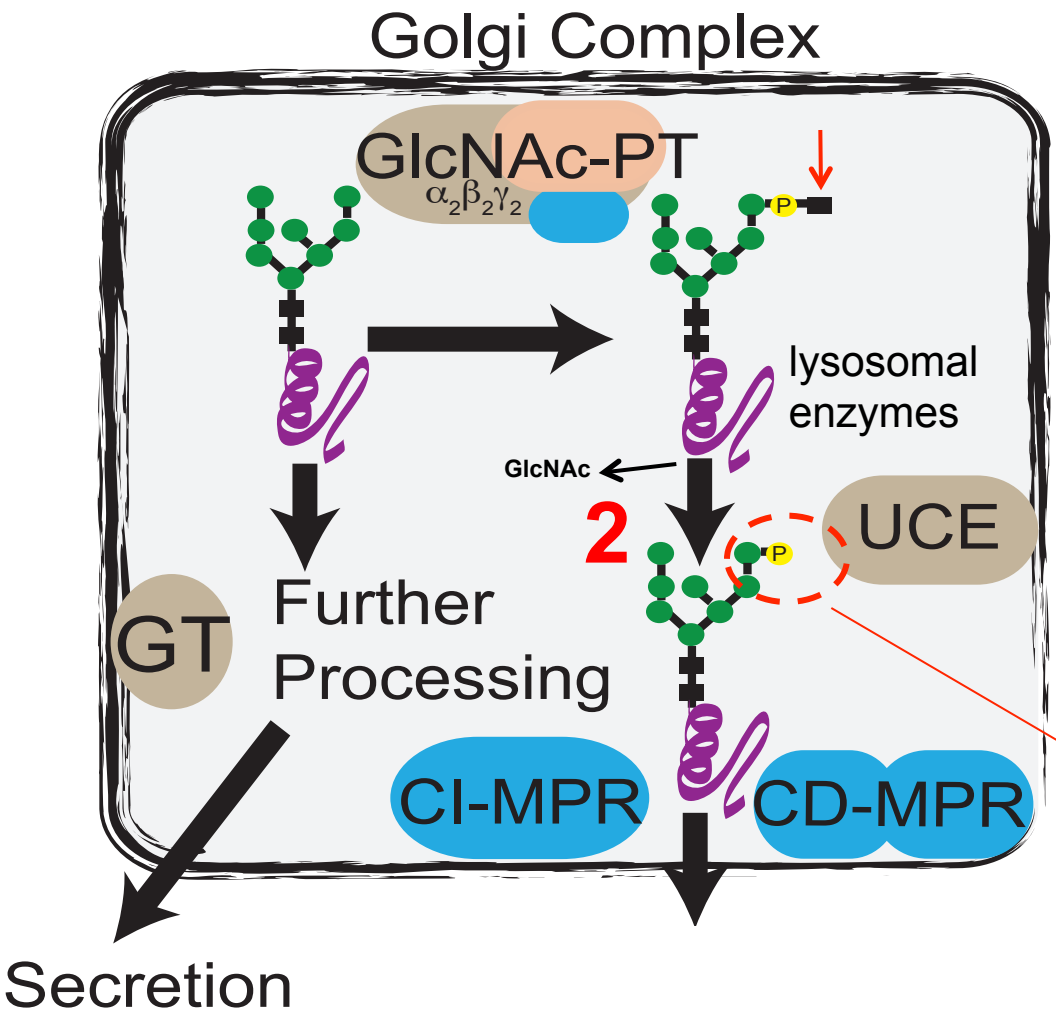


1) GlcNAc-1-phosphotransferase
   - *cis* Golgi glycosyltransferase
   - recognizes ~60 lysosomal enzymes via a conformation-dependent region
   - modifies mannose ➔ phosphodiester Man-6-P-GlcNAc



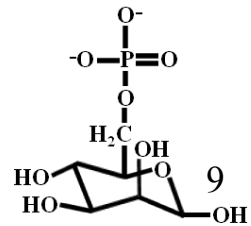
Lysosomal enzymes are glycoproteins with N-linked glycans

Delivery of lysosomal enzymes to lysosomes by Mannose 6-Phosphate Receptors (MPRs) – Man-6-P tag (*N*-glycans)

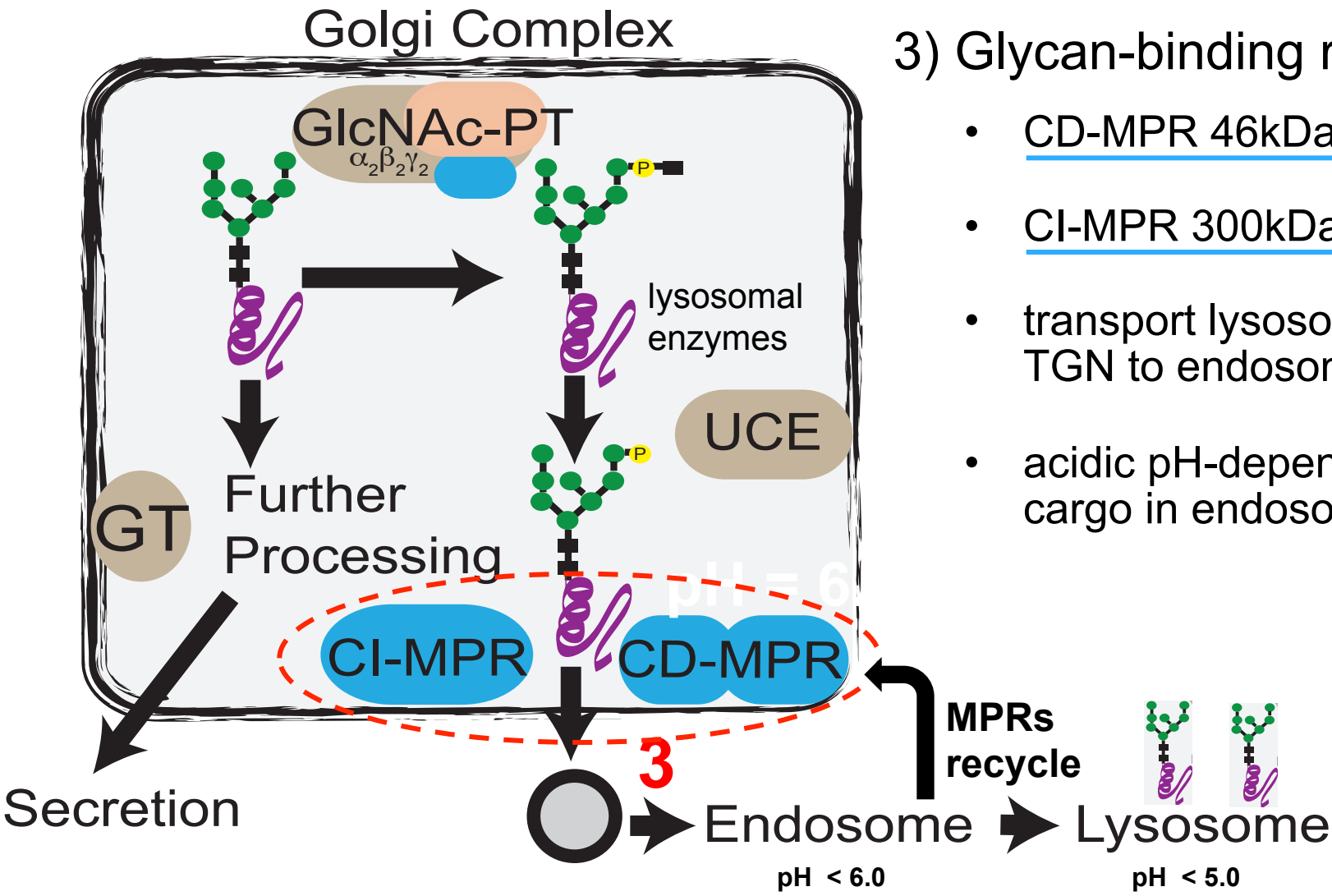


1) GlcNAc-1-phosphotransferase
   - *cis* Golgi
   - recognizes ~60 lysosomal enzymes via a conformation-dependent region
   - modifies mannose ➔ phosphodiester Man-6-P-GlcNAc

2) UCE = "uncovering" enzyme
   α-*N*-acetylglucosaminidase
   - Glycosidase *in trans* Golgi network
   - removes GlcNAc ➔ phosphomonoester Man-6-P



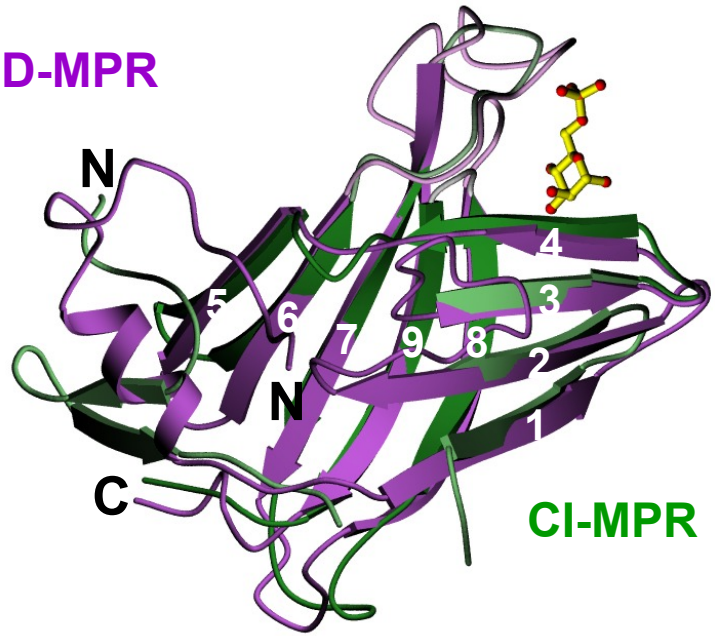
Delivery of lysosomal enzymes to lysosomes by Mannose 6-Phosphate Receptors (MPRs) – Glycan-binding receptors



3) Glycan-binding receptors/**P-type lectins**

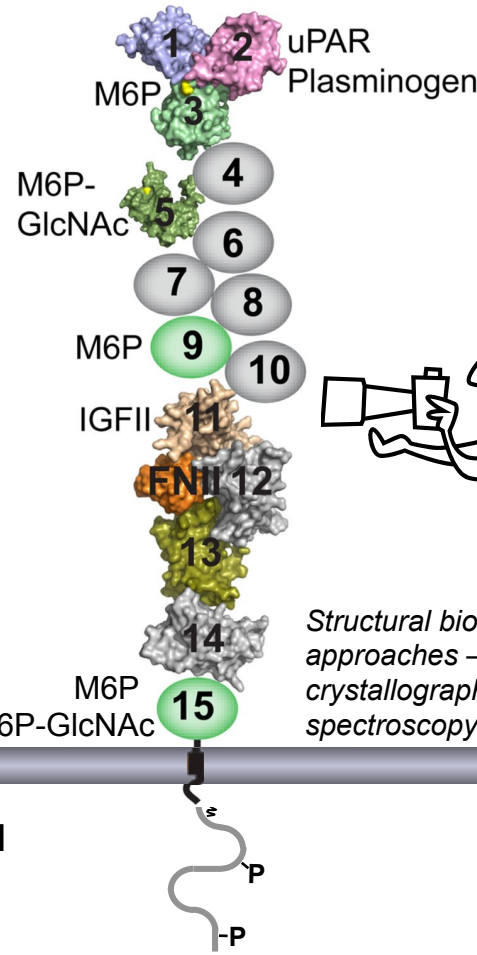
- CD-MPR 46kDa (cation-dependent)
- CI-MPR 300kDa (cation-independent)
- transport lysosomal enzymes from TGN to endosomes
- acidic pH-dependent release of cargo in endosomes

P-type Lectins “unusual”: bind with high affinity to Man-6-P

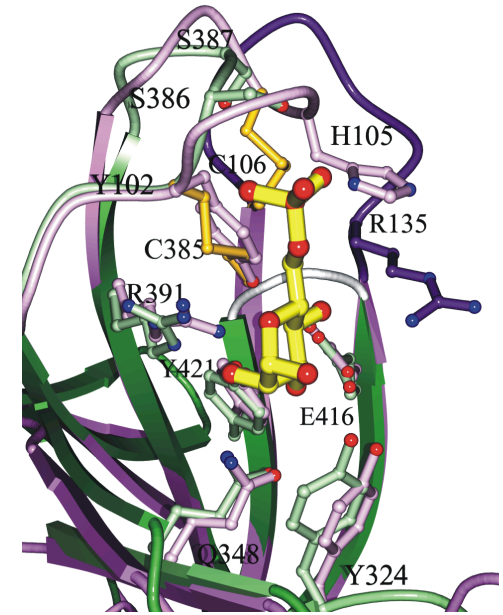


CD-MPR and CI-MPR 150-residue domains have a similar fold

Structures of 8 out of the 15 domains have been determined



Structural biology approaches – X-ray crystallography & NMR spectroscopy



Mannose ring

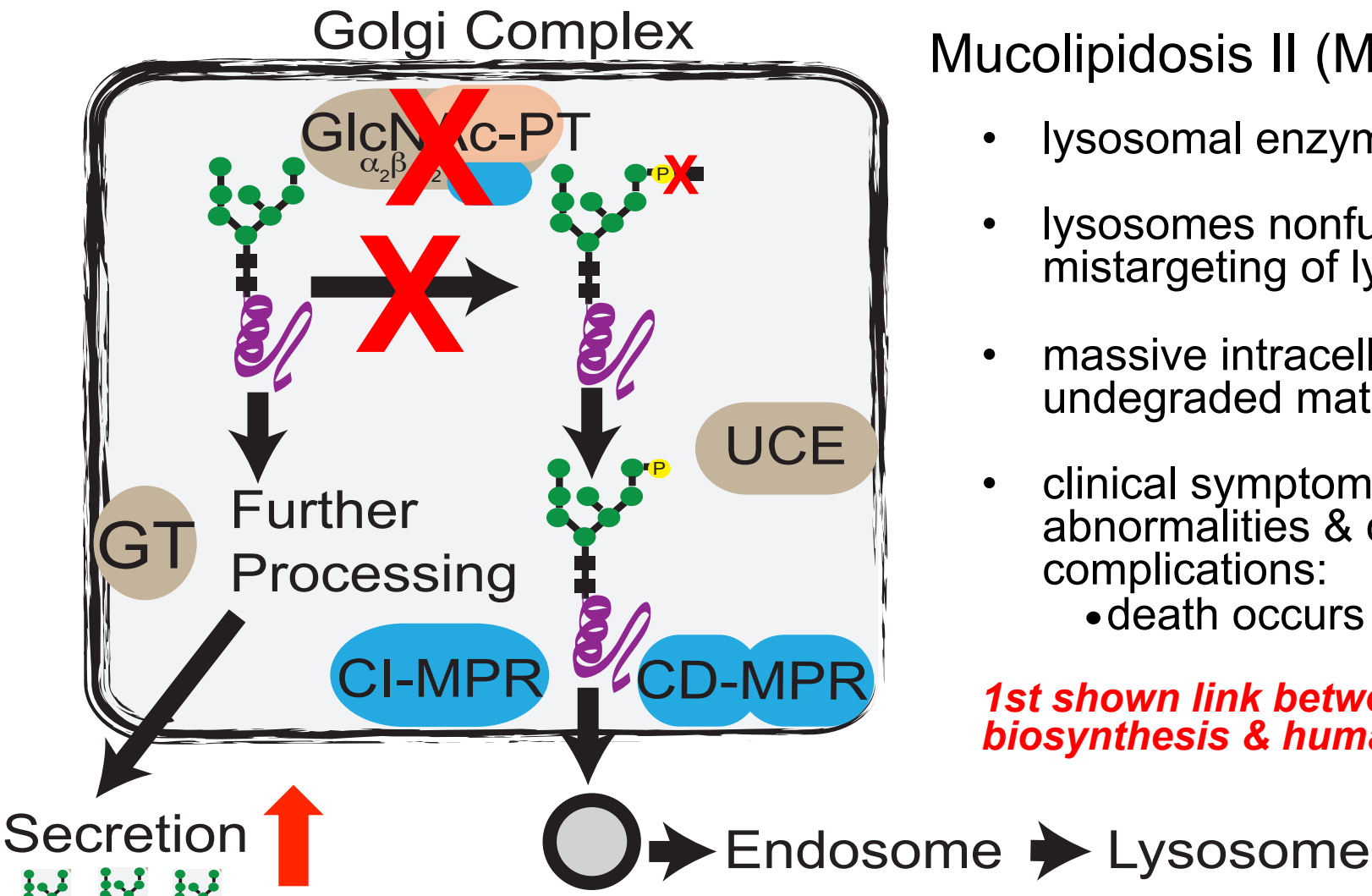
Conserved residues are located in identical positions within the binding pocket (Gln, Arg, Glu, Tyr)

“signature motif”

CI-MPR domains 11-14: Y Jones, EMBO J, 2008
CI-MPR domain 11: G Sheldrick, Acta Crystallogr D Biol Crystallogr, 2003

Roberts et al., Cell, 1998
Olson et al., EMBO J, 2004
Olson et al., PNAS, 2010
Kim, Olson, Dahms, Curr Opin Struct Biol, 2009

Deficiency of GlcNAc-1-phosphotransferase causes mucolipidosis II (MLII), a lysosomal storage disease

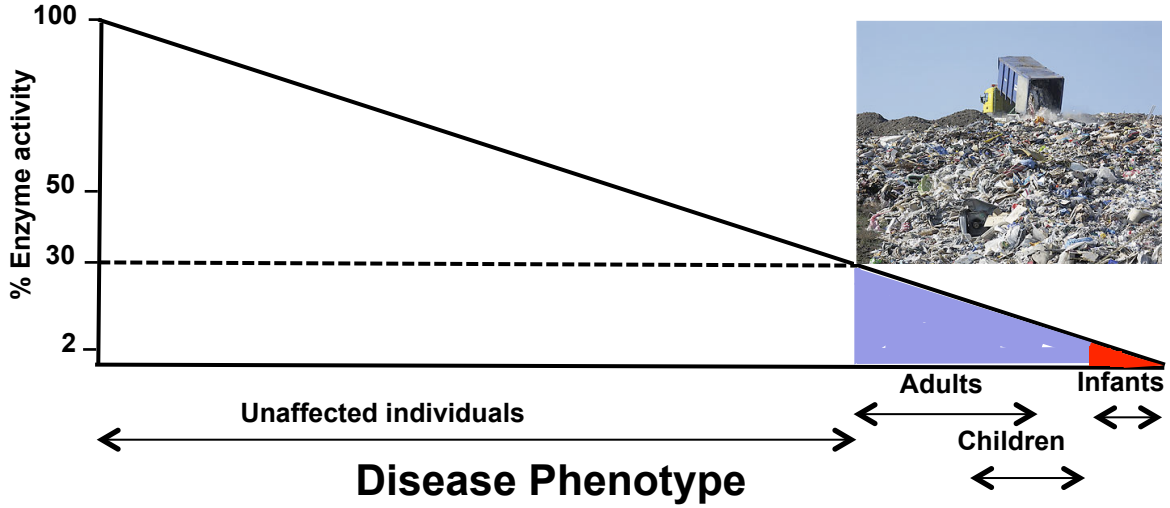


Mucolipidosis II (MLII)

- lysosomal enzymes lack Man-6-P tag
- lysosomes nonfunctional due to mistargeting of lysosomal enzymes
- massive intracellular storage of undegraded material
- clinical symptoms include skeletal abnormalities & cardiorespiratory complications:
  - death occurs by 10 years of age

1st shown link between glycoprotein biosynthesis & human disease

Lysosomal Storage Diseases (LSDs) – family of progressive, degenerative disorders with multi-organ involvement



Intracellular accumulation of undegraded material

↓

Skeletal deformities, mental retardation, heart disease, decreased life expectancies are common features.

Lysosome contains ~60 different hydrolytic enzymes

- Most caused by a deficiency of a single hydrolytic enzyme (glycosidase)
  ~50 different diseases (Fabry, Hurler, Pompe)
- Inheritance:
  - *Autosomal recessive* *(two copies of an abnormal gene must be present in order for the disease or trait to develop; both carrier, but non-infected, parents have to pass on their defective gene to progeny; 1 in 4 chance of happening)*
  - *X-linked* *(males have only one X chromosome … defective gene on this chromosome (one copy) will induce disease … e.g., Fabry disease)*
- Prevalence: individually rare, altogether incidence is ~1 in 6,000 live births (as a group they are among the most common genetic disorders in children)
- Heterogeneous:
  - ✓ Disease severity/age of onset is inversely proportional to residual enzyme activity
  - ✓ Siblings with same mutation can experience different symptoms

Glycosylation & Disease: Lysosomal Storage Diseases

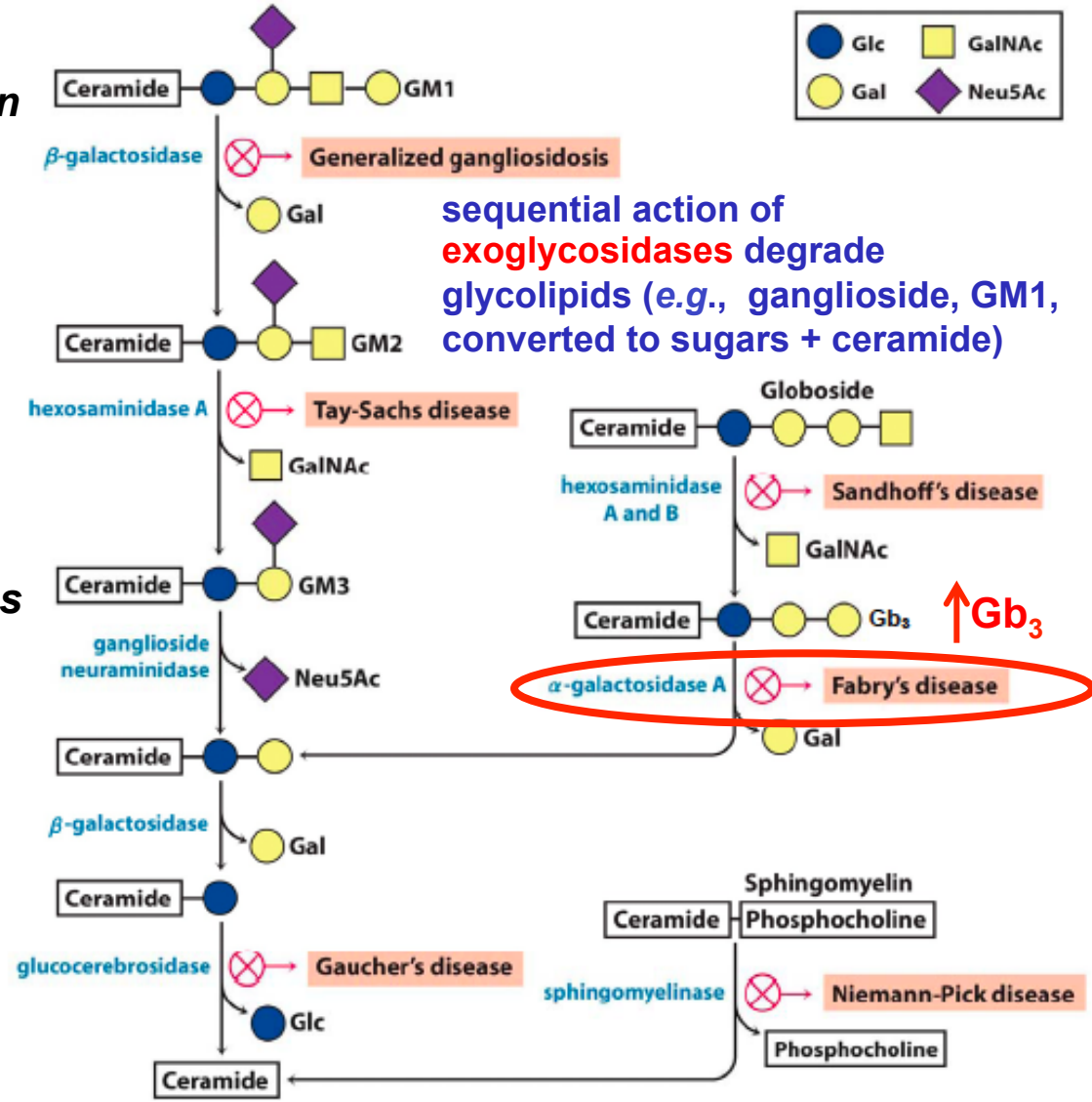
- ❑ *~ 50 different lysosomal storage diseases*
- ❑ *Grouped according to the substrate that accumulates:*
  - ✓ *Glycosaminoglycans - Mucopolysaccharidoses*
  - ✓ *Glycosphingolipids - Sphingolipidoses*
  - ✓ *Lipids – Lipidoses*
  - ✓ *Glycoproteins*
  - ✓ *Enzyme localization - Mucolipidosis*

Disorder	Enzyme Deficiency	Storage Product(s)	Major Organs Involved
Mucopolysaccharidoses			
MPS / Hurler and Scheie syndromes	α-iduronidase	Dermatan sulfate, heparan sulfate	Central nervous system, connective tissue, heart, skeleton, cornea
MPSII Hunter syndrome	Iduronate sulfatase	Dermatan sulfate, heparan sulfate	Central nervous system, connective tissue, heart, skeleton, cornea
Sphingolipidoses			
GM, gangliosidosis	β-Galactosidase	GM, ganglioside, oligosaccharides	Central nervous system, skeleton, viscera
Krabbe's disease	Galactosylcerebroside β-Galactosidase	Galactosylsphingosine	Central nervous system
Tay-Sachs disease	Hexosaminidase A	GM_2, ganglioside	Central nervous system
Fabry disease	α-Galactosidase A	Gb_3, globotriaosylceramide	Kidney, heart, cornea
Lipidoses			
Wolman's disease	Acid lipase	Triglycerides, cholesteryl esters	Liver, spleen, adrenal
Cholesteryl ester storage disease	Acid lipase	Triglycerides, cholesteryl esters	Liver, spleen, heart
Disorders of Glycoprotein Degradation			
Fucosidosis	α-Fucosidosis	Fragments of glycoproteins and glycolipids	Central nervous system
Mannosidosis	α-Mannosidase	Fragments of glycoproteins	Central nervous system, skeleton, liver, spleen
Sialidosis (mucolipidosis I)	Oligosaccharide neuramindase	Fragments of glycoproteins	Central nervous system, skeleton, liver, spleen
Disorders of Enzyme Localization			
Mucolipidosis II (I-cell disease)	*N*-Acetylglucosaminyl-phosphotransferase	Mucopolysaccharidoses, lipids, glycoproteins	Central nervous system, connective tissue, skeleton, heart
Mucolipidosis III (Pseudo-Hurler polydystrophy	*N*-Acetylglucosaminyl-phosphotransferase	Mucopolysaccharidoses, lipids, glycoproteins	Joint and connective tissue problems predominantly

http://www.lysosomaldiseasenetwork.org/official-list-lysosomal-diseases/

Sphingolipidoses (Lysosomal Storage Disease)

- ***Degradation occurs in lysosomes by the action of hydrolytic enzymes, exoglycosidases, from non-reducing end of glycan.***
- ***Genetic mutation causes decrease or absent activity of hydrolytic lysosomal enzyme.***
- ***Substrate accumulates upstream of the catabolic block.***
- ***Clinical complications are related to the organs where accumulation of undegraded substrate occurs in large amounts.***
  - ✓ *Gangliosides abundant in neurons, gangliosidoses predominantly neurological disorders*
  - ✓ *GAGs abundant in cartilage, MPS predominantly skeletal disorders*



Box 10-1 figure 1
Lehninger Principles of Biochemistry, Seventh Edition

Fabry Disease (X-linked) is the Most Common Lysosomal Storage Disease

Australia: 1 in 117,000

Meikle et al. *JAMA*. 1999

Recent newborn screening

Italy: 1 in 3,100

Spada et al. *Am J Hum Genet*. 2006

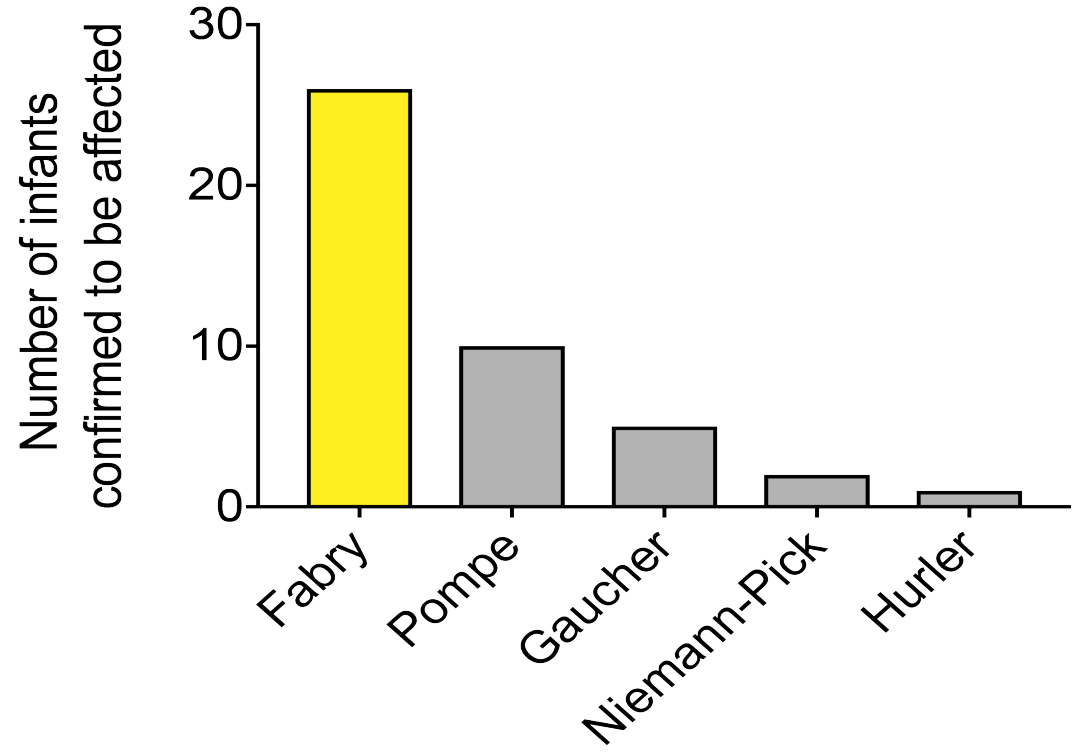
Washington: 1 in 5,495

Elliott et al. *Mol Genet Metab*. 2016

Illinois: 1 in 8,454

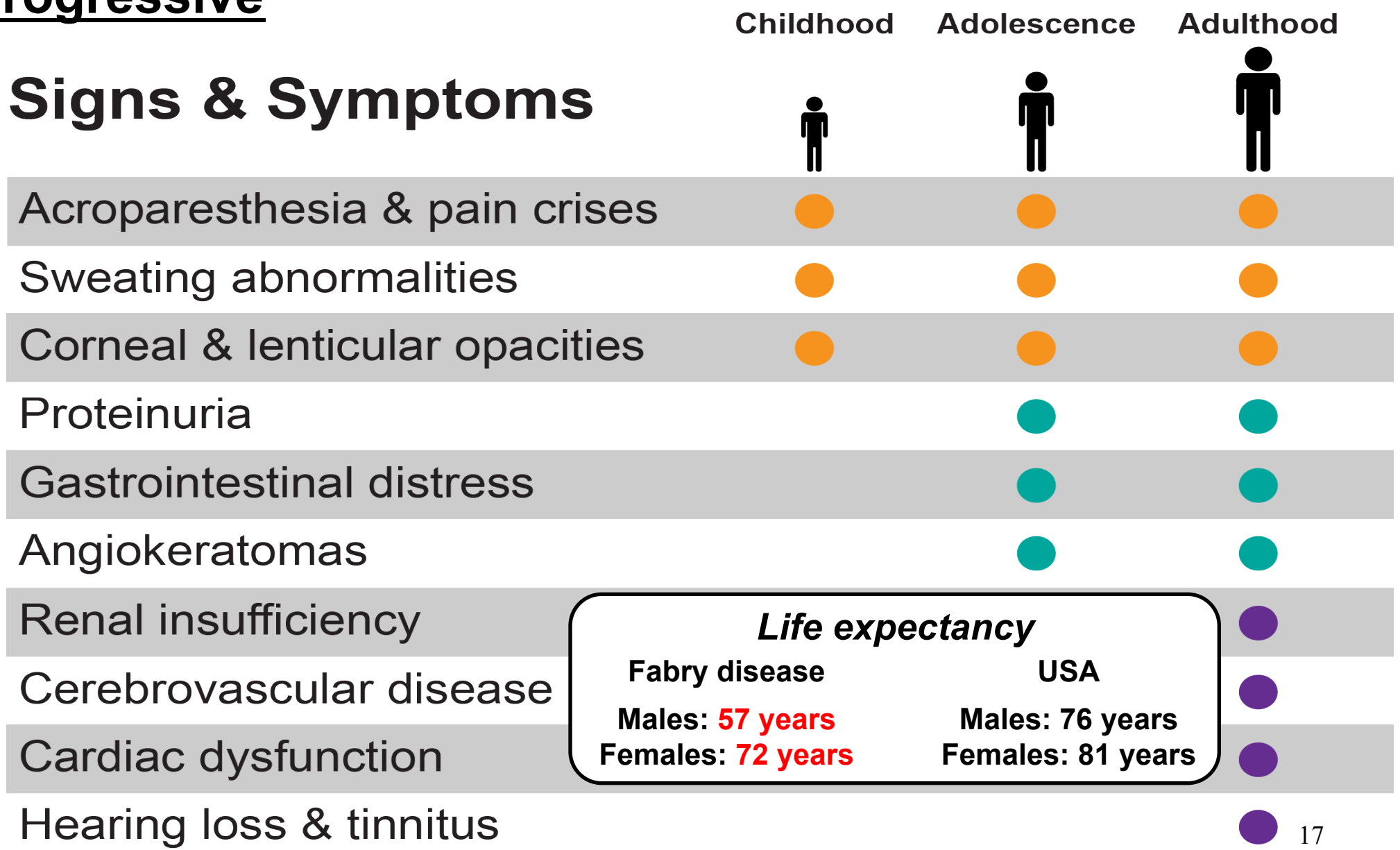
Burton et al. *J Pediatr*. 2017

Illinois incidence of five lysosomal storage diseases
(n = 219,793 infants screened)

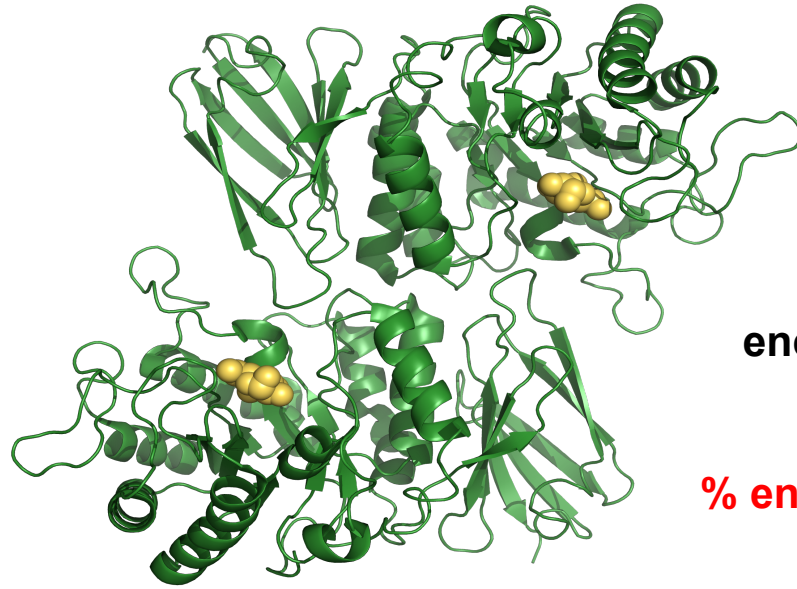


Adapted from Burton et al. *J Pediatr.* 2017

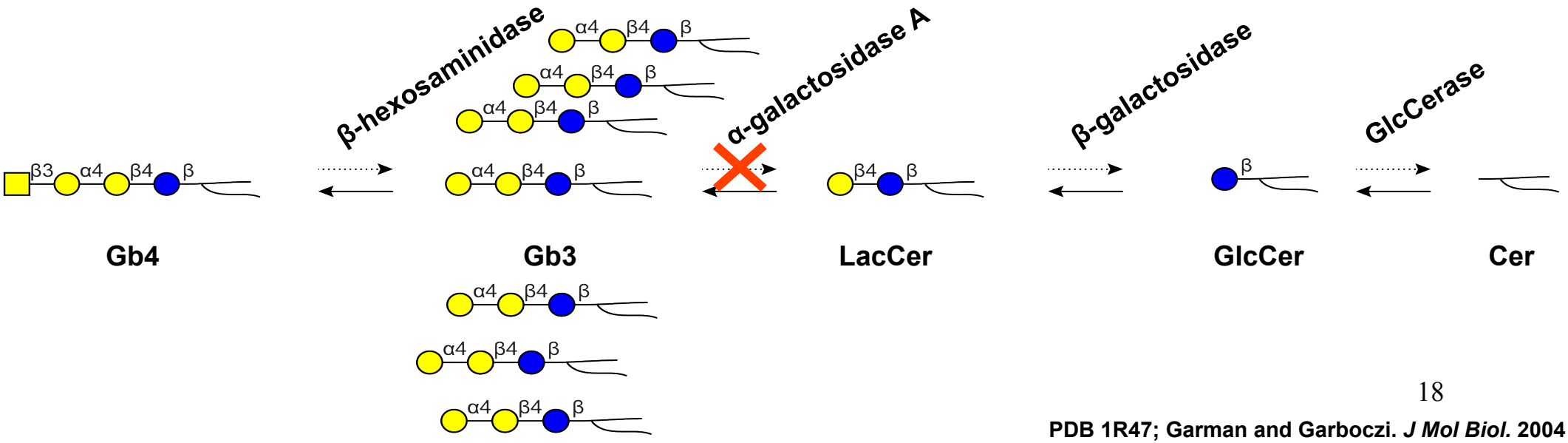
Fabry Disease Affects Multiple Organ Systems and is Progressive



A-Galactosidase A Deficiency causes Accumulation of Gb3



α-galactosidase A
encoded by *GLA gene on* X-chromosome
>700 mutations
% enzyme activity range 0-100%



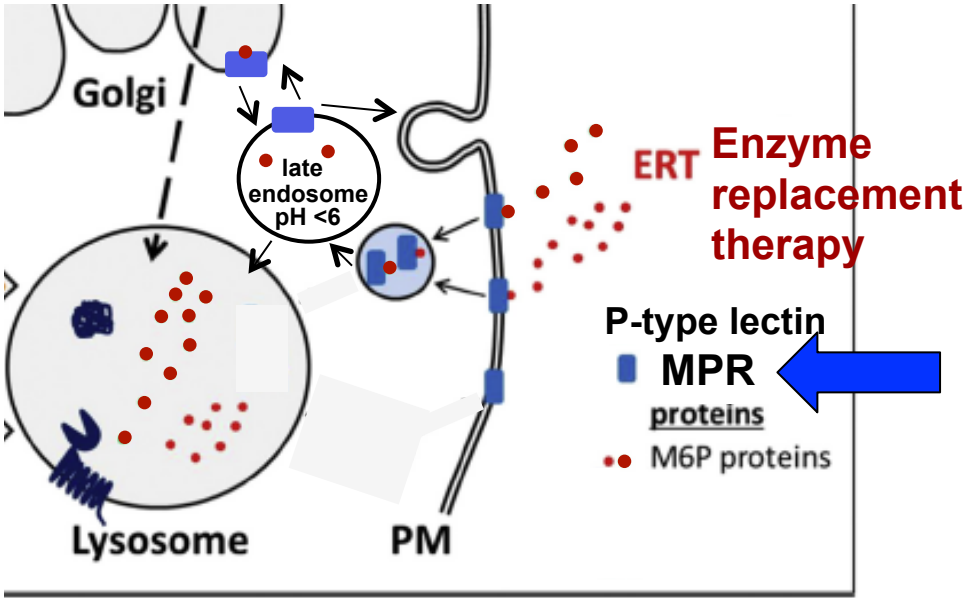
PDB 1R47; Garman and Garboczi. *J Mol Biol.* 2004

FDA-approved Treatments are Limited to a Few Lysosomal Storage Diseases

- ***Enzyme Replacement Therapy (ERT)***
  - ***Frequent treatment of recombinant enzyme (protein is given weekly, bi-weekly)***
  - ***Does not cross blood-brain barrier***
  - ***Not a cure, may slow symptom development***
  - ***Immune reactions often development, neutralizing the recombinant infused enzyme***

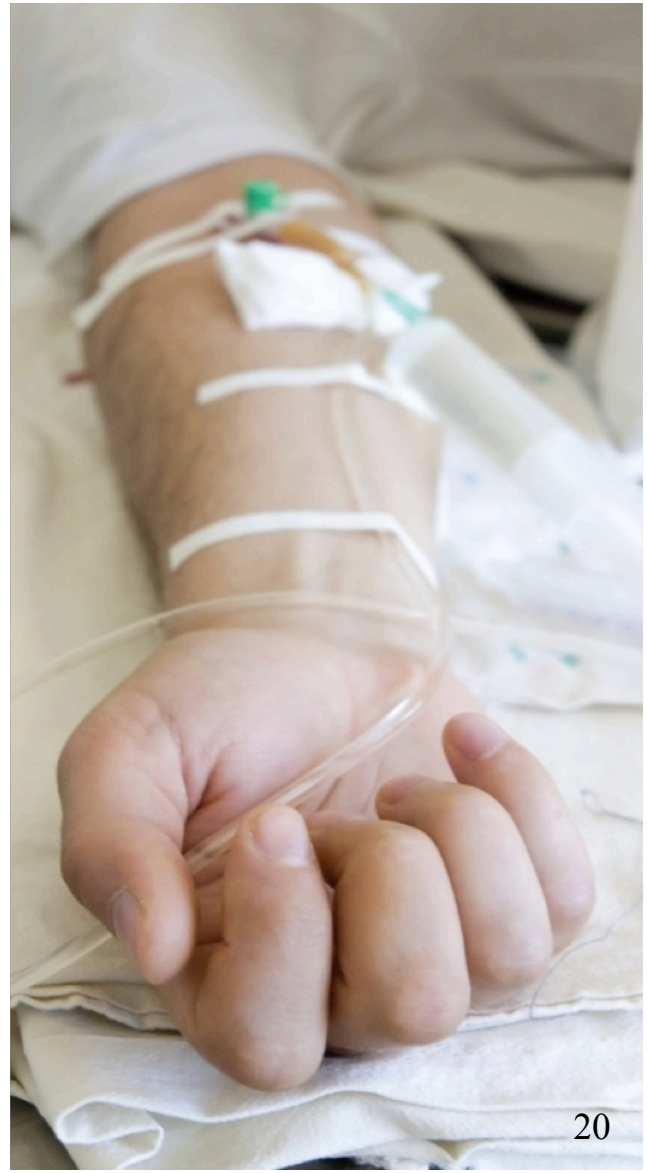
- ***Hematopoietic Stem Cell Transplantation (HSCT)***
  - ***Stem cell and bone marrow transplantation … possible resolution***
  - ***Stem cells can cross blood-brain barrier … but significant risks remain***
  - ***Stem cells have been used to some extent in Krabbe disease, MPS I, VI and VII***

FDA-approved enzyme replacement therapy (ERT) available for only a few (9) lysosomal storage diseases



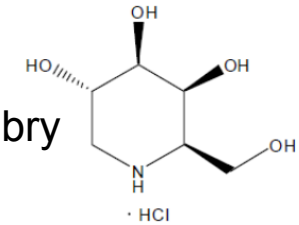
IV infusion every 2 weeks of recombinant Man-6-P-tagged enzyme: internalized by cell surface mannose 6-phosphate receptor (MPR)

[note: annual cost to patient $250,000 - $400,000]



Preclinical & Clinical Trials Underway for Numerous Lysosomal Storage Diseases:

- Improve efficacy of enzyme replacement therapy
- Pharmacological chaperone therapy
  - oral once every other day migalastat (Galafold, an iminosugar) for Fabry disease, FDA approved in 2018; stabilizes mutant enzyme



- Substrate reduction therapy
- Gene therapy
- mRNA therapy
- Gene editing zinc finger nuclease gene editing approach:
  - 8 Hunter patients [MPSII, express in liver cells IDS GAGs build-up]
  - 3 Hurler patients [MPSI, express in liver cells IDUA, GAGs build-up]; 1st patient treated in 2017

Much interest by biopharma industry in developing treatment

- *… 5 year exclusivity guaranteed by the FDA*
- *… crippling diseases*
- *… treatment using biologicals, e.g., enzyme, that can be more easily developed in comparison to traditional development of drugs*
- *… biogenerics (biosimilars) difficult to produce*
- *… apparently justifiable high cost of treatment (~$5,000 - $100,000 per dose)*

List of FDA-approved Treatments

- **Enzyme Replacement Therapy (ERT)**

Gaucher disease	1st ERT 1991 developed by Rosco Brady at NIH
	imiglucerase (Cerezyme)
	velaglucerase alfa (VPRIV)
	taliglucerase alfa (Elelyso)
Fabry disease	agalsidase beta (Fabrazyme)
Lysosomal acid lipase deficiency	sebelipase alfa (Kanuma)
MPSI / Hurler	laronidase (Aldurazyme)
MPSII / Hunter	ldursulfase (Elaprase)
MPSIVA / Morquio	elosulfase alfa (VIMIZIM)
MPSVI	galsulfase (Naglazyme)
MPSVII / Sly	vestronidase alfa (Mepsevii)
Pompe disease	alglucosidase alfa (Lumizyme)

- **Substrate Reduction Therapy (oral)**

Gaucher disease	eliglustat (Cerdelga) & miglustat (Zavesca)

- **Chaperone Therapy (oral)**

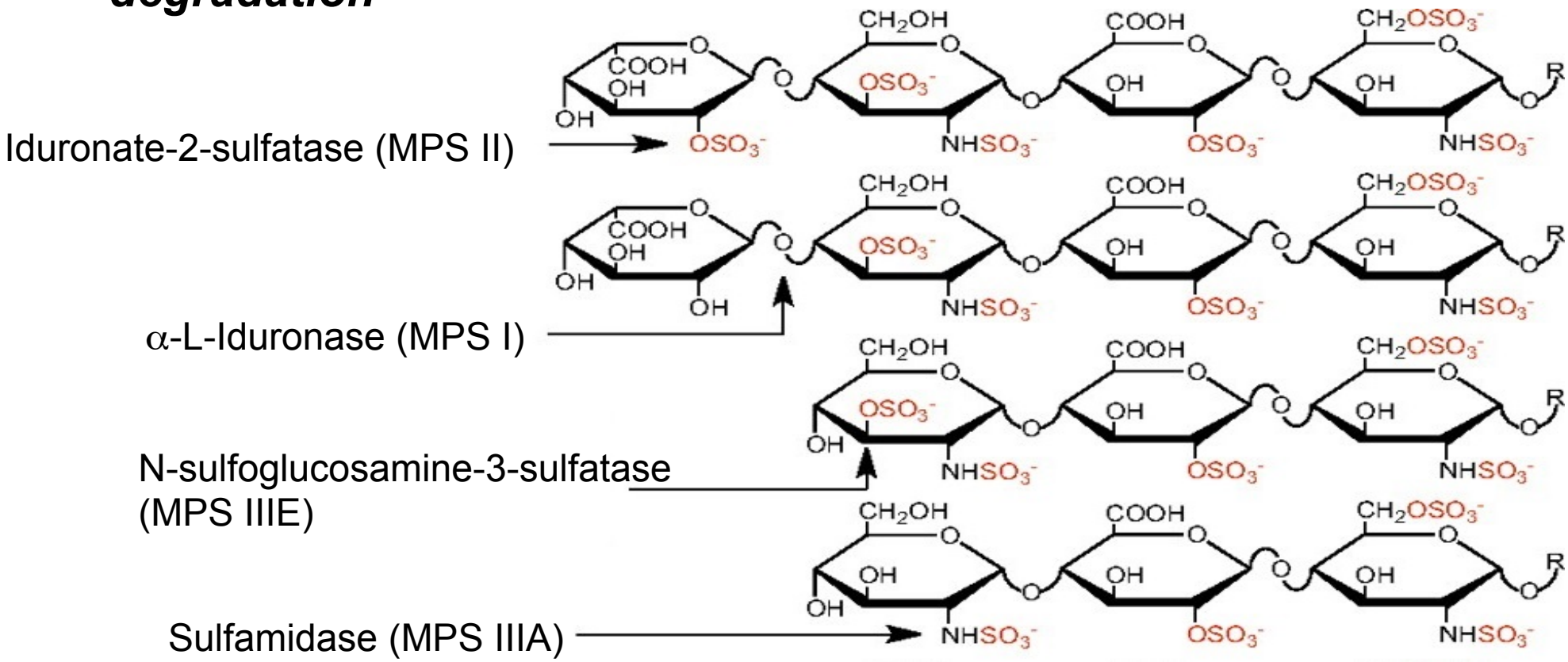
Fabry disease	migalastat (Galafold)

- **Storage Reduction Therapy (oral)**

Cystinosis	cysteamine (Cystagon)

Mucopolysaccharidoses (MPS)

- ***Enzymes involved in heparan/dermatan sulfate degradation***



Mucopolysaccharidoses (MPS)

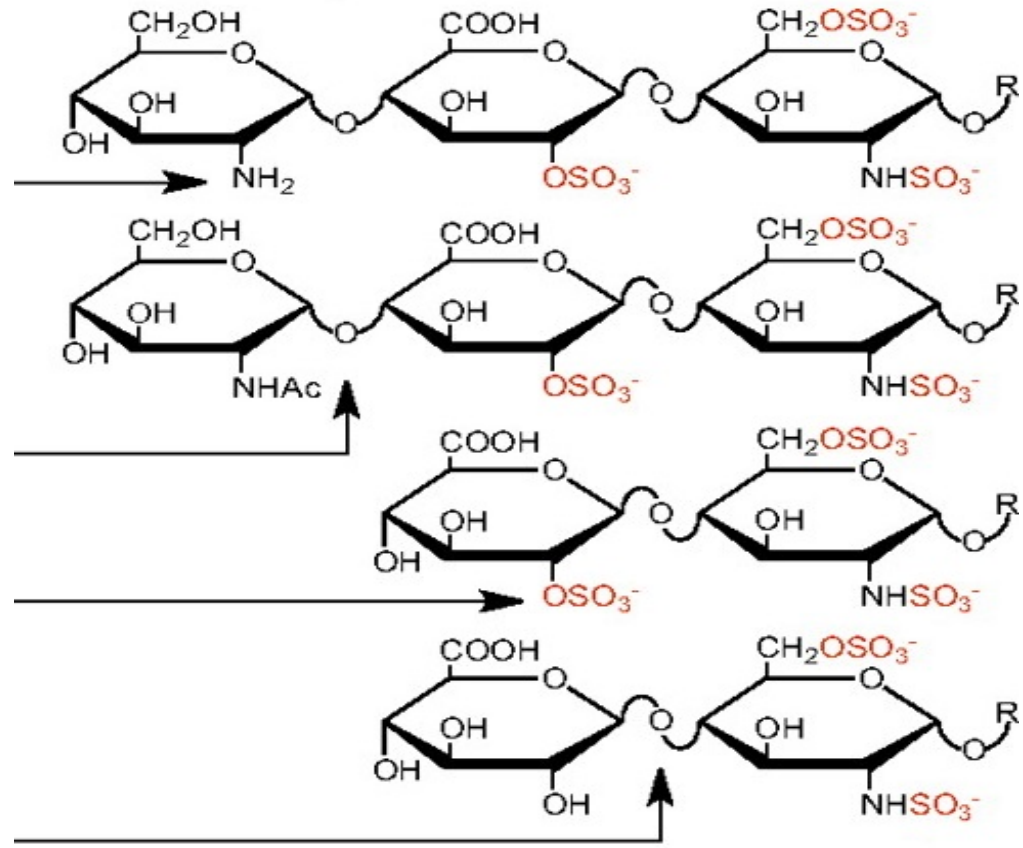
- ***Enzymes involved in heparan/dermatan sulfate degradation***

Glucosamine-acetyltransferase (MPS IIIC)

α-N-acetyl glucosaminidase (MPS IIIB)

Glucuronate-2-sulfatase

β-D-glucuronidase (MPS VII)



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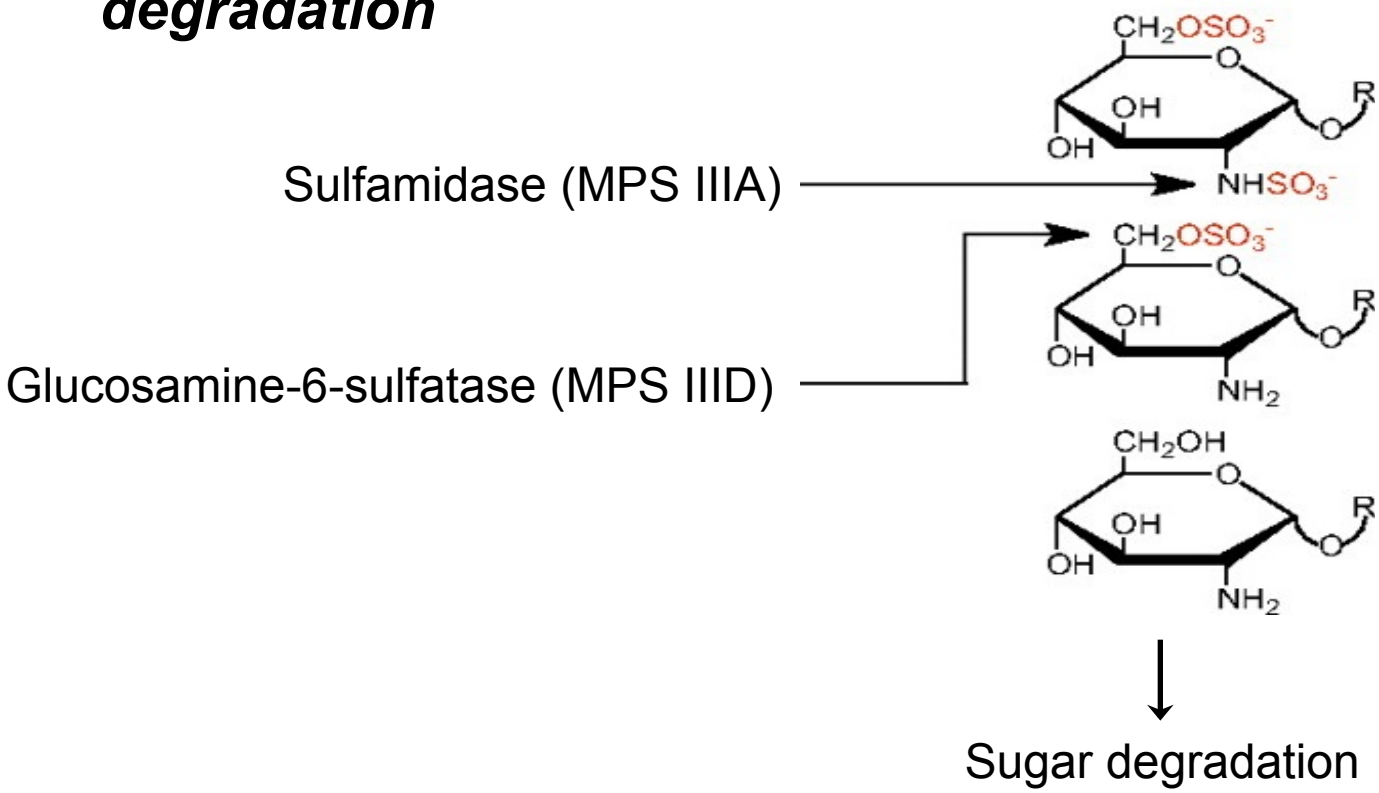
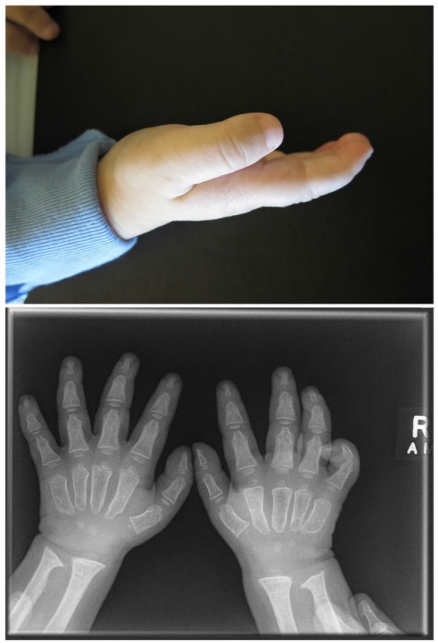
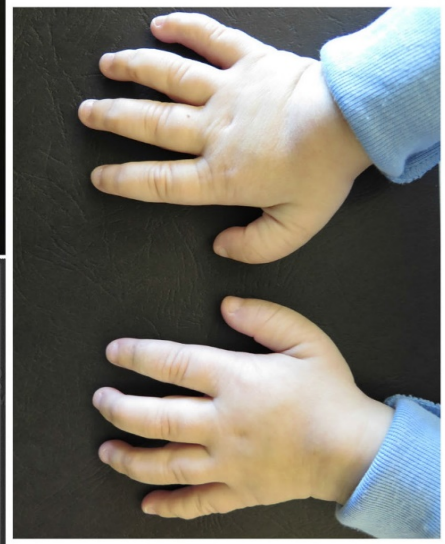
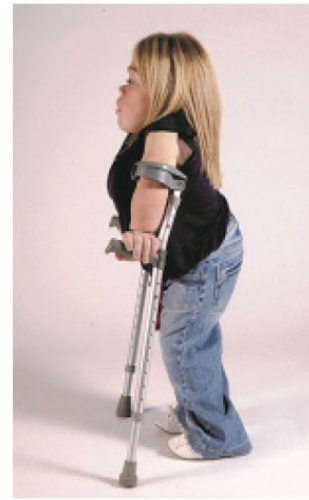


Clinical Manifestations

- ***MPS***
  - ✓ *Musculoskeletal … joint stiffness, reduced range of movement, progressive claw-like appearance … carpal tunnel syndrome … nocturnal wrist pain; degenerative joint disease … short stature*
  - ✓ *Extra-skeletal … coarse facies with thickened skin*
  - ✓ *Cognitive impairment … developmental effects … regression*
  - ✓ *Cardiomyopathy … coronary artery disease, lung infiltration …*







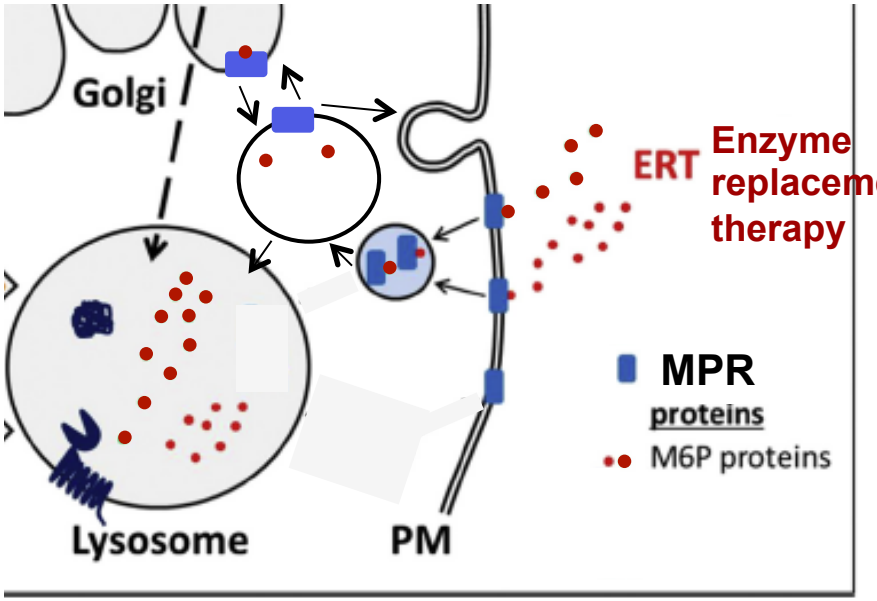
James et al. *J. Paediatr. Child Health* 52 (2016) 262 – 271; Mitchell et al. *Eur. J. Internal Med.* 34 (2016) 2 – 10.

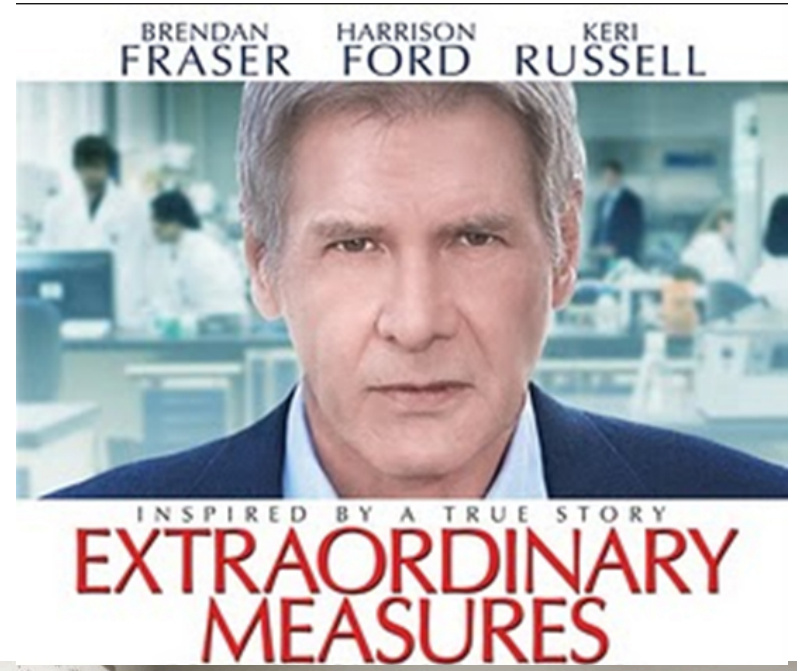
Mucopolysaccharidoses (MPS)

- ***Summary***

MPS I	**α-*L*-Iduronidase**	**coarse facial features, intellectual disability, ….**
MPS II	**Iduronate sulfatase**	**milder intellectual disability, …**
MPS III	**Sulfamidase, *N*-acetylglucosaminidase α-glucosamine-*N*-acetyltransferase *N*-acetylglucosamine-6-sulfatase**	**developmental delay, motor dysfunction, …**
MPS IV	**Galactose-6-sulfatase β-galatosidase**	**skeletal dysplasia, motor dysfunction,…**
MPS VI	***N*-acetylgalactosamine-4-sulfatase**	**skeletal dysplasia, heart defects**
MPS VII	**β-glucuronidase**	**short stature, developmental delay**
MPS IX	**Hyaluronidase**	**inflammed joints, pain, short stature**

Hollywood loves glycans





Harrison Ford explains enzyme replacement therapy to treat Pompe disease, a lysosomal storage disease

Glycogen accumulation in muscle (heart, diaphragm), patients on ventilator, death prior to age 20 without ERT

Additional details of MPS I, MPS II, & MPS IVA are on the following slides

MPS I

- ***The Disease***
  - ✓ *Hurler disorder (severe); Hurler – Scheie disorder (intermediate); Scheie disorder (attenuated)**
  - ✓ *Symptoms of Hurler … mental retardation, hydrocephalus, physical disability, deformity, clawed hands, sleep apnea, respiratory insufficiency, cardiac disease, corneal clouding, deafness, short stature, coarse facial features, fatigue …. death by 10 years*
  - ✓ *Symptoms of Hurler-Scheie … later onset of disease; death in teenage yrs; … rest of symptoms similar but less severe*
  - ✓ *Symptoms of Scheie … even later onset of disease; life span normal to slightly reduced; … much reduced symptoms*
  - ✓ *ERT proposed in 1964 by De Duve*

*Wraith et al. BioDrugs 2005; 19:1-7

Laronidase (Aldurazyme™)

- ***Enzyme Therapy … α-L-iduronase (Laronidase)***
  - ✓ *…. is a recombinant version of the human enzyme that was approved by the FDA in 2003*
  - ✓ *…. 83 kDa …. produced in CHO cells*
  - ✓ *…. only one randomized clinical trial so far (2013), which did not include many patients … shows improved ability to walk, reduced excretion of abnormal GAGs in urine and reduced cessation of breathing during sleep (see Cochrane Data. System. Rev. 2013; CD009354)*
  - ✓ *…. administered at 0.58 mg/kg/wk as IV infusion*
  - ✓ *…. mean half-life in the range of 1.5 to 3.6 hr*
  - ✓ *…. most patients develop antibodies by week 12 … routinely pre-medicated with anti-histamines and anti-pyretics before infusion*
  - ✓ *…. side effects include vomiting, nausea, arthralgia, tachycardia, abdominal pain, hypertension, erythema, …*
  - ✓ *…. has man-6-phosphate on glycans of the enzyme that help its uptake into lysosomes*
  - ✓ *…. two glutamic acids separated by a distance of 5.5 A organize around the scissile interglycosidic bond for optimal hydrolysis (see Curr. Opin. Struct. Biol. 1994; 4:885-892; Structure 1995; 3:853-859)*
  - ✓ *…. catalytic mechanism involves nucleophilic attack followed by hydrolysis*

MPS II

- ***The Disease***
  - ✓ *Hunter disorder*
  - ✓ *Some individuals have a more severe form of disease*
  - ✓ *Symptoms of Hurler … neurologic involvement, severe airways obstruction, skeletal deformities, cardiomyopathy, …. death by 10 - 30 yrs*
  - ✓ *Estimated incidence of 1 in 162,000 live births*

*da Silva et al. Cochrane Data. System. Rev. 2016; CD008185

Idursulfase (Elaprase™)

- ***Enzyme Therapy … iduronate-2-sulfatase (Idursulfase)***
  - ✓ *…. is a recombinant version of the human enzyme that was approved by the FDA in 2006 … costs $567,412 per year per patient*
  - ✓ *…. produced in a human cell line (HEK most probably)*
  - ✓ *…. administered at 0.5 mg/kg/wk as IV infusion*
  - ✓ *…. only one randomized clinical trial so far, which included 96 patients followed for 53 weeks … shows improved ability to walk for 6 min*
  - ✓ *.... generally well tolerated … most frequent serious adverse reactions were hypoxic episodes …. Other reactions include cardiac arrhythmia, pulmonary embolism, cyanosis, respiratory failure, infection, arthralgia, headache, pruritus, musculoskeletal pain, urticaria, diarrhea, cough, ….*
  - ✓ *…. a particular Cys (C84) is post-translationally modified to C-α-formylglycine for optimal hydrolysis of IdoA2S (see Biochem J 326(Pt 1), 243–247 (1997); Biochem J 386(Pt 2), 395–400 (2005))*
  - ✓ *…. multiple N-glycosylation sites … all occupied but may not be critical for activity*
  - ✓ *… sialic acid groups present … may mask immunogenicity*
  - ✓ *…. has man-6-phosphate on glycans of the enzyme that help its uptake into lysosomes*
  - ✓ *…. ~32% patients develop antibodies … results in smaller reduction in the levels of GAGs in urine*

MPS IVA

- ***The Disease***
  - ✓ *Morquio A syndrome*
  - ✓ *Continuum of severity … have a more severe form of disease*
  - ✓ *Symptoms … skeletal deformities, shortness of stature, bony changes in arms, …. Reach adulthood …*
  - ✓ *May not exhibit cognitive decline … major difference from other forms of MPS*
  - ✓ *Estimated incidence of 1 in 71,000 to 1 in 1,179,000 live births*

*Regier and Tanpaiboon, *Appl. Clin. Genet.* 2016; 9:67–74.

Elosulfase alfa (Vimizim™)

- ***Enzyme Therapy … N-acetylgalactosamine-6-sulfatase (Elosulfase alfa)***
  - ✓ *…. is a recombinant version of the human enzyme that was approved by the FDA in 2014 … costs $380,000 per year per patient*
  - ✓ *…. produced in CHO cells*
  - ✓ *…. administered at 2.0 mg/kg/wk as IV infusion*
  - ✓ *…. several clinical trials …. one trial included 176 patients followed for 24 weeks … shows improvements in 6-min walk test, 3-min stair climb test and respiratory function*
  - ✓ *…. generally well tolerated*
  - ✓ *.... most frequent serious adverse …. anaphylaxis (cough, erythema, throat tightness, urticarial, hypotension, dyspnea, GI issues) ….*
  - ✓ *…. a particular Cys (C79) is post-translationally modified to C-α-formylglycine for optimal hydrolysis of GalN6S*
  - ✓ *…. multiple N-glycosylation sites*
  - ✓ *… sialic acid groups were not detected by the European Medicines Agencies*
  - ✓ *…. has man-6-phosphate on glycans of the enzyme that help its uptake into lysosomes*
  - ✓ *… by week 16, ~96% of patients developed neutralizing antibodies that inhibit the drug from binding the mannose-6-phosphate receptor on cells*