Engineered GlcNAc-1-Phosphotransferase (S1S3 PTase) Dramatically Alters Glycosylation of Lysosomal Enzymes Leading to Enhanced Phosphorylation for Improved CI-MPR Binding

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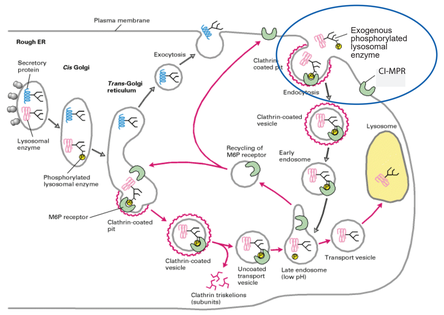
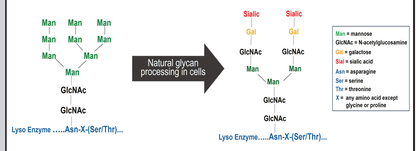
Natural M6PR Pathway Enables Exogenous Phosphorylated Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Storage Diseases



Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 17.7

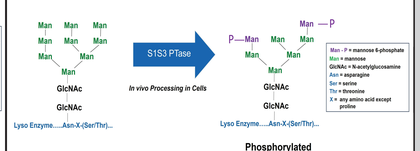
Most N-glycans are Unphosphorylated and Processed to Complex-Type Structures During Over-Expression. S1S3 PTase Co-expression Ensures Production of Lysosomal Enzymes with High Levels of M6P

The Problem:



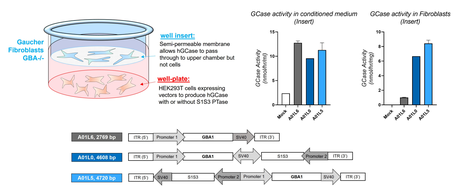
- Identical Man9 N-glycan structure is added to glycoproteins in endoplasmic reticulum during synthesis; N-glycan processing primarily occurs in Golgi
- Phosphorylation of recombinant lysosomal enzymes is highly inefficient in cells leading to mostly complex-type structures
- Complex-type N-glycans are typical of plasma proteins and do not enable cellular uptake or delivery of exogenous lysosomal enzymes to lysosomes
- No current technology can increase phosphorylation within cells

The Solution:

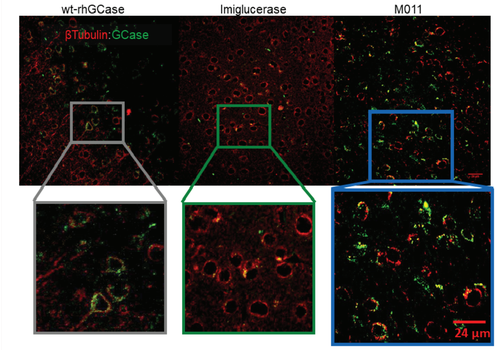


- Co-expression of S1S3 PTase with therapeutic protein produces lysosomal enzyme with high levels of M6P to enable much improved drug targeting
- Cation-independent M6P receptor (CI-MPR) is present on nearly all cells for cellular uptake of exogenous M6P-bearing lysosomal enzymes

M6P Enables Efficient Cellular Uptake of hGCase for Cross-Correction of Gaucher Fibroblasts



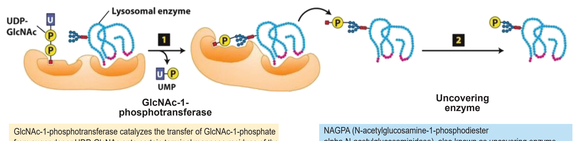
Phosphorylation Enables Signficantly Better Cellular Uptake of M011 in Neurons than Imiglucerase after ICM Injection



Study Details

Animal: $Gba^{D409V/D409V}$ Mouse
Age: 8 weeks
Dose: 140 mg
Dose route: ICM
Dose Volume: 10 uL
EOL: 24 hrs post dose

Phosphorylation of Lysosomal Enzymes is Mediated by GlcNAc-1-Phosphotransferase that is Inherently Inefficient in Cells



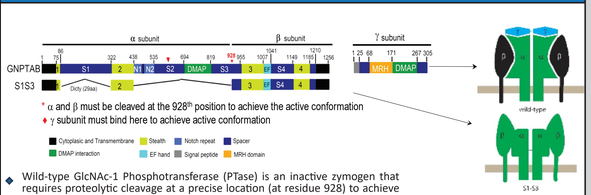
GlcNAc-1-phosphotransferase catalyzes the transfer of GlcNAc-1-phosphate from sugar donor UDP-GlcNAc onto certain terminal mannose residues of the N-linked oligosaccharides in cis-Golgi

NAGPA (N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase), also known as uncovering enzyme, removes covering GlcNAc group to reveal mannose 6-phosphate (M6P)

Section 17.7 Protein Glycosylation in the ER and Golgi Complex, Molecular Cell Biology, 8th edition, 2016

- GlcNAc-1-Phosphotransferase is a large and complex protein that is responsible for adding phosphate groups to mannose residues on N-linked glycans of newly synthesized lysosomal enzymes in Golgi to create mannose 6-phosphate (M6P)
- Phosphorylation of lysosomal enzymes is an inherently inefficient process in nature, it is further compromised when overexpressing lysosomal enzyme
- Increasing GlcNAc-1-Phosphotransferase activity within cells for producing therapeutic lysosomal enzymes with higher M6P content has not been achievable thus far with traditional ERT and gene therapy approaches

S1S3 Variant Has Key Attributes That Enable Its Use for Development of Best-In-Class Recombinant Enzymes and Gene Therapies

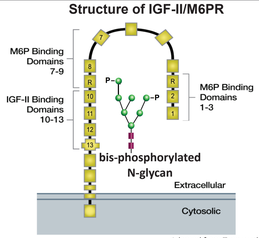


* α and β must be cleaved at the 928th position to achieve the active conformation
♦ γ subunit must bind here to achieve active conformation

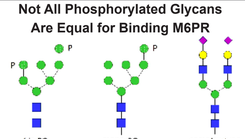
- Wild-type GlcNAc-1 Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at a precise location (at residue 928) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving phosphorylation
- S1S3 PTase is a truncated, pre-activated variant with significant advantages:
  - Has proper conformation without need for γ -subunit
  - Has 20-fold higher specific activity
  - Enables efficient phosphorylation of soluble lysosomal enzymes
  - Small gene size enables its incorporation into expression constructs for GTx

Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65

Structure of IGF-II/Cation-Independent M6P Receptor (IGF-II/CI-MPR) and Measured Binding Affinities of Carbohydrate Ligands



Adapted from Tong et al., 1989

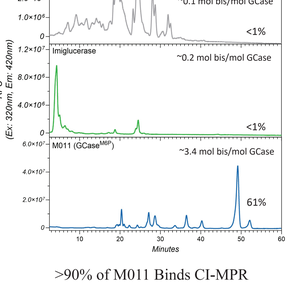


Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

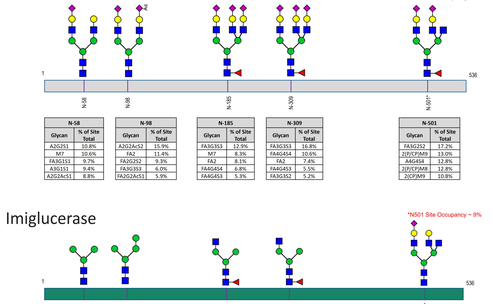
M011: $GCase^{M6P}$ (GCase Co-Expressed with S1S3 PTase) is an ERT Therapy with Increased Phosphorylation, Superior Receptor Binding and Higher CI-MPR Affinity

- β-Glucocerebrosidase (GCase) is a lysosomal enzyme that cleaves by hydrolysis the β-glycosidic linkage of the chemical glucocerebroside
- It is an ~60 kDa glycoprotein with 5-6 N-linked glycosylation sites (6th site engineered into M011 for added stability)
- Gaucher Disease is caused by reduced or absent GCase activity resulting in glucosylceramide accumulation in the liver, spleen, and macrophage lineage cells.

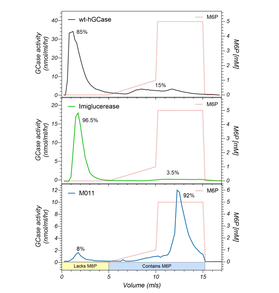
Improved Bis-PO_4 Glycan Content (2AB Oligosccharide Chromaotography)



Funadamental Change in GCase Glycosylation (LC-MS/MS Site Specific Glycosylation)



>90% of M011 Binds CI-MPR (CI-MPR Affinity Chromatography)

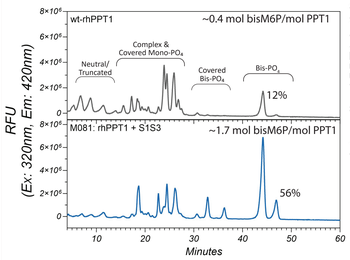


Same S1S3 PTase Approach Can be Utilized for Increasing M6P Levels on Vast Majority of Soluble Lysosomal Enzymes

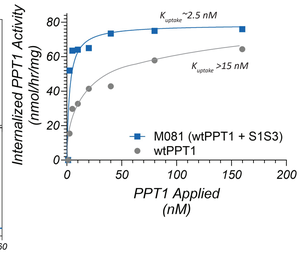
M081: Batten CLN1 ERT Development

- Palmitoyl-protein thioesterase 1 (PPT1) is a lysosomal enzyme that removes thioester-linked fatty acyl groups during catabolism of lipid-modified proteins
- It is a 32.7 kDa glycoprotein with 2 N-linked glycosylation sites
- Batten CLN1 is caused by diminished or missing PPT1 Activity in the CNS

Improved Bis-PO_4 Glycan Content (2AB Oligosccharide Chromatography)



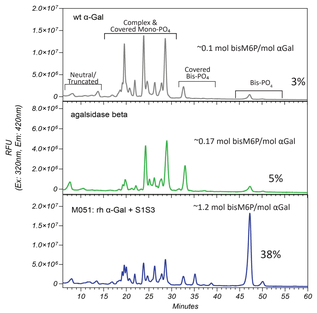
Improved Cellular Uptake (PPT1 KO Fibroblast)



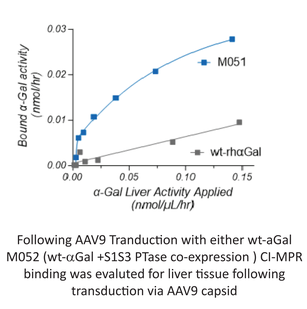
M052: Fabry GTx Development

- α-Galactosidase (α-Gal A) is a lysosomal enzyme that hydrolyses globotriaosylceramide (GL-3) in kidney and other cells
- α-Gal A is a 60 kDa glycoprotein homodimer with 3 N-linked glycosylation sites
- Fabry Disease is caused by reduced or absent α-Gal A activity resulting in GL3 accumulation in the kidney, heart and skin

Improved Bis-PO_4 Glycan Content (2AB Oligosccharide Chromatography)



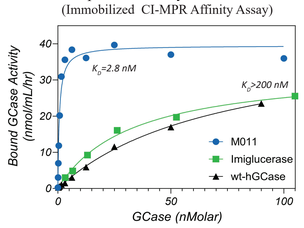
Improved CI-MPR Binding for M052 Over wt-αGal AAV9 from Tranduced Liver



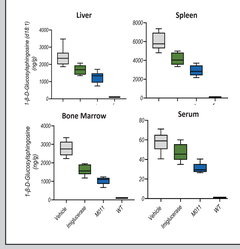
Following AAV9 Tranduction with either wt-aGal M052 (wt-αGal +S1S3 PTase co-expression) CI-MPR binding was evaluated for liver tissue following transduction via AAV9 capsid

Highly Phosphorylated M011 has a high CI-MPR Affinity

Improved Affinity for CI-MPR (Immobilized CI-MPR Affinity Assay)



M011 is Significantly Better than SOC for Reducing Accumulated Substrate



Study Details

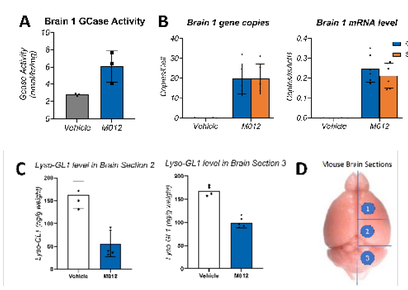
Animals
Strain: D409V/null Gaucher Mouse
Gender: Mixed
Age: 8-14 weeks
N: 8 per group – 4 male/4 female

Treatment
Age matched Wild-type
PBS
Dosed Weekly – 1.5 mg/kg
PBS
Imiglucerase
M011: $GCase^{M6P}$

EOL: 1 week following last dose

Analysis: GC content in serum and tissues

GBA^{D409V} mice treated with M012 AAV9 Enhances GCase Activity, Gene Expression and Substrate Reduction in Brain



AAV9 M012 treated GaucherD409V mice display enhanced GCase Activity (A), DNA copy and mRNA copy (B), and reduced Lyso-GL1 levels (C). (D) shows brain sections that were analyzed.

Conclusions

- CI-MPR is a receptor pathway that enables cellular uptake of exogenous lysosomal enzymes in nearly all cells and tissues including CNS neurons
- Phosphorylation is inherently inefficient for many recombinant lysosomal enzymes which limits in vivo efficacy for both ERTs and GTx
- Increasing GT dosage does not fix the lysosome targeting problem since protein overexpression exacerbates poor phosphorylation
- S1S3 PTase, a truncated highly active GlcNAc-1-phosphotransferase, is a major scientific breakthrough that overcomes this problem
- Co-expression of S1S3 PTase ensures that lysosomal enzymes are produced with high levels of bis-M6P resulting in more potent ERTs and GTs
- Highly phosphorylated lysosomal enzymes have superior CI-MPR binding and cellular uptake leading to better substrate clearance

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M6P Therapeutics
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