M161 (rhGALNS) with Enhanced Phosphorylation with S1S3 Phosphotransferase may Introduce a More Efficacious MPS IVA ERT

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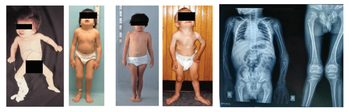
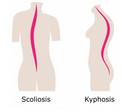
MPS IV A Disease

- Morquio Syndrome A, also known as Mucopolysaccharidosis Type IV A (MPS IV A) is a rare metabolic disorder in which the body cannot process keratan sulfate and chrondriotin-6-sulfate.
- MPS IV A is caused by a mutation in the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) leading to low or absent GALNS enzyme activity.
- In the severe disease form, symptoms typically appear between ages 1 and 3 and include knock-knees, scoliosis or kyphosis, short stature, heart and vision problems, enlarged liver and a reduced life span of 20-30 years.
- Incidence rate in the US is estimated at between 1 in 200,000-300,000 live births.
- Treatment addressing MPS IVA disease include:
  - Enzyme replacement therapy (ERT)
  - Hematopoietic stem cell transplantation (HSCT)
- Current ERT is ineffective in addressing clinical complications due to inefficient cellular uptake.
  - This is more reflected in avascular tissues, such as corneal, cartilage and heart valvular tissue





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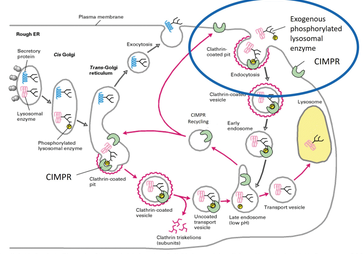
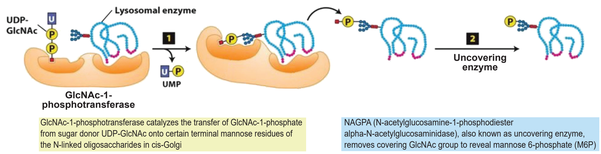
Natural M6P Receptor Pathway Enables Phosphorylated Exogenous Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Storage Diseases



- CI-MPR is a receptor pathway that enables cellular uptake of exogenous lysosomal enzymes in nearly all cells and tissues.
- Phosphorylation is inherently ineffecient for many recombinant lysosomeal enzymes which limits in vivo efficacy for both ERTs and GTx.
- Increasing GTx dosage does not fix the lysosomal targeting problem; recombinent protein over-expression exacerbates poor phosphorylation.

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

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

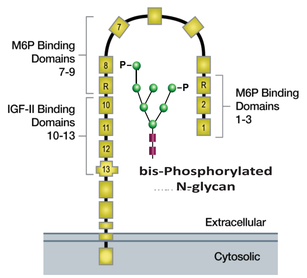


Section 17.7 Protein Glycosylation in the ER and Golgi Complex, Molecular Cell Biology, 8th edition, 2016

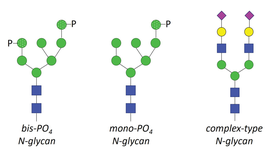
- GlcNAc-1-Phosphotransferase is a large and complex protein that is responsible for adding phosphate groups to mannose residues on N-linked glycans of newly synthesized lysosomal enzymes in Golgi to create mannose 6-phosphate (M6P)
- Phosphorylation of lysosomal enzymes is an inherently inefficient 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

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

Structure of IGF-II/M6PR



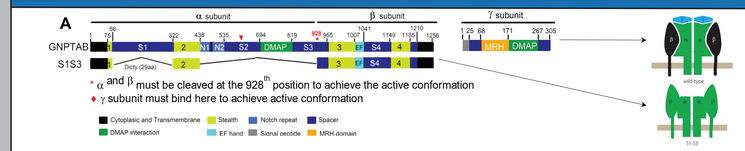
Not All Phosphorylated Glycans Are Equal for Binding M6PR



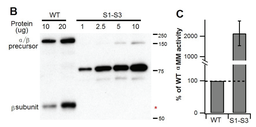
Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

Adapted from Tong et al., 1989

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

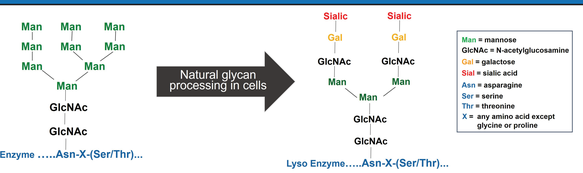


- Wild-type GlcNAc-1 Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at a precise location (at residue 928) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving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



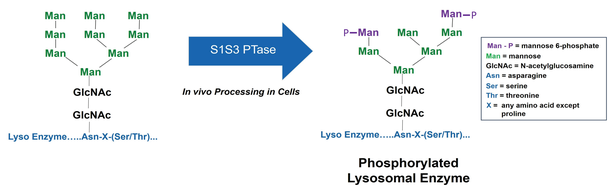
Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.

The Problem: Most N-glycans on Lysosomal Enzymes are Not Phosphorylated and Processed to Complex-Type Structures During Over-Expression



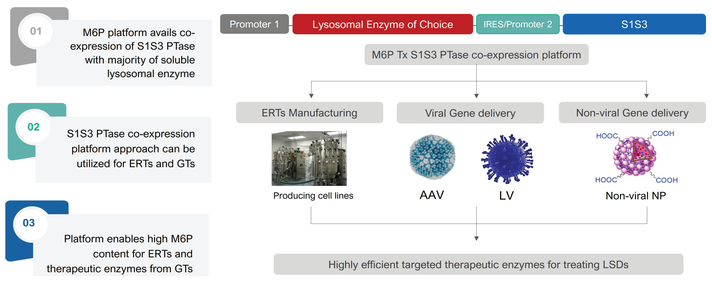
- Identical Man9 N-glycan structure is added to glycoproteins in endoplasmic reticulum during synthesis; N-glycan processing primarily occurs in Golgi
- Phosphorylation of recombinant lysosomal enzymes is highly inefficient in cells leading to mostly complex-type structures
- Complex-type N-glycans are 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: S1S3 PTase Co-Expression Platform Ensures Production of Therapeutic Lysosomal Enzymes with High Levels of M6P

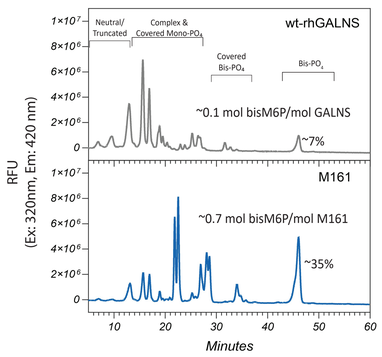


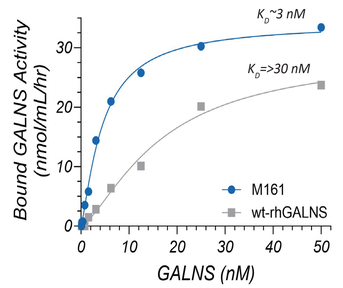
- Co-expression of S1S3 PTase with therapeutic protein produces lysosomal enzyme with high levels of M6P to enable much improved drug targeting
- Cation-independent M6P receptor (CI-MPR) is present on nearly all cells for cellular uptake of exogenous M6P-bearing lysosomal enzymes

M6P Therapeutics' Unique S1S3 PTase Co-Expression Platform for Producing Therapeutic Lysosomal Enzymes with High M6P Levels M6P for ERTs and from GTs



CI-MPR Affinitiy of rhGALNS + S1S3 PTase (M161) is Dramatically Improved with Optimal Glycan Structure

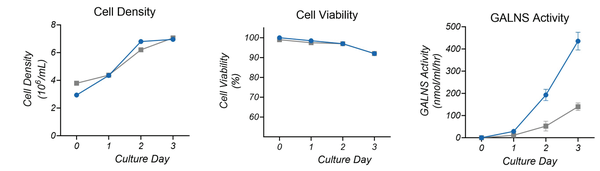




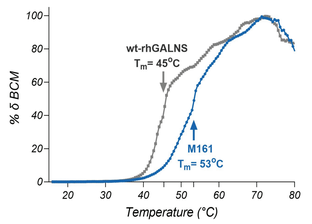
- wt-rhGALNS contains ~7% bis-M6P and thus, only this minor fraction is able to bind to CI-MPR to enable cellular uptake. The poor affinity of wt-rhGALNS (>30 nM) for the CI-MPR dramatically limits cellular uptake following therapy.
- In contrast, M161 contains ~35% bis-M6P and has 10X stronger affinity (~3 nM) for the CI-MPR which enables more efficient cellular uptake at relevant tissue levels following therapy.

rhGALNS (+ SUMF) +/- S1S3 Phosphotransferase Expression

- Expression System: Expi293 HEK cells/Gibco Expi293 transient expression system
- Incubator Settings: 8% CO_2, 37C, 85% humidity
- Cell viability and density were determined using 0.4% Trypan Blue and Invitrogen Countess II FL cell counter
- Cells were centrifuged twice at 4,122 x g, 4C



Phosphorylation of GALNS Increases the Stability of the Enzyme



- Phosphorylation of GALNS not only increases the cell uptake but also increases the stability of the enzyme
- The melting point increased by ~8°C and the aggregation temperature increased by ~4°C in M161 compared to wt-rhGALNS

Enzyme	T_m (°C)	T_{onset} (°C)	T_{agg} (°C)
M-161	53.4	36.2	43.4
wt-rhGALNS	45.6	35.1	39.2

Conclusions

- M6P Therapeutics uses S1S3 phoshotransferase to efficiently phosphorylate high mannose glycans which increases the % bis-phosphorylated glycans by ~30% (5-fold) and binding affinity to CI-MPR by 10-fold.
- Enhanced bidning to the CI-MPR increases the efficiency of trafficking of the ERT into the lysosome via CI-MPR.
- Phosphorylation of GALNS by S1S3 increases the integrity of the GALNS structure as evident by the higher melting point (T_m) and increases the stability as evident by the higher aggregation temperature (T_{agg}) compared to wt-rhGALNS.
- Increased stability increases the efficacy of the drug, especially in ERT.
- Overall, GALNS co-expressed with S1S3 could potentially be an efficient ERT as well as Gene therapy.