I. Introduction:
Fabry disease is X-linked and occurs from a deficiency of α-galactosidase A, a lysosomal enzyme that normally degrades the ganglioside globotriaosylceramide (Gb3). Lysosomal accumulation of Gb3 results in cardiovascular, renal, and neurological pathologies, and hemizygous males with Fabry disease tend to have the most severe presentations. The cation-independent mannose 6-phosphate receptor (CI-MPR) recognizes lysosomal enzymes bearing a mannose 6-phosphate (M6P) tag, via the amino acids Gln644, Arg687, Glu709, and Tyr714. In its fifth domain, and transports these enzymes from the trans-Golgi network to the lysosome. Four out of the CI-MPR’s 15 domains are able to bind M6P or M6P conjugated to N-acetylgalcosamine. CI-MPR is also present at the cell surface, and this localization can be exploited to deliver exogenous M6P-tagged biomolecules (i.e., α-galactosidase A) to the lysosome. Intravenous enzyme replacement therapy (ERT) for Fabry disease is extremely expensive, inefficient, and immunogenic. Determining the 3-D structure of CI-MPR is crucial in improving the efficiency of Fabry ERT because this knowledge will allow for the rational design of recombinant α-galactosidase A with optimally placed M6P moieties. Further structural studies of CI-MPR will not only lead to a reduction in ERT cost, but will also improve the lives of those suffering from Fabry disease.

II. Symptoms of Fabry
A. Figure A shows angokeratomas, small dark bumps on the skin that usually occurs on the lower back, groin, and thighs. They increase in size and number with age of those diagnosed with Fabry.
B. Figure B is a cornea that is opaque. The buildups of Gb3 cause the clear areas of the cornea, to the left of the arrow, to turn cloudy. This normally does not limit vision, but helps diagnose Fabry.
C. Figure C shows magnified images of kidney glomeruli of a patient with Fabry Disease. The image on the left shows a glomerulus with Gb3 inclusions, while the image on the right shows the glomerulus after ERT. Easily seen, there is less Gb3 accumulation after the treatment started.

III. Binding of M6P tag to CI-MPR
1) Initially, the enzyme α-galactosidase A travels to the cis face of the Golgi apparatus. Shown in more detail in diagram A, GlcNAc-phosphotransferase attaches a phosphate and a GlcNAc to a mannose residue on α-galactosidase A. Uncovering enzyme then removes the GlcNAc, leaving the M6P tag.
2) α-galactosidase A then arrives at the trans-Golgi network, and here CI-MPR binds to the M6P moiety. Diagram B represents the attachment of CI-MPR to the M6P tag on α-galactosidase A.
3) Finally, CI-MPR transports α-galactosidase A to the early endosome. The endosome then acidifies and transforms into a lysosome. This drop in pH causes a conformational change in CI-MPR and triggers its release from α-galactosidase A. α-galactosidase A can now degrade Gb3 and CI-MPR is recycled to transport additional lysosomal enzymes to the lysosome.

IV. Active Residues
Using nuclear magnetic resonance (NMR) spectroscopy, the above graph shows which specific residues bind to M6P and Man-P-GlcNAc in domain 5 of CI-MPR. First, 1H and 2H chemical shifts are measured in ligand-free domain 5. Next, M6P is titrated into domain 5 (top) or Man-P-GlcNAc is titrated into domain 5 (bottom). CI-MPR domain 5 residues that bind to these small molecules will experience the greatest changes in chemical shift. In these NMR experiments, chemical shift perturbations occur most notably in residues around the 680’s and the 710’s. These data allowed researchers to discover the four main residues that bind M6P and Man-P-GlcNAc: Gln644, Arg687, Glu709, and Tyr714. Also notably, there is a large spike at residue 653. This is the amino acid responsible for positioning the M6P ring for the binding.

V. Enzyme Replacement Therapy
When there is a defect in α-galactosidase A, Fabry disease develops. Currently, the only FDA-approved treatment for Fabry disease is ERT. To obtain wild type enzyme, mammalian cells are genetically engineered to produce α-galactosidase A tagged with M6P. The Fabry patient is then intravenously infused with this recombinant enzyme. Tens of milligrams are required for each patient every two weeks. This frequent infusion of massive amounts of protein is incredibly expensive (~$200,000/patient/year) and can cause life-threatening immune reactions. Although ERT reverses some of the complications of Fabry disease, further research still needs to be completed to determine if events like strokes, renal dysfunction, and cardiac events can be prevented.

VI. Conclusion
Fabry disease affects 1 out of 50,000 males, and while less prevalent in females, can cause significant pathology in heterozygotes as well. ERT is the only FDA-approved treatment for Fabry disease, but is very expensive and does not cure the pathology. To improve the lives of those affected, modeling the domains of CI-MPR is essential to better understand enzyme trafficking to the lysosome. With an improved understanding of the M6P binding sites of CI-MPR, we will be able to create enzymes that have the correct “key” to connect with this receptor. Lysosomal enzymes can then proceed to the lysosome, where they can perform their functions and cure patients with lysosomal storage diseases.

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Citations

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