While post translational modifications do not exist in bacterial expression systems, plant derived expression systems do facilitate these modifications known to be crucial for protein expression and activity. One of the major differences between mammalian and plant protein expression system is the variation of protein sugar side chains, caused by the differences in biosynthetic pathways. Glycosylation was shown to have a profound effect on activity, folding, stability, solubility, susceptibility to proteases, blood clearance rate and antigenic potential of proteins. Hence, any protein production in plants should take into consideration the potential ramifications of plant glycosylation.

Protein glycosylation is divided into two categories: N-linked and O-linked modifications (2). The two types differ in amino acid to which the glycan moiety is attached to – N-linked are attached to Asn residues, while O-linked are attached to Ser or Thr residues. In addition, the glycan sequences of each type bears unique distinguishing features. Of the two types, N-linked glycosylation is the more abundant, and its effect on protein function has been extensively studied. O-linked glycans, on the other hand are relatively scarce, and less information is available regarding their affect on proteins.

Summary ~~of the Invention~~

The background art does not teach or suggest a device, system or method for selectively producing glycosylated proteins in plant culture. The background art also does not teach or suggest such a device, system or method for producing high mannose proteins in plant culture. The background art also does not teach or suggest a device, system or method for producing proteins in plant culture through the endoplasmic reticulum (ER). The background art also does not teach or suggest such a device, system or method for producing proteins in plant culture through the endoplasmic reticulum (ER) while by-passing the Golgi body. The background art also does not teach or suggest such a device, system or method for producing proteins in plant culture by using an ER signal to by-pass the Golgi body.

The present ~~invention~~disclosure overcomes these disadvantages of the background art by providing a device, system and method for producing glycosylated proteins in plant culture, particularly proteins having a high mannose glycosylation, while optionally and

preferably targeting (and/or otherwise manipulating processing of) such proteins with an ER signal. Without wishing to be limited by a single hypothesis, it is believed that such targeting causes the proteins to by-pass the Golgi body and thereby to retain the desired glycosylation, particularly high mannose glycosylation. It should be noted that the term "plant culture" as used herein includes any type of transgenic and/or otherwise genetically engineered plant cell that is grown in culture. The genetic engineering may optionally be permanent or transient. Preferably, the culture features cells that are not assembled to form a complete plant, such that at least one biological structure of a plant is not present. Optionally and preferably, the culture may feature a plurality of different types of plant cells, but preferably the culture features a particular type of plant cell. It should be noted that optionally plant cultures featuring a particular type of plant cell may be originally derived from a plurality of different types of such plant cells.

The plant cells may be grown according to any type of suitable culturing method, including but not limited to, culture on a solid surface (such as a plastic culturing vessel or plate for example) or in suspension.

The ~~invention~~disclosure further relates to vectors and methods for expression and production of enzymatically active high mannose lysosomal enzymes using transgenic plant root, particularly carrot cells. More particularly, the ~~invention~~disclosure relates to host cells, particularly transgenic suspended carrot cells, vectors and methods for high yield expression and production of biologically active high mannose Glucocerebrosidase (GCD). The ~~invention~~disclosure further provides for compositions and methods for the treatment of lysosomal storage diseases.

The present ~~invention~~disclosure is also of a device, system and method for providing sufficient quantities of biologically active lysosomal enzymes, and particularly, human GCD, to deficient cells. The present ~~invention~~disclosure is also of host cells comprising new vector compositions that allow for efficient production of genes encoding lysosomal enzymes, such as GCD.

The present ~~invention~~disclosure therefore solves a long-felt need for an economically viable technology to produce proteins having particular glycosylation requirements, such as the high mannose glycosylation of lysosomal enzymes such as GCD for example. The present ~~invention~~disclosure is able to solve this long felt need by using plant cell culture.

In order to further explain the present ~~invention~~disclosure, a brief explanation is now provided of the biosynthetic pathway of high-mannose proteins. The basic biosynthesis pathway of high-mannose and complex N-linked glycans is highly conserved among all eukaryotes. Biosynthesis begins in the Endoplasmic Reticulum (ER) with the transfer of the glycan precursor from a dolichol lipid carrier to a specific Asn residue on the protein by the oligosaccharyl transferase. The precursor is subsequently modified in the ER by glycosidases I and II and a hypothetical mannosidase to yield the high mannose structures, similar to the process occurring in mammals.

Further modifications of the glycan sequence to complex and hybrid structures occur in the Golgi. Such modifications include removal of one of the four mannose residues by α-mannosidase I, addition of an *N*-acetylglucosamine residue, removal of the two additional mannose residues by α-mannosidase II, addition of *N*-acetylglucosamine and optionally, at this stage, xylose and fucose residues may be added to yield plant specific N-linked glycans. After the transfer of xylose and fucose to the core, complex type N-glycans can be further processed via the addition of terminal fucose and galactose. Further modifications may take place during the glycoprotein transport.

Several approaches are currently used in the background art to control and tailor protein glycosylation in plants, all of which have significant deficiencies, particularly in comparison to the present ~~invention~~disclosure. Gross modifications, such as complete inhibition of glycosylation or the removal of glycosylation sites from the peptide chain is one strategy. However, this approach can result in structural defects. An additional approach involves knock-out and introduction of specific carbohydrate processing enzymes. Again, this approach is difficult and may also have detrimental effects on the plant cells themselves.

The present ~~invention~~disclosure overcomes these deficiencies of the background art approaches by using an ER signal and/or by blocking secretion from the ER to the Golgi body. Without wishing to be limited by a single hypothesis, since a high mannose structure of lysosomal enzymes is preferred, if secretion can be blocked and the protein can be maintained in the ER, naturally occurring high mannose structures are obtained without the need for remodeling.

As indicated above, proteins transported via the endomembrane system first pass into the endoplasmic reticulum. The necessary transport signal for this step is represented by a signal sequence at the N-terminal end of the molecule, the so-called signal peptide. As

soon as this signal peptide has fulfilled its function, which is to insert the precursor protein attached to it into the endoplasmic reticulum, it is split off proteolytically from the precursor protein. By virtue of its specific function, this type of signal peptide sequence has been conserved to a high degree during evolution in all living cells, irrespective of whether they are bacteria, yeasts, fungi, animals or plants.

Many plant proteins, which are inserted into the endoplasmic reticulum by virtue of the signal peptide do not reside in the ER, but are transported from the endoplasmic reticulum to the Golgi and continue trafficking from the Golgi to the vacuoles. One class of such sorting signals for this traffic resides are signals that reside on the C-terminal part of the precursor protein [Neuhaus and Rogers, (1998) Plant Mol. Biol. 38:127-144]. Proteins containing both an N-terminal signal peptide for insertion into the endoplasmic reticulum and a C-terminal vacuolar targeting signal are expected to contain complex glycans, which is attached to them in the Golgi [Lerouge et al., (1998) Plant Mol. Biol. 38:31-48]. The nature of such C- terminal sorting signals can vary very widely. US 6,054,637 describes peptide fragments obtained from the region of tobacco basic chitinase, which is a vacuolar protein that act as vacuolar targeting peptides. An example for a vacuolar protein containing a C-terminal targeting signal and complex glycans is the phaseolin storage protein from bean seeds [Frigerio et al., (1998) Plant Cell 10:1031-1042; Frigerio et al., (2001) Plant Cell 13:1109-1126.].

The paradigm is that in all eukaryotic cells vacuolar proteins pass via the ER and the Golgi before sequestering in the vacuole as their final destination. Surprisingly, the transformed plant root cells of the present disclosure~~invention~~ produced an unexpected high mannose GCD. Advantageously, this high mannose product was found to be biologically active and therefore no further steps were needed for its activation. Without wishing to be limited by a single hypothesis, it would appear that the use of an ER signal with the recombinant protein being produced in plant cell culture was able to overcome transportation to the Golgi, and hence to retain the desired high mannose glycosylation. Optionally, any type of mechanism which is capable to produce high mannose glycosylation, including any type of mechanism to by-pass the Golgi, may be used in accordance with the present disclosure~~invention~~.

In a first aspect, there is provided a human glucocerebrosidase protein which comprises the amino acid sequence as set forth in SEQ ID NO: 8, wherein said human glucocerebrosidase protein is glycosylated and comprises at least one exposed mannose, at least one fucose residue having an alpha (1-3) glycosidic bond and at least one xylose, and is linked at its C terminus to a vacuolar targeting signal peptide as set forth in SEQ ID NO: 2.

In a second aspect, there is provided an isolated nucleic acid sequence encoding the human glucocerebrosidase protein as defined above.

In a third aspect, there is provided a pharmaceutical composition comprising the human glucocerebrosidase protein as defined above and a pharmaceutically acceptable carrier.

In a fourth aspect, there is provided a plant cell expressing the human glucocerebrosidase protein as defined above.

In a fifth aspect, there is provided a method of producing a human glucocerebrosidase protein comprising: preparing a culture of plant cells as defined above; and culturing said cell culture under conditions permitting the expression of said protein.

In a sixth aspect, there is provided a human glucocerebrosidase protein which comprises the amino acid sequence set forth in SEQ ID NO: 8, wherein said human glucocerebrosidase protein is glycosylated and comprises at least one exposed mannose, at least one fucose having an alpha (1-3) glycosidic bond and at least one xylose, and is linked at its C terminus to a vacuolar targeting signal peptide as set forth in SEQ ID NO: 2, wherein said linkage is defined by the respective amino acid coordinates of SEQ ID NO: 14.

In a seventh aspect, there is provided a pharmaceutical composition comprising the human glucocerebrosidase protein as defined above and a pharmaceutically acceptable carrier.

In an eighth aspect, there is provided a plant cell expressing the human glucocerebrosidase protein as defined above.

In a ninth aspect, there is provided the use of the glucocerebrosidase protein as defined above, in the manufacture of a medicament for the treatment or prevention of Gaucher's disease.

In a first embodiment~~aspect~~, the present disclosure~~invention~~ relates to a host cell producing a high mannose recombinant protein of interest. This cell may be transformed or transfected with a recombinant nucleic acid molecule encoding a protein of interest or with an expression

vector comprising the nucleic acid molecule. Such nucleic acid molecule comprises a first nucleic acid sequence encoding the protein of interest operably linked to a second nucleic acid sequence encoding a vacuolar targeting signal peptide. The first nucleic acid sequence may be optionally further operably linked to a third nucleic acid sequence encoding an ER (endoplasmic reticulum) targeting signal peptide. The host cell of the ~~invention~~disclosure is characterized in that the protein of interest is produced by the cell in a highly mannosylated form.

The host cell of the ~~invention~~disclosure may be eukaryotic or prokaryotic cell.

In one embodiment, the host cell of the ~~invention~~disclosure is a prokaryotic cell, preferably, a bacterial cell, most preferably, an *Agrobacterium tumefaciens* cell. These cells are used for infecting the preferred plant host cells described below.

In another preferred embodiment, the host cell of the ~~invention~~disclosure may be eukaryotic cell, preferably, a plant cell, and most preferably, a plant root cell selected from the group consisting of *Agrobacterium rihzogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cell.

In a preferred embodiment, the plant root cell is a carrot cell. It should be noted that the transformed carrot cells of the ~~invention~~disclosure are grown in suspension. As mentioned above and described in the Examples, these cells were transformed with the *Agrobacterium tumefaciens* cells.

In another embodiment, the recombinant nucleic acid molecule comprised within the host cell of the ~~invention~~disclosure, comprises a first nucleic acid sequence encoding a lysosomal enzyme that is in operable linkage with a second nucleic acid sequence encoding a vacuolar targeting signal peptide derived from the basic tobacco chitinase A gene. This vacuolar signal peptide has the amino acid sequence as denoted by SEQ ID NO: 2. The first nucleic acid sequence may be optionally further linked in an operable linkage with a third nucleic acid sequence encoding an ER (endoplasmic reticulum) targeting signal peptide as denoted by SEQ ID NO: 1. In one embodiment, the recombinant nucleic acid molecule comprised within the host cell of the ~~invention~~disclosure further comprises a promoter that is functional in plant cells. This promoter should be operably linked to the recombinant molecule of the ~~invention~~disclosure.

In another embodiment, this recombinant nucleic acid molecule may optionally further comprise an operably linked terminator which is preferably functional in plant

cells. The recombinant nucleic acid molecule of the ~~invention~~disclosure may optionally further comprise additional control, promoting and regulatory elements and/or selectable markers. It should be noted that these regulatory elements are operably linked to the recombinant molecule.

In a preferred embodiment, the high mannose protein of interest produced by the host cell of the ~~invention~~disclosure may be a high mannose glycoprotein having exposed mannose terminal residues.

Such high mannose protein may be according to another preferred embodiment, a lysosomal enzyme selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase and sialidase. In a preferred embodiment, the lysosomal enzyme may be the human glucocerebrosidase (GCD). Hereinafter recombinant GCD, rGCD, rhGCD all refer to various forms of recombinant human GCD unless otherwise indicated.

As previously described, Gaucher's disease, the most prevalent lysosomal storage disorder, is caused by point mutations in the hGCD (human glucocerebrosidase) gene (GBA), which result in accumulation of GlcCer in the lysosomes of macrophages. The identification of GCD deficiency as the primary cause of Gaucher's disease led to the development of enzyme replacement therapy as a therapeutic strategy for this disorder. However, glycosylation plays a crucial role in hGCD activity and uptake to target cells.

Therefore, according to other preferred embodiments of the present ~~invention~~disclosure, suitably glycosylated hGCD is preferably provided by controlling the expression of hGCD in plant cell culture, optionally and more preferably by providing an ER signal and/or otherwise by optionally and more preferably blocking transportation to the Golgi.

Optionally and preferably, the hGCD has at least one oligosaccharide chain comprising an exposed mannose residue for the treatment or prevention of Gaucher's disease.

Still further, in a particular embodiment, this preferred host cell is transformed or transfected by a recombinant nucleic acid molecule which further comprises an ^{35}S promoter from Cauliflower Mosaic Virus, an octopine synthase terminator of *Agrobacterium tumefaciens* and TMV (Tobacco Mosaic Virus) omega translational enhancer element. According to a preferred embodiment, this recombinant nucleic acid

molecule comprises the nucleic acid sequence substantially as denoted by SEQ ID NO: 13 and encodes a high mannose GCD having the amino acid sequence substantially as denoted by SEQ ID NO: 14.

It should be appreciated that the present ~~invention~~disclosure further provides for an expression vector comprising a nucleic acid molecule encoding a biologically active lysosomal enzyme.

In one preferred embodiment, the expression vector of the ~~invention~~disclosure comprises a nucleic acid molecule encoding a biologically active high mannose human glucocerebrosidase (GCD). Preferably, this preferred expression vector comprises a nucleic recombinant nucleic acid molecule which having the nucleic acid sequence substantially as denoted by SEQ ID NO: 13.

In a second ~~aspect~~embodiment, the present ~~invention~~disclosure relates to a recombinant high mannose protein produced by the host cell of the ~~invention~~disclosure.

In a preferred embodiment, this high mannose protein may be a biologically active high mannose lysosomal enzyme selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase and sialidase. Most preferably, this lysosomal enzyme may be human glucocerebrosidase (GCD).

Still further, the ~~invention~~disclosure provides for a recombinant biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue.

According to a preferred embodiment, the recombinant lysosomal enzyme of the ~~invention~~disclosure can bind to a mannose receptor on a target cell in a target site. Preferably, this site may be within a subject suffering from a lysosomal storage disease.

It should be noted that the recombinant lysosomal enzyme has increased affinity for the target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme for the target cell. In a specific embodiment, the target cell at the target site may be a Kupffer cell in the liver of the subject.

In a preferred embodiment, the recombinant lysosomal enzyme may be selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase,

hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase.

Most preferably, this recombinant lysosomal enzyme is glucocerebrosidase (GCD).

In a third ~~aspect~~embodiment, the ~~invention~~disclosure relates to a method of producing a high mannose protein. Accordingly, the method of the ~~invention~~disclosure comprises the steps of: (a) preparing a culture of recombinant host cells transformed or transfected with a recombinant nucleic acid molecules encoding a recombinant protein of interest or with an expression vector comprising the recombinant nucleic acid molecules; (b) culturing these host cell culture prepared by step (a) under conditions permitting the expression of the protein, wherein the host cells produce the protein in a highly mannosylated form; (c) recovering the protein from the cells and harvesting the cells from the culture provided in (a); and (d) purifying the protein of step (c) by a suitable protein purification method.

According to a preferred embodiment, the host cell used by this method is the host cell of the ~~invention~~disclosure.

In another preferred embodiment, the high mannose protein produced by the method of the ~~invention~~disclosure may be a biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue.

This recombinant enzyme can bind to a mannose receptor on a target cell in a target site. More particularly, the recombinant enzyme produced by the method of the ~~invention~~disclosure has increased affinity for the target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme to the target cell. Accordingly, the target cell at the target site may be Kupffer cell in the liver of the subject.

In a specific embodiment, this lysosomal enzyme may be selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase and sialidase. Most preferably, this lysosomal enzyme may be glucocerebrosidase (GCD).

In another preferred embodiment, the host cell used by the method of the ~~invention~~disclosure may be a plant root cell selected from the group consisting of *Agrobacterium rihzogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cell. Most preferably, the plant root cell is a carrot cell. It should be particularly noted that in the method of the ~~invention~~disclosure, the transformed host carrot cells are grown in suspension.

In a further ~~aspect~~embodiment, the present ~~invention~~disclosure relates to a method for treating a subject having lysosomal storage disease using exogenous recombinant lysosomal enzyme, comprising: (a) providing a recombinant biologically active form of lysosomal enzyme purified from transformed plant root cells, and capable of efficiently targeting cells abnormally deficient in the lysosomal enzyme. This recombinant biologically active enzyme has exposed terminal mannose residues on appended oligosaccharides; and (b) administering a therapeutically effective amount of the recombinant biologically active lysosomal enzyme to the subject. In a preferred embodiment, the recombinant high mannose lysosomal enzyme used by the method of the ~~invention~~disclosure may be produced by the host cell of the ~~invention~~disclosure. Preferably, this host cell is a carrot cell.

In another preferred embodiment, the lysosomal enzyme used by the method of the ~~invention~~disclosure may be a high mannose enzyme comprising at least one oligosaccharide chain having an exposed mannose residue. This recombinant enzyme can bind to a mannose receptor on a target cell in a target site within a subject. More preferably, this recombinant lysosomal enzyme has increased affinity for these target cells, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme to the target cell.

More specifically, the lysosomal enzyme used by the method of the ~~invention~~disclosure may be selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase. Preferably, this lysosomal enzyme is glucocerebrosidase (GCD).

According to a preferred embodiment, the method of the ~~invention~~disclosure is therefore intended for the treatment of a lysosomal storage disease, particularly Gaucher's disease.

In such case the target cell at the target site may be a Kupffer cell in the liver of the subject.

The ~~invention~~disclosure further provides for a pharmaceutical composition for the treatment of a lysosomal storage disease comprising as an active ingredient a recombinant biologically active high mannose lysosomal enzyme as defined by the ~~invention~~disclosure. The composition of the ~~invention~~disclosure may be optionally further comprise pharmaceutically acceptable diluent, carrier or excipient.

In a specific embodiment, the composition of the ~~invention~~disclosure is intended for the treatment of Gaucher's disease. Such composition may preferably comprise as an effective

ingredient a biologically active high mannose human glucocerebrosidase (GCD), as defined by the ~~invention~~disclosure.

The ~~invention~~disclosure further relates to the use of a recombinant biologically active high mannose lysosomal enzyme of the ~~invention~~disclosure in the manufacture of a medicament for the treatment or prevention of a lysosomal storage disease. More particularly, such disease may be Gaucher's disease.

Accordingly, this biologically active lysososomal enzyme is a biologically active high mannose human glucocerebrosidase (GCD), as defined by the ~~invention~~disclosure.

According to the present ~~invention~~disclosure, there is provided a host cell producing a high mannose recombinant protein, comprising a polynucleotide encoding the recombinant protein and a signal for causing the recombinant protein to be produced as a high mannose protein. Preferably, the polynucleotide comprises a first nucleic acid sequence encoding the protein of interest operably linked to a second nucleic acid sequence encoding a signal peptide. Optionally, the signal peptide comprises an ER (endoplasmic reticulum) targeting signal peptide. Preferably, the polynucleotide further comprises a third nucleic acid sequence for encoding a vacuolar targeting signal peptide.

Preferably, the signal causes the recombinant protein to be targeted to the ER. More preferably, the signal comprises a signal peptide for causing the recombinant protein to be targeted to the ER. Most preferably, the polynucleotide comprises a nucleic acid segment for encoding the signal peptide.

Optionally and preferably, the signal causes the recombinant protein to by-pass the Golgi. Preferably, the signal comprises a signal peptide for causing the recombinant protein to not be targeted to the Golgi. More preferably, the polynucleotide comprises a nucleic acid segment for encoding the signal peptide.

Optionally and preferably, the host cell is any one of a eukaryotic and a prokaryotic cell. Optionally, the prokaryotic cell is a bacterial cell, preferably an *Agrobacterium tumefaciens* cell. Preferably, the eukaryotic cell is a plant cell. More preferably, the plant cell is a plant root cell selected from the group consisting of *Agrobacterium rihzogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cell. Most preferably, the plant root cell is a carrot cell.

Preferably, the recombinant polynucleotide comprises a first nucleic acid sequence encoding the protein of interest that is in operable link with a second nucleic acid sequence

Preferably, the high mannose protein is a biologically active high mannose lysosomal enzyme selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase and sialidase.

More preferably, the lysosomal enzyme is human glucocerebrosidase (GCD).

According to other preferred embodiments of the present ~~invention~~disclosure, there is provided a recombinant biologically active high mannose lysosomal enzyme having at least one oligosaccharide chain comprising an exposed mannose residue.

According to still other preferred embodiments, there is provided a recombinant protein, comprising a first portion having signal peptide activity and a second portion having lysosomal enzyme activity, the first portion causing the second portion to be processed in a plant cell with at least one oligosaccharide chain comprising an exposed mannose residue.

Preferably, the lysosomal enzyme comprises a protein for the treatment or prevention of Gaucher's disease.

More preferably, the protein comprises hGCD.

Preferably, the first portion comprises a plant cell ER targeting signal peptide. More preferably, the recombinant enzyme can bind to a mannose receptor on a target cell in a target site within a subject suffering from a lysosomal storage disease. Most preferably, the recombinant lysosomal enzyme has increased affinity for the target cell, in comparison with the corresponding affinity of a naturally occurring lysosomal enzyme for the target cell.

Also most preferably, the recombinant lysosomal enzyme is selected from the group consisting of glucocerebrosidase (GCD), acid sphingomyelinase, hexosaminidase, α-N-acetylgalactosaminidise, acid lipase, α-galactosidase, glucocerebrosidase, α-L-iduronidase, iduronate sulfatase, α-mannosidase or sialidase.

Preferably, the recombinant lysosomal enzyme is glucocerebrosidase (GCD).

Also preferably, the target cell at the target site is a Kupffer cell in the liver of the subject.

According to still other preferred embodiments there is provided the use of a recombinant biologically active high mannose lysosomal enzyme as described above, in the manufacture of a medicament for the treatment or prevention of a lysosomal storage disease. Preferably, the disease is Gaucher's disease. More preferably, the biologically active lysososomal enzyme is a biologically active high mannose human glucocerebrosidase (GCD).

The ~~invention~~disclosure will be further described on the hand of the following figures, which are illustrative only and do not limit the scope of the ~~invention~~disclosure which is also defined by the appended claims.

Brief Description of the Figures

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

Figure 1A-1B

1A shows the resulting expression cassette comprising ^{35}S promoter from Cauliflower Mosaic Virus, TMV (Tobacco Mosaic Virus) omega translational enhancer element, ER targeting signal, the human GCD sequence (also denoted by SEQ ID NO: 7), vacuolar signal and octopine synthase terminator sequence from *Agrobacterium tumefaciens*.

1B shows a schematic map of pGreenII plasmid backbone.

Figure 2 shows Western blot analysis of hGCD transformed cell extracts using anti hGCD specific antibody. Standard Cerezyme (lane 1) was used as a positive control, untransformed callus was used as negative control (lane 2), various selected calli extracts are shown in lanes 3-8.

Figure 3A-3C shows the first step of purification of rhGCD on a strong cation exchange resin (Macro-Prep high-S support, Bio-Rad), packed in a XK column (2.6x20cm). The column was integrated with an AKTA prime system (Amersham Pharmacia Biotech) that allows conductivity monitoring, pH and absorbency at 280nm. Elution of the rh-GCD was obtained with equilibration buffer containing 600mM NaCl.

9. The isolated nucleic acid sequence of claim 8, comprising the nucleic acid sequence as set forth in SEQ ID NO: 7.

10. The isolated nucleic acid sequence of claim 8, comprising the nucleic acid sequence as set forth in SEQ ID NO: 13.

11. A pharmaceutical composition comprising the human glucocerebrosidase protein of any of claims 1 to 7 and a pharmaceutically acceptable carrier.

12. A plant cell expressing the human glucocerebrosidase protein of any of claims 1 to 7.

13. The plant cell of claim 12, wherein the plant cell is a carrot cell.

14. The plant cell of claim 12, wherein the main glycan structure of said glucocerebrosidase protein produced by said plant cell comprises exposed mannose, as measured by linkage analysis.

15. The plant cell of any one of claims 12-14, wherein said plant cell is lyophilized.

~~16. A pharmaceutical composition comprising plant cells of any of claims 12 to 15 and a pharmaceutically acceptable carrier.~~

~~17. The pharmaceutical composition of claim 16, formulated for oral administration.~~

16~~18~~. A method of producing a human glucocerebrosidase protein comprising:

preparing a culture of plant cells of any of claims 12 to 14; and

culturing said cell culture under conditions permitting the expression of said protein.

1719. The method of claim 1618, wherein said cell culture is cultured in suspension.

1820. The method of claim 1719, further comprising:

purifying said protein.

19~~21~~. A human glucocerebrosidase protein which comprises the amino acid sequence set forth in SEQ ID NO: 8, wherein said human glucocerebrosidase protein is glycosylated and comprises at least one exposed mannose, at least one fucose having an alpha (1-3) glycosidic bond and at least one xylose, and is linked at its C terminus to a vacuolar targeting signal peptide as set forth in SEQ ID NO: 2, wherein said linkage is defined by the respective amino acid coordinates of SEQ ID NO: 14.

20~~22~~. A pharmaceutical composition comprising the human glucocerebrosidase protein of claim 19~~21~~ and a pharmaceutically acceptable carrier.

21~~23~~. A plant cell expressing the human glucocerebrosidase protein of claim 19~~21~~.

22~~24~~. Use of the glucocerebrosidase protein as defined by any one of claims 1 to 7 and 19~~21~~, in the manufacture of a medicament for the treatment or prevention of Gaucher's disease.

~~25. The use of the plant cell as defined by any of claims 12 to 15 in the manufacture of a medicament for the treatment or prevention of Gaucher's disease.~~