Vitamin C prevents more diseases than scurvy?

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Key words: Ascorbic acid/Copper/Dehydroascorbic acid/Glypican/Heparan sulfate/Hypoxia/Nitric oxide/Vitamin C

Novel findings provide possible explanations for observed beneficial effects of a high intake of vitamin C on cell growth, gene transcription, host resistance to infection, uptake of polyamines and clearance of misfolded proteins. Vitamin C exerts its effects indírectly via hypoxia-inducible factor, nitric oxide synthase and the heparan sulfate proteoglycan glypican-1, which is deglycanated in a vitamin C- and copper-dependent reaction. In fibroblasts from patients with Niemann-Pick C disease, heparan sulfate degradation is defective but can be restored by supplementation with ascorbate. Likewise, accumulation of Alzheimer amyloid β can be reduced by ascorbate-induced heparan sulfate degradation. Other conditions that can be improved upon vitamin C supplementation include cancer, cardiovascular disease, stroke and a rare peripheral neuropathy called Charcot-Marie-Tooth disease.

How much vitamin C do we need?

The reduced form of vitamin C, ascorbic acid, is well-known for its role in preventing scurvy. It is also believed to be a general antioxidant that protects cells from damage by reactive oxidant species. Ascorbate reactivates various dioxygenases, such as the protocollagen prolyl and lysyl hydroxylases, as well as enzymes involved in the biosynthesis of carnitine or the conversion of dopamine to noradrenaline (norepinephrine). A progressive deficiency of vitamin C is expected to first inactivate dioxygenases with a low affinity for ascorbic acid. Inactivation of prolyl hydroxylase is therefore the prime effect of vitamin C deficiency, which results in insufficiently hydroxylated collagen. The abnormal, less-stable fibers formed by such collagens contribute to the skin lesions and blood-vessel fragility seen in scurvy; for reviews, see [1-3].

Most mammalian species synthesize L-ascorbic acid from D-glucose in the liver; for review, see [4]. The enzyme catalyzing the final step, L-gulono-1,4-lactone oxidase (GULO, EC 1.1.3.8) is missing or mutated in humans, other primates, the guinea pig and certain fruit bats. In human males, a daily intake of 200 mg vitamin C saturates the plasma level at 70-80 μM, while females require twice that amount to reach a comparable level [5].

Ablation of the *Gulo* gene has been performed in mice [6]. As expected, these mice depend on dietary supplementation of vitamin C for survival. However, the daily requirement was several-fold greater than that observed for humans. When plasma ascorbic acid decreased to subnormal levels, small, but significant increases in total cholesterol and decreases in high density lipoprotein cholesterol were observed as the animals aged. The mechanism behind this effect is not known. More severe vitamin C deficiency resulted in aortic wall damage that could be ascribed to defects in the crosslinking of elastin, which partly depends on the formation of hydroxylysine. Hence, the symptoms of vitamin C deficiency in humans and in *Gulo*$^{-/-}$ mice are clearly different.

Ingestion of mega-doses of vitamin C has been claimed to be necessary to keep in good health but also questioned. However, many recent studies do point to functions for vitamin C in mammalian cells other than as a general antioxidant. Scurvy is prevented by a relatively low intake of vitamin C, but is there another higher level required for other functions? Are the results of mild but prolonged deficiences only apparent at an advanced age? To discuss these issues we need a brief summary of the uptake and interconversions of the redox forms of vitamin C.

Is it important to drink fresh fruit juice?

The reduced form of vitamin C, ascorbic acid (Fig. 1 a, formula **I**), is utilized in cell metabolism as an electron donor, capable of donating one or two electrons. In the first

oxidation step, ascorbyl radical (**II**) is generated, followed by dehydroascorbic acid (**III**) in the second step. The ascorbyl radical is not believed to accumulate *in vivo*, because two ascorbyl radicals can dismutate into one ascorbic acid and one dehydroascorbic acid molecule.

As shown in Fig. 1 b, intracellular accumulation of vitamin C is mediated either by the specific, high-affinity, low-capacity sodium-ascorbate transporters SVCT1 and –2, which transport ascorbate (**I** in Fig. 1a), or by the unspecific, low-affinity, high-capacity hexose transporters GLUT1 and –3, which transport the hexose-like, bicyclic hemiketal of dehydroascorbic acid or its hydrated form (**IV** and **V** in Fig. 1 a). Both transport mechanisms occur in many cells in the body, but fibroblasts have only SVCT-mediated uptake and neutrophils have only GLUT-mediated uptake [7-11].

Mice deficient in the mouse ortholog of the ascorbate-transporter SVCT2 have been created [12]. $SVCT2^{-/-}$ mice died within a few minutes of birth with respiratory failure and intraparenchymal brain hemorrhage, although their skin had normal 4-hydroxyproline levels This points to a previously unrecognized requirement for ascorbate in the perinatal period.

Vitamin C concentrations in the brain exceed those in blood by 10-fold. GLUT1, expressed on the endothelial cells at the blood-brain barrier, transports dehydroascorbic acid into the brain [13]. Also GLUT3 is able to transport dehydroascorbic acid [14], as well as the insulin-sensitive GLUT4 [15]. Dehydroascorbic acid can then be converted to ascorbic acid by various intracellular reducing agents (Fig. 1 b). In this way, neurons can accumulate high amounts of ascorbate, up to 10 mM while the cerebrospinal fluid contains approx. 1 mM; for more comprehensive reviews, see [2, 3, 11].

In human intestinal brush-border membranes, the maximal rates of uptake are similar for dehydroascorbic acid and ascorbate when glucose is absent. Surprisingly, glucose

inhibits ascorbate uptake but not dehydroascorbic acid uptake. Hence, it is possible that glucose derived from food may increase the bioavailibility of dehydroascorbic acid relative to ascorbate. Although glucose does compete with dehydroascorbic acid uptake in many cells there are other notable exceptions besides human enterocytes, namely rat astrocytes and human renal epithelial cells [11].

Ascorbate is easily converted to dehydroascorbic acid by contact with air (Fig. 1 a). If dehydroascorbic acid is taken up and distributed to various organs, equally well or sometimes better than ascorbate, the freshness of the morning fruit juice is perhaps not so critical. However, dehydroascorbic acid can be irreversibly hydrolyzed to 2,3-diketogulonic acid by heating (**VI** in Fig. 1 a).

Vitamin C, oxygen and nitric oxide may have a complicated relationship

Although there appear to be few, if any, documentations that ascorbate interacts with antioxidant response elements in the genome, ascorbate can affect gene transcription indirectly. Again, ascorbate-dependent dioxygenases are involved. One well-studied target of hydroxylation is the hypoxia-inducible transcription factor (HIF-1). In the α subunit of HIF-1, prolyl and asparaginyl residues are hydroxylated by ascorbate-dependent dioxygenases (Fig. 1 c, ***i***). Formation of hydroxyproline targets HIF-1 for destruction in the proteasomes, while asparaginyl hydroxylation modulates its interaction with transcriptional co-activators. Non-hydroxylated HIF-1α increases the expression of most glycolytic enzymes as well as the glucose transporters GLUT1 and GLUT3 [16]. Hypoxia may thus increase cellular uptake of dehydroascorbic acid. The increased reducing power associated with hypoxia would then lead to formation of ascorbate inside cells.

Cardiovascular disease is associated with endothelial dysfunction. It is often manifested as a loss of vasodilation associated with decreased generation of nitric oxide (NO)

by the endothelial cells. Vitamin C infusion or ingestion improves endothelial function in these diseases. The mechanisms believed to account for this improvement include decrease in LDL oxidation, scavenging of intracellular superoxide, release of NO from S-nitroso groups (SNO) and activation of NO synthase [17].

The enhancement of NO production induced by ascorbate is partly due to a direct tetrahydrobiopterin-dependent activation of NO synthase (Fig. 1 c, ***ii***) [18]. Ascorbate prolongs the half-life of the tetrahydrobiopterin cofactor by protecting it from oxidation [19]. This effect has also been confirmed by overexpression of GULO, the enzyme that converts gulonolactone to ascorbate. This enhances NO production in endothelial cells by elevating the intracellular tetrahydrobiopterin content [20]. Moreover, expression of NO-synthase (NOS) is also stimulated by HIF-1α [21].

Increased NO production raises the level of S-nitrosylation (-SNO) of specific Cys residues in many proteins. This modulates protein function, as was first shown with the N-methyl-D-aspartate (NMDA) receptor [22]. Today, over a hundred different proteins, forming a nitroso-proteome, have been described. They include haemoglobin, a number of cell surface receptors and channel proteins, signal transducers, nuclear proteins and transcription factors, cyclins, cytoskeletal proteins and proteins/enzymes involved in apoptosis, vesicular transport, cholesterol and steroid synthesis, as well as general metabolism and even NO-production [23-27]. Ascorbate could thus indirectly affect them all.

It has been debated whether vitamin C can protect against common cold and other infections. Many investigations have been undertaken to elucidate the mechanism by which vitamin C might enhance systemic immunity, particularly in defense of viral diseases [28]. Some feeding studies show that vitamin C has a positive effect on host defense. The antioxidant function, which attenuates activation of the transcription factor NFκB, would be generally anti-inflammatory. For example, as ascorbate stimulates expression of inducible

NOS and supports NO production by protecting tetrahydrobiopterin, the generated NO represses inhibitory I-κB kinase through S-nitrosylation, whereby NFκB is inhibited [29]. Ascorbate also decreases T cell death and increases natural killer cell activity [28].

NO formation by NOS requires oxygen (Fig. 1 c) and HIF-1α itself is also a target for S-nitrosylation [26, 30]. NO prevents destruction of HIF-1α by inhibiting hydroxylation and facilitates binding of the transactivator cAMP-response-element-binding protein (CREB) by nitrosylating a key Cys residue in HIF-1α. However, ascorbate could counteract these effects both by supporting hydroxylation of HIF-1α, and thereby facilitating HIF-1α destruction, and by attenuating S-nitrosylation, presumably by inducing de-nitrosylation [30, 31]. Hence, there may be a complex interplay between intracellular oxygenation, NO production and available vitamin C. For example, 3α-dihydroxysteroid dehydrogenase, which reduces dehydroascorbic acid to ascorbate, is itself also a target of S-nitrosylation [3, 26].

Ascorbate also affects the turn-over of glypican- 1, a complex protein conjugated with both lipid and glycosaminoglycan and involved in cell growth and differentiation; for review, see [32]. Glypican-1 is a glycosylphosphatidylinositol (GPI)-anchored, lipid raft-associated heparan sulfate proteoglycan that is internalized via a caveolin-associated route and recycles via endosomes and the Golgi back to the cell surface (Box 1). Before or during endocytosis glypican-1 is S-nitrosylated in a copper-dependent reaction [33]. Free Zn(II) ions, which are particularly abundant in neural cells, can block Cu(II) binding to glypican-1 and thereby attenuate S-nitrosylation [33-36]. We have identified three copper-binding proteins that can accomodate the Cu(II)-to-Cu(I) reduction that is required to support formation of glypican-1-SNO in the presence of free Zn(II) ions. These are the GPI-linked cellular prion protein [34, 37], the GPI-linked, brain-specific splice variant of ceruloplasmin

[35] and the amyloid precursor protein of Alzheimer´s disease [36], all of which may thus be regarded as catalysts of S-nitrosylation [26].

De-nitrosylation of SNO-containing proteins can be carried out by thioredoxin, superoxide dismutase and possibly protein disulfide isomerase [26]. Nitrosothiols are also decomposed in the presence of ascorbate [23, 38]. We have found that ascorbate can induce autodegradation of the heparan sulfate side-chains in glypican-1-SNO (Fig. 1 c, ***iii***). Ascorbate decomposes glypican-1-SNO to NO and glypican-1-SH (Box 1). NO is probably converted to nitroxyl (HNO) in a copper-dependent redox reaction [39, 40]. Then NO/HNO cleaves the heparan sulfate chains at $GlcNH_3^+$ residues. This results in release of heparan sulfate as glycans or oligosaccharides carrying anhydromannose (anMan) at their reducing termini (Box 1). Such degradation products, which can be detected by a specific mAb directed to the anMan –containing terminal sequence, are generated both constitutively and after addition of ascorbate in a variety of cells [33, 36, 41]. We have recently reported that the NO-copper-dependent deaminative degradation of glypican-1 heparan sulfate is defective in fibroblasts from patients with Niemann-Pick C1 (NPC-1) disease [41]. In NPC diseases, which are neurovisceral degenerative disorders, transport of lipid raft-derived cholesterol and sphingolipids through late endosomes is impeded resulting in lysosomal accumulation. By supplying ascorbate to NPC-1 fibroblasts, the NO-catalyzed, deaminative heparan sulfate degradation was restored (Fig. 2). A side-effect, resulting in nitrosative stress, was observed but could be minimized by inhibition of heparanase-catalyzed degradation of heparan sulfate by using suramin.

Cells may grow well and live long in the presence of vitamin C

Ascorbate protects cells from oxidative assults and this is generally believed to be the main reason for the beneficiary effects of vitamin C. However, the effects could also be mediated

via glypican-1 autoprocessing. Two possible functions for the ascorbate-induced heparan sulfate autodegradation in glypican-1 have emerged. One is related to uptake of the growth-promoting and differentiation-inducing polyamine spermine from the environment. The other concerns clearance of potentially misfolding proteins and aggregating peptides.

Recycling glypican-1 can transport basic compounds bound to its polyanionic heparan sulfate side chains (Box 2). Examples include polyamines, like spermine [42], basic peptides [43] and net cationic DNA/heparan sulfate-basic peptide complexes [44]. The latter are ultimately delivered to the nucleus as indicated by functional transcription of the DNA. For the intracellular release of spermine from the heparan sulfate carrier, NO-catalyzed cleavage of heparan sulfate is necessary [42]. Enzymatic degradation of a heparan sulfate chain already covered by cargo should be hampered for steric reasons. NO suffers no such restraints. Continuous supply of vitamin C may be important to maintain the capacity to degrade heparan sulfate in this manner and thereby facilitate uptake and delivery of basic compounds and complexes.

Age-related neurodegenerative disorders resulting in dementia are increasing rapidly. One of the most common causes of dementia is Alzheimer´s disease. Numerous epidemiological studies have evaluated the beneficiary effects of various nutrients, including vitamin C. One prospective epidemiological cohort study suggested a protective effect of vitamins C and E in Alzheimer´s disease [45].

Amyloid aggregates derived from misfolded proteins or peptides are usually associated with heparan sulfate chains or chain fragments [46]. We have found that glypican-1 expression and autoprocessing enhances amyloid precursor protein metabolism and amyloid β clearance in Tg2576 fibroblasts and N2a neuroblastoma cells [47]. Scrapie prion-infected GT-1 cells exhibit augmented glypican-1 autoprocessing (Löfkvist *et al.*, unpublished). Stimulation of NO-dependent autoprocessing of glypican-1 by ascorbate may be a mechanism

to enhance clearance of protein aggregates. How heparan sulfate degradation products facilitate clearance of aggregation-prone polypeptides remains a challenge for future research.

Vitamin C prevents more diseases than scurvy

The benificial effects of mega-dose vitamin C supplementation for the prevention of cancer, cardiovascular disease, stroke and neurodegeneration associated with amyloid deposition, such as prion, Alzheimer´s and Niemann-Pick C diseases, have been vehemently debated. Whereas the antioxidant properties of vitamin C are recognized as positive, its pro-oxidant activity has been considered to be negative. *In vitro*, ascorbate reduces Fe(III) ions to Fe(II) and Cu(II) to Cu(I), which in turn can reduce H_2O_2 to hydroxyl radicals, which can induce oxidative damage to DNA, proteins and lipids. However, oxidative damage to DNA by vitamin C supplementation has not been documented by numerous *in vivo* studies; most studies show either a reduction in oxidative DNA damage or a null effect [3].

In cardiovascular diseases, a benificial effect of vitamin C ingestion has been documented [17]. It is probably due to the ability of vitamin C to increase production of atheroprotective NO. Potent cerebroprotection by dehydroascorbic acid in experimental stroke in mice has also been reported [48]. By intravenous infusion of dehydroascorbic acid, which can pass the blood-brain barrier and be converted to ascorbate inside the brain cells (Fig. 1 b), infarct volume was reduced up to 9-fold depending on dose and time of administration postischemia. Ascorbate had no effect on infarct volumes, mortality and neurological deficits.

Charcot-Marie-Tooth disease (CMT) is a hereditary peripheral neuropathy characterized by abnormal myelinization. Vitamin C in the ascorbate form corrects the phenotype of a mouse model of CMT-1A, the most frequent form of the disease [49].

Ascorbate seems to modulate, probably indirectly, the expression of a Schwann cell gene (PMP22), the product of which has not been identified biochemically.

Selective killing of cancer cells by high concentrations of ascorbate has also been reported [50]. Normal cells were unaffected by 20 mM ascorbate, whereas several cancer cell lines had EC_{50} values below 4 mM. Ascorbate-mediated cell death seems to be caused by protein-dependent generation of H_2O_2 in the extracellular space. The ascorbyl radical produced in this reaction should be eliminated by dismutation (see above). The nature of the proteins involved in the ascorbate-promoted generation of H_2O_2 is unknown, as is the mechanism for the selective killing by H_2O_2.

Results obtained when studying therapeutic effects of vitamin C in mice models of human diseases may not be directly applicable to humans in view of the fact that mice can synthesize ascorbate (see above). The crossing of *Gulo-/-* mice [6] with *e.g.* a mouse overexpressing mutant amyloid precursor protein or some other amyloid-generating protein may generate a ”humanized” mouse model. In summary, it appears highly warranted to further explore the mechanisms behind the benificiary effects of vitamin C on a variety of diseases by exploiting the novel functions for vitamin C.

Ackowledgements

Research carried out in the authors´ laboratory was supported by grants from the Swedish Science Council (VR-M), the Bergvall, Crafoord, Hedborg, Kock, Segerfalk, Zoega and Österlund Foundations, and the Medical Faculty of Lund University. We thank Prof. Catharina Svanborg at the Department of Microbiology, Immunology and Glycobiology, Lund University, for use of microscope facilities and Prof Gunnar Peijler, Uppsala University,

and Prof Peter Påhlsson, Linköping University, Sweden for generous gifts of monoclonal antibodies.

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Figure legends

Figure 1. Structure, uptake, interconversion and function of vitamin C redox forms. **(a)** L-Ascorbic acid (**I**) is oxidized, in two steps, first to ascorbyl radical (**II**) and then to dehydroascorbic acid (**III**). Dehydroascorbic acid can also be hydrated (**IV**) and converted to a bicyclic hemiketal (**V**). These reactions are reversible. Dehydroascorbic acid, which is a lactone, can be irreversibly hydrolyzed to 2,3-diketogulonic acid (**VI**). **(b)** L-Ascorbic acid is taken up by the sodium-vitamin C transporter (SVCT) and dehydroascorbic acid by glucose transporters (GLUT), presumably as the bicyclic hemiketal (**V** in **a**). Inside cells, dehydroascorbic acid is reduced to ascorbic acid. In the liver as well as in the brain, a glutathione-dependent dehydroascorbic acid reductase converts dehydroascorbic acid to ascorbate [51]. This enzyme is present mainly in the cytosol of neurons and not associated with any organelles except for the nucleus. Rat liver 3α-dihydroxysteroid dehydrogenase (oxidoreductase) reduces dehydroascorbic acid in an NADPH-dependent manner [52]. Also other reductases can convert dehydroascorbic acid to ascorbate using either glutathion or the thioredoxin system. **(c**) L-Ascorbate ***(i)*** stimulates hydroxylation of the hypoxia-inducible transcription factor (HIF) which targets HIF for destruction by the proteasome. In the absence of oxygen, HIF escapes hydroxylation and destruction and increases the expression of glucose transporters as well as nitric oxide-synthase (NOS) [16, 21]. Ascorbate ***(ii)*** also stimulates NOS to generate more nitric oxide (NO) from arginine and oxygen. NO activates guanylate cyclase, raising the level of cyclic GMP [53]. Another pathway by which NO signals is by modifying Cys thiols in proteins to Cys-SNO in a copper-dependent reaction.

HIF itself is one of the targets for S-nitrosylation. NO prevents destruction of HIF by inhibiting hydroxylation and modulates its transcriptional activities by nitrosylating a key Cys residue in HIF. The heparan sulfate proteoglycan glypican-1 is another target for S-nitrosylation. Ascorbate ***(iii)*** induces release of NO from glypican-1-SNO resulting in deaminative cleavage of its heparan sulfate side-chains (see Box 1). Glypican-1 is a carrier during spermine uptake and ascorbate-induced cleavage of its side-chains is required for unloading of the cargo (see Box 2).

Figure 2. Ascorbate-induced, NO-copper-dependent heparan sulfate degradation in Niemann-Pick C1 fibroblasts. The panels show confocal immunofluorescence microscopy images of subconfluent **(a-b)** normal HFL-1 and **(c-f)** NPC-1 fibroblasts **(a-d)** left untreated or treated with **(e)** ascorbate (ASC) or **(f)** suramin (SUR) and ascorbate (ASC) and then stained for glypican-1 (GPC-1, red) and anMan-containing heparan sulfate degradation products (AM, green). Bar, 20 μM. Copied from [41] by permission from Oxford University Press.

Box texts

Box 1. Glypican-1 and ascorbate-induced heparan sulfate degradation

Plasma membrane (PM)-associated glypican-1 is depicted (top left) as a protein with Cys thiols (SH) in the N-terminal globular domain, three heparan sulfate chains (long black bars) in which the glucosamine residues are sometimed N-unsubstituted ($GlcNH_3^+$, green star) and extending from the C-terminal part of the protein, and a C–terminal glycosyl-phosphatidyl-inositol-anchor (black oval with two small bars) plugged into a lipid raft (blue rectangle).

Glypican-1 is probably endocytosed via caveolae and recycles via endosomes and the Golgi back to the cell surface.

S-nitrosylation (SNO) of Cys in the glypican-1 protein part is a redox reaction that requires nitric oxide (NO) and Cu(II) ions. Cu(II) is delivered by cuproproteins and reduced to Cu(I). The heparan sulfate chains of recycling glypican-1 can be degraded either by heparanase or by nitroxyl (HNO) generated from NO that is released from the SNO groups in the glypican-1 core protein. In this step Cu(II) is regenerated from Cu(I). Release of NO is induced by an unknown reducing agent during passage from early to late endosomes [54] or by exogenously supplied ascorbate or dehydroascorbic acid depending on the cell type (Fig. 1 b). Late endosomes contain a strong reducing power of unknown nature [55] which may participate in the deaminative cleavage of heparan sulfate. The deaminative cleavage takes place at the $GlcNH_3^+$ residues and generates anMan-containing free heparan sulfate oligosaccharides (short black bars with a green pentagon at the end). Recycling endosomes transport the truncated glypican-1 to the Golgi, where new heparan sulfate chains are extended on the remaining stubs (black arrowheads).

Box 2. Uptake of spermine via recycling glypican-1

Lipid raft-associated glypican-1, substituted with polyanionic heparan sulfate chains in which the glucosamine residues are sometimes N-unsubstituted ($GlcNH_3^+$, green dot) binds the cationic polyamine spermine present in the external environment. Via caveolar endocytosis of glypican-1, spermine is internalized to endosomal compartments. Heparanase can cleave heparan sulfate chains that are free of cargo but the presence of spermine should exert steric hindrance. However, the heparan sulfate chains carrying spermine are accessible to deaminative cleavage by NO released from SNO groups in the glypican-1 core protein by

ascorbate. Spermine then exits the endosomes into the cytosol and can reach the nucleus. The confocal image shows cells that have taken up spermine from the environment. After 10 min of ascorbate treatment free spermine can be seen using a mAb specific for free spermine. Uptake of spermine is abrogated in cells where glypican-1 formation is silenced by RNAi. In mutant polyamine uptake-deficient cells, spermine uptake was partially restored by providing spermine NONOate and ascorbate. Bar, 20 μM.

Glossary box

Amyloid β – Short peptides (40-42 amino acids) derived from the transmembrane amyloid precursor protein by cleavage at a β-site located in the N-terminal external portion near the transmembrane segment and at a γ-site located within the transmembrane segment.

Anhydromannose- Mannose with an internal ether bond between C-2 and C-5.

Caveolae – Flask-shaped invaginations of the plasma membrane with a lipid composition similar to that of lipid rafts and containing caveolins attached to the cytoplasmic face.

Deaminative cleavage- In the present context it is the NO/nitrite-catalyzed cleavage of heparan sulfate at N-unsubstituted glucosamine residues. It involves the initial formation of an azido-derivative of the glucosamine, followed by ring contraction to an anhydro-manno configuration and subsequent cleavage the glycosidic bond to the preceding sugar in the chain [56].

Deglycanation – Removal of the glycan portion of a proteoglycan.

Glycosaminoglycan – The glycan portion of a proteoglycan. The basic structure is a disaccharide repeat, usually containing a uronic acid and a hexosamine, often substituted with sulfate groups at different positions.

Heparanase – An endoglucuronidase that cleaves heparan sulfate and heparin at sites where the glucuronic acid is located in a sulfated domain.

Heparan sulfate – A glycosaminoglycan with the basic disaccharide repeat, glucuronic acid (GlcA)-glucosamine (GlcN), where the GlcN may be N-acetylated or N-sulfated and the GlcA may be epimerized to iduronic acid (IdoA). The sugars are sometimes sulfated at C-3 or C-6 in GlcN and at C-2 in the uronic acids.

Lipid raft – A plasma membrane domain rich in cholesterol, sfingolipids and GPI-linked proteins.

Proteoglycan – A protein substituted with glycosaminoglycans.

S-nitrosylation – A reaction where Cys-SH and NO form Cys-SNO.

Spermine – H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2

Suramin – A urea substituted at both amino groups with a polysulfonated complex structure consisting of two benzene and one naphthalene rings joined by amide bonds. The compound serves as a heparan sulfate antagonist.

Tetrahydrobiopterin – A cofactor of amino acid catabolism that is similar to the heterocyclic, fused two-ring pterin moiety of tetrahydrofolate, but it is not involved in one-carbon transfers; instead it participates in oxidation reactions.

(a)

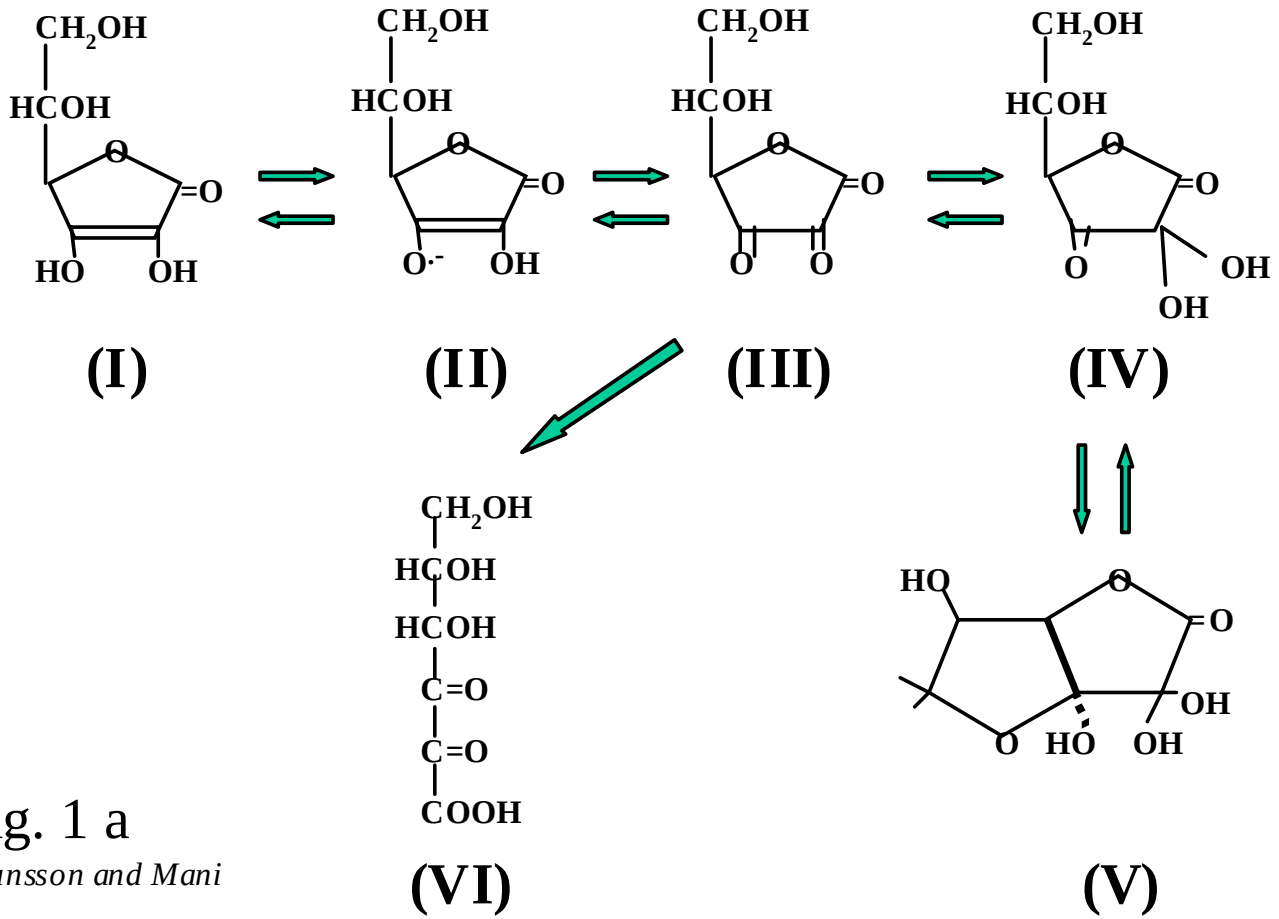

Fig. 1 a

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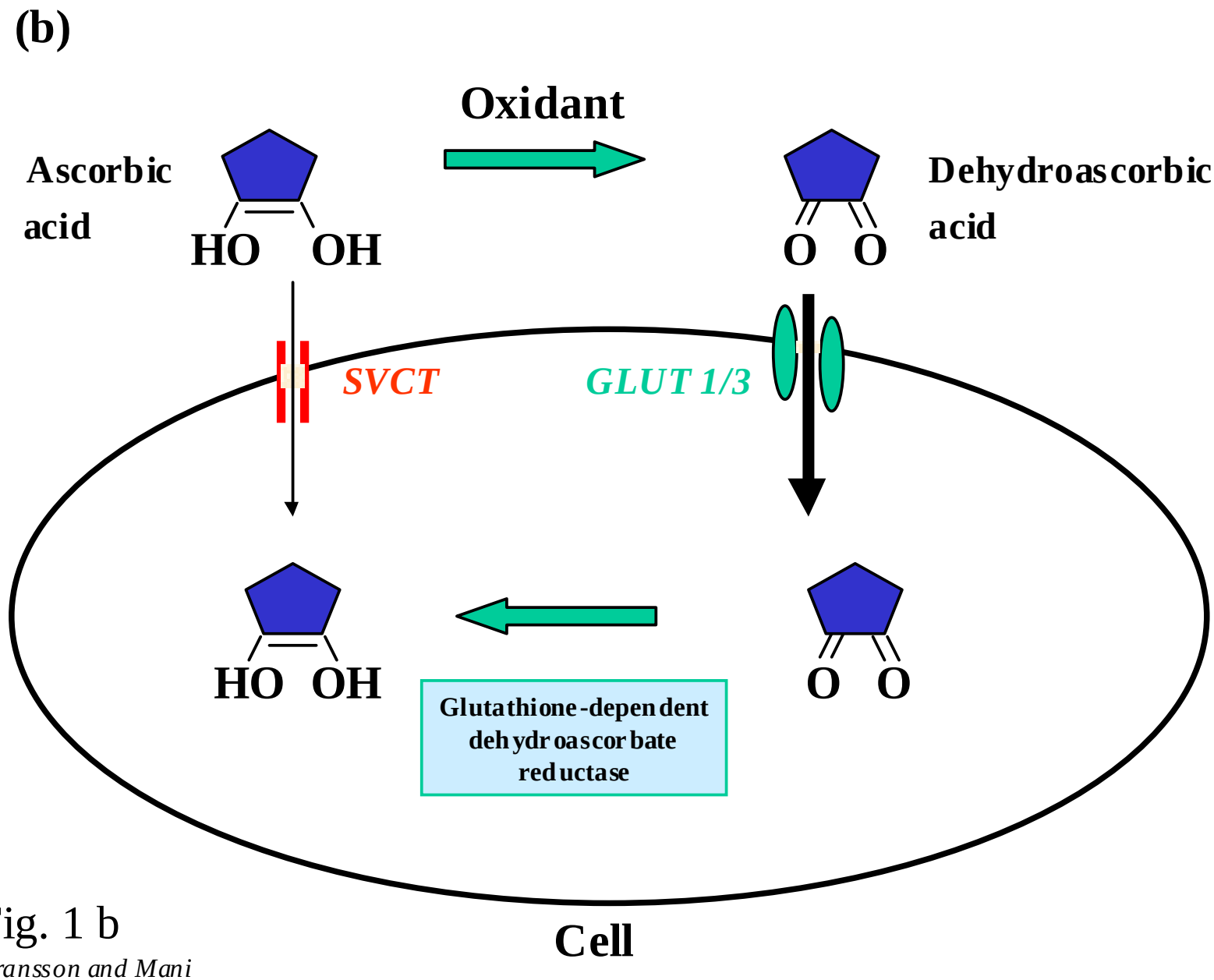

Fig. 1 b

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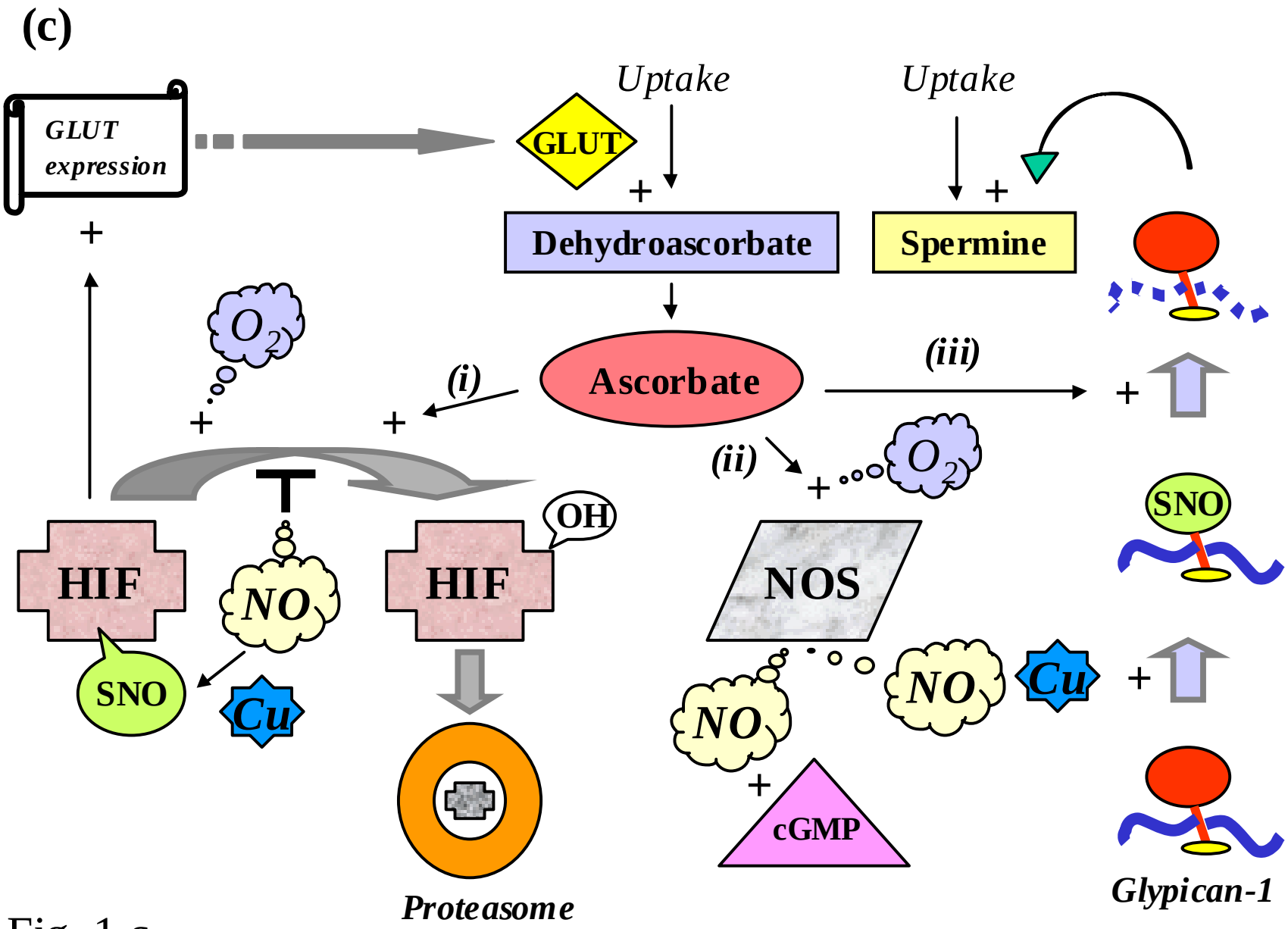

Fig. 1 c

Fransson and Mani

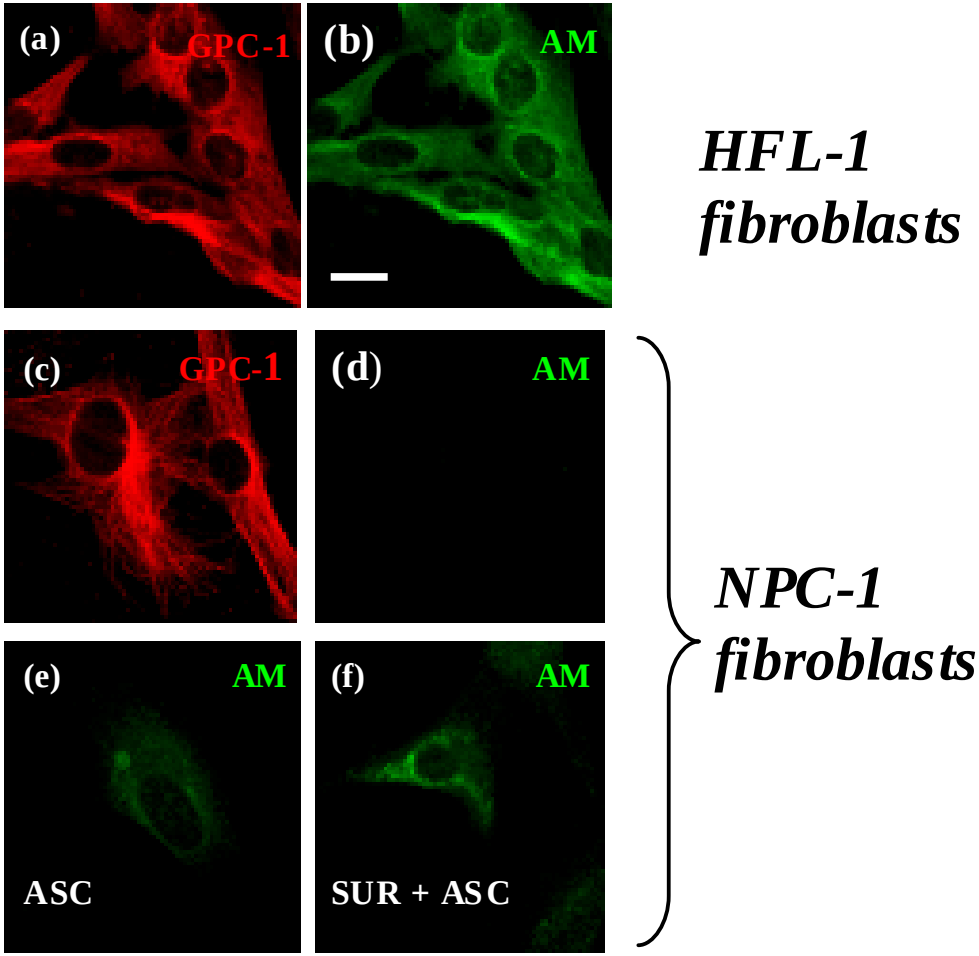

Fig. 2

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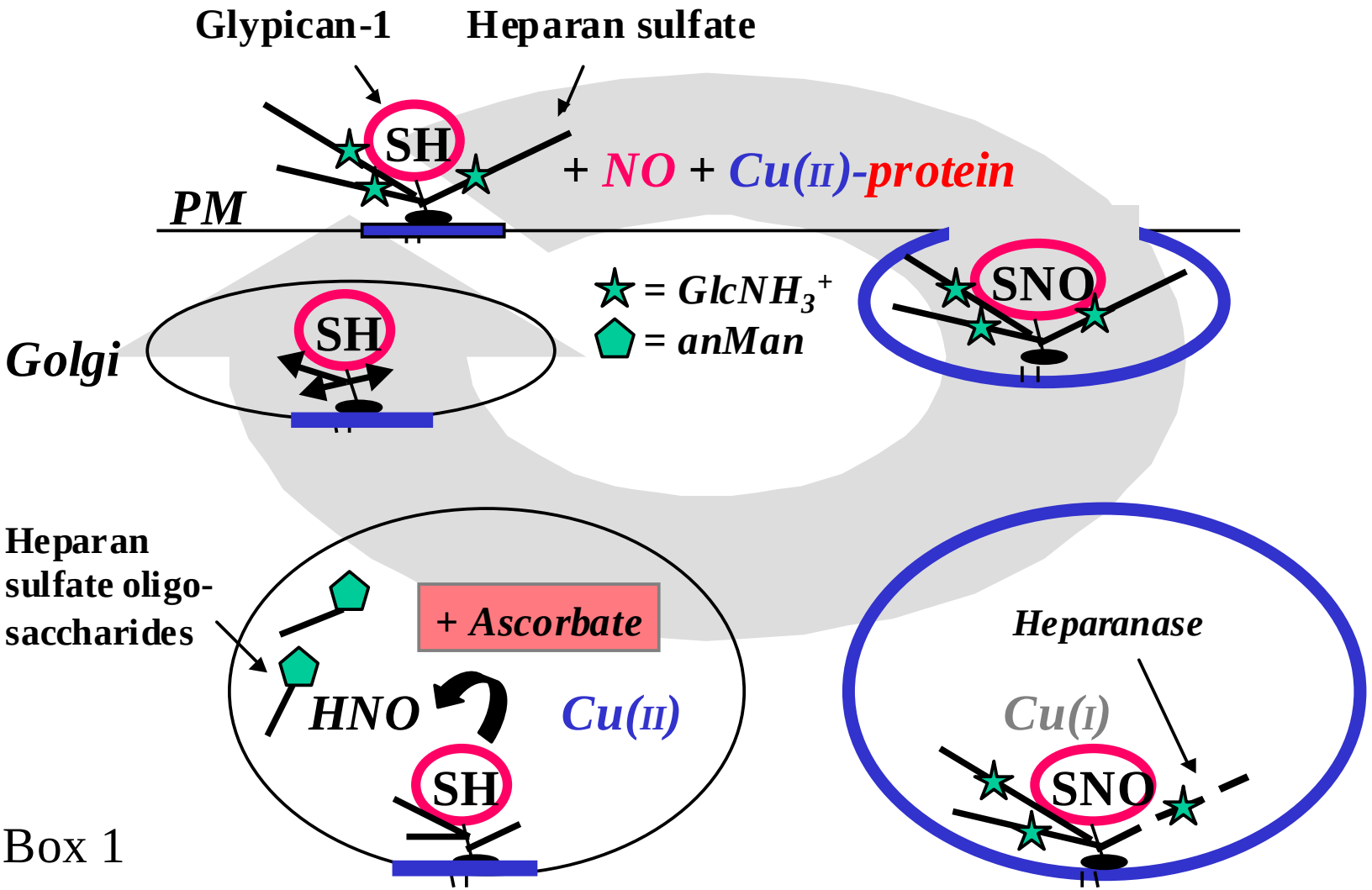

Box 1

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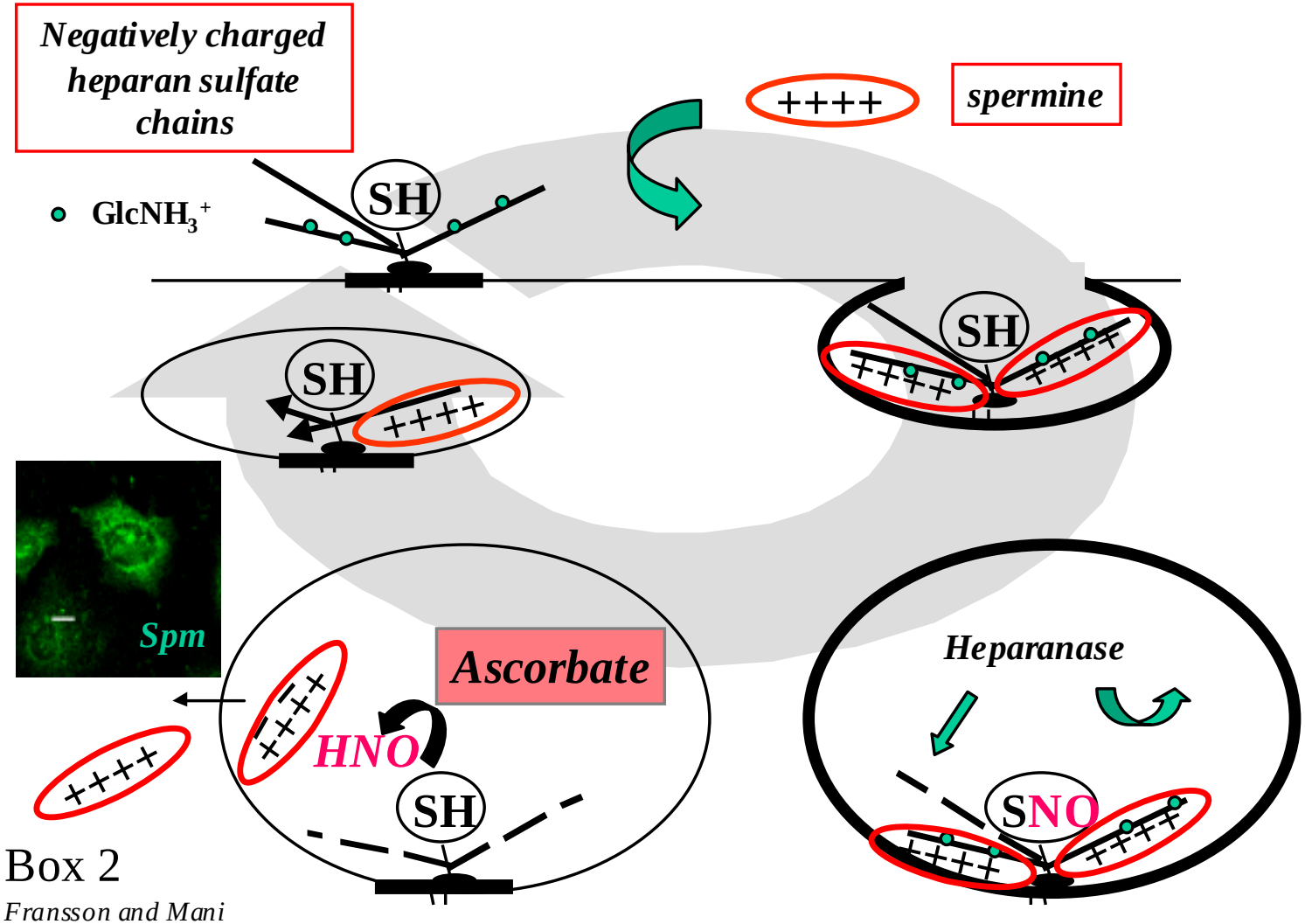

Box 2

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