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## **Carbohydrate nanocarriers in biomedical applications: functionalization and construction**

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## Abstract

The specific targeting of either tumor cells or immune cells *in vivo* by carefully designed and properly surface-functionalized nanocarriers may become effective therapeutics for the treatment of a variety of diseases. Carbohydrates, as prominent biomolecules, have shown their outstanding ability in balancing the biocompatibility, stability, biodegradability, and functionality of nanocarriers. The recent applications of sugar (mono/oligosaccharides and/or polysaccharides) for the development of nanomedicines are summarized in this review, including the application of carbohydrates for the surface-functionalization of various nanocarriers and for the construction of the nanocarrier itself. Current problems and challenges are also addressed.

**Keywords:** carbohydrate, sugar, saccharide, nanoparticle, drug delivery, polysaccharide, nanocarrier.

## 1. Introduction

Since Paul Ehrlich has coined the term of the "magic bullet" for modern medicine in the beginning of 20<sup>th</sup> century, the development of targeted drug delivery has received immense interdisciplinary attention, ranging from chemistry over biology to medicine.<sup>1</sup> In the last decades, the idea has gradually evolved to the application of nanometer-sized vehicles for the delivery of drugs, due to their advantages including *i*) to protect the payload from degradation *in vivo*, *ii*) to allow specific targeting to the diseased tissue and thus *iii*) to reduce the risk of systemic toxicity, and, finally, *iv*) to release the drug, while the carrier is eliminated from the body without trace. All these properties have been realized partly in today's nanomedicine, however, have still not been accomplished completely. The innovative design and chemical functionalization of suitable nanocarriers is thus still the challenge to finally generate "magic bullets", selective drug delivery systems, of the 21<sup>st</sup> century.

The early-stage nanocarriers were mostly prepared from artificial polymeric<sup>2-5</sup> or inorganic materials.<sup>4, 6-9</sup> To increase their blood circulation times poly(ethylene glycol) (PEG) is often attached to their surface as the so called "stealth layer" decreasing protein adsorption.<sup>4-6, 10</sup> These nanocarriers suffered several intrinsic drawbacks, especially regarding their biocompatibility and biodegradability. More recently, the research focus shifted to use natural materials for the fabrication of nanocarriers, which are inherently compatible with the metabolic system and have a high potential in their biological and biomimetic effects.

Together with lipids, proteins, and nucleic acids, carbohydrates (or saccharides) are one of the four major classes of biomolecules. The combination of several advantages of carbohydrates makes them unique candidates for application in nano-medicine:

- i) chemically well-defined structure
- ii) biocompatible/ biodegradable
- iii) available on large scale
- iv) protein-repellent
- v) high water solubility
- vi) no aggregation
- vii) natural targeting agents

In contrast to proteins and nucleic acids, when oligo/poly saccharides are formed through chain elongating and branching, the linkage points between sugar units are not restricted to constant positions. Regioisomers can be formed by elongating the sugar chain at different hydroxy groups, resulting in a significantly enhanced code capacity. While 20 amino acids yield  $6.4 \times 10^7$  hexapeptide isomers, the same amount of hexose repeating units in an oligosaccharide will result in  $1.44 \times 10^{15}$  different isomers.<sup>11</sup> In addition, most of the carbohydrates are located on the outer surface of the cell, in the extracellular fluid and blood,<sup>12</sup> which is the biological environment for the intravenously injected nano-medicines. The molecular understanding of the peculiarities of carbohydrates will help to pave the road for the translation of “sweet” nano-medicines to the clinic. Besides their role in biological signaling, carbohydrates also have other biological functions, including energy storage, protection of cell organelles<sup>13</sup>, modification of the properties of peptides or proteins<sup>14</sup>, etc., which might grant the nano-medicine additional advanced properties. In addition, carbohydrates are responsible for cell/cell, and cell/matrix communications and interactions in cellular organelles or multicellular organs.<sup>15-17</sup> Studying and utilizing the information from this natural “glyco-code” and exploiting the differences between healthy and malignant cells is a promising strategy for the diagnosis and treatment of cancer.<sup>18</sup>

Besides the biological origin of oligo- and polysaccharides and their important role in biological communication, their inherent hydrophilicity makes them even

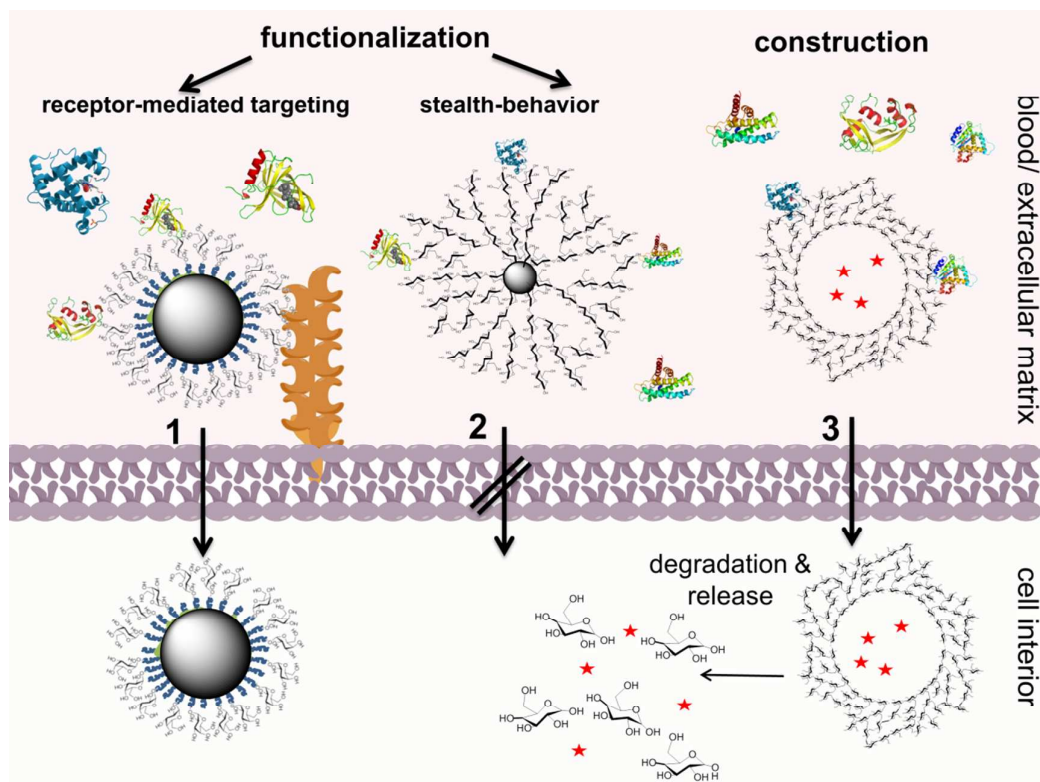
more attractive for biomedical polymer science. They are currently discussed as potential biodegradable substitutes for PEG, to reduce unspecific protein adsorption.<sup>19</sup> It has been reported that hydroxyethyl starch (HES),<sup>20-24</sup> a synthetically modified starch derivative, but also dextrin<sup>25-27</sup> or other saccharides<sup>28, 29</sup> can reduce the protein adsorption on nanocarriers and prolong their circulation time in the blood stream similar to PEG. This protein-repellent property, together with their active biological function to interact with certain proteins/ cell surfaces, renders carbohydrates very promising elements for the construction of future therapeutics.

Another feature making carbohydrates interesting for drug delivery is their biodegradability. This does not only ensure the eventual body clearance of the materials, but is an additional handle to trigger drug release or activation by certain enzymes.<sup>23-27</sup> For HES, for example, degradation kinetics can be precisely adjusted by varying the degree of hydroxyethylation.<sup>23, 24</sup> In summary, the i) biological activity, combined with ii) the potential stealth properties, and iii) the enzymatic stimulus makes carbohydrates interesting materials for the design of nanocarriers for biomedical applications. Both, surface-modification of preformed nanoparticles with carbohydrates or the direct construction of the nanocarriers from mono-, oligo-, or polysaccharides have attracted considerable attention during the last decade over the borders of single disciplines.

There are some reviews concerning the use of polysaccharides in nanomedicine, such as an - at that time comprehensive - article covering sugar-decorated nanoparticles from 2004.<sup>21</sup> More recent reviews cover peptide- and saccharide-conjugated dendrimers (from 2012),<sup>30</sup> and nanoparticles based on polysaccharides (2008<sup>31</sup> and 2014<sup>32</sup>), which mainly focus on the synthetic methods. In 2013, a review disclosed comprehensively the application of carbohydrate functionalized nanoparticles as sensitive detection agents, inhibitors of bacterial adhesion, cancer vaccines in

therapeutic systems, drug delivery agents, with focus on their imaging and detecting properties.<sup>33</sup> Two interesting reviews about glyco-nanoparticles have also been published in 2013; both focused on inorganic nanoparticles, like carbon nanomaterials, metal nanoparticles, quantum dots (QDs), magnetic (MNPs) and silica nanoparticles (SNPs). Marradi et al. elaborately discussed the density and orientation of sugars and their influence on the multivalency of binding,<sup>34</sup> while Reichardt et al. summarized applications of glyco-nanoparticles in molecular imaging, biosensors for lectin/glycan, new concepts for the affinity separation and analysis, and vaccine development,<sup>35</sup> all of which will not be the focus of the present review. A more recent review on glyco-nanoparticles was published in 2014,<sup>36</sup> which also focused on inorganic nanoparticles and their application for imaging and diagnostics.

Herein, recent design strategies for carbohydrate-based nanocarriers will be reviewed: our collection covers the surface-functionalization of nanoparticles with as well as the full construction of nanocarriers from saccharides. In addition, both monomeric and oligo/polymeric carbohydrate-motifs are reviewed, as depicted in Scheme 1. The main focus of this review is to comprehensively present the advantages of carbohydrates as major components in drug delivery systems.



Scheme 1. Schematic summary of the content of this review. 1. Surface modification of the nanocarriers by certain sugars, e.g. mannose, can enhance the receptor mediated uptake of the nanocarrier. 2. Surface modification of the nanocarriers with other saccharides, e.g. dextrin (polysaccharide of glucose), can decrease the unspecific uptake of the nanocarriers by cells. 3. Nanocarriers fully based on saccharides can be degraded by cells, which will result in the release of the payload.

The review is structured as follows: the fundamental part will discuss the properties of carbohydrates for drug delivery, the chemical relations between different sugars, and how nature functionalizes proteins with saccharides to produce glycoproteins. The subsequent part will address the applications of carbohydrates for i) the surface-functionalization of nanocarriers and ii) the construction of nanocarriers. In the final part, the overall benefits gained from the application of carbohydrates are summarized and we give an outlook on



potential future applications (Figure 1 gives an overview about the structural relations of carbohydrates, which are discussed herein).

## 2. Fundamentals

### 2.1 Recognition of saccharides by cell surface receptors and the use for targeting of specific cells.

Cells of higher organisms are in constant communication and interaction with their environment. In order to survive and maintain the appropriate functions, external signals must be received by the cell-surface, and subsequently delivered into the cell's interior.<sup>37</sup> While many of these biological informations are encoded and delivered by protein-protein interactions, carbohydrates also play a significant role.<sup>18, 38, 39</sup> Carbohydrates act as recognition markers in different pathological and physiological processes, most of them occurring on the surfaces of cells. Three classes of proteins serve as receptors for the carbohydrate ligands: enzymes (for the synthesis, remodeling and degradation of carbohydrate), immunoglobulins and, most importantly, lectins<sup>40</sup> which are membrane-bound receptors and assist during the process of endocytosis.<sup>41</sup>

Through the binding with these receptors, many types of carbohydrates, including mono-, oligo-, and polysaccharides have been found to specifically bind to certain cell types. Mono/oligosaccharides like mannose derivatives exhibit strong binding to the C-type lectin DC-SIGN on the surface of dendritic cells,<sup>42</sup> C-type lectin receptors on alveolar macrophages,<sup>43</sup> and the plant lectin concanavalin A.<sup>44</sup> Galactose can also bind selectively to C-type lectin receptors on alveolar macrophages<sup>43</sup> and carbohydrate receptors on *E. coli* cells.<sup>45</sup> Lactobionic acid can bind to asialoglycoprotein receptors (ASGP-R) of hepatic tumor cells.<sup>46</sup> For rhamnose, a specific targeting effect to human skin cells was demonstrated.<sup>47</sup> Polysaccharides like hyaluronic acid or chitosan

have been found to specifically bind to ocular mucosa.<sup>48-50</sup> Functionalized dextran has proven to specifically target vascular smooth muscle cells<sup>51</sup> and human endothelial cells.<sup>52</sup> Many cellular events are regulated by these sugar codes, including cell adhesion, proliferation, and cell death.<sup>53-56</sup>

Cancer still is one of the most prevalent deadly diseases worldwide and constitutes one of the two major causes of death in industrialized countries. While the complete eradication of malignant tumor is severely complicated by a tendency to form metastases, all malignant cells have special biological signatures which distinguish them from their healthy counterparts.

Carbohydrates, in particular glycoconjugates, play an essential role for cancer metastasis and communication, through the interaction with endogenous lectins present on the cancer cells.<sup>57-59</sup> Presumably due to the fast metabolism of the tumor tissue, some of these lectins, e. g. galectins, are found expressed at an elevated level on malignant cells while they are not expressed detectably by their healthy counterparts.<sup>12, 60</sup> Defined by their role as  $\beta$ -galactose receptors,<sup>61</sup> galectins have been reported as indicator for malignancies in stomach,<sup>62</sup> liver,<sup>63</sup> and the corresponding colon cancer.<sup>64-66</sup> A high galectin-1 level was reported in papillary carcinomas, but not in the healthy tissues.<sup>67, 68</sup> A significant increase in the galectin-1 expression in adenocarcinoma cells was also reported, in contrast to the adjacent normal endometrium.<sup>69</sup> In addition, other carbohydrates, like hyaluronic acid also shows specific binding to CD44 receptors<sup>70, 71</sup> which are expressed at low levels on hematopoietic, epithelial, and neuronal cells but at much higher levels in various tumor cells like lymphomas, melanomas, colorectal, and lung tumor cells.<sup>72, 73</sup> Thus, many carbohydrate-related biomarkers have been developed which individually exhibit specific binding to different cancer cells,<sup>74</sup> and may open up possibilities to specifically target cancer cells by an appropriate carbohydrate functionalization of nanocarriers.

## 2.2 Protein repellent properties of carbohydrates

When a nanocarrier enters a biology fluid, e.g. is intravenously injected into the bloodstream, it will adsorb proteins on its surface, due to hydrophobic interactions and the high surface energy of most types of nanocarriers.<sup>75, 76</sup> This procedure, known as opsonization, can lead to phagocytosis of the nanocarrier by the Mononuclear phagocyte system (MPS). The adsorbed proteins will determine the fate of the nanocarrier *in vivo* (this process is often called the formation of a “biological identity”),<sup>75</sup> typically resulting in the fast clearance of the nanocarriers from the blood. This makes any *in vivo* specific targeting a challenging task.<sup>75</sup> In order to prolong the *in vivo* plasma half-life times of the nanocarriers, the opsonization needs to be reduced, either by the material of the nanocarrier itself, or by surface modification (the dress of the nanocarriers). Currently, PEGylation is the “gold standard” to achieve long blood circulation times and reduced unspecific cellular uptake due to the hydrophilicity and the steric repulsion by PEG-modified surfaces and proteins.<sup>19</sup> PEGylation has achieved numerous successes in the past decades, and many PEG-related products both in consumer care and biomedical applications have improved the quality of life.<sup>19, 77</sup> In spite of these achievements, recent studies reported several drawbacks of PEG. The occurrence of renal tubular vacuolization in animal models have raised concerns that a prolonged therapy with PEGylated drugs may lead to an accumulation of PEG in the cytoplasm of kidney cells as the polymer is not biodegradable.<sup>78, 79</sup> In addition, PEG potentially forms toxic degradation products upon storage which could provoke adverse effects.<sup>19</sup> These setbacks of PEG could be circumvented by using polysaccharides as substitutes which often show low hypersensitivity even after chemical functionalization.<sup>80</sup> The structural similarity of many polysaccharides, for example HES or dextran, to the sugar component of glycogen, which is the form for the storage of sugar in animals, is a probable explanation why they lack immunogenicity. Moreover,

the biodegradability of polysaccharides is advantageous over many other synthetic polymers that are currently discussed as alternatives for PEG.<sup>81</sup> Not only the post-injection clearance of the nanocarriers is enhanced by its biodegradability, but also enzymatic induced masking-unmasking or encapsulation-release cascades of the payload are possible.<sup>23-27, 44</sup> However, care has to be taken depending on the chemical modification, e.g. anchoring or polymerizable groups that may alter both the degradation process and the cytotoxicity of the carbohydrates.

Numerous studies have already proven that polysaccharide or their derivatives like HES<sup>20-22, 82</sup> or dextran exhibit a low protein affinity.<sup>28, 29</sup> Furthermore, the microbial polysaccharide pullulan, glycolipids, and dextran have shown their ability to decrease the uptake of nanocarriers into the MPS,<sup>83, 84</sup> while HES has been proven to suppress the unspecific uptake of the nanocarriers *in vitro*,<sup>85</sup> and prolong the plasma halftime *in vivo*, the process of its attachment also being called HESylation.<sup>23, 24</sup> While several mono- or oligosaccharides are responsible for the communication of biological information in the organism, some of them are capable of impeding the phagocytosis of native cells by the MPS. Sialic acid is one example of these saccharides and red blood cells without surface sialic acid are immediately removed from the blood by the MPS.<sup>86</sup> It has been proven, that when sialic acid is coupled to the surface of quantum dots, the *in vivo* plasma half-life time of the latter is prolonged.<sup>87</sup>

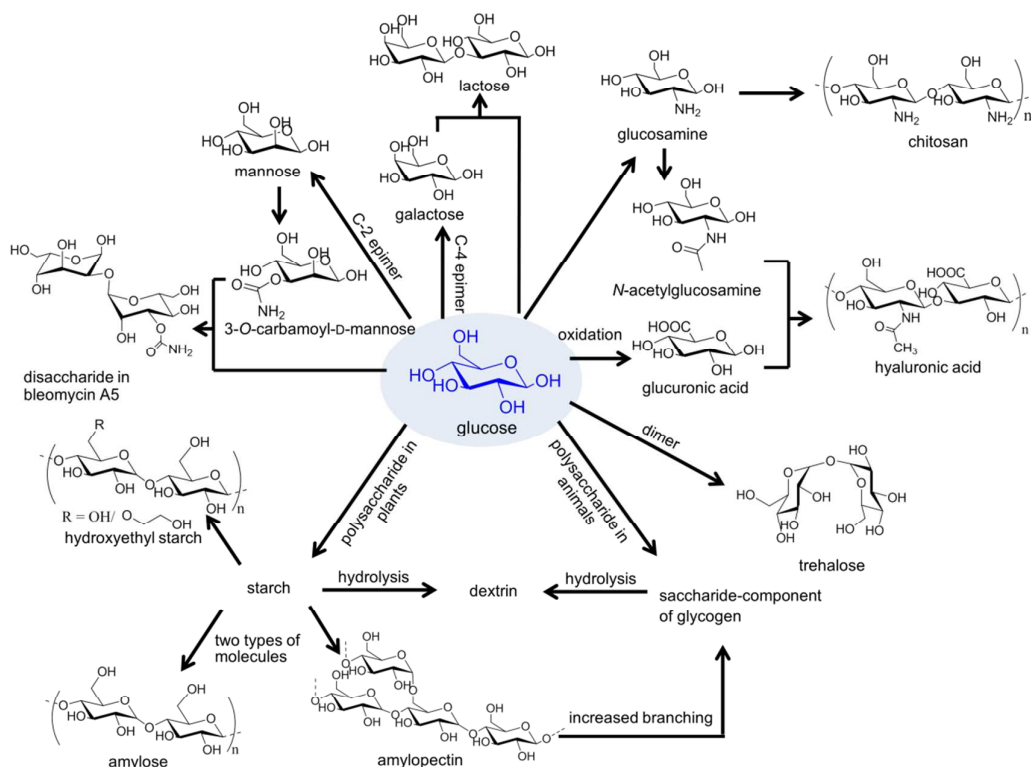


Figure 1. Relations between different carbohydrates, which are relevant for this review.

### 2.3 Glycoproteins: how nature uses carbohydrates

In nature, glycoproteins, i.e. glycosylated polypeptides, are of high importance and function as hormones,<sup>88</sup> antibodies,<sup>89</sup> antifreeze proteins,<sup>90</sup> and proteins in the cell membrane.<sup>14</sup> After glycosylation, the attached (oligo)saccharides provide additional properties for the protein, such as facilitating the protein folding and stabilizing the conformation of the peptidic backbone,<sup>91</sup> protection,<sup>92</sup> elongation of the *in vivo* plasma half-life,<sup>93</sup> communication with the immune system,<sup>94</sup> and adhesion to cognate receptors on other cell surfaces.<sup>95, 96</sup>

Inspired by these natural strategies, various researchers have prepared neoglycoproteins for diverse applications. Pharmacologically active peptides have been used for the treatment of various diseases.<sup>77, 97</sup> A major drawback, however, is their usually rapid degradation *in vivo*. To optimize the pharmacokinetic properties of such drugs, artificial polymers are frequently coupled to their surface. Typically PEG is used for this purpose but in modern literature, an increasing percentage of biodegradable biopolymers are coupled to proteins to optimize their therapeutic performance. For example, hyaluronan-functionalized insulin showed a prolonged and enhanced hypoglycemic effect, demonstrating the potential of hyaluronan for increasing the plasma half-life of peptides.<sup>98</sup>

Anakinra, a synthetically generated interleukin-1 antagonist, is used for the treatment of rheumatic arthritis, but has a plasma halftime of only 108 min; after conjugation with HES its blood circulation time was increased by a factor of 6.5.<sup>99</sup>

Dextrin, a glucose polymer with a molecular weight of 7,700 and 47,200 g/mol and a degree of succinylation of 9-32 mol% was used to functionalize trypsin (a serine protease) and thus masking its activity. The activity of the enzyme can be restored after degradation of the polysaccharide by  $\alpha$ -amylase.<sup>25-27</sup> Also hyaluronic acid was used for the functionalization of trypsin, resulting in an increase of its activity to 145% over the native protein, while exhibiting a 52% higher stability in the presence of elastase (a protease).<sup>100</sup> Although these works are beyond the scope of this review, the idea of mimicking nature to utilize the advantageous properties of different sugars is identical.

### 3. Carbohydrate-functionalized Nanocarriers

Being a C-2-epimer of glucose, mannose is an important monosaccharide for the glycosylation of proteins. Mannose-containing glycoproteins are produced in the liver and secreted into the blood, hence mannose is distributed throughout the body.<sup>101</sup> Many mannose-binding proteins, like the C-type lectins, are crucial for cell-surface recognition and other communication events.<sup>102</sup> Recently, mannose has been applied to functionalize mesoporous silica nanoparticles,<sup>44</sup> magnetic nanoparticles,<sup>18</sup> gold nanoparticles,<sup>42</sup> and polyanhydride nanoparticles<sup>43</sup> (Table 1, entry 1) to specifically target cells; distinct biological functionalities have been achieved in each case.

When thiol-functionalized mannose is reacted with alkenyl-terminated silanes in a radical thiol-ene addition (Table 1, entry 1, a), surface functionalization of mesoporous silica nanoparticles can be achieved, whose pores can be sealed by adding concanavalin A, a carbohydrate-binding protein, to the dispersion. The pores can be re-opened under acidic conditions (pH < 5.5) or in a glucose-rich environment. Release of the payload in the tumor tissue, where the pH value is typically lower than that in healthy tissue, or under high blood sugar level is thus possible.<sup>44</sup> In another work, mannose-functionalized silica nanoparticles have been prepared, which showed specific binding to MCF-7 human breast cancer cells.<sup>103</sup>

Carboxylated derivatives of mannose (Table 1, entry 1, b), galactose (Table 1, entry 2, a), fucose (Table 1, entry 3), and sialic acid (Table 1, entry 4, a) have been coupled to amino-functionalized magnetic nanoparticles via an amide linkage. When these nanoparticles were incubated with different malignant and non-malignant cells and investigated via magnetic resonance imaging, it was shown that the malignant cells can be differentiated by the changes of  $T_2$  relaxation time ( $\% \Delta T_2$ )<sup>18</sup>, as shown in Figure 2. Similar work was conducted using sialic acid-functionalized magnetic nanoparticles to detect the levels of

$\beta$ -amyloid, which is a pathological hallmark of Alzheimer's disease, both *in vitro* and *ex vivo*.<sup>104</sup>

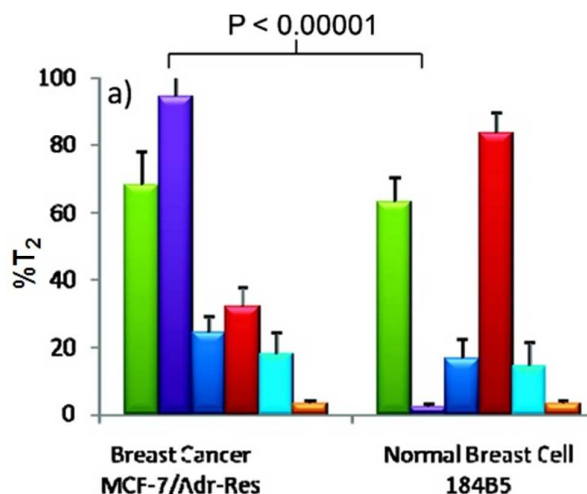


Figure 2. Discrimination of the breast cancer cells from their healthy counterparts by the changes of  $T_2$  relaxation time ( $\% \Delta T_2$ ) in magnetic resonance imaging, by magnetic particles functionalized with: mannose (green), galactose (violet), fucose (dark blue), sialic acid (red), glucose (light blue), compared to unmodified magnetic particles (orange), (adapted with permission from reference<sup>18</sup>. Copyright 2010 American Chemical Society).

Different (oligo)mannosides have also been functionalized with thiols and coupled to gold nanoparticles.<sup>42</sup> The obtained glycosylated gold nanoparticles show stronger binding to DC-SIGN (a C-type lectin) on the surface of dendritic cells compared to gp120, which is a protein essential for the entry of HI virus into cells, and thus could serve as a potential carbohydrate-based drug against HIV (Table 1, entry 1, c). Similar glycosylated gold nanoparticles have also been prepared in another work and observed to cross the blood–brain barrier (BBB) nearly 3-fold faster / more efficiently than unmodified gold nanoparticles.<sup>105</sup>

Both,  $\alpha$ -1,2-linked dimannose (Table 1, entry 1, d) and galactose (Table 1,



entry 2, g) have been coupled to polyanhydride nanoparticles through an amidation reaction via 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) as the coupling agent. The obtained mannose surface-functionalized particles, which were termed “pathogen-like” nanocarriers, exhibited specific binding to alveolar macrophages through the surface C-type lectin and enhanced the expression of the macrophage mannose receptor.<sup>43</sup>

It is the concern of some recent publications that the protein adsorption after contact with blood will hamper all specific targeting of nanocarriers due to shielding of targeting groups, which might reduce the efficiency of “targeted” drug delivery systems remarkably.<sup>106-113</sup> A current challenge is to understand the interaction of blood proteins with nanocarriers which carry additional targeting groups. The adsorption of plasma proteins onto the targeting agent could hinder the recognition of the targeting agent by the respective cells and hence could make any *in vivo* targeting impossible.<sup>75</sup> The interactions of mannose-functionalized nanocarriers with plasma proteins have been studied to address this problem. It turned out that, in comparison to a PEGylated nano-carrier, additional functionalization of the PEGylated nanocarrier with mannose did not significantly change its protein corona formation. Furthermore, these mannose functionalized nanocarriers showed the same binding affinity to dendritic cells (DCs) both in the presence and absence of the plasma protein corona.<sup>114</sup>

*Galactose* is the C-4 epimer of glucose and is for example essential for the antigen structure of red blood cells which is the determinant of the blood type. For O and A antigens, two galactose units are contained in the saccharide portion while for the B antigen, three galactose units are contained.<sup>115</sup> Galactose functionalized with an azide group at the C1-position, was coupled to pillar[5]arene by a Huisgen-type cycloaddition, while the latter is self-assembled into nanorods (Table 1, entry 2, c), which have proven a high

affinity for the carbohydrate receptors on *E. coli*. as well as low toxicity, and can be utilized as excellent cell glues to agglutinate these bacteria.<sup>45</sup> In another work, different statistical glycol-dithiocarbamate copolymers were prepared and used to functionalize gold nanoparticles on the surface, which were further coupled with gold(I) triphenylphosphine as an anticancer agent. Among these glyconanoparticles, the galactose-functionalized ones were found to be 4-fold more cytotoxic to HepG2 cells, in comparison with glucose and lactose functionalized particles.<sup>116</sup>

*Sialic acid* is a monosaccharide, which is widely distributed in animal tissues and mostly bound in form of glycoproteins.<sup>117</sup> It plays an important role in recognition and communication with the immune system,<sup>118-120</sup> which is also proven by the fact that red blood cells without sialic acid on the surface are immediately removed from blood by the MPS.<sup>86</sup> Ketone-functionalized sialic acid is reacted with aminoxy-functionalized quantum dots, namely phosphorylcholine self-assembled monolayer-coated quantum dots (PC-QDs), and their *in vivo* half-life times are extended compared to quantum dots functionalized by other monosaccharides (Table 1, entry 4, b; and Figure 3).<sup>87</sup>

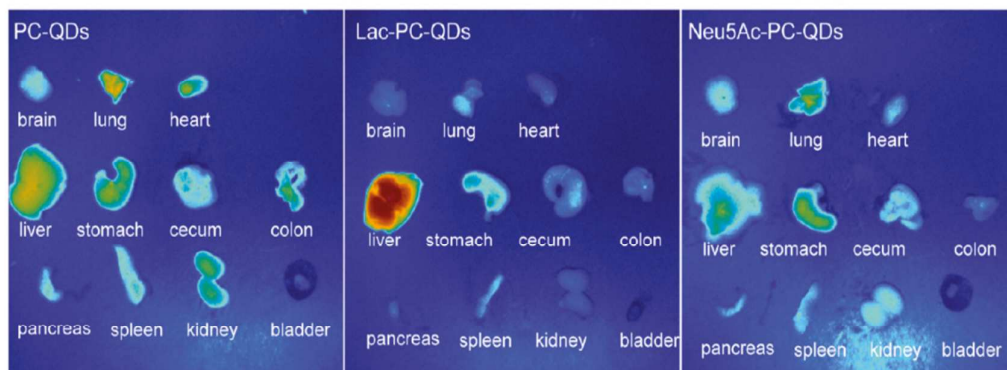


Figure 3. Images of major organs isolated from three tested mice, 2 h after the administration of phosphorylcholine quantum dots (PC-QDs), lactose-functionalized quantum dots (Lac-PC-QDs), and sialic acid-functionalized

quantum dots (Nue5Ac-PC-QDs). (Reprinted with permission from reference<sup>87</sup>. Copyright 2011 American Chemical Society).

*Sialyl-Lewis<sup>X</sup>*, one of the most important blood group antigens, which is displayed on the terminus of glycolipids that are present on the cell surface, has been used to functionalize superparamagnetic silica nanoparticles (Table 1, entry 4, c), with the functionalization strategy shown in Figure 4. These nanoparticles have diameters of around 18 nm and carry NH<sub>2</sub>-groups.<sup>121</sup> Subsequent functionalization of these particles with an NHS-ester allows coupling to amino-functionalized *Sialyl-Lewis<sup>X</sup>*. The obtained glycosylated nanoparticles bind specifically to the inflammation-associated endothelial transmembrane proteins E and P selectin, both cell adhesion molecules. *In vivo* studies have shown an accumulation in the brain vasculature by measuring the relaxing time of the nanoparticles via MRI.<sup>121</sup>

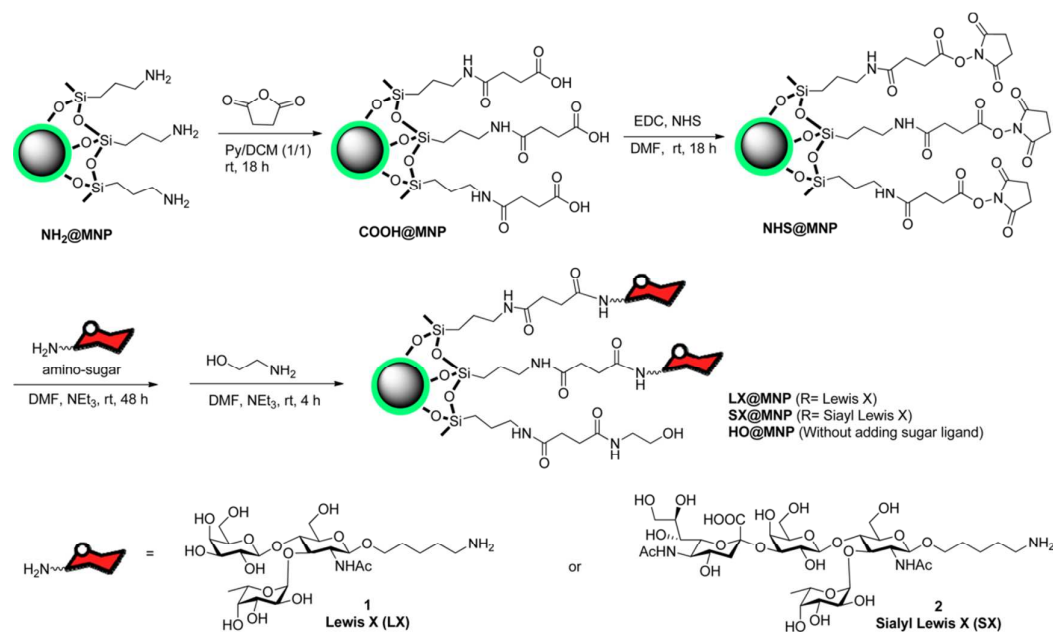


Figure 4. functionalization of superparamagnetic nanoparticles with a silica core by *Sialyl-Lewis<sup>X</sup>*. (Reprinted with permission from reference<sup>121</sup>. Copyright 2014 American Chemical Society).

*Lactobionic acid* (4-O- $\beta$ -D-galactopyranosyl-D-gluconic acid, (Table 1, entry 6))

specifically bind to hepatocytes.<sup>122</sup> Thiolated lactobionic acid was used to functionalize block copolymers, which were prepared by the ring-opening copolymerization of  $\epsilon$ -caprolactone and a pyridyl disulfide containing cyclic carbonate, followed by post polymerization modification with thiolated lactobionic acid via the thiol-disulfide exchange reaction. The post-modified block copolymers then self-assembled into micelles with lactobionic acid on the surface. These micelles were shown to target liver cancer through asialoglycoprotein receptors (ASGP-R), furthermore, the saccharide shells is cleavable under a reductive environment mimicking the interior of a cell.<sup>46</sup>

*Rhamnose* is a mannose-related 6-deoxy hexose which naturally occurs in the L-form. It is found mainly in bacteria and plants and is often present in the cell walls and is essential for the survival of bacteria.<sup>123</sup> Phosphonated rhamnose has been prepared (Table 1, entry 7) and anchored to magnetic nanoparticles through the strong binding of phosphonates groups to metals. The rhamnose-functionalized magnetic nanoparticles exhibited targeting effect to human skin cells. Since the iron oxide nanoparticles are superparamagnetic, they can be used as MRI contrast agent with specific cell targeting.<sup>47</sup>

*Bleomycin* is a glycopeptide-type antibiotic, bleomycin (BLM, Figure 5), has strong antitumor abilities and is used for the treatment of malignant lymphomas and squamous cell carcinomas.<sup>124, 125</sup> Additionally, BLM also has shown a specific tumor cell targeting effect, and hence has been used for tumor imaging.<sup>126</sup> In order to understand the origin of the tumor targeting effect, it has been split into the BLM-analogue deglycobleomycin (devoid of the disaccharide moiety) and the disaccharide moiety itself. The difference for the ability for specific tumor targeting of these two derivatives has been investigated, and the disaccharide motif was found to be responsible for selective binding to MCF-7 human breast carcinoma cells and BxPC-3 pancreatic cancer cells, while having their healthy counterparts not being targeted.<sup>127</sup> In contrast, deglycobleomycin (*Bleomycin* without the disaccharide

moiety) did not show any specific targeting. Furthermore, after coupling the disaccharide moiety to the surface of microbubbles (Table 1, entry 8), which consist of an empty core and a lipid shell, and originally used as contrast agents for ultrasonography,<sup>128</sup> specific targeting of MCF-7 human breast carcinoma cells has been observed. Furthermore, the subsequent study verified that it is a single sugar unit from this disaccharide, namely the carbamoylmannose moiety, which is responsible for the tumor cell specific targeting effect.<sup>129</sup>

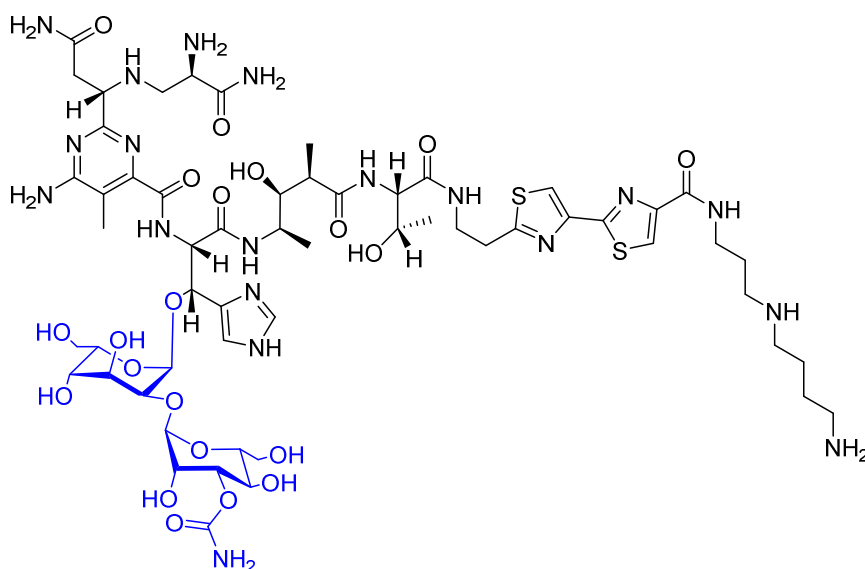


Figure 5. Structure of Bleomycin A<sub>5</sub> highlighting the disaccharide (blue).

*Trehalose* ( $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 1)- $\alpha$ -D-glucopyranoside) is composed of two  $\alpha$ -glucose units which are linked by an  $\alpha$ , $\alpha'$ -1,1-glucosidic bond. It is widely found in animals, plants, and microorganisms. It is the blood-sugar of many insects, including locust, butterflies and bees. It is believed to transfer into a gel phase under dehydrating condition, protecting the cell internal organelles and hence the whole cells against desiccation.<sup>13</sup> A monomer, namely methacrylamido trehalose (Table 1, entry 9) was polymerized followed by chain extension with aminoethyl methacrylamide (AEMA). The obtained

polymer was used to complex siRNA to polyplexes which carry trehalose on the surface. These polyplexes show high stability in the presence of high salt concentrations and serum proteins and are specifically internalized into a brain tumor cell line (U-87 cells) as can be seen in Figure 6.<sup>130</sup>

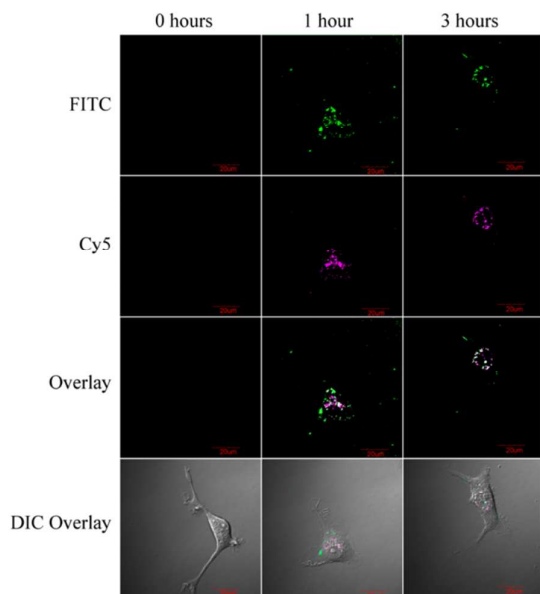
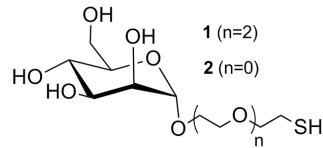
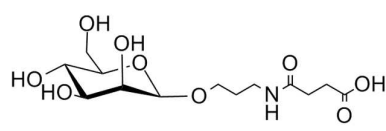
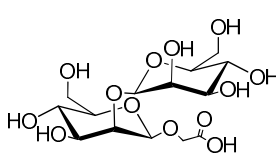
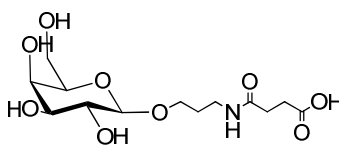


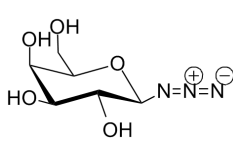
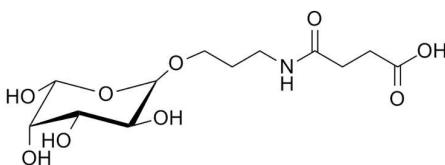
Figure 6. Confocal microscopy of U-87 cells transfected with siRNA containing polyplexes, both fluorescent intensity from Cy5-labeled siRNA (magenta) and FITC-labeled poly(methacrylamidotrehalose) is detected (Reprinted with permission from reference<sup>130</sup>. Copyright 2013 American Chemical Society)

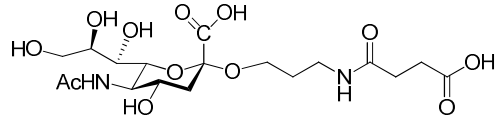
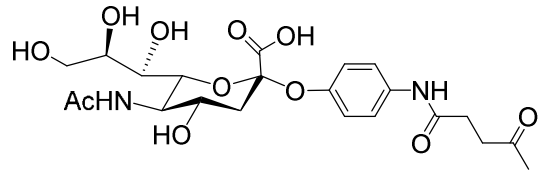
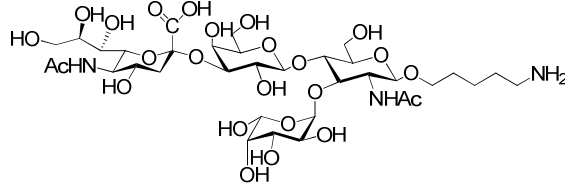
Table 1: Carbohydrates used for surface-functionalization of nanocarriers.

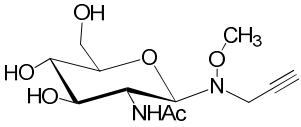
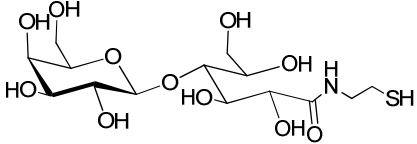
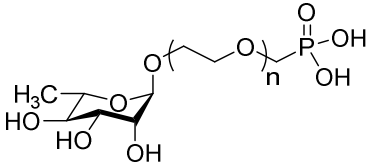
#	Type of sugar	Chemical structure	Nanocarrier	Properties	Chemistry	note	ref
1	mannose	<p>a</p> 	mesoporous silica nanoparticles	pore closure by coupling of mannose with concanavalin A	thiol-ene reaction	re-opening of the pore controlled by pH or glucose level	44
		<p>b</p> 	magnetic nanoparticles	increased binding affinity with different cell surface	amidation	selective binding to cancer cells	18
		c	Different (oligo)mannosides functionalized	gold	inhibition of	reaction	a potential

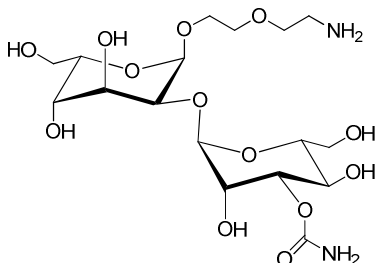
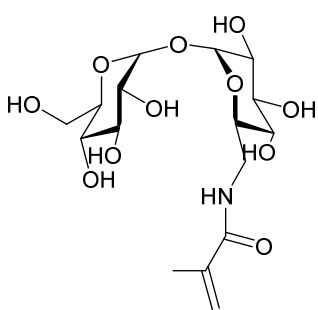
			with thiol groups	nanoparticles	DC-SIGN/gp120 binding	between the thiol group and gold surface	anti-HIV system	
		d		poly-anhydride nanoparticles	targeting C-type lectin receptors on alveolar macrophages	EDC coupling	enhanced expression of mannose receptor	43
2	galactose	a		magnetic nanoparticles	increased binding affinity with different cell surface	amide coupling reaction	differentiates normal cells from cancer cells	18

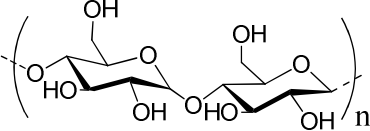
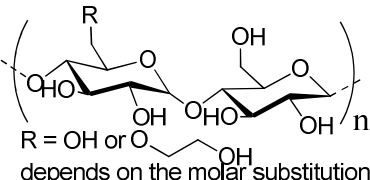
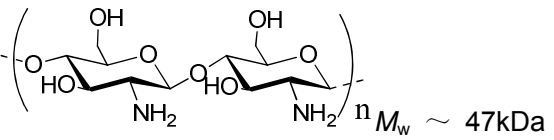


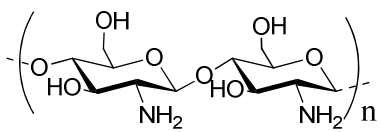
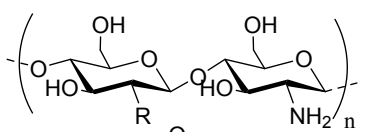
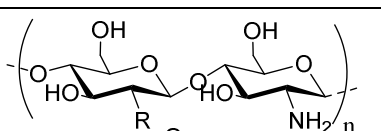
		b	<p>polyanhydride nanoparticles</p>	<p>targets C-type lectin receptors on alveolar macrophages</p>	<p>EDC coupling</p>	<p>enhanced expression of galactose lectin</p>	43
		c	 <p>self-assembled nanorods</p>	<p>high binding affinity to carbohydrate receptors on E. coli.</p>	<p>Huisgen azide alkyne cycloaddition</p>	<p>decreasing the toxicity of the nanorods</p>	45
3	fucose		<p>magnetic nanoparticles</p>	<p>Increased binding affinity with</p>	<p>amidation</p>	<p>distinction between isogenic</p>	18

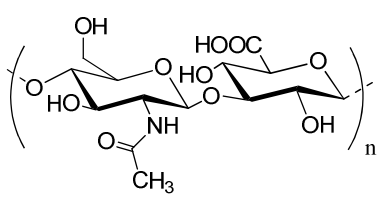
4	Sialic acid	a			different cell surfaces		sublines of cancer cells	
		b		quantum dots	prolonged <i>in vivo</i> lifetime	Huisgen azide alkyne cycloaddition	87	
		c	 Sialyl-Lewis <sup>x</sup>	core shell silica magnetic nanoparticles	bind specifically to the endothelial transmembrane inflammatory proteins E and P selectin	coupling between amine and NHS ester	nanoparticles accumulated in the brain vasculature	121

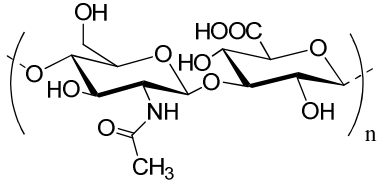
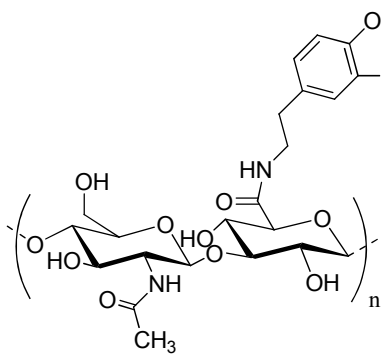
5	glucose		magnetic nanoparticles	Increased binding affinity with different cell surfaces	Huisgen azide alkyne cycloaddition		18
6	lactobionic acid		micelles	targeting liver cancer cells through asialoglycoprotein receptors (ASGP-R)	thiol-disulfide exchange reaction	un-coating in a reductive environment (mimicking the cell interior)	46
7	Rhamnose		Fe <sub>3</sub> O <sub>4</sub> nanoparticles	targeting of human skin cells	binding of phosphonate to Fe		47

					oxide		
8	Disaccharide Moiety of Bleomycin A <sub>5</sub>		microbubbles	selective binding to different cancer cell types	reacted with a NHS-ester coupled Cy5 dye		127
9	trehalose		block copolymer self-assembly and siRNA complex	colloidal stability of polyplexes at high salt concentration and specific internalization into glioblastoma cells	RAFT block copolymerization, with aminoethyl methacrylamide	the amount of siRNA delivered can be controlled	130

10	starch		copper nanoparticles	lower toxicity	reduction of copper nitrate solution by ascorbic acid, starch as stabilizer for the nano-particle	excellent bactericidal action	131
11	Hydroxyethyl starch (HES)		DNA-polyplexes micelles	reduced unspecific cell uptake	Schiff base formation and reductive amination	deshielding of the nanocarrier possible	23, 24
12	chitosan	a		polylactic-co-glycolic acid nanoparticles	significantly increased (>5-fold) uptake by	electrostatic interactions	132

			e	MCF-7 cells			
b	 <p>Molecular weight of 100,000 g/mol</p>	hyaluronic-paclitaxel nanoparticle	protection of the payload	electrostatic interactions	pH responsive release of paclitaxel	133	
c	 <p>R = NH<sub>2</sub> or HN-C(=O)-CH<sub>3</sub> depends on the deacetylation degree &gt; 75% deacetylated</p>	silver nanoparticle	lower toxicity	chitosan as a stabilizer during preparation	higher rate of killing cancer cell compared to PEGylated gold nanorod	134	
d	 <p>R = NH<sub>2</sub> or HN-C(=O)-CH<sub>3</sub> depends on the deacetylation degree degree of</p>	gold nanoparticles	low unspecific cell uptake, enhanced	glycol-modified chitosan is used as reducing	tomography of liver tissues with metastatic	135	

			deacetylation=82.7%; $M_w = 250,000$		stability and tumor targeting ability	agent for Gold(III) chloride <i>in situ</i>	cancer	
13	Hyaluronic Acid	a		micelles prepared from branched poly(ethylene imine)	Increased transfection efficiency and decreased cytotoxicity	reductive amination		136

		<p>b</p>  <p>The structure shows a repeating unit of a chitosan derivative in its cyclic Haworth projection. It features an acetamido group (-NHCOCH<sub>3</sub>) at the C2 position and a carboxylic acid group (-COOH) at the C6 position. The unit is enclosed in brackets with a subscript 'n'.</p>	<p>re-constituted high density lipoprotein loaded with lovastatin</p>	<p>lower accumulation in liver and higher atherosclerotic lesions targeting efficiency</p>	<p>electrostatic interactions</p>	<p>efficiently suppressed the advancement of atherosclerosis</p>	<p>137</p>
		<p>c</p>  <p>The structure shows a repeating unit of a chitosan derivative in its cyclic Haworth projection. It features an acetamido group (-NHCOCH<sub>3</sub>) at the C2 position and a catechol group (-NHCH<sub>2</sub>CH<sub>2</sub>-3,4-dihydroxyphenyl) at the C6 position. The unit is enclosed in brackets with a subscript 'n'.</p>	<p>gold nanocage</p>	<p>specific binding to cancer cells via interaction with CD44, release in lysosome</p>	<p>Au-catechol bonds</p>	<p>near-infrared irradiation accelerates the release</p>	<p>138</p>



*Starch* is a polysaccharide based on glucose as the monomer, which is coupled via glycosidic linkages. Two forms of starch are found in nature: amylose, a linear and helical polysaccharide with  $\alpha$ -1,4-glycosidic bonds, and amylopectin, a branched poly(glucose) with 1,4- and 1,6- glycosidic bonds. It is the energy storage medium of green plants and the most common carbohydrate in human diets.<sup>139</sup> The sugar part of glycogen, as another glucose polymer, is used to store glucose in animals with a similar structure as amylopectin, but with a higher degree of branching. Starch is used as the stabilizer during preparation of copper nanoparticles, while ascorbic acid is used as reducing agent, and copper nitrate as the source of copper (Table 1, entry 10), which will result in starch-functionalized copper nanoparticles with a reduced toxicity, while retaining high antibacterial potential against both gram negative and gram positive strains.<sup>131</sup>

*Hydroxyethyl starch.* For some applications, the degradation kinetics of starch is too fast; starch is rapidly hydrolyzed by plasma amylases. In order to balance the biodegradability and stability, hydroxyethyl starch (HES) was introduced. It is prepared by ethoxylation of the hydroxyl groups with ethylene oxide, resulting in decreased biodegradation kinetics. The degree of hydroxyethyl-substitution is expressed by the molar substitution, which is the mean number of hydroxyethyl groups per glucose unit, and ranges between 0 and 3. The higher the molar substitution, or the higher the C2/C6 ratio of hydroxyethylation, the lower the rate of metabolization.<sup>80</sup> Moreover, HES exhibits low hypersensitivity<sup>80</sup> and depressed protein adsorption<sup>20-22, 82</sup> rendering it an interesting substitute for PEG for the preparation of stealth nanocarriers. HESylation of proteins and nanocarriers is of high potential for future drug delivery vehicles as it combines adjustable degradation with stealth properties.<sup>140</sup> HES with different molecular weights and degrees of substitution were coupled to poly(ethylene imine) via Schiff base formation and reductive amination (Table 1, entry 11). Subsequent complexation of the polymer with

DNA generated so called DNA-polyplexes, which presented HES on their surface. These polyplexes proved to exhibit stealth properties, as the nanocarrier is protected against  $\alpha$ -amylase. The effect of deshielding is also affected by the degree of substitution of HES, as can be seen in Figure 7.<sup>23, 24</sup>

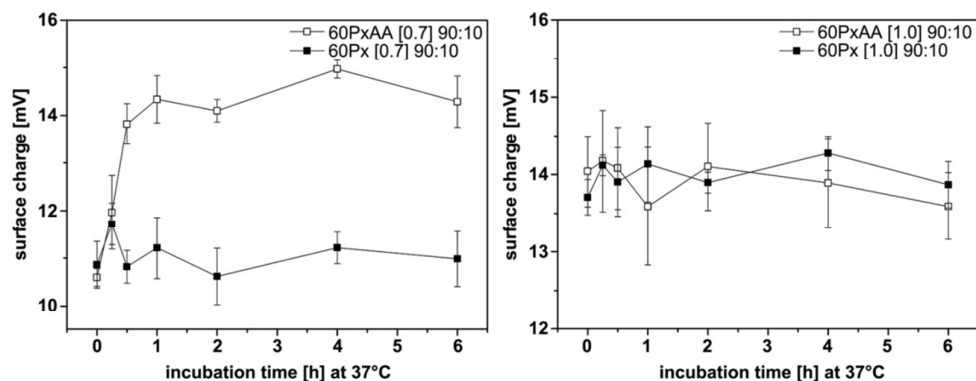


Figure 7. The effect of alpha amylase (AA) on biophysical characteristics of HESylated polyplexes. Two different HES species are coupled with poly(ethylene imine) (Px). Both of them have the molecular weight of 60 kDa, and the molar substitution of 0.7 and 1.0 respectively. The surface charge of HES60[0.7]-shielded (left) and HES60[1.0]-shielded (right) DNA-polyplexes under the effect of AA as a function of time at 37 °C (Reprinted with permission from reference 19).

*Chitosan* is a linear cationic polysaccharide, mainly prepared from the shells of shrimps or other crustaceans, composed of randomly distributed *N*-acetyl-D-glucosamine (acetylated unit) and glucosamine (deacetylated unit), with the ratio being referred as the degree of deacetylation.<sup>141</sup> Due to the cationic charges of chitosan, it can be electrostatically anchored onto the surface of anionically charged polymers or particles, such as poly(lactic-co-glycolic acid) nanoparticles (Table 1, entry 12, a). Compared to the unmodified nanoparticles, the chitosan-coated particles proved a

significant increased (>5-fold) uptake by MCF-7 cells, while the proapoptotic effect of chitosan providing synergistic cytotoxic activity with docetaxel, an anti-mitotic chemotherapeutic.<sup>132</sup> In another work, chitosan was adsorbed to the surface of nanoparticles, which were formed by the self-assembly of hyaluronic-paclitaxel conjugates, by electrostatic interaction (Table 1, entry 12, b). This enables the protection of the ester bond between hyaluronic acid and paclitaxel at acidic pH, and allows a controlled *in vitro* release of paclitaxel from the nanocarrier, which makes it suitable for oral administration.<sup>133</sup>

Nanoparticles based on effective Au and Ag photothermal transducers can be used to trigger localized hyperthermia of tumors. Chitosan has been used for the surface functionalization of silver nanoparticles (Table 1, entry 12, c), and gold nanoparticles (Table 1, entry 12, d). To a mixture of aqueous solutions of trisodium citrate, ascorbic acid, chitosan, and preformed Ag nanoparticles, a solution of AgNO<sub>3</sub> was added dropwise, and chitosan surface functionalized Ag nanoparticles are obtained. These Ag nanoparticles show a lower toxicity compared to PEGylated gold nanorods, which are a common hyperthermia agent.<sup>134</sup> Ethylene glycol-modified chitosan is used as reducing agent to produce gold nanoparticles along the polymer chain by reducing gold (III) chloride trihydrate *in situ*, as can be seen in Figure 8. The obtained gold nanoparticles exhibited stealth properties, enhanced stability and tumor targeting ability.<sup>135</sup>

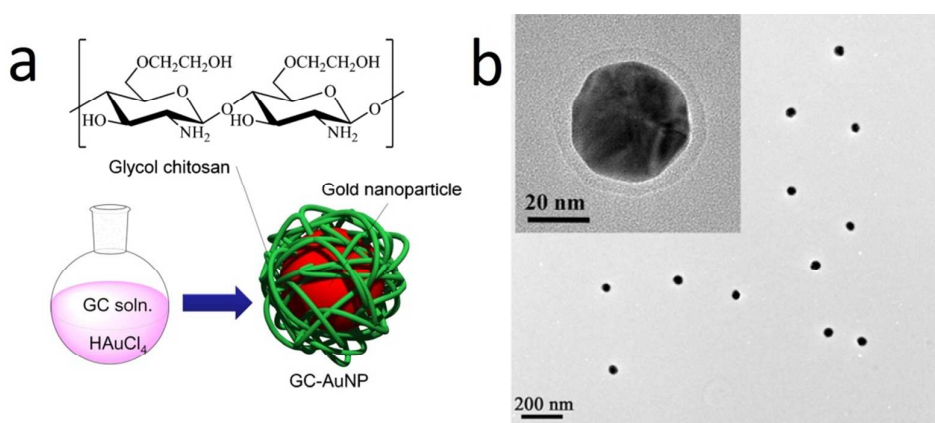


Figure 8. (a) Chemical structure of glycol chitosan (GC) and surface-modified AuNPs (GC-AuNP). (b) TEM images of GC-AuNP (inset : magnified images). (adapted from ref.<sup>135</sup> with permission)

*Gum Arabic* is the dried gum of acacia tree branches and stems. As a mixture of polysaccharides and proteins, it is mainly composed of galactose (44%), rhamnose (13%), arabinose (27%), glucuronic acid (16%) and peptides (2-3%),<sup>142</sup> and possesses excellent emulsifying properties.<sup>143</sup> Gum Arabic capped gold nanoparticles (GNP) have been prepared by using the leaf extract of *Vitex negundo* as a reducing agent and gum Arabic as a capping agent.<sup>144</sup> Epirubicin was encapsulated in the GNP, while the surface of the GNP was functionalized by folic acid. These GNP showed increased stability at pH 7.4, together with enhanced cytotoxicity against A549 cells in comparison to free epirubicin. In another work, in-vivo studies of gum Arabic functionalized GNP resulted in significant alterations in lung tumors in mice upon laser irradiation, including cyto-toxicity, apoptosis, decreased inflammation and angiogenesis, and enhanced lipid peroxidation.<sup>145</sup>

*Hyaluronic acid* is a polysaccharide distributed widely in all tissues and body fluids of vertebrates and is most abundantly found in the connective tissues, serves many physiological functions, including lubrication, filtering, water homeostasis, and regulation of plasma protein distribution. It is metabolized by receptor-mediated endocytosis, and subsequent lysosomal degradation.<sup>146</sup> Hyaluronic acid was conjugated with branched poly(ethylene imine) via reductive amination (Table 1, entry 13, a). Then, the polymer was self-assembled into micelles which were surface-modified by hyaluronic acid and proved increased transfection efficiency and decreased cytotoxicity.<sup>136</sup> A reconstituted high density lipoprotein loaded with lovastatin (a statin which blocks the de novo-synthesis of cholesterol) was functionalized by hyaluronic acid (Table 1, entry 13, b), through electrostatic adsorption of hyaluronic acid to a cationic lipid core of the nanoparticle. After surface-modification, the

nanocarrier has lower accumulation in liver and better atherosclerotic lesions targeting efficiency, and efficiently suppressed advancement of atherosclerosis.<sup>137</sup> Dopamine coupled hyaluronic acid has also been used to surface functionalize a gold nano-cage (Table 1, entry 13, c), as can be seen in Figure 9.

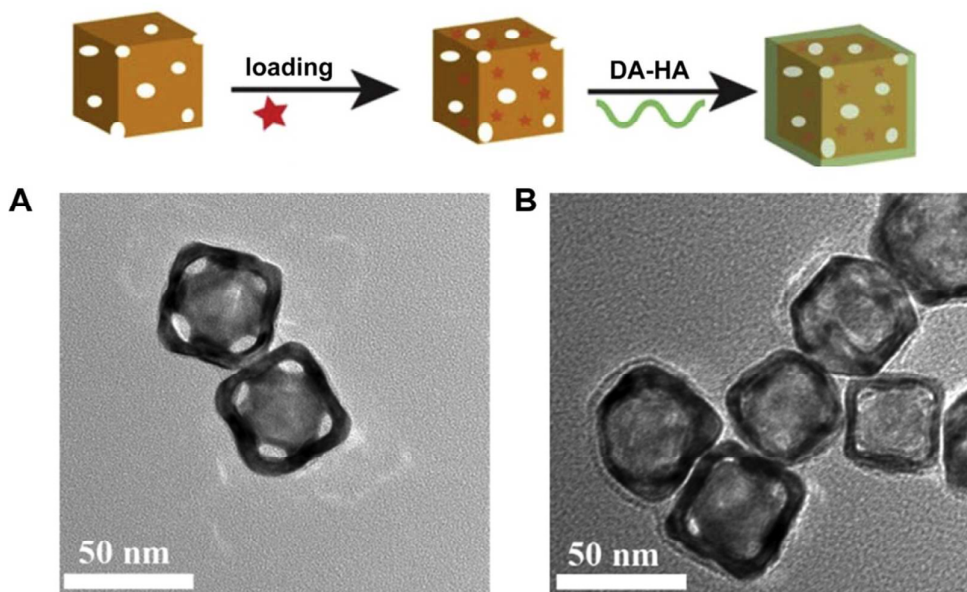


Figure 9. Schematic representation of the coating of doxorubicin loaded gold nano-cage with hyaluronic acid, and the TEM image of the gold nano-cage before (A), and after (B) hyaluronic acid coating. (adapted from reference <sup>138</sup> with permission)

The hyaluronic acid layer can seal the nanoporous in the gold nanocage to protect the encapsulated dopamine, while the interaction between hyaluronic acid and the excess CD44 receptors on the cancer cells can lead to specific cellular internalization of the nanocage.<sup>138</sup> After the functionalized gold nanoparticles enter the lysosomes, the degradation of the hyaluronic acid layer *in situ* will result in the release of the payload, furthermore, the release can be accelerated upon near-infrared (NIR) irradiation.

#### 4. Carbohydrate-constructed Nanocarriers

Due to their outstanding biocompatibility, biodegradability, high diversity of chemical functionalities, and versatile biological functions, carbohydrates are also useful for the construction of nanocarriers for biomedical applications.

Amphiphilic dendrimers carrying both long alkyl chains and mono- or disaccharides as the hydrophilic part (Table 2, entry 1a, 2 and 3 with mannose, galactose, and lactose, respectively) can be formulated into vesicles by the addition of their THF or ethanolic solution into water. So called “glycodendrimersomes” (Figure 10) are generated via self-assembly of the amphiphile. They exhibited multivalent binding with lectins from both plants and humans.<sup>147</sup>

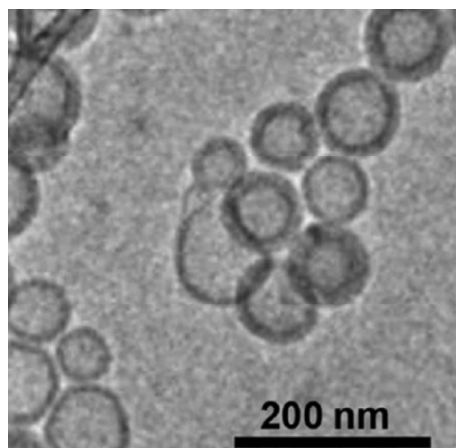


Figure 10. Cryo-TEM images of glycodendrimersomes assembled from amphiphilic glycodendrimer composed of mannose (adapted with permission from reference<sup>147</sup>. Copyright 2013 American Chemical Society).

Apart from self-assembly, emulsion techniques are interesting and versatile methods for the *in situ* formation of carbohydrate-based nanocarriers: Nanocapsules can be prepared in an inverse miniemulsion (i.e., a stable

dispersion of water droplets in an organic solvent, compare Figure 11) by the polyaddition of the sugar-hydroxyls (dissolved inside the aqueous droplets) at the interface to strong electrophiles. Mannose-nanocapsules were prepared by the polyaddition of mannose to toluene diisocyanate (TDI) which occurs exclusively at the interface of a water-in-oil miniemulsion (Table 2, entry 1, a). By the inverse miniemulsion technique, hydrophilic guests can be encapsulated with high efficiencies in the aqueous core of the capsule if they do not take part in the polyaddition reaction. After intravenous injection mannose nanocapsules are preferentially deposited in the lung.<sup>148</sup>

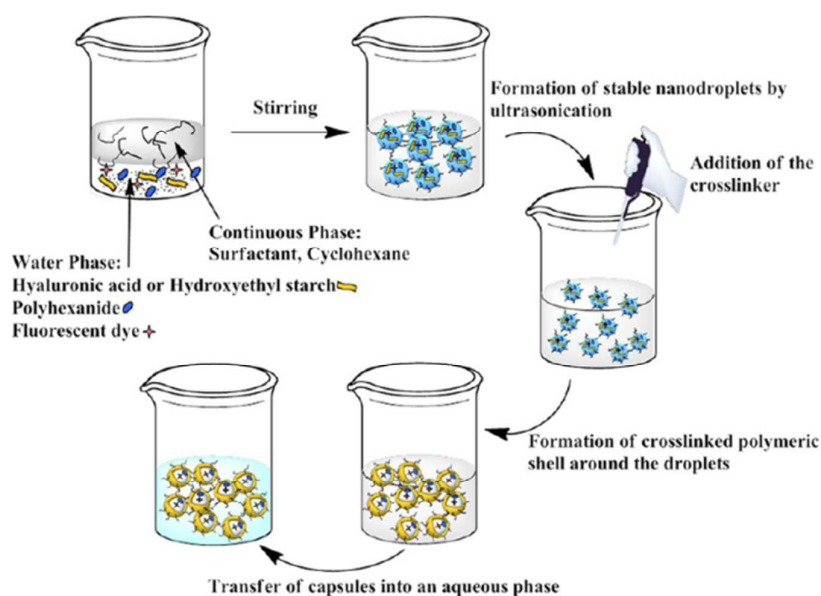


Figure 11. Schematic illustration of the nanocapsule formation through interfacial polyaddition in the inverse miniemulsion system with hyaluronic acid as the polyol component and toluene diisocyanate (TDI) served as the bifunctional electrophilic crosslinker (Reprinted with permission from reference<sup>149</sup>. Copyright 2013 American Chemical Society).

Water-soluble potato starch has been used to prepare nanocapsules (Table 2, entry 7a) by the above mentioned inverse miniemulsion technique, while silver

nanoparticles were generated *in situ* in the core to serve as an antibacterial agent.<sup>150</sup>

HES with a molecular weight of  $M_w = 200,000 \text{ g}\cdot\text{mol}^{-1}$  and a degree of substitution of 0.5 has also been used to prepare nanocapsules through the inverse miniemulsion technique. The obtained HES nanocapsules showed a suppressed uptake into HeLa cells<sup>85</sup> and a preferential deposition in the liver (Table 2, entry 9).<sup>148</sup>

The *in vivo* plasma half-life times of the HES nanocapsules obtained by this strategy can be further tailored by different surface functionalization methods. PEGylation of the capsule surface by isocyanate-terminated PEG results in increased plasma half-life times with 20% and 5% of the nanocapsules remaining in the blood plasma after 24 h and 72 h, respectively.<sup>151</sup>

Despite the straightforward reaction setup, this strategy has limited feasibility, when used to encapsulate and protect pharmaceutical agents, which often contain nucleophiles like amines, thiols, or alcohols, and consequently will participate in the polycondensation reaction with the diisocyanate electrophile. Recent work presents strategies to use bioorthogonal reactions to generate the nanocarriers allowing the encapsulation of more complex molecules.

Two different strategies have been developed to meet this demand so far. In the first strategy, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) is utilized in an oil-in-water miniemulsion: an aqueous solution of azide-functionalized sucrose and a miglyol solution of a dialkyne (bis-(propargyloxy)butane) as oil phase were allowed to react at the interface of surfactant-stabilized hydrophobic droplets.<sup>152</sup> Sucrose was functionalized with azide groups under Mitsunobu conditions (Table 2, entry 5). The obtained nanocapsules have a diameter below 200 nm, and a core filled with miglyol, as can be seen in Figure 12, allowing loading of the nanocarriers with



hydrophobic molecules. However, the removal of the copper catalyst may be problematic with this protocol and copper-free click chemistry could be used in future studies to prevent this, which was demonstrated for non-carbohydrate systems in miniemulsion recently.<sup>153</sup>

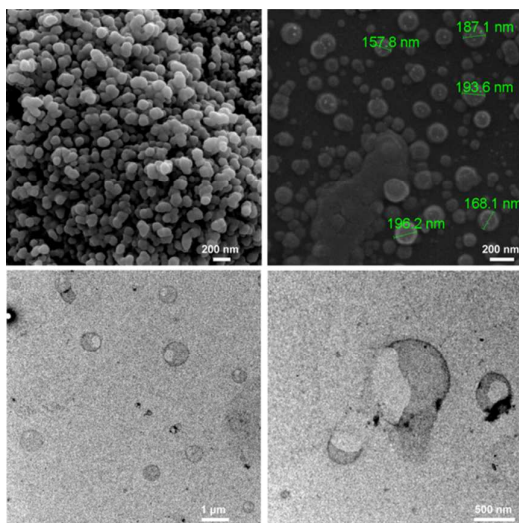


Figure 12. SEM (top) and TEM (bottom) images of the sucrose nanocapsules generated by interfacial CuAAC polyaddition. (Reprinted with permission from reference<sup>152</sup>. Copyright 2012 American Chemical Society)

In the second strategy, olefin cross metathesis was carried out in a water-in-oil miniemulsion by the reaction of acrylated dextran dissolved in water droplets and dispersed in a cyclohexane solution of phenyldi(undec-10-en-1-yl)-phosphate as the oil phase (Table 2, entry 6).<sup>154</sup> The TEM and SEM images of the obtained capsules can be seen in Figure 13. The ruthenium catalyst for the olefin metathesis can be easily removed by centrifugation as it is only soluble in the continuous (outer) phase. These nanocapsules offer the possibility to be degraded by enzymes that cleave dextran or the phosphate crosslinkers and in addition by pH changes due to ester cleavage.

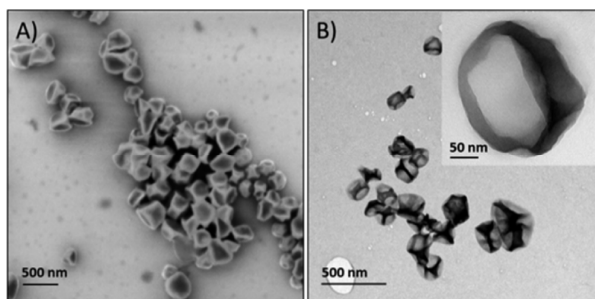


Figure 13. (a) Scanning electron microscopy image and (b) transmission electron microscopy image of the nanocapsules prepared in a minimulsion process by olefin cross metathesis. (Reprinted with permission from reference<sup>154</sup>. Copyright 2014 American Chemical Society).

Another emulsion technique has been used to encapsulate hydrophilic guests (e.g. proteins) into inverse micelles of trehalose (Table 2, entry 4, Figure 14): Dioctyl sodium sulfosuccinate (AOT) is dissolved in isooctane as the oil phase, while different proteins are dissolved together with trehalose in the water phase. After mixing of the two phases, stable water-in-oil micelle dispersions will be obtained, which can be subsequently freeze-dried by flash-freezing, and result in AOT-coated sugar-glass nanoparticles. The surfactant coating on the nanoparticle surface provides colloidal stability in organic solvent–polymer solutions. The trehalose, which transfers into gel phase under dehydrating conditions, protects the cell internal organelles and hence protect the cells in desiccation, serves to protect the protein from chemical and physical degradation during storage.<sup>155</sup>

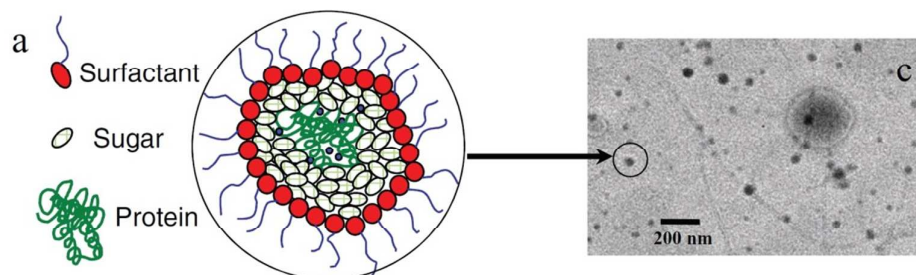


Figure 14. Schematic representation and TEM image of sugar-glass nanoparticles. (Reprinted with permission from reference<sup>155</sup>)

Multilayered polysaccharide vesicles are generated from starch as the core and hyaluronic acid (HA) as the shell (Table 2, entry 7b). The hydroxyl groups of starch were activated by succinic anhydride and then reacted with an excess of cysteamine by Steglich esterification (i.e. *N,N*-dimethylamino pyridine (DMAP), dicyclohexyl carbodiimide (DCC), and *N*-hydroxy succinimide (NHS) in dimethyl sulfoxide (DMSO)) to produce amino-functionalized starch with additional disulfide bonds. The amines were then reacted with the activated ester groups of HA. Rehydration of a thin film of this core-shell HA-starch conjugate in PBS will result in self-assembled nanoparticles with starch core and hyaluronic acid shell, which are subsequently treated by amylase, and result in vesicles with a hollow inner core in the end. Proteins/peptides can be encapsulated in these vesicles, when they are dissolved in the PBS buffer used. In addition, The enzymatic degradation of the HA shell by hyaluronidase (HYAL) enzyme contributed to accelerate the release of the payload.<sup>156</sup> In another work, starch modified with ammonium groups is complexed with siRNA by electrostatic interaction to self-assemble into nanocarriers (Table 2, entry 7, c), the starch can protect the siRNA from enzymatic degradation on its delivery route. It has high cellular

uptake into a human ovarian adenocarcinoma cell line and efficiently induced P-glycoprotein (P-gp) gene silencing.<sup>157</sup>

Antibacterial nanodevices are interesting for coatings and wound dressings if the release of antibacterial agents can be triggered by the presence of bacteria. HA-nanocapsules (Table 2, entry 8a) containing the antimicrobial agent Polyhexanide were prepared by the interfacial polycondensation with TDI in an inverse miniemulsion. They can be specifically cleaved in the presence of the enzyme hyaluronidase, a factor of pathogenicity and invasion for bacteria like *Staphylococcus aureus* and *Escherichia coli*.<sup>149</sup>

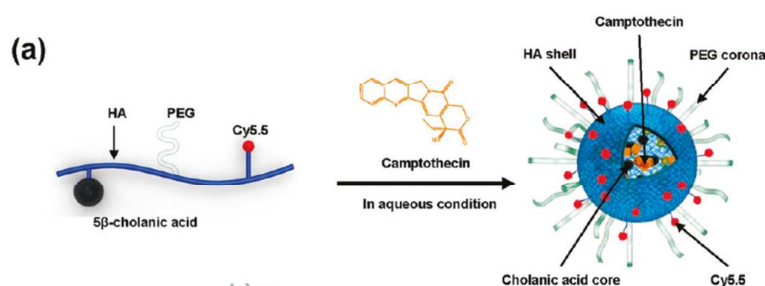


Figure 15. Schematic illustration of the formation of drug-loaded HA-NPs. (Reprinted with permission from reference<sup>158</sup> Copyright 2011 American Chemical Society).

Hyaluronan-cholanic acid conjugates (HA-CA conjugates) were synthesized by the chemical conjugation of the hydrophobic bile acid (a steroidal acid) to the hydrophilic HA backbone through amide formation (Table 2, entry 8b, Figure 15). The amphiphilic HA-CA can self-assemble into nanoparticles and can be loaded with doxorubicin and camptothecin, both strongly cytotoxic compounds, and exhibited an efficient intracellular uptake into SCC7 cancer cells. The size of the nanoparticles was varied between 200 and 400 nm by varying the degree of substitution. Enzyme-triggered drug release was induced by the enzyme Hyal-1, as can be seen in Figure 16.<sup>158-161</sup>

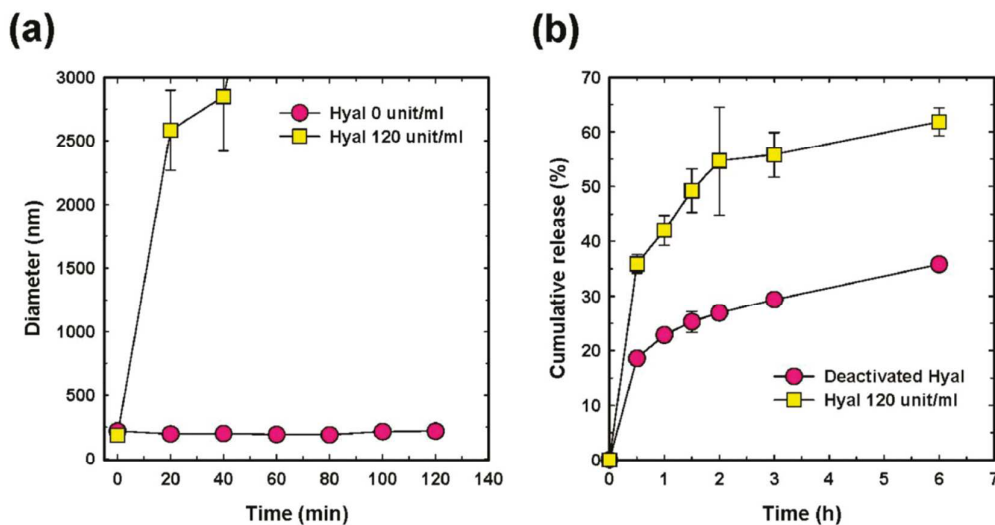


Figure 16. (a) Particle size changes of P-HA-NPs and (b) release patterns of CPT from P-HA-NPs in the presence and absence of Hyal-1. Error bars represent the standard deviation ( $n = 5$ ). (Reprinted with permission from reference<sup>158</sup>. Copyright 2011 American Chemical Society).

Esterification between hyaluronic acid and paclitaxel, a mitotic inhibitor used in cancer chemotherapy, was conducted via dicyclohexyl carbodiimide (DCC) coupling in anhydrous DMSO (Table 2, entry 8c). These conjugates then self-assemble in water to nanoparticles, which demonstrated higher cellular uptake than free paclitaxel against HepG2 cells, a human liver carcinoma cell line. The oral administration of this nanoparticles can result in remarkable accumulation of paclitaxel into the tumor (Figure 17).<sup>133</sup>

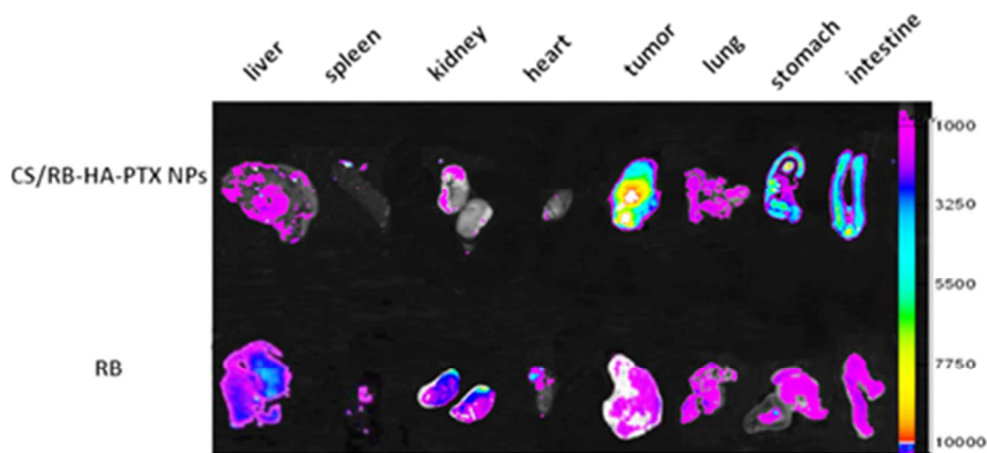


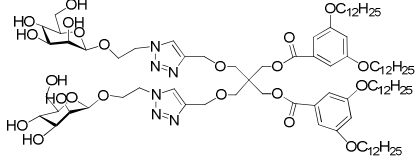
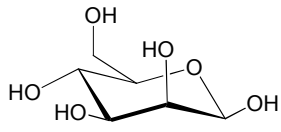
Figure 17. *Ex vivo* fluorescence intensity images of the tumors and major organs after oral administration of rhodamine B labelled hyaluronic acid-paclitaxel nanoparticles (CS/RB-HA-PTX CNPs) and free Rhodamine B (RB). (Taken from reference <sup>133</sup> with permission).

Amphiphilic hexadecylated polysaccharides were synthesized (Table 2, entry 8d) and self-assembled in water into nanoparticles. Doxorubicin can be encapsulated in these nanoparticles, which showed specific binding with CD44 over-expressing cancer cells. Higher therapeutic potential in the presence of a green tea polyphenol, epigallocatechin-3-gallate, was also observed for this nanoparticle system.<sup>162</sup>

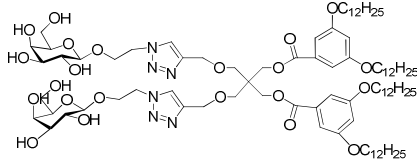
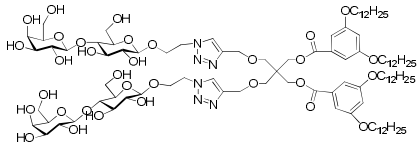
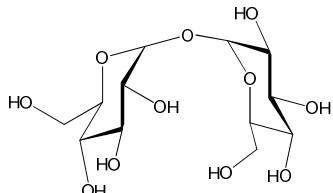
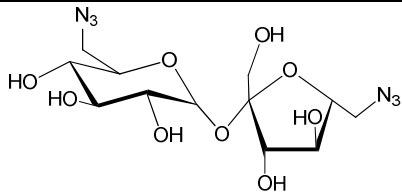
Other polysaccharides like cellulose and chitin, which intrinsically are water insoluble, have also been used to construct nanocarriers. In some cases, their water solubility has been increased by chemical modifications, for example, carboxymethylation. Carboxymethyl cellulose has been conjugated with docetaxel and Poly(ethylene glycol) through acetylation, self-assembled into nanoparticles, which was stable against dilution, and induced significantly higher toxic effects against EMT-6 murine mammary carcinoma cells and murine Pan02 tumors cells.<sup>163</sup> Carboxymethyl chitin nanoparticles have also been prepared by crosslinking of the polysaccharide with  $\text{CaCl}_2$  and  $\text{FeCl}_3$ .<sup>164</sup> The obtained nanoparticle showed anti-bacterial activity by itself, and a sustained and controlled release of the payload. While different functions are

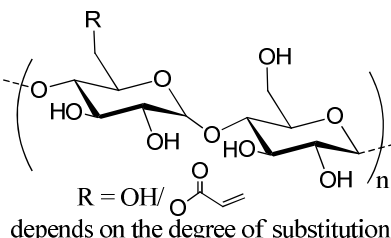
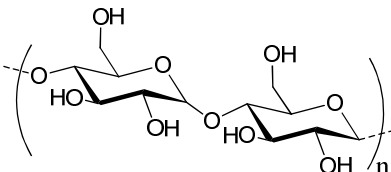
enabled by cellulose and chitin, the intrinsic low water solubility and poor bio-degradability in animals rendered them to be used more as wound dressing, scaffolds for tissue engineering and medical implants, which is not the focus of this review and is comprehensively reviewed in detail elsewhere.<sup>165, 166</sup>

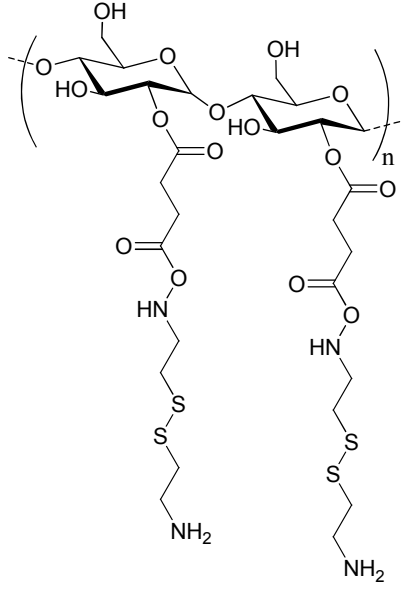
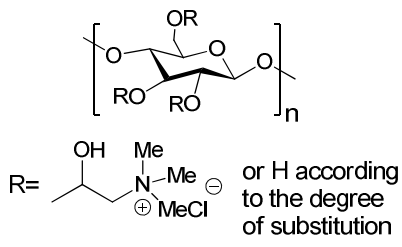
**Table 2.** Application of (poly)saccharides to construct nanocarriers.

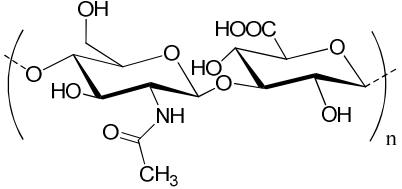
#	Type of sugar	Chemical structure	nanocarrier	Properties	Chemistry	note	Ref.
1	mannose	<p>a</p> 	glyco-dendrimer	multivalent binding with human and plant lectins	self-assembly in water into glycodendrimers	controlled over the size by adjusting the concentration	147
		<p>b</p> 	nanocapsule	encapsulation of hydrophilic guest in the core of the capsule	polycondensation with diisocyanate in a miniemulsion system	a preferential deposition in the lung	148

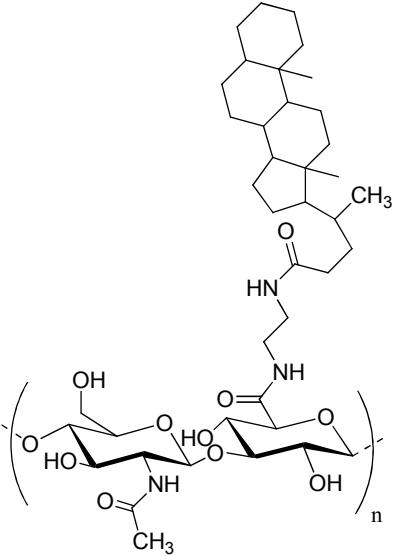
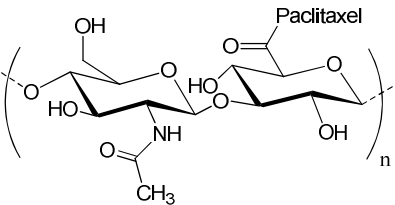


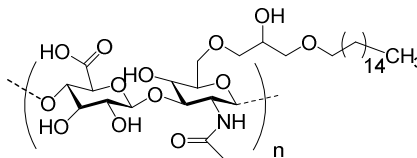
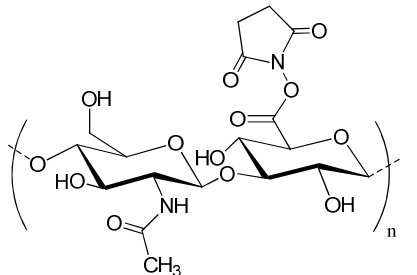
2	galactose		glyco-dendri mersome	multivalent binding with human and plant lectins	self-assembly in water into Glycodendri mersomes	controlled over the size by adjusting the concentration	147
3	lactose		glyco-dendri mersome	multivalent binding with human and plant lectins	self-assembly in water into Glycodendri mersomes	controlled over the size by the concentration	147
4	trehalose		nanoparticle	protect the protein from chemical and physical degradation during storage	self-assembly into inverse micelles		155
5	sucrose		nanocapsule	encapsulation of hydrophobic guest in the core of the	Huisgen azide alkyne cycloaddition	prepared by bio-orthogonal reactions	152

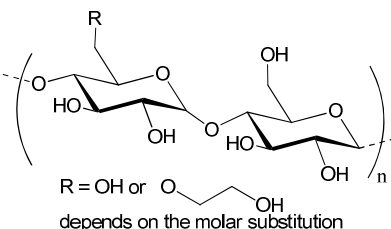
				capsule			
6	dextran	 <p>R = OH / <math>\text{O}-\text{C}(=\text{O})-\text{CH}=\text{CH}_2</math> depends on the degree of substitution</p>	nanocapsule	encapsulation of hydrophilic guest in the core of the capsule	olefin cross metathesis on the interface of miniemulsion	prepared by bio-orthogonal reactions	154
7	starch	 <p>water-soluble potato starch MW 15,000 g mol<sup>-1</sup></p>	nanocapsule	encapsulation of water soluble guests in the core and functionalization of the shell possible	polycondensation with diisocyanate in a miniemulsion system	silver nanoparticles in the aqueous core, as antibacterial agent	150

	<p>b</p> 	<p>multilayered polysaccharide vesicle</p>	<p>the amylase treatment of the nanoparticles allows the presence of a void / hollow inner core (resulting from the degradation starch molecules) within the fabricated particles</p>	<p>rehydration of a thin film of hyaluronate-starch to form vesicles</p>		156
	<p>c</p>  <p>or H according to the degree of substitution</p>	<p>siRNA complex</p>	<p>protect siRNA from enzymatic degradation</p>	<p>self-assembled with siRNA to form nanocarriers</p>	<p>efficiently induced P-glycoprotein gene silencing in the human ovarian</p>	157

							adenocarcino macell line	
8	Hyaluronic Acid	a	 <p><math>M_w = 140\ 000\ \text{g}\cdot\text{mol}^{-1}</math></p>	nanocapsule	encapsulation of polyhexanide in the core of the capsule	polycondensation with diisocyanate in a miniemulsion system	release of polyhexanide upon the contact with bacterial	149

		<p>b</p> 	nanoparticle	specific uptake by SCC7 cancer cells, Encapsulation of doxorubicin and camptothecin	amphiphilic HA-CA is self-assembled to nanoparticles in PBS	size can be tuned between 237–424 nm , rapid drug release in the presence of enzyme Hyal-1	158-161
		<p>c</p> 	nanoparticle	higher cellular uptake than free paclitaxel in HepG2 cells	hyaluronic - paclitaxel self-assemble in water to form the nano-particles	paclitaxel could accumulate remarkably into tumor sites after oral	133

						administration	
		<p>d</p> 	nanoparticle	binding of the particle with CD44 over-expressed cancer cells	self-assembled in water into nanoparticles	higher therapeutic potential in the presence of a green tea polyphenol, epigallocatechin-3-gallate	162
		<p>e</p> 	multilayered polysaccharide vesicle	incubation with hyaluronidase contributed to accelerate the release	rehydration of a thin film of hyaluronate-starch to form vesicles	drug release in the presence of hyaluronidase	156

9	HES	<p>a</p>  <p>R = OH or O-CH<sub>2</sub>-CH<sub>2</sub>-OH depends on the molar substitution</p> <p><math>M_w = 200,000 \text{ g}\cdot\text{mol}^{-1}</math></p>	nanocapsule	encapsulation of hydrophilic guest in the core of the capsule	polycondensation with diisocyanate in a miniemulsion system	suppressed unspecific uptake into HeLa cells, preferential deposition in the liver	85, 148
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## 5. Summary and Outlook

This review summarizes current approaches on the use of (poly)saccharides in nanoparticulate drug delivery systems. Two major strategies have been discussed: a) the surface-functionalization and b) the construction of nanocarriers with/from carbohydrates.

In summary, functionalization of nanocarriers with mono/oligosaccharides has proven the following advantageous properties:

- (i) specific targeting to different cell types;<sup>43, 45-47, 127, 130</sup>
- (ii) diagnostics and differentiation of healthy and malignant population of the same cell type;<sup>18, 127</sup>
- (iii) protecting the payload<sup>130</sup> and prolonged *in vivo* plasma half-life time (due to reduced protein adsorption);<sup>87</sup>
- (iv) controlled release of a payload by specific protein interaction;<sup>44</sup>
- (v) competing with and inhibiting the binding of other saccharide containing bacteria, virus, or pathogen with the corresponding cells.<sup>42</sup>

For nanocarriers that are constructed from carbohydrates, similar properties are obtained, however, the major difference is their potential full degradation, which is not guaranteed for other nanocarriers that are functionalized with carbohydrates:

- (i) protecting the payload<sup>133</sup> and increasing the plasma half-life times;<sup>23, 24, 137</sup>
- (ii) specific targeting;<sup>132, 136, 137, 167</sup>
- (iii) enzyme-induced released or activation of a therapeutic agent;<sup>23-27</sup>
- (v) decreased toxicity of the payload;<sup>131, 134, 136, 167</sup>



(vi) kinetics of metabolization are tunable by chemical functionalization to balance biocompatibility and stability.<sup>23, 24</sup>

As carbohydrates are omnipresent as functional surface coatings in nature, their use in biomedical applications is obvious. They have been used to construct nanocarriers, which enable the encapsulation and protection<sup>155, 157</sup> of different (mainly) water-soluble guests,<sup>85, 148-150, 158-162</sup> while keeping the biological properties such as specific targeting,<sup>133, 147, 158-162, 168</sup> suppressed unspecific cell uptake,<sup>85</sup> and enzyme-triggered release.<sup>149, 156, 158-161</sup> In addition, the inherent high chemical functionality of different polysaccharides (mainly: hydroxyls, carboxylic acids, amines) allows straightforward crosslinking or on top functionalization of these molecules which is often a major factor of their application compared to synthetic macromolecules. However, it always must be considered, that the molecular weight distributions of these biopolymers are typically rather broad, making fractionation necessary in some cases. Nevertheless, due to the biodegradability of polysaccharides, their non-uniform molecular weight might not be too problematic for nanoparticulate drug delivery devices, however, the toxicity after any chemical modification and also that of the degradation products after chemical modification need to be considered.

A major feature of all nanocarriers, either modified or constructed of carbohydrates is their low protein interaction: the high level of hydrophilicity induces for many of them a “stealth” behavior, and the unspecific cell uptake due to opsonization is low. Furthermore, targeting is achieved by carbohydrates in multiple cases. This behavior plus the inherent biodegradability renders carbohydrate-based nanocarriers as a high potential platform for developing the “magic bullet” that was coined by Paul Ehrlich more than 100 years ago and makes research in this direction promising for many scientists. It is certain that

several new developments in treating diseases or enabling sophisticated diagnostics will rely on carbohydrates in future.

## Acknowledgments

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