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Oligosaccharide-Mediated Nuclear Transport of Nanoparticles

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The transport of nanoparticles into the cellular nucleus is a potentially important technique because it can open the way to a wide range of applications, including the sequence-specific detection of genomic DNA, efficient DNA transfection, and the specific entry of drugs into the nucleus.[1] It has been reported that the nuclear import of proteins larger than 40 kD does not occur by passive diffusion.[2] Similarly, the nuclear import of macromolecules or particles is strictly regulated. Therefore, the nuclear import of nanoparticles that contain gold nanoparticles and quantum dots has been achieved by coating the surface with classical nuclear localization signals (NLS), that is, short, highly positively charged peptides.[3] However, the problem remains that positively charged particles can interact with serum protein, resulting in rapid clearance from the plasma compartment.[4] Because the cationic NLS interacts with negatively charged DNA, NLS peptides do not work as efficient signals for transport of DNA into the nucleus;[5] this implies that the use of peptide-based cationic NLS might be limited when using DNA-displaying nanoparticles.

Monsigny et al. have shown that sugars can also work as nuclear localization signals.[6–9] The neoglycoproteins, BSA (bovine serum albumin)–glucose, BSA–mannose and BSA–fucose are rapidly transported into the nucleus of HeLa cells, whereas BSA without chemical modifications is not. Because carbohydrates normally show high biocompatibility and water solubility, they are suitable for use in the modification of synthetic carriers and nanoparticles. Previously, however, only the application of these carbohydrate signals to the nuclear import of proteins was examined, and there are no reports on the effectiveness of carbohydrate signals for the nuclear import of artificial materials, such as nanoparticles and polymers. Previous reports that used BSA have focused only on monosaccharides as a signal. In this paper, we expand the varieties of carbohydrates tested from monosaccharides to oligosaccharides in the search for an efficient signal that is applicable to the nuclear import of nanoparticles. Herein, we present our unique finding that nanoparticles (quantum dots) that display oligo α-glucopyranoside on their surface are readily transported into the nucleus of digitonin-permeabilized HeLa cells. Semiconductor QDs have a diameter of several nanometers and their specific transport inside the cell can be readily achieved through the display of multiple ligands on their surface. As far as we know, this is the first report to describe the import of nanoparticles into the nucleus without the use of cationic NLS.

Because BSA that has been substituted with α-glucopyranoside has been reported to be efficiently transported into the cell nucleus,[6] we synthesized neoglycolipids that contain various carbohydrates comprised of different numbers of glucose units (Scheme 1). In addition to α-monoglucopyranoside–lipid **3**, we synthesized maltose(Glcα1-4Glc)–lipid **4**, maltotriose(Glcα1-4Glcα1-4Glc)–lipid **5**, and panose(Glcα1-6Glcα1-4Glc)–lipid **6**. The cellotriose(Glcβ1-4Glcβ1-4Glc)–lipid **7** is a trisaccharide that is composed of only a β-linked glucose, thus lipid **7** was used as a control to α-linked glucose. Neoglycolipids were synthesized based on a previously described method[10,11] by starting from fully acetylated carbohydrates, hexa(ethyleneglycol) and 11-bromoundecene.

CdTe nanocrystals that had been stabilized with mercaptopropionic acid (MPA) were prepared in water as described by Yang et al.[12] Sugar-displaying CdTe QDs were synthesized by surface exchange from MPA to the neoglycolipid in water (Figure 1 A), and then purified by using spin filtration.[11] Ligand exchange occurring on the surface of the QDs was confirmed by MALDI-ToF mass spectrometry. Ligands immobilized on inorganic nanoparticles, such as QDs, were detached from the surface during the laser deposition process, and the mass corresponding to the molecular weight of neoglycolipids was clearly detected (Figure 1 B). Furthermore, the display of sugars on the QDs was visually confirmed by trapping on a lectin-immobilized column. For example, maltotriose **5**–QDs were specifically trapped on a ConA (concanavalin A; α-mannose and α-glucose specific) agarose column, whereas they were not trapped on WGA (wheat germ agglutinin; GlcNAc specific) or LCA (lens culinaris agglutinin; branched-fucose specific) agarose columns (data not shown). We explored the interactions between sugar-displaying CdTe QDs and digitonin-permeabilized HeLa cells. Digitonin treatment causes partial damage to the plasma membrane[13] and increases the permeability of cells. Digitonin permeabilization has been used often in the study of biochemical processes that are related to the import and export of nuclear proteins.[14–16] Although live cells become semi-intact upon digitonin treatment due to the leakage of cytoplasmic proteins through the plasma membrane, the nuclear membrane is left intact. Import buffer (pH 7.3, 20 mM HEPES, 110 mM KOAc, 5 mM NaOAc, 2 mM $Mg(OAc)_2$, 0.5 mM EGTA) that contained the sugar-displaying CdTe QDs (0.6 $mg\,mL^{-1}$,

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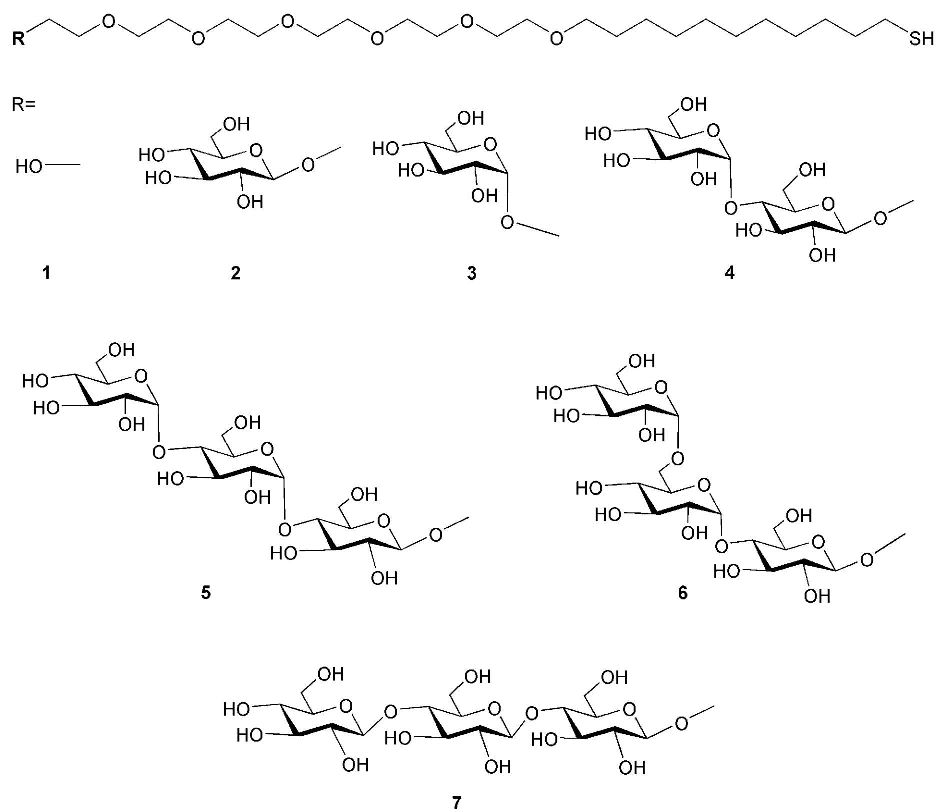

Scheme 1. Chemical structures of neoglycolipids.

10 μL per well) was added to the permeabilized cells and incubated for 1 h at 37 °C. After being washed with buffered solution, the cells were observed by confocal laser scanning microscopy (Figure 2). Monoglucopyranoside-displaying QDs (both α-glucose **3**– and β-glucose **2**–QDs) were retained in the cytosol and not translocated into the nucleus. Although only a few maltose **4**–QDs were seen in the nucleus, a marked accumulation of maltotriose **5**–QDs in the nucleus was observed. Similarly, panose **6**–QDs were readily imported into the nucleus. The digitalized fluorescent intensities from the sugar-displaying QDs in the nuclei were in the following order: trisaccharide > disaccharide ≫ PEG (Figure 2 H). The transport of maltotriose-displaying QDs into the nucleus can be explained by the hypothesis that α-glucose residues on the unreduced terminus are more accessible than when the same residues are directly linked to the lipid moiety; this indicates that the putative receptor can recognize more than a single glucose residue. The sugar-free PEG **1**–QDs, which were considered to have the smallest diameter, did not enter the nucleus and were only detected in the cytosol; this indicates that the neoglycolipid-coated QDs used in this study were large enough so as not to freely penetrate the nucleus pores by passive diffusion. The cellotriose **7**–QDs also remained in the cytosol. These control experiments support the hypothesis that the α-glucopyranoside-containing oligosaccharides are important for the specific delivery of quantum dots into the nucleus. In our previous study, we have shown that classical NLS-coated QDs are transported into the nucleus only in the presence of additional cytosolic factors.[11] In contrast, the nuclear import of sugar-displaying QDs did not require additional cytosolic factors. This corresponds closely to the results of a previous report on the nuclear import of glycosylated BSA.[6] Unlike the results that were obtained by using glycosylated BSA, however, the addition of ATP to the import buffer did not affect the efficiency of nuclear import of the sugar-displaying QDs in our study; this indicates that the transport of sugar-displaying QDs occurred in an ATP-independent manner.

To test the general applicability of maltotriose-dependent nuclear import, we prepared maltotriose-modified BSA. Commercially available Texas Red-labeled BSA (TR–BSA) was modified with maltotriose derivatives by using heterobifunctional crosslinker SMCC (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate). Attachment of the maltotriose residues

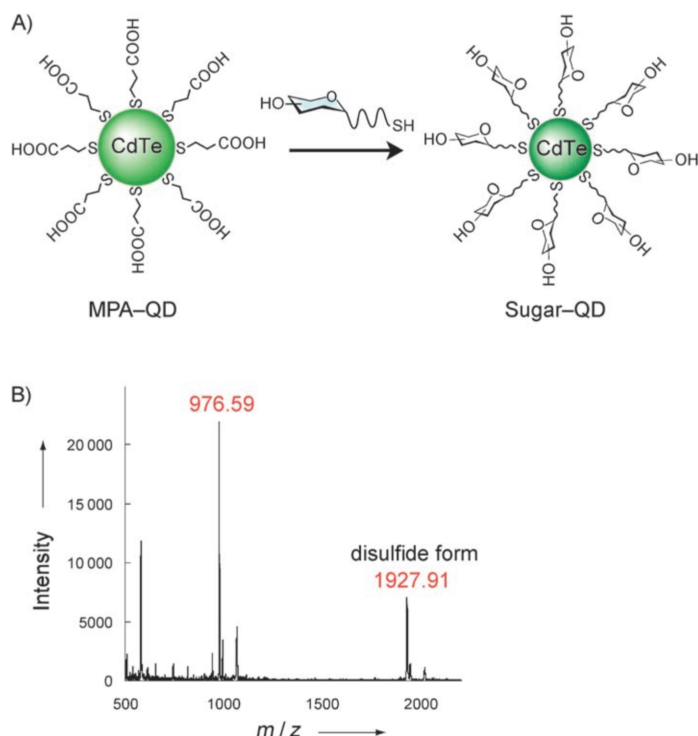

Figure 1. A) Synthesis of sugar-displaying QDs by a thiol exchange reaction, and B) confirmation of maltotriose–lipid **5** immobilization on QDs by mass spectrometry. The calculated molecular weight of maltotriose–lipid **5** ($C_{41}H_{78}O_{22}SNa$) is 977.46.

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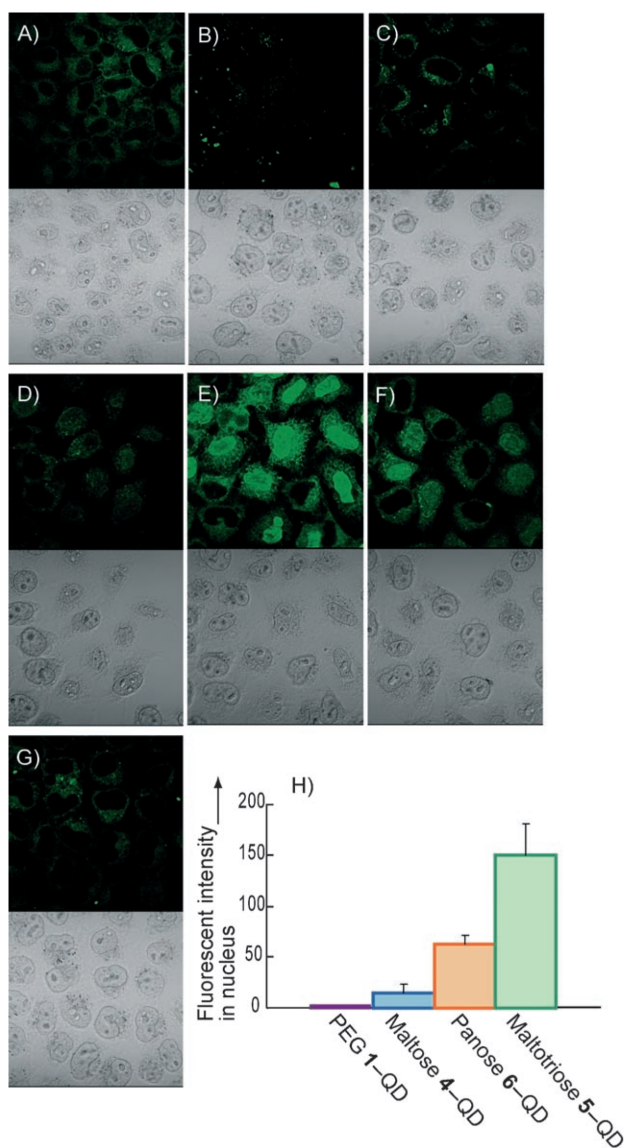
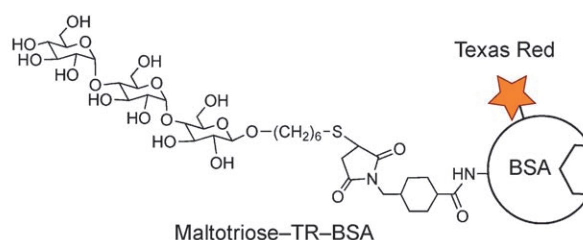


Figure 2. Confocal fluorescence (top panels) and differential interference contact (DIC; lower panels) images of digitonin-permeabilized HeLa cells incubated with sugar-displaying QDs. A) PEG **1**–QDs; B) β-Glc **2**–QDs; C) α-Glc **3**–QDs; D) maltose **4**–QDs; E) maltotriose **5**–QDs; F) panose **6**–QDs; G) cellotriose **7**–QDs; H) digitalized fluorescence intensity of PEG **1**–QDs, maltose **4**–QDs, maltotriose **5**–QDs and panose **6**–QDs in the nucleus. The mean of the fluorescence intensity in the nucleus of each cell was digitalized.

onto the TR–BSA was confirmed by MALDI-ToF mass spectrometry. The mass spectra indicated that the average molecular weight of the BSA was increased from 66 800 to approximately 92 500 after the coupling reaction, which corresponds to the attachment of about 28 maltotriose residues. Nuclear transport of maltotriose–TR–BSA was investigated under the same conditions as those of the quantum dot experiment by using the digitonin assay. Interestingly, maltotriose–TR–BSA was incorporated into the nucleus of all observed cells, whereas TR-labeled BSA did not diffuse into the nucleus. Thus, maltotriose appears to be a nuclear import signal not only for QDs but also for proteins. In the case of quantum dots, mono α-glucopyranoside did not work as a nuclear import signal; however, trisaccharides, such as maltotriose or panose-displaying QDs, did mediate nuclear import. In the case of BSA however, both mono α-glucopyranoside and trisaccharide mediated nuclear transport. Thus, we speculate that in the case of nanoparticles it might be important to increase signal efficiency by oligomerization of monosaccharides.

To exclude the possibility that the maltotriose-lipid **5** displayed on the QDs works as a surfactant to destroy the nuclear membrane, TR–BSA was co-incubated with permeabilized HeLa cells in the presence of maltotriose **5**–QDs (at the same concentration as in Figure 2; 0.6 $mg\,mL^{-1}$) or free maltotriose–lipid **5** (20 mM). As shown in Figures 3 D and E, no accumulation of TR–BSA was detected in the nucleus of HeLa cells even in the presence of lipid **5** or maltotriose **5**–QDs; this indicates that



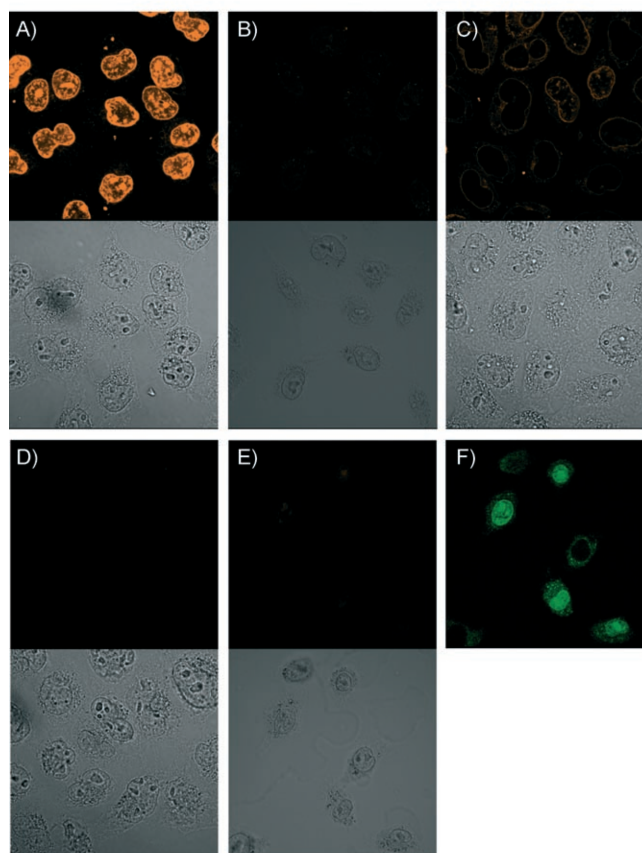


Figure 3. Confocal fluorescence (top panels) and DIC (lower panels) images of digitonin-permeabilized HeLa cells incubated with maltotriose-attached Texas Red-labeled BSA (maltotriose–TR–BSA) or Texas Red-labeled BSA (TR–BSA). A) Maltotriose–TR–BSA; B) TR–BSA; C) maltotriose–TR–BSA after WGA treatment; D) TR–BSA with maltotriose–lipid **5**; E) and F) TR–BSA incubated with maltotriose **5**–QDs; E) fluorescence from TR–BSA; F) fluorescence from maltotriose **5**–QDs.

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the free or clustered neoglycolipid does not damage the nuclear envelope and supports the hypothesis that the maltotriose moiety attached on the cargo molecule acts as a molecular-recognition site. WGA is known to bind to the *O*-GlcNAc residue of the nuclear-pore complex and to inhibit the nuclear import of macromolecules through the nuclear pores.[17] In the presence of WGA, the nuclear import of maltotriose–TR–BSA was clearly inhibited; this supports the idea that the sugar-mediated nuclear import occurs across nuclear pores. The mechanism by which the sugar-mediated nuclear transport occurs is unknown; however, because cytosolic factors are not required, we speculate that the sugar-displaying QDs or proteins engage in direct interaction with nuclear-pore proteins or proteins adjacent to the pore without the need of a carrier.

Lastly, we changed the density of maltotriose residues on the surface of quantum dots and BSA. The concentration of maltotriose residues on QDs was reduced by diluting the concentration of the maltotriose–lipid **5** with PEG–lipid **1** during the ligand-exchange reaction on the surface of MPA–QDs. The number of maltotriose residues on the BSA was controlled by changing the ratio of SMCC to TR–BSA. In the case of BSA, the average number of maltotriose residues was accurately estimated from the molecular weight, which was confirmed by MALDI-ToF MS. Figure 4 shows confocal fluorescence (top panels) and DIC (lower panels) images of digitonin-permeabilized HeLa cells after treatment with QD and BSA, respectively. In both cases, decreasing the density of maltotriose residues drastically reduced the nuclear import of QDs and BSA. The digitalized fluorescent intensities in the nuclei are summarized in Figure 4 I and J. In both cases, the fluorescent intensities in the nuclei were exponentially increased with increases in the density of maltotriose residues on the QDs and BSA. Generally, in the case of NLS peptide, only one signal sequence is required to mediate the nuclear import of a cargo, whereas the oligosaccharide-mediated nuclear import of proteins or nanoparticles requires a highly multivalent signal display. This difference implies that oligosaccharide-driven nuclear import is not mediated by a carrier protein that recognizes oligosaccharide signals, but rather the display of oligosaccharides might facilitate passive diffusion through increased affinity with nucleus pores by multivalent contacts. In fact, the nuclear import of maltotriose **5**–QDs occurred in a dose-dependent manner; this indicates the existence of an energy-independent passive diffusion mechanism.

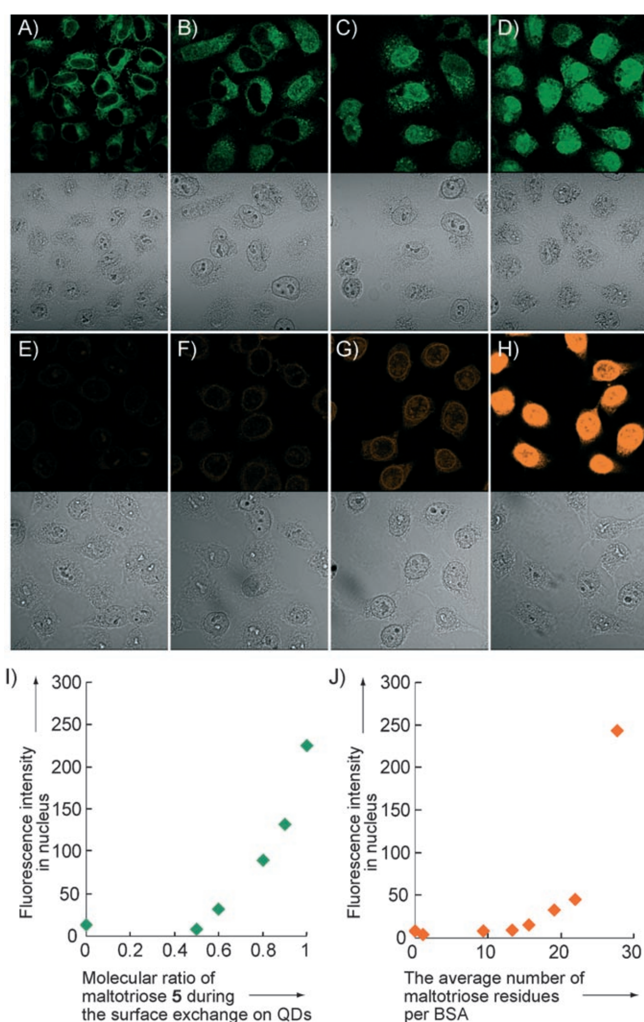

Figure 4. Confocal fluorescence (top panels) and DIC (lower panels) images of digitonin-permeabilized HeLa cells incubated with maltotriose-displaying QDs and maltotriose–TR–BSA that had a variety of maltotriose residues. The molecular ratio ([maltotriose–lipid **5**]/([maltotriose–lipid **5**] + [PEG–lipid **1**]) in the solution during the surface-exchange reaction was: A) 0, B) 0.6, C) 0.8, D) 1.0. In the case of BSA, the average number of maltotriose residues on a single BSA was: E) 0, F) 16, G) 22, H) 28. I) Digitalized fluorescence intensity of QDs in the nucleus was plotted as a function of the molecular ratio of maltotriose–lipid **5**. J) Digitalized fluorescence intensity of TR–BSA in the nucleus was plotted as a function of the average number of maltotriose residues per BSA.

In summary, nanoparticles that displayed α-glucose-containing oligosaccharides on their surface were imported into the nucleus. Unlike with proteins, mono-α-glucopyranoside did not work as a nuclear-import signal for QDs; however, α-glucopyranoside-terminated trisaccharides, such as maltotriose or panose, strongly mediated the nuclear import of QDs. The physiological significance of oligo α-glucopyranoside as a signal for nuclear import is still unclear; however, this finding could be widely applicable to the transport of a variety of nanoparticles into the cell nucleus, and could lead to new techniques for imaging or for the detection of molecular-recognition events in the nucleus. We assume that a carbohydrate-based strategy alone is not sufficient because the carbohydrate-coated nanoparticles cannot penetrate the cell membrane without the aid of chemical perforation. Therefore, we are currently engaged studying the encapsulation of the sugar-displaying nanoparticles in positively-charged liposomes to allow the passage of nanoparticles into the cytosol in live cells.

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