Site-Specific Multivalent Carbohydrate Labeling of Quantum Dots and Magnetic Beads

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Cell-surface carbohydrates act as receptors for a variety of protein ligands and thereby play a significant role in a wide range of biological processes, including immune-recognition events[1] and the interaction of viruses and bacteria with host cells[2] as well as tissue growth and repair.[3] As such, binding interactions of carbohydrates and proteins provide a starting point for the development of novel diagnostic agents and a framework for new therapies.[4] It is notable that the low affinity and specificity that are typical of monomeric carbohydrate–protein interactions are dramatically enhanced when the carbohydrate component is presented as a multivalent ligand; a phenomenon referred to as the "cluster-glycoside effect".[5–7] In response to this observation, considerable effort has focused on the design of unique, multivalent carbohydrate ligands in the form of linear polymers,[8–13] liposomes,[14,15] dendrimers,[16–18] beads,[19,20] or nanoparticles.[21–23] In this regard, we have recently described a useful route for the synthesis of glycopolymers by a cyanoxyl-mediated free-radical polymerization scheme that can be performed under aqueous condition and is tolerant of a wide range of monomer functionalities, including $-OH$, $-COOH$, $-NH_2$, and $-OSO_3H$ groups.[24] Conveniently, this synthetic approach facilitates selective derivatization of the polymer-chain terminus.[25] Herein, we report site-specific multivalent carbohydrate labeling of nanocrystal (quantum-dot) and magnetic-bead surfaces using a biotin chain-end-functionalized glycopolymer and demonstrate the potential value of these multivalent carbohydrate polymers in both imaging and biocapture applications (Figure 1).

Semiconductor nanocrystals are a new class of size-tunable optical probe.[26,27] Recently, nanocrystal surfaces have been functionalized with DNA,[28] peptides,[29] proteins,[30] and other small ligands[31] with intended applications as biological reagents and probes. Nanocrystal–streptavidin conjugates, for example, have been used to stain tissues, cells, and intracellular organelles.[32,33] Likewise, nanocrystal–avidin–antibody conjugates have improved the sensitivity of conventional fluoroimmunoassays.[34] To the best of our knowledge, carbohydrate-conjugated nanocrystals have yet to be explored in bioimaging applications although a few nanocrystal–carbohydrate conjugates have been reported (see also note added in proof).[35,36]

In the present study, nanocrystal–multivalent carbohydrate conjugates were produced by incubating nanocrystal–streptavidin (50 μL, 120 $\mu g\,mL^{-1}$ streptavidin in phosphate buffered saline (PBS), Qdot™ 565 streptavidin conjugate, Quantum Dot Corp., Hayward, CA) with biotin end-terminated glycopolymer **1** (50 μL, 1 $mg\,mL^{-1}$ in PBS) bearing ten pendant lactose groups for one hour at room temperature. RCA_{120} is a lectin that binds to terminal β-D-galactose.[37] As a model system, RCA_{120}-immobilized agarose beads (100 μL, 2 $mg\,mL^{-1}$, Sigma) were incubated with nanocrystal–carbohydrate conjugates in PBS (100 μL) for 1 h at room temperature and subsequently washed three times with PBS. Confocal microscopy confirmed fluorescent staining of the lectin-modified bead surfaces (Figure 2A). Of particular interest was that staining intensity was dramatically enhanced by the initial exposure of RCA_{120} beads to biotin end-terminated glycopolymer **1** followed by incubation of the mixture with streptavidin–nanocrystal conjugates (Figure 2B). The weak-intensity staining observed when using the first approach might have been due to the presence of free glycopolymer along with the nanocrystal–carbohydrate conjugates. As a two-step procedure, the sensitivity of staining was increased through the formation in situ of nanocrystal–carbohydrate complexes on the bead surface without the need to purify the conjugate. The absence of staining on treatment with streptavidin nanocrystals alone or with the use of nonbiotinylated glycopolymer **2** confirms the necessity and specificity of both carbohydrate–lectin and streptavidin–biotin interactions (Figure 2C).

Biotin end-terminated glycopolymers were also used to extend the versatility of magnetic-bead-based biocapture assays that have been employed for the rapid isolation of a variety of lectin-bearing cells and biomolecules.[38] Indeed, Rye and Bovin have demonstrated that glycopolymer-derivatized magnetic beads provide a useful tool for the selection of cells expressing a specific carbohydrate-binding phenotype.[39] Bundy and Fenselau have also reported that glycopolymer-based affinity capture surfaces are more sensitive than lectin-based systems for microbial capture.[40] While in both reports glycopolymers were effectively attached to the bead and

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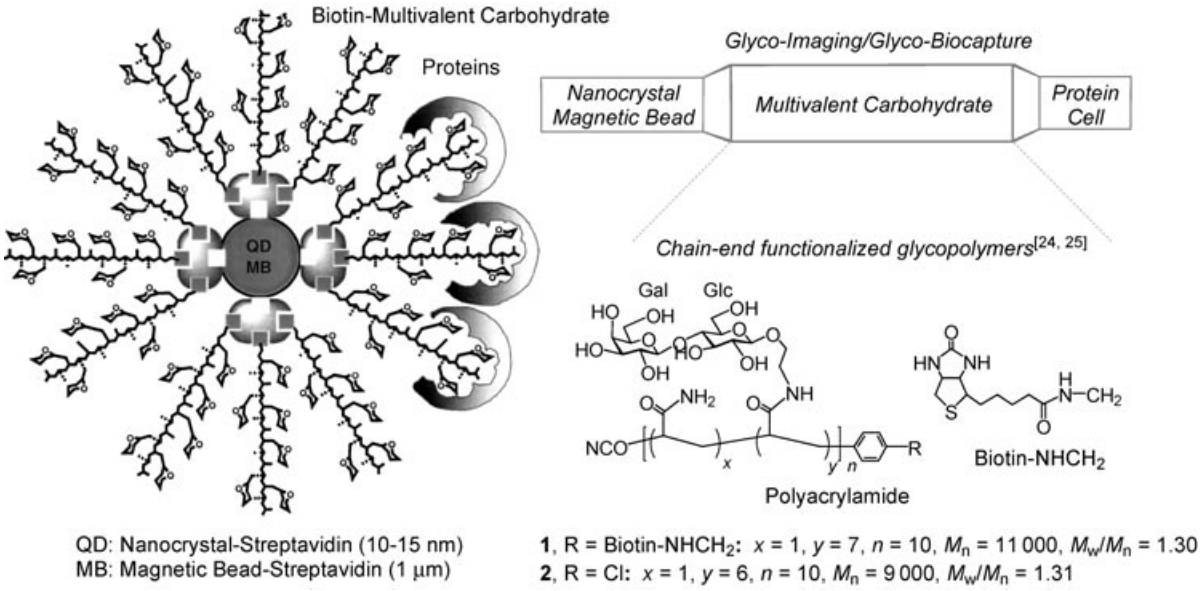

Figure 1. *Schematic illustration of a streptavidin-derivatized nanocrystal or magnetic bead that presents an oriented, high-density array of surface-bound glycopolymers.*

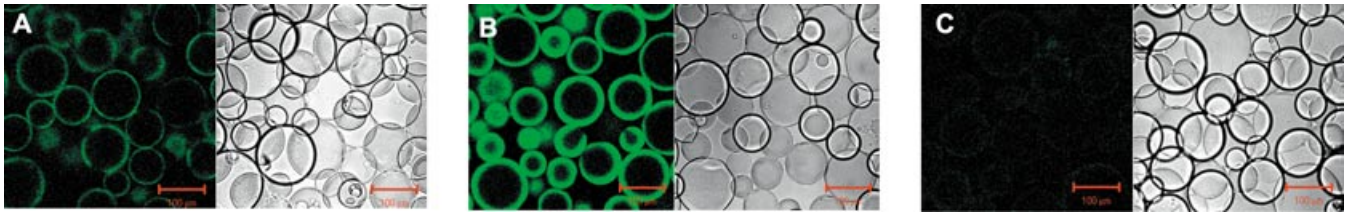

Figure 2. *Glyco-imaging with streptavidin nanocrystals and biotin end-terminated glycopolymers. A) Lectin beads stained with preformed nanocrystal–glycopolymer conjugates. B) Lectin beads initially exposed to biotin end-terminated glycopolymers followed by staining with streptavidin-nanocrystal conjugates. C) Lectin beads incubated with streptavidin nanocrystals alone. A similar image was observed when beads were incubated with nonbiotinylated glycopolymer* ***2****. Filter set ex 480 ± 20 nm/em 560 ± 20 nm). Bar: 100 μm.*

membrane surface at multiple backbone binding sites, we believe that immobilization of glycopolymers through a single chain terminus will increase ligand surface density and accessibility with a concomitant enhancement in biocapture potential and performance of particle-based biosensors.

Biotin end-terminated glycopolymer **1** was bound onto the surface of streptavidin-derivatized magnetic beads (MagPrep™ Streptavidin Beads, EMD Biosciences, Inc., San Diego, CA) by incubating 40 μg (4×10^{-9} mol) of **1** with 5 mg of beads in 0.5 mL of PBS, followed by thorough washing in PBS. To evaluate their lectin-binding capacity, the beads were incubated with a fluorescein (FITC)-labeled galactose-binding lectin (*Psophocarpus tetragonolobus*, 164 μg, 4×10^{-8} mol, Sigma) in 0.5 mL of PBS. Lectin capture was confirmed by confocal microscopy with visualization of lectin-induced bead assembly due to interactions with and between multivalent carbohydrate-coated beads (Figure 3). The lectin-binding capacity was 2.4×10^{-9} mol of lectin per mg of magnetic beads, as determined by fluorescence-intensity analysis.[41]

In order to assess capture specificity, glycopolymer-coated magnetic beads were incubated with galactose-specific binding lectin (*Psophocarpus tetragonolobus* and RCA_{120}) and glucose/mannose-specific (Concavalin A) lectin (20 μM) in 0.5 mL

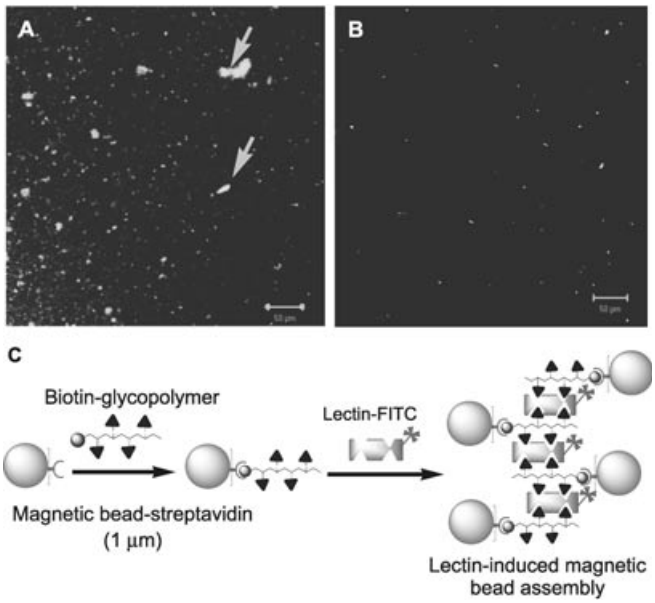

Figure 3. *Glyco-biocapture: A) FITC–lectin captured on glycopolymer-coated streptavidin magnetic beads. Arrows indicate lectin-induced bead assembly. B) Minimal nonspecific binding of FITC–lectin to uncoated streptavidin magnetic beads was observed. C) Schematic illustration of glyco-capture and lectin-induced bead assembly. (Bar: 50 μm).*

of PBS. Subsequent incubation with an excess of free biotin in combination with gel electrophoresis allowed the release and identification of the captured lectin. As demonstrated in Figure 4, capture of galactose-binding lectins was confirmed (*Psophocarpus tetragonolobus*: Gel A, lane 8; RCA_{120}: Gel B, lane 3), while capture did not occur in the absence of immobilized glycopolymer **1** (Gel A, lane 5) or with use of nonbiotinylated glycopolymer **2** (Gel A, lane 6). Likewise, beads coated with a galactose-based glycopolymer were not able to capture Concavalin A, a glucose-binding lectin (Gel B, lanes 4, 5). MALDI mass matrix solution[42] or addition of free carbohydrate ligand[39] provide alternate approaches for inducing the release of the captured target. Moreover, dissociation of biotin from streptavidin can also be achieved by incubating conjugates in formamide, phenol, or SDS, thereby releasing the captured lectin from the bead surface. Nevertheless, use of free biotin yields both the target and biotin-glycopolymer under very mild conditions and thereby facilitates further analysis of the releasate, including further structural characterization of carbohydrate-binding sites.[43]

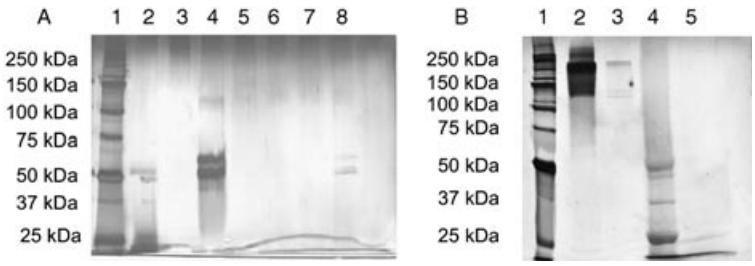

Figure 4. *Lectins released from magnetic beads by addition of free biotin (12% SDS-PAGE gel/silver staining). Gel A) Bead capture of galactose-specific lectin (*Psophocarpus tetragonolobus*). Lane 1: Molecular markers; 2: streptavidin alone; 3: biotin glycopolymer **1** alone; 4:* P. tetragonolobus *alone (M_W = 41 kDa); 5: beads without glycopolymer; 6: beads with nonbiotinylated glycopolymer **2**; 7: elution of bead-captured* P. tetragonolobus *(20 min); 8: elution of bead-captured* P. tetragonolobus *(2 h). Gel B) Bead capture of galactose-specific (RCA_{120}, M_W = 120 kDa) and glucose-specific (Concavalin A, M_W = 27 kDa) lectins. Lane 1: Molecular markers; 2: RCA_{120} alone, M_W = 120 kDa; 3: elution of bead-captured RCA_{120}; 4: Concavalin A alone (M_W = 27 kDa); 5: no evidence of captured Concavalin A.*

In summary, we have presented a strategy for oriented and high-density labeling of streptavidin–nanocrystal and magnetic-bead surfaces with biotin end-terminated carbohydrate-bearing polymers. Through variation of the polymer pendant group, nanocrystals and magnetic beads can be derivatized with a range of natural or synthetic carbohydrate ligands. In the process, the applicability of these important reagents as fluorescent probes and in biocapture applications has been extended.

Note added in proof

During manuscript submission, one report about a quantum dot-conjugated sugar ball and its cellular uptake came out: F. Osaki, T. Kanamori, S. Sando, T. Sera, Y. Aoyama, *J. Am. Chem. Soc.* **2004**, *126*, 6520–6521.

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