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Glycan multivalency effects toward albumin enable *N*-glycan-dependent tumor targeting

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ABSTRACT

Multivalent interactions play an essential role in molecular recognition in living systems. These effects were employed to target tumor cells using albumin clusters bearing ~10 molecules of asparagine-linked glycans (*N*-glycans). Noninvasive near-infrared fluorescence imaging clearly revealed A431 tumors implanted in BALB/cA-nu/nu mice after 1 h in an *N*-glycan structure-dependent manner, thereby demonstrating the efficient use of glycan multivalency effects for tumor targeting in vivo.

A variety of strong ligand–receptor interactions, such as the antigen–antibody and biotin–avidin interactions,[1,2] have been explored for their utility in medical and chemical biology applications. Relatively weak interactions can also play an important role in ligand–receptor interactions in living systems. These interactions are often strengthened by cluster effects: a single interaction that is too weak to invoke a biological reaction may be bolstered by forming ligand clusters that engage in multivalent interactions to increase the overall interaction strength and initiate a biological process. For example, the glycan–lectin interaction is relatively weak, but these moieties may be clustered to play an indispensable role in molecular recognition processes.[3–6] The artificial introduction of glycan into a template can mimic clustered molecular recognition to the benefit of biological studies.[7–11] Since the pioneering synthesis of glycopolymers by Whitesides,[12] glycocluster strategies have been extensively studied. Significant multivalency effects have been obtained among glycan dendrimers, which act as anti-adhesion molecules,[13–15] and among glycan-coated liposomes, which act as drug delivery systems.[16] Kato and Fujita recently developed a novel self-assembled spherical complex of gangliosides and characterized its interactions with amyloid β and α-sinuclein.[17]

Our group has also investigated the in vivo profiles of glycoclusters by synthesizing a polylysine-based *N*-glycan dendrimer.[18] The low stability of this dendrimer in serum resulted in rapid excretion from the mouse body. To circumvent this problem, we developed an efficient strategy for constructing *N*-glycan clusters on a protein surface using a combination of strain-promoted alkyne–azide cyclization[19] and 6π-azaelectrocyclization[20] reactions (Fig. 1).[21] The *N*-glycans **2a–f** were connected to the unsaturated aldehyde **1** linker to yield **3a–f**, which were reacted with fluorescence-labeled human serum albumin (HSA) **4** to afford the glycoalbumins **5a–f** (Figs. 1 and 2). As many as ten *N*-glycans, both homogeneous glycans and heterogeneous glycans, could be introduced onto a single molecule of HSA in a few simple steps. The in vivo kinetics of these glycans in nude mice depended strongly on the structure of the *N*-glycan: a sialic acid-terminated glycocluster was excreted via the urinary bladder, whereas a galactose-terminated glycocluster was selectively excreted via the intestines. The excretion pathway could, therefore, be controlled by the glycan structure. Microscopy studies revealed that the glucosamine-terminated glycoclusters were specifically localized in non-parenchymal cells in the liver, which might find future use in the detection of liver cirrhosis. These results suggested that receptor-mediated molecular

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