ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

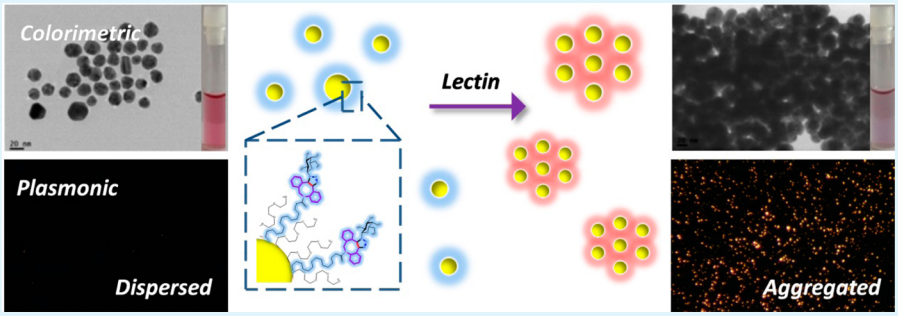
Colorimetric and Plasmonic Detection of Lectins Using Core–Shell Gold Glyconanoparticles Prepared by Copper-Free Click Chemistry

Xi-Le Hu,[†] Hong-Ying Jin,[†] Xiao-Peng He,*[,†] Tony D. James,[‡] Guo-Rong Chen,[†] and Yi-Tao Long*[,†]

[†]Key Laboratory for Advanced Materials & Institute of Fine Chemicals, East China University of Science and Technology, Shanghai 200237, PR China

[‡]Department of Chemistry, University of Bath, Bath, BA2 7AY, U.K.

Supporting Information



ABSTRACT: This study describes the simple preparation of core–shell glycosyl gold nanoparticles (AuNPs) using stepwise, copper-free click chemistry-promoted self-assembly. The as-formed glyco-AuNPs can be used for the selective detection of sugar–lectin interactions, which are vital to many important physiological and pathological processes. The approach uses AuNPs as bioprobes since they produce, sensitively, changes in both color visible to the naked eye and surface plasmon resonance (SPR), on aggregation. Strain-promoted click reaction of an azido galactoside with a lipid cyclooctyne affords a galactolipid that can be embedded into polyethylene glycol (PEG)-coated AuNP via self-assembly. Subsequently, using naked-eye and plasmon resonance scattering spectroscopy, we were able to observe the colorimetric and plasmonic variations of the glyco-AuNPs, respectively, in the presence of a selective lectin over other proteins.

KEYWORDS: *sugar, lectin, Au-NP, click chemistry, SPAAC, plasmon resonance scattering spectroscopy*

INTRODUCTION

The copper-free click chemistry was first developed by Bertozzi et al.[1] for the bioorthogonal fluorogenic imaging of species in complex biological systems. The method eliminates the employment of the catalyst, copper, used in conventional copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) click reactions[2,3] within living systems.[4−7] The removal of the catalyst might not only reduce the potential deleterious effect to the human body but also simplify the detection process.

In recent years, strain-promoted azide–alkyne cycloaddition (SPAAC),[4−7] among others,[4] has become popular for bioorthogonal imaging. The distorted bond angles of the sp-hybridized carbons in cyclooctynes (~160°) can dramatically accelerate the AAC reaction rate.[8] Following the first successful example of SPAAC unraveled by Bertozzi et al.,[1] many cyclooctyne derivatives have been effectively developed with improved reaction rate and simplified synthetic procedure.[9−13] Among them, the azadibenzocyclooctynes represent the most reactive and stable cyclooctyne, for which sophisticated[11,12] and scale-up[13] synthetic routes have been developed.

Despite the extensive use of the SPAAC for in vivo imaging, SPAAC-based sensory systems for biorecognition in vitro have been scarcely described. Popik et al.[11] reported a SPAAC-based surface functionalization method for confining a variety of molecules to the surface. SPAAC has also been employed in the conjugation of functional liposomes.[14,15] However, to the best of our knowledge, solution-based probes constructed by SPAAC for biorecognition have been elusive.

This study describes the preparation of core–shell glyco-gold nanoparticles (AuNPs) using stepwise SPAAC-promoted self-assembly. We use AuNPs as bioprobes[16−22] since they are able to produce sensitive changes in both color that is visible to the naked eye and surface plasmon resonance,[23,24] on aggregation. These NPs are then used for the detection of sugar–lectin interactions which are vital to many important physiological and pathological processes.[25−30] SPAAC was used to couple an azido galactoside with a lipid cyclooctyne, producing an amphiphilic glycolipid that can be embedded into polyethylene glycol (PEG)-coated AuNPs via self-assembly (Figure 1). This stepwise assembly is spontaneous and does not involve or generate any impurities, making it suitable for the in situ detection of lectins. Furthermore, the glyco-shell on the surface

Received: November 1, 2014
Accepted: December 22, 2014
Published: December 22, 2014

DOI: 10.1021/am5076293
ACS Appl. Mater. Interfaces 2015, 7, 1874−1878

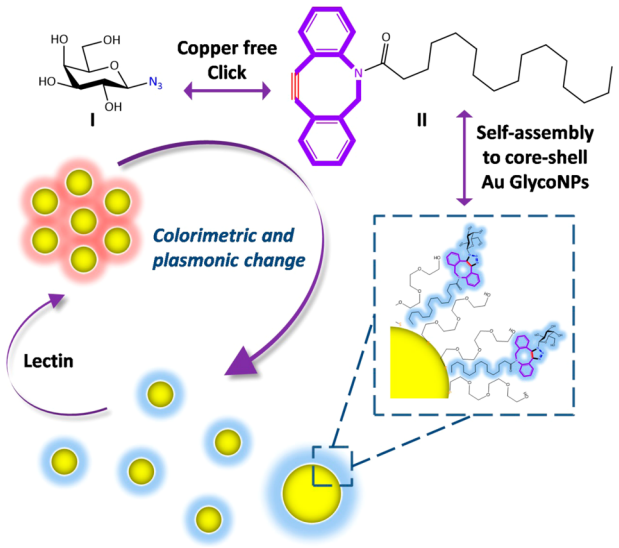


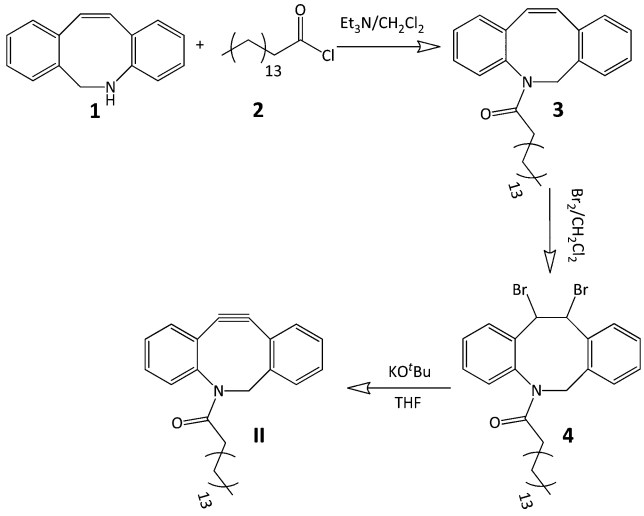
Figure 1. Cartoon depicting the simple formation of core−shell glyco AuNPs promoted by a copper-free click chemistry.

of the AuNP core has fluidity similar to cell-surface glycolipids, hence facilitating binding with the lectin.

RESULTS AND DISCUSSION

Synthesis of cyclooctyne **II** was achieved by amidation of dihydrodibenzo[*b*,*f*]azocine **1**[11] with chloride **2**, followed by a bromination-debromination procedure (Scheme 1).[11−13] With

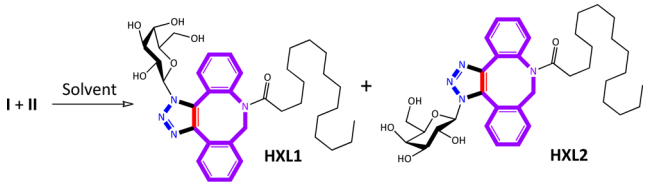
Scheme 1. Synthesis of Cyclooctyne II



II in hand, we investigated the optimal reaction condition for the SPAAC with azido galactoside **I** (Figure 1). As shown in Table 1, among several mixed solvents, MeCN/MeOH (1:1, v/v) gave mixed regioisomers[11−13] with the highest yield (89%) at room temperature with overnight stirring. Increasing the temperature to 40 °C improved the overall yield to 97% (without purification), producing the isomers **HXL1** and **HXL2** in a 1:1.6 molar ratio (as determined by HPLC).

Although it has been demonstrated that the generation of regioisomers does not impact in vivo bioorthogonal imaging,[4−7] the regioisomeric effect on the sensing properties of SPAAC-based bioprobes in vitro has never been investigated.

Table 1. Investigation of the Reaction Conditions for the Copper-Free Click Reaction for Azido Galactoside I with Cyclooctyne II



solvent (1:1, v/v)	*T* (°C)	time (h)	yield (%)[a]	HXL1/HXL2[a]
DMSO/MeOH	20	12	84	1/1.6
CH_2Cl_2/MeOH	20	12	87	1/1.6
MeCN/MeOH	20	2	18	1/1.6
	20	12	89	1/1.2
	35	12	94	1/1.4
	40	12	97	1/1.6

[a]Yield (without purification) and molar ratio monitored by HPLC.

Note that isomerism may sometimes impact the binding affinity of a ligand with its receptor. Therefore, we isolated the two isomers by column chromatography to explore if the two separate species and their mixtures would have different sensing abilities. AuNPs were coated with a thiol-PEG (2-(2-(2-(2-mercaptoethoxy)ethoxy)ethoxy)ethanol) to both enhance the water dispersibility and facilitate the embedding of the amphiphilic galactolipids into the PEG layer of the particles (Figure 1). Then, the isolated isomers **HXL1** and **HXL2** and mixtures with different ratios (**HXL1**/**HXL2** = 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and a molar ratio of 1:1.6 which is the ratio produced by the SPAAC reaction without purification) were embedded into the AuNPs in a Tris-HCl buffer (0.01 M, pH 7.4) to generate the core−shell glyco-AuNPs, which were used for lectin detection.

Using UV spectroscopy, the recognition of the glyco-AuNPs toward a galactose-selective peanut agglutinin (PNA) was carried out. As shown in Figure 2, addition of increasing PNA to the buffer solution of the particles with the single regioisomers (Figure 2a and Figure 2d for **HXL1** and **HXL2**, respectively) or with mixed isomers (Figure 2b and Figure 2c with ratios of 5:5 and 1:1.6, respectively) led to similar concentration-dependent bathochromic shifts in the UV spectra. These observations are in accordance with previous literature reports suggesting the aggregation of glyco-AuNPs upon complexation with the polymeric lectin.[16−22]

Plotting the UV absorbance change of the four glyco-AuNPs as a function of PNA concentration led to linear plots in a concentration range of 0.01−0.05 μM (Figure 2e), and the limits of detection (LODs) of **HXL1**, **HXL2**, **HXL1**/**HXL2** (5:5) and **HXL1**/**HXL2** (1:1.6) embedded probes were determined to be 4.4, 4.7, 3.5, and 3.5 nM, respectively. Additionally, the LODs of glyco-AuNPs with other **HXL1**/**HXL2** mixing ratios were determined to range from 3.4 to 4.7 nM (Figure 2f, Supporting Information Figure S1 and Figure S2). These observations suggest that (1) AuNPs with the pure isomers show similar sensing properties, (2) those with the mixed isomers (irrespective of the mixing ratio) show slightly better sensitivity than with the pure isomers (which provides a direct evidence that SPAAC-produced isomerism does not impact largely the sensitivity of the resulting bioprobe), and importantly (3) the regioisomeric products obtained from the SPAAC reaction can be used without purification for self-

DOI: 10.1021/am5076293
ACS Appl. Mater. Interfaces 2015, 7, 1874−1878

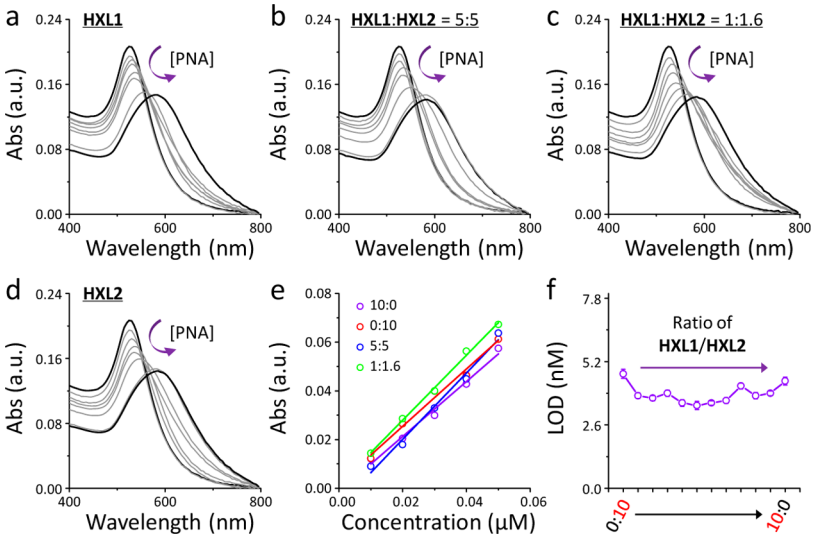


Figure 2. UV spectra of AuNP embedded with (a) **HXL1**, (b) **HXL1**/**HXL2** = 5:5, (c) **HXL1**/**HXL2** = 1:1.6 and (d) **HXL2** in the presence of increasing PNA (0–0.2 μM). (e) Plotting the UV absorbance (Abs) change of AuNP with pure **HXL1** (10:0), pure **HXL2** (0:10), **HXL1**/**HXL2** = 5:5 or **HXL1**/**HXL2** = 1:1.6 as a function of PNA concentration. (f) Plotting the limit of detection (LOD) of AuNP as a function of **HXL1** to **HXL2** ratio. (Solvent: 0.01 M Tris-HCl, pH 7.4.) The LOD difference of different mixing ratios is statistically significant ($P < 0.05$).

assembly with PEG-AuNPs and are suitable for the detection of lectin. These observations imply that the simple SPAAC-based assembly strategy developed is well-suited for biosensing.

To investigate the sensing behavior of the probe, dynamic light scattering (DLS) and scanning electron microscope (SEM) were carried out. The raw AuNPs (Figure 3a and Figure 3b) and glyco-AuNPs (Figure 3c and Figure 3d, with embedded **HXL1**/**HXL2** of 1:1.6 molar ratio) possessed similar particle sizes (10–100 nm) and showed good dispersibility in aqueous buffer solution (with the typical reddish color of

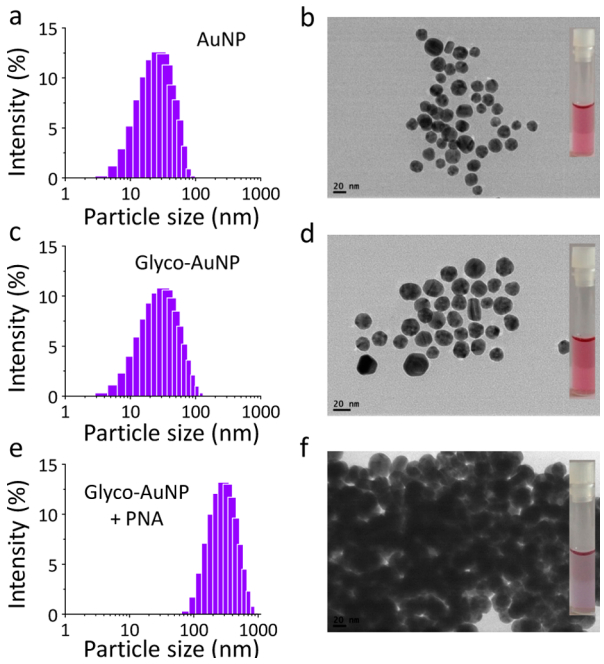


Figure 3. Dynamic light scattering of (a) AuNP, (c) glyco-AuNP, and (e) glyco-AuNP in the presence of 0.2 μM PNA. Scanning electron microscope images of (b) AuNP, (d) glyco-AuNP, and (f) glyco-AuNP in the presence of 0.2 μM PNA. (Insets: color of the corresponding nanoparticles in 0.01 M Tris-HCl, pH 7.4).

AuNPs). In contrast, addition of PNA to the glyco-AuNP solution led to particle size enlargement (100–1000 nm) and aggregation, along with a color change from red to violet. Moreover, addition of increasing PNA to the solution led to a concentration-dependent increase of the particle size (Supporting Information Figure S3). These observations are in agreement with the UV spectral variations and suggest the successful self-assembly of the core–shell glyco-AuNPs for recognition of lectins via gold-aggregation-induced colorimetric and UV absorbance changes.[16−22]

In addition, thermogravimetric and anthrone/sulfuric acid-based colorimetric analyses (well-established methods for quantification of sugars)[31−33] were carried out to quantify the galactolipids coated on the AuNP surface. Both analyses (Supporting Information Figure S4) suggested that the galactolipids were immobilized on the particle surface with a high surface coverage rate (82% and 91% as determined by the thermogravimetric and anthrone method, respectively).

We then investigated the selectivity of the glyco-AuNPs toward a range of lectins and proteins including the *N*-acetyl-glucosamine-selective wheat germ agglutinin (WGA), mannose/glucose-selective concanavalin A (ConA), mannose-selective *Lens culinaris* (LCA), bovine serum albumin (BSA) and pepsin (PeP) (Figure 4). These proteins did not cause substantial color (Figure 4a) and UV spectral (Figure 4b) variations of the glyco-AuNPs, suggesting the good selectivity of the glyco-AuNPs.

By taking advantage of the plasmonic resonance scattering (PRS) property of AuNP,[23,24] we also used PRS spectroscopy to test the selectivity of the glyco-AuNPs. The glyco-AuNPs without or with incubation with the different proteins were immobilized on indium tin oxide (ITO) glass slides, and then their dark-field images were recorded.[23,24] We observed that the glyco-AuNPs in the presence of PNA showed evident surface scattering (Figure 5b). However, addition of other unselective proteins (Figure 5c–5g) did not cause the scattering of the AuNPs, which is similar to the property of glyco-AuNP alone (Figure 5a). This suggests that the selective sugar-lectin complexation that produces AuNP aggregates could enhance

DOI: 10.1021/am5076293
ACS Appl. Mater. Interfaces 2015, 7, 1874–1878

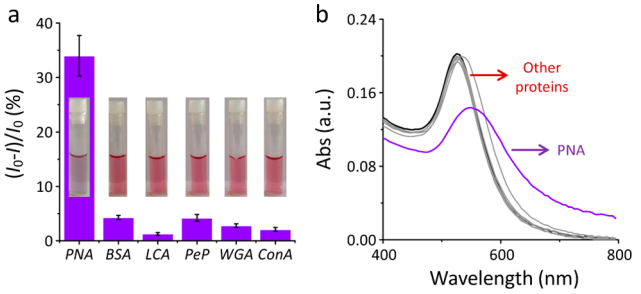


Figure 4. (a) Histograms (where I_0 and I are the UV absorbance of the particles in the absence and presence of a protein at 525 nm, respectively) and (b) UV spectra depicting the selectivity of the self-assembled glyco-AuNP (in 0.01 M Tris-HCl, pH 7.4) in the presence of selective and unselective lectins and proteins [peanut agglutinin (PNA), bovine serum albumin (BSA), *Lens culinaris* (LCA), pepsin (PeP), wheat germ agglutinin (WGA), and concanavalin A (ConA)].

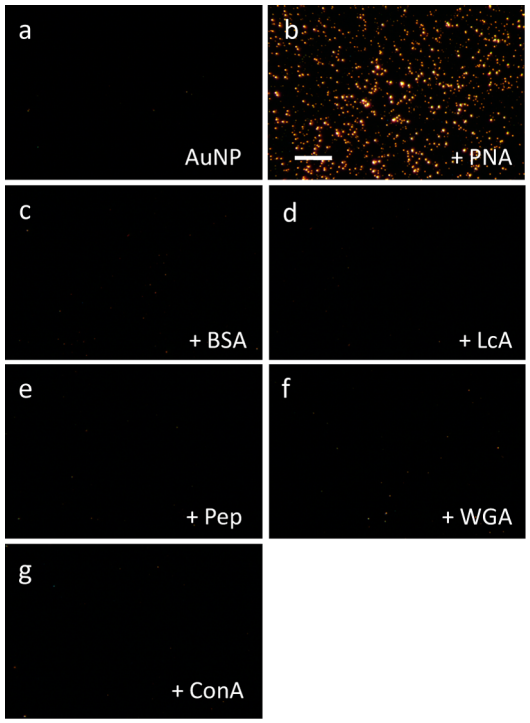


Figure 5. Dark-field images of the self-assembled glyco-AuNP in the absence and presence of selective and unselective lectins and proteins [peanut agglutinin (PNA), bovine serum albumin (BSA), *Lens culinaris* (LCA), pepsin (PeP), wheat germ agglutinin (WGA). and concanavalin A (ConA)] (scale bar: 10 μm).

the PRS signal of the glyco-AuNPs upon aggregation. These observations are in agreement with the SEM and UV results obtained.

CONCLUSION

In summary, we have developed a simple strategy for the self-assembly of core−shell glyco-AuNPs using copper-free click chemistry. The glyco-nanoparticles produced by SPAAC (i.e., the reaction ratio of **HXL1**/**HXL2** = 1:1.6) could be used directly for the sensitive and selective detection of lectins using both the UV and plasmonic resonance scattering spectroscopic techniques. This study provides a unique insight into the simple production of nanoparticles for biorecognition.

ASSOCIATED CONTENT

Supporting Information

Experimental section, additional figures, and NMR copy of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: xphe@ecust.edu.cn.
*E-mail: ytlong@ecust.edu.cn.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the 973 project (2013CB733700), the National Natural Science Foundation of China (21176076, 21202045), the Key Project of Shanghai Science and Technology Commission (13NM1400900), and the Fundamental Research Funds for the Central Universities. The Catalysis And Sensing for our Environment (CASE) network is thanked for research exchange opportunities.

REFERENCES

(1) Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. A Strain-Promoted [3 + 2] Azide−Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems. *J. Am. Chem. Soc.* **2004**, *126*, 15046−15047.
(2) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596−2599.
(3) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* **2002**, *67*, 3057−3064.
(4) Chang, P. V.; Bertozzi, C. R. Imaging Beyond the Proteome. *Chem. Commun.* **2012**, *48*, 8864−8879.
(5) Jewett, J. C.; Bertozzi, C. R. Cu-Free Click Cycloaddition Reactions in Chemical Biology. *Chem. Soc. Rev.* **2010**, *39*, 1272−1279.
(6) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. In Vivo Imaging of Membrane-Associated Glycans in Developing Zebrafish. *Science* **2008**, *320*, 664−667.
(7) Sletten, E. M.; Bertozzi, C. R. From Mechanism to Mouse: A Tale of Two Bioorthogonal Reactions. *Acc. Chem. Res.* **2011**, *44*, 666−676.
(8) Wittig, G.; Krebs, A. Zur Existenz Niedergliedriger Cycloalkine, I. *Chem. Ber.* **1961**, *94*, 3260−3275.
(9) Chadwick, R. C.; Van Gyzen, S.; Liogier, S.; Adronov, A. Scalable Synthesis of Strained Cyclooctyne Derivatives. *Synthesis* **2014**, *46*, 669−677.
(10) Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. Aza-dibenzocyclooctynes for Fast and Efficient Enzyme PEGylation via Copper-Free (3 + 2) Cycloaddition. *Chem. Commun.* **2010**, *46*, 97−99.
(11) Kuzmin, A.; Poloukhtine, A.; Wolfert, M. A.; Popik, V. V. Surface Functionalization Using Catalyst-Free Azide−Alkyne Cycloaddition. *Bioconjugate Chem.* **2010**, *21*, 2076−2085.
(12) Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. Visualizing Metabolically Labeled Glycoconjugates of Living Cells by Copper-Free and Fast Huisgen Cycloadditions. *Angew. Chem., Int. Ed.* **2008**, *47*, 2253−2255.
(13) Sletten, E. M.; Bertozzi, C. R. A Hydrophilic Azacyclooctyne for Cu-Free Click Chemistry. *Org. Lett.* **2008**, *10*, 3097−3099.
(14) Jølck, R. I.; Sun, H.; Berg, R. H.; Andresen, T. L. Catalyst-Free Conjugation and in Situ Quantification of Nanoparticles Ligand

DOI: 10.1021/am5076293
ACS Appl. Mater. Interfaces 2015, 7, 1874−1878

Surface Density Using Fluorogenic Cu-Free Click Chemistry. *Chem.—Eur. J.* **2011**, *17*, 3326−3331.
(15) Bostic, H. E.; Smith, M. D.; Poloukhtine, A. A.; Popik, V. V.; Best, M. D. Membrane Labeling and Immobilization via Copper-Free Click Chemistry. *Chem. Commun.* **2012**, *48*, 1431−1433.
(16) Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, G.-F.; Chen, Y.-C.; Wu, Y.-C.; Chen, C.-C. Quantitative Analysis of Multivalent Interactions of Carbohydrate-Encapsulated Gold Nanoparticles with Concanavalin A. *Chem. Commun.* **2003**, *39*, 2920−2921.
(17) Marín, M.; Rashid, A.; Rejzek, M.; Fairhurst, S. A.; Wharton, S. A.; Martin, S. R.; McCauley, J. W.; Wileman, T.; Field, R. A.; Russell, D. A. Glyconanoparticles for the Plasmonic Detection and Discrimination Between Human and Avian Influenza Virus. *Org. Biomol. Chem.* **2013**, *11*, 7101−7107.
(18) Marradi, M.; Chiodo, F.; García, I.; Penadés, S. Glyconanoparticles as Multifunctional and Multimodal Carbohydrate Systems. *Chem. Soc. Rev.* **2013**, *42*, 4728−4745.
(19) Nagy, J. O.; Zhang, Y.; Yi, W.; Liu, X.; Motari, E.; Son, J. C.; Lejeune, J. T.; Wang, P. G. Glycopolydiacetylene Nanoparticles as a Chromatic Biosensor To Detect Shiga-Like Toxin Producing *Escherichia coli* O157:H7. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 700−703.
(20) Schofield, C. L.; Mukhopadhyay, B.; Hardy, S. M.; McDonnell, M. B.; Field, R. A.; Russell, D. A. Colorimetric Detection of *Ricinus communis* Agglutinin 120 Using Optimally Presented Carbohydrate-Stabilised Gold Nanoparticles. *Analyst* **2008**, *133*, 624−634.
(21) Wei, J.; Zheng, L.; Lv, X.; Bi, Y.; Chen, W.; Zhang, W.; Shi, Y.; Zhao, L.; Sun, X.; Wang, F.; Cheng, S.; Yan, J.; Liu, W.; Jiang, X.; Gao, G. F.; Li, X. Analysis of Influenza Virus Receptor Specificity Using Glycan-Functionalized Gold Nanoparticles. *ACS Nano* **2014**, *8*, 4600−4607.
(22) Adak, A. K.; Kin, H.-J.; Lin, C.-C. Multivalent Glycosylated Nanoparticles for Studying Carbohydrate−Protein Interactions. *Org. Biomol. Chem.* **2014**, *12*, 5563−5573.
(23) Shi, L.; Jing, C.; Ma, W.; Li, D.-W.; Halls, J. E.; Marken, F.; Long, Y.-T. Plasmon Resonance Scattering Spectroscopy at the Single-Nanoparticle Level: Real-Time Monitoring of A Click Reaction. *Angew. Chem., Int. Ed.* **2013**, *52*, 6011−6014.
(24) Jing, C.; Gu, Z.; Ying, Y.-L.; Li, D.-W.; Zhang, L.; Long, Y.-T. Chrominance to Dimension: A Real-Time Method for Measuring the Size of Single Gold Nanoparticles. *Anal. Chem.* **2012**, *84*, 4284−4291.
(25) Zhang, Y.; Guo, Y.; Xianyu, Y.; Chen, W.; Zhao, Y.; Jiang, X. Nanomaterials for Ultrasensitive Protein Detection. *Adv. Mater.* **2013**, *25*, 3802−3819.
(26) Li, Z.; Deng, S.-S.; Zang, Y.; Gu, Z.; He, X.-P.; Chen, G.-R.; Chen, K.; James, T. D.; Li, J.; Long, Y.-T. Capturing Intercellular Sugar-Mediated Ligand−Receptor Recognitions via a Simple Yet Highly Biospecific Interfacial System. *Sci. Rep.* **2013**, *3*, 2293.
(27) Zhang, H.-L.; Zang, Y.; Xie, J.; Li, J.; Chen, G.-R.; He, X.-P.; Tian, H. A "Clicked" Tetrametric Hydroxamic Acid Glycopeptidomimetic Anatagonizes Sugar−Lectin Interactions on the Cellular Level. *Sci. Rep.* **2014**, *4*, 5513.
(28) Ma, W.; Liu, H.-T.; He, X.-P.; Zang, Y.; Li, J.; Chen, G.-R.; Tian, H.; Long, Y.-T. Target-Specific Imaging of Transmembrane Receptors Using Quinonyl Glycosides Functionalized Quantum Dots. *Anal. Chem.* **2014**, *86*, 5502−5507.
(29) Li, K.-B.; Zang, Y.; Wang, H.; Li, J.; Chen, G.-R.; James, T. D.; He, X.-P.; Tian, H. Hepatoma-Selective Imaging of Heavy Metal Ions Using a "Clicked" Galactosylrhodamine Probe. *Chem. Commun.* **2014**, *50*, 11735−11737.
(30) He, X.-P.; Li, R.-H.; Maisonneuve, S.; Ruan, Y.; Chen, G.-R.; Xie, J. Fluorogenic Supramolecular Complexes Formed Between Pyrenyl-β-Cyclodextrin and Glyco-Rhodamine for the Selective Detection of Lectins. *Chem. Commun.* **2014**, *50*, 14141−14144.
(31) Koehler, L. H. Differentiation of Carbohydrates by Anthrone Reaction Rate and Color Intensity. *Anal. Chem.* **1952**, *24*, 1576−1579.
(32) Chien, Y.-Y.; Jan, M.-D.; Adak, A. K.; Tzeng, H.-C.; Lin, Y.-P.; Chen, Y.-J.; Wang, K.-T.; Chen, C.-T.; Chen, C.-C.; Lin, C.-C. Globotriose-Functionalized Gold Nanoparticles as Multivalent Probes for Shiga-Like Toxin. *ChemBioChem.* **2008**, *9*, 1100−1109.
(33) Wang, X.; Ramström, O.; Yan, M. A Photochemically Initiated Chemistry for Coupling Underivatized Carbohydrates to Gold Nanoparticles. *J. Mater. Chem.* **2009**, *19*, 8944−8949.

DOI: 10.1021/am5076293
ACS Appl. Mater. Interfaces 2015, 7, 1874−1878