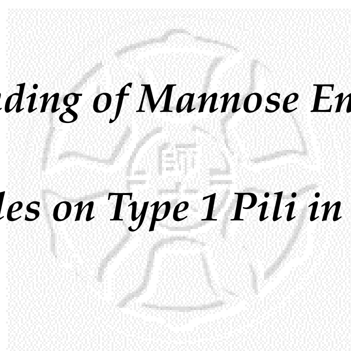
Chapter 3.

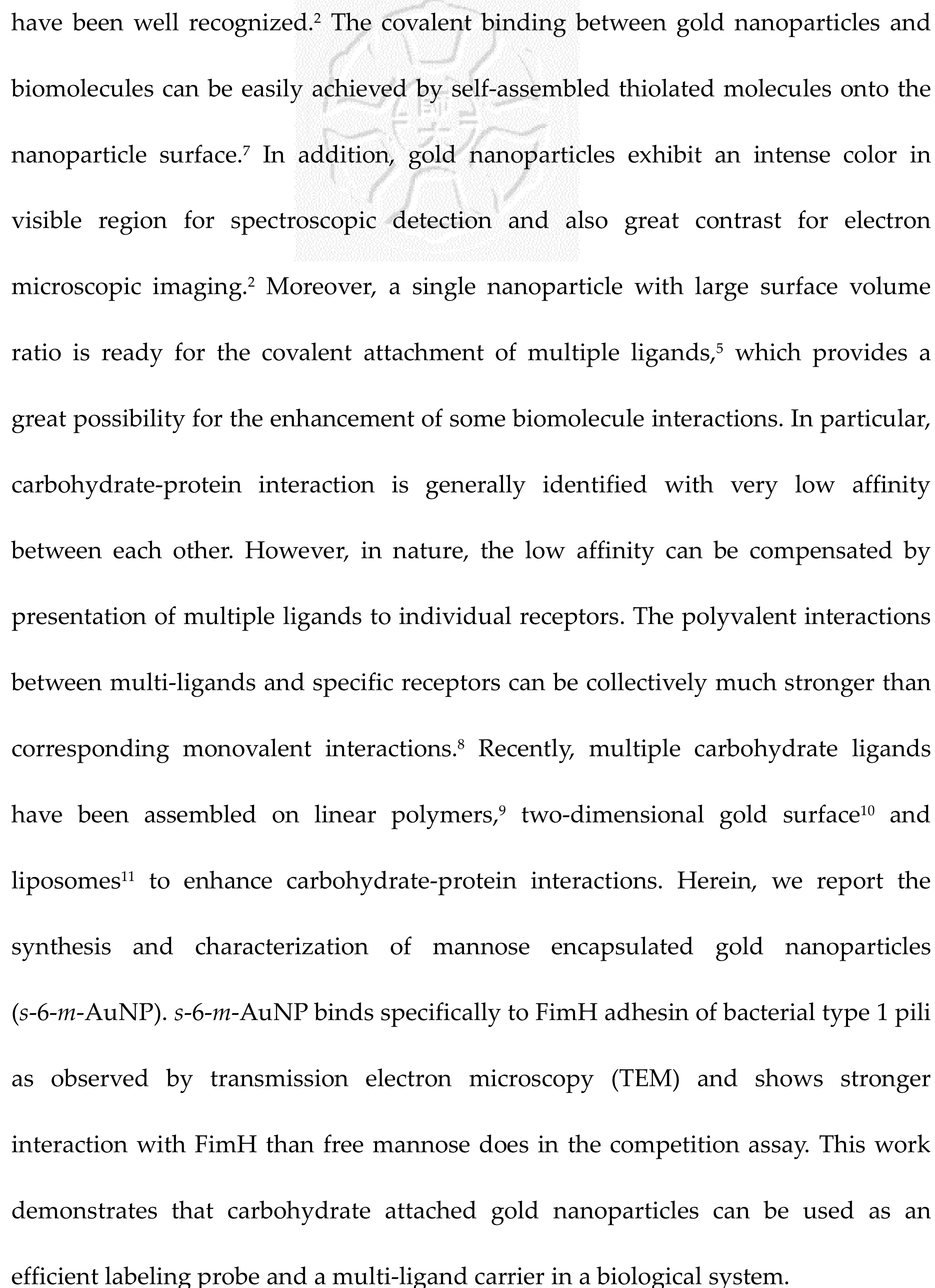


Selective Binding of Mannose Encapsulated Gold Nanoparticles on Type 1 Pili in Escherichia coli

3.1. Introduction

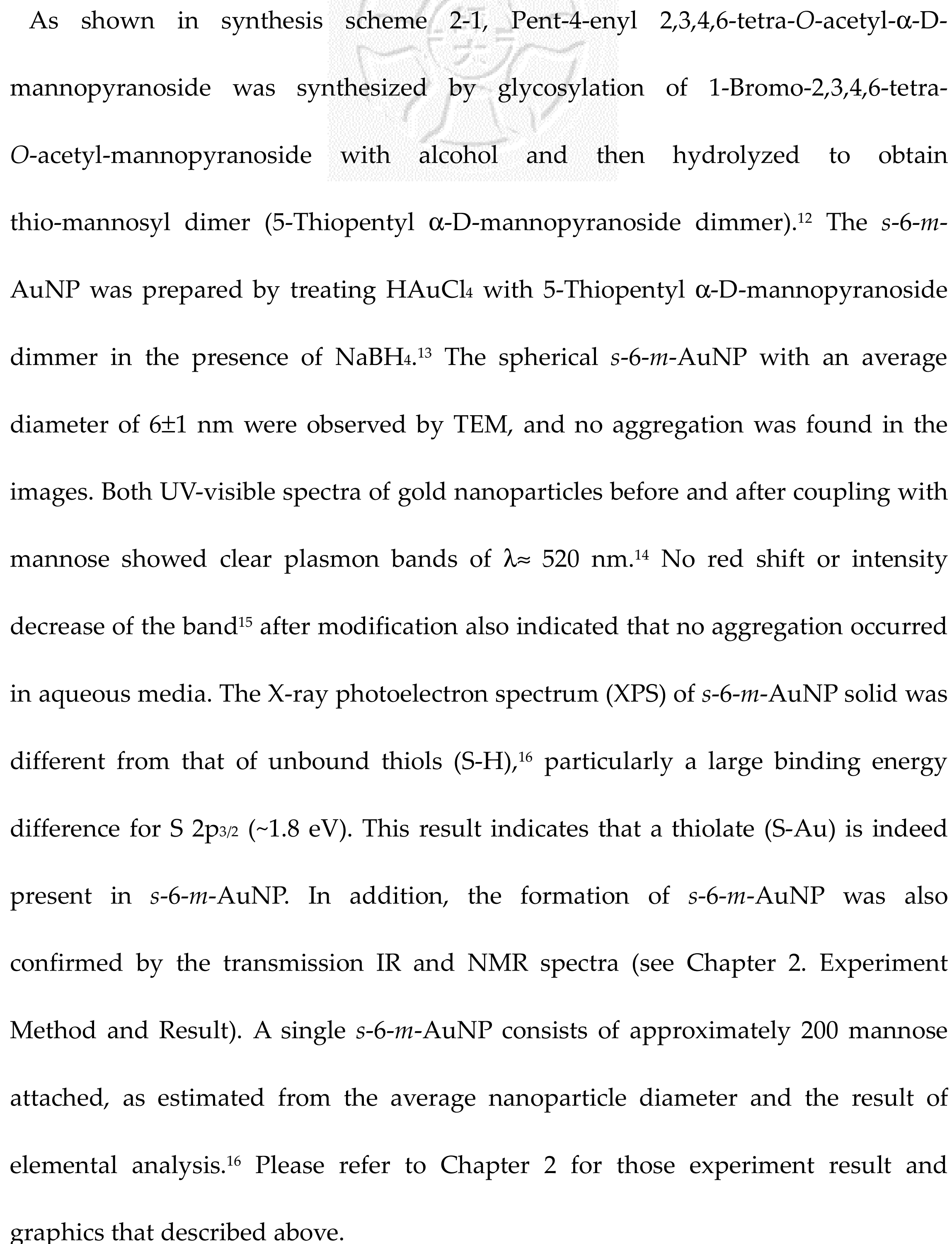
Metal and semiconductor nanoparticles coupling with biomolecules have attracted great interests recently because the resulting materials may bring new applications in biological systems.[1] In protein-based recognition, many current diagnostic kits have been long developed on the basis of the interaction between antibody conjugated gold nanoparticles and their receptors.[2] Of late, gold nanoparticles attached with DNA have been applied for the detection of target sequence through complementary hybridization.[3] Also, functional organic ligands have been encapsulated on gold nanoparticles for the fabrication of new chemical probes.[4] Besides gold nanoparticles, semiconductor nanoparticle bioconjugates as selective fluorescent biological labels have shown great potential in biological studies and medical applications.[5] However, the biomolecules on functionalized nanoparticles of recent studies have been mostly limited in DNA and protein, and very few examples[6] were reported on carbohydrates. Moreover, the application of carbohydrate-conjugated nanoparticles in biological assays has not been explored.

The technical advantages of applying gold nanoparticles in biological systems



have been well recognized.[2] The covalent binding between gold nanoparticles and biomolecules can be easily achieved by self-assembled thiolated molecules onto the nanoparticle surface.[7] In addition, gold nanoparticles exhibit an intense color in visible region for spectroscopic detection and also great contrast for electron microscopic imaging.[2] Moreover, a single nanoparticle with large surface volume ratio is ready for the covalent attachment of multiple ligands,[5] which provides a great possibility for the enhancement of some biomolecule interactions. In particular, carbohydrate-protein interaction is generally identified with very low affinity between each other. However, in nature, the low affinity can be compensated by presentation of multiple ligands to individual receptors. The polyvalent interactions between multi-ligands and specific receptors can be collectively much stronger than corresponding monovalent interactions.[8] Recently, multiple carbohydrate ligands have been assembled on linear polymers,[9] two-dimensional gold surface[10] and liposomes[11] to enhance carbohydrate-protein interactions. Herein, we report the synthesis and characterization of mannose encapsulated gold nanoparticles (*s*-6-*m*-AuNP). *s*-6-*m*-AuNP binds specifically to FimH adhesin of bacterial type 1 pili as observed by transmission electron microscopy (TEM) and shows stronger interaction with FimH than free mannose does in the competition assay. This work demonstrates that carbohydrate attached gold nanoparticles can be used as an efficient labeling probe and a multi-ligand carrier in a biological system.

3.2. Result

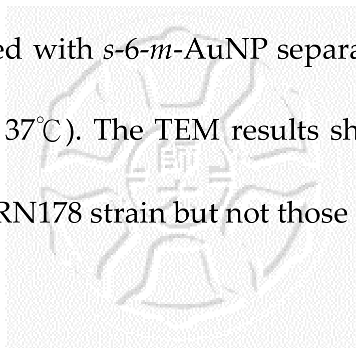


As shown in synthesis scheme 2-1, Pent-4-enyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside was synthesized by glycosylation of 1-Bromo-2,3,4,6-tetra-*O*-acetyl-mannopyranoside with alcohol and then hydrolyzed to obtain thio-mannosyl dimer (5-Thiopentyl α-D-mannopyranoside dimmer).[12] The *s*-6-*m*-AuNP was prepared by treating $HAuCl_4$ with 5-Thiopentyl α-D-mannopyranoside dimmer in the presence of $NaBH_4$.[13] The spherical *s*-6-*m*-AuNP with an average diameter of 6±1 nm were observed by TEM, and no aggregation was found in the images. Both UV-visible spectra of gold nanoparticles before and after coupling with mannose showed clear plasmon bands of $\lambda \approx 520$ nm.[14] No red shift or intensity decrease of the band[15] after modification also indicated that no aggregation occurred in aqueous media. The X-ray photoelectron spectrum (XPS) of *s*-6-*m*-AuNP solid was different from that of unbound thiols (S-H),[16] particularly a large binding energy difference for S $2p_{3/2}$ (~1.8 eV). This result indicates that a thiolate (S-Au) is indeed present in *s*-6-*m*-AuNP. In addition, the formation of *s*-6-*m*-AuNP was also confirmed by the transmission IR and NMR spectra (see Chapter 2. Experiment Method and Result). A single *s*-6-*m*-AuNP consists of approximately 200 mannose attached, as estimated from the average nanoparticle diameter and the result of elemental analysis.[16] Please refer to Chapter 2 for those experiment result and graphics that described above.

3.3. Discussion

The stability of *s*-6-*m*-AuNP in various media was further examined before its application in a biological system. Previous reports have shown that gold nanoparticles stabilized by surfactants or polymers were not effective in preventing aggregation of the nanoparticles particularly under high concentrations of salt medium.[17] *s*-6-*m*-AuNP was found to be very stable in deionized water and phosphate buffer solution (PBS) as suggested by the absorption spectra, and its stability was independent of high ion strength and pH values in the range from 1.5 to 12 of solutions. *s*-6-*m*-AuNP is easily redissolved in aqueous media without aggregation. These properties have made application of *s*-6-*m*-AuNP in biological systems feasible.

s-6-*m*-AuNP was then tested for its ability to bind mannose-specific adhesin FimH of type 1 pili in *E. coli*. Type 1 pili are filamentous proteinaceous appendages that extend from the surface of many gram-negative organisms and are composed of FimA, FimF, FimG and FimH proteins.[18] The FimA accounts for more than 98% of the pilus protein, and FimH is uniquely responsible for the binding to D-mannose.[19] Two *E. coli* strains ORN178 and ORN208 were used in experiments to examine the affinity of *s*-6-*m*-AuNP to FimH. The ORN178 strain expresses wild-type type 1 pili, whereas the ORN208 strain is deficient of the FimH gene and expresses abnormal type 1 pili that fail to mediate D-mannose-specific binding.[19a] Two bacterial strain



mixtures were incubated with *s*-6-*m*-AuNP separately in buffers (PBS, LB, H_2O) and at temperatures (4, 25, 37℃). The TEM results showed that *s*-6-*m*-AuNP selectively bound the pili of the ORN178 strain but not those of the ORN208 strain (Figure 3-1).

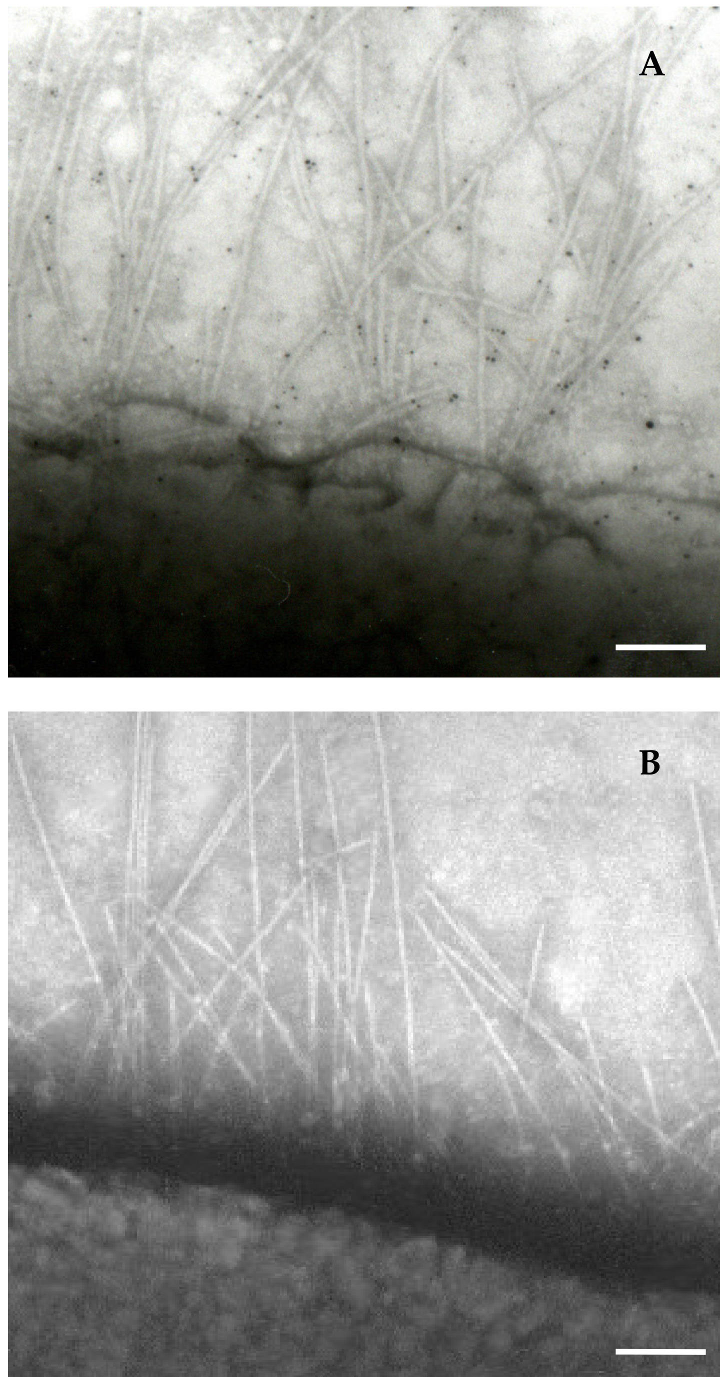


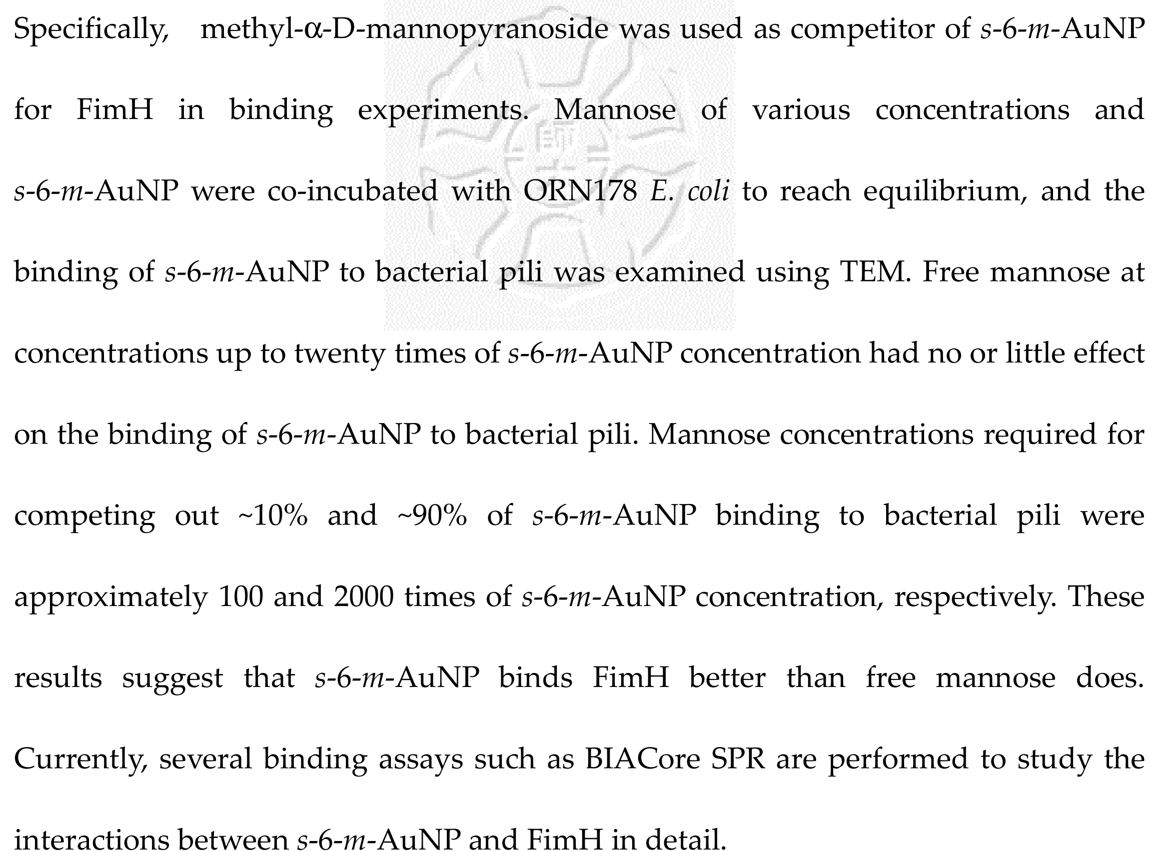
Figure 3-1. Typical TEM images of sectioned areas of (A) pili of the *E. coli* ORN178 strain bound with *s*-6-*m*-AuNP, (B) the *E. coli* ORN208 strain deficient of the fimH gene without *s*-6-*m*-AuNP bound. The experiments were performed in LB at room temperature. Scale bar = 100 nm.

The selective binding was observed in all buffers tested. On the other hand, the temperatures significantly affected *s*-6-*m*-AuNP binding. We found that 25℃ was the best temperature tested for *s*-6-*m*-AuNP binding and that more nonspecific binding and less specific binding were observed in TEM at 4℃ and 37℃ than 25℃, respectively. The nanoparticles were localized at the lateral ends and distributed at intervals along the shaft of the pili (on the average of 100 to 150 nm interval) in ORN178 strain, consistent with the lateral localization and similar distribution interval of FimH protein along type 1 pili by immunoelectron microscopy.[19b]

Further experiments were performed to test the binding ability of *s*-6-*m*-AuNP to FimH with respect to free mannose in solution, see Table 3.

m-AuNP : mannose[a]	Percentage of m-AuNP binding to pili [b]
1:1	~100%
1:10	~100%
1:20	>90%
1:100	>50%
1:200	<50%
1:2000	<10%
1:20000	0%

Table 3. Competition binding assay of *s*-6-*m*-AuNP. a Concentration ratio of m-AuNP and methyl α-D-mannopyranoside. b The percentages were calculated from the ratios of the numbers of m-AuNP binding.



Specifically, methyl-α-D-mannopyranoside was used as competitor of *s*-6-*m*-AuNP for FimH in binding experiments. Mannose of various concentrations and *s*-6-*m*-AuNP were co-incubated with ORN178 *E. coli* to reach equilibrium, and the binding of *s*-6-*m*-AuNP to bacterial pili was examined using TEM. Free mannose at concentrations up to twenty times of *s*-6-*m*-AuNP concentration had no or little effect on the binding of *s*-6-*m*-AuNP to bacterial pili. Mannose concentrations required for competing out ~10% and ~90% of *s*-6-*m*-AuNP binding to bacterial pili were approximately 100 and 2000 times of *s*-6-*m*-AuNP concentration, respectively. These results suggest that *s*-6-*m*-AuNP binds FimH better than free mannose does. Currently, several binding assays such as BIACore SPR are performed to study the interactions between *s*-6-*m*-AuNP and FimH in detail.

3.4. Conclusion

The strong and selective binding of *s*-6-*m*-AuNP to bacterial type 1 pili presents a novel method of labeling specific protein on the cell surface using carbohydrate-conjugated nanoparticles. Moreover, in comparison with the conventional sandwich immunoassay, the biomolecule-conjugated nanoparticles can provide a relatively easy and direct method to visualize the target receptors on the cell surface under an electron microscope. Other potential applications of carbohydrate encapsulated nanoparticles, for example, to intervene the protein-carbohydrate adhesion may provide new insights for the design of pharmaceutical active agents.