Supplementary materials

Multivalent mannose displaying nanoparticles constructed from poly{styrene-*co*-[(maleic anhydride)-*alt*-styrene]}

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Content

Titration of mannose and galactose in NP-mannose and NP-glalactose

The amount of sugar were titrated according a published procedure[1]. Briefly, various aliquots of mannose /galactose solution (100 mM) were added into test tubes and the final volumes were brought to 1mL with deionized water. To the tubes were added 80% phenol in water (50 μL) followed by concentrated sulfuric acid (5 mL). The solutions were incubated at ambient temperature for 10minutes, and then shaken and placed for 20 minutes in a water bath at 25 ^{o}C. The absorbance at 490nm was measured as the function of monosaccharide concentrations on a spectrophotometer using distilled water as the control (Figure 1S and Figure 2S). The nanoparticles were titrated using the same procedure as described above, and the amounts of sugar in the nanoparticles were determined based on the standard curves (Table 1S). According to the equations in Figure 7S and Figure 8S, the percentages of mannose or galactose in NP-mannose and NP-galactose (*P*) were calculated as following:

Mannose/NP-mannose

$$P = \frac{0.516 - 0.1525}{0.0609} \times \frac{1}{17.094} = 34.9\%$$

Galactose/NP-galactose

$$P = \frac{0.297 - 0.0804}{0.0406} \times \frac{1}{17.094} = 31.2\%$$

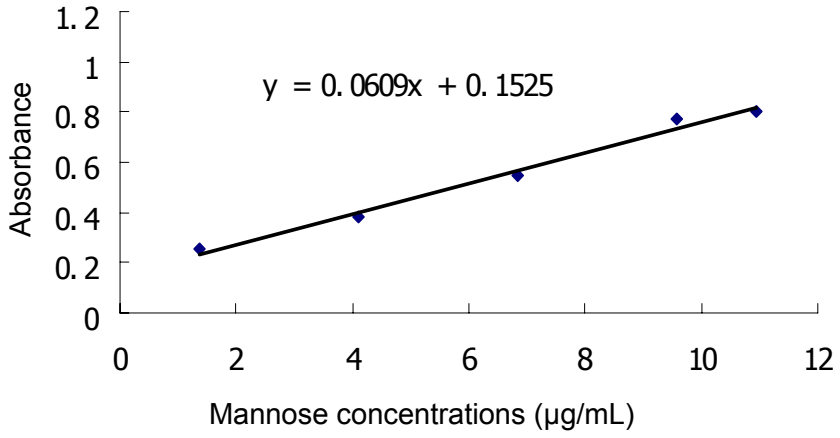


Figure 1S: Titration curve of mannose by the phenol/sulfuric assay

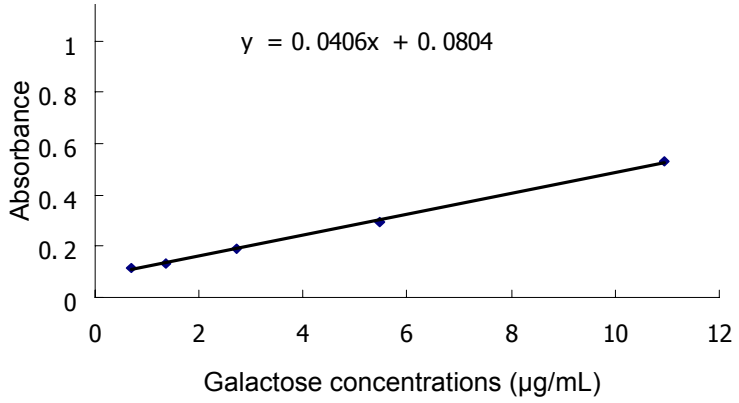
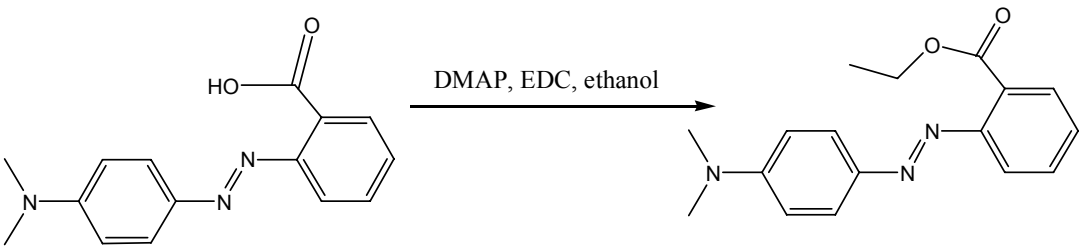


Figure 2S: Titration curve of galactose by the phenol/sulfuric assay

Table 1S: Determination of the sugar contents of NP-mannose and NP-galactose by the reported colorimetric assay[1]

samples	Final concentration(μg/mL)	Absorbance (
NP-galactose	17.094	0.297
NP-mannose	17.094	0.516



Scheme 1S: Synthetic route of the ethyl ester of methyl red

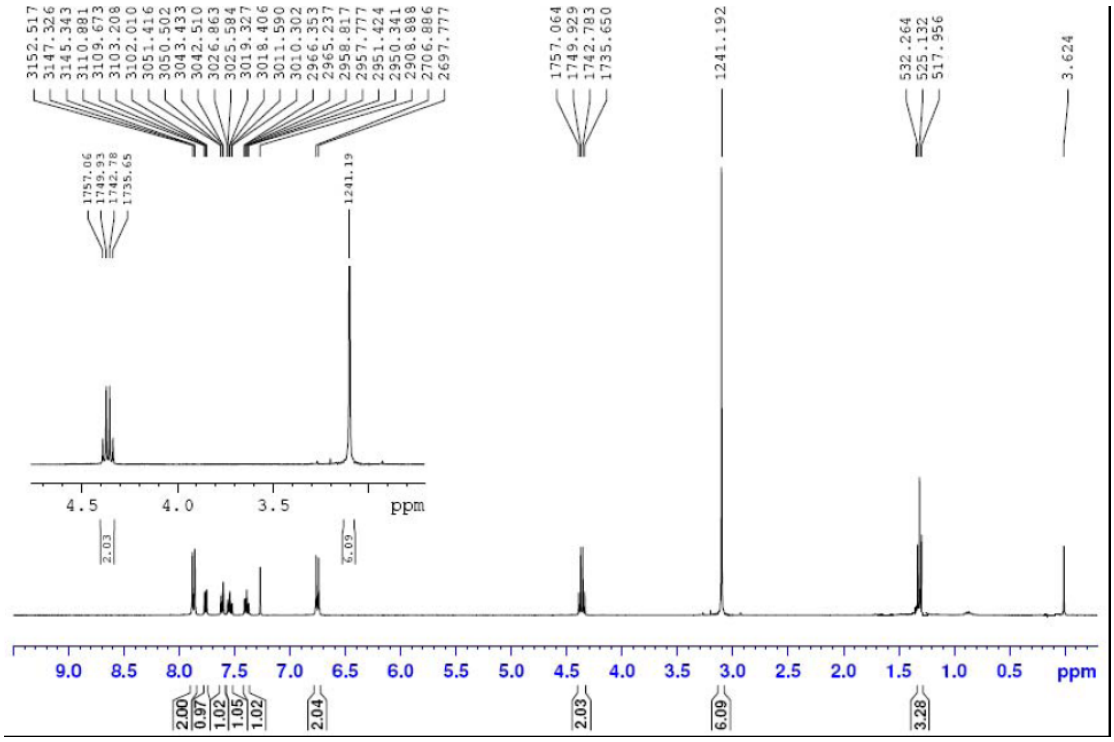


Figure 3S: ^{1}H-NMR spectrum of ethyl ester of methyl red

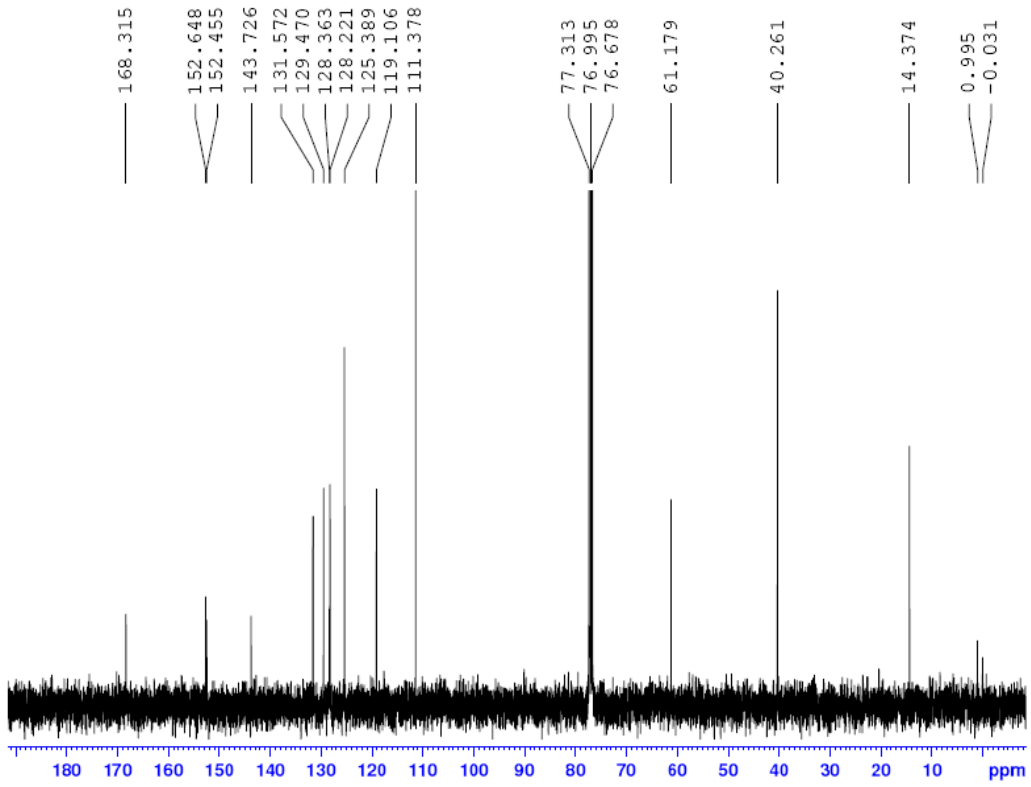


Figure 4S: ^{13}C-NMR spectrum of ethyl ester of methyl red

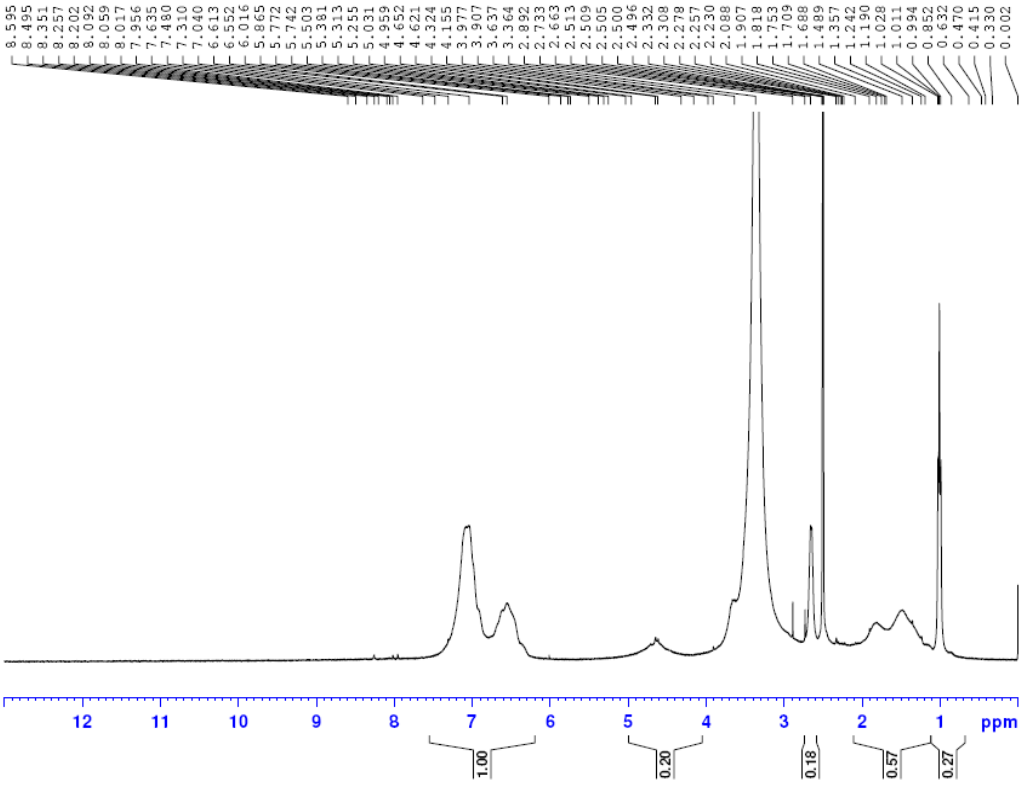


Figure 5S: ^{1}H-NMR spectrum of PS-mannose in deuterated DMSO

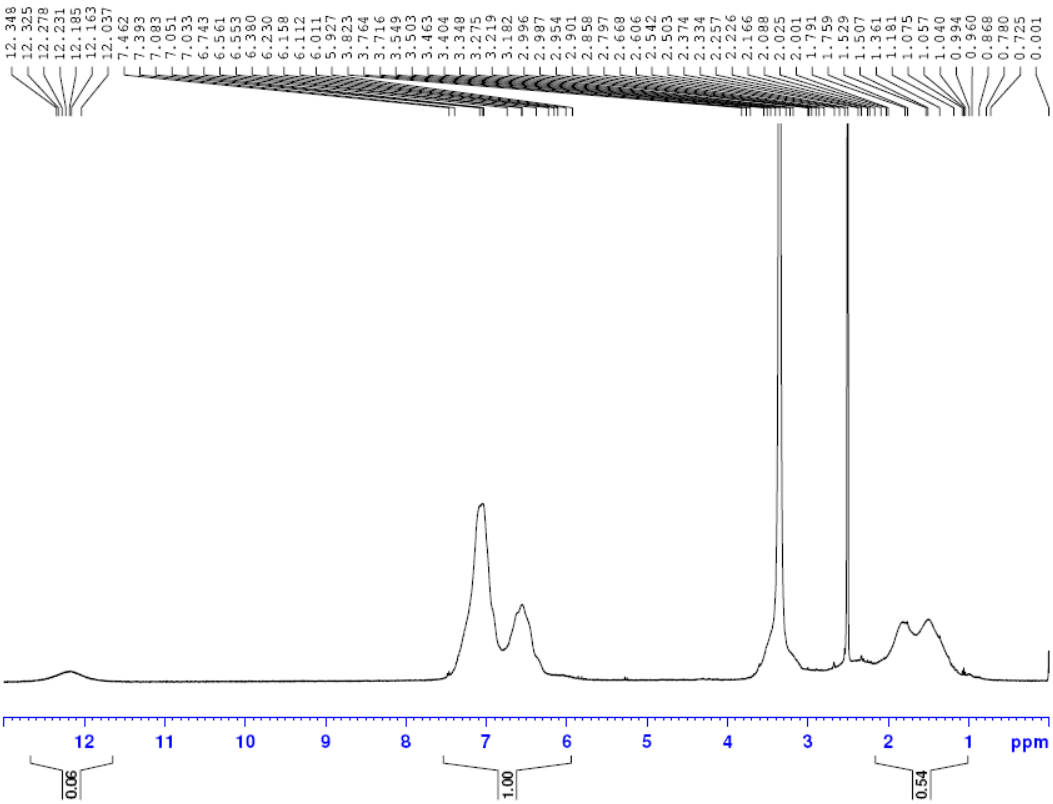


Figure 6S: ^{1}H-NMR spectrum of PS-COONa in deuterated DMSO

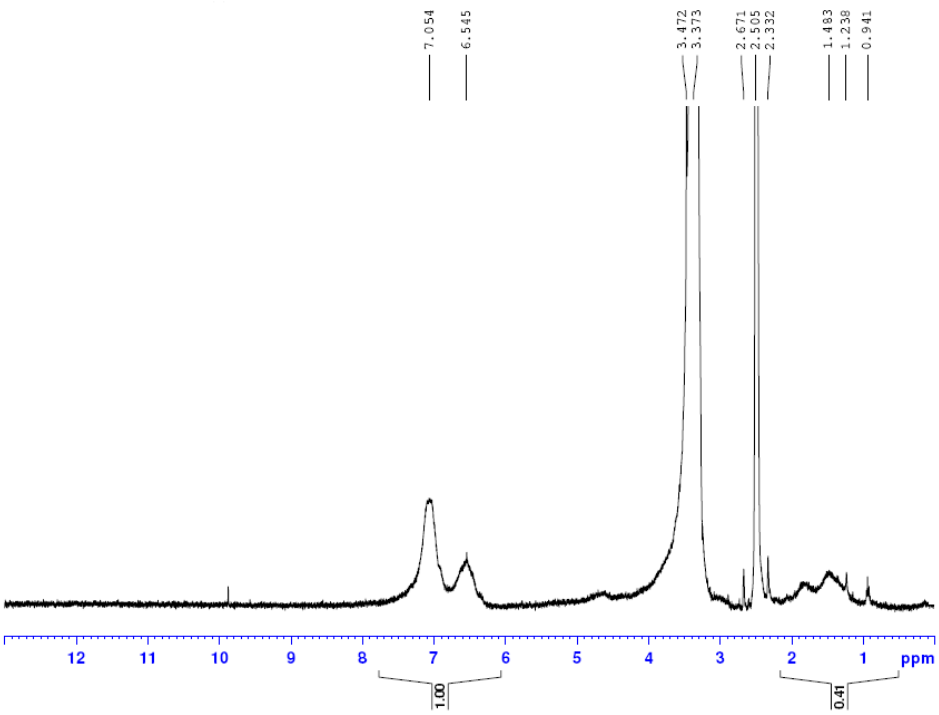


Figure 7S: ^{1}H-NMR spectrum of PS-galactose in deuterated DMSO

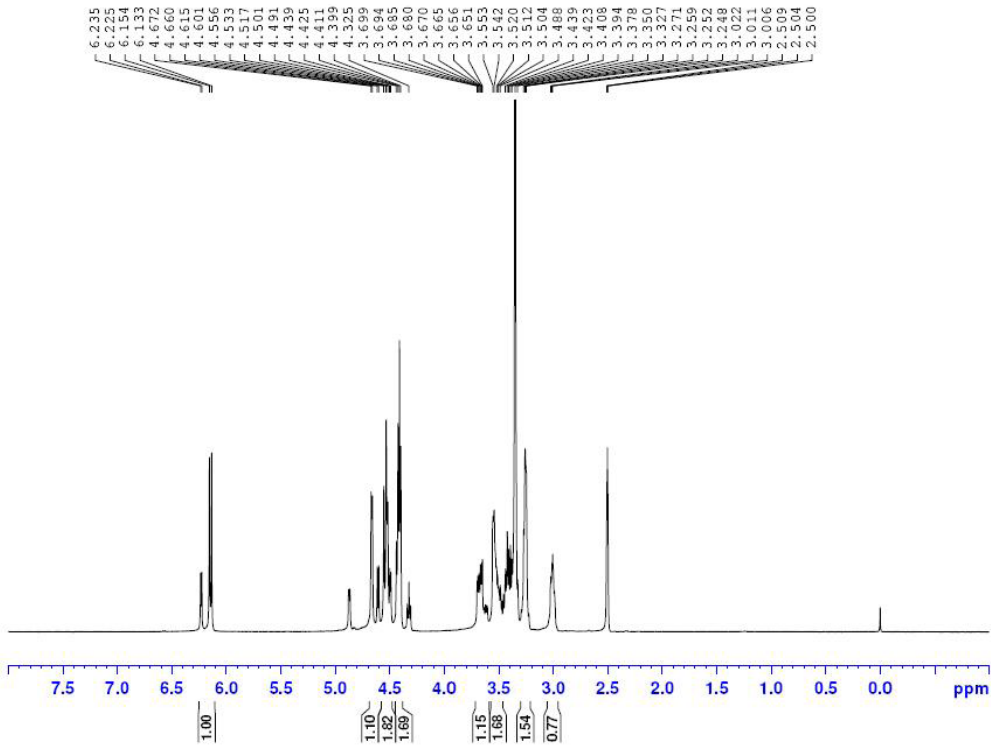


Figure 8S: ^{1}H-NMR spectrum of mannose in deuterated DMSO

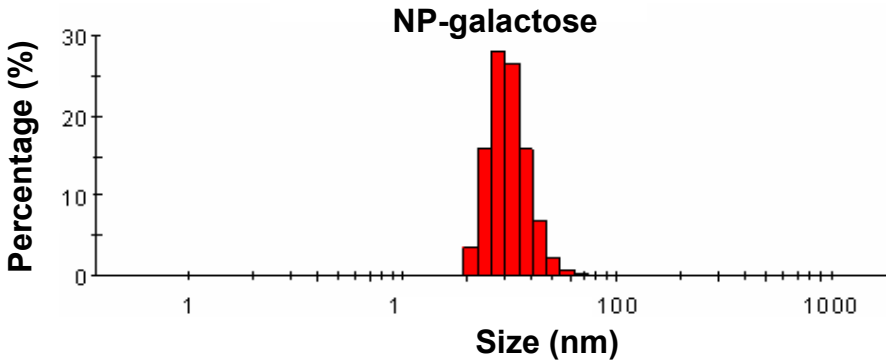


Figure 9S: Diameter size of NP-galactose measured by dynamic light scattering.

FRET studies of tetrameric FITC-Con A / NP-galactose interaction

Tetrameric FITC-Con A/NP-galactose complex was formed *via* incubation of solutions containing NP-mannose (0.4μg), FITC-labeled Con A (0.4μg) in Tris-HCl buffer (50 mM, pH 7.2, 50μL) containing calcium chloride (2mM) (buffer A) and manganese chloride (2mM) at room temperature for 15 minutes. The final volume was adjusted to 3 mL with addition of buffer A. The fluorescence emission spectra were recorded as using tetrameric FITC-Con A as the control.

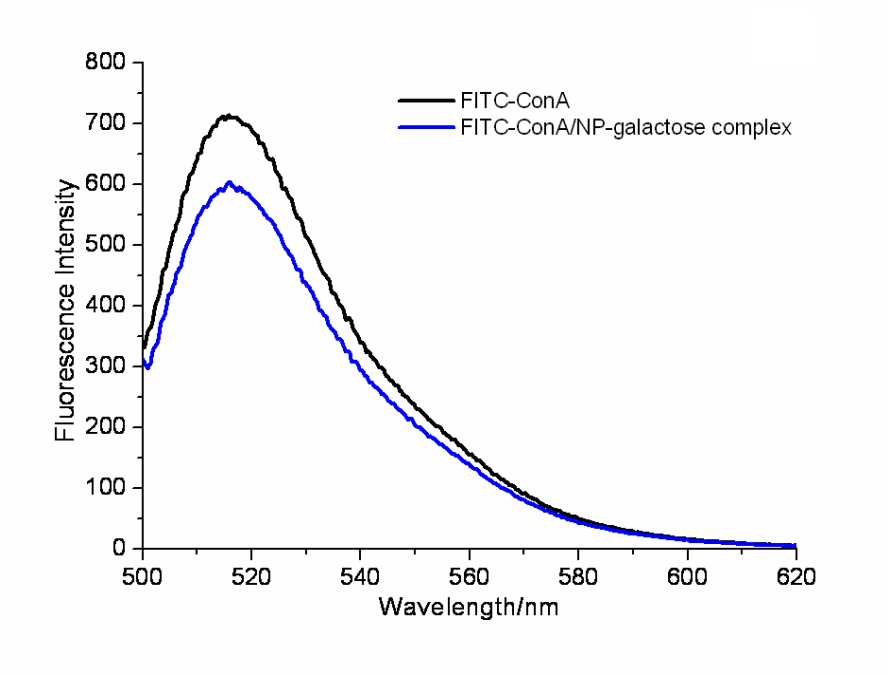


Figure 10S: Inhibitory effects of D-mannose on the fluorescence emission of tetrameric FITC-Con A /NP-galactose complexes doped with methyl red.

Recovery of the fluorescence of FITC-Con A/glyconanoparticle complex in presence of mannose monosaccharide

To the tetrameric FITC-Con A/NP-mannose complex, formed *via* incubation of solutions containing mannose-NP (0.4μg), FITC-labeled Con A (0.4μg) in Tris-HCl buffer (50 mM, pH 7.2, 50μL) containing calcium chloride (2mM) and manganese chloride (2mM) at room temperature for 15 minutes, were added various volumes of D-mannose solution. The final volume was adjusted to 3 mL with addition of Tris-HCl buffer. The fluorescence intensities at 520nm were recorded as a function of the concentrations of D-mannose monosaccharide.

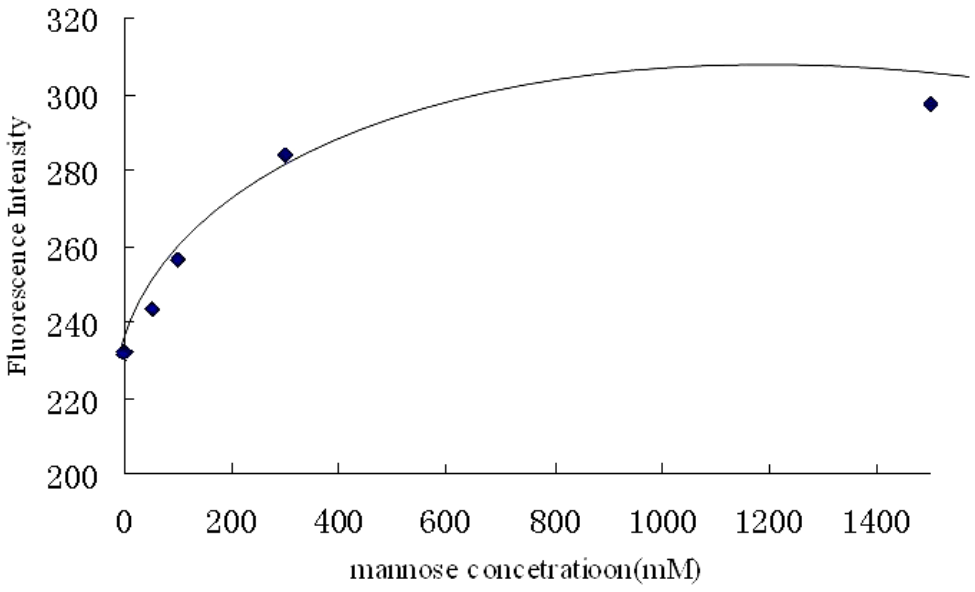


Figure 11S: Correlation of the fluorescence intensity of FITC-Con A/NP-mannose with various concentrations of mannose monosaccharide

Binding of NP-mannose with human spermatozoa in presence of mannose

Human spermatozoa samples were stained with a slight modification of the reported procedure[2]. NP-mannose (0.4μg/ml) doped with rodamine 6G were incubated with human spermatozoa for 20 min at 37 °C in HEPES buffer (30 mM, pH 7.0) containing NaCl (150 mM), $MgCl_2$ (0.5 mM), $CaCl_2$ (20 mM) and bovine serum albumin (1%, w/v) in the presence of various amount of mannose monosaccharide. Spermatozoa were then pipetted on glass sides and fixed on cover glass. The morphology of the sperm cells was visualized using phase-contrast microscope. The picture on the left was taken from samples incubated with 0.5M of mannose, the middle picture was obtained from samples incubated with 1.0M of mannose and the right picture was obtained from samples incubated with 2.0M of mannose. With the increased amount of mannose, the aggregation of sperms by NP-mannose was inhibited, as compared to the sample without addition of mannose.

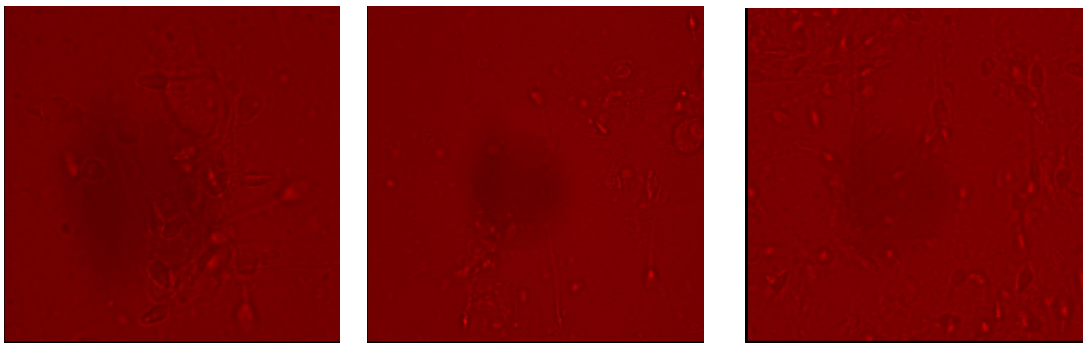


Figure 12S: Morphology of human sperms incubated with PS-mannose in presence of mannose monosaccharide (0.5M, left; 1.0M, middle, and 2.0M, right)..

References

1. Michel. DuBois; K. A. Gilles; J. K. Hamilton; P. A. Rebers; Smith, F. *analytical chemistry* **1956,** *28*, 350-356.
2. Gabriele, A.; D'Andrea, G.; Cordeschi, G.; Properzi, G.; Giammatteo, M.; De Stefano, C.; Romano, R.; Francavilla, F.; Francavilla, S. *Mol Hum Reprod* **1998,** *4*, 543-53.