Supporting Information

Glyco-gold nanoparticle shapes enhance carbohydrate-protein interactions in mammalian cells.

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1. Materials and Methods

1.1. General Instructions. All chemicals were reagent grade and used as supplied except where noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mmol). Compounds were visualized by UV irradiation or dipping the plate in CAM/ninhydrin solution followed by heating. Column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on Jeol 400 MHz, with cryo probe using residual solvents signals as an internal reference ($CDCl_3$ δ_H, 7.26 ppm, δ_C 77.3 ppm and CD_3OD δ_H 3.31 ppm, δ_C 49.0 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. UV-visible measurements were performed with Evolution 300 UV-visible spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence spectra were recorded in FluoroMax-4 spectrofluorimeter (Horiba Scientific, U.S.A.). DC-SIGN transfected and Knockdown HeLa was obtained from UCSD cell bank. MDA-MB-231 and HepG2 cell lines were purchased from NCCS, Pune.

2. Uv-visible and physical characteristic of the complexes.

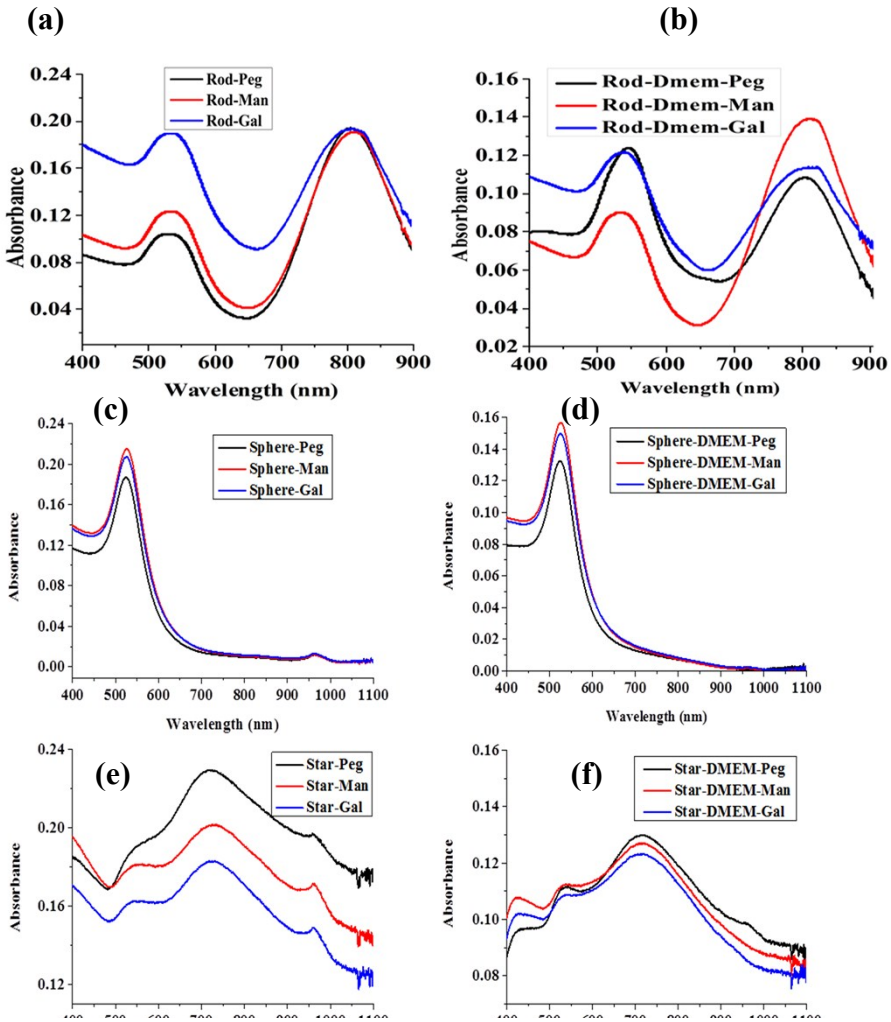


Fig. S1 UV-visible spectra of rod, sphere and star shaped AuNPs in water (**a, c, e)** and DMEM medium without phenol red (**b, d, f**).

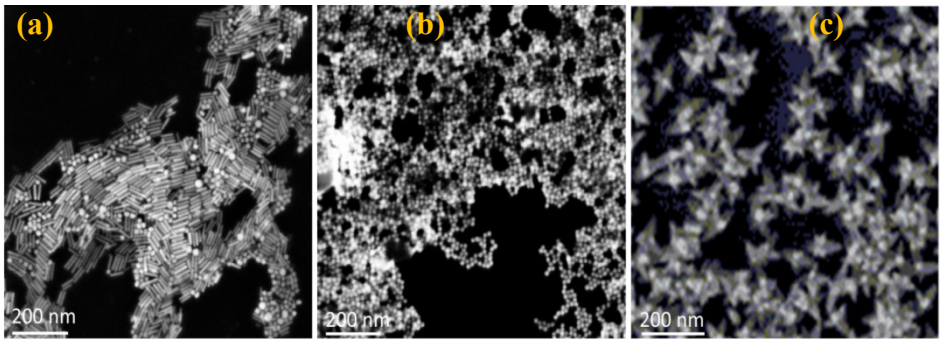


Fig. S2 SEM images of (a)rod, (b)sphere and (c)star shaped AuNPs.

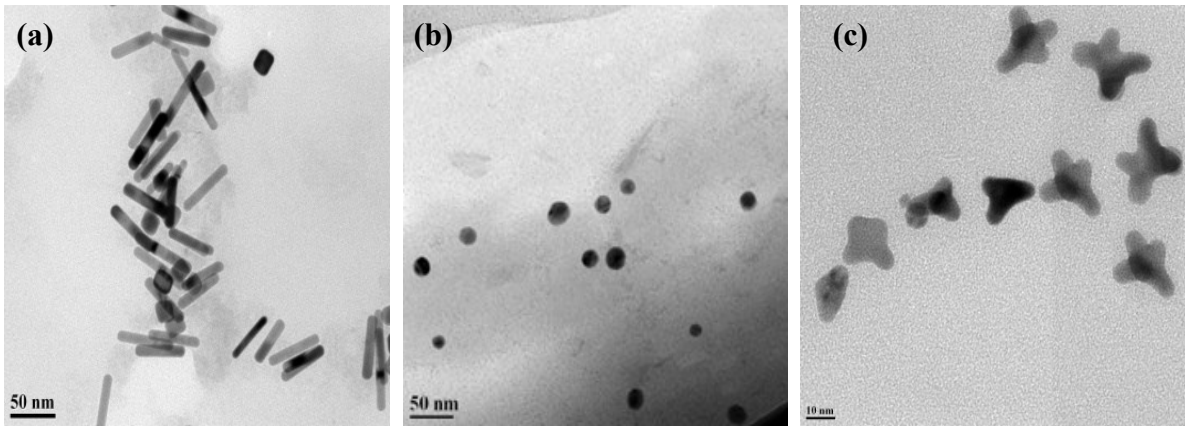


Fig. S3 TEM images of (a)rod, (b)sphere and (c)star shaped AuNPs.

a) Aspect ratio of AuNPs:

Aspect ratio of Rod, Sphere, Star shape nanoparticles calculated by the ratio of major axis to the minor axis[1]

Particle	**Aspect ratio**
Rod	3.8 : 1
Sphere	1 : 1
Star	2.4 : 1

b) Volume of AuNPs.

Particle	**Volume (nm^3)**
Rod	4185 ± 455
Sphere	3945 ± 564
Star	3687 ± 157

3. Zeta potential studies:

We used a zeta-potential analyzer to measure the surface potential of AuNPs. The electrostatic potential on the particle surface is called the zeta potential. In the measurement, we applied unit field strength (1 Volt per metre) to the AuNP solution. We measured zetapotential of different shapes of AuNPs in water. In case of DMEM medium, We incubated all AuNps in DMEM medium for 24 h and purified by centrifugation and measured zetapotential.

Table S1. Zeta potential values of rod, sphere and star AuNPs.

Particle	ζ-potential (mV)	
	Water	**DMEM**
Rod	32.7 ± 1.5	5.75 ± 0.4
Sphere	-22.2 ± 0.9	-17.4 ± 0.2
Star	-29.9 ± 0.4	-7.5 ± 0.7
Rod-Man	-9.8 ± 1.2	-6.66 ± 0.5
Rod-Gal	-18.4 ± 0.3	-8.95 ± 0.8
Rod-PEG	-29.2 ± 1.7	-3.64 ± 0.3
Sphere-Man	-22.1 ± 1.1	-5.3 ± 0.1
Sphere-Gal	-18.2 ± 0.8	-2.82 ± 0.4
Sphere-PEG	-16.1 ± 0.4	-2.45 ± 0.3
Star-Man	-23.1 ± 0.9	-3.53 ± 0.5
Star-Gal	-24.2 ± 0.5	-3.97 ± 0.1
Star-PEG	-15.5 ± 1.2	-5.72 ± 0.2

4. Phenol-sulfuric acid method to quantify sugars on AuNPs.

The concentration of mannose/ galactose sugars on AuNPs were determined by the phenol-sulfuric acid method. 100 μL sugar functionalized-AuNPs were were added to concentrated sulfuric acid (750 μL, 100%) and aqueous phenol solution (5% w/v, 100 μL) in the test tube and heated to 80°C. After 5 min, solution was cooled to room temperature and the absorbance coefficient at 490 nm was measured. AuNPs as such in sulfuric acid was used as a control. The sugar concentration was estimated by comparing the absorption of the sample with a standard curve.

Table S2. Quantification of sugar conjugation on rod, sphere and rod shape AuNPs.

S.No	Nanoparticles	Concentration(mg/mL)
1	Rod-Man	1.6 ± 0.21
2	Rod-Gal	1.9 ± 0.17
3	Sphere-Man	1.8 ± 0.11
4	Sphere-Gal	2.2 ± 0.25
5	Star-Man	1.52 ± 0.09
6	Star-Gal	1.67 ± 0.12

5. MTT assay.

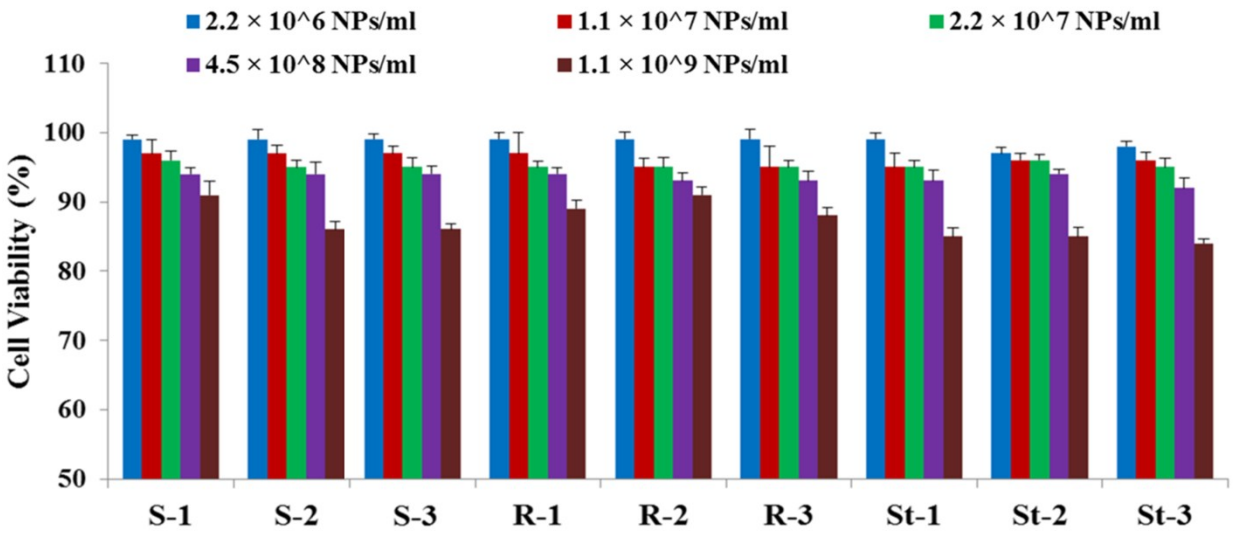


Fig. S4 MTT assay showing the cell viability of sphere, rod and star shape AuNPs at 37 ºC for 48 h incubation with NIH 3T3 cells.

6. Inhibition Assay.

96-well ELISA plates were treated immobilized mannose, galactose-BSA (1 mg/ml) as reference ligand and incubated with horesradish peroxide (HRP) labeled ConA or PNA (0.5 mg/ml) or DC-SIGN in the presence of different shapes of G-AuNPs (5.69 X 10^5 -10^8) in varying concentrations. After incubation for 2 h, the plates were washed and remaining labeled lectin bound to the reference ligand was quantified by a HRP-catalysed color reaction using 2, 2'-azinobis(3-ethyl-benzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) as substrate. In case of DC-SIGN and anti-IgG-HRP was added to quantify the binding of lectin. Logerthemic curve for inhibition of lectin binding to immobilized Mannose or galactose are shown in the figure below. From these curves the concentration that reduce the binding of labeled lectin to the microtiter plates by 50% (IC_{50} values) were determined as a meassure of potency of the synthesized inhibitors.

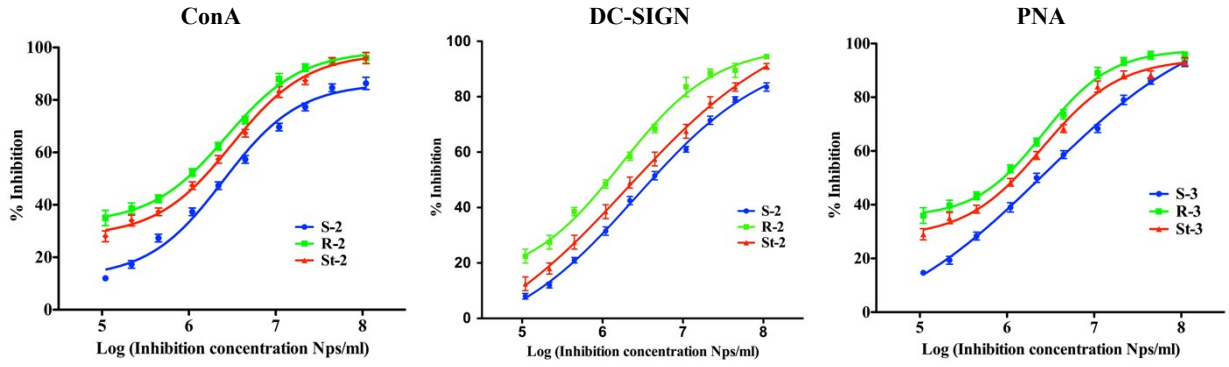
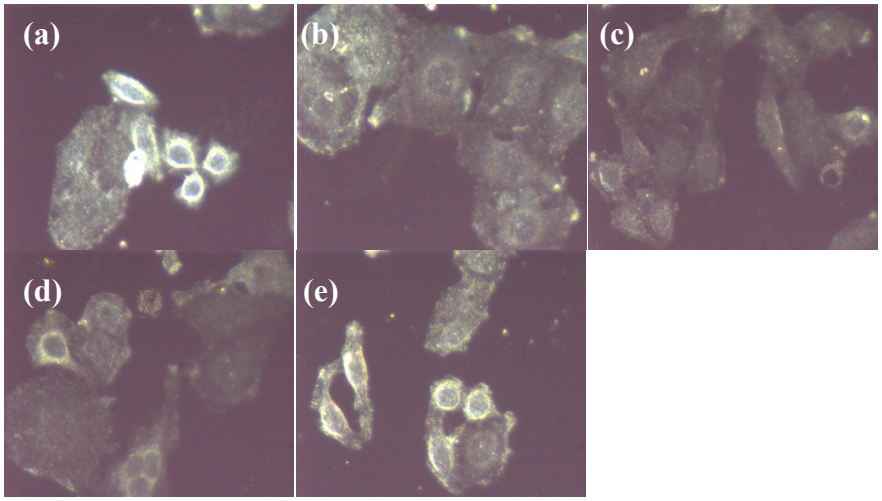


Fig. S5 Inhibition assay of G-AuNPs with (a) ConA; (b) DC-SIGN; and (C) PNA.

7. Mechanisitic studies of HepG2 cellines.

(i)



(ii)

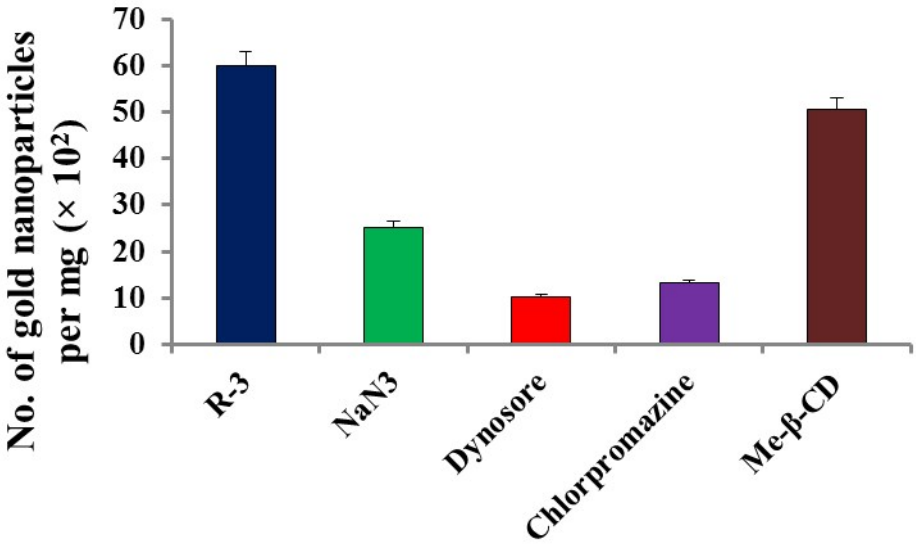


Fig. S6 (i) Dark field microscopic images of HepG2 cells treated with inhibitor for 30 mins followed by R-3 after 4 h. (a) control R-3 after 4 h; (b) NaN_3 (50 mM); (c) dynosore (50 µM); (d) chlorpromazine (25 µM); (e) Me-β-cyclodextrin (10 µM). (ii) Statistical analysis of ICP-MS data in presence and absence of inhibitor after 4 h. Data are presented as mean ±SEM for three independent experiments (***P<0.001, **P<0.01 *P<0.05 and n.s = not significant

References

1 P. M. Chaudhary, S. Sangabathuni, R. V. Murthy, A. Paul, H. V. Thulasiram and R.Kikkeri, *Chem. Commun*., 2015, **51,** 15669.