SUPPORTING INFORMATION

Glycans as Biofunctional Ligands for Gold Nanorods: Stability and Targeting in Protein-Rich Media

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Materials and methods:

Tetrachloroauric acid ($HAuCl_4$), trisodium citrate, hexadecyltrimethylammonium bromide (CTAB), silver nitrate ($AgNO_3$), hydrochloric acid (HCl), ascorbic acid and O-[2-(3-mercaptopropionylamino)ethyl]-O′-methylpolyethylene glycol 5 KDa (PEG) were purchased from Sigma-Aldrich. Wheat germ agglutinin 36 KDa (WGA) and the β-galactoside-binding lectin galectin-3 (Gal-3) were purchased from Abcam. Dialysis membranes with a molecular weight cut-off of 100 kDa (cellulose ester) and ultra-filtration membranes (regenerated cellulose) were purchased from Millipore. Milli-Q H_2O (Millipore, Billerca, MA, USA) was used as the solvent. J774 macrophages were purchased from the ATCC and were cultured in DMEM media supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). DLD-1 colorectal adenocarcinoma cells were obtained from American Type Culture Collection (ATCC) and were grown in RPMI media supplemented with 10% FBS. Cells were maintained in a humidified atmosphere at 37 °C, 5% CO_2 and passaged using pipetting (J774) or trypsin-EDTA (DLD-1). All reagents were purchased from Invitrogen. UV-Vis spectra were measured on a Beckman Coulter DU 800 spectrometer. High-resolution mass spectra (HR-MS) were obtained using the matrix-assisted laser desorption/ionization (MALDI) technique with a 4700 Proteomics Analyzer (Applied Biosystems) with MALDI-time-of-flight (TOF) configuration. TEM analysis was carried out in a Philips JEOL JEM-2100F working at 200 kV.

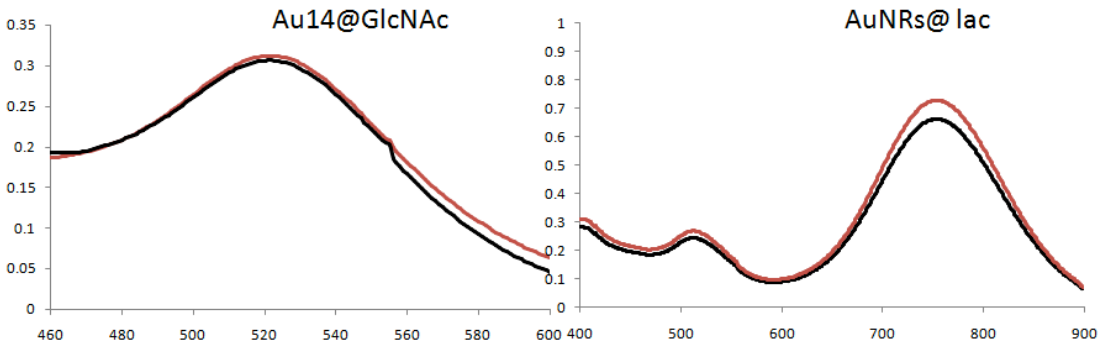


Figure S1. UV-Vis spectra of Au_{14}@GlcNAc and AuNRs@ Lac before (black) and after (red) incubation with a standard cell culture medium (DMEM) supplemented with 10% fetal bovine serum (FBS 10%), 2 hours at 37 ºC.

Determination of protein concentration by Bradford analysis:[1] To 5 µL of fetal bovine serum (FBS) placed into microplate wells (triplicates) were added 250µL of Bradford reagent (Sigma), mixed with plate shaker for 30 seconds, incubated for 10 minute at room temperature and then, absorbance at 595 was measured with a plate reader (TECAN, Genious Pro). The protein concentration was then determined using a bovine serum albumin calibration curve.

LC-MS/MS analysis. Gel bands were first sliced to small pieces, and washed in milli-Q water. Reduction and alkylation was applied by incubation with ditiothreitol (DTT, 10 mM in 50 mM ammonium bicarbonate) at 56 ºC for 20 min, followed by an incubation in Iodoacetamide (IA, 50 mM in 50 mM ammonium bicarbonate) for another 20 min in the dark. Gel pieces were dried and incubated with trypsin (12.5 µg/mL in 50 mM ammonium bicarbonate) for 20 min in ice. After rehydration, the trypsin supernatant was discarded; spots were covered with 50 mM ammonium bicarbonate and incubated overnight at 37 ºC. After digestion, acidic peptides were further extracted with TFA 0.1% and dried out in a RVC2 25 Speedvac concentrator (Christ). Peptides were resuspended in 10 µL 0.1% FA and sonicated for 5 min. prior to their analysis.

Peptide mixtures obtained from the digestion were separated by on-line nanoLC and analyzed by electrospray tandem mass spectrometry. Peptide separation was performed

[1] Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.

on a nanoAcquity UPLC system (Waters) connected to an LTQ Orbitrap XL mass spectrometer (Thermo Electron). Sample was loaded onto a Symmetry 300 C18 UPLC Trap column, 180 µm x 20 mm, 5 µm (Waters). The precolumn was connected to a BEH130 C18 column, 75 µm x 200 mm, 1.7 µm (Waters) equilibrated in 3% acetonitrile and 0.1% formic acid, and peptides were eluted at 300 nl/min using a 60 min linear gradient of 3–50% acetonitrile directly onto the nanoelectrospray ion source (Proxeon Biosystems). The mass spectrometer automatically switched between MS and MS/MS acquisition in DDA mode. Survey full scan MS spectra (m/z 400–2000) were acquired in the orbitrap with 30,000 resolution at m/z 400. The 6 most intense ions were sequentially subjected to CID fragmentation in the linear ion trap. Precursors with charge states of 2 and 3 were specifically selected for collision-induced dissociation. Collision-energy applied to each peptide was automatically normalized as a function of the m/z and charge state. Analyzed peptides were excluded for further analysis during 30 s using dynamic exclusion lists. Searches were performed using Mascot Search engine (Matrix Science) on Proteome Discoverer 1.2. software (Thermo Electron). Carbamidomethylation of cysteines as fixed modification, and oxidation of methionines as variable modifications, 5 ppm of peptide mass tolerance, and 0.5 Da fragment mass tolerance were adopted as search parameters. Two missed cleavages were allowed. Spectra were searched against all entries from UniprotKB/Swiss-Prot version 2013_09 (540,958 entries). Only proteins identified with high confidence peptides ($p<0.01$) were considered for further comparisons. For differential analysis, a Normalized Spectral Abundance Factor analysis (NSAF) was carried out, following the strategy described by Zhang et al.[2] Briefly, spectral counts were normalized against protein length (Spectral Abundance Factor, SAF), and then the SAF values obtained for each protein were normalized against the sum of the SAF values obtained in each run (NSAF).

[2] Y. Zhang, Z. Wen , M.P . Washburn , L. Florens , *Anal Chem.* **2010**, *82*, 2272-2281.

Table S1. The 10 most abundant proteins (ppm) detected after incubation of NPs with FBS (10 % in PBS) for 2 hours.

	Au_{14}@PEG	**Au_{14}@GlcNAc**	**Au_{14}@Lac**
1	Serum albumin	Serum albumin	Serum albumin
2	Alpha-2-macroglobulin	Alpha-2-macroglobulin	Alpha-2-macroglobulin
3	Alpha-2-HS-glycoprotein	Alpha-2-HS-glycoprotein	Alpha-1-antiproteinase
4	Serotransferrin	Complement C3	Complement C3
5	Apolipoprotein A-I	Apolipoprotein A-I	Apolipoprotein A-I
6	Alpha-1-antiproteinase	Hemoglobin fetal subunit beta	Hemoglobin fetal subunit beta
7	Hemoglobin fetal subunit beta	Serotransferrin	Alpha-2-HS-glycoprotein
8	Alpha-fetoprotein	Alpha-1-antiproteinase	Serotransferrin
9	Vitamin D-binding protein	Vitamin D-binding protein	Inter-alpha-trypsin inhibitor heavy chain H4
10	Apolipoprotein A-II	Hemoglobin subunit alpha	Inter-alpha-trypsin inhibitor heavy chain H3

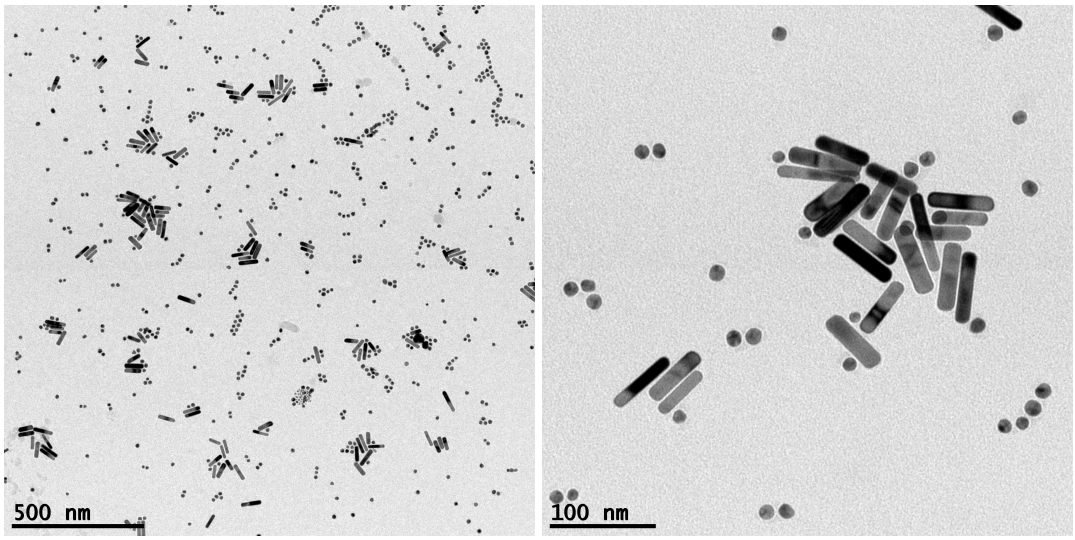


Figure S2. Low and high magnification TEM images of binary mixture containing AuNRs@GlcNAc and Au_{14}@Lac after addition of WGA.

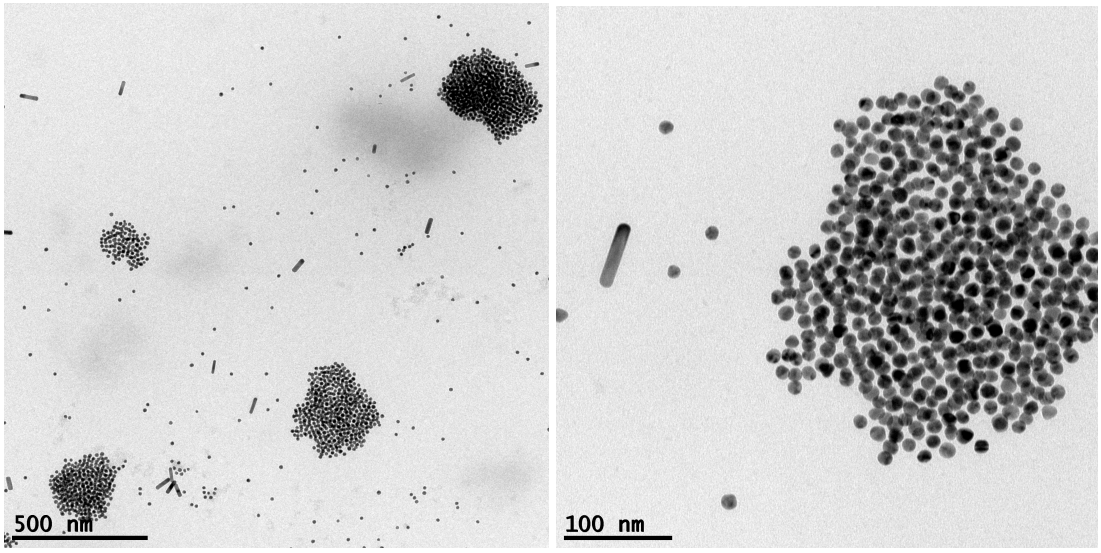


Figure S3. Low and high magnification TEM images of binary mixture containing AuNRs@GlcNAc and Au_{14}@Lac after addition of Gal-3.

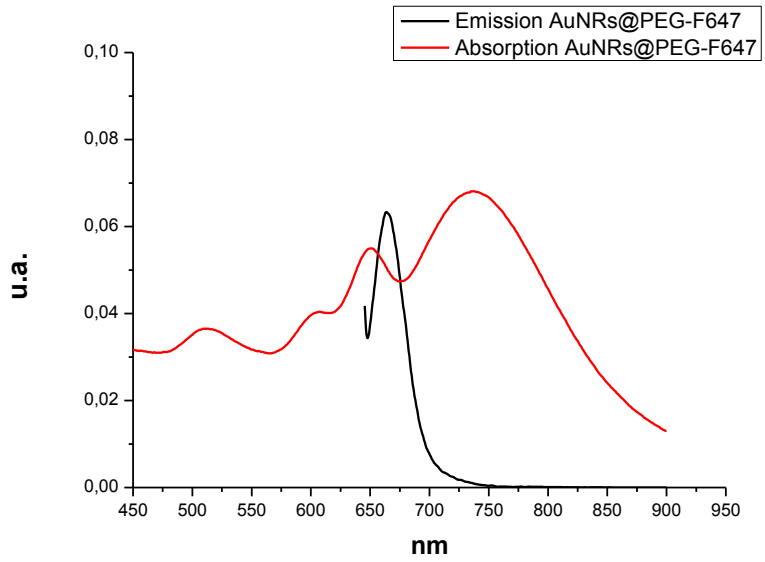


Figure S4. UV-vis-NIR spectra of AuNRs@PEG-F647 (red) and corresponding emission spectra with excitation at 640 nm (black).

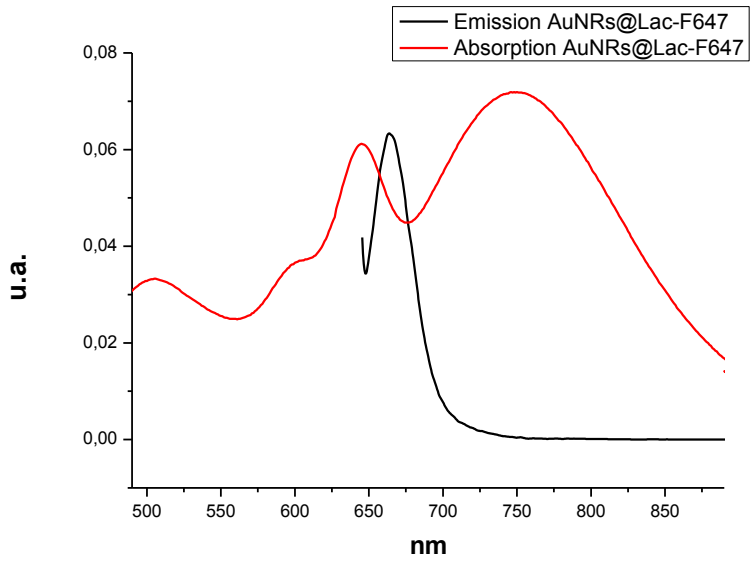


Figure S5. UV-vis-NIR spectra of AuNRs@Lac-F647 (red) and corresponding emission spectra with excitation at 640 nm (black).

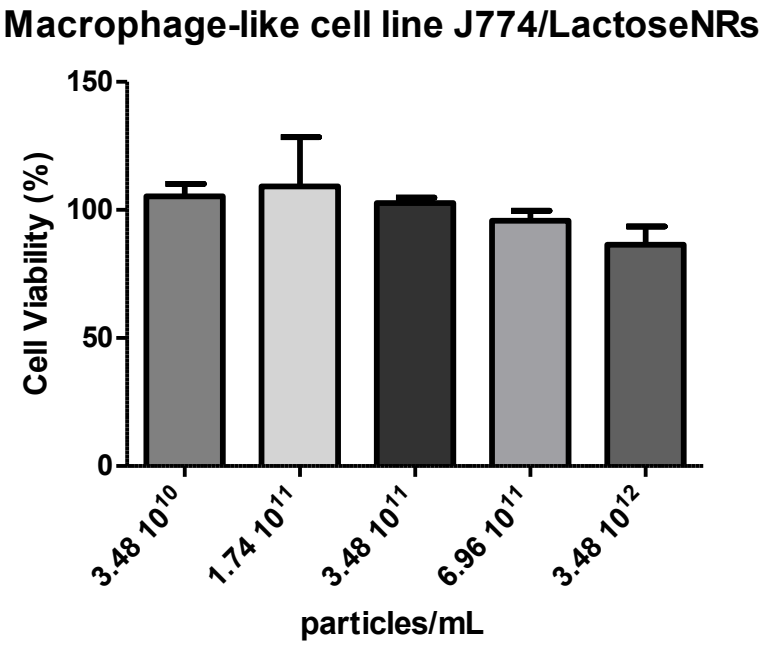


Figure S6. MTT cell viability assay at 24 hours for AuNRs@Lac in J774 cell line.

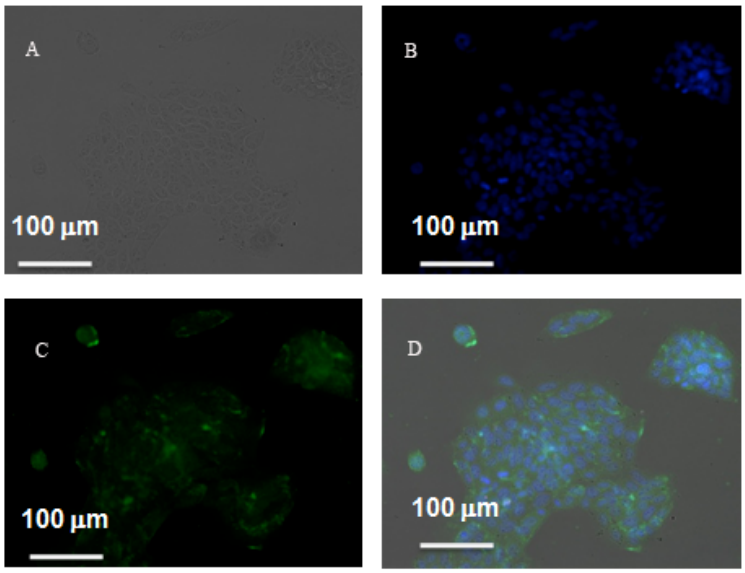
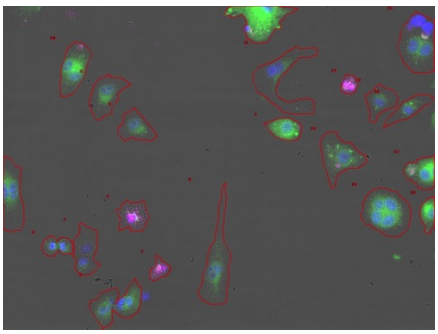


Figure S7. Gal-3 staining in DLD-1. Bright field image of DLD-1 cells (A), fluorescence image of nuclei stained with DAPI (B, blue), fluorescence image obtained after incubating DLD-1 cells with rabbit polyclonal anti-galectin 3 antibody for 1 hour at 37 ºC and Alexa-fluor 488 anti-rabbit as secondary antibody (green fluorescence, C) and bright-field fluorescence overlay images (D).



$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\,n\Sigma x^2 - (\Sigma x)^2\,]\,[\,n\Sigma y^2 - (\Sigma y)^2\,]}}$$

	J774 Lactose
Region / Cell	Pearsons
1	0,1351
2	0,0967
3	0,5764
4	0,0000
5	0,0000
6	0,0000
7	0,0000
8	0,0000
9	0,9488
10	0,6126
11	-0,1195
12	0,1345
13	0,0000
14	-0,1476
15	0,5155
16	0,0000
17	0,5022
18	0,0000
19	0,0056
20	0,3921
21	0,0000
Average	**0,1739**
Stdeva	*0,2938*

Colocalization analysis was procesed using Pearsons Coefficient of Colocalization. In brief, a ROI was drawn around each cell taking care to limit presence of background which can skew results (Dunn et al,. 2011). The PCC was calculated using Axiovision software (Zeiss). Results are expressed as mean with SD of all cells present in the image.

Figure S8. Description of how Pearson coefficients were calculated.[3]

[3] K. W. Dunn, M.M. Kamocka, H.H. MacDonadl, *Am J Physiol Cell Physiol.* **2011**, *300*, C723–C742.