Electronic Supplementary Information

for

Factors Affecting T Cell Responses Induced by Fully Synthetic Glyco Gold-Nanoparticles

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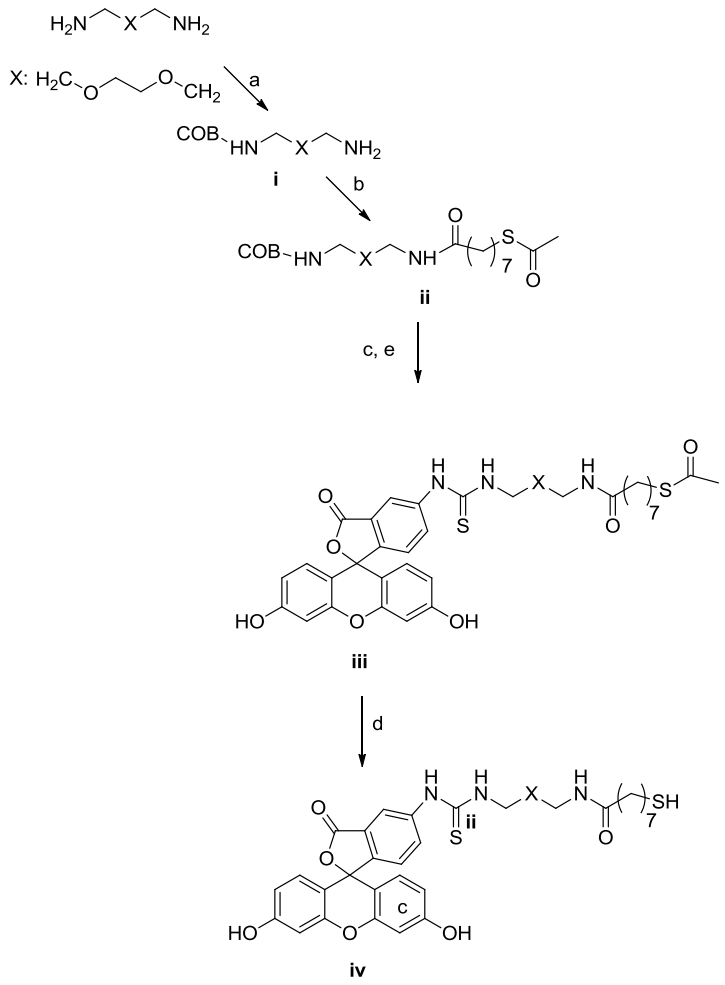
Further Experimental Details

The Scheme for the preparation of the Fluorescein-tagged thiol (**iv**) is reported below.



Scheme. a: BOC (0.6 equiv), dioxane, rt, 12 h. b: 8-acetylsulfanyl-octanoic acid pentafluorophenyl ester (0.9 equiv), DIPEA (1.0 equiv), DCM, rt, 12 h. c: TFA/DCM (1:1), 0 ^{0}C – rt, 30 min. d: 6 M HCl/EtOH (1:1), 60 ^{0}C, 3 h. e: Fluorescein 5-isothiocyanate (0.9 equiv), DIPEA (1.63 equiv), THF, rt, 12 h.

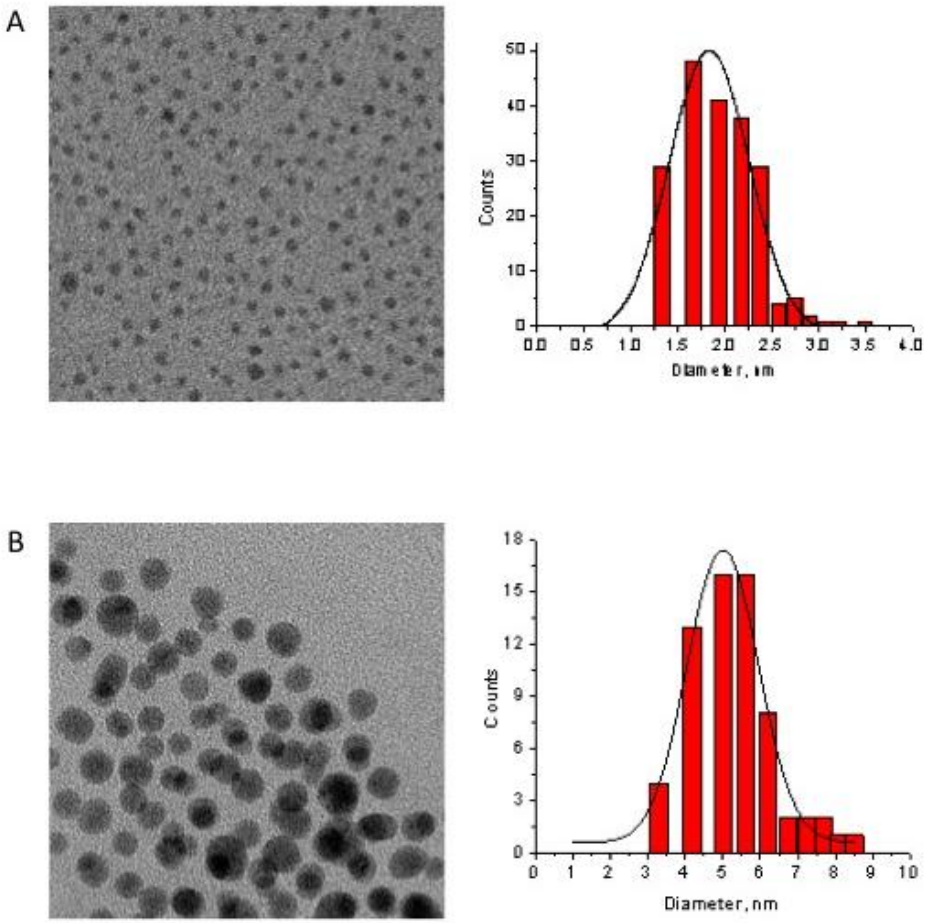


Figure S1. Nanoparticle characterizations. (A) TEM image of **1/3** (the bar corresponds to 5 nm) and size distribution: average diameter = 1.8 ± 0.5 nm. (B) TEM image of **2/4** (the bar corresponds to 5 nm) and size distribution: average diameter = 4.9 ± 0.9 nm.

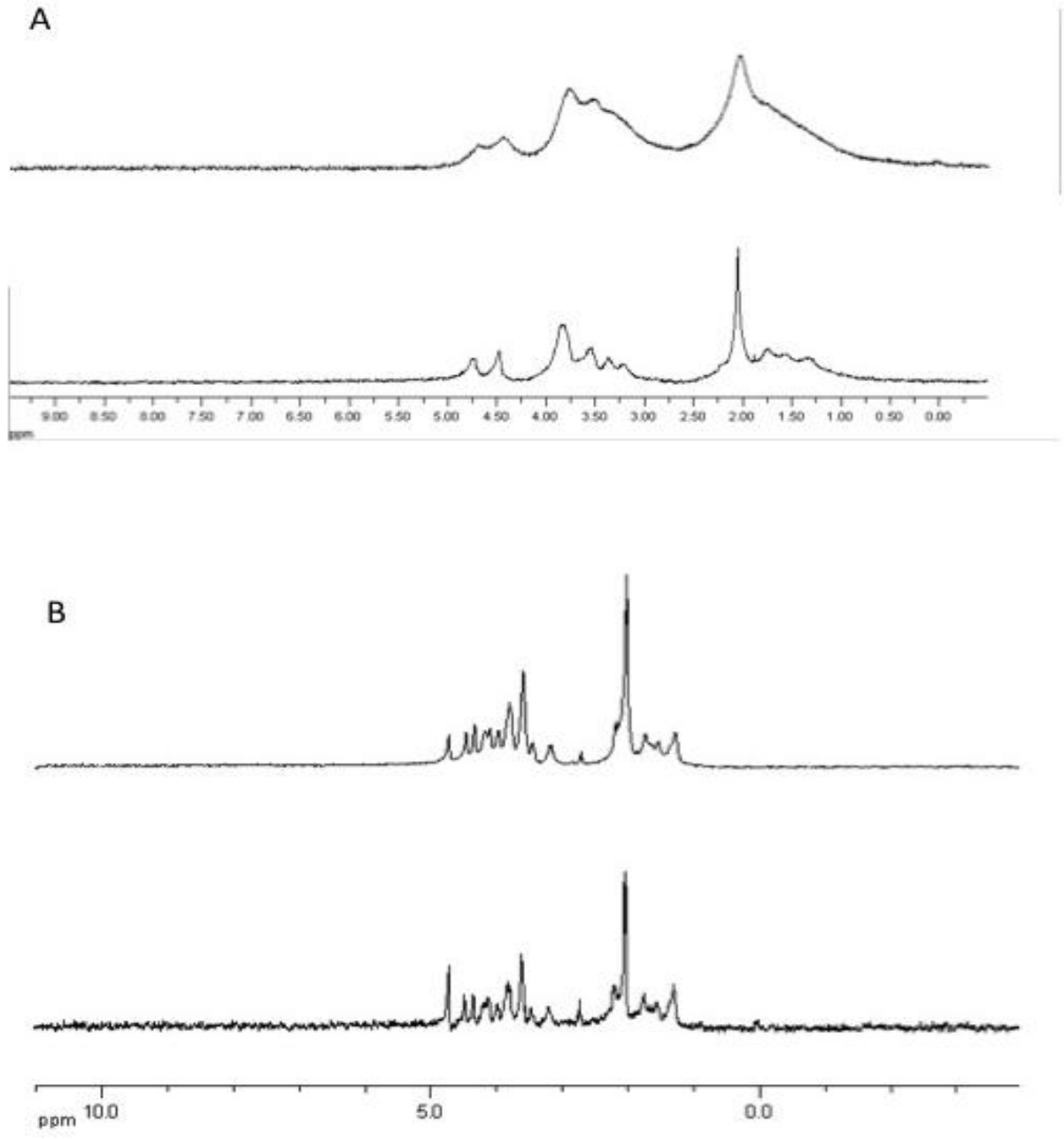


Figure S2. ^{1}H-NMR spectra. Diffusion filtered ^{1}H-NMR (300 MHz) spectra of the Au-NPs **1** and **2** (monosaccharide-coated) (A), and **3** and **4** (disaccharide-coated) (B) in D_2O, mixing time 0.2 sec, T = 28 °C.

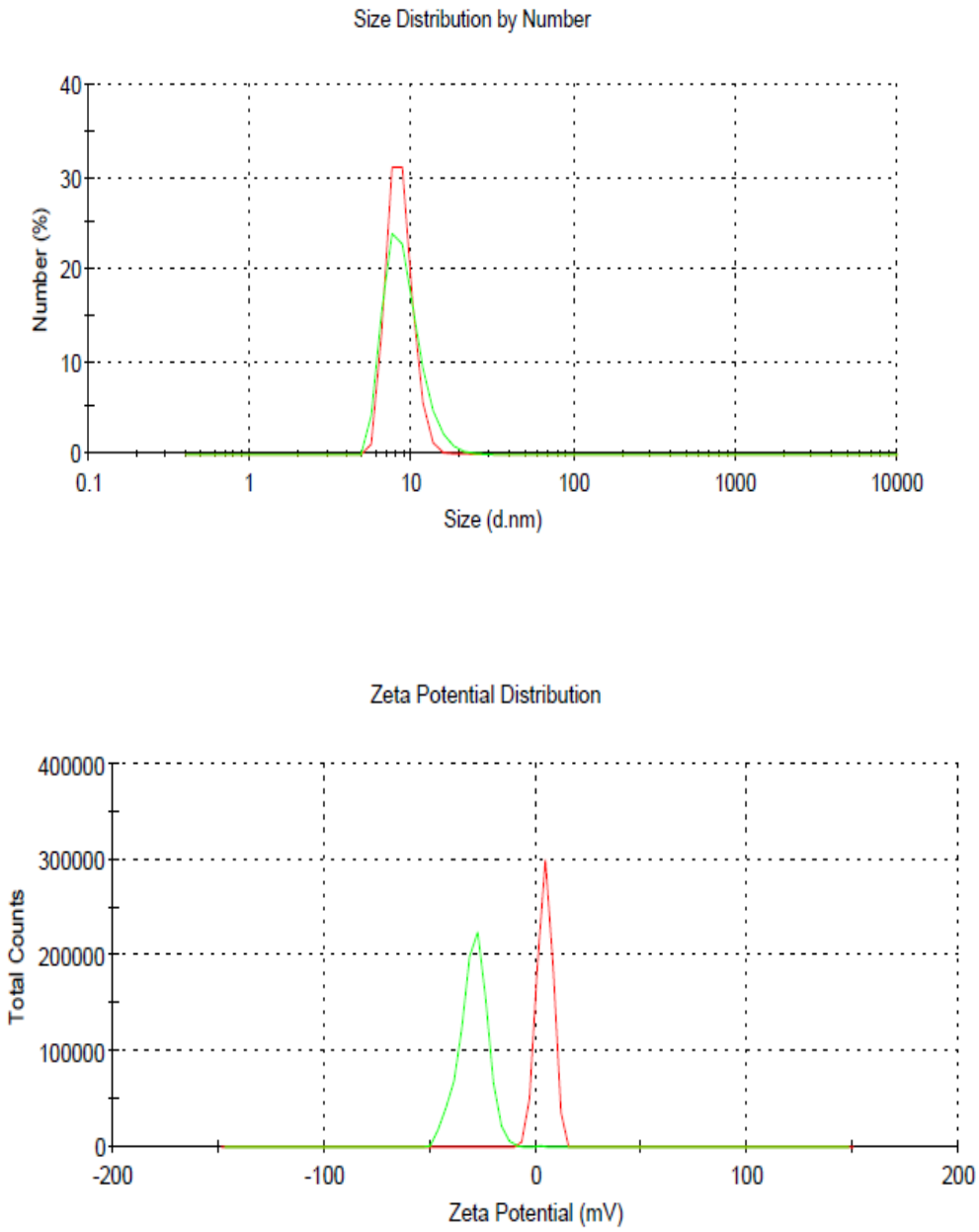
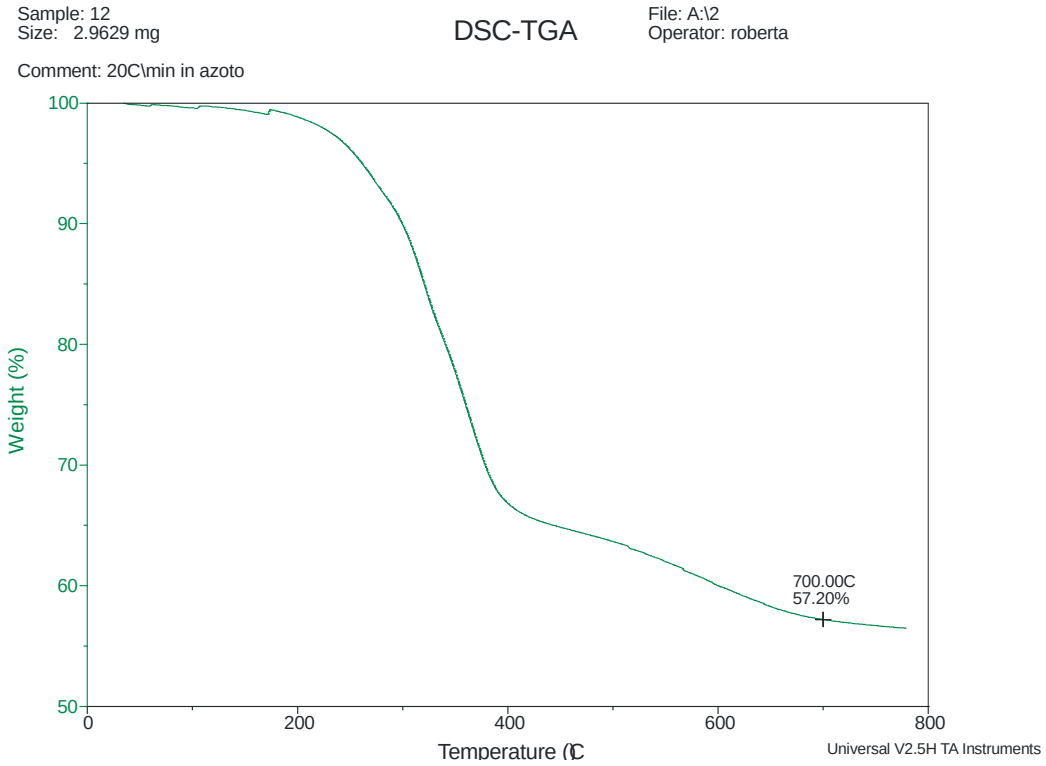


Figure S3. Characterization of 5 nm Glyco-Au-NPs by dynamic light scattering hydrodynamic diameter, top, and Z potentials, bottom, (Glyco-Au-NPs **2** in red, **4** in green)



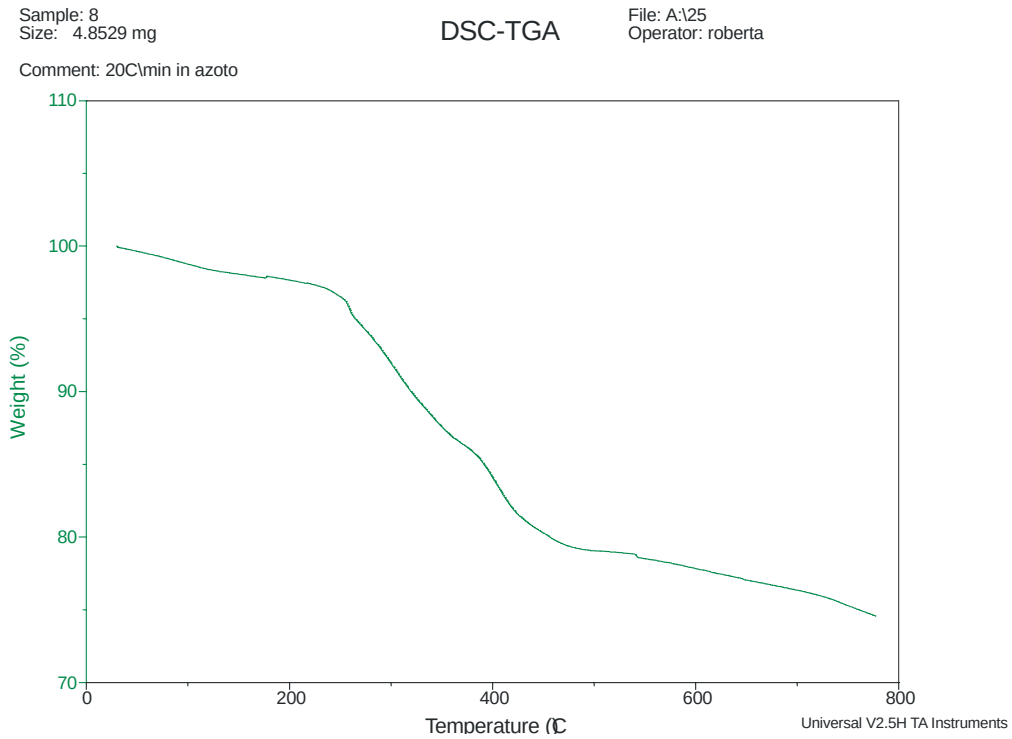
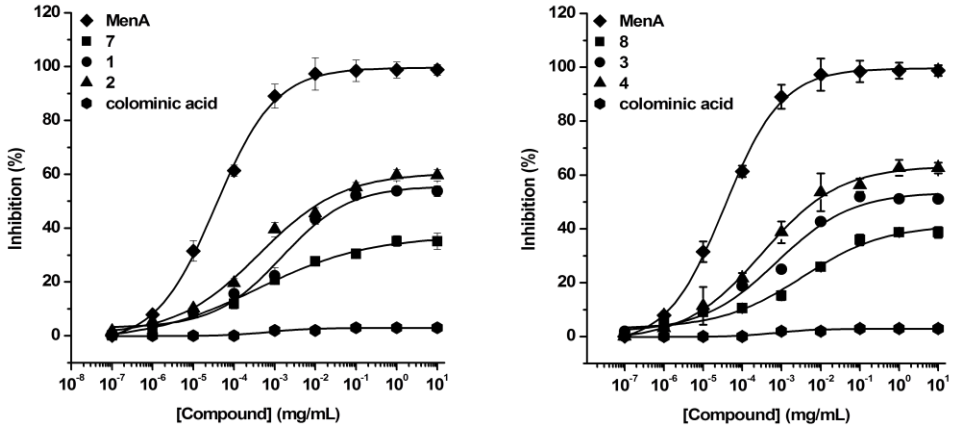


Figure S4. Characterization of 5 nm Glyco-Au-NPs by therogravimetry; top: Glyco-Au-NPs **3** ; bottom: Glyco-Au-NPs **1**.

A

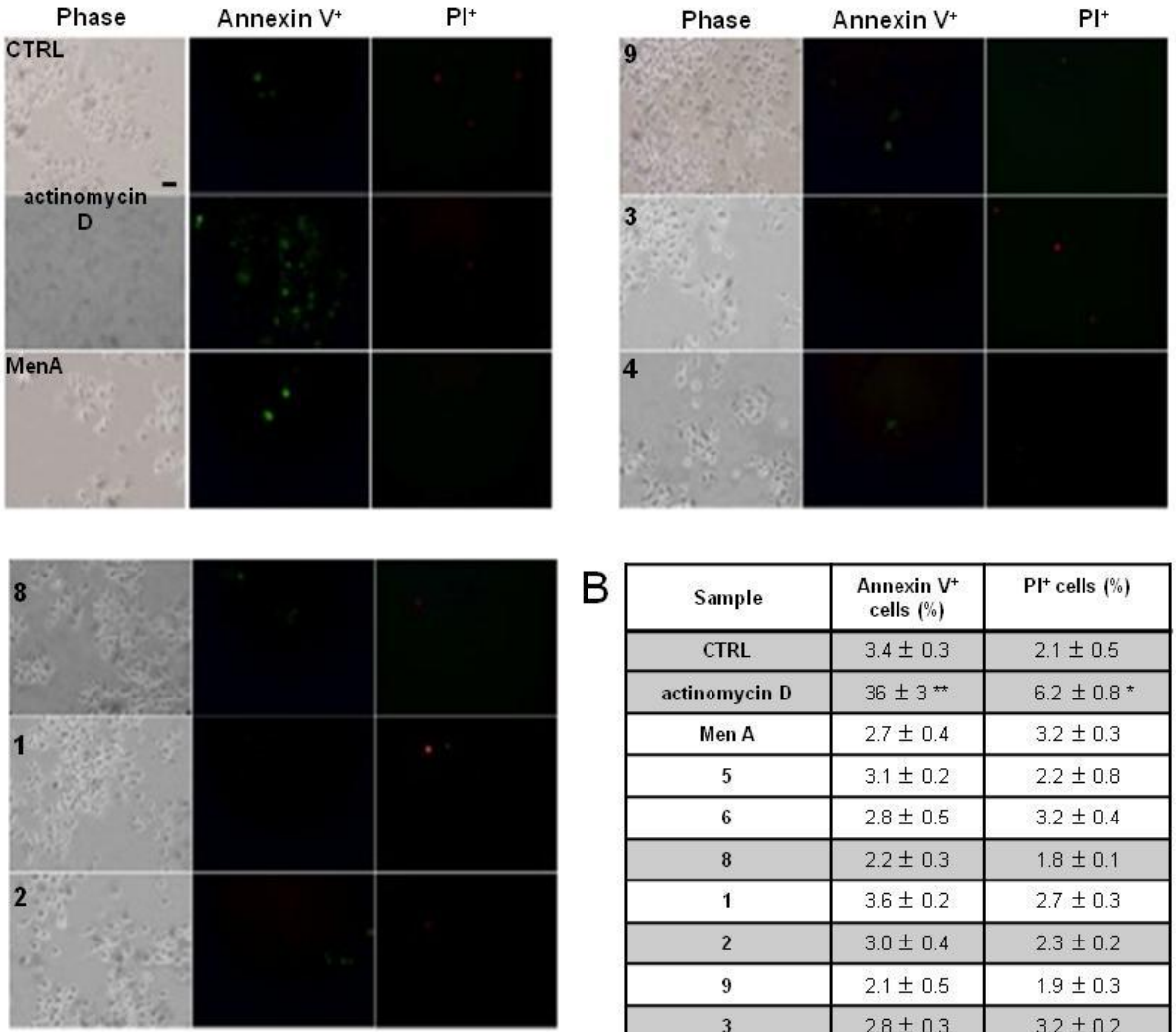


B

Compound	Maximum inhibition (%)[a]	IC_{50} (mg/mL)
MenA	100 ± 3	3.58×10^{-5}
7	33 ± 1	4.06×10^{-1}
1	53 ± 3	1.73×10^{-3}
2	60 ± 2	4.32×10^{-4}
8	38 ± 3	3.01×10^{-1}
3	51 ± 2	9.33×10^{-4}
4	63 ± 3	3.24×10^{-4}

Figure S5: Glyco-Au-NPs ability to bind specific anti-MenA Ab. (A) Concentration-response curves of monosaccharide **8**, Glyco-Au-NPs **1** and **2** (left panel) or disaccharide **9**, Glyco-Au-NPs **3** and **4** (right panel) for the inhibition of the binding between MenA and the anti-MenA mouse polyclonal Ab evaluated by competitive ELISA. (B) The maximum inhibition elicited at 1 mg/ml by each compound in the ELISA. The concentration of each compound that produce the 50% of the maximum inhibition (IC_{50}). The data represent means ± SEM of five experiments run in triplicate.

A



B

Sample	Annexin V+ cells (%)	PI+ cells (%)
CTRL	3.4 ± 0.3	2.1 ± 0.5
actinomycin D	36 ± 3 **	6.2 ± 0.8 *
Men A	2.7 ± 0.4	3.2 ± 0.3
5	3.1 ± 0.2	2.2 ± 0.8
6	2.8 ± 0.5	3.2 ± 0.4
8	2.2 ± 0.3	1.8 ± 0.1
1	3.6 ± 0.2	2.7 ± 0.3
2	3.0 ± 0.4	2.3 ± 0.2
9	2.1 ± 0.5	1.9 ± 0.3
3	2.8 ± 0.3	3.2 ± 0.2
4	3.2 ± 0.2	2.5 ± 0.2

Figure S6. A. Effects of the test compounds on apoptotic or necrotic cell death. Representative images include cell morphology (left panels), apoptotic cells stained with annexin V (green; middle panels), and necrotic cells stained with PI (red; right panels) after (24 h) treatment with saccharides **8** and **9** or Glyco-Au-NPs **1-4**. Images are representative of four different cell preparations. (Scale bar = 30 nm). B. Apoptotic and necrotic cells were counted under epifluorescence microscopy in six different fields of each sample. In all sample, the number of annexin V^+ and PI^+ cells were expressed as percentage over the total counted cells. The data represent means ± SEM of five experiments. Cells treated (2 h) with 0.5 mg/ml actinomycin D were considered as positive control of apoptosis, while TEG-functionalized Au-NPs **5** and **6** were used as Au-NP controls. *$P \leq 0.05$; **$P \leq 0.01$ versus compound-untreated cells (CTRL).

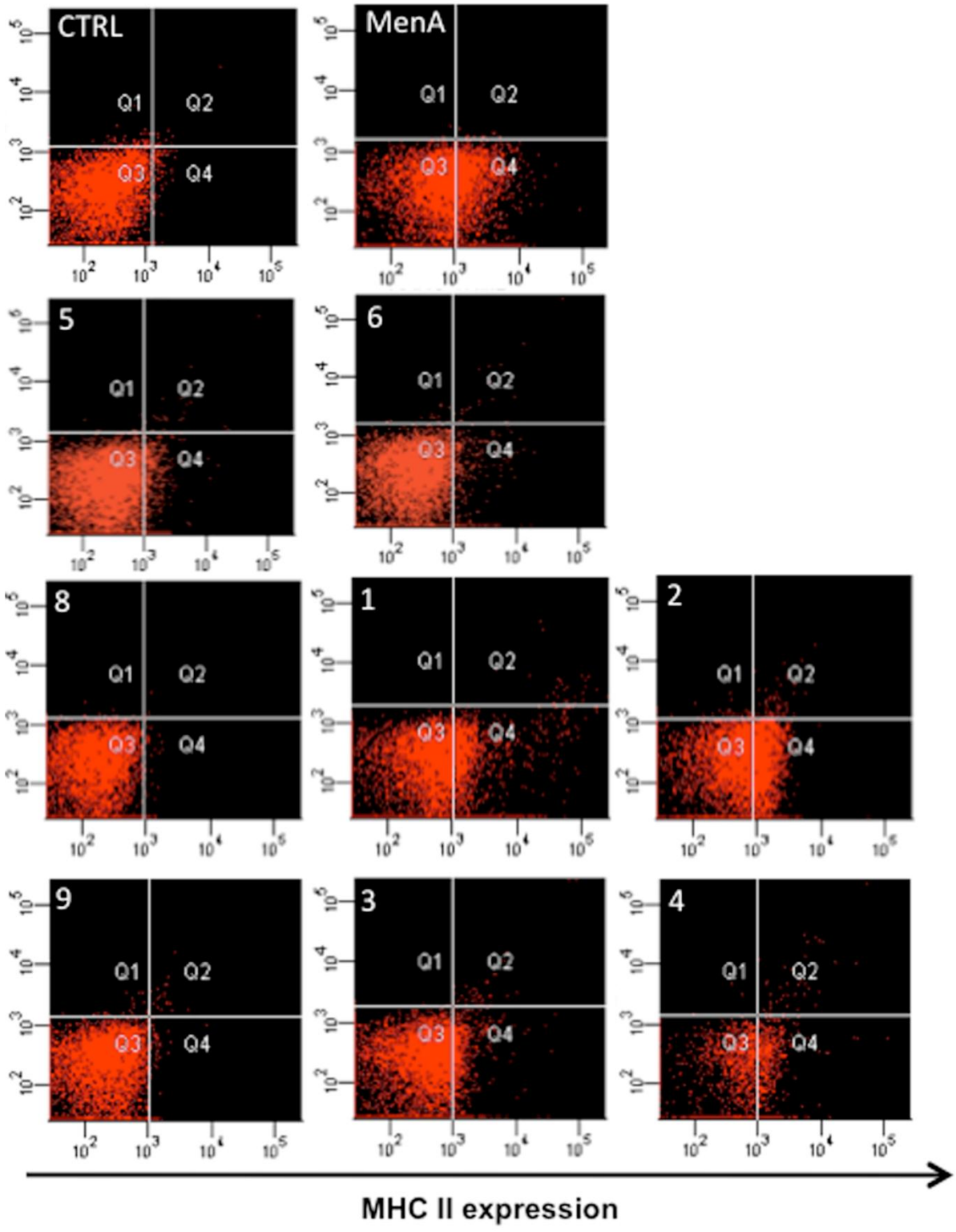


Figure S7. Glyco-Au-NPs activate macrophage effector functions. Effects of the test compounds on MHC II expression. FACS dot-plots show the expression of MHC II induced on RAW 264.7 cells by test compounds. Au-NPs **5** and **6** were used as Au-NP controls, while compound-untreated cells (CTRL) as negative controls. The y-axis represents fluorescence intensity.

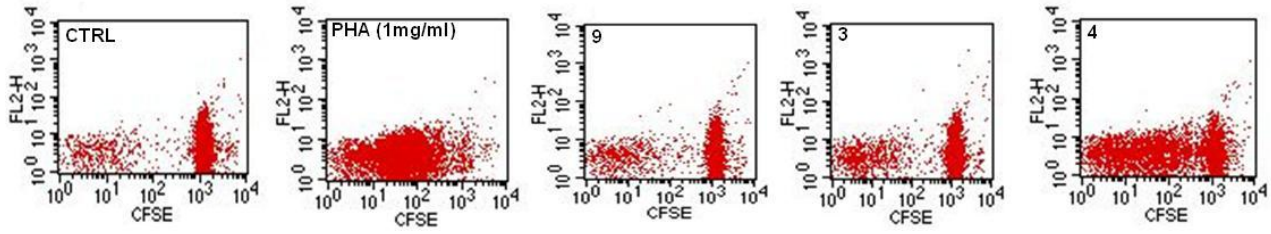


Figure S8. T cell proliferation measured by FACS as reduction of CFSE fluorescence intensity due to cell division. Representative dot plots of CFSE-labelled T cells treated (6 days) with PHA (positive control), disaccharides **9**, Glyco-Au-NPs **3** and **4.**

Release IL-2 (pg/ml)						
[compound](μM)	0	1×10^{-2}	1×10^{-1}	1×10^{0}	1×10^{1}	1×10^{2}
PHA	-	-	-	386 ± 18 **	-	-
CTRL	12 ± 1.7	-	-	-	-	-
5		13 ± 2.5	14 ± 1.3	12 ± 1.9	15 ± 1.3	15 ± 2.3
6		14 ± 2.4	17 ± 1.8	15 ± 1.4	13 ± 1.4	13 ± 1.5
8	-	12 ± 2.2	15 ± 1.9	12 ± 1.7	18 ± 2.1	17 ± 1.3
1	-	13 ± 2.2	19 ± 1.5	13 ± 1.8	14± 2.2	15 ± 1.8
2	-	14 ± 1.9	13 ± 1.7	15 ± 2.1	19 ± 2.3	15 ± 1.8
9	-	8 ± 1.3	12 ± 2.4	14 ± 1.9	13 ± 2.2	15 ± 2.9
3	-	20 ± 3.5	61 ± 7.1 *	33 ± 4.2	16 ± 5.1	12 ± 3.3
4	-	8 ± 2.3	66 ± 7.2 *	102 ± 8.1**	144 ± 16.6**	132 ± 12.6**

Figure S9. Ability of test compounds to induce IL-2 release from activating T cells evaluated by ELISA. Data represent means ± SEM of seven experiments run in triplicate. *P≤0.05; **P≤0.01 vs. compound-untreated controls.