Electronic Supplementary Material (ESI) for Nanoscale.

ELECTRONIC SUPPLEMENTARY INFORMATION

Acid anhydride coated carbon nanodots: Activated platforms for engineering clicked (bio)nanoconstructs

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1. Experimental procedures

Fluorescence quantum yield

Fluorescence quantum yield (FLQY) was estimated using quinine sulfate dissolved in 0.1 M H2SO4 as a standard (FLQYst = 0.54) and applyieng the following equation

$$FLQY = FLQY_{st}\left(\frac{I_{sm}}{I_{st}}\right)\left(\frac{OD_{st}}{OD_{st}}\right)\left(\frac{\eta^2{}_{sm}}{\eta^2{}_{st}}\right)$$

where I is the integrated emission intensity measured at the excitation maxima (454 nm and 326 nm respectively), OD denotes the optical density, η represents the refractive index. The subscript “sm” stands for sample and the subscript “st” indicates the standard fluorescence of a known fluorophore. The absorbance value of the carbon dot solution was restricted to <0.01, and integrated emission intensities of those solutions were recorded in a luminescence spectrometer.

Cell culture

CHO-k1 (ATCC CCL-61), Hela (ATCC CCL-2), RAW 264.7 (ATCC TIB-71) and CT26.WT (ATCC CRL-2638) cells were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM glutamine plus 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Cell were seeded at 1.5 x 104 cells/well density in 48 well plates and incubated at 37 ºC for 24 h to reach a cell confluence of 80–90%.

2. Figures

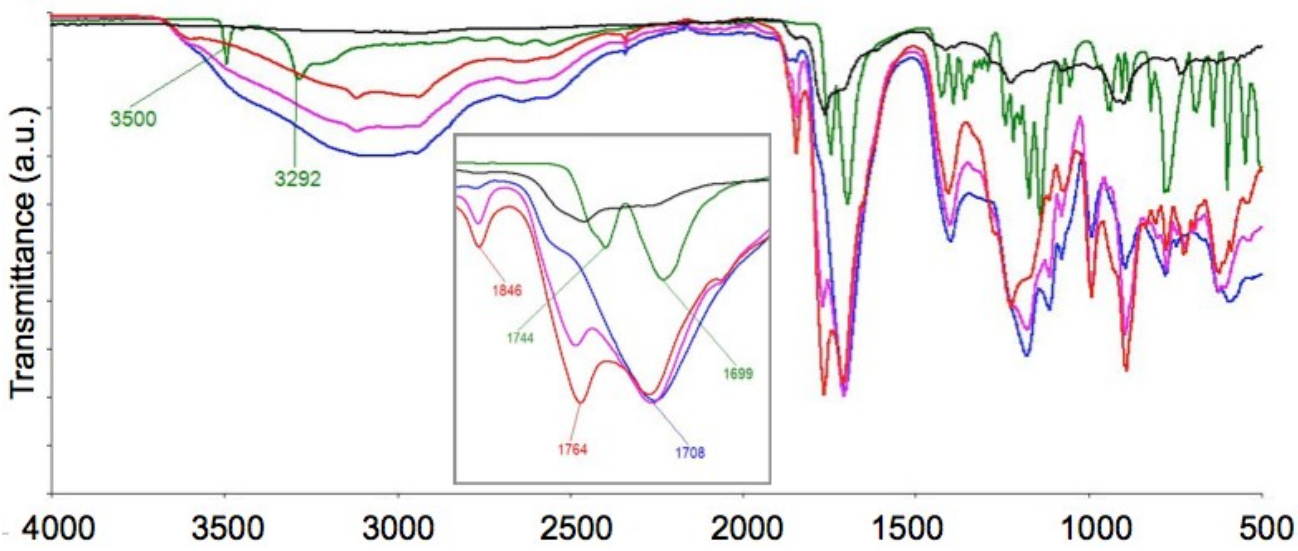

Figure S1. FTIR of the CA (green) and the resulting products from the thermolysis at 180 ºC for 20 min (blue),1 h (magenta), 2 h (red) and 48 h (black). Insert, detail of the 1650-1850 cm^{-1} region.

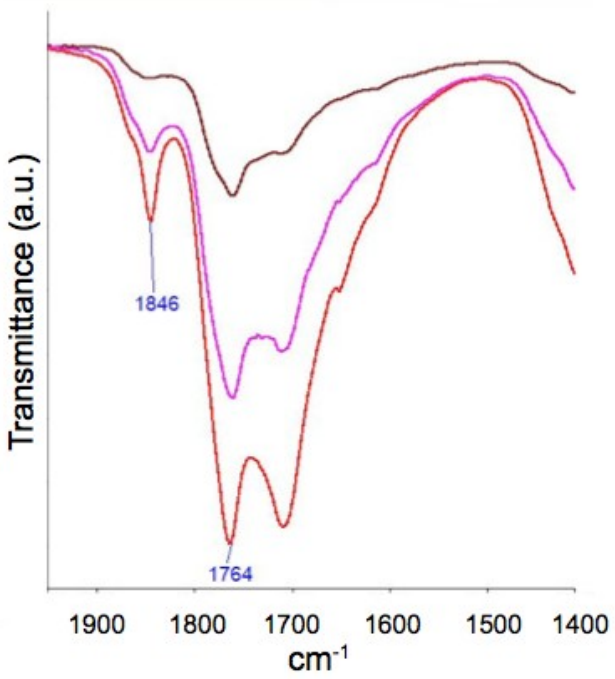

Figure S2. Evolution of the anhydride band at long times of thermolysis. Detail of the FTIR of samples heated at 180º C during 2 h (red), 20 h 40 min (magenta) and 10 h (brown).

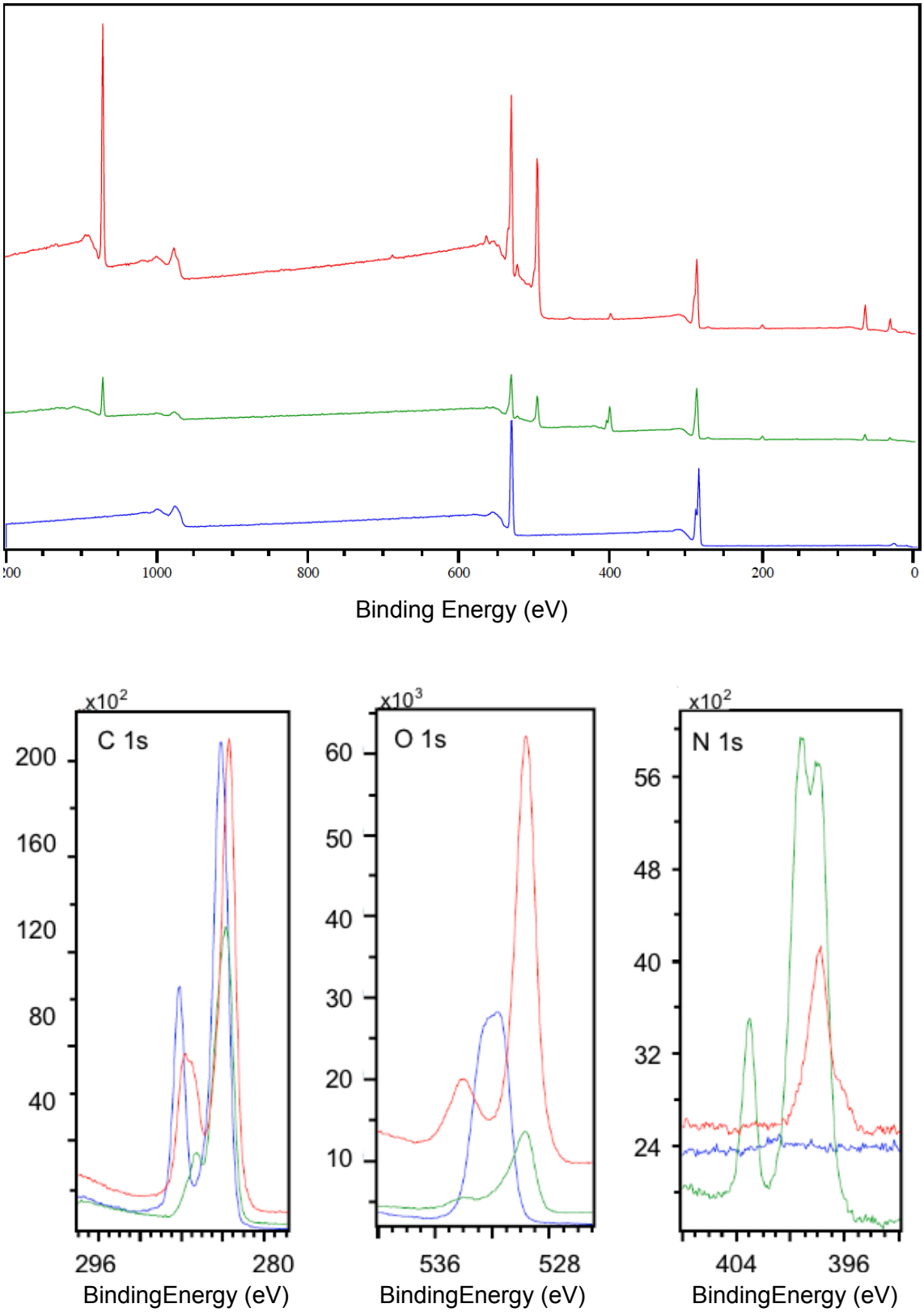

Figure S3. Full XPS spectra (top) and high resolution (bottom) C 1s, O 1s and N 1s spectra of **AA-CNDs** (blue), **Ak-CNDs** (red) and **Az-CNDs** (green)

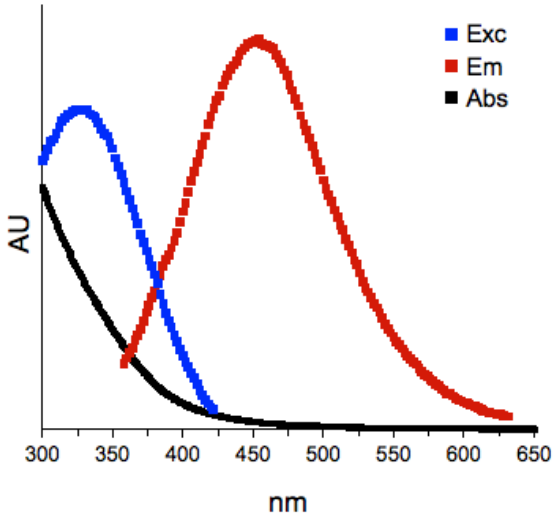

Figure S4. UV–Vis absorption & photoluminescence excitation/emission spectra of **AA-CNDs(Na)**

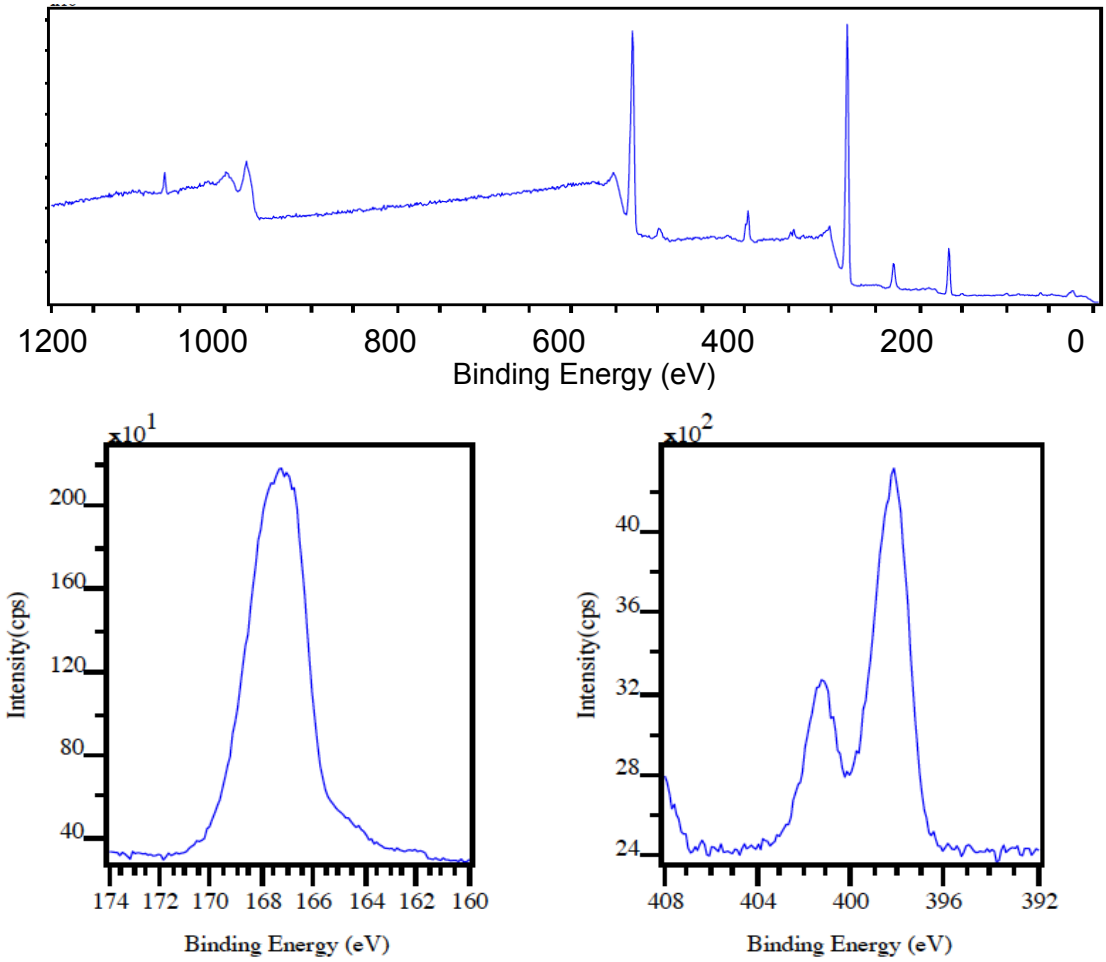

Figure S5.-Full XPS spectrum (top) and high resolution (bottom) S 2p and N 1s spectra of **VS-CNDs**

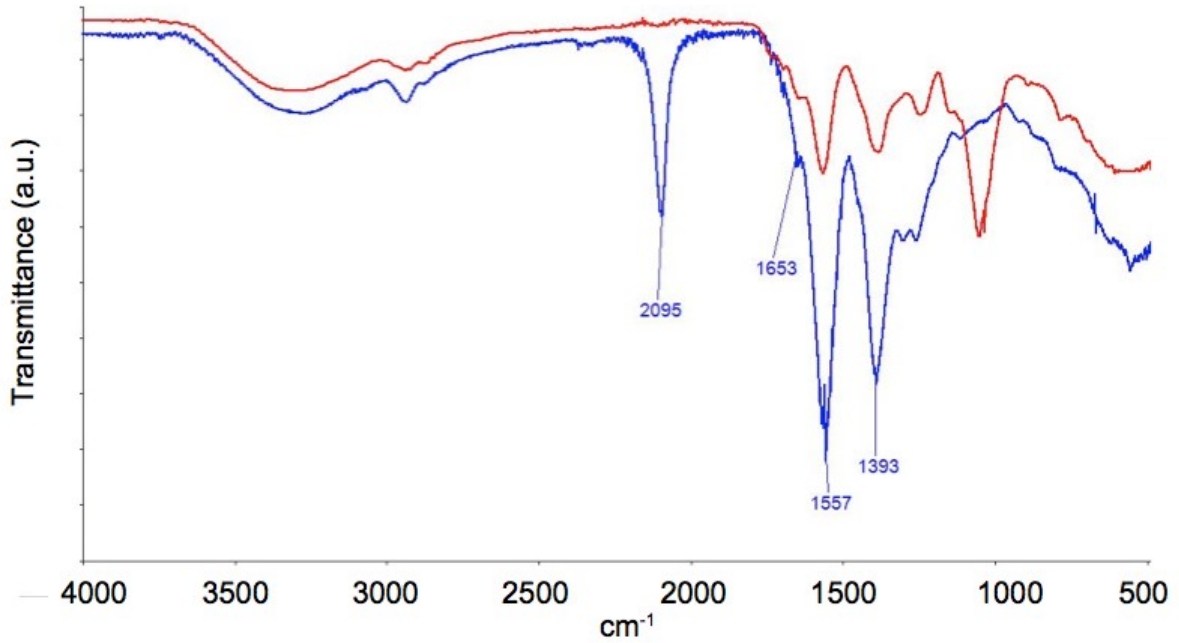

Figure S6. FIR of **Az-CNDs** (blue) and the **glyco-CNDs** resulting from the click reaction with alkynyl sugar

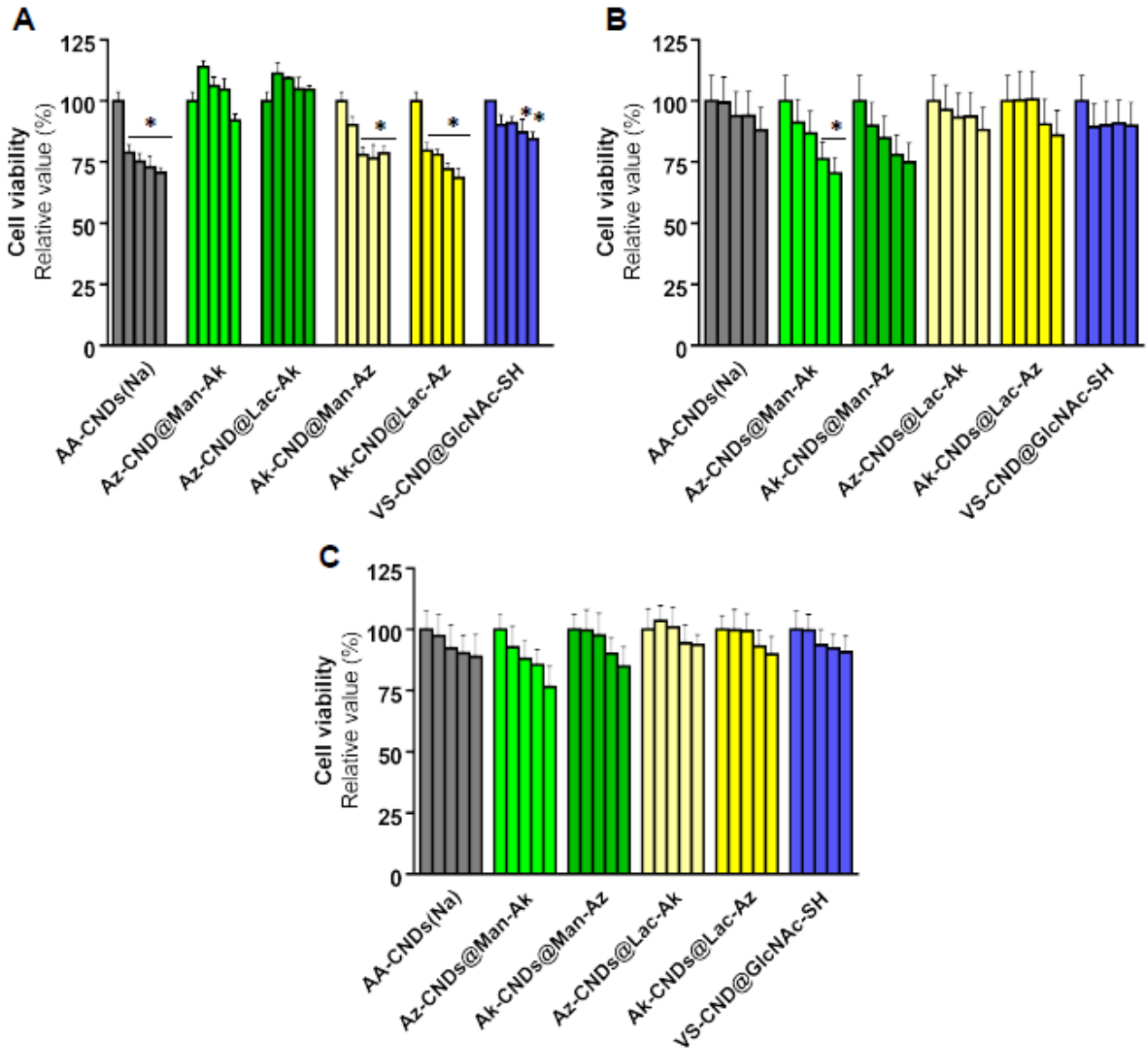

Figure S7. Citotoxycity assay. Cytotoxicity was evaluated in CHO-K1 (A), CT26wt (B) and Hela (C) cells at 0, 10, 50, 100 and 250 µg/mL concentrations (bars from left to right for each sample). Results are reported as % viability based on the untreated control cells normalized to 100% viable. Results are expressed as means ± SEM (n=6). * p <0,05 vs 0 µg/mL for each compound

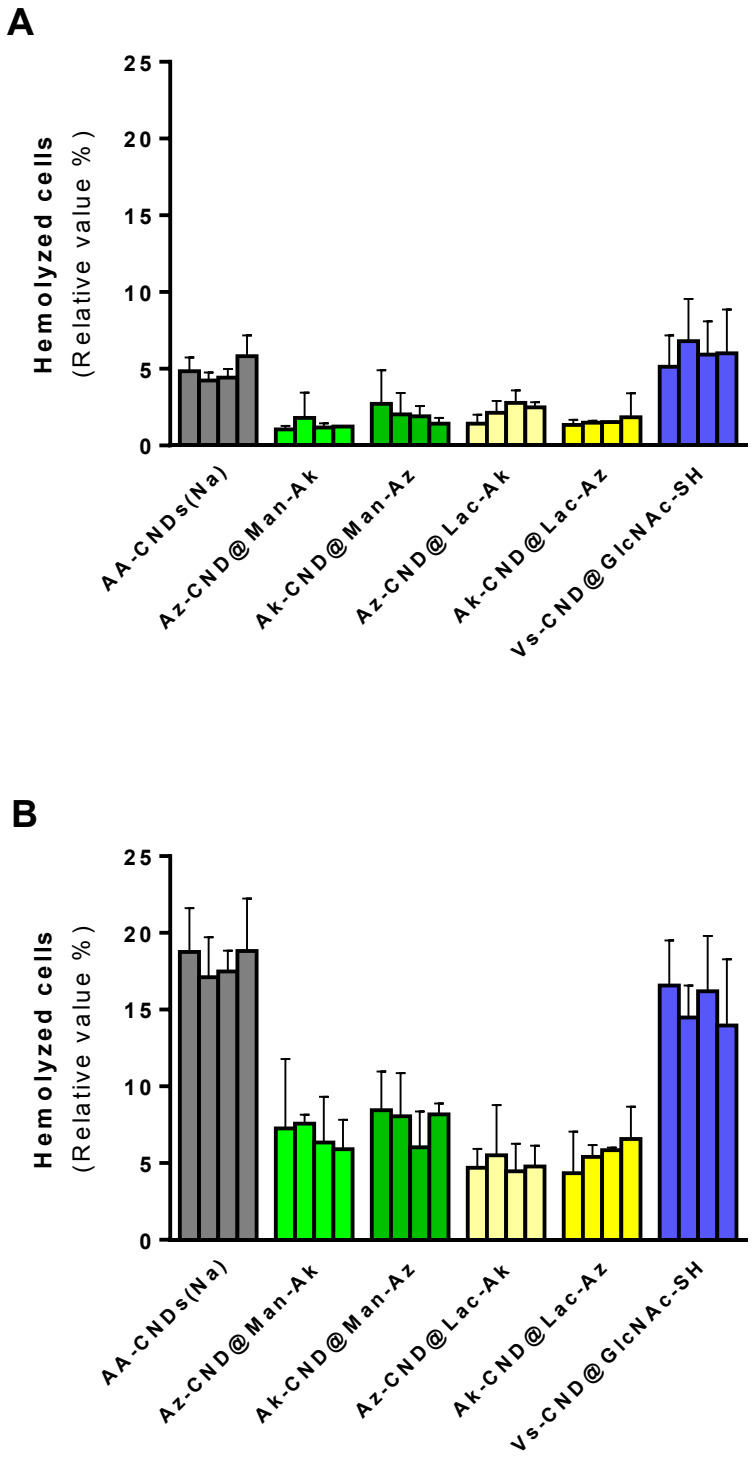

Figure S8. Hemolysis assay. Hemolytic assay was carried out at 250 (A) and 500 μg/mL (B) concentrations for 1, 3, 6 and 24 hours (bars from left to right for each sample). Results are expressed as means ± SEM (n=6).

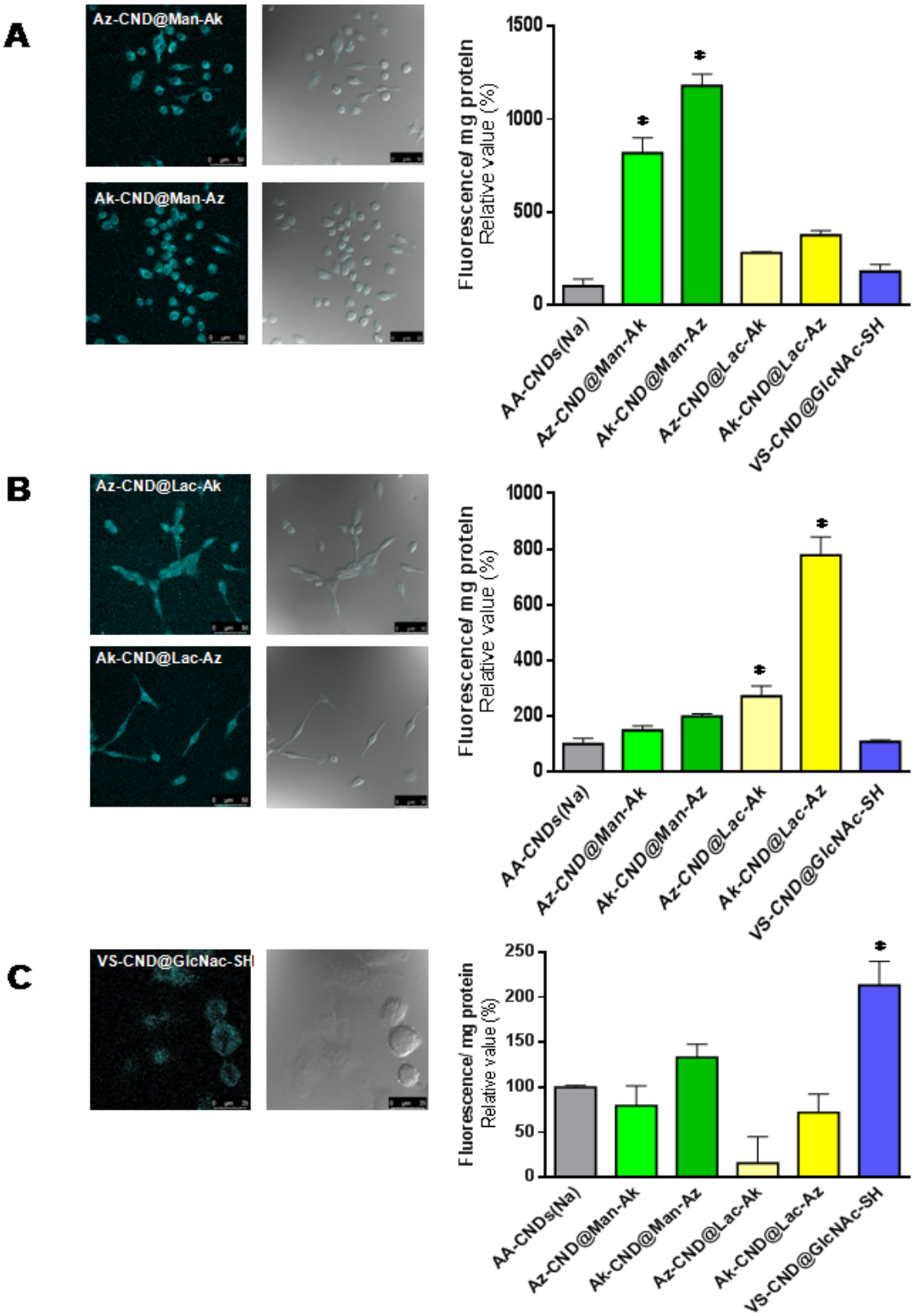

Figure 9. Cellular uptake. (A) RAW264.7, (B) CT26.WT and (C) Hela cells were incubated with 250 µg/mL CNDs derivatives for 6 hours and then cell uptake was visualized by confocal microscopy (representative images corresponding to maximum projections and Nomarsky images are shown) under conditions where no signals were detected in non-incubated cells. To quantitate cell uptake, fluorescence was measured and was normalized with protein concentration and the background fluorescence of non-incubated cells was subtracted. Results are expressed as means ± SEM (n=6). * p <0,05 vs AA-CNDs(Na).

3. Tables

Table S1.- Quantification (%atomic concentration) of the most significant peaks detected in clickable CNDs by XPS.

Peak	AA-CNDs	Az-CNDs	Ak-CNDs	VS-CNDs
C 1s	68.03	57.19	44.34	64.93
O 1s	31.87	17.64	35.35	25.23
N 1s	-	18.24	1.66	4.38
Na 1s	-	5.29	17.49	1.11
S 2p	-	-	-	3.41
Cl 2p	-	1.16	0.61	-

Table S2.- Elemental analysis of the clickable CNDs.

Sample	%C	%N	%H
Az-CND	27.53 ± 0.856	7.71 ± 0.410	3.31 ± 0.219
Ak-CND	17.37 ± 1.393	0.86 ± 0.047	2.34 ± 0.184
VS-CND	39.65 ± 0.141	4.49 ±0.163	6.12 ± 0.049

Table S3. Quantification of the sugar coating byphenol-sulfuric acid method and rotatory power of glyco-CNDs**.**

Sample	μmol sugar/mg glyco-CNDs	$[\alpha]_D$
Az-CND@Man-Ak	1.3 ± 0.11	+23º
Az-CND@Lac-Ak	0.7 ± 0.10	+2º
Ak-CND@Man-Az	1.0 ± 0.04	+13º
AK-CND@Lac-Az	0.7 ± 0.09	+8º
VS-CND@GlcNAc-SH	—	-4º

Table S4. Fluorescence quantum yield (FLQY) using quinine sulfate as standard (QY=0.54) of glyco-CNDs and their precursors

Sample	FLQY(%)
AA-CNDs(Na)	4
Ak-CNDs	7
Ak-CND@Man-Az	4
AK-CND@Lac-Az	1
Az-CNDs	3
Az-CND@Man-Ak	3
Az-CND@Lac-Ak	2
VS-CNDs	3
VS-CND@GlcNAc-SH	4

Table S5. IC_{50} of the glyco-CNDs

Compound	IC_{50} (mg/mL)	IC_{50} (uM)	$IC_{sugar}/IC_{glyco\text{-}CNDs}$
Met-Man		18000	1
Az-CND@Man-Ak	0.02	26.2	687
Ak-CND@Man-Az	0.015	14.9	1208
Lac		2300	1
Az-CND@Lac-Ak	0.02	13.9	165
AK-CND@Lac-Az	0.011	8.1	284
GlcNAc		335000	1
VS-CND@GlcNAc-SH	3.35	4300	78