Supporting Information

Mixed-charge pseudo-*zwitterionic* mesoporous silica nanoparticles with low-fouling and reduced cell uptake properties

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Equipment

Solution state nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE AV-250 and a Bruker AVANCE AV-500 spectrometers. Chemical shifts (δ) are reported in ppm. ^{1}H and ^{13}C resonances were measured relative to internal deuterated solvent peaks considering TMS = 0 ppm.

HR-MAS NMR spectra were obtained on a Bruker AMX500MHz spectrometer equipped with a semisolid state probe. Measurements were performed in the solid state, by suspending the hybrid materials in a small amount of D2O.

Solid state magic angle spinning nuclear magnetic resonance (MAS NMR) and cross polarization magic angle spinning nuclear magnetic resonance (CP MAS NMR) spectra were obtained on a Bruker Avance AV-400WB spectrometer equipped with a solid state probe using a 4 mm zirconia rotor. Typical measurement conditions were as follows: ^{13}C NMR experiments were conducted with proton decoupling, the spectrometer frequency was set to 100.62 MHz (^{13}C) and 400.13 MHz (^{1}H) and MAS rotation rate was maintained at 6 kHz. The NMR spectra consisted of 15000 to 17500 acquisitions with cross-polarization contact times of 2 ms, 2.5 μs pulse wide and a 5 s recycle delay. Chemical shift values were referenced at 176.1 ppm to carbonyl carbon of glycine. ^{29}Si NMR quantitative spectra were obtained at 79.49 MHz with MAS rotation rate of 12 kHz, a pulse wide of 4.5 μs, a recycle delay of 5 s, and the number of scans was 17000 to 20000. ^{29}Si CP MAS NMR experiments were done setting the transmitter frequency to 79.49 MHz (^{29}Si) and 400.13 MHz (^{1}H), samples were spun to 12 kHz and a 5 s recycle delay, a 4.5 μs pulse wide and a cross-polarization contact time of 3.5 ms were employed. All chemical shift values reported were externally referenced at 0 ppm to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS sodium salt).

Fourier transformed infrared (FTIR) spectra were collected in a Thermo Nicolet Nexus spectrometer equipped with a Goldengate attenuated total reflectance (ATR) device.

Chemical microanalyses were performed with a Perkin Elmer 2400 CHN and a LECO CHNS-932 thermo analyzers. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were carried out in air between 30 and 800 °C with a flow rate of 100 mL/min and a heating rate of 5 °C/min using a Perkin Elmer Pyris Diamond thermobalance.

Ordered mesoporous materials were analyzed using powder X-ray diffraction at small angles (SA-XRD) in a Philips X'Pert Plus diffractometer (Philips Electronics NV, Eindhoven, Netherlands) with Bragg-Brentano geometry, operating with CuKα radiation (λ = 1.5406 Å) at 40 kV and 20 mA. The diffractograms were collected over the range between 0.6° and 10.0° 2θ with a step size of 0.02° 2θ and a contact time of 5 s per step.

The surface characterisation of materials was carried out by N_2 adsorption/desorption analysis at −196 °C on a Micromeritics ASAP2020 analyser (Micromeritics Co, Norcross, USA). In all cases, 30-50 mg of the functionalized materials were outgassed under a vacuum lower than 10^{-5} Torr during 24 h at 60 °C prior to the measurements. The Brunauer-Emmett-Teller (BET) equation was used to calculate the surface area S_{BET}. Single-point total pore volume (V_T, cm^3/g) was measured at $P/P_0 \approx 0.97$. The average mesopore size (D_P) between 0.5 and 40 nm was obtained from the maximum of the pore size distribution curve calculated from the adsorption branch of the isotherm using the BJH (Barrett-Joyner-Halenda) method [1,2].

Electrophoretic mobility measurements of the materials suspended in water (0.1 mg/mL) were used to calculate the zeta-potential (ζ) values of the nanoparticles. Measurements were performed in a Zetasizer Nano-ZS instrument (Malvern) equipped with a 633 nm "red" laser. ζ-potential can be described by Smoluchowski's equation [3]: $\zeta = 4\pi\eta U/\varepsilon$, where U is the electrophoretic mobility, η is the viscosity of water, and ε is the dielectric constant of water. The hydrodynamic size of mesoporous nanoparticles was measured by means of Dynamic Light Scattering (DLS) with the same Malvern instrument. Values presented are mean ± SD from triplicate measurements.

SEM-EDS analysis was performed on a JEOL JSM 6335F microscope. For this purpose, samples were dispersed in ethanol and dried onto a copper stud and coated with a film of Cr previous to observation.

Transmission electron microscopy (TEM) was carried out with a JEOL JEM 1400 and JEOL JEM 2100 instruments equipped with a CCD camera (KeenView Camera) and operated at 120 and 200 kV, respectively. Sample preparation was performed by dispersing in ethanol and subsequent deposition onto carbon-coated copper grids.

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[1] S. J. Gregg, K. S. W. Sing. Adsorption Surface Area and Porosity, 2nd ed. Academic Press, New York, 1982.

[2] S. Lowell, J. E. Shields, M. A. Thomas, M. Thommes. Characterization of Porous Solids and Powders: Surface Area, Pore Size and Density. Particle Technology Series, Vol. 16, 2004, Springer Science+Business Media B.V.

[3] M. V. Smoluchowski, Handbuch der Electrizitat und des Magnetismus, Barth, Leipzig, Germany 1921, p. 366.

Characterization of the samples:

TGA studies have been carried out in order to determine the organic content (functionalization and LEVO loaded) of the samples. In the case of non-functionalized MSNs (Fig. S1A), a weight loss of approximately 16.1% is observed up to 100 °C, attributed to the calcination of remaining organic matter (i.e. CTAB template). Functionalized ZMSN-1.5 and PEGylated nanoparticles present a first loss step below 100 °C (about 2%) corresponding to the elimination of adsorbed water on the silica surface. Further weight loss takes place in the range of 100-250 °C (22.4% for ZMSN-1.5 and 30.1% for PEG) confirming the effective functionalization of the nanoparticles. Furthermore, drug-loaded nanoparticles increase (Fig. S2B) the weight loss up to 23 and 26% (@MSN and @ZMSN-1.5, respectively). On the contrary, PEGylated NPs present a lower weight loss of about 20%, showing a higher loaded yield in *zwitterionic* materials.

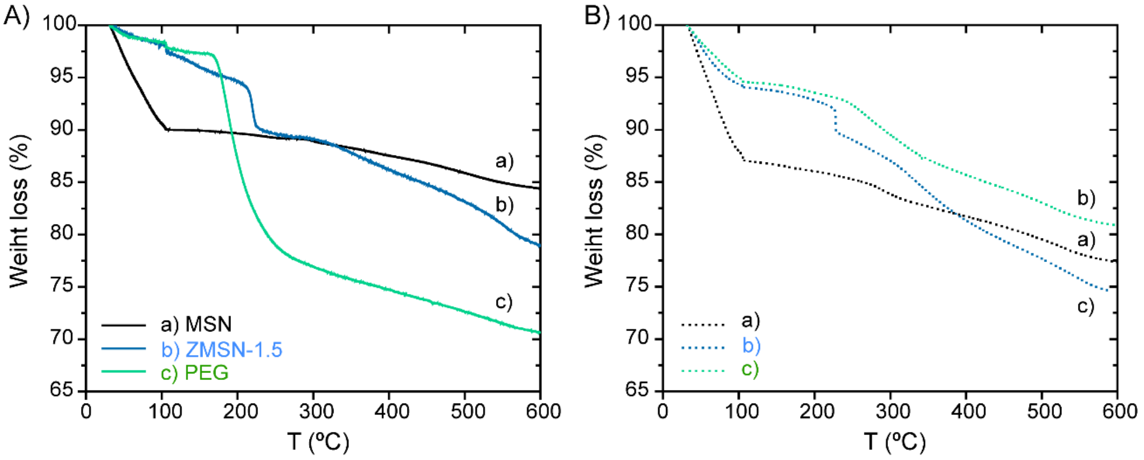


Figure S1. Thermogravimetic (TGA) analysis of the A) unloaded and B) LEVO containing a) pristine MSN, b) *zwitterionic* ZMSN-1.5 and c) PEGylated synthesized systems.

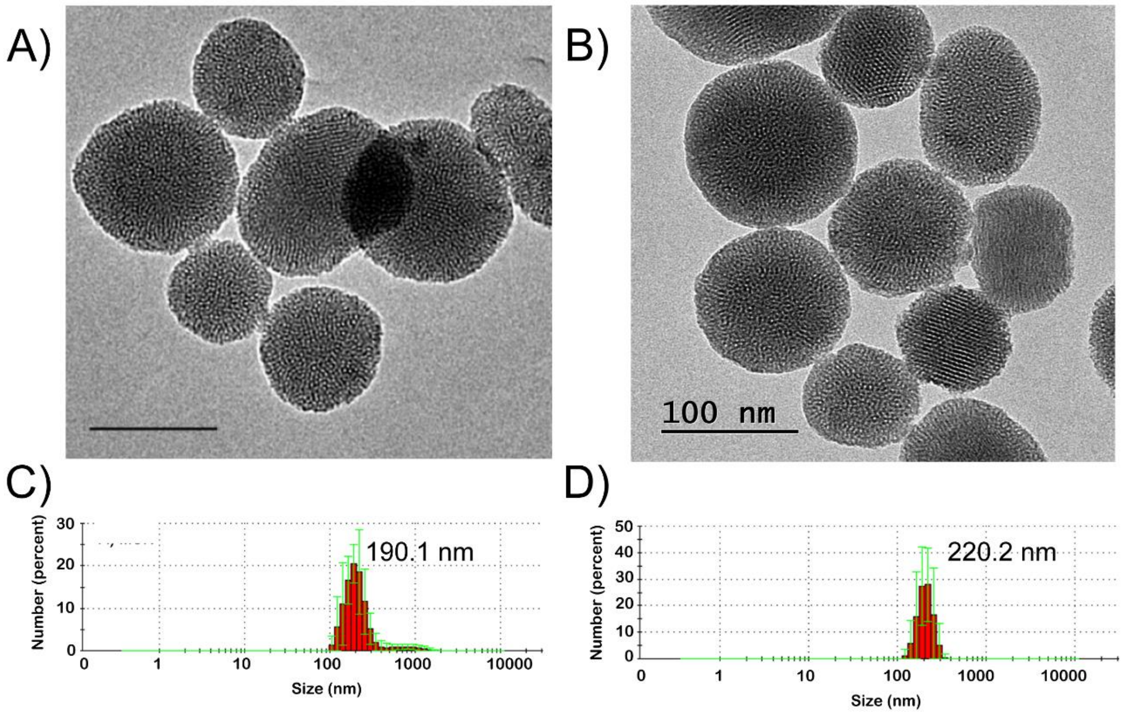


Figure S2. TEM images of A) bare MSNs and B) ZMSN-1.5 (*upper row*). DLS size histograms are displayed (*lower row*) for C) naked and D) modified nanoparticles.

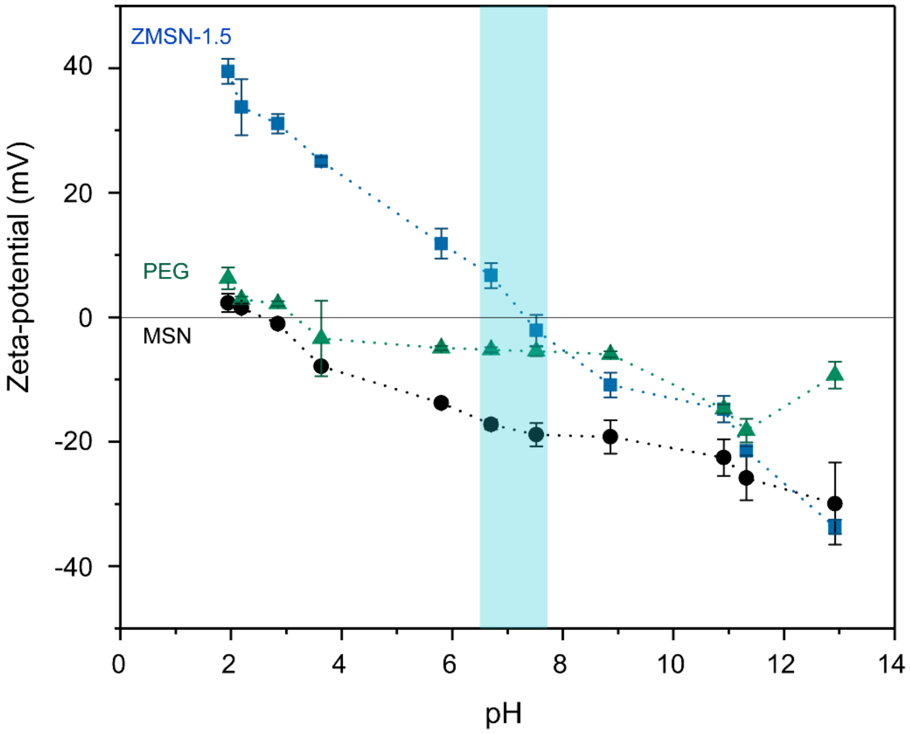
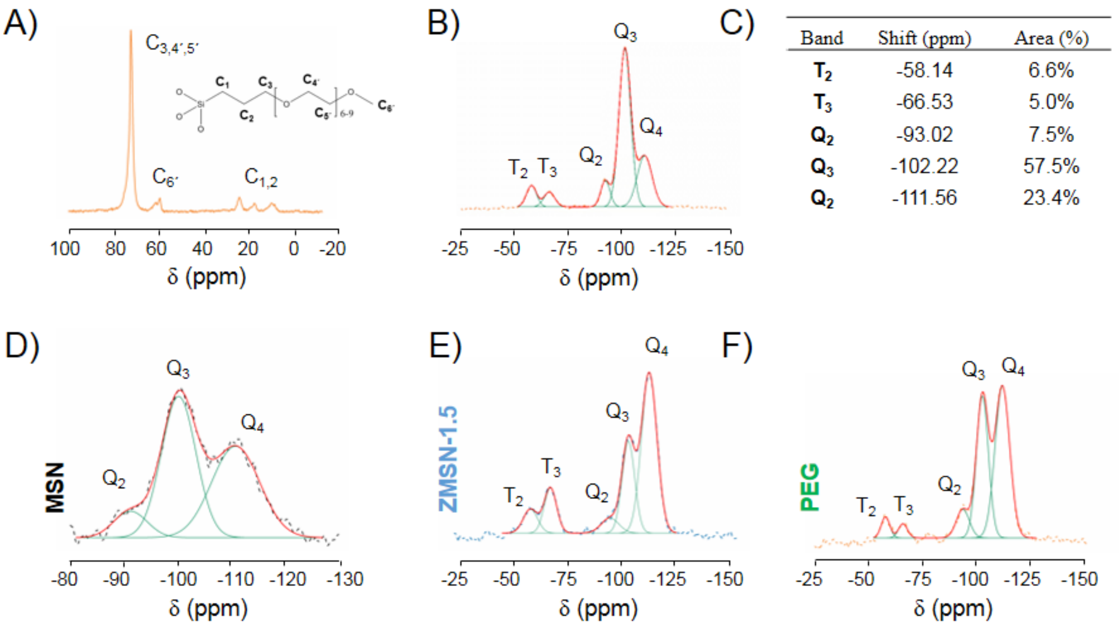


Figure S3. ζ-potential variation with pH values for MSNs (black circles), ZMSN-1.5 (blue squares) and PEGylated (green triangles) systems.



Band	Shift (ppm)	Area (%)
T_2	-58.14	6.6%
T_3	-66.53	5.0%
Q_2	-93.02	7.5%
Q_3	-102.22	57.5%
Q_2	-111.56	23.4%

Figure S4. A) $^1H \rightarrow ^{13}C$ CP-MAS NMR (showing the carbon assignment the structure scheme). B) ^{29}Si MAS NMR spectra PEGylated nanoparticles, showing C) the chemical shift and peak area (%) of deconvoluted silicon Q_n and T_n ^{29}Si MAS NMR bands. Single-pulse ^{29}Si MAS NMR spectra of D) MSN, E) ZMSN-1.5 and F) PEGylated systems.

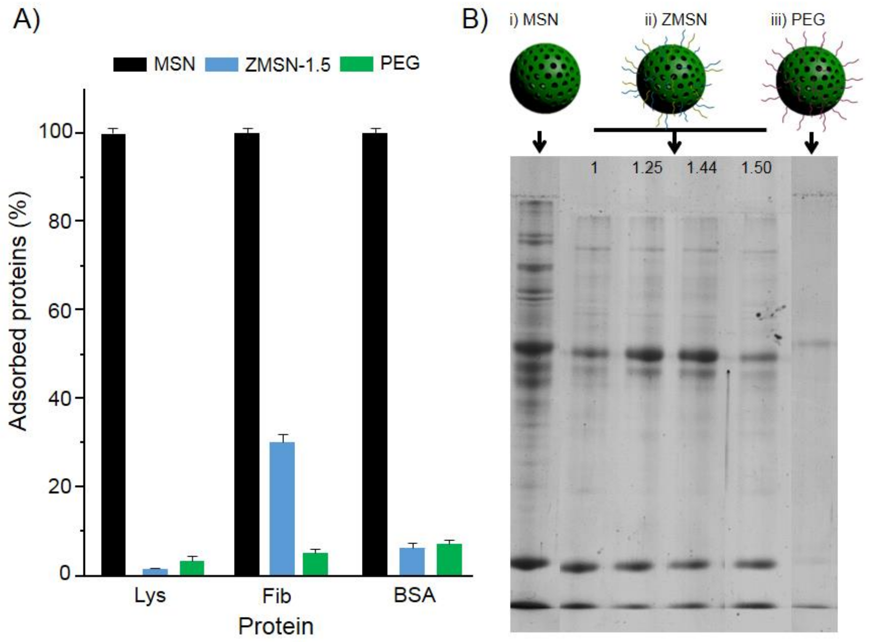


Figure S5. A) Percentage of adsorbed lysozyme (Lys), fibrinogen (Fib) and bovine serum albumin (BSA) on pristine MSNs, ZMSN-1.5 and PEGylated MCM-41 nanoparticles. B) Laemmli buffer gel electrophoresis results performed in fetal calf serum (FCS).

Table S1. Estimation of the ratio of total average fluorescence intensity from LSCM images arising from the nanoparticles (I_{Total}) between those within the cells (I_{Cell}) calculated by image analysis (FiJi software).

Sample	Estimated averaged I_{Total} / I_{cell}
MSN	37.1 ± 6.5
ZMSN-1.5	16.8 ± 6.8
PEG	7.7 ± 2.8

Table S2. Textural features (S_{BET}: surface area; D_P: pore diameter; V_P: pore volume) of pristine MSN and bi-functionalized zwitterionic ZMSN-1.5 obtained by N_2 adsorption porosimetry.

Sample	S_{BET} (m^2g^{-1}) [a]	D_p (nm) [b]	V_P (cm^3g^{-1}) [c]
MSN	1041	2.4	1.20
MSN@LEVO	331	2.3	0.41
ZMSN-1.5	500	2.3	0.48
ZMSN-1.5@LEVO	143	1.9	0.22