Programmable Drug Release Using Bioresponsive Mesoporous Silica Nanoparticles for Site-specific Oral Drug Delivery

Amirali Popat [a,b,c], Siddharth Jambhrunkar[a], Jun Zhang[a], Jie Yang[a], Honwei Zhang[a], Anand Meka[a], Chengzhong Yu[a]*

[a] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia

[b] The School of Pharmacy, The University of Queensland, Brisbane, QLD 4072, Australia

[c] Mucosal Diseases Group, Mater Research Institute – The University of Queensland, Translational Research Institute, 37 Kent St, Woolloongabba, QLD 4102

Electronic Supporting Information (ESI)

Experimental part:

Materials:

Cetyltrimethylamonium bromide (99%) (CTAB), tetraethylorthosilicate (98%) (TEOS), Pluronic F127 (P-F127, $EO_{106}PO_{70}EO_{106}$), (3-Aminopropyl)triethoxysilane (≥ 98%, APTES), EDC (N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, Sulfasalazine (SZ, >99%), Pepsin, and Pancreatine, were purchased from Sigma-Aldrich Australia. Soy protein isolate (SPI) was received as a gift sample from ADM Australia. All other chemicals and solvents were analytical grade and were used without further purification.

Methods:

Preparation of MCM-48 nanoparticles: MCM-48 nanoparticles were synthesized by slight modification of Kim *et al* procedure.[1] In a typical synthesis, 2.0 g of CTAB and 8.0 g of P-F127 were dissolved in 596 mL of H_2O/NH_3/EtOH (NH_4OH(aq) (2.8 wt %)/EtOH) = 2.5/1 (v/v)). Then, TEOS (7.2 g) was added quickly to the solution and stirred at 850 rpm for 1 min. The reaction mixture was further kept 24 h in static conditions (RT). The resulting white, solid product was isolated by high speed centrifugation, re-dispersed twice in deionized water and then ethanol, isolated again by centrifugation and finally dried overnight in air at 60 °C. MSNs were finally calcined (air, 550 °C, 1 °C/min) for 5.5 hours.

Amino group grafting and SZ loading: To functionalise primary amino groups inside and outside porous structure of MCM-48, 500 mg of calcined MCM-48 were mixed in 50 mL of dry toluene in a round bottom flask then 750 mg of APTES was added at once and the reaction was refluxed at 115°C overnight under nitrogen atmosphere. Functionalised particles were then centrifuged (20 min at 12000 rpm), washed twice with ethanol and once with acetone. Finally, particles were dried overnight at 50 °C. To load the SZ into pores of amino functionalized particles, MCM-NH (200 mg) were soaked into concentrated SZ solution (10mM-100mM) in DMSO. Suspension was sonicated for one minute in a

bath sonicator and kept in shaker bath for 24 hours at RT in dark. The suspension was then centrifuged and the resulting supernatant was removed.

Succinylation of SPI (SSPI): SPI was succinylated using a modified literature method.[2, 3] In a typical experiment, 200 mg of SPI was dissolved in 20 mL of phosphate buffer at pH 7.5. Then 100 mg of succinic anhydride was added in 5 times (20 mg each time) with stirring at room temperature. The solution was stirred over 1 hour while the pH was maintained between 8 and 9 by addition of 2 M NaOH. After pH stabilization around 8.5, the solution was further stirred for another 20 min. The solution was then dialyzed (10,000 NMWCO, dialysis tubing, benzoylated, avg. flat width 32 mm, Sigma-Aldrich) in nanopure water for 24 hours at 4 °C (water was changed 10 times, sample-to-volume ratio 1:100). Finally solution was freeze-dried for 2 days.

Grafting of SSPI onto MCM-NH_2-SZ: For protein grafting, succinylated SPI (50 mg) was first dispersed in pH 5 MES buffer (0.1 M, 8 mL, low moisture content ≥ 99%, Sigma-Aldrich). To activate the carboxylic acid groups, EDC (6 mg, (*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, crystalline) was added to above solution and the resulting mixture was stirred for 20 min at RT. Then, a suspension of MCM-NH-SZ (25 mg) in MES buffer pH 5 (0.1 M, 2 mL) was added at once and the white obtained solution was further stirred for 2 hours at RT. Particles were finally centrifuged (20 min at 12000 rpm), washed twice with buffer to remove excess EDC and unreacted protein followed by freeze drying under vacuum. The supernatant was analysed for any leaked SZ during coating.

Drug Release:

pH dependent SZ release: To monitor SZ release from all the nanoformulations, 10mg equivalent of SZ loaded nanoparticles were added in 10ml of 0.1N HCl (pH 1.2). At predetermined time intervals, 1 ml of the samples were withdrawn and immediately replaced with an equal volume of dissolution medium to keep the volume constant. These samples were then properly diluted and analysed for SZ content at 360 nm using UV-Vis spectrophotometer. After 2 hours, gastric emptying time after oral delivery the suspension was centrifuged and particles were re dispersed with 10ml of pH 5.5 buffer to simulate duodenum and similar procedure is followed. After one hour, particles are transferred to pH 7.4 buffer and drug release was monitored for 48 hours. Samples where enzyme is needed Pepsin and Pancreatin were added in pH 1.2 and pH 7.4 respectively to simulate stomach and small intestine.

5-ASA release in presence of azo-reductase:

Phosphate buffer (50 mL, 50 mM, pH 7.4) was prepared and D-glucose (25 mg/mL) was added. Nitrogen gas was bubbled through the solution for 10 min to deoxygenate it. The bottle was sealed to ensure no exposure of oxygen. A male rat was euthanized and the cecum was exposed. Cecum content was added to the above solution and nitrogen gas was again bubbled to remove any oxygen that entered while adding the cecum content. The container was sealed and incubated at 37^{o}C for 24 h to grow the anaerobic bacteria responsible for reduction of the azo bond. This process will ensures

removal of aerobic bacteria present in the cecum hence avoiding the interference. After 24 h the solution (5 mL) was added to SZ (2.5 mg), MSN-NH-SZ (10 mg) and MSN-NH-SZ-SPI (10 mg). After 2 h of exposure, the samples were centrifuged and supernatant was analysed by HPLC to evaluate the degradation of SZ.[4] Similar experiments were carried out in PBS without cecum content as a control.

Characterization:

Transmission electron microscopy (TEM) images of materials were obtained using a JEOL JEM-1010 electron microscope operated at 100 kV. High-resolution images were taken using a FEI Tecnai F20 electron microscope with an acceleration voltage of 300 kV. Powdered small angle X-ray diffraction (XRD) analyses were carried out on a Rigaku miniflex diffractometer using Co (λ = 1.792 Å and 1.541 Å) radiations and operated at 30 kV with a variable slit width. The scanning rate was set at 1 °/minute over 2θ from 0.5 - 10 °. The N_2 adsorption isotherms were measured at 77 K on a nitrogen adsorption apparatus (Tristar II) after degassing the samples at 200 °C for 24 h. Before the sorption measurements, the MCM-48 were outgassed for 6 h at 200 °C. MCM-NH and MCM-NH-SZ were outgassed for 8 h at 80 °C. MCM-NH-SPI were outgassed 24 h at room temperature. Specific surface area, SBET, were determined using the BET equation in the range $0.05 \geq P/P_0 \geq 0.20$ and the total pore volume was measured at $P/P_0 = 0.95$. Zeta-potential and dynamic light scattering (DLS) measurements were performed using a Malvern DTS Nano zetasizer ZS 173^o (equilibration time set to 2 min; 3 measurements taken on each sample; only quality criteria data accepted as valid results). FTIR spectra were recorded using a Nicolet 6700 FTIR spectrometer with a narrow band MCT detector. The spectra were obtained from 128 scans with a resolution of 4 cm^{-1}. Solid-state magic-angle spinning (MAS) solid-state nuclear magnetic resonance (NMR) spectra were obtained on a Bruker DRX300 MHz NMR spectrometer. The 75.4 MHz ^{13}C CP-MAS spectra were obtained using a 7 mm rotor spinning at 4 kHz. The chemical shifts are reported in ppm relative to tetramethylsilane (TMS). UV–visible absorption spectra were recorded using a Shimadzu UV-1700 spectrophotometer In both cases, measurements were taken in a quartz cuvette with a 1 cm path length.

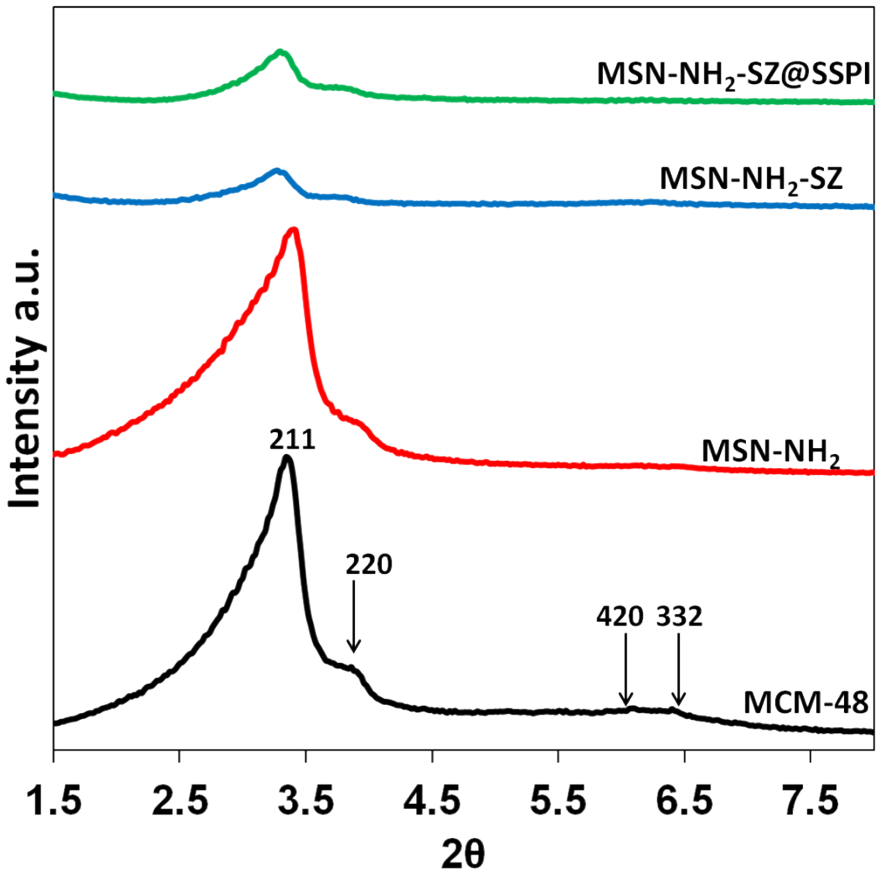


Fig S1. Small angle powder X-Ray diffraction patterns obtained for MCM-48, MSN-NH_2, MSN-NH_2-SZ, and MSN-NH_2@SSPI

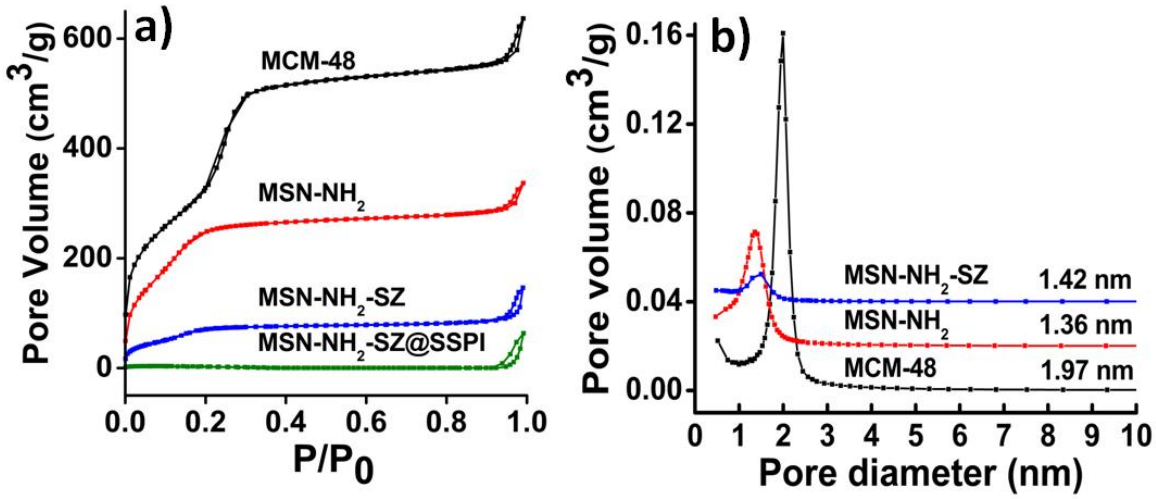


Fig S2. a) N_2 physisorption adsorption-desorption isotherms (at – 196 °C) and **b)** corresponding BJH pore size distributions for various nanoparticles.

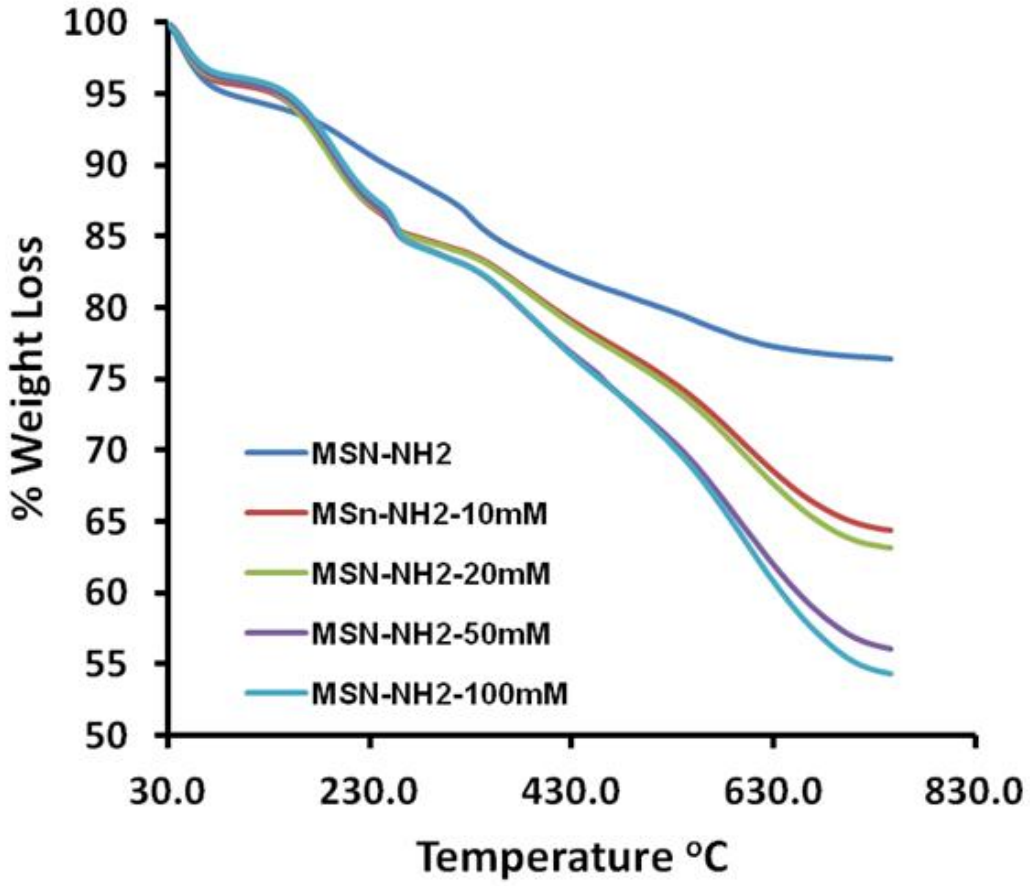


Fig S3. TGA analysis of SZ adsorbed onto MSN-NH_2 at different initial concentration (10-100mM) in DMSO

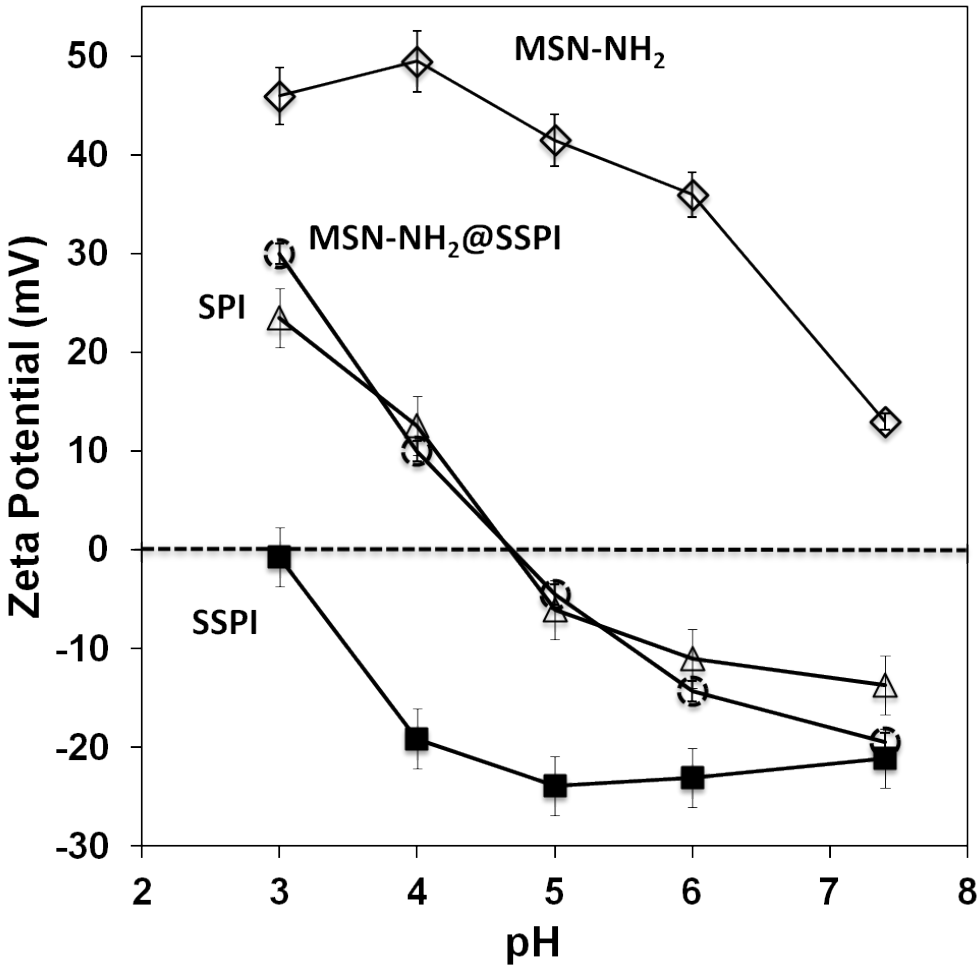


Fig S4. Zeta potential of MSN-NH_2, SPI, SSPI and MSN-NH_2@SSPI (control test, See experimental part of the ESI) as a function of pH

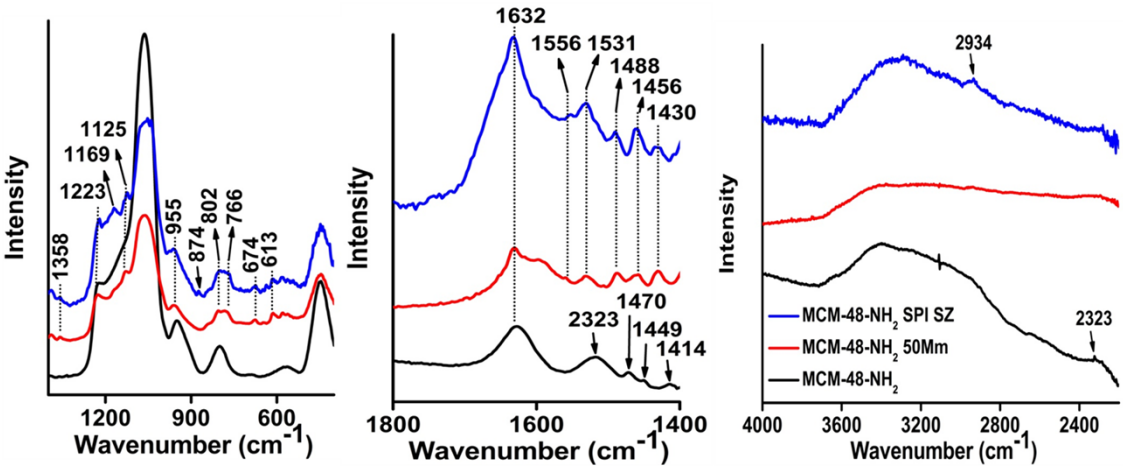


Fig S5. FTIR spectra of MSN-NH_2, MSN-NH_2-SZ and MSN-NH_2-SZ@SSPI

Spectra for MSN-NH_2, MSN-NH_2-SZ and MSN-NH_2@SSPI further confirmed the drug loading and grafting. MSN-NH showed bands due to Si-O-Si vibrations at 450 cm^{-1} (rocking), 800 cm-1 (bending), and 1000-1300 cm^{-1} (stretching); a band at 1630 cm^{-1} from H-O-H bending vibration. Vibrations around 1470-1530 cm^{-1} confirmed the presence of primary amino groups on the silica (Figure S5b).[5] After SZ loading, vibrations around 1400-1600 increased both in number and intensity. For instance, peak near 1488 cm^{-1} confirms presence of pyridine ring of sulfasalazine and vibrations around 1358 cm^{-1} affirms presence of C-N bond in SZ. One characteristics of SZ is presence of sulphur in the form of SO_2 which is validated by vibrations around 1125 cm^{-1}(Fig S5a). After protein modification a peak around 2900 cm^{-1} appears which is due to presence of large amount of primary and secondary amine in protein. Also, intensity of vibrations around 3000-3500 cm^{-1} also increased due to presence of numerous hydroxyl groups in succinylated protein (SSPI). A broad and high intensity peak around 1630-1700 cm^{-1} is due to C=O of number of amide bonds present in protein.

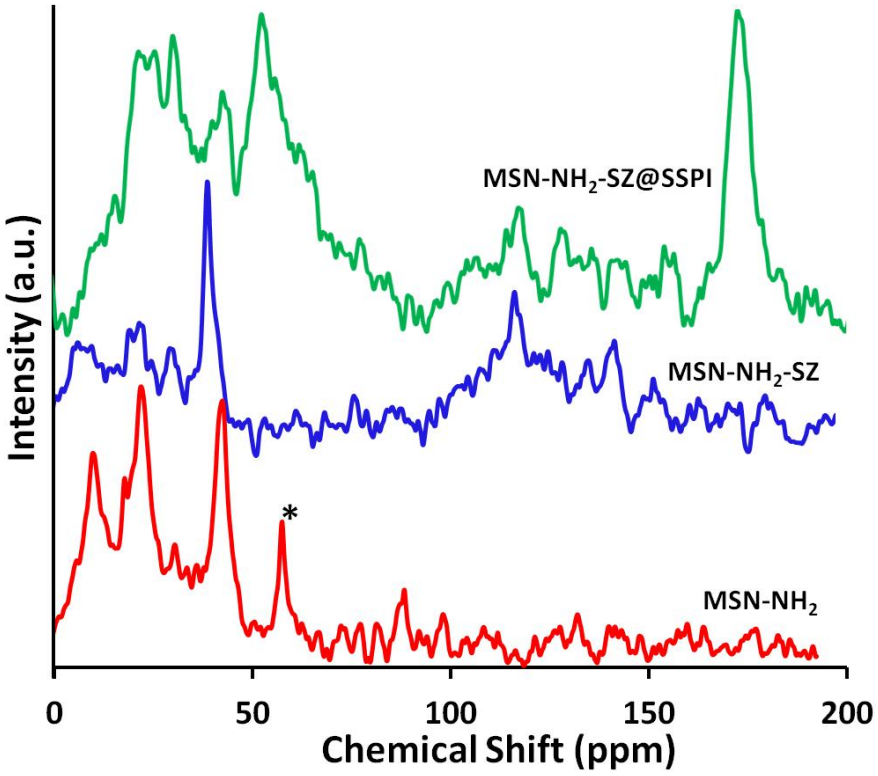


Fig S6. ^{13}C NMR spectra of MSN-NH_2, MSN-NH_2-SZ, MSN-NH_2-SZ@SSPI. Picks due to residual DMSO and APTES are denoted with *.

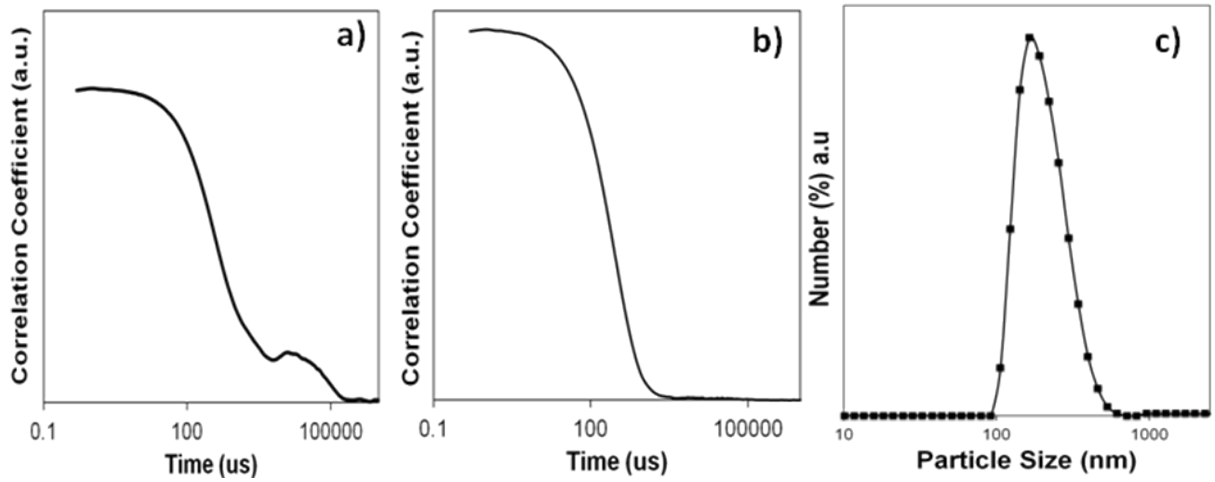
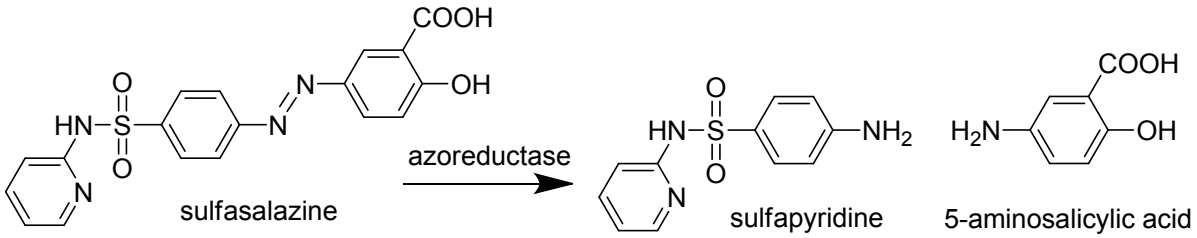


Fig S7. Correlograms of MSN-NH_2 (a) and MSN-NH_2@SSPI (b) obtained by dynamic light scattering in PBS at pH = 7.4 and corresponding particle size of MSN-NH_2-SPI (c). As hypothesised MSN-NH_2 are not stable in PBS due to hydrogen bonding between amine and silanol groups supported by correlogram (Fig S7a). Conversely, MSN-NH_2@SSPI showed good correlation coefficient and a single sharp peak in DLS and a low poly dispersity index (PDI) of 0.2 was observed in PBS (Figs S7b,c). Furthermore, zeta potential of MSN-NH is +10 mV suggesting electro kinetic instability in PBS while, zeta potential of MSN-NH_2@SSPI was found to be -22 mV confirming adequate colloidal stability.



Reaction S1: Reduction of sulfasalazine (SZ) into sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA) in presence of azoreductase

Table S1: Porosity data obtained from N_2 physisorption

Sample	BET (m^2g^{-1})	Pore Vol. (cm^3g^{-1})	BJH Pore size (nm)
MCM-48	1505	0.99	2.0
MSN-NH_2	829	0.51	1.4
MSN-NH_2-SZ	260	0.22	1.4
MSN-NH_2-SZ@SSPI	120	-	-

Table S2: Mathematical modelling to describe the release kinetics

	Regression Coefficient R^2	
Sample	**Higuchi**	**Korsmeyer and peppas**
MCM-48-SZ	0.94	0.84
MSN-NH2-SZ	0.84	0.72
MSN-NH2-SZ@SSPI-E	0.92	0.97

We also studied the mechanism of release from protein coated and uncoated nanoparticles. The in-vitro release data (Fig 3) were fitted to various kinetic models, e.g, Higuchi's model (cumulative % drug release vs. square root of time) or Korsmeyer-Peppas plot (log of cumulative % drug release vs. log time).[6] R^2 (coefficient of correlation) values were calculated for the linear curve obtained by regression analysis of the above plots. From the R^2 values listed in Table S2, is apparent that the drug release from MCM-48-NH_2 and MSN-NH_2-SZ mainly follows a model based on diffusion (Higuchi model) from the porous nanoparticles, while SSPI coated nanoparticles MSN-NH_2-SZ@SSPI show a combination of "erosion"-controlled (due to protein degradation in presence of pancreatine enzyme) and diffusion-controlled release of SZ from the nanoparticles, which further corroborate successful grafting of the protein on the particle surface. Further studies to delineate full kinetics are underway and are out of scope of this manuscript.

References:

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