Supporting Information

**Encapsulation of polyprodrugs enables an efficient and controlled release of dexamethasone**

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**EXPERIMENTAL SECTION**

**Materials.** Dexamethasone sodium phosphate (DXM, >98%), acetic acid (98%), adipic dihydrazide (ADH) (>98%), and fluorescein isothiocyanate (FITC) (99%) were purchased from Sigma Aldrich. Tetraethoxysilane (TEOS, 98%), cetyltrimethylammonium chloride (CTMA-Cl, 99%), ethanol (99%), cyclohexane (>99%), ammonium hydroxide (30% in water), and 3-aminopropyltrimethoxysilane (APTES, >98%) were purchased from Alfa Aesar. Lutensol AT50 (LUT, 99%) was supplied by BASF. The oil soluble surfactant poly((ethylene-co-butylene)-b-(ethylene oxide)), P(E/B-b-EO), consisting of a poly(ethylene-co-butylene) block ($M_w = 3,700 \text{ g mol}^{-1}$) and a poly(ethylene oxide) block ($M_w = 3,600 \text{ g mol}^{-1}$) was synthesized starting from $\omega$-hydroxypoly-(ethylene-co-butylene), which was dissolved in toluene after addition of ethylene oxide via anionic polymerization.[20] All other chemicals were purchased from Sigma-Aldrich and used as received.
**Synthesis of PDXM.** ADH (174 mg, 1 mmol) and DXM (393 mg, 1 mmol) were first dissolved in water (5 mL). Acetic acid (0.5 mL) was added to the solution. The mixture was purged with argon and stirred at 50 °C for different reaction times (12 h, 24 h, 48 h, 72 h, 96 h, and 144 h). The products were purified by dialyzing the reaction mixture in 20 mL of milli-Q water. Dialysis tube with a molecular weight cutoff of 1 kDa was used to remove unreacted DXM and ADH from the mixture. The dialysis medium was changed with fresh media every 12 h until no UV absorption of DXM was detected in the supernatant. PDXM powder was then obtained by freeze-drying the dialyzed solutions. In this study, PDXM with four different molecular weights, namely PDXM2k, PDXM7k, PDXM50k, PDXM100k, were obtained by changing the reaction time.

**Release of DXM from PDXM at Different pH Values.** PDXM powder (10 mg) was first dissolved in 2 mL phosphate buffered saline (PBS, 0.01 M, pH 7.4). The solution was subsequently sealed in a dialysis bag with a molecular weight cut-off of 1 kDa. The dialysis bag was immersed in 20 mL PBS at room temperature in a shaking culture incubator. 2 mL of supernatant was taken from the incubation medium at given intervals and equal volume of fresh PBS was added to keep the volume constant. The same procedure was performed for the degradation of PDXM in acetate buffer solution at pH 5.4. The release of cleaved DXM in the dialysis medium was quantified by measuring its characteristic absorbance at \( \lambda = 240 \) nm using UV-Vis spectroscopy.

**Encapsulation of DXM/PDXM in SiO\(_2\) NCs.** The SiO\(_2\) NCs loaded with DXM or PDXM were synthesized in a water-in-oil miniemulsion. Firstly, 18 mg of DXM or 20 mg of PDXM with different molecular weights and 20 µL of 25 wt% CTMA-Cl aqueous solution were dissolved in 0.5 g of milli-Q water. Ammonium hydroxide was added to the mixture to adjust the pH value of the aqueous phase to pH 12. In parallel, 35.7 mg of P(E/B-b-EO) were dissolved in 7.5 g of cyclohexane and the mixture was added to the aqueous phase under stirring at 500
rpm for 1 h at room temperature. The emulsion was then sonicated under ice cooling for 180 s at 70% amplitude in a pulse regime (20 s sonication, 10 s pause) using a Branson 450 W sonifier and a 1/2″ tip. A cyclohexane solution containing 10.7 mg of P(E/B-b-EO) and 250 µL TEOS in 5 g of cyclohexane was then added dropwise over a period of 20 min to the miniemulsion. The mixture was stirred at 25 °C for 24 h.

For fluorescent labeling of the SiO2 NCs, 0.25 mg of Cy5-NHS ester was dissolved in 1 mL of anhydrous ethanol and then 0.16 mg of APTES was added. The mixture was stirred for 12 h in the dark at room temperature. Afterwards, 800 µL of the mixture was added to 3 mL of the SiO2 NC dispersion and stirred for 12 h. The fluorescently labeled SiO2 NCs were purified by repetitive centrifugation (3 times for 20 min, RCF 1664) and redispersion in cyclohexane to remove the excessive amount of precursors and surfactant. For transferring the nanocapsules into aqueous media, 600 µL of SiO2 NCs dispersion in cyclohexane were added dropwise to 5 g of Lutensol AT50 aqueous solution (1 wt%) under mechanical stirring, and the samples were placed in an ultrasound bath for 3 min at 25 °C (25 kHz). Subsequently, the samples were stirred openly at 25 °C for 24 h to evaporate cyclohexane.

**Encapsulation Efficiency of DXM/PDXM in SiO2 NCs.** After their transfer to the aqueous media, the SiO2 NCs were washed 5 times with PBS (pH 7.4) by centrifugation to remove the non-encapsulated drug. At each purification step, the supernatant was collected and its pH was adjusted to pH 5.4 by adding an aqueous solution of 0.1 M HCl. The mixture was incubated at 37 °C for 24 h to depolymerize PDXM. The concentration of DXM was then determined by UV-Vis spectroscopy. Encapsulation efficiency was calculated as the remaining percentage after purification.

**Release of DXM from SiO2 NCs.** Release of DXM from SiO2 NCs was performed by using dialysis membranes with a molecular weight cut-off of 14 kDa. 2 mL of dispersion of SiO2 NCs
encapsulating DXM or PDXM with different molecular weights were placed in a dialysis bag that was immersed in 20 mL PBS (pH 7.4) or acetate buffer solutions (pH 5.4) at 37 °C. 2 mL of the supernatants were taken from the incubation media at given intervals and an equal volume of fresh buffer solution was added to keep the volume constant. The pH-responsive release of DXM from SiO2 NCs was expressed as the cumulative ratio of released drug to encapsulated drug. The drug release experiments were performed in triplicates for each sample. To investigate the release mechanism of DXM from nanocapsules, the release profiles were fitted with different mathematical models including zero order, first order, and Higuchi kinetics by using DDsolver, a menu-driven add-in program for Microsoft Excel written in Visual Basic.[21]

**Analytical Tools.** 1H NMR spectra were measured at 300 MHz on a Bruker Avance 300 Spectrometer with deuterated water as solvent. 1H NMR spectra were processed with the MestReNova 9.0.1-13254 software. The 1H kinetics (200 experiments, D1 of 10s, sweep width 17000 Hz), COSY (17000 Hz, 90° pulse 9ms, 298K), NOESY, 1H,13C-HSQC (1J - (1H-13C) 145 Hz) and the DOSY (Diffusion Ordered Spectroscopy) 1 experiments with water suppression (stebppp1s19) were executed with a 5 mm TXI 1H/13C/15N z-gradient probe and a gradient strength of 5.516 [G/mm] on the 850 MHz spectrometer. The gradient strength was calibrated with the diffusion coefficient of a sample of 2H2O/1H2O at a defined temperature of 298K and compared with the literature. The gradient strength was varied in 32 steps from 2 % to 100 % and for each gradient 64 number of scans was used. The diffusion time d20 was optimized to 50 ms and the gradient length p30 was kept at 1.4 ms. The temperature was defined with a standard 1H methanol NMR sample. Controlling the temperature was realized with a VTU (variable temperature unit) and an accuracy of +/- 0,1K, which was checked with the standard Bruker Topspin 3.6 software. For the 1H NMR measurement of the PDXM7k in acidic solution, 10 mg PDXM7k powder was dissolved in acetate buffer solution at pH 5.4 and incubated at 37 °C for 48 h. The solution was then freeze-dried and the obtained powder was dissolved in
deuterated water (99.9 atom % D). For the kinetics study of degradation, the PDXM7k polymer was acidified by adding hydrochloride solution to pH below 5.5 and the mixture was subjected to the NMR immediately. The degradation process was monitored for 20 h.

Gel permeation chromatography (GPC) measurements were performed to determine the apparent molecular weight of the synthesized polymer prodrugs and their molar weight distribution. The polymers were first dissolved in water to reach a concentration of 5 mg·mL⁻¹ and were then filtered through a 0.45 μm Teflon filter. The measurements were performed on a Waters 515 pump with a refractive index detector (ERC RI 101). Three columns (0.8 × 30 cm, 10 μm) with different porosities (10⁶, 10⁴ and 500 Å) from SDV (PSS, Germany) were used at room temperature. The elution rate of water was 1.0 mL·min⁻¹. The resulting apparent molecular weights were calculated using poly(ethylene oxide) with narrow molecular weights as standards. The UV-Vis absorption spectra of DXM and PDXM were obtained using a UV-Vis spectrometer (Lambda 16, Perkin Elmer). The calibration curve of DXM in water is displayed in Figure S15. The average size and size distribution of SiO₂ NCs were measured by dynamic light scattering (DLS) at 25 °C on a Nicomp 380 submicron particle sizer (Nicomp Particle Sizing Systems, USA) at a fixed scattering angle of 90°. For the determination of the size of PDXM, dynamic light scattering measurements were performed on an ALV spectrometer equipped with a thermostat and consisting of a goniometer and an ALV-5004 multiple-tau full-digital correlator (320 channels). A He-Ne Laser operating at a laser wavelength of 632.8 nm was used as light source. Measurements were performed at 20 °C at different angles ranging from 30° to 150°. PDXM were dissolved at a concentration of 2 mg mL⁻¹ were used for the measurement. The aggregation behavior of the SiO₂ NC-PDXM in blood plasma was studied by multi-angle DLS using a method reported previously.[¹³] Before the measurements, PBS or undiluted human plasma was filtered through Millex-LCR filters (Merck Millipore, Billerica, USA) with 450 nm pore size into quartz cuvettes with an inner
radius of 9 mm for light scattering from Hellma (Müllheim, Germany). 10 μL of SiO₂ NC-PDXM100k dispersion (solid content: 0.2 wt%) was unfilteredly pipetted in a light scattering cuvette containing 200 μL undiluted human plasma. The mixture was then diluted with filtered PBS up to 1 mL total sample volume in the light scattering cuvette. Prior to use, the quartz cuvettes were cleaned with acetone using a Thurmond apparatus. For DLS analysis of the SiO₂ NCs alone, 10 μL of the SiO₂ NC-PDXM100k solution (0.2 wt%) was added (without filtration) in 990 μL of filtered PBS. Plasma alone was prepared by adding 800 μL of PBS to 200 μL undiluted human plasma. After mixing, the samples were incubated for 20 min on a shaker at room temperature (20 °C) prior to the measurement. DLS measurements were performed at 20 °C. For data analysis, a robust multicomponent fit method was used as previously reported.[13]

The morphology of nanocapsules was characterized with a JEOL 1400 (JEOL Ltd., Tokyo, Japan) transmission electron microscope (TEM) operating at an accelerating voltage of 120 kV. Typically, the samples were prepared by diluting the dispersions in demineralized water to obtain a solid content of ~ 0.01 wt%. One drop of diluted dispersion was placed on 300 mesh carbon-coated copper grids and left to dry overnight at room temperature. SEM measurements were performed with a Gemini 1530 (Carl Zeiss AG, Oberkochen, Germany) field emission scanning electron microscope at an accelerating voltage of 170 V. Samples were prepared by diluting dispersions in demineralized water to obtain a solid content of ~ 0.01 wt%. One drop of diluted dispersion was deposited on silica wafers and left to dry. Nitrogen adsorption–desorption measurements were carried out on a Quantachrome Autosorb-1 analyzer (Boynton Beach, FL) at 77.3 K. The capsule dispersions were freeze-dried for 48 h and degassed at 70 °C for 12 h under high vacuum before measurements. The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) equation based on adsorption data points in the \( P/P_0 \) range of 0 < \( P/P_0 \) < 0.25. Pore size distributions were estimated from adsorption branches of the isotherms using the Barrett-Joyner-Halenda (BJH) method. Prior to HPLC analysis (Agilent
Technologies 1200 Series) at 20 °C, samples were filtered gently through a 0.45 μm filter. DXM was detected with a photodiode array detector at 240 nm. An Agilent Eclipse plus C18 column was used with a flow rate of 1 mL min⁻¹. The gradient was composed of acetonitrile/water with 0.1% trifluoroacetic acid, starting with 20%/80% and reaching 100% acetonitrile after 10 min. Sample (PDXM100k@pH=5.4) was prepared by incubating PDXM100k in acetate buffer solution at pH 5.4 sealed in a dialysis bag with a molecular weight cut-off of 14 kDa for 48 h. The dialysis medium was taken for HPLC measurements. DXM-hexanohydrazide conjugate was synthesized as follows. DXM (1.55 g, 3 mmol) and hexanohydrazide (26.0 mg, 2 mmol) were first dissolved in milli-Q water (25 mL). Acetic acid (2.5 mL) was added to the solution. The mixture was purged with argon and stirred at 50 °C for 96 h. The mixture (DXM-hexanohydrazide and unreacted DXM) powder was then obtained by freeze-drying the obtained solution and the product was measured by HPLC.

Mice. For the in vitro study, six to eight-week old female C57BL/6J mice were obtained from Janvier (Le Genest-Saint-Isle, France). For the in vivo study, 15 weeks old BALB/c mice were obtained from Charles River (Boston, USA). All mice were kept under a 12 h dark, 12 h light cycle (with food and water supply ad libitum) in the animal facility of the Translational Animal Research Center, University Medical Center Mainz, Germany. The animals were treated in accordance with NIH publications entitled “Principles for Use of Animals” and “Guide for the Care and Use of Laboratory Animals”. All protocols have been approved by the local Animal Care and Use Committee (“Landesuntersuchungsamt Rheinland-Pfalz”); Reference: 23 177-07/G 17-1-0068.

Human Plasma. Human citrate plasma was obtained from the Department of Transfusion Medicine Mainz from healthy donors in accordance with the Declaration of Helsinki. All experiments were performed in compliance with the relevant laws and institutional guidelines. The institutional ethics committee approved the study (Landesärztekammer Rheinland-Pfalz,
Written informed consent was obtained for any experimentation with materials from human subjects. A plasma pool from ten volunteers was prepared and stored at -80 °C.

**Protein Corona Analysis.** SiO₂ NCs (1 mg) were incubated with 1 mL of human citrate plasma 1 h at 37 °C under constant agitation. Hard corona-coated nanocapsules were isolated via centrifugation and washing as previously described.[22] To detach the corona proteins from the nanocapsules’ surface, the pellet was incubated with 2% sodium dodecyl sulfate (SDS) and in Tris-HCl (62.5 mM) for 5 min at 95 °C. The dispersion was centrifuged (20,000 g, 1 h, 4 °C) and the supernatant containing hard corona proteins was recovered. Subsequently, the isolated proteins were analyzed by proteomics.

**Liquid Chromatography Coupled to Mass Spectrometry (LC-MS).** For proteomic analysis, SDS was removed from the protein sample via Pierce Detergent Removal Spin Columns. Digestion of corona proteins was performed as described in our previous reports.[23] Finally, isolated peptides were diluted with 0.1% formic acid spiked with 50 fmol µL⁻¹ Hi3 Ecoli (Waters) for absolute protein quantification by LC-MS. Measurements were performed on a nanoACQUITY UPLC system coupled to a Synapt G2- Si mass spectrometer. Data was analyzed with the MassLynx 4.1 software and Progenesis QI (2.0). A reviewed human database was downloaded from Uniprot for protein identification.

**Drug Release Experiments with Protein Corona.** 2 mL of nanocapsule dispersions incubated with human citrate plasma or PBS were placed in a dialysis bag with molecular weight cut-off of 1 kDa. The dialysis bag was immersed in 20 mL acetate buffer solutions (pH 5.4) at 37 °C. 2 mL of the supernatants was taken from the incubation media at given intervals and an equal volume of fresh buffer solution was added to keep the volume constant. The release of cleaved DXM in the dialysis medium was quantified by measuring its characteristic
absorbance at $\lambda = 240$ nm using UV-Vis spectroscopy. The drug release experiments were performed in triplicates for each sample.

**In vivo Fluorescence Imaging (IVIS).** 15 weeks old BALB/c mice were anesthetized with isoflurane. Cy5-NHS ester-conjugated SiO$_2$ NC (final concentration of 0.5 wt%) were administered intravenously with 22 mg/kg body weight (approx. 500 µg) using a tail vein catheter system. The Cy5-NHS ester control was diluted with PBS to achieve the similar fluorescence intensity as the Cy5-SiO$_2$ NC solution. A standard was generated by using the SPARK Multimode Microplate Reader (TECAN, Männedorf, Switzerland).

The animals were measured using the IVIS SpectrumCT (Perkin Elmer, Waltham, USA) for epifluorescence of Cy5 (settings: Excitation filter: 640 nm; Emission filter: 700 nm; Exposure time: 7 s). Mice were sacrificed by cardiac puncture after 120 minutes.

The dissected organs were analyzed using the IVIS SpectrumCT (Excitation filter: 640 nm; Emission filter: 700 nm, Exposure time: 2 s). Rectangular regions of interest were placed around each organ for obtaining the radiant efficiency using the living image software (Perkin Elmer).

**Flow cytometer staining.** One third of the liver was prepared for flow cytometric analyses using the liver dissociation kit (Miltenyi Biotec). One third of the spleen was dissociated by grinding through a 40 µm cell strainer. After red blood cell lysis and washing, $7 \times 10^6$ liver cells and $5 \times 10^6$ spleen cells were transferred into tubes for the following staining procedure. Cells were washed with PBS and stained with Fixable Viability Dye (FVD) eFluor 506 (ThermoFisher; 1:2,500) for 20 minutes at 4 °C. After washing, cells were incubated with Fc-block (anti-CD16/32; clone 2.4G2) 1:50 in buffer (PBS, 1% FBS, 2mM EDTA). Cells were incubated with antibodies using the concentrations indicated in Table SS1) in a total volume of 100 µl per tube for 45 min on the shaker at 4 °C. After washing, cells were fixed with “FACS-Fix” (PBS, 0.7% paraformaldehyde, 2 mM EDTA).
Table SS1. The table lists the antibodies used for flow cytometric analyses (antigens, clone, fluorophore, the applied concentration, and supplier).

Liver

| antigen | clone | fluorophore      | applied concentration (µg/ml) | purchased from |
|---------|-------|------------------|-------------------------------|----------------|
| CD3     | REA641| PE               | 0.225                         | Milteny        |
| CD49b   | DX5   | PE               | 0.225                         | Milteny        |
| Ly6G    | 1A8   | PE               | 0.225                         | Miltenyi Biotec|
| Ly6C    | HK1.4 | PerCP-Cyanine5.5 | 0.5                           | ThermoFisher   |
| CD163   | TNKUPJ| Super Bright 702  | 0.5                           | ThermoFisher   |
| CD11c   | N418  | PE-eFluor 610    | 0.5                           | ThermoFisher   |
| CD68    | FA-11 | PE-Cyanine7      | 0.5                           | ThermoFisher   |

Spleen

| antigen | clone  | fluorophore     | applied concentration (µg/ml) | purchased from |
|---------|--------|-----------------|-------------------------------|----------------|
| CD3     | 17A2   | FITC            | 1.25                          | ThermoFisher   |
| CD49b   | HMa2   | FITC            | 1.25                          | ThermoFisher   |
| Ly6G    | Rea526 | FITC            | 0.225                         | Miltenyi Biotec|
| Ly6C    | HK1.4  | PerCP-Cyanine5.5| 0.5                           | ThermoFisher   |
| F4/80   | BM8    | eFluor 450      | 0.5                           | ThermoFisher   |
| CD68    | FA-11  | PE-Cyanine7     | 0.5                           | ThermoFisher   |
| CD11b   | M1/70  | Super Bright 600| 0.7                           | ThermoFisher   |
| CD11c   | N418   | PE-eFluor 610   | 0.5                           | ThermoFisher   |

Flow Cytometric Analyses. Cell samples were analyzed under a flow speed of 500 µl/min with a FSC/SSC of 210/310 using the Attune Nxt Flowcytometer (ThermoFisher). Fluorescence
Compensations were calculated using compensation beads (ThermoFisher). Populations were defined as described in Table SS2 and S11/S12. The median fluorescence intensity of Cy5 and the frequency of Cy5 positive gated cells of each population of interest were exported as csv-file and evaluated using Excel (Microsoft) and Rstudio.

**Table SS2.** The table describes the populations of liver and spleen investigated by flow cytometry.

| liver      | Abb. | Population          | Combination of flow cytometry markers |
|------------|------|---------------------|---------------------------------------|
|            |      | lineage             | CD3 +, Ly6G +, CD49b +                |
|            | KC   | Kupffer cells       | lineage -, CD11c -, CD163 +           |
|            | Mono | monocytes           | lineage -, CD11c -, CD163 -, Ly6C +    |
|            | MO   | macrophages         | lineage -, CD11c -, CD163 -, Ly6C -, CD68 + |
|            | DC   | dendritic cells     | lineage -, CD11c +                     |

| spleen     | Abb. | Population          | Combination of flow cytometry markers |
|------------|------|---------------------|---------------------------------------|
|            |      | lineage             | CD3 +, Ly6G +, CD49b +                |
|            | MZM  | marginal zone       | lineage -, CD11c -, CD11b +, SSC^low, Ly6C -, F4/80 -, CD209b + |
|            | RPM  | red pulp macrophages| lineage -, CD11c -, CD11b +, SSC^low, Ly6C -, CD68 +, F4/80 + |
|            | WPM  | white pulp macrophages| lineage -, CD11c -, CD11b -, SSC^low, Ly6C -, CD68 +, F4/80 - |
|            | Mono | monocytes           | lineage -, CD11c -, CD11b +, Ly6C +    |
|            | DC   | dendritic cells     | lineage -, CD11c +                     |

**Immunofluorescence.** 10 µm thick cryosections were dried for 20 minutes on glass slides and fixed with 4% paraformaldehyde in PBS for 20 minutes at RT. Slides were washed twice with 0.05% Triton X-100 in PBS and once with 30 mM glycine + 0.05% Triton X-100 in PBS.
Sections were blocked with blocking buffer (PBS, 0.05% Triton X-100, 2.5% goat serum, 5% bovine serum albumin (BSA)) for 1 h at RT. Primary antibody (Rat anti-CD68, FA-11, ThermoFisher) was added and incubated overnight at 4 °C, at a concentration of 1 µg mL⁻¹ in staining buffer (PBS, 0.05% Triton X-100, 2.5% BSA, and 1.24% goat serum). Antibodies were washed off thoroughly and the secondary antibody (Goat anti-Rat Alexa Fluor 488, ThermoFisher) was added and incubated for 2 h at a concentration of 0.2 µg mL⁻¹ in staining buffer. After washing, sections were mounted with Prolong Gold Antifade Mountant with Dapi (ThermoFisher).

Images were acquired using the confocal laser scanning microscope LSM510 (Zeiss) using pinhole width of one airy unit. Laser Power and digital gain were adjusted on the maximum intensity of a Cy5-SiO₂ NC-treated liver section using the range indicator. The images were then further processed in Fiji ImageJ.

**Isolation of Non-Parenchymal Liver Cells and Stimulation with PDXM-Containing SiO₂ NCs for Functional Experiments.** For the functional experiments (Figure 7), murine non-parenchymal liver cells (NPCs) were isolated from livers as described previously.[24] Briefly, mice were anesthetized with Ketamin/Xylazin and livers were perfused with 20 mL Ca²⁺- and Mg²⁺-free Hank’s Balanced Salt Solution (HBSS, Sigma) containing 100 UꞏL⁻¹ collagenase A (Roche Diagnostics GmbH, Mannheim, Germany), 5% heat-inactivated fetal calf serum (FCS, GE Healthcare), and 10 µg·mL⁻¹ DNase I (AppliChem). Following dissection, the livers were incubated for 15 min at 37 °C and subsequently grinded through a 70 µm cell strainer to generate single cell suspensions. Hepatocytes were pelleted and discarded after centrifugation for 15 min at 4 °C and 30 x g. The non-parenchymal cell fraction remaining in the supernatant was further purified by centrifugation at 300 x g, resuspended in Histodenz solution in HBSS to reach a final concentration of 20% and overlaid with HBSS, followed by centrifugation at 1500 x g for 20 min. NPCs were collected at the Histodenz/HBSS interface.
and washed with RPMI 1640 medium containing 5% FCS and 1% penicillin/streptomycin. Cell suspensions were then cultured in HEPES-buffered RPMI 1640 medium containing 10% FCS, 1% penicillin/streptomycin, 1 mM l-glutamine, 1% essential and non-essential amino acids, 1 mM sodium pyruvate, 50 nM β-mercaptoethanol, and with or without 2.5 µg·mL⁻¹ lipopolysaccharide (LPS) at a concentration of 10⁶ NPCs·mL⁻¹. Different formulations of SiO₂ NCs containing DXM or PDXM, or free DXM or PDXM were added at different concentrations and incubated for 24 h.

**Analysis of Cytotoxicity and SiO₂ NC-induced Cytokine Secretion by NPCs.** Toxicity of SiO₂ NC formulations by NPCs was analyzed by propidium iodide (PI; BD Pharmingen) staining (5 µl per sample) and subsequent flow cytometric quantification (BD LSR II) of PI positive cells. Interleukin-6 (IL-6) levels in cell culture supernatants were analyzed using an enzyme-linked immunosorbent assay kit (ThermoFisher) according to manufacturer’s instructions.
Results

Table S1. Number-average molecular weight ($M_n$), weight-average molecular weight ($M_w$), and molecular weight distribution (MWD) of PDXM obtained at various temperatures, reaction times, and concentrations of catalyst. For gel permeation chromatography (GPC) measurements, the PDXM polymers were dissolved in water to reach a concentration of 5 mg·mL$^{-1}$ and the solutions were filtered through a 0.45 μm Teflon filter before measurement.

| Entry   | Temperature [°C] | Reaction time [h] | Catalyst [mmol/mL] | $M_n$ [g/mol] | $M_w$ [g/mol] | MWD |
|---------|-----------------|-------------------|--------------------|---------------|---------------|-----|
| PDXM2k  | 50              | 12                | 1.59               | 2,000         | 3,100         | 1.50|
| PDXM7k  | 50              | 24                | 1.59               | 7,100         | 10,000        | 1.43|
| PDXM50k | 50              | 36                | 1.59               | 50,900        | 117,100       | 1.53|
| PDXM100k| 50              | 72                | 1.59               | 101,100       | 193,800       | 2.32|
| PDXM130k| 50              | 96                | 1.59               | 133,400       | 170,800       | 1.28|
| PDXM*   | 50              | 144               | 1.59               | Precipitation |               |     |
| PDXM10k | 50              | 72                | 0.43               | 10,300        | 14,200        | 1.37|
| PDXM40k | 50              | 72                | 0.83               | 36,400        | 49,700        | 1.37|
| PDXM120k| 70              | 72                | 1.59               | 122100        | 151400        | 1.24|
Figure S1. (a) Number average molecular weight of PDXM synthesized with varied reaction times at 50 °C with 1.59 mmol mL⁻¹ catalyst; (b) Number average molecular weight of PDXM synthesized at varied temperatures by keeping reaction time of 72 h and catalyst concentration at 1.59 mmol mL⁻¹; (c) Number average molecular weight of PDXM synthesized with varied amounts of catalyst with temperature kept at 50 °C and reaction time of 72 h.
Figure S2. Multiangle DLS results of (a) PDXM7k, (b) PDXM50k, and (c) PDXM100k measured at 20 °C at angles ranging from 30° to 150°.

Figure S3. Encapsulation efficiency of DXM/PDXM in SiO₂ NCs after different steps of purification. The lines represent a visual guide.
Table S2 Hydrodynamic diameters of SiO$_2$ NCs containing DXM and PDXM with different $M_w$.

| Entry                | Diameter (PDI) (nm) |   |   |
|----------------------|---------------------|---|---|
|                      | In cyclohexane      | In water |
| SiO$_2$ NC-DXM       | 342 (0.32)          | 357 (0.40) |
| SiO$_2$ NC-PDXM2k    | 317 (0.41)          | 331 (0.36) |
| SiO$_2$ NC-PDXM7k    | 341 (0.44)          | 329 (0.33) |
| SiO$_2$ NC-PDXM50k   | 350 (0.39)          | 325 (0.50) |
| SiO$_2$ NC-PDXM100k  | 303 (0.50)          | 338 (0.41) |

Table S3 First order kinetics for the release of DXM from SiO$_2$ NCs at different pH values.

In this one-order kinetic equation of $M_t = M_\infty (1 - e^{-kt})$, $M_t$ is the DXM concentration at time $t$, $M_\infty$ is the final DXM concentration outside the dialysis membrane and $k$ is the release rate.

| Entry                | First-order: $M_t = M_\infty (1 - e^{-kt})$ | $R^2_{\text{adjusted}}$ | $t_{1/2}$ [min] |
|----------------------|-----------------------------------------------|--------------------------|-----------------|
| SiO$_2$ NC-DXM (pH=7.4) | 99.09±0.86 0.007±0.0004 | 0.987 | 86±5 |
| SiO$_2$ NC-DXM (pH=5.4) | 98.93±0.89 0.007±0.0007 | 0.985 | 90±9 |
| SiO$_2$ NC-PDXM2k (pH=5.4) | 98.81±1.49 0.005±0.0005 | 0.986 | 132±13 |
| SiO$_2$ NC-PDXM7k (pH=5.4) | 97.54±0.81 0.004±0.0005 | 0.992 | 158±20 |
| SiO$_2$ NC-PDXM50k (pH=5.4) | 97.18±0.81 0.003±0.0004 | 0.994 | 212±30 |
| SiO$_2$ NC-PDXM100k (pH=5.4) | 97.96±1.08 0.002±0.0003 | 0.989 | 247±41 |
Figure S4. The release rate of DXM as a function of its concentration in the release medium; $\alpha$ is the slope of linear function.

Figure S5. Release profiles of DXM from PDXM and SiO$_2$ NC-PDXM (at pH 5.4 and 7.4, 37°C, mean ± SD, $n = 3$).
Figure S6. Dynamic light scattering analysis of SiO₂ NC-PDXM100k incubated in 100% citrate plasma. Upper graphs: exemplary autocorrelation functions $g_1(t)$ (black dots) of the mixture of plasma/NCs at a scattering angle of (a) 30°, (b) 60°, and (c) 90°. Temperature for the measurement was 20 °C. The blue line represents the fit of the sum of the individual components with an additional aggregation function. Lower graphs: corresponding residuals resulting from the difference between the data and the two fits.

Figure S7. a) Hard corona proteins were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS) and classified into seven different groups depending on their biological
functions. b) List of the 20 most abundant corona proteins for both nanocapsule types and their relative amounts. For both nanocapsules, fibrinogen was the most abundant protein (~ 60%) followed by apolipoprotein A-I (~ 10%). In literature, both proteins have also been identified in the protein corona of silica nanoparticles\cite{25} and other nanomaterials.\cite{14, 26}

![Graph](image-url)

**Figure S8.** Release profiles of DXM from SiO$_2$ NC-PDXM7k and SiO$_2$ NC-PDXM100k with and without protein corona (at 37 °C, at pH = 5.4, mean ± SD, n = 3).

**Table S4.** Characteristics of the Cy5-labelled SiO$_2$ NCs for *in vivo* biodistribution and cellular uptake studies.

| Entry                                | Value  |
|--------------------------------------|--------|
| Hydrodynamic diameter by DLS (nm)    | 370    |
| PDI of size distribution            | 0.19   |
| Zeta potential (mV)                  | + 8.7  |
| Solid content of NCs in dispersion (wt%) | 1.0    |
| Cy5 concentration in dispersion (mg mL$^{-1}$) | 0.01   |
Figure S9. CLSM images of Cy5-SiO2 NCs and CD68 staining on liver sections. Mice were sacrificed 2 h after NC injection and liver sections were generated. Two magnifications of liver sections of a Cy5-SiO2 NC-treated animal as well as a PBS-treated one are shown ((a) 63x oil, (b, c) 10x, at separate spots). Co-localization of Cy5-SiO2 NC (red) and CD68 (green) appears in yellow on the merged image. (c) The unspecific binding of the secondary antibody was evaluated in the control staining.
Figure S10. Flow cytometric analysis of liver cells. Mice were sacrificed 2 h after NC injection and non-parenchymal liver cells were isolated for flow cytometric analyses. (a) Dead cells were excluded by live/dead staining. Doublets were excluded upon a high FSC-W. For all following gates, the region of interest was chosen according to the FMO (fluorescence minus one) control. Lineage negative cells were gated for CD11c and defined as dendritic cells (DCs). The CD11c negative population was gated for CD163, which is a marker expressed on Kupffer cells (KC).\textsuperscript{116} The negative population was gated for Ly6C, a marker for monocytes (Mono). The pan-macrophage marker CD68 was used to determine a further macrophage population (MO). (b) Cy5-SiO\textsubscript{2} NC uptake into NPC population. The histogram represents the distribution of Cy5 intensity of the defined cell populations (in (a)) for a representative Cy5-SiO\textsubscript{2} NC treated (red) and PBS treated mouse (black).
Figure S11. Flow cytometric analysis of splenocytes. Mice were sacrificed 2 h after NC injection and splenocytes were isolated for flow cytometric analyses. (a) Gating scheme. Dead cells were excluded by live/dead staining. For following gates, the region of interest was adapted to the FMO (fluorescence minus one) control. Doublets were excluded upon a high FSC-W. CD11c high expressing cells were defined as the dendritic cells (DC) population.
CD11c low/negative cells were separated into CD11b positive and CD11b negative cells. CD11b positive cells were gated for Ly6C, determining a monocyte (Mono) population. The Ly6C negative cells, were gated for co-expression of CD68 and F4/80 and classified as red pulp macrophages (RPM). The CD68 negative/F4/80 positive population was gated for expression of CD209b and assigned as marginal zone macrophages (MZM). In order to obtain the population of white pulp macrophages (WPM), the CD11b negative population was gated for Ly6C negative and following gated for CD68 positive and F4/80 negative cells. The gating strategy is based on Borges da Silva et al. and Shawn Rose et al.\textsuperscript{[27]} (b) Cy5-SiO\textsubscript{2} NC uptake in spleen cell populations. The histogram represents the distribution of Cy5 intensity of the defined cell populations (in (a)) for a representative Cy5-SiO\textsubscript{2} NC-treated (red) and PBS-treated mouse (black).

Figure S12. Confocal microscopy of Cy5-SiO\textsubscript{2} NC and CD68 staining on spleen sections. Mice were sacrificed 2 h after NC injection and spleen sections were generated. Depicted is a confocal image (10x) of a representative section of the spleen of a Cy5-SiO\textsubscript{2} NC-treated and PBS-treated animal, stained for CD68. The images show: CD68 (green, left column), Cy5-SiO\textsubscript{2} NC (red, middle column) and the overlaid channels (right column). Using the same laser settings for the Cy5 channel as for the liver staining (Figure S10), the distribution of Cy5-SiO\textsubscript{2} NC in the spleen is representatively depicted and appears significantly lower compared to the liver section (Figure S10).
Figure S13. In vivo biodistribution of Cy5-NHS ester and Cy5-SiO2 NCs in organs and cell populations. Mice were sacrificed 2 h after NC injection and organs were dissected for ex vivo IVIS imaging. (a) Fluorescence images of organs after intravenous injection of Cy5-NHS ester (free dye, 9.7 µg/ml) and Cy5-SiO2 NCs. The same fluorescence intensity of Cy5 for the free and conjugated formulation was used. Parts of the figure are presented in figure 5b. (b) Radiant efficiency of organs from the mice sacrificed at 2 h post injection. Data are partly presented in Figure 5 for the conditions “PBS” and “Cy5-SiO2 NC”. $P < 0.05$ (*) (Kruskall-Wallis test). (c) Percentage of Cy5-NHS ester positive cells compared to the Cy5-SiO2 NC positive ones. The graph shows means ± SD of the frequency of Cy5 positive cells for the cell populations of interest (described in Figure 5e, S11 and S12). If the population included less than 50 cells, the
value was excluded. In each group 3 animals were analyzed. (d) Confocal microscopy of Cy5-NHS ester and CD68 staining of liver sections. For the acquisitions the same laser settings as for the liver sections (Figure 5d, S10) has been used.

Figure S14. Calibration curve for the determination of the concentration of DXM in water (at pH 7.4) by UV-spectroscopy.

Figure S15. Toxicity of PDXM-loaded SiO₂ NCs on non-parenchymal liver cells in vitro. NPCs were incubated with different concentrations of PDXM-SiO₂ NC formulations for 24 h and dead cells were subsequently stained with propidium iodide for 5 min followed by flow cytometric analyses to determine the frequency of dead cells.
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