Supplementary Information

Brush Conformation of Polyethylene Glycol Determines the Stealth Effect of Nanocarriers in the Low Protein Adsorption Regime

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Table of Contents

1. EXPERIMENTAL SECTION

1.1 Materials

Albumin from chicken egg white (lyophilized powder, ≥98%, Sigma Aldrich), sodium chloride (NaCl, ≥99.0%, Sigma Aldrich), cyanine 5-5'-CCA CTC CTT TCC AGA AAA CT (Cy5-oligo, 0.1 nmol μL^{-1} in PBS buffer, IBA Lifesciences), cyclohexane (HPLC grade, VWR), 2,4-toluene diisocyanate (TDI, 99%, Sigma Aldrich), sodium dodecyl sulfate (SDS, 99%, Alfa Aesar), and isocyanate-mPEG (PEG molecular weight = 2 kDa, 3.4 kDa, and 5 kDa, Nanocs, USA) were used as received. The surfactant poly((ethylene-*co*-butylene)-*block*-(ethylene oxide)), P((E/B)-*b*-EO), consisting of a poly(ethylene-*co*-butylene) block (NMR: M_n = 3900 g mol^{-1}) and a poly(ethylene oxide) block (NMR: M_n = 2700 g mol^{-1}) was synthesized according to a reported procedure.[1] Milli-Q water was used for the synthesis of nanocapsules.

1.2 Synthesis of Ovalbumin Nanocapsules (OVA and OVA-PEG NCs)

The OVA-NCs were synthesized in an inverse (water-in-oil) miniemulsion. First, the water phase was prepared by dissolving 50 mg ovalbumin and 7.2 mg NaCl in 0.4 g Milli-Q water, followed by addition of 100 μL Cy5-oligo solution. Separately, the continuous phase was prepared by dissolving 35.7 mg of P(E/B-*b*-EO) surfactant in 7.5 g cyclohexane. The continuous phase was poured to the aqueous phase under stirring at 500 rpm. The pre-emulsion was then homogenized by ultrasonication for 180 s (30 s ultrasonication, 10 s pause) with ice cooling at 70% amplitude using a Branson 450W sonifier and a 1/2' tip. Separately, 10.7 mg P((E/B)-*b*-EO) was dissolved in 5 g cyclohexane and 12 mg TDI was added to the solution. The TDI solution was added dropwise to the obtained miniemulsion in 5 min and the reaction was proceeded for 24 h at 25 °C. Afterwards, the excess of surfactant was removed from the dispersion by repetitive centrifugation and replacement of the supernatant with fresh cyclohexane.

For the PEGylation of OVA-NCs, 2.5 mL OVA-NC dispersion (as synthesized) was purified by centrifugation for 30 min at a RCF of 1664 and redispersed in anhydrous cyclohexane to remove excess surfactant. The dispersion was then sonicated for 30 s at 10% amplitude in a pulse regime (5 s sonication, 5 s pause) to hinder aggregation. Afterwards, 2.5 mL anhydrous cyclohexane was added to the dispersion under stirring at 500 rpm and 25 °C. Different amounts of monoisocyanate-mPEG (16 mg PEG for OVA-PEG2k$_{0.2}$, 32 mg PEG for OVA-

PEG2k$_{0.5}$, 128 mg PEG for OVA-PEG2k$_{2.5}$, 27 mg PEG for OVA-PEG3.4k$_{0.2}$, 54 mg PEG for OVA-PEG3.4k$_{0.8,}$ 40 mg PEG for OVA-PEG5k$_{0.2}$) were dissolved in 800 μL anhydrous acetone and the solution was added dropwise to the nanocapsule dispersion. The reaction was allowed to proceed for 4 h, followed by centrifugation at 3000 rpm for 30 min (Eppendorff centrifuge 5417C) to remove non-reacted PEG. The pellets were re-dispersed in 600 μL cyclohexane.

600 µL of dispersions in cyclohexane were added dropwise to 5 g SDS aqueous solution (0.1 wt%) under stirring and the samples were transferred to an ultrasound bath for 3 min at 25 °C (25 kHz). The samples were then stirred for 24 h at 25 °C to evaporate the cyclohexane in open vials. To further remove non-reacted PEG and excess of SDS, the dispersions were then purified by dialysis (MWCO of 100 kDa) and centrifugation (3×30 min, RCF 1664 g) using a centrifugal filter (MWCO = 100 kDa). The supernatant from the last centrifugation of OVA-PEG2k$_{2.5}$ dispersions was freeze-dried and kept for ^{1}H NMR spectroscopy measurements.

1.3 Quantification of PEG Chains Grafted on NCs

The PEGylated NC dispersions were freeze-dried and redispersed in D_2O at the concentration of 1 mg mL^{-1} for the ^{1}H NMR measurement. A coaxial set-up was used where an inner glass tube containing 5 wt% dichloromethane (DCM) in deuterated DCM was used as the reference (see Figure S6a).[2] The average number of PEG chains per NC and chains per nm^2 surface were calculated from ^{1}H NMR spectra. Integration of the resonance corresponding to ethylene glycol units (δ = 3.5~3.6 ppm) was compared with signal of external standard DCM at δ = 5.5 ppm to quantify the number of ethylene glycol units in the dispersion (Figure S6a). The number of NCs in the dispersion was calculated based on the solid content of the dispersion, hydrodynamic diameter of the NCs, density of ovalbumin, and mass ratio between ovalbumin and water in the NCs with the consideration that the OVA-NCs have a core-shell structure, which contains water in the core. Therefore, only the weight of shell materials and grafted PEG chains was measured during the determination of solid content of the dispersions by the freeze-drying method. A standard curve was plotted from the measurement of a series of NCO-PEG2k solution in D_2O with concentrations from 0.1 to 1 mg mL^{-1} for describing the relationship between PEG concentration and the ratio of the integral between the -CH_2CH_2- protons in PEG and the protons from DCM (Figure S6b). Detailed calculation of the chain number per NC and chain number per nm^2 surface are shown in **Section 2.1** in the Supporting Information.

1.4 NMR Relaxation Time Measurement

For the ^{1}H- (1D and 2D) measurements, a 5 mm QXI ^{1}H/^{13}C/^{15}N/^{19}F probe equipped with a z-gradient on the 700.02 MHz Bruker AVANCE III system was used. All samples were prepared in D_2O and referenced with the remaining HDO signal at 4.67 ppm ($\delta(^{1}H)$). For a quantitative ^{1}H NMR (700 MHz) measurements 64 transients were used with an 9.1 µs long 90° pulse and a 12600 Hz (18 ppm) spectral width together with a recycling delay of 8 s. The spin-lattice relaxation time constants (T_1) were measured using a standard inversion-recovery pulse sequence (180–τ–90–acquire)[3] with 16 delays and 16 number of scans with a sweep width of 10 ppm (7000 Hz). The recovery time (τ) was varied from 1 ms to 5 s and the temperature was set at 298 K. The area of the NMR peak at each recovery time was fitted with a mono-exponential decay to calculate the relaxation time T_1. The relaxation delay used in this experiment was fixed at 30 s. The temperature was kept at 298 K and the control of the temperature was realized with a variable temperature unit and an accuracy of +/- 0.1 K.

1.5 Protein Corona Studies

1.5.1 Purification of Hard Protein Corona

Human citrate plasma was obtained from the Department of Transfusion Medicine Mainz from healthy donors in accordance with the Declaration of Helsinki. A plasma pool from ten volunteers was prepared and stored at -80 °C.

Nanocapsules (with totally calculated surface area of 0.05 m^2) were incubated with 1 mL of human plasma at 37 °C for 1 h. Hard corona coated nanocapsules were centrifuged (20 000 g, 30 min, 4 °C) and washed with 1 mL PBS solution. This step was repeated three times. To detach the hard corona proteins from the nanocapsule surface, 100 µL of an aqueous solution containing 2 wt% SDS supplemented with 62.5 mM Tris-HCL was added and the sample was incubated for 5 min at 95 °C. After centrifugation, the supernatant containing hard corona proteins was isolated and the protein concentration was analyzed by Pierce 660 nm Protein Assay (Thermo Fisher, Germany) according to manufacturer's instructions. Percentage error of the protein assay was determined from triplicate measurements of representative nanocapsule samples (OVA-NCs and OVA-PEG5k$_{0.2}$).

1.5.2 Digestion in Solution

Prior to protein digestion, SDS was removed *via* Pierce detergent removal columns (Thermo Fisher, Germany). Tryptic digestion was carried out according to a previously reported protocol.[4] Briefly, proteins were precipitated using ProteoExtract protein precipitation kit (Merck Millipore, Germany) according to manufacturer's instructions. The protein pellet was resuspended in RapiGest SF (Waters, USA) dissolved in 50 mM ammonium bicarbonate (Sigma Aldrich, Germany) and further incubated at 80 °C for 15 min. Proteins were reduced with dithithreitol (5 mM, Sigma Aldrich, Germany) for 45 min at 56 °C and alkylated with idoacetoamide (15 mM, Sigma Aldrich, Germany) for 1 h in the dark. Tryptic digestion was carried out with a protein:trypsin ratio of 50:1 for 16 h at 37 °C. Digestion was stopped by adding 2 µL hydrochloric acid (Sigma Aldrich, Germany). The sample was centrifuged (14,000g, 15 min, 4 °C) to remove degradation products of RapiGest SF.

1.5.3 Liquid Chromatography-Mass Spectrometry (LC-MS)

For absolute protein quantification, 50 fmol of Hi3 EColi Standard (Waters, USA) was added to the peptide samples. A nanoACQUITY UPLC system equipped with a C18 nanoACQUITY Trap Column (5 µm, 180 µm x 20 mm, Waters, USA) and C18 analytic reversed phase column (1.7 µm, 75 µm x 150 mm, Waters, USA) was used for peptide separation and coupled to a Synapt G2-Si mass spectrometer (Waters, USA). Electrospray ionization (ESI) was carried in positive ion mode using a nanoLockSpray source. The mass spectrometer was operated in resolution mode performing data-independent experiments (MS^E). Acquired data was processed with MassLynx 4.1 and proteins were identified with Progenesis QI using a reviewed human data base downloaded from Uniprot. For absolute protein quantification the peptide sequence of Hi3 Ecoli standard (Chaperone protein CLpB, Water, USA) was added to the database and the amount of each protein was calculated based on the TOP3/Hi3 approach.[5]

1.6 Cellular Uptake Studies

1.6.1 Generation of Bone-Marrow-Derived Dendritic Cells

Bone marrow-derived dendritic cells (BMDCs) were differentiated from bone marrow progenitors of 8- to 10-weeks old C57BL/6 mice as described by Bros *et al.*[6] Initially, the mice were sacrificed by cervical dislocation and cleaned with ethanol. Both femur and tibia bones as well as the Os Ilium were removed by carefully peeling off the fur from the knee joint up to the back and dissecting the legs. BM cells were obtained by flushing the isolated bones

with washing buffer (Iscove's Modified Dulbecco's Solution, IMDM; Sigma Aldrich, Deisenhofen, Germany) supplemented with 5% fetal calf serum (FCS; Sigma Aldrich) and 50 μM β-mercaptoethanol (Roth, Karlsruhe, Germany). Potential bone fragments were removed with a cell strainer (Greiner Bio-One, Frickenhausen, Germany). Following centrifugation (300 x g, 10 min, 4 °C), the cell pellet was resuspended in 1 ml lysis buffer (pH 7.4, 155 mM NH_4Cl, 10 nM $KHCO_3$, 100 µM EDTA-Na_2) to eliminate erythrocyte contamination *via* osmotic shock. After stopping the lysis by adding 49 mL washing buffer, the cells were centrifuged again as described before. The cells were then counted with a Neubauer chamber and the cell concentration adjusted to 2 x 10^6 BM cells per ml. To analyze nanoparticle interaction *via* flow cytometry, the BM cells were seeded in 12 well suspension culture plates (Greiner Bio-One) with BMDC culture medium (IMDM supplemented with 5% FCS, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg mL^{-1} streptomycin (all from Sigma Aldrich) and 5% GM-CSF (granulocyte-macrophage colony-stimulating factor; derived from X63.Ag8-653 myeloma cells stably transfected with murine GM-CSF expression construct [7]) with 2 x 10^5 cells per 1.25 mL, and cultured at 37 °C and 10 % CO_2. At day 3, 500 µL of the same medium was added into each well. On day 6, 1 ml of the old medium was replaced with 1 mL fresh medium per well. On day 7, the BMDCs differentiation was complete. Because the presence of FCS proteins could influence the artificial corona composition, the remaining FCS was removed by repetitive washing with PBS prior to the addition of NCs. We added human plasma (5 vol%) together with nanocapsules to the cells, simulating an immediate corona formation in the cell culture. After 4 h of incubation, the binding/uptake of the OVA-NCs was assessed by flow cytometry. Additionally, all NP formulations were tested for potential endotoxin contaminations by Pierce LAL assay (Thermo Fisher Scientific, Waltham, USA). In accordance with recommendations of the FDA, an endotoxin concentration of 0.5 EU/ml was considered as permitted maximum.[8]

1.6.2 Flow Cytometry

To detect cell-nanoparticle-interactions, cells were harvested and washed in staining buffer (phosphate buffer saline [PBS]/2 % FCS). To block Fc receptor-mediated staining, cells were incubated with rat anti-mouse CD16/CD32 Ab (clone 2.4G2), purified from hybridoma supernatant for 15 min at room temperature. Afterwards, cells were incubated with eFluor450-conjugated Ab specific for MHC class II I-$A^{b,d,q}$/I-$E^{d,k}$ (clone M5/114.15.2), APC-, FITC- or PE-Cy7-labelled anti-CD11c (clone N418) (all from eBioscience, San Diego, USA), for 30 min

at 4 °C. Samples were measured with an Attune NxT flow cytometer equipped with Attune NxT software (Thermo Fisher Scientific, Waltham, USA). Data were generated based on defined gating strategy and analyzed using FlowJo software (FlowJo, Ashland, USA).

1.7 Biodegradability Study of OVA and OVA-PEG NCs

In order to evaluate the influence of PEGylation on the biodegradability of OVA-NCs, the release of Cy5-oligo from OVA and OVA-PEG NCs was performed by using dialysis membranes with a molecular weight cutoff of 14 kDa. 2 mL of dispersion of OVA and OVA-PEG NCs encapsulating Cy5-oligo were placed in a dialysis bag that was immersed in 20 mL PBS containing 2 mg mL^{-1} trypsin at 37 °C. 2 mL of supernatants were taken from the incubation medium at given intervals and an equal volume of fresh buffer solution was added to keep the volume constant. The release of Cy5-oligo, which shows an absorption maximum at 649 nm and an emission maximum at 666 nm, was quantified by infinite M1000 plate reader (Tecan, Crailsheim, Germany). The trypsin-responsive release of Cy5-oligo from OVA and OVA-PEG NCs was expressed as the cumulative released Cy5-Oligo. The release experiments were performed in triplicate for each sample. To investigate the release mechanism, the release profiles were fitted with the first order model, and a menu-driven add-in program for Microsoft Excel written in Visual Basic.[9]

1.8 Characterization techniques

^{1}H NMR spectra were measured at 250 MHz on a Bruker Avance 250 Spectrometer and processed with the MestReNova 9.0.1-13254 software. The OVA-NCs samples were freeze-dried and dissolved in D_2O at a concentration of 1 mg NC per mL prior to the NMR measurement. Average sizes and size distribution of NCs were measured by dynamic light scattering (DLS) at a concentration of 20 μg/mL in water at 25 °C on a Nicomp 380 submicron particle sizer (Nicomp Particle Sizing Systems, USA) at a fixed scattering angle of 90°. Zeta potential measurements were performed at a concentration of 20 μg/mL in 10^{-3} M potassium chloride aqueous solution at pH 6.8 and 25 °C with Malvern Zeta sizer (Malvern Instruments, U.K.). The morphologies of nanocapsules were 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 (for TEM) and left to dry overnight at room temperature.

2. Calculation of Surface PEG Density and Conformation

2.1 Derivation of the Equations for Calculating PEG Grafting Density per nm^2 Surface

$$D_{PEG/nm^2} = \frac{N_{PEG\ per\ NC}}{S_{per\ NC}} = \frac{N_{PEG}/N_{NC}}{S_{per\ NC}}$$

$$N_{PEG} = \frac{m_{OVA-PEG} \times W_{t_{PEG}}}{M_{w_{PEG}}} \times N_A$$

$$N_{NC} = \frac{m_{OVA}}{m_{per\ NC}} = \frac{m_{OVA-PEG} \times (1 - W_{t_{PEG}})}{\frac{1}{11} \times \rho_{H_2O} \times \frac{4}{3}\pi(D_h/2)^3}$$

$$S_{per\ NC} = 4\pi(D_h/2)^2$$

where $D_{PEG/nm2}$ represents the grafting density of PEG chains per nm^2 surface of OVA-NCs, $N_{PEG\ per\ NC}$ represents the number of PEG chains per NC, $S_{per\ NC}$ represents the surface area per NC, N_{PEG} is the total number of PEG chains in the sample for NMR measurement, N_{NC} is the total number of NCs in the NMR sample, $m_{OVA\text{-}PEG}$ is the mass of dried OVA-PEG samples submitted to NMR measurement, M_{wPEG} is the molecular weight of PEG, N_A is the Avogadro constant (6.02*10^{23} mol^{-1}), m_{OVA} is the mass of OVA-NC in the OVA-PEG sample, $m_{per\ NC}$ is the mass per NC, the use of 1/11 is due to the fact that only the OVA shell was measured after freeze drying the core-shell capsules and the OVA counts for 1/11 of the mass of the whole capsule, and D_h is the hydrodynamic diameter of nanocapsules.

2.2 Calculation for Determining the PEG Conformational Regime

Flory radius (R_F), grafting distance (D) and thickness of PEG layer (L) were determined using the following equations (1-3).[10]

$$R_F = \alpha N^{3/5} \quad (1)$$

$$D = 2(A/3.14)^{1/2} \quad (2)$$

$$L = N(\alpha^{5/3})/D^{2/3} \quad (3)$$

where α is the monomer length (0.35 nm for PEG),[11] N is the number of PEG repeating unit, and A is the area occupied per PEG chain. A is calculated from the measured grafting density (chains per nm^2) by using equation A=1/density.

(1) For OVA:

Hydrodynamic radius (r) = 147 nm; Surface area (S_{OVA}) = $4\times3.14\times r^2 = 2.71\times10^5$ nm^2

(2) For OVA-PEG2k$_{0.2}$:

α = 0.35 nm, N = 44 $R_F = aN^{3/5}$ = 3.39 nm

A = 5.56 nm^2 D = 2.66 nm

N = 44, α = 0.35 nm, D = 2.66 nm $L = N(\alpha^{5/3})/D^{2/3} = 44\times0.17/1.92 = 3.90$ nm

(3) For OVA-PEG2k$_{0.5}$:

α = 0.35 nm, N = 44 $R_F = \alpha N^{3/5}$ = 3.39 nm

A = 1.79 nm^2 D = 1.51 nm

N = 44, α = 0.35 nm, D = 1.51 nm $L = N(\alpha^{5/3})/D^{2/3} = 44\times0.17/1.32 = 5.67$ nm

(4) For OVA-PEG2k$_{2.5}$:

α = 0.35 nm, N = 44 $R_F = \alpha N^{3/5}$ = 3.39 nm

A = 0.40 nm^2 D = 0.72 nm

N = 44, α = 0.35 nm, D = 0.72 nm $L = N(\alpha^{5/3})/D^{2/3} = 44\times0.17/0.80 = 9.35$ nm

(5) For OVA-PEG3k$_{0.2}$:

α = 0.35 nm, N = 75 $R_F = \alpha N^{3/5}$ = 4.67 nm

A = 4.55 nm^2 D = 2.4 nm

N = 75, α = 0.35 nm, D = 2.4 nm $L = N(\alpha^{5/3})/D^{2/3} = 75\times0.17/1.79 = 7.12$ nm

(6) For OVA-PEG3k$_{0.8}$:

α = 0.35 nm, N = 75 $R_F = \alpha N^{3/5}$ = 4.67 nm

A = 1.20 nm^2 D = 1.24 nm

N = 75, α = 0.35 nm, D = 1.24 nm $L = N(\alpha^{5/3})/D^{2/3} = 75\times0.17/1.15 = 11.09$ nm

(7) For OVA-PEG5k$_{0.2}$:

α = 0.35 nm, N = 110 $R_F = \alpha N^{3/5}$ = 5.87 nm

A = 5.26 nm^2 D = 2.59 nm

N = 110, α = 0.35 nm, D = 2.59 nm $L = N(\alpha^{5/3})/D^{2/3}$ = 110×0.17/1.88 = 9.95 nm

3. Results

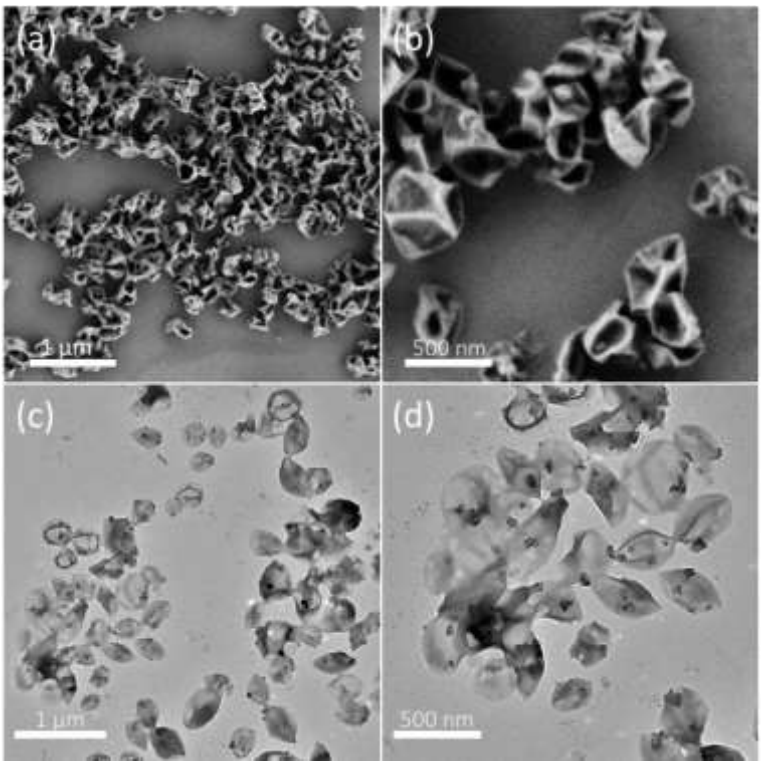


Figure S1. (a-b) SEM and (c-d) TEM micrographs of OVA-NCs. (c) is shown as Figure 1a in the main manuscript. Samples were purified by centrifugation in cyclohexane.

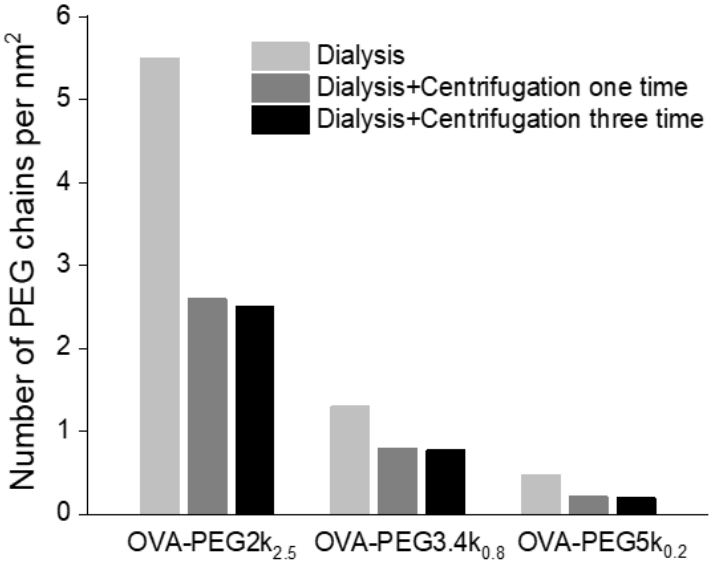


Figure S2. PEG density of OVA-PEG2k$_{2.5}$, OVA-PEG3.4k$_{0.8}$, and OVA-PEG5k$_{0.2}$ after different purification processes (only dialysis, one centrifugation step after dialysis, and three centrifugation steps after dialysis).

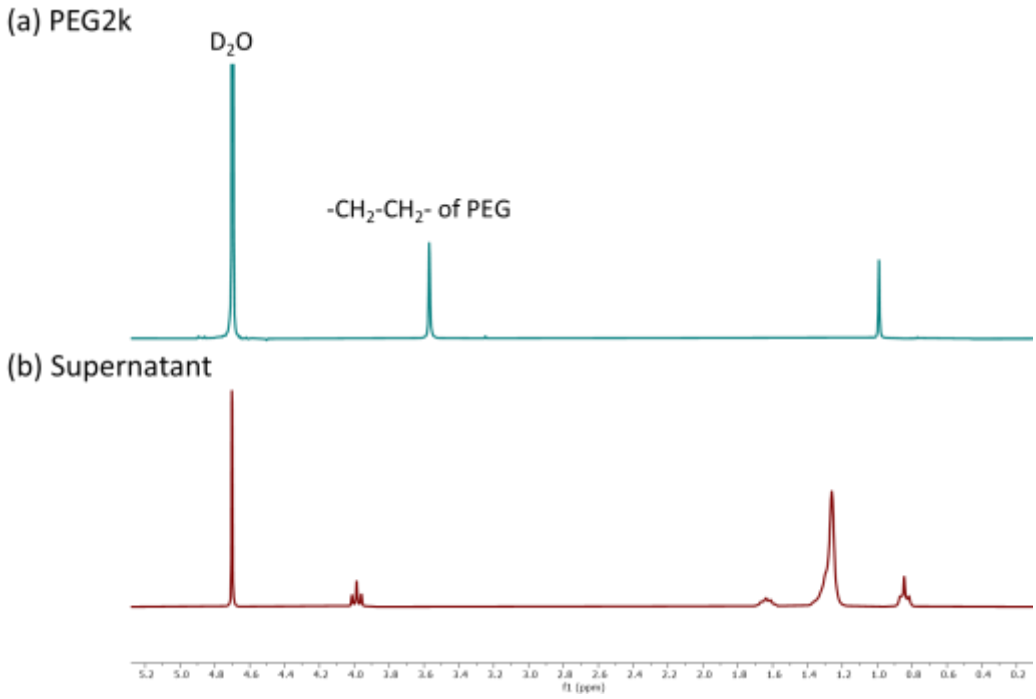


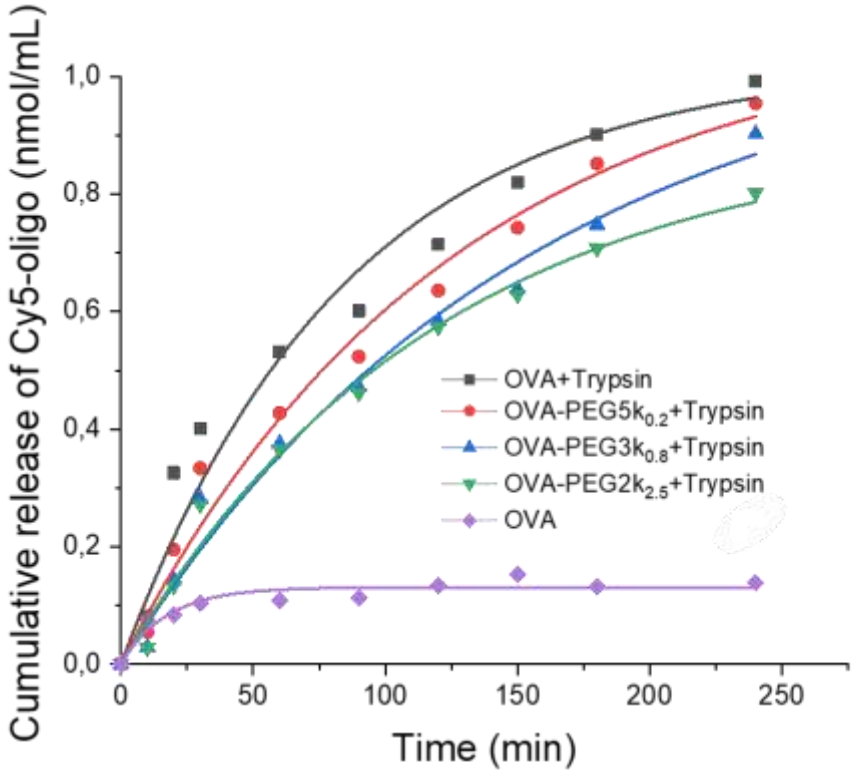
Figure S3. ^{1}H NMR (250 MHz, 298K) spectra of PEG2k and the supernatant from the centrifugation of OVA-PEG2k$_{2.5}$ NC dispersion. The supernatant was freeze-dried and dissolved in HDO at a concentration of 1 mg mL^{-1} for ^{1}H NMR spectroscopy measurement.

Table S1. Characteristics (hydrodynamic diameter D_h in water and in blood plasma, zeta potential in water) of OVA-NCs PEGylated by grafting PEG chains with defined molecular weights and densities.

Entry	D_h in water		ξ-potential [a]	D_h in plasma	
	[nm]	PDI	mV	[nm]	PDI
OVA	254	0.5	-19	303	0.3
OVA-PEG2k$_{0.2}$	291	0.4	-26	297	0.3
OVA-PEG2k$_{0.5}$	261	0.4	-27	259	0.4
OVA-PEG2k$_{2.5}$	259	0.4	-32	260	0.4
OVA-PEG3.4k$_{0.2}$	301	0.4	-25	290	0.4
OVA-PEG3.4k$_{0.8}$	289	0.4	-31	311	0.4
OVA-PEG5k$_{0.2}$	305	0.4	-24	323	0.4

[a] Measured in 10^{-3} M potassium chloride solution at pH 6.8 and 25 °C.

Biodegradability of OVA-NCs was preserved after PEGylation. Enhanced release of payload Cy5-oligo was induced upon incubation with a protease trypsin (Figure S4). Release of payload from OVA-PEG NCs was slightly slower than release from non-PEGylated NCs due to the existence of a hydrophilic polymer shell around the capsules.



Entry	First-order: $M_t = M_\infty(1\text{-}e^{-kt})$		$R^2_{adjusted}$	$t_{1/2}$ [min]
	M_∞	k		
OVA+Trypsin	0.99 ± 0.86	0.004	0.965	60±5
OVA-PEG2k$_{2.5}$+Trypsin	0.98 ± 0.89	0.002	0.983	81±4
OVA-PEG3k$_{0.8}$+Trypsin	0.98 ± 1.49	0.001	0.985	104±7
OVA-PEG5k$_{0.2}$+Trypsin	0.97 ± 0.81	0.001	0.988	121±4

Figure S4. Release profiles of Cy5-oligo for non-PEGylated and PEGylated OVA-NCs in PBS buffer without or with 2 mg mL^{-1} trypsin. The release experiments were performed at 37 °C in an incubator. Average results of three experiments were reported. The table shows the first order kinetics for the release of Cy5-oligo from OVA and OVA-PEG NCs upon the incubation with trypsin. In this first-order kinetic equation $M_t = M_\infty(1\text{-}e^{-kt})$, M_t is the Cy5-oligo concentration at time t, M_∞ is the final Cy5-oligo concentration outside the dialysis membrane and k is the release rate constant.

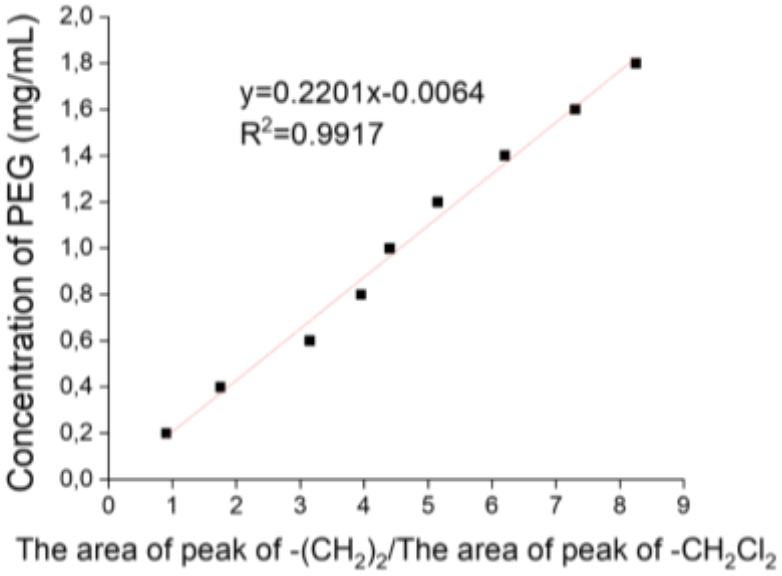


Figure S5. (a) Schematic representation of the method used for the quantification of surface attached PEG chains by ^{1}H NMR spectroscopy in HDO (250 MHz, 298K). A glass tube containing 5wt% dichloromethane (DCM) in deuterated DCM was used as the reference. (b) The standard curve describing the relationship between the concentration of PEG and the ratio between the integral of -CH_2CH_2- and the integral of peak for the reference CH_2Cl_2.

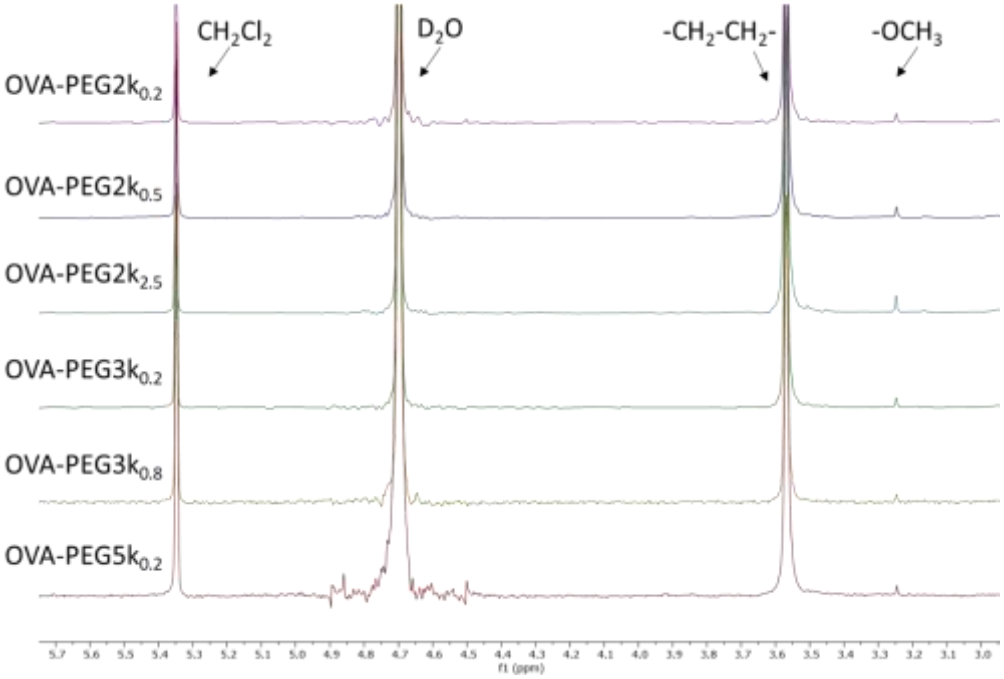


Figure S6. ^{1}H NMR (250 MHz, 298K) spectra of OVA-PEG NCs modified with varied PEG molecular weight and densities. The NC dispersions were freeze-dried and redispersed in HDO at the concentration of 1 mg mL^{-1} for the ^{1}H NMR measurement. A glass tube containing 5 wt% CH_2Cl_2 in deuterated dichloromethane (CD_2Cl_2) was used as reference.

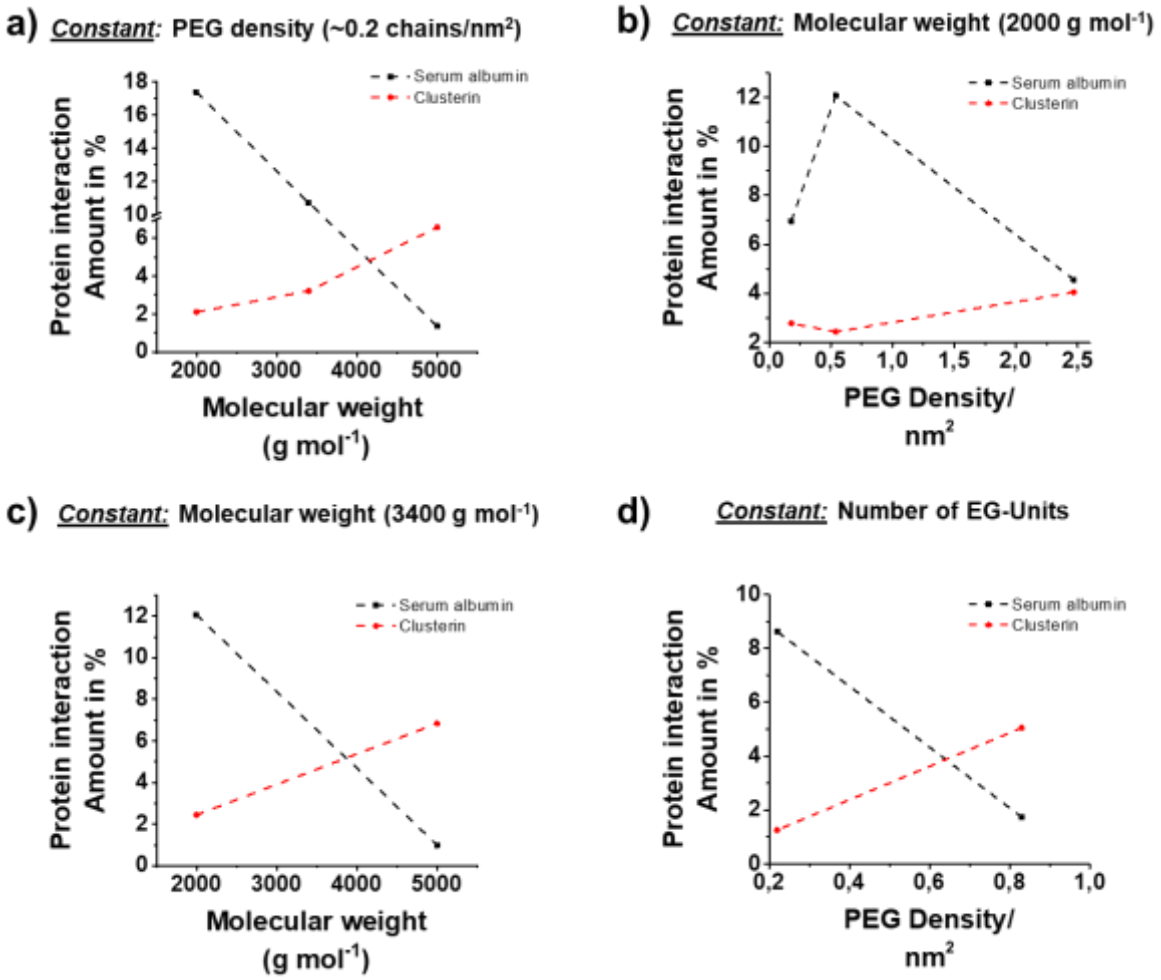


Figure S7. (a-c) Relative amount of serum albumin and clusterin in the protein corona of OVA-PEG NCs after incubation with human serum. (a) Increased molecular weight at the same grafting density (0.2 chains nm^{-2}). (b, c) Increased PEG density at constant molecular weight (2000 or 3400 g mol^{-1}). (d) Same number of ethylene glycol units per nm^2 but from different combinations of PEG molecular weight and densities (PEG5k at 0.2 chains nm^{-2} *vs* PEG2k at 0.5 chains nm^{-2}). Values are expressed as the mean ± SD of technical triplicates.

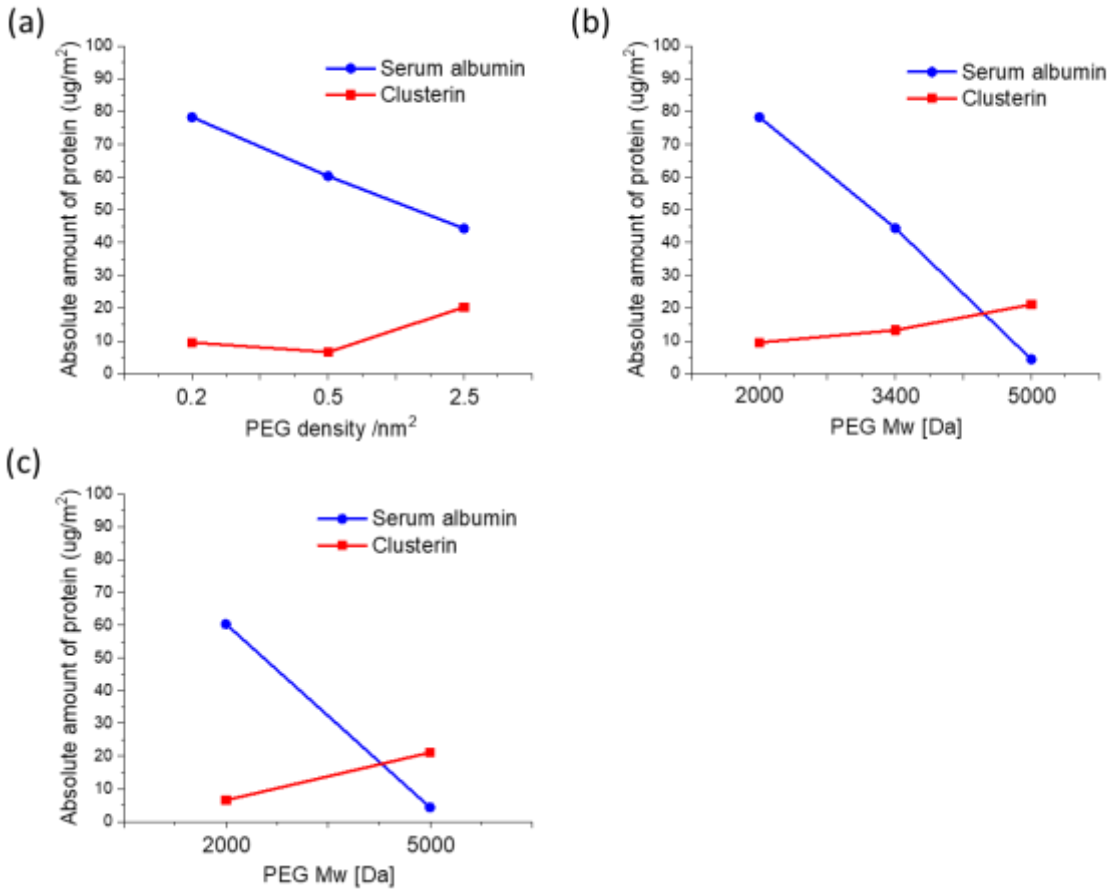


Figure S8. Absolute amount of serum albumin and clusterin in the protein corona of OVA-PEG NCs: (a) Increasing PEG density at a fixed molecular weight of 2000 g mol^{-1}; (b) Increasing molecular weight at a fixed grafting density of 0.2 chains nm^{-2}; (c) Constant number of ethylene glycol units per nm^2 but with different PEG molecular weight and densities (Mushroom-brush intermediate conformation: PEG2k at 0.5 chains/nm^2 *vs* Brush conformation: PEG5k at 0.2 chains/nm^2).

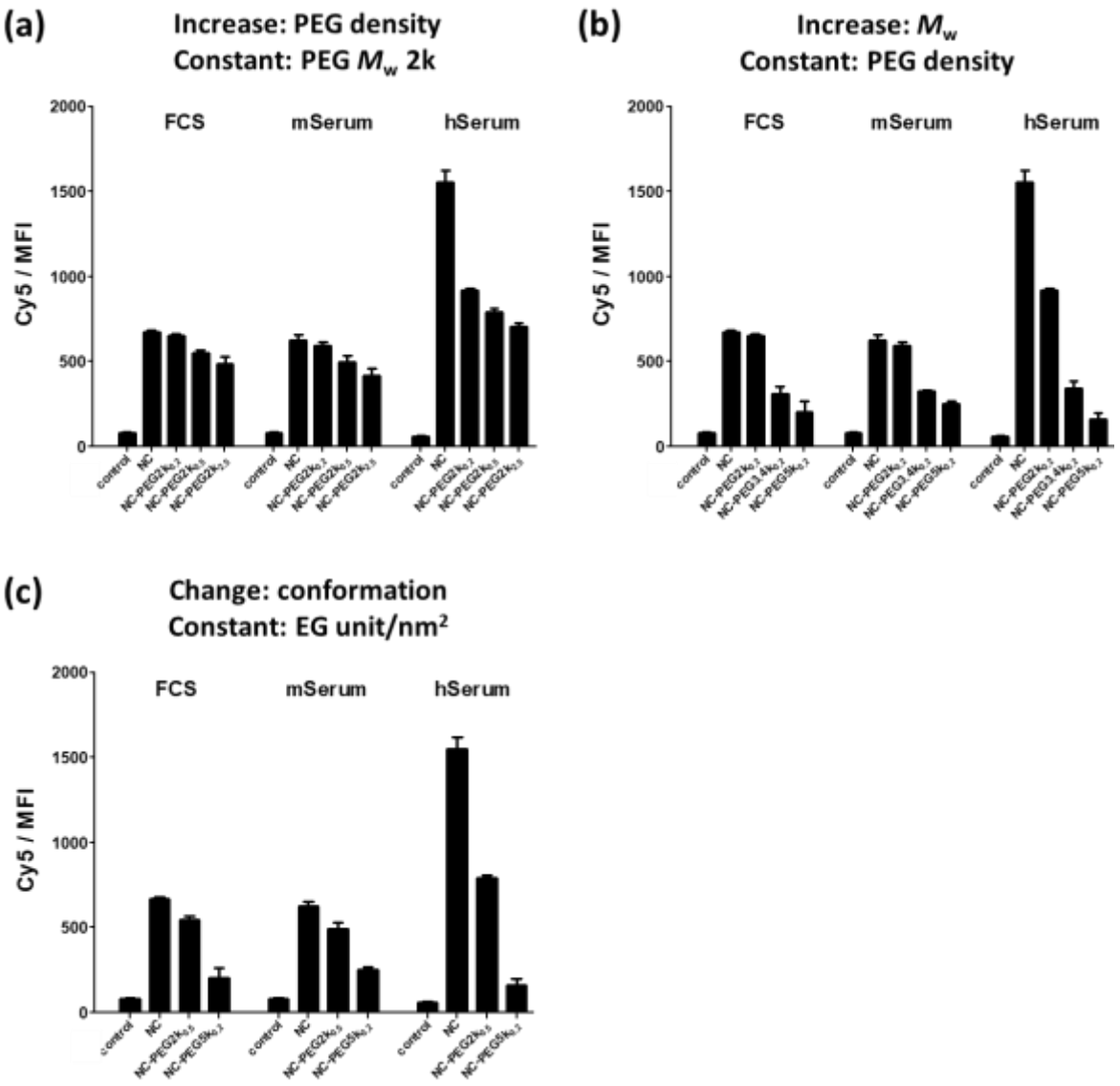


Figure S9. Cellular uptake of OVA and OVA-PEG NCs with varied PEG M_w and surface densities after incubation with different protein sources (FCS, mSerum, and hSerum). (a) Increased PEG density at constant M_w (2000 g mol^{-1}). (b) Increased M_w at constant grafting density (0.2 chains nm^{-2}). (c) Constant number of ethylene glycol units per nm^2 but from different combinations of PEG M_w and densities (PEG2k at 0.5 chains nm^{-2} *vs* PEG5k at 0.2 chains nm^{-2}). BMDCs (1 x 10^6 cells mL^{-1}) were incubated with differently PEGylated OVA-NCs in the absence (no proteins) or presence of proteins for 4 h. The median fluorescence intensity (MFI) was measured by flow cytometry. Cells treated with non-PEGylated OVA-NCs and untreated cells were used as control.

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