Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supporting Information

Anti-PEG antibodies enriched in the protein corona of PEGylated nanocarriers impact the cell uptake

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Content

Experimental Section

Materials:

All plasma samples were obtained from the Transfusion Medicine Department at the University Medical Centre of the Johannes Gutenberg University Mainz. Blood samples for the plasma screening were collected from 500 healthy donors after obtaining informed consent. All experiments containing human blood plasma from these donors were approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz, Mainz No. 2019-14748. To obtain pooled plasma for protein corona formation, blood was taken from 10 healthy donors after obtaining informed consent and pooled subsequently. All experiments containing human blood plasma from these donors were approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz, Mainz No. 837.439.12 (8540-F). Accordingly, all experiments involving human material were performed in compliance with all relevant laws and guidelines.

After collection, the citrate plasma (either individual samples or pooled plasma) was centrifuged at 20,000 g for 1 h at room temperature to remove residual protein precipitates and stored at -80 °C until further use.

Chimeric human anti-PEG IgG (clone no. c3.3 IgG) and chimeric human anti-PEG IgM (clone no. cAGP4-IgM) were purchased from IBMS Academia Sinica (Taipei, Taiwan) and used without further purification. Anti-Human IgG (F_c specific) − peroxidase antibody, HRP-conjugated goat F(ab')2 antihuman IgM F_c5µ, poly(ethylene glycol) diamine (average M_n = 10,000 g mol^{-1}) and poly(ethylene glycol) (average M_n = 10,000 g mol^{-1}) were acquired from Sigma-Aldrich, USA. Dulbecco's phosphate buffered saline (PBS, Thermo Fisher Scientific, USA), skim milk powder (VWR International, USA), 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, Merck, Germany), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, Merck, Germany) and QuantaBlu Fluorogenic Peroxidase Substrate Kit (Thermo Fisher Scientific, Waltham, USA) were further used for assay experiments. Methoxy-terminated poly(ethylene glycol)-fluorescein (2k, 5k, 10k, 20k) (Creative PEGWorks, USA) and fluorescein isothiocyanate-dextran (Sigma-Aldrich, USA) were purchased for binding studies. Tetraethoxysilane (TEOS, 98%, Alfa Aesar, Germany), hexadecane (98%, TCI, Germany), olive oil (highly refined, low acidity, Sigma-Aldrich, USA) and cetyltrimethylammonium chloride (CTMA-Cl, Acros Organics, 99%), (3-aminopropyl)triethoxysilane (APTES, 99%, Sigma-Aldrich, USA), amine-reactive fluorescent dye Cyanine5 NHS ester (Cy5-NHS, Lumiprobe GmbH, Germany), Lutensol® AT50 (poly(ethylene glycol)-hexadecyl ether) (BASF SE, Germany), Lutensol® AT25 (BASF SE, Germany) and Lutensol® AT80 (BASF SE, Germany), were used for nanocarrier synthesis. Sodium dodecyl sulfate (SDS, SERVA Electrophoresis GmbH, Germany), tris(hydroxymethyl)aminomethan hydrochlorid (Tris*HCl), Pierce 660 nm Assay (Thermo Scientific, Germany), bovine serum albumin (BSA, Serva, Germany) were used as received for protein corona experiments. For the cell uptake, Dulbecco Modified Eagle Medium (DMEM, Gibco, USA), Roswell Park Memorial Institute (RPMI)-1640 (Gibco, Germany), fetal bovine serum (FBS, Gibco, Germany), penicillin (Gibco, Germany), streptomycin (Gibco, Germany), glutamine (Gibco, Germany), phorbol-12-myristate-13-acetate (PMA) (Sigma-Aldrich, Germany), trypsin (Gibco, Germany), Zombie Aqua (BioLegend, USA), anti-CD64, CD16, and/or CD32 (BioLegend, USA) were used.

ELISA:

The ELISA experiments were performed based on a previously published procedure from Chen et al.[1] Maxisorp 96-well microplates (Thermo Fisher Scientific, Waltham, USA) were coated with 0.5 µg NH_2-PEG10000-NH_2 in 50 µL 0.1 M $NaHCO_3$/Na_2CO_3 buffer (adjusted to pH 9.5) per well overnight at 4 °C. Afterwards, the content of the wells was discarded and the well blocked with 200 µL of 5% (w/v) skim milk powder in Dulbecco's phosphate-buffered saline (PBS, Thermo Fisher Scientific) per well at room temperature for 2 h. Plates were washed once with 100 µL PBS per well immediately before use. In wells where no competition took place, 50 µL of 2% (w/v) skim milk in PBS was added. The wells were incubated for 30 min at room temperature. Human plasma samples were pre-diluted to 20%, 50%, 80%, or no dilution in 2% (w/v) skim milk in PBS. Serial dilutions of chimeric anti-PEG antibodies c3.3-IgG in 2% (w/v) skim milk powder in PBS were prepared starting from 8 µg mL^{-1}. For each sample (including standards), 60 µL of the sample were diluted with 120 µL of 2% (w/v) skim milk in PBS (33%-dilution). 50 µL of these 33%-diluted samples were added to the respective well and incubated for 1 h at room temperature. Unbound antibodies were removed by washing the plates twice with 0.1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) in PBS and once with pure PBS. Afterwards, 50 µL of anti-human IgG-peroxidase antibody (0.25 mg L^{-1} in 2% (w/v) skim milk in PBS, Fc-specific) was added to each well and incubated for 1 h at room temperature. The content of the wells was discarded, and each well was washed four times with 100 µL 0.1% (w/v) CHAPS in PBS and once with 100 µL pure PBS. Then, 100 µL ABTS substrate was added per well and incubated for 30 min in the dark. The absorbance was measured at λ = 405 nm in a microplate reader (Tecan infinite M1000 plate reader, Switzerland).

For the competition experiment, the well plate was prepared as described. After blocking with skim milk for 2 h, 50 µL of poly(ethylene glycol) (5 g L^{-1} in 2% (w/v) skim milk in PBS) was added to the wells and incubated for 30 min. Afterwards, the same procedure was followed.

The analysis of anti-PEG IgM was performed accordingly. Chimeric human anti-PEG IgM (clone no. cAGP4-IgM) was used as a standard in black maxisorp 96-well microplates. (HRP)-conjugated goat F(ab')2 antihuman IgM Fc5µ was used as secondary antibody. QuantaBlu Fluorogenic Peroxidase Substrate Kit (Thermo Fisher Scientific, Waltham, USA) was used according to the manufacturer's instructions and the fluorescence measured at λ = 325/420 nm in a microplate reader (Tecan infinite M1000 plate reader).

For the determination of the assay's limit of detection (LOD) and limit of quantification (LOQ), ten blank samples (2% (w/v) skim milk in PBS) were measured. LOD was calculated as the mean background absorbance of the blank samples plus three times its standard deviation and LOQ was calculated as the mean background absorbance plus ten times its standard deviation according to DIN 32645.

Positive responses were defined as samples with absorbance values greater than LOQ. The relative concentrations of anti-PEG IgG or IgM in positive samples were calculated by comparison with c3.3-IgG or cAGP4-IgM standard curves, respectively. Additionally, the absorbance reading after the addition of PEG10k (competition experiment) needed to be reduced by at least 35% as compared to the reading without the addition of PEG10k to confirm specific binding.

For the measurement of protein corona samples, 120 µL of each sample was diluted with 240 µL of 2% (w/v) skim milk in PBS (33% dilution) and added to the wells as described.

MST measurements:

For the MST measurements, a target solution (methoxy-terminated poly(ethylene glycol)-fluorescein (mPEG-FITC)) and a ligand solution (chimeric anti-PEG c3.3 IgG) were prepared in PBS buffer. The target was prediluted to twice the concentration used in the measurement (Table S1). The obtained ligand concentration varied with a maximum concentration of 1500 µg mL^{-1} (10 µM). The used ligand concentration in each measurement can be seen in Table S1. A serial dilution of the ligand was prepared by mixing 10 µL PBS buffer with 10 µL ligand solution. This was repeated 15 times to obtain 16 different concentrations. 10 µL of target solution was added to each of the 16 samples and mixed by pipetting. The solutions were transferred into premium-coated capillaries and analyzed with a blue excitation laser and medium MST power (Nanotemper Monolith NT.115, Germany). The excitation power for each measurement is given in Table S1, which displays all conducted measurements and their conditions. For data evaluation, all performed measurements were analyzed individually with the software MO.Control 2, using the automatic response evaluation. Resulting dose response curves were fitted individually due to different measurement parameters as shown in Table S1. K_d values were averaged after fitting all measurements individually for each PEG chain length ± standard deviation except for $mPEG_{2k}$, where n=1. For $mPEG_{2k}$ only one measurement was conducted due to limited availability of the expensive anti-PEG IgG as because of the lower binding affinity high antibody concentrations were needed. Ideally, K_d evaluation should be performed with averaged data from at least three experiments with identical measurement parameters. Thus, our procedure results in larger errors, but can still give information about the binding affinity trends.

Table S1: Settings used for microscale thermophoresis analysis. Target: methoxy-terminated poly(ethylene glycol) (mPEG) with different chain lengths, ligand: anti-PEG IgG.

target	target concentration / nM	ligand concentration / µM	excitation power / %	excitation color	MST power
mPEG20k-FITC	20	2.5	100	blue	medium
mPEG20k-FITC	20	1	80	blue	medium
mPEG20k-FITC	20	1	60	blue	medium
mPEG10k-FITC	25	0.75	60	blue	medium
mPEG10k-FITC	25	0.75	80	blue	medium
mPEG10k-FITC	25	0.75	100	blue	medium
mPEG10k-FITC	20	1	60	blue	medium
mPEG10k-FITC	20	4	40	blue	medium
mPEG10k-FITC	20	4	40	blue	medium
mPEG5k-FITC	50	1.65	20	blue	medium
mPEG5k-FITC	25	0.75	80	blue	medium
mPEG2k-FITC	50	5	40	blue	medium
dextran-FITC (neg. control)	50	1.65	80	blue	medium

Synthesis of silica nanocapsules (SiNCs):

SiNCs were likewise synthesized according to the previously described procedure in an oil-in-water miniemulsion by using the surface of oil nanodroplets as a template for the hydrolysis and condensation of alkoxysilanes.[2] Specifically, 2.0 g (9.6 mmol) of TEOS was first mixed with 125 mg of hexadecane and 1 g of olive oil to form the oil phase. In the second step, 30 mL of a 0.77 mg mL^{-1} aqueous solution of CTMA-Cl was poured into the oil mixture while stirring. After a pre-emulsification step by stirring at 1000 rpm for 1 h, the obtained emulsion was sonicated by using a Branson 450 W sonifier with a 1/2'' tip at 70% amplitude for 180 s (30 s of sonication, 10 s of pause) with ice cooling. The resulting miniemulsion was stirred at 1000 rpm for 12 h at room temperature to obtain an aqueous dispersion of SiNCs. For the fluorescent labeling of SiNCs, Cy5-NHS was first coupled with APTES at a molar ratio of 1:1.1 to obtain fluorescently labeled silica precursors. The APTES-Cy5 conjugates were then mixed with TEOS as the silica source. The molar ratio of Cy5 with TEOS was 1:14,000.

SiNCs were PEGylated by replacing the templating surfactant CTMA-Cl by the nonionic surfactant Lutensol® AT25, AT50, or AT80. Specifically, 35 mg, 70 mg, or 140 mg of Lutensol® AT50 were added to 2 mL of SiNCs dispersion to obtain different PEG densities. Accordingly, 39 mg Lutensol® AT25 or 108 mg Lutensol® AT80 was added to 2 mL of SiNCs dispersion. The dispersion was stirred at 1,000 rpm for 2 h and then dialyzed against MilliQ water in a dialysis tube with a MWCO of 1,000 g mol^{-1}. In this case, CTMA-Cl (M_w = 320 g mol^{-1}) could diffuse through the dialysis membrane into the aqueous dialysis medium while the Lutensol® AT25 (M_w = 1,230 g mol^{-1}) was kept inside. Afterwards, the dialyzed dispersion was centrifuged at 12,000 g to remove the excess of Lutensol® surfactant. The pellet was redispersed in water and the dispersion was stirred at 1,000 rpm for 24 h. The samples were stored at room temperature and protected from light under constant agitation.

Transmission electron microscopy (TEM):

TEM micrographs were taken on an FEI Tecnai F20 transmission electron microscope operated at 200 kV. Micrographs were taken using a 2k charge-coupled device camera from Gatan (Type: Ultrascan 1000).

Dynamic light scattering (DLS):

DLS measurements of SiNCs were performed using an instrument from ALV (Langen, Germany) consisting of an electronically controlled goniometer and an ALV-5000 multiple τ full-digital correlator with 320 channels having a measurement range between 10^{-7} s and 10^{3} s. A helium-neon laser (Type 1145 P) from JDS Uniphase (Milpitas, USA) of 632.8 nm wavelength and 25 mV output power was used as a source of light. Before measurements, samples were filtered into quartz cuvettes for light scattering from Hellma (Müllheim, Germany) with an inner radius of 9 mm. Millex-SV filters (Merck Millipore, Billerica, USA) with 5 µm pore size were used for filtration. Prior to use, the quartz cuvettes were cleaned with acetone using a Thurmond apparatus.

Zeta potential measurements:

Zeta potential measurements were performed using a Nano Z Zetasizer (Malvern Instruments GmbH, Herrenberg, Germany). 20 µL of the sample were diluted with 1 mL of a 1 mM KCl solution and

measured at 25 °C after two minutes of equilibration. Each measurement was repeated in triplicate and mean values as well as standard deviations were calculated.

Protein corona preparation:

The protein source of pooled or human citrate plasma was used for the experiments. For each sample, an aqueous NC suspension (0.05 m^2 of NC surface area in a total volume of 300 µL) was mixed in an Eppendorf-tube with 1 mL of the respective plasma source. After an incubation period of 1 h, while shaking at a temperature of 37 °C, the remaining free proteins were removed using centrifugation (always 20,000 g, for 1 h at 4 °C). The supernatant was discarded, and the pellet was resuspended in 1 mL of PBS. The suspension was again centrifuged for 1 h at 20,000 g and 4 °C. These washing steps were repeated in total three times. Before the last washing step, the suspension was transferred into a new Eppendorf-tube.

After the last washing step of the corona preparation, the pellet was suspended in 100 µL of a 0.0625 M Tris*HCl solution containing 2 wt% of SDS for protein detachment. The suspension was incubated at 95 °C for 5 min and was centrifuged again for 1 h at 20,000 g and 4 °C. The supernatant was further used for analysis.

For the protein corona preparation for cell uptake experiments, 5 plasma screening samples of similar anti-PEG IgG concentration were each pooled to obtain batches of low (<0.5 µg mL^{-1}), medium (9.6 µg mL^{-1}) and high (110.5 µg mL^{-1}) anti-PEG IgG concentration. The NC surface was normalized to a surface area of 0.0125 m2 (in 75 µL volume) and incubated with 250 µL of plasma for 1 h at 37 °C. The remaining free proteins were removed using centrifugation (20,000 g, for 1 h at 4 °C). The supernatant was discarded, and the pellet resuspended in the appropriate cell culture medium with a resulting NC concentration of 0.5 mg mL^{-1}.

Pierce assay:

The protein quantification of desorbed corona proteins was quantified with a Pierce 660 nm Assay (Thermo Scientific, Germany) according to the manufacturer's instructions. Bovine serum albumin (BSA) was used as a standard (Serva, Germany). The absorption was measured at 660 nm with a Tecan infinite M1000 plate reader.

Liquid chromatography-mass spectrometry (LC-MS):

Proteomic analysis was carried out as previously described.[3] Briefly, SDS was removed from the protein samples via Pierce Detergent Removal Spin Columns (Thermo Fisher). Further, proteins were precipitated using a ProteoExtract protein precipitation kit (CalBioChem, Merck, Germany) overnight. Afterwards, the protein pellets were isolated via centrifugation (14 000 g, 10 min, 4 °C) and resuspended with RapiGest SF (Waters) in ammonium bicarbonate buffer (50 mM). The protein solution was reduced with dithiothreitol (Sigma Aldrich) at a concentration of 5 mM for 45 min at 56 °C and alkylated with 15 mM iodoacetoamide (Sigma Aldrich) for 1 h in the dark. Tryptic digestion (protein : trypsin ratio 50:1) was carried out for 18 h at 37 °C. Afterwards, the reaction was quenched with 2 µL hydrochloric acid (0.1 vol%, (Sigma Aldrich).

Tryptic peptides were diluted with 0.1% formic acid spiked with 50 fmol $µL^{-1}$ Hi3 *E. coli* (Waters) for absolute protein quantification. The peptide solution was injected into a nanoACQUITY UPLC system

coupled to a Synapt G2-Si mass spectrometer. The system was operated in resolution mode, with a NanoLockSpray source in positive ion mode. Data-independent acquisition (MSE) experiments were performed, and data was analyzed with MassLynx 4.1.

Proteins were identified with Progenesis GI (2.0) using a reviewed human database downloaded from Uniprot. For analysis, the following criteria were chosen: max. protein mass 600 kDa, one missed cleavage, fixed modifications for carbamidomethyl and cysteine, variable oxidation for methionine, and a false discovery rate of 4%. Peptide identification required three identified fragments and for protein identification, five identified fragments and two peptides were needed. Based on the TOP3/Hi3 quantification, the amount of each protein in fmol is provided.

Cell culture:

The murine macrophage cells from the cell line RAW 264.7 were cultured in Dulbecco Modified Eagle Medium (DMEM, Gibco, USA). The human cells from the cell line THP-1 were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium. Both were supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin and 2 mM glutamine (all from Gibco, Germany) at 37 °C with 5% CO_2 in an incubator.

THP-1 macrophage differentiation:

The human monocyte cell line THP-1 was differentiated into macrophages for 5 days prior to the experiments with the nanocarriers. On day 0 the cells were stimulated with 100 ng mL^{-1} of phorbol-12-myristate-13-acetate (PMA) (Sigma-Aldrich, Germany) and seeded at a density of 200,000 cells per well in 24-well plates. After 2 days, the medium was changed to fresh RPMI without PMA and the cells rested for the following 3 days before the experiment. This differentiation yields variable levels of differentiated cells and especially their activation state. Therefore, one has to treat these as different batches as the ones used for the 4 °C and for the 37 °C experiments were done at different time points.

Cell uptake experiments and flow cytometry measurements:

For the cell uptake experiments, cells were seeded at a density of 150,000 cells per well in 24-well plates in cell culture medium with 10% FBS. After overnight incubation, the medium was changed to serum-free medium. The cells were incubated in fresh serum-free medium with the nanocarrier dispersions added at a concentration of 10 µg mL^{-1} to the cells for 2 h (RAW 264.7) or 75 µg mL^{-1} for 2 h (THP-1) at 37 °C. RAW 264.7 macrophages tend to show a high uptake behavior. In order not to not overload the cells and see differences in the uptake, a lower nanocarrier concentration was used as for THP-1 cells.

For flow cytometry experiments, adherent cells were washed with PBS and detached from the culture vessel with 2.5% trypsin (Gibco, Germany) and measurements were performed on the Attune Nxt cytometer (Invitrogen, Germany) with a 670 nm laser for excitation of Cy5-NHS-Ester (Cy5). The data analysis of the cell uptake was conducted using Attune™ NxT Software. The cells were selected using the respective FSC/SSC plot, thereby excluding cell debris. The gated cells were analyzed for the fluorescent signal (RL1) and a threshold was set such that less than ~1% was more fluorescent than the threshold. This threshold was used for all gated events and given as percentage of gated events or the median fluorescence intensity (MFI) is given.

The viability of the cells was measured by staining with the viability dye Zombie Aqua (BioLegend, USA) according to the manufacturer's instructions, prior to the flow cytometry measurements. The 405 nm laser was used for the excitation of the Zombie Aqua dye.

Receptor blocking experiments with antibodies:

For the receptor blocking experiments, purified anti-mouse CD16/32, purified anti-mouse CD64 (FcγRI), purified anti-human CD16, purified anti-human CD32 or purified anti-human CD64 (Biolegend, USA) were added to the cells at 1 μg mL^{-1} in fresh serum-free medium for 30 min at 4 °C before the respective NC samples were added. After the incubation, the nanoparticles were added to the wells and the cells incubated for 1 h at 4 °C.

Plasma screening additional information

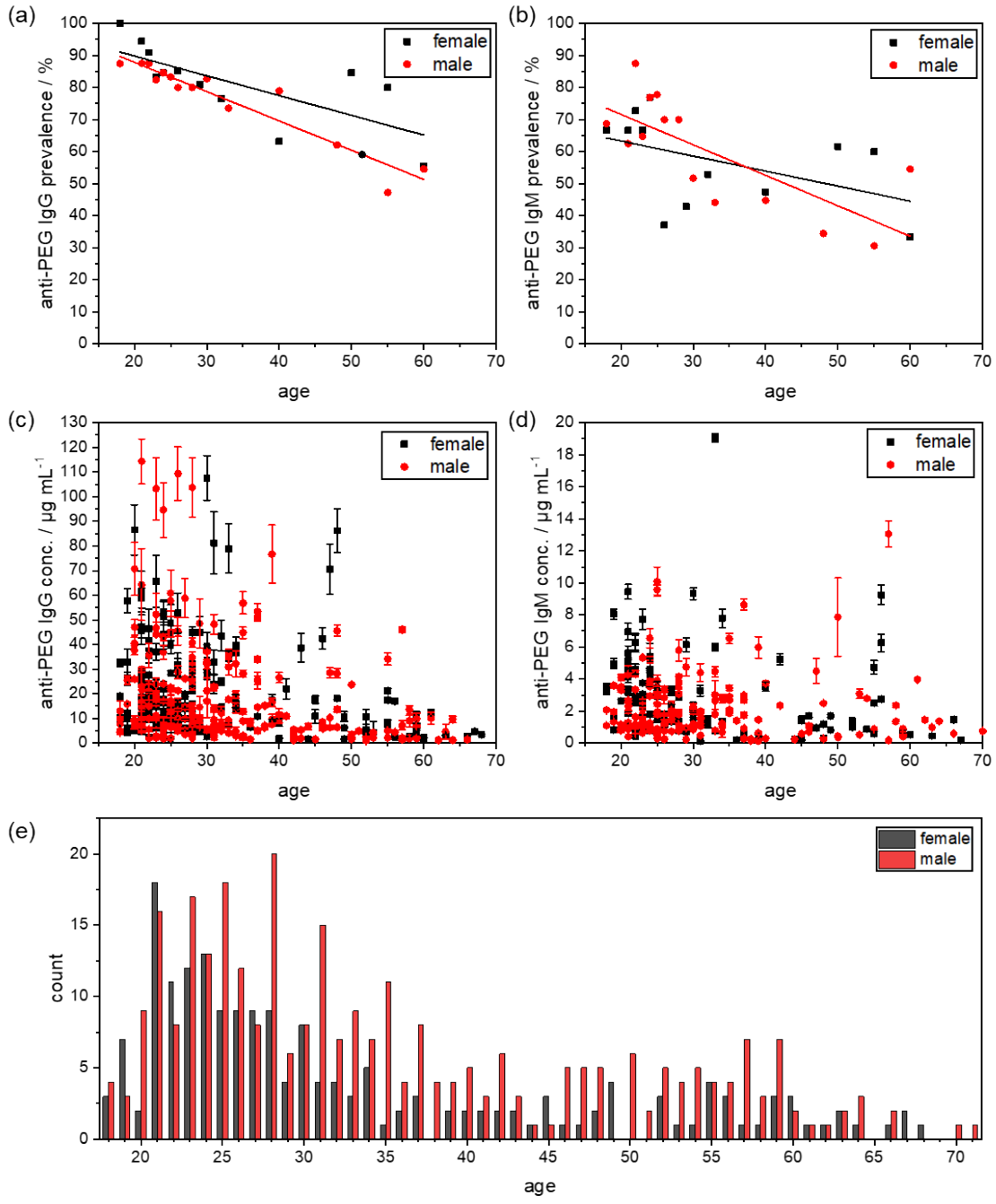

Figure S1: (a, b) Prevalence of anti-PEG IgG and IgM antibody distribution depending on age and separated by sex (black: female, red: male samples). (c, d) Concentration of anti-PEG IgG and IgM antibodies of all measured samples depending on age without sample grouping for individual patients. Error bars represent standard deviation from triplicates. (e) Age distribution of all collected plasma samples (black: female, red: male samples).

Microscale thermophoresis binding curves

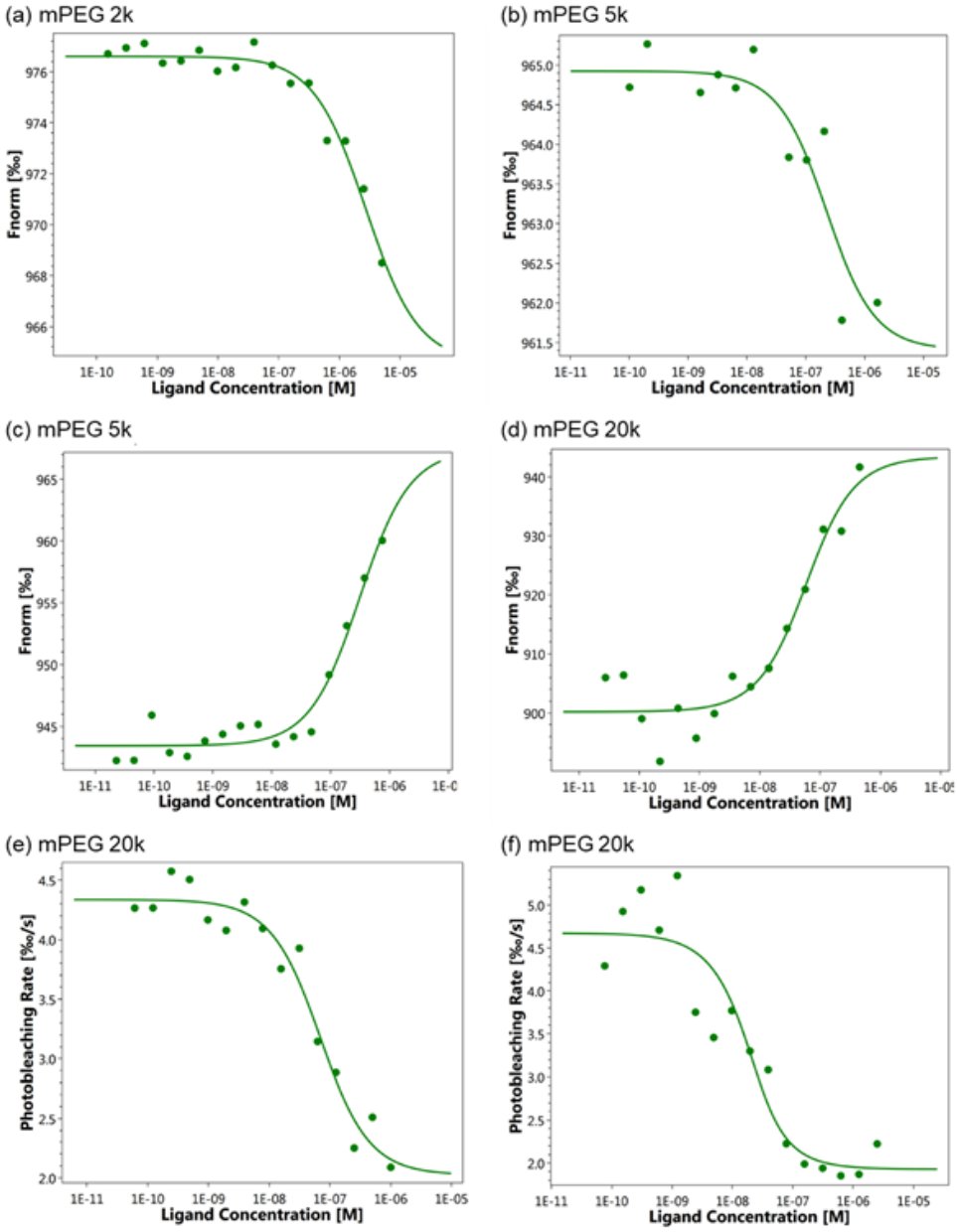

Figure S2: Microscale thermophoresis data analysis. Dose response curves for binding experiments between anti-PEG IgG as ligand and FITC-conjugated mPEG with different chain lengths as target: (a) $mPEG_{2k}$-FITC, (b-c) $mPEG_{5k}$-FITC, (d-f) $mPEG_{20k}$-FITC. Evaluation of raw MST traces was performed according to automatic response evaluation. Due to different experimental parameters (see Table S1), dose response curves were fitted individually according to the law of mass action to obtain K_d values, which were then averaged.

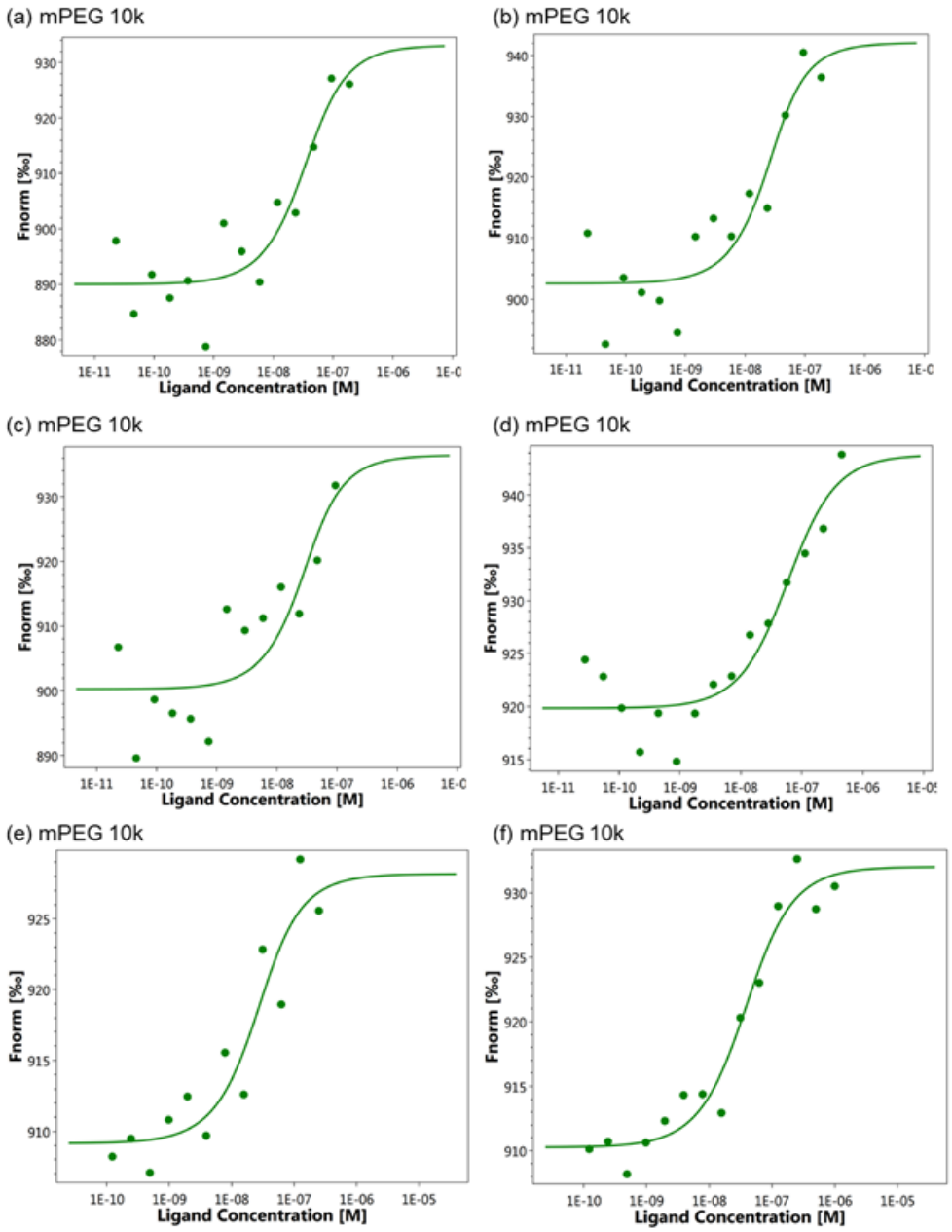

Figure S3: Microscale thermophoresis data analysis. Dose response curves for binding experiments between anti-PEG IgG as ligand and FITC-conjugated mPEG with different chain lengths as target: (a-f) $mPEG_{10k}$-FITC. Evaluation of raw MST traces was performed according to automatic response evaluation. Due to different experimental parameters (see Table S1), dose response curves were fitted individually according to the law of mass action to obtain K_d values, which were then averaged.

Transmission electron microscopy (TEM) imaging of nanocarriers

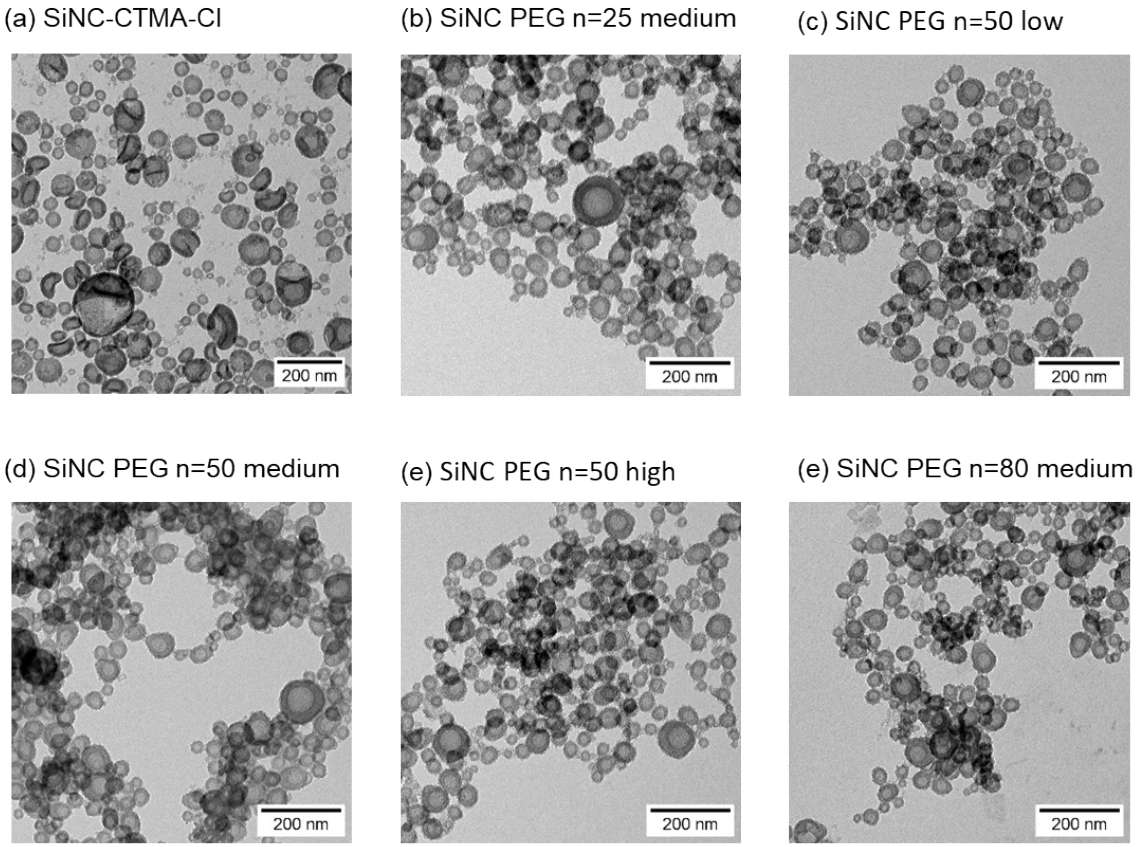

Figure S4: TEM micrographs of SiNCs with different surface functionalization, corresponding to the samples described in the main manuscript Table 2.

LC-MS results of protein corona composition

Table S2: LC-MS identification of proteins found on NC with different stabilization. Proteins were grouped according to function and pure pooled citrate plasma is shown as a reference. Values are represented in % based on all identified proteins. The protein group with the highest abundance was highlighted in grey. *n* corresponds to the number of PEG repeating units.

	plasma (reference)	CTMA-Cl	PEG *n*=50 low	PEG *n*=50 medium	PEG *n*=50 high	PEG *n*=25 medium	PEG *n*=80 medium
Acute Phase	6.03	0.01	0.06	0.13	0.21	0.09	0.04
Coagulation	4.39	79.62	7.58	13.63	13.74	6.18	10.52
Complement	4.54	2.65	1.33	2.58	1.84	2.87	2.54
Immunoglobulins	25.28	1.87	2.41	2.44	2.70	1.34	2.52
Lipoproteins	1.57	8.99	56.12	67.35	63.95	79.72	72.25
Other Plasma Components	7.84	1.63	5.53	2.95	3.63	1.36	2.53
Serum Albumin	50.06	1.14	14.61	1.24	1.70	0.29	0.79
Tissue Leakage	0.30	4.10	12.36	9.67	12.24	8.16	8.81

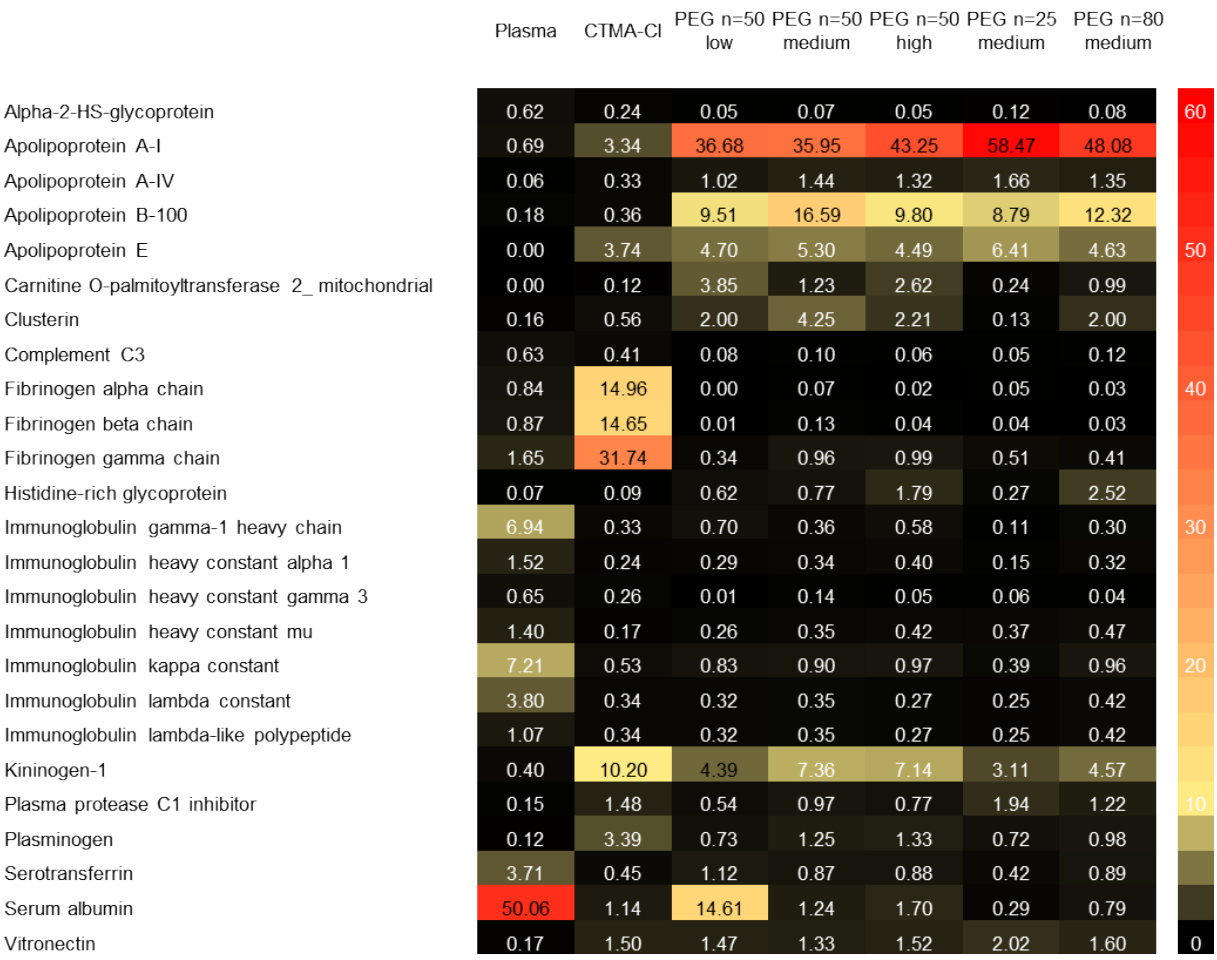

Figure S5: LC-MS identification of proteins found on NC with different stabilization. Heatmap indicating the most abundant proteins (Top 25) in the pure plasma (reference) and protein corona. Values are represented in % based on all identified proteins. *n* corresponds to the number of PEG repeating units.

Cellular uptake additional information

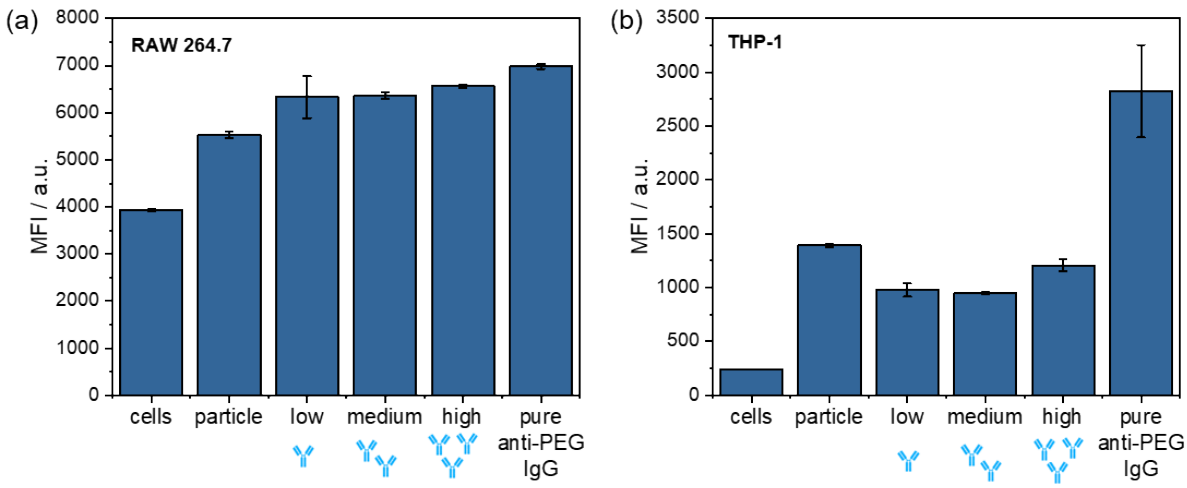

Figure S6: Cellular uptake **at 37 °C** of SiNCs coated with proteins including an increasing concentration of anti-PEG IgG. Plasma samples with a low (<0.5 µg mL^{-1}), medium (9.6 µg mL^{-1}), and high (110.5 µg mL^{-1}) anti-PEG IgG concentration respectively were used for the protein corona preparation. Additionally, pure anti-PEG IgG was used as a positive control. For the negative control, only cells without any addition of NCs were measured. (a) Uptake in murine RAW 264.7 macrophages, displayed in median fluorescence intensity (MFI). (b) Uptake in human THP-1 macrophages, displayed in median fluorescence intensity (MFI).

Fc blocking experiments

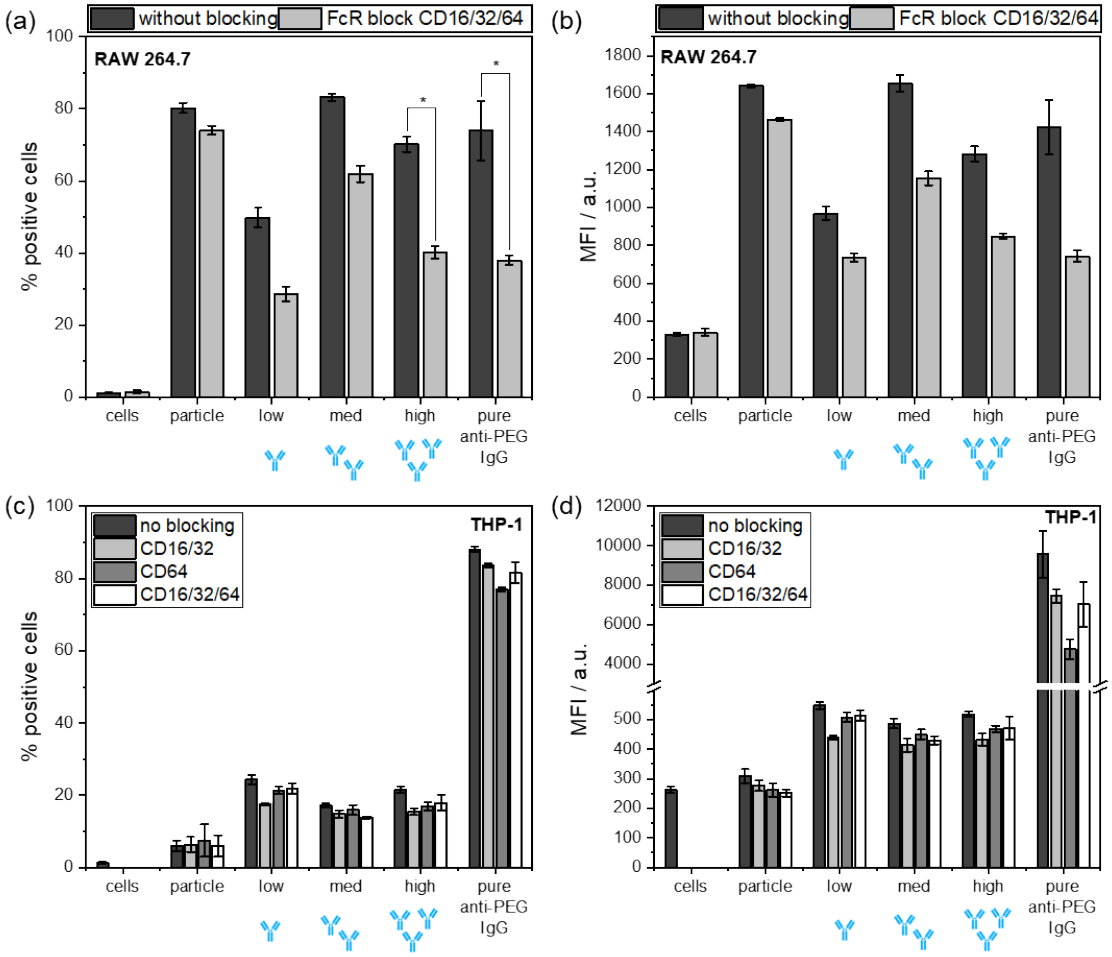

Figure S7: F_c blocking experiments of SiNCs **at 4 °C** with protein corona including an increasing concentration of anti-PEG IgG. Plasma samples with a low (<0.5 µg mL^{-1}), medium (9.6 µg mL^{-1}) and high (110.5 µg mL^{-1}) anti-PEG IgG concentration were used for the protein corona preparation. Additionally, pure anti-PEG IgG was used as a positive control. For the negative control, only cells without any addition of NCs were measured. CD16/CD32 (binding aggregated IgG with low affinity for the ligand) and CD64 (binding monomeric IgG with high affinity for the ligand) receptors were either blocked individually or all three at the same time. Uptake at 4 °C in murine RAW 264.7 macrophages, displayed in % positive cells (a) and median fluorescence intensity (MFI) (b). Uptake at 4 °C in human THP-1 macrophages, displayed in % positive cells (c) and median fluorescence intensity (MFI) (d). Values are mean values with standard deviation of three biological replicates. The analysis of variance (ANOVA) two-way test was used for statistical analysis yielding *$p < 0.05$ corresponding to blocked/unblocked receptors.

References

1. Chen, B.-M., et al., *Measurement of Pre-Existing IgG and IgM Antibodies against Polyethylene Glycol in Healthy Individuals.* Analytical Chemistry, 2016. **88**(21): p. 10661-10666.
2. Jiang, S., et al., *Controlling protein interactions in blood for effective liver immunosuppressive therapy by silica nanocapsules.* Nanoscale, 2020. **12**(4): p. 2626-2637.
3. Simon, J., et al., *Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers.* Angewandte Chemie International Edition, 2018. **57**(19): p. 5548-5553.