Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supplementary Information for

Fully Degradable Protein Nanocarriers by Orthogonal Photoclick Tetrazole-ene Chemistry for the Encapsulation and Release of Functional Drugs

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Experimental section

Materials

All chemicals and materials were used as received. Human serum albumin (>97% purity) and albumin from chicken egg white (grade VI) were purchased from Sigma Aldrich as well as benzene, dimethylsulfoxide (DMSO) (>99%), 4-formylbenzoic acid (97%), benzenesulfonohydrazide (98%) and 5-norbornene-2-carboxylic acid (98%, *endo/exo* mixture), 1,6-hexanediol (99%). Cyclohexane (HPLC grade) and HCl (37%) were purchased from VWR. The block copolymer poly((ethylene-*co*-butylene)-*b*-(ethylene oxide) P((E/B)-*b*-EO) used as the oil soluble surfactant was synthesized as described in literature[1] and consists of a poly((ethylene-*co*-butylene) block (NMR: M_n = 3,900 g/mol) and a poly(ethylene oxide) block (NMR: M_n = 2,700 g/mol). The anionic surfactant sodium dodecyl sulfate (SDS) was purchased from Alfa Aesar. DQ™ ovalbumin (D-12053) was purchased from Molecular Probes and hydrophilic CdTe quantum dots from PlasmaChem. Sulforhodamine 101 (SR101) was purchased from BioChemica, Aldrich. Amicon Ultra-2 centrifugal filter devices were purchased from Merck Millipore (100,000 nominal molecular weight limit (NMWL)). The human blood plasma, prepared according to the standard guidelines, was obtained from the University Clinic of Mainz (Germany). Due to the high variation of protein composition of

different patients, a pool of plasma obtained by the mixture of serum of several healthy donors was used for all measurements. For cell experiments, Histopaque-1077 and Hank´s Balanced Salt Solution from Sigma Aldrich was used. Magnetic cell separator (MACS) was obtained from Miltenyi Biotec. Propidium iodide solution was purchased from eBiosciences. The immunostimulant lipopolysaccharide (LPS) and R848 were obtained from Invivogen (Toulouse, France). The magnesium and calcium free phosphate-buffered saline, was purchased from Life Technologies. Demineralized water was used for all experiments.

Instrumentation.

For ultrasonication, a Branson Sonifier W-450-Digital was used with a ½" tip, operating under ice cooling for 3 min at 70% amplitude with pulse cycles of 20 s sonication and 10 s pauses.

Sigma 3 k-30 from Sigma Centrifuges, UK was used for centrifugation.

Morphological studies were performed with transmission electron microscopy (TEM). For the measurements, 20 µL of diluted PNC dispersion was placed onto a 300 mesh carbon-coated copper grid and allowed to dry under ambient conditions. The TEM measurements were performed with a Jeol 1400 transmission electron microscope was used with an accelerating voltage of 120 kV. For trehalose embedding, 8 µL sample were mixed with 2 µL OsO4 solution and 8µL trehalose solution (1 wt% in water), placed on a lacey copper grid and blotted using filter paper.

The average size and size distribution of the PNCs were measured via dynamic light scattering (DLS) at 25 °C using a Nicomp 380 submicron particle sizer (Nicomp Particle Sizing Systems, USA) at an angle of 90°.

For Zeta potential measurements in 10^{-3} M potassium chloride solution at pH 6.8 and 25 °C, the Malvern Zeta sizer (Malvern Instruments, U.K.) was used.

All dynamic light scattering experiments in human blood plasma were performed on a commercially available instrument from ALV GmbH consisting of a goniometer and an ALV-

5000 multiple-tau full-digital correlator with 320 channels. A helium-neon laser (JDS Uniphase with a single mode intensity of 25 mW operating at a laser wavelength of $\lambda_0 = 632.8$ nm) was used as the light source. All solutions for light scattering experiments were prepared in dust-free quartz light scattering cuvettes (inner diameter 18 mm, Hellma, Müllheim), which were cleaned prior to use with distilled acetone.

Fluorescence intensity and absorbance measurements were performed with the Infinite M1000 plate reader from Tecan, Austria using 96-well plates.

UV/Vis spectroscopy was performed with a PerkinElmer Lambda 25 spectrophotometer.

For nuclear magnetic resonance (NMR) analysis, ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance spectrometers operating with 250, 500 and 700 MHz frequencies. Deuterated chloroform or deuterated DMSO was used as the solvent.

Flow cytometric analysis was performed with a BD FACS Canto II flow cytometer equipped with BD FACSDiva software (BD Biosciences). Data were analyzed using FlowJo software (FlowJo, Ashland, USA) and are presented as means ± SEM of the values (GraphPad Software, La Jolla, USA).

Methods

Synthesis DN1. Dinorbornene cross-linker was synthesized by esterification reaction of 2.28 g (19.3 mmol) 1,6 hexanediol with 8.05 g (58.3 mmol) 5-norbornene-2-carboxylic acid. The starting compounds were dissolved in 200 mL of benzene, pTsOH was used in catalytic amounts and the mixture was stirred at 100 °C under reflux using a Dean-Stark apparatus. Afterwards, the product was washed with twice with 30 mL saturated $NaHCO_3$ solution and twice with 30 mL of water to remove unreacted norbornene acid. The product was purified after solvent removal by column chromatography (PE:EtOAc 3:1, R_F value: 0.71) and verified by TLC prior to NMR analysis. Yield: 3.54 g (9.88 mmol, 52%).

DN1: ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.24 – 5.85 (m, 4 H, CH=CH), 4.20 – 3.88 (m, 4 H, OCH_2), 3.20 – 2.90 and 2.21 (m, 6 H, CH), 1.90 (m, 2 H, CH_2), 1.74 – 1.28 (m, 14 H, CH_2,

CH) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 176.26-174.76 (C=O), 137.99-132.29 (C=C), 64.31-64.07 (C-O), 49.59 (CH-COO), 46.58-25.61 (C_{aliph}) ppm. FT-IR ν=3061 (=CH), 2970 (C-H), 2869 (CH_2), 1732 (C=O), 1178 (C-O) cm^{-1}.

Synthesis of 4-(2-phenyl-2H-tetrazol-5-yl)benzoic acid (TET). The synthesis of TET was achieved in three steps, following previously reported procedures.[2, 3] In the first step, benzenesulfonohydrazide (1.72 g, 10.0 mmol) was added to 1.5 g (10.0 mmol) 4-formylbenzoic acid in 100 mL EtOH and the mixture stirred for 30 min. The resulting hydrazone adduct was precipitated with 200 mL DI-water, filtered and dried overnight. Yield: 2.74 g (9.13 mmol, 91.3%). Next, the diazonium salt was prepared by dissolving 0.79 g (11.4 mmol) $NaNO_2$ in 4.6 mL water and adding it to a cooled mixture of 1.05 g (11.4 mmol) aniline in 18 mL water/EtOH (1:1) with 3 mL conc. HCl. The obtained diazonium salt was added dropwise to the hydrazone dissolved in 68 mL pyridine and cooled with an ice-salt-bath to -20 °C. The mixture was stirred for 6 h and extracted afterwards for three times with 150 mL EtOAc. The organic solution was washed with 100 mL 3 M HCl. After removal of the solvent, the product was isolated by recrystallization from hot EtOH. Yield: 2.23 g (8.35 mmol, 91.5%).

^{1}H NMR (250 MHz, DMSO-d6) δ 13.28 (s, 1 H, COOH), 8.30 (d, J = 8.5 Hz, 2 H, C_6H_4), 8.23-8.10 (m, 4 H, C_6H_4, C_6H_5), 7.76-7.58 (m, 3 H, C_6H_5) ppm.

Coupling of 4-(2-phenyl-2H-tetrazol-5-yl)benzoic acid to proteins. For the coupling reaction of 4-(2-phenyl-2*H*-tetrazol-5-yl)benzoic acid to ovalbumin and human serum albumin, 500 mg of the respective protein were added to 5 mL DMSO. TET and about 10 mol-% DMAP respective to the TET amount were added and the mixture stirred until a homogeneous solution was obtained. In the next step, equivalent amounts of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) respective to TET were added and the reaction was allowed to proceed under ambient conditions for 48 h. The product was precipitated with 50 mL i-PrOH, filtered and dissolved in water. The side-products were removed by dialysis

for 4 days (MWCO 1000 Da) under frequent water exchange. After lyophilization, 430 mg of the product was obtained. The amount of TET/protein was determined by UV/vis spectroscopy in DMSO, using the absorption maximum of TET in DMSO at 280 nm for calibration and unmodified proteins as blanks to exclude their contribution to the absorption intensity (Figure S2). The amounts used for the reaction and the obtained degrees of modification are depicted in Table S1.

Protein Nanocarrier (PNC) synthesis. First, 12.5 mg of the TET-functionalized protein were dissolved in 0.25 g buffer (Na_2HPO_4/KH_2HPO_4 at pH 7.6). In case of encapsulation of R848, 50 µL with a concentration of 10 mg/mL in DMSO was added to the aqueous solution. 17.9 mg of surfactant P((E/B)-*b*-EO) were dissolved in 3.75 g of cyclohexane and the mixture was added drop-wise to the stirred aqueous solution. The pre-emulsion was homogenized by ultrasound. A third solution containing of 1.0 mg P((E/B)-*b*-EO) and 0.5 g of cyclohexane and a 100-fold molar excess (to TET in the system) of the dinorbornene cross-linker was then added drop-wise to the stirred miniemulsion. The mixture was transferred to a quartz glass tube attached to a peristaltic pump with a flow rate of 2.5 mL/min. The miniemulsion was irradiated with a hand-held UV lamp at 254 nm for 30 min.

After preparation, the PNCs were purified by repetitive centrifugation (3 times for 20 min, RCF 1664) to remove excess surfactant and cross-linker and re-dispersed in pure cyclohexane. For transfer into the aqueous phase, 500 µL of PNC dispersion from the cyclohexane phase were added drop-wise to 5 g of an aqueous SDS solution (0.1 wt-%) under mechanical agitation in an ultrasound bath over 3 min at 25 °C (25 kHz). Subsequently, the samples were stirred in open vials for 24 h at 25 °C to evaporate cyclohexane. For removal of excess SDS, the dispersion was purified via Amicon Ultra-2 centrifugal filter (3 times for 20 min, RCF 1664) and redispersed in DI-water. The successful removal of excess SDS could be monitored by the concentration-dependent color change of stains-all dye (see Figure S8).[4]

Determination of PNC permeability and encapsulation efficiency. The permeability of the PNC-shell was studied by the encapsulation of the fluorescent dye SR101. SR101 shows an absorption maximum at 550 nm and an emission maximum at 605 nm.

Protein Nanocarrier Preparation: 0.54 mg of SR101 was mixed with the aqueous phase and the nanocarrier preparation was carried out as described above. After re-dispersion in the aqueous SDS solution, the PNCs were sedimented by centrifugation with the Amicon Ultra-2 centrifugal filter devices and the fluorescence intensity of the permeate was measured at the plate reader. The measured value quantifies the non-encapsulated amount of SR101, which is located in the continuous phase. The encapsulation efficiency was calculated by correlating the obtained fluorescence intensity to the intensity of the starting concentration of SR101. In order to rule out any bleaching effect during the PNC preparation, a calibration curve was recorded by treating the dye solution with UV-light using the same conditions as for PNC synthesis.

To determine the permeability, the dispersion was stored at room temperature in the dark over several days and the experiment was repeated over a given period of time and the fluorescence signal was compared to the initial value. The experiment was conducted two times with three single measurements.

Biodegradability of PNCs. The degradation of DQ ovalbumin labeled PNCs (encapsulation of 150 µL with a concentration of 1 mg/mL in PBS) was studied with the serine protease trypsin. 20 µL of trypsin with a concentration of 0.5 mg/mL in PBS buffer were added to 100 µL PNC dispersion. Control measurements of the PNCs in PBS buffer were carried out additionally. The changes in fluorescence intensity were recorded over 2.5 h at 37 °C. For the proteolytic release of hydrophilic CdTe quantum dots, 3 mg of the quantum dots were encapsulated in HT-DN1. After purification and transfer to water, the PNC dispersion was treated with trypsin (amounts as above), aliquots were taken over time, centrifuged for 10 min and the supernatant

was used for measurement. The control measurement (0 min) was performed by adding water instead of trypsin.

Toxicity evaluation of PNCs. Human monocyte-derived Dendritic cells (moDCs) were generated as previously described.[5] Briefly, adult peripheral blood mononuclear cells (PBMCs) were isolated from fresh buffy coats, obtained from healthy voluntary donors upon informed consent (University of Mainz, Germany), by centrifugation through Histopaque®-1077 density gradient media for 20 min at 900 x g and 20 °C. The interphase consisting of PBMCs was subsequently extracted and washed with Hank´s Balanced Salt Solution. $CD14^+$ monocytes were isolated from the PBMC fraction by positive selection using CD14 MicroBeads, MACS LS columns and a magnetic cell separator in accordance with the manufacturer's instructions. Purified $CD14^+$ monocytes were cultured in X-Vivo 15 medium supplemented with L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 200 U/mL GM-CSF and 200 U/mL IL-4 for 6 days. Subsequently, immature moDCs were collected and co-cultured in presence of different concentrations of the PNCs (1.0 / 10 / 100 µg/mL) or without PNCs for 24 h. Subsequently, moDCs were harvested and incubated with 10 µL propidium iodide solution for 2 min in order to stain nuclei of dead cells, followed by flow cytometric analysis.

Dynamic Light Scattering in human blood plasma. For stability measurements in human blood plasma, first DLS analysis of the pure PNCs was performed by dilution of 100 µL of PNC dispersion with 1 mL filtered PBS buffer solution at physiological pH (pH 7.4) and salinity (0.125 M). For the measurement of PNCs in human blood plasma, 100 µL of the PNC dispersion were added to 1 mL plasma filtered through a 0.2 µm GS filter and the mixture was incubated at 37 °C for 30 min. DLS analysis of plasma was performed similarly to maintain the same dilutions. All DLS measurements were performed at 37 °C.

Protein corona analysis. Human blood plasma was obtained from the Department of Transfusion Medicine Mainz from healthy donors. To remove any aggregated plasma samples were centrifuged for 30 min at 20000 g before usage.

Protein nanocarriers (1 mg) were incubated with human blood plasma (1 mL) for 1 h at 37 °C with constant agitation (300 rpm). Unbound proteins were removed via centrifugation at 20000 g for 30 min. The remaining nanocarrier pellet was resuspended in PBS (1 mL) and washed by three centrifugation steps (20000 g, 30 min). After the last washing step, protein nanocarriers bound with proteins were resuspended in urea-thiourea buffer (7 M urea, 2 M thiourea, 4% CHAPS) and bound proteins were quantified by Pierce assay and identified with SDS-PAGE and LC-MS/MS.

SDS-PAGE. Protein solutions (6 µg in 26 µL total volume) were added to LDS NuPAGE sample buffer (4 µL) and NuPAGE sample reducing agent (10 µL). This mixture (total volume: 40 µL) was heated up for 10 min at 70 °C, loaded onto a 10% NuPAGE bis-tris gel (10-well) in a chamber with NuPAGE MES SDS running buffer and SeeBlue Plus2 prestained standard as protein marker. Proteins were separated at 100 V for 1 h and washed with distilled water. Gels were stained with Simply Blue™ for at least 4 h and de-stained with distilled water overnight.

Protein quantification. Protein concentration were quantified with the Pierce 660 nm protein Assay (Thermo Scientific; Germany) according to manufacturer's instructions and bovine serum albumin (Serva, Germany) was used a standard. Absorption was measured at 660 nm at the plate reader.

In solution digestion. Tryptic digestion was performed after the protocol of Tenzer et al.[6] with the following adjustments. Proteins were precipitated overnight using ProteoExtract protein precipitation kit (CalBioChem) according to the manufacturer's instructions. The resulting protein pellet was isolated via centrifugation (14000 g, 10 min) and washed twice with washing buffer (500 µL, CalBioChem). The resulting protein pellet was resuspended in

RapiGest SF (Waters Cooperation) dissolved in 50 mM ammonium bicarbonate (Sigma Aldrich) and incubated at 80 °C for 15 min. Proteins were reduced by adding dithiothreitol (Sigma Aldrich) to gain a final concentration of 5 mM and incubated for 45 min at 56 °C. For alkylation, iodoacetoamide (final concentration 15 mM, Sigma Aldrich) was added and the solution was incubated in the dark for 1 h. Tryptic digestion with a trypsin:protein ratio of 1:50 was carried out over 14 h at 37 °C. The reaction was quenched by adding 2 µL hydrochloric acid. To remove degradation products of RapiGest SF, peptide samples were centrifuged (14.000 g, 15 min) and the remaining supernatant containing digested peptides was applied to LC-MS/MS.

Liquid-chromatography mass-spectrometry (LC-MS) analysis. For absolute quantification, peptide samples were spiked with 25 fmol/µL of Hi^3 EColi Standard (Waters Cooperation), applied to a C18 nanoACQUITY Trap Column (5 µm, 180 µm x 20 mm,) and separated on a C18 analytic reversed phase column (1.7 µm, 75 µm x 150 mm) using a nanoACQUITY UPLC systems. The mobile system consisted of phase (A) 0.1% (v/v) formic acid in water and phase (B) acetonitrile with 0.1% (v/v) formic acid. The flow rate was set to 300 µL/min and with a gradient of 2 – 37% mobile phase (A) to (B) over 70 min. As a reference component, Glu-Fibrinopeptide (150 fmol/µL, Sigma) was infused at a flow rate of 500 µL/min. Eluted peptides were infused into a Synapt G2Si (Waters).

The mass spectrometer was operated in resolution mode performing data-independent acquisition (MS^E) and electrospray ionization (ESI) was performed in positive ion mode with nanoLockSpray source. Data acquisition was carried out over 90 min with a mass to charge range (m/z) over 50-2000 Da, scan time of 1 s and ramped trap collision energy from 20 to 40 V. Data processing was conducted with MassLynx 4.1 and proteins were identified with Progenesis QI for Proteomics Version 2.0 with continuum data using a reviewed human data base (Uniprot). The peptide sequence of ovalbumin (chicken) and Hi^3 Ecoli standard

(Chaperone protein CLpB, Waters Cooperation) was added to the database for absolute quantification. Data was processed with high energy, low energy and peptide intensity values of 120, 25, and 750 counts. For protein and peptide identification the following search criteria were applied: one missed cleavage, maximum protein mass 600 kDa, fixed carbamidomethyl modification for cysteine, variable oxidation for methionine and protein false discovery rate of 4%. Two assigned peptides and five assigned fragments are required for protein identification and three assigned fragments are necessary to identify a peptide. Further, peptides with a score below 4 were excluded and based on the TOP^3/Hi^3 approach[7] the absolute amount of each protein in fmol was generated. A total list of all identified proteins in each sample is provided in Table S3.

Cell experiments. Bone marrow-derived dendritic cells (BMDCs) were differentiated from bone marrow progenitors (BM cells) of 8- to 10-week-old C57BL/6 mice as first described by Scheicher et al.[8] and modified by Gisch et al.[9] Briefly, the bone marrow was obtained by flushing the femur, tibia, and hip bone with Iscove's Modified Dulbecco's Medium (IMDM) containing 5% FCS (Sigma-Aldrich, Deisenhofen, Germany) and 50 µM β-mercaptoethanol (Roth, Karlsruhe, Germany). For the BMDC stimulation analysis via flow cytometry the bone marrow cells (2 x 105 cells/1.25 mL) were seeded in 12 well suspension culture plates (Greiner Bio-One, Frickenhausen, Germany) with culture medium (IMDM with 5% FCS, 2 mM L-Glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin (all from Sigma-Aldrich), and 50 µM β-mercaptoethanol), supplemented with 5% of GM-CSF containing cell culture supernatant derived from X63.Ag8-653 myeloma cells stably transfected with a murine GM-CSF expression construct.[10] On 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, BMDCs were treated with nanocarrier formulations and LPS (positive control) as indicated in the figure legends. Before usage, all nanoparticle solutions were checked for endotoxin

contaminations by limulus amebocyte lysate (LAL) assay (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions.

To analyze the expression of the BMDC surface maturation marker CD86, BMDCs were harvested and washed in staining buffer (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. After that, the cells were incubated with phycoerythrin (PE)-conjugated anti-CD86 (clone GL-1) and PE-Cy7-labelled anti-CD11c (clone N418) (all from eBioscience, San Diego, USA) for 30 min at 4 °C.

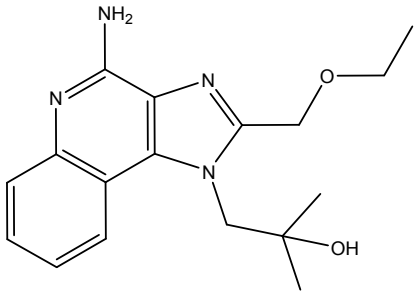

Figure S1: Structure of Resiquimod (R848).

Table S1: Reagents used for protein modification with TET.

	Protein/ mol	TET/ mol	DMAP/ mol	EDC/ mol	Spectroscopically determined amount of TET/protein
OVA	$5.83 \cdot 10^{-6}$	$1.17 \cdot 10^{-4}$	$1.17 \cdot 10^{-5}$	$1.17 \cdot 10^{-4}$	13
HSA	$2.26 \cdot 10^{-6}$	$4.52 \cdot 10^{-5}$	$4.52 \cdot 10^{-6}$	$4.52 \cdot 10^{-5}$	12

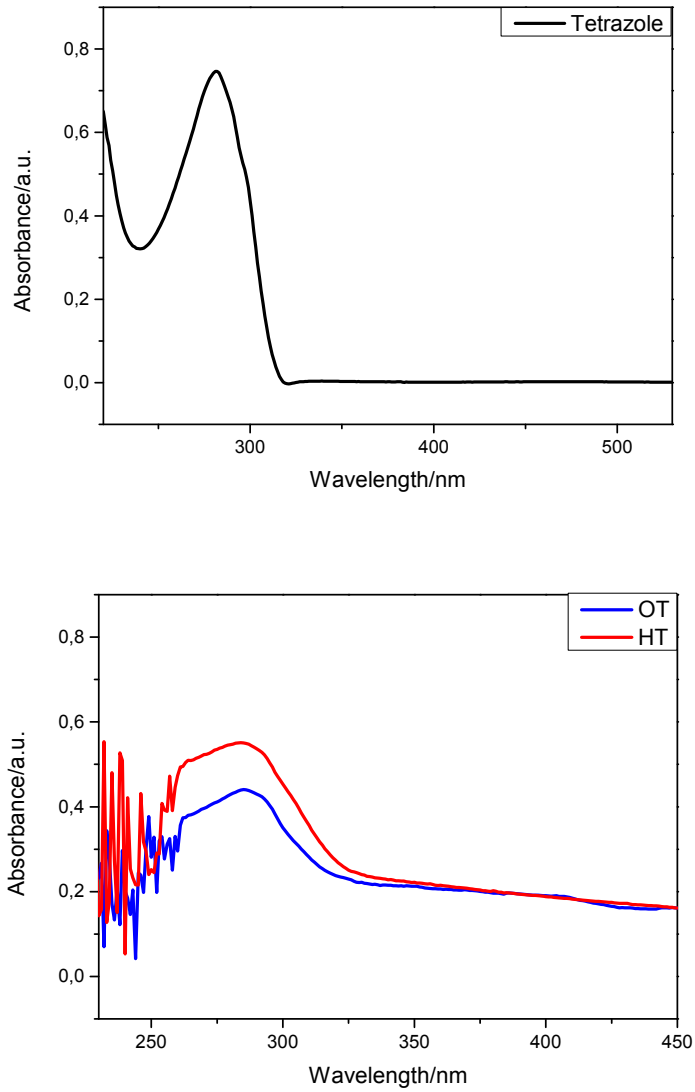

Figure S2: Top: absorption spectrum of TET. Bottom: absorption spectra of TET-coupled proteins after blanking with native proteins to exclude their contribution to the absorption intensity (OT for OVA-TET, HT for HSA-TET).

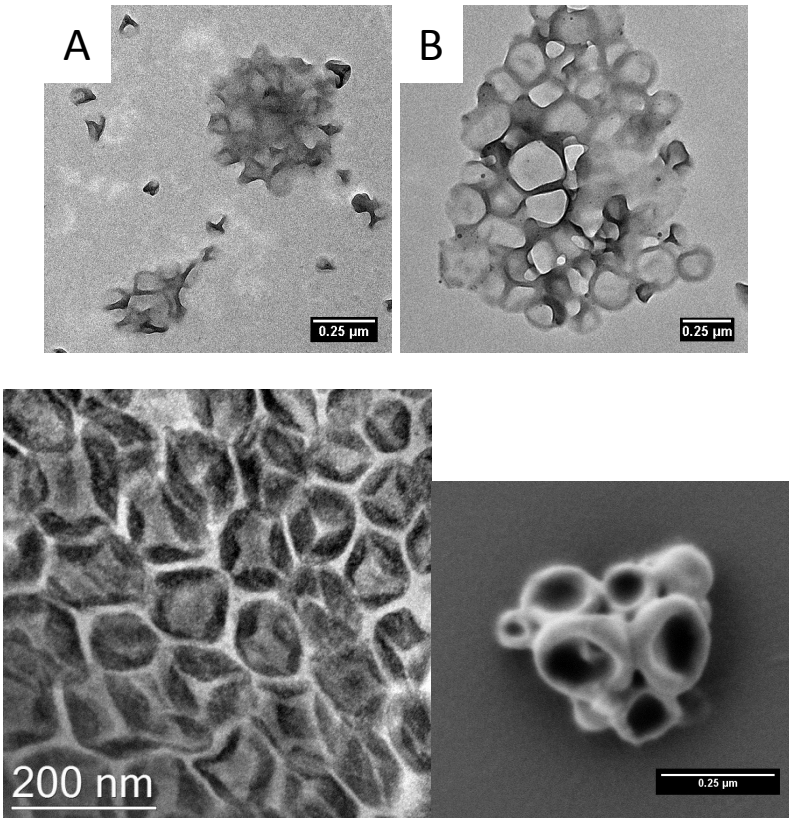

Figure S3: *top*: Drop-cast transmission electron microscopy images of PNCs. A: HT-DN1, B: OT-DN1. *Bottom*: left: TEM in trehalose of OT-DN1. *Right:* SEM image of OT-DN1.

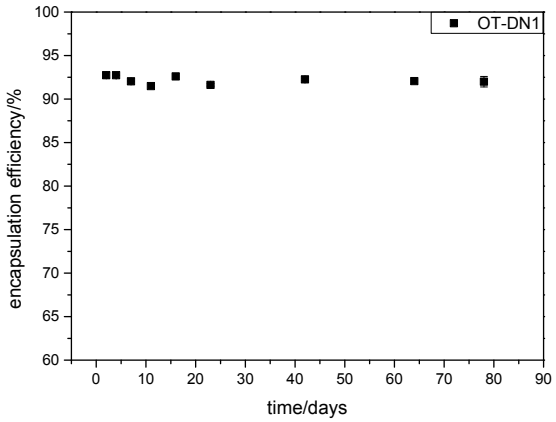

Figure S4: Encapsulation efficiency of OT-DN1 PNCs determined by measurement of the fluorescence intensity of sulforhodamine SR101 dye. Day 2 as the starting day means 36 h after transfer of the PNCs from the organic to the aqueous phase. The experiment was conducted twice with two single measurements per sample.

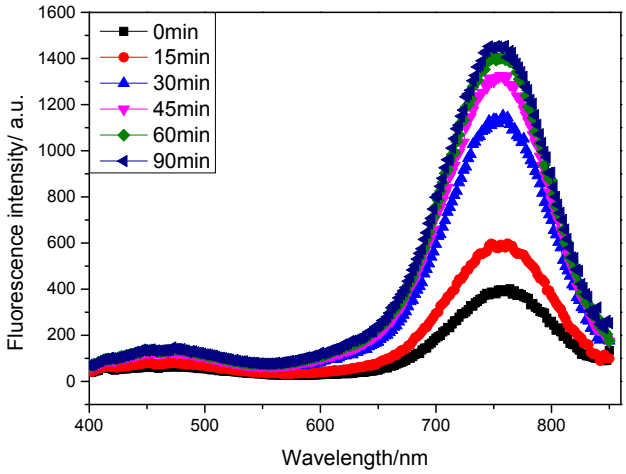

Figure S5: Fluorescence spectra of HT-DN1 PNC supernatant with hydrophilic, carboxyfunctionalized CdTe quantum dots recorded with an excitation wavelength of 364 nm. 0 min is before trypsin addition.

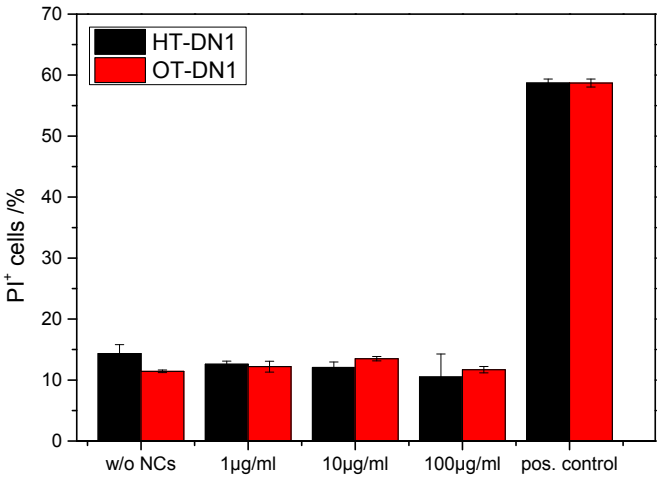

Figure S6: Toxicity analysis of OT-DN1 and HT-DN1 PNCs incubated with human moDCs for 24 h. Dead moDCs are displayed as PI^+, obtained after staining with propidium iodide.

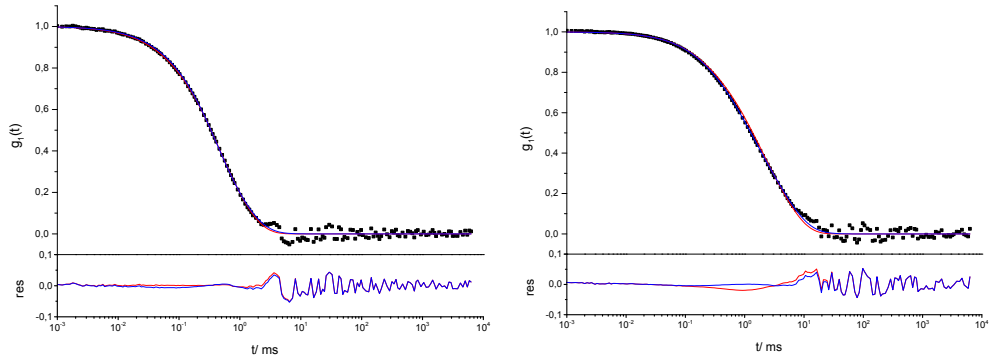

Figure S7: Dynamic light scattering measurement of PNCs in human blood plasma after a method developed by Rausch et al.[11] Self-autocorrelation function of the mixture of PNCs with human blood plasma (left: with HT-DN1, right: OT-DN1). The data points in black derive from the PNC/plasma mixture. The red curve represents the force fit and its residue without the intensity contribution from aggregates. It fits well with the blue curve which considers intensity contribution from aggregates. The measurement was performed at an angle of 90° and 37 °C.

Table S2: Zeta-potential of PNCs (OT for OVA-TET, HT for HSA-TET conjugates) after transfer to the aqueous phase and purification and zeta-potential of these samples after incubation in human blood plasma.

Sample	ζ [mV]	ζ [mV] in plasma
OT-DN1	-37 ±12	-14±4
HT-DN1	-30±10	-16±5

Table S3: Detailed description of the protein corona composition (20 major proteins) of OT-DN1 and HT-DN1 PNCs.

Description (% based on all identified proteins)	**OT-DN1**
Clusterin	23.26

Ig kappa chain C region	7.93
Fibronectin	7.94
Apolipoprotein A-IV	8.32
Ig mu chain C region	5.85
Complement C1q subcomponent subunit C	4.59
Serum albumin	3.65
Complement C1q subcomponent subunit B	3.16
Ig gamma-1 chain C region	2.54
Zinc finger protein basonuclin-2	2.53
Ig lambda-3 chain C regions	2.14
Ig gamma-2 chain C region	1.96
Complement factor H	1.56
Actin, cytoplasmic 2	1.64
Ig alpha-1 chain C region	1.48
Complement C1q subcomponent subunit A	1.38
Apolipoprotein A-I	1.3
Fibrinogen beta chain	1.27
Complement C3	1.21

Description (% based on all identified proteins)	**HT-DN1**
Ig kappa chain C region	12.14
Ig mu chain C region	10.81
Serum albumin	4.55
C4b-binding protein alpha chain	3.94
Complement C1q subcomponent subunit C	8.72
Fibrinogen beta chain	2.04

Complement factor H	2.15
Ig gamma-1 chain C region	2.51
Complement C1q subcomponent subunit B	5.14
Fibrinogen gamma chain	1.7
Ig lambda-3 chain C regions	2.7
Fibrinogen alpha chain	1.04
Fibronectin	3.22
Ig gamma-2 chain C region	1.79
Complement C3	1.96
Immunoglobulin lambda-like polypeptide 5	1.36
Ig gamma-3 chain C region	1.16
Complement C1q subcomponent subunit A	2.35
Ig heavy chain V-III region	1.05
Platelet-activating factor acetylhydrolase IB subunit beta	1.52

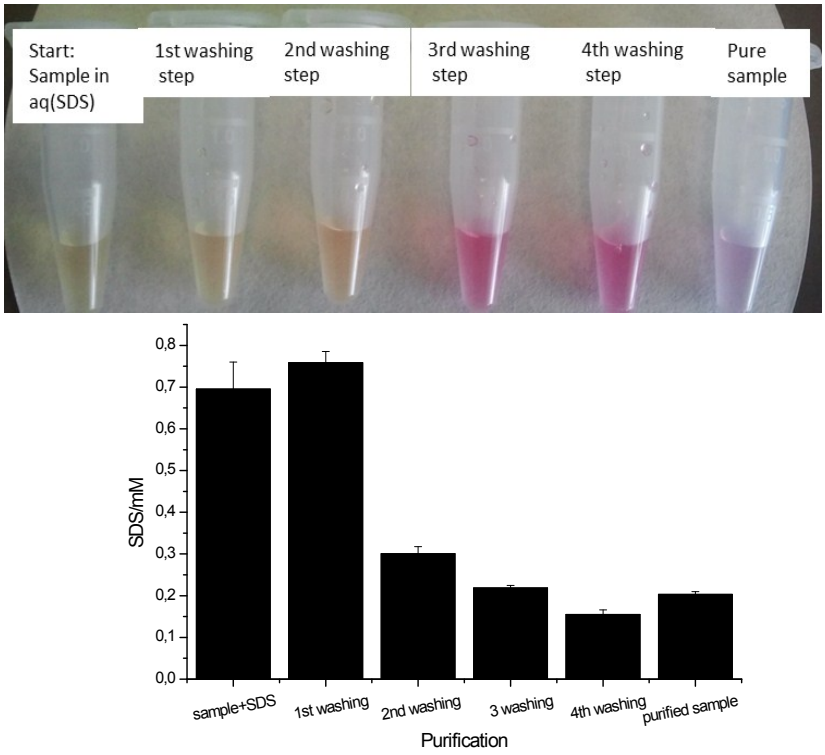

Figure S8: SDS quantification of OT-DN1 with stains-all dye. Top: colorimetric change of the dye from yellow (high SDS content) to purple (low SDS content). Bottom: SDS amount in the sample before and after purification as well as the amount in the respective supernatants.

Spectra

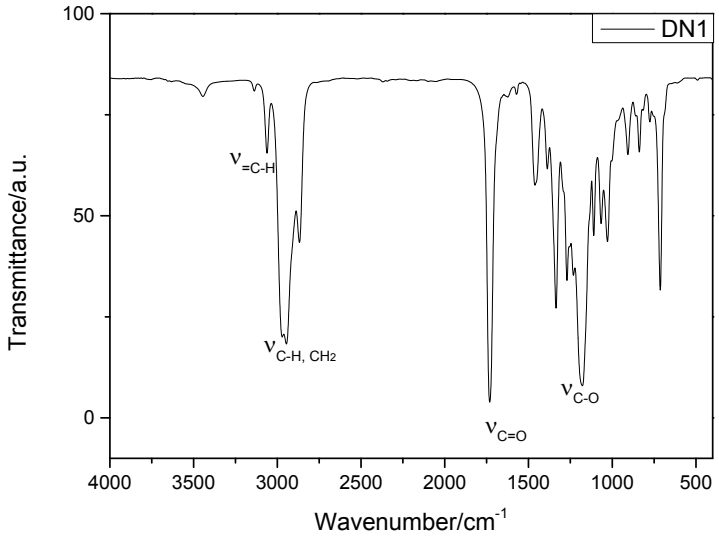

Figure S9: FT-IR spectrum of dinorbornene cross-linker DN1.

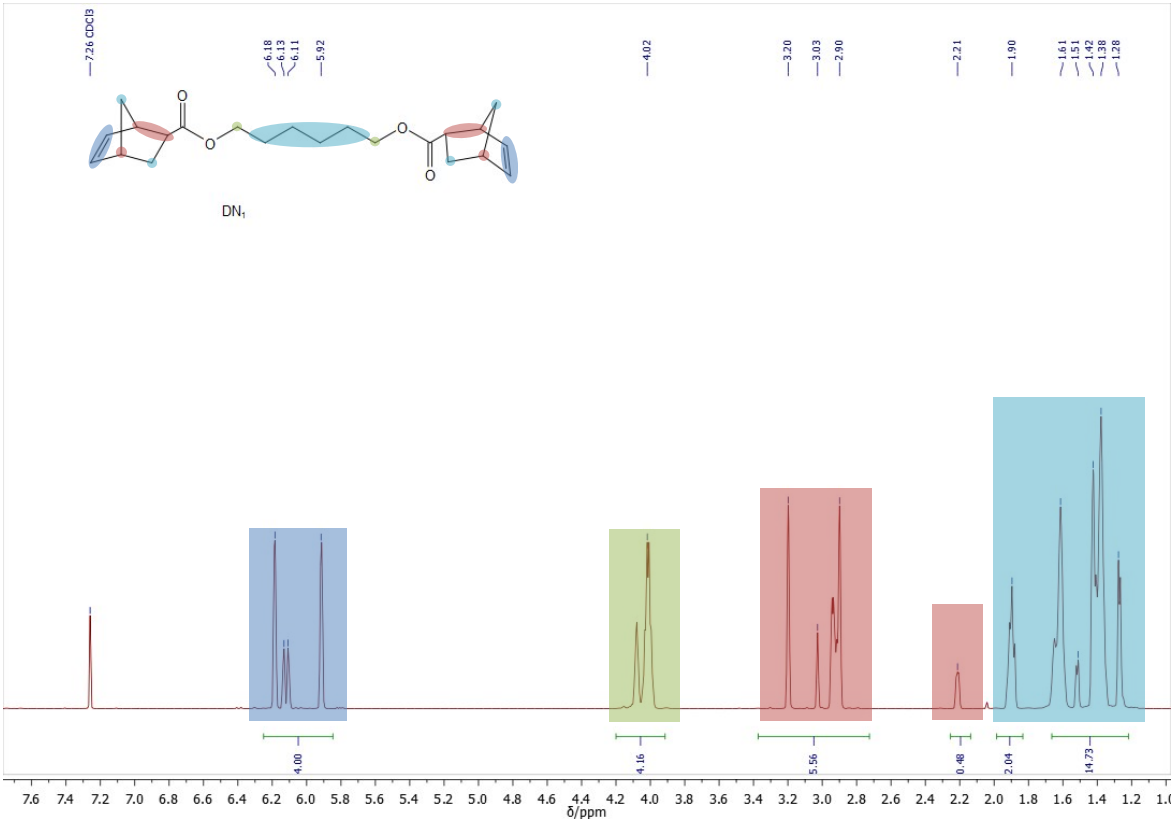

Figure S10: ^{1}H NMR of DN1 (700 MHz, 298K, $CDCl_3$).

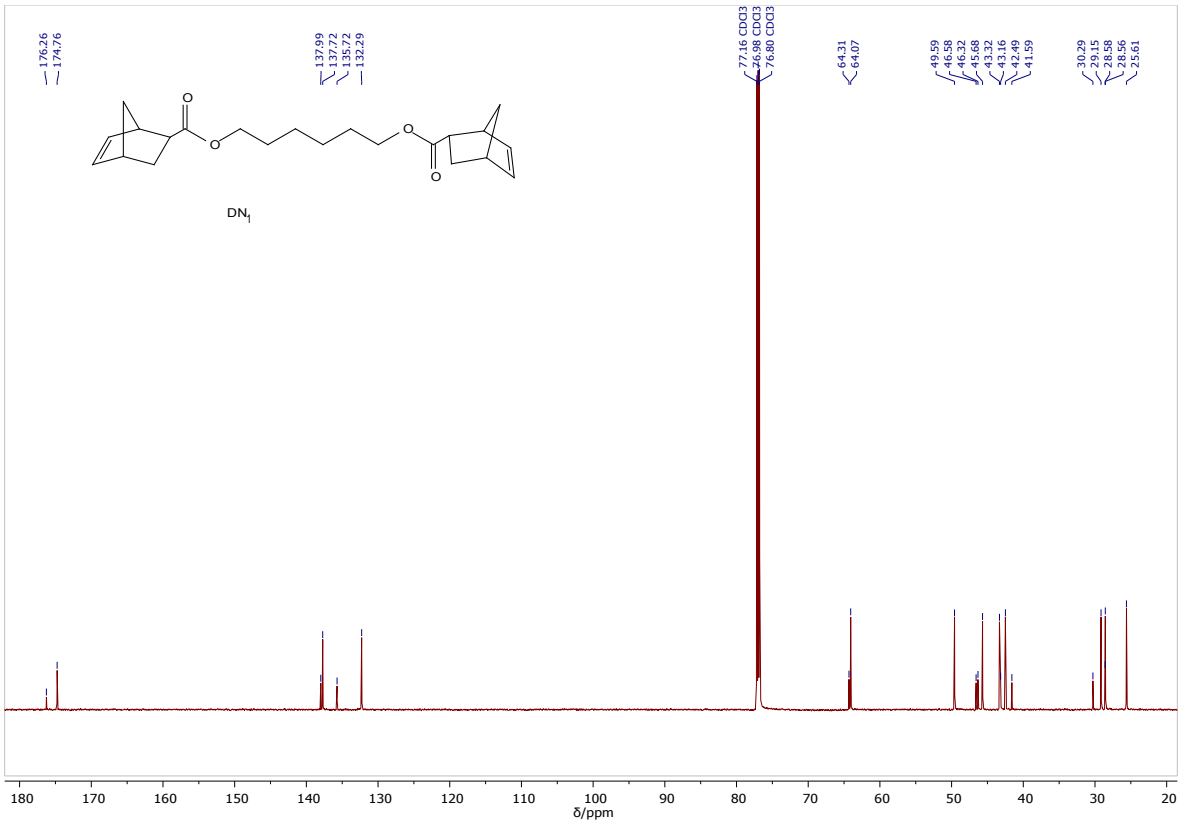

Figure S11: ^{13}C NMR of DN1 (176 MHz, 298 K, $CDCl_3$).

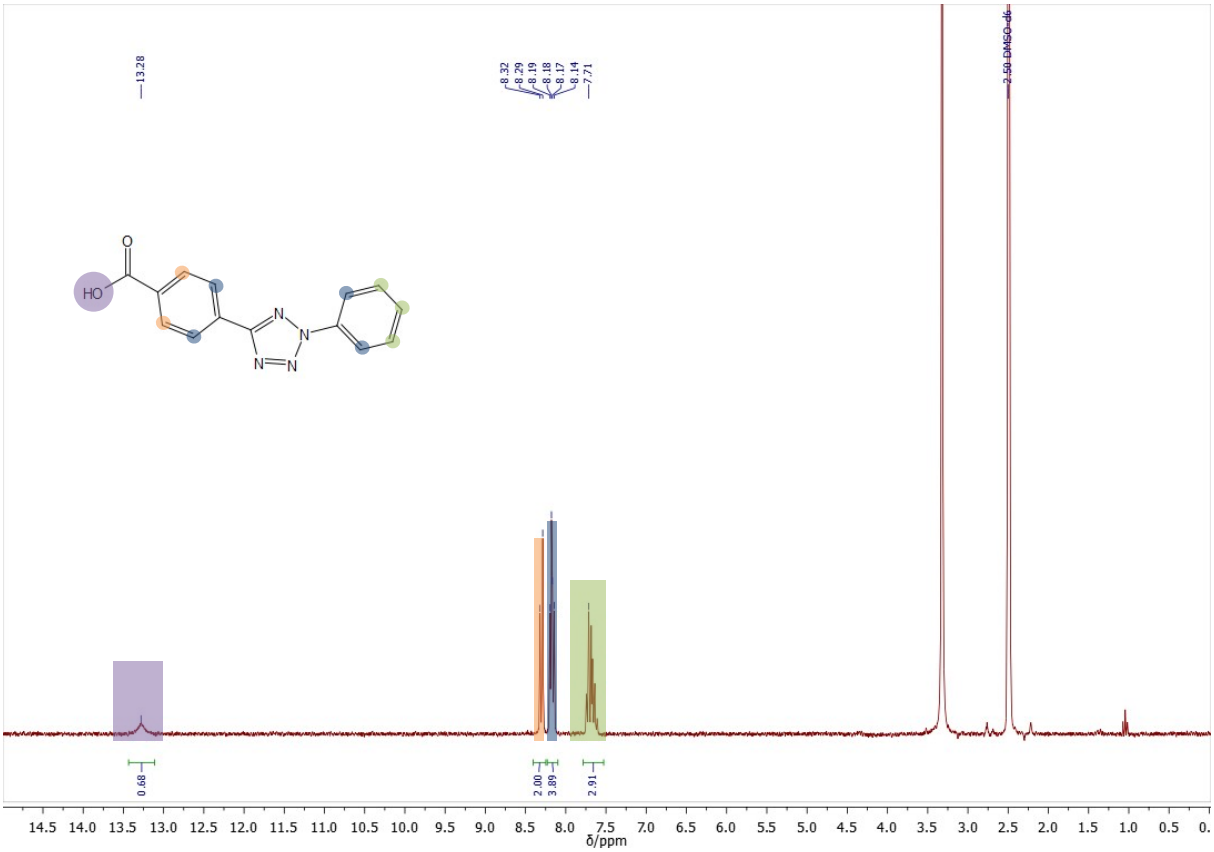

Figure S12: ^{1}H NMR of 4-(2-phenyl-2*H*-tetrazol-5-yl)benzoic acid (250 MHz, 298 K, DMSO-d6).

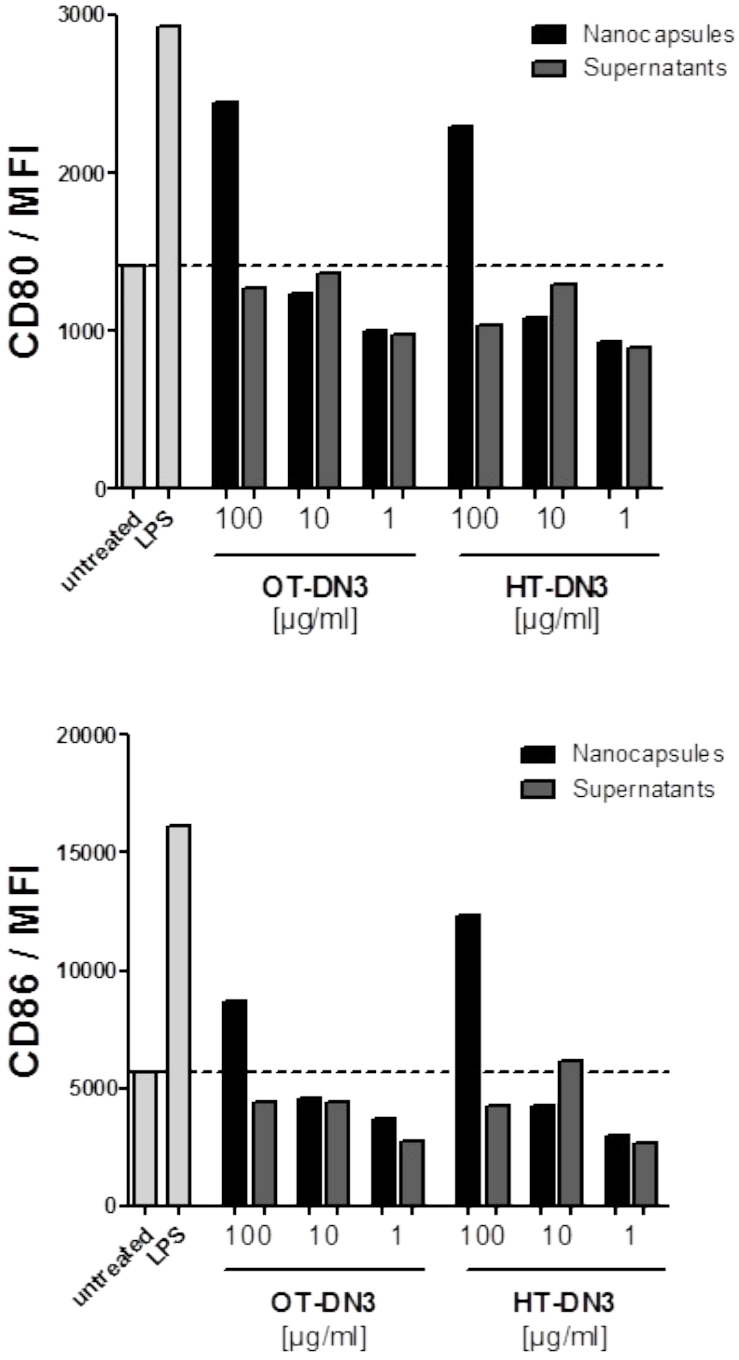

Figure S13: R848 shows high encapsulation and no leakage in TET-clicked protein nanocarriers: Comparison of nanocarriers and supernatant in the efficiency of the upregulation of CD80/ CD86 expression by bone marrow derived dendritic cells (BMDCs; (treatment with PNCs, crosslinked via TET, loaded with R848 vs. supernatant (PNCs were removed by centrifugation. Results indicate a successful intracellular cleavage of the PNCs after 8 months of storage.

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