Supporting Information

Patchy Amphiphilic Dendrimers Bind Adenovirus and Control Its Host Interactions and In Vivo Distribution

Yuzhou Wu†,*,1,2, Longjie Li†,1, Larissa Frank†,3, Jessica Wagner2,4, Patrizia Andreozzi5,6, Brenton Hammer2, Marco D’Alicamasso7, Maria Pelliccia5,7,9, Weina Liu2,8, Sabyasachi Chakrabortty2,8, Silke Kroī8,10,11, Johanna Simon2, Katharina Landfester2, Seah Ling Kuan2, Francesco Stellacci12,13, Klaus Müllen2, Florian Kreppel*,3,14 and Tanja Weil*,2,8

1 Hubei Key Laboratory of Bioinorganic Chemistry and Materia Medica, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, 430074 Hongshan, Wuhan, P.R. China
2 Max Planck Institute for Polymer Research, 55128 Mainz, Germany
3 Department of Gene Therapy, Ulm University, 89081 Ulm, Germany
4 Graduate School Materials Science in Mainz, 55128 Mainz, Germany
5 IFOM - FIRC Institute of Molecular Oncology, IFOM-IEO Campus, 20139 Milan, Italy
6 CIC biomaGUNE Soft Matter Nanotechnology Group San Sebastian-Donostia, 20014 Donastia San Sebastián, Spain
7 Fondazione Centro Europeo Nanomedicina (CEN), 20133 Milan, Italy
8 Institute for Inorganic Chemistry I, Ulm University, 89081 Ulm, Germany
9 Fondazione IRCCS Istituto Neurologico “Carlo Besta”, 20133 Milan, Italy
10 IRCCS Istituto Tumori “Giovanni Paolo II”, 70124 Bari, Italy

11 IRCCS Ospedale Specializzato in Gastroenterologia "Saverio de Bellis", 70013 Castellana Grotte (BA), Italy

12 Institute of Materials, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

13 Interfaculty Bioengineering Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

14 Lehrstuhl für Biochemie und Molekulare Medizin, Universität Witten/Herdecke (UW/H), Center for Biomedical Research and Education (ZBAF), Fakultät für Gesundheit/Department für Humanmedizin, 58453, Witten, Germany

Email: wuyuzhou@hust.edu.cn, Florian.Kreppel@uni-wh.de, weil@mpip-mainz.mpg.de

† These authors contributed equally to this work.
1. Materials, instruments, and animals

Chemicals were purchased from Sigma Aldrich unless otherwise stated. Cell lines were obtained from ATCC unless otherwise specified. 

\(^1\)H and \(^{13}\)C NMR spectra were recorded on Bruker AMX250, AC300, AMX500, and AMX700 NMR spectrometers using the residual proton or the carbon signal of the deuterated solvent as an internal standard. MALDI-TOF mass spectra were measured using a Bruker Reflex II, which was calibrated against poly(ethylene glycol) (3000 g/mol). Samples for MALDI-TOF MS were prepared by mixing the analyte with the matrix (dithranol) in THF in a ratio of 1:50. In some cases, cationization by mixing the matrix with potassium trifluoroacetate (K) or silver trifluoroacetate (Ag) was performed. All reported MALDI-TOF MS measurements were within the experimental error, characteristic for the applied technique. Flow cytometry was performed with a Beckman-Coulter Gallios 3L10C instrument. For in vivo studies, C57BL/6J mice were purchased from China Three Gorges University. Bicinchoninic acid (BCA) protein assay kit was purchased from Suzhou Comin Biotechnology. The enhanced chemiluminescence (ECL) kit and PVDF membrane were purchased from Millipore. Antibodies against EGFP, \(\beta\)-Actin, GAPDH and secondary antibody were all obtained from Cell Signaling Technology. Western blots were scanned and bands quantified using Tanon-5500 Chemiluminescent Imaging System.

2. General procedure for the synthesis of polyphenylene dendrimers (PPDs)

All dendrimers were synthesized by previously reported procedures.\(^1\)\(^2\)

**PPD 1** was recovered as a faint yellow solid in 71% yield. \(^1\)H NMR (300 MHz, CD\(_2\)Cl\(_2\), 298 K, \(\delta\)): 0.75-0.90 (12H, t), 1.45-1.60 (8H, m), 2.35-2.40 (8H, t), 6.95-
7.05 (8 H, m), 7.15-7.30 (32 H, m), 7.40-7.50 (32 H, m), and 7.70-7.85 (10H, m) ppm. $^{13}$C NMR (75 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 13.7, 16.2, 23.3, 25.2, 32.4, 38.3, 121.5-144.1, and 148.2 ppm. MALDI – MS found [M/Z] = 2210.91 g/mol with theoretical [M/Z] = 2210.45 g/mol.

PPD 2 was recovered as a faint yellow solid in 59% yield. $^1$H NMR (300 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 0.80-0.90 (24 H, t), 1.50-1.60 (16H, m), 2.40-2.45 (16H, t), and 7.05-7.75 (234 H, s) ppm. $^{13}$C NMR (75 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 13.7, 16.2, 24.1, 25.5, 36.5, 119.6-143.2, and 147.1 ppm. MALDI – MS found [M/Z] = 5745.12 g/mol with theoretical [M/Z] = 5744.14 g/mol.

PPD 3 was achieved in 63% yield as a yellow solid. $^1$H NMR (300 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 0.70-0.85 (48 H, m), 1.45-1.60 (32H, m), 2.35-2.50 (32H, m), and 7.10-7.80 (378 H, m) ppm. $^{13}$C NMR (75 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 13.7, 24.1, 37.6, 121.1-143.4, and 151.2 ppm. MALDI – MS found [M/Z] = 9767.15 g/mol with theoretical [M/Z] = 9766.23 g/mol.

PPD 4 was recovered as a yellow solid in 69% yield. $^1$H NMR (300 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 7.15 - 7.90 (306 H, d) ppm. $^{13}$C NMR (75 MHz, CD$_2$Cl$_2$, 298 K, $\delta$): 121.1-145.2, 146.5, 148.2, 149.1, and 152.3 ppm. MALDI – MS found [M/Z] = 7265.03 g/mol with theoretical [M/Z] = 7264.32 g/mol.

3. Ad5-based vectors

Virus vectors were prepared according to standard protocols$^3$ and stored at –80 °C. All vectors were purified by double CsCl banding and subsequent desalting by PD-10 columns (GE Healthcare). Vectors were stored in 50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol. Vector titers were determined by OD260.$^3$ Vector purity was confirmed by SDS-PAGE and silver staining.

4. Characterization of PPD/Ad5 interaction
4.1 Light scattering

Light scattering was used to determine interaction between Ad5 and PPD 3 by means of measuring the polydispersity index (PDI) and the hydrodynamic diameter of the particles in case of a monodisperse suspension. A monodisperse suspension was characterized by a PDI ≤ 0.2 and a polydisperse suspension by a PDI ≥ 0.2. Complex formation was performed in a volume of 1 mL PBS with \(10^{11}\) Ad5 particles. PPD 3 was added in defined ratios to Ad5, then mixed and incubated for 10 min. After transfer to a cuvette, it was filled up with PBS to a total volume of 2 mL. All samples were tested for an appropriate scattering intensity before measurement (at least \(5 \times 10^4\)) and measured at an angle \(\theta = 90^\circ\). For intensive cleaning of the cuvette, ethanol and acetone was used to avoid measurement errors by dust particles.

Table S1. Light scattering measurement of the complex formation with PPD and Ad5.

| Sample       | Only Ad5 | PPD:Ad5 100:1 | PPD:Ad5 200:1 | PPD:Ad5 1000:1 | PPD:Ad5 10,000:1 |
|--------------|----------|---------------|---------------|----------------|------------------|
| PPD(P16S16) | HD\(^1\) 108 nm | 205 nm | 275 nm | 729 nm | -- |
| + Ad5        | PI\(^2\) 0.03 | 0.12 | 0.01 | 0.17 | -- |
| PPD(P0S16)  | HD\(^1\) -- | 108 nm | -- | 109 nm | 110 nm |
| + Ad5        | PI\(^2\) -- | 0.10 | -- | 0.09 | 0.03 |

\(^1\)HD = Hydrodynamic Diameter.

\(^2\)PDI = Polydispersity Index. When PDI < 0.2, the complex was considered as monodisperse.

4.2 Transmission electron microscopy (TEM)

For each sample, complex formation with different ratio of PPD 3 was prepared. The following procedure was performed similarly to Chen et al.\(^4\) The incubation mixtures were incubated for 10 min and immediately one grid per sample was
coated with 20 μL (2 × 10⁹ Ad5 particles complexed with PPD 3) of the particular incubation mixture. Afterwards, samples were stained with 20 μL of 2% phosphotungstic acid (PTA) solution and incubated for 3 min. Grids were dried overnight and analyzed with Jeol JEM-1400 TEM after 24 h.

5. Investigation of PPD/Ad5 transfection with different cell lines
5.1 General experimental protocol for transduction assay
For transduction assays, 96 well plates were used containing 2 × 10⁴ cells per well, which were seeded as before. Cells were infected with pMOI 1000 unless otherwise specified and incubated for 24 h (CAR positive cell lines such as A549 cells, Hela cells and MDCK-2 cells) or 72 h (CAR negative cell lines, such as SKOV-3 cells, CHO-K1 cells and PTK1 cells) at 37 °C. EGFP positive cells and the overall mean fluorescence intensity (MFI) of EGFP expression was measured with flow cytometry.

**Figure S1:** Infection Assay using Ad5 and defined concentrations of PPD3. Three steps are involved in the procedure. First, an incubation mixture was prepared with 5 × 10⁹ Ad5 particles, a defined concentration of PPD3 and filled up to a volume of 50 μL with PBS. Second, cells were infected with Ad5 that had interacted with PPD3. And finally, after an incubation time according to the
cell type the overall intensity of EGFP expression was measured with flow cytometry.

5.2 General gating strategy for flow cytometry

**Figure S2:** Example of gating strategy used for flow cytometry. The live single cells were gated according to their FSC/SSC profile (left panel), and the mean fluorescence intensity in channel FL-1 (FITC) was measured. The upper panel exemplarily shows the results after transduction of SKOV-3 cells with vector only, the lower panel after incubation of the vector with PPD-3.

5.3 Preformed PPD3/Ad5 complexes are essential for increased transduction.

The PPD3 and Ad5 was pre-incubated at different times and the transduction efficiencies were tested (Figure S3). Within 20 min, the transduction efficiency increased according to the increasing of PPD3/Ad5 pre-incubation time.
Prolonging the incubation time by more than 20 min resulted in no additional increase in transduction efficiency. Thus, the 20 min pre-incubation is optimal for sufficient complex formation, and this condition has also been applied in the following experiments. To further prove that the complex formation by pre-incubating PPD3 and Ad5 together is essential, PPD3 and Ad5 were added to SKOV-3 cells by various application procedures. Figure S3 depicts the efficiencies after three different treatment protocols. Pre-incubation of PPD3 and Ad5 for 20 min before addition of the complexes to cell culture yielded about an eight times higher transduction efficacy. When Ad5 was first added to SKOV-3 cells and could interact with the cell membrane receptors, followed by the removal of excess Ad5 in solution by washing with PBS buffer and subsequent addition of PPD3, there was no increase in transduction efficiency compared with the Ad5 control experiment. This finding indicates the inability of the dendrimers to complex the virus once it is already attached to the cellular membrane (Figure S3). When PPD3 was first added to SKOV-3 cells, allowing to accumulate at the cellular membrane and removing excess PPD3 before Ad5 was added to the cells, again, no transduction enhancement was detected (Figure S3). This experiment suggests that only pre-formed the PPD3/Ad5 complexes are able to significantly enhance Ad5 uptake and transduction efficiency.
Figure S3. The influence of PPD and Ad5 pre-incubation time to the EGFP expression in CHO-K1 cells (n = 3). 500 pMOI Ad5 was used in the experiment.

Figure S4. Ad5 transduction assay showed that PPD3/Ad5 complex formation is essential for enhancing transduction. Cond. 1: PPD3 pre-incubated with Ad5 for complex formation before adding to SKOV-3 cells. Cond. 2: Ad5 incubated with cells for 1 h and removed before adding PPD3. Cond. 3: PPD3 incubated with cells for 1 h and removed before adding Ad5. The cells were then incubated for 24 h for all three conditions and the EGFP expressing was analyzed by flow cytometer to evaluate the transfection efficiency.
5.4 PPD3/Ad5 transduction to different CAR positive and CAR negative cells

Hela and MDCK-2 cells were maintained in MEM (Minimum Essential Medium) (Gibco®) supplemented with 10% heat inactivated fetal calf serum (FCS), 1% L-glutamine and 1% Penicillin/Streptomycin. CHO-K1 cells were maintained in F-12 Nut Mix (HAM) 1X (Gibco®) supplemented with 10% heat inactivated FCS, 1% Penicillin/Streptomycin. PTK1 cells were maintained in DMEM supplemented with 10% heat inactivated FCS, 1% glutamine, 1% Penicillin/Streptomycin. All the cells were maintained at 37 °C under 5% CO$_2$.

50 μL of dendrimers and Ad5 solutions were left equilibrate for 1 h at 37 °C and the added to 1 mL of medium in a 12-well flat bottom plate containing $10^5$ cells (MOI = 10–100) for 24–48 h at 37 °C with medium supplemented with 2% of FCS. The same experimental conditions were used in other cell lines. The cells were harvested 24–48 h post-transduction, fixed in 1% paraformaldehyde, stored at 4 °C in PBS and protected from light. The fraction of infected cells was quantified by Fluorescence-Activated Cell Sorting (FACS), measuring GFP signal of infected cells. The sample acquisitions were performed with, FACSCalibur (BD Biosciences; USA) and $10^4$ events were acquired for each sample. Untreated cells were set as negative control. Each experiment was performed in triplicate and the data were analyzed from two independent experiments.
Figure S5. PPD3/Ad5 transduction efficiency in different (+)CAR and (-)CAR cell lines. Infectivity of Ad5 virus is expressed in % of infected cells (GFP-Positive Cells). Different cell lines were tested with Ad5 with and without pre-incubation with PPD3 dendrimers at 10^{-7} M. Grey and yellow bars represent the % of infected cells in CAR receptor cell lines (+CAR). Cyan bars and violet bars represent the % of infected cells in a CAR negative receptor cell lines (-CAR). Grey bar represent the % of infected Hela cells (+CAR) after 48 h post-infection. Hela cells were infected with Ad5 (MOI = 10) with and without PPD3 at 10^{-7} M. Yellow bars represent the % of infected MDCK-2 (+CAR) cells after 48h post-infection of Ad5 (MOI = 10) with and without PPD3 at 10^{-7} M. Cyan bars represent the % of infected CHO-K1 (-CAR) cells after 48 h post-infection of Ad5 (MOI = 100) with and without PPD3 at 10^{-7} M. Violet bars represent the % of infected PTK1 (-CAR) cells after 48 h post-infection of Ad5 (MOI = 10) with and without PPD3 at 10^{-7} M. Ad5 with/without PPD3 were pre-incubated 1 h at 37 °C 5% of CO₂. These experimental conditions were applied to all cell lines. The experiments were performed in duplicate. The error bars present the standard deviation.

6. Testing the wide type Ad5 (wtAd5) activity when complexed with PPD3

In this experiment we used replication-competent wild-type viruses instead of replication-defective vectors. Upon entry the viruses replicated in the cells,
specific particles were formed and the cells were lysed. Therefore, to study cell viability after treatment with wtAd5, the CellTiter-Glo (Luminescent Cell Viability Assay by Promega) was applied. In brief, metabolic activity of the cells was measured via the ATP content according to the manufacturer's instructions. After cell lysis, the ATP amount measured decreased, indicating successful virus replication and release of progeny virions. As shown in the figure below, SKOV-3 cells were hardly lysed by Ad5 due to the fact that the virus did not efficiently enter the cells. After incubation with PPD3, however, more virus particles entered the cells and the cells could be lysed by replicating virus.

**Figure S6.** Wild type Ad5 activity when complexes with PPD3. (a) A549 cells were infected with MOI 1000 with and without PPD3 for 72 h. (b) SKOV-3 cells were infected with MOI 3000 with and without PPD3 for 72 h. The luminescence result (in relative light units (RLU)) represents cell viability (n = 3). * represents p-value \( \leq 0.05 \), *** represents p-value \( \leq 0.001 \).

7. **Stability of PPD3/Ad5 complex in serum**

CHO-K1 cells were used for these experiments. Firstly, Ad5 was pre-incubated with different concentrations of PPD3 for 20 min. Then, incubation was performed in FCS at 25 °C for 20 min (as a positive control), 4 °C for 48 h, 37 °C for 1hr or 37 °C for 48 h. Thereafter, Ad5 or PPD3/Ad5 were added into 24
well plates, which were seeded with CHO-K1 cells (the pMOI is 1000). All further steps were performed as described in section 5.1. After incubating of the PPD3/Ad5 complexes in FCS at 4 °C for 48 h or at 37 °C for 1 h, there was no significant reduction in their transduction efficiency. These results indicate that PPD3/Ad5 complexes remain stable in FCS.

![Figure S7](image)

**Figure S7.** The stability of PPD3/Ad5 complex in FCS. Black columns represent the condition of 25 °C for 20 min, red columns represent 4 °C for 48 h and blue columns represent 37 °C for 1 h and green columns represent 37 °C for 48 h (the columns are so low that they do not appear in the diagram) (n = 3).

### 8. Real-time quantitative PCR (qPCR) analysis

We extracted the DNA of mice livers after 7 days and 14 days with TIANamp Genomic DNA Kit (DP304, Tiangen Biotech (Beijing), China). Then EGFP DNA level in livers was quantified by real-time quantitative PCR via StepOne Plus (Life Technologies, USA). 10 μL of SYBR Green mixture (FastSYBR Mixture, CW2622M, CWBIO (Beijing), China), 0.4 μL of 10 pmol/μL primer forward and reverse (for EGFP: forward 5'-TTCAAGGACGACGGCAACTACAAG-3'; reverse 5'-GAACTCCAGCAGCAGGCACTACAAG-3'; for murine β-actin: forward
5'-ATCACTATTGGCAACGAGCGGTTC-3'; reverse 5'CAGCACTGTGTTGGCATAAGGTC-3'), 0.4 μL 50×high Rox and 2 μL sample were mixed in a final volume of 20 μL. The results were consistent with western blot. We also observed significantly decrease of EGFP DNA in liver when mice were infected by PPD3/Ad5 complex instead of raw Ad5.

**Figure S8.** Quantification of EGFP DNA levels in the liver with qPCR (n = 3) after 7 days and 14 days. * represents p-value ≤ 0.05.

9. Hematoxylin and eosin staining:

For hematoxylin and eosin staining, thin sections of the embedded tissues were then stained with hematoxylin and eosin. After staining, the sections were dehydrated in ascending grades of ethyl alcohol, cleared with xylene, and covered with a coverslip.
Figure S9. Hematoxylin and eosin (H&E) staining of liver tissues (10 time of magnification).

10. Kinetic binding analysis

The interaction between PPD3 and Ad5 was studied by Bio-Layer Interferometry assays (BLI) from Octet96 (Pall ForteBio, CA, USA). In order to receive a significant signal for this binding event, we immobilized PPD3 dendrons at the sensor surface and applied Ad5 as binding molecule. To immobilize PPD at the surface of streptavidin-coated biosensors, we used a biotinylated dendrimer branch of PPD3 with the same surface structure. The basic experiment contains four steps: Step 1: Hydration of the biosensor to record the baseline. Step 2: Immobilization of PPD3 dendrons on the streptavidin (SA) biosensor. Step 3: Washing and establishing the baseline. Step 4: Association of the Ad5. Step 5: Dissociation (Figure S10).

A significant interaction signal was seen even in the presence of only 2 pM Ad5. The $K_D$ (equilibrium dissociation constant) determined by this method is $1.27 \times 10^{-12}$ M. We believe that this very strong binding could be a result of multivalent interactions between the large virus particles providing large numbers of binding sites and the sensor surface densely coated with PPD3 dendrons. These results clearly support that there is a strong binding between PPD3 and Ad5 viruses.
Figure S10. Workflow for dendron loading and dendron-Ad5 interaction assay.

Figure S11. BLI analysis of Ad5 binding to biotinylated PPD3 dendrons immobilized on streptavidin-coated biosensors. Association and dissociation curves are shown at different concentrations. Red lines represent regression modelling.
Table S2. Kinetics analysis results.

| Conc. (pM) | Response | KD (M)     | kon(1/Ms) | kdis(1/s) | kobs(1/s) | Full R²     |
|------------|----------|------------|-----------|-----------|-----------|-------------|
| 2          | 0.076    | 1.27×10⁻¹² | 5.87×10⁸  | 7.47×10⁻⁴ | 1.92×10⁻³ | 0.967526    |
| 1          | 0.0558   | 1.27×10⁻¹² | 5.87×10⁸  | 7.47×10⁻⁴ | 1.33×10⁻³ | 0.967526    |
| 0.5        | 0.0129   | 1.27×10⁻¹² | 5.87×10⁸  | 7.47×10⁻⁴ | 1.04×10⁻³ | 0.967526    |
| 0.25       | 0.0048   | 1.27×10⁻¹² | 5.87×10⁸  | 7.47×10⁻⁴ | 8.94×10⁻⁴ | 0.967526    |

Conc. (nM): Molar concentration of the sample used in the association step. Response: Response calculated from the time window entered in the Steady State Analysis section. K_D (M): Equilibrium dissociation constant. k_on (1/Ms): Rate of association. k_dis (1/s): Rate of dissociation. k_obs (1/s): Observed binding rate. Full R²: R² is the coefficient of determination, which is an estimate of the goodness of the curve fit.

11. Interaction of coated PPD3 with serum proteins

Coating of model nanoparticles with PPD3. Amino-functionalized nanoparticles⁵ (PS-NP, concentration = 10 mg/mL, diameter 100 nm, zeta-potential ~ + 40 mV) were incubated with dendrimers in a defined ratio (1 mg NP: 1 mg PPD3) for 1 h at room temperature. Afterwards the suspension was diluted with water (1 mL), centrifuged (20,000 g, 15 min) and resuspended in water.

Human serum and plasma. Blood serum and citrate plasma were collected from ten healthy donors at the Transfusion Center of the University Clinic of Mainz, Germany after obtaining informed consent. All serum and plasma samples were pooled and stored at −20 °C.
**Protein corona analysis.** The hard protein corona was analyzed as described in previous reports. A defined ratio of nanoparticles (0.05 m²) and human blood plasma or serum (1 mL) was used. After 1 h of incubation at 37 °C, the nanoparticles were centrifuged and washed with PBS (three times, 1 mL, 20,000 g, 30 min, 4 °C) to remove unbound proteins. In the final step, the hard corona proteins were detached from the nanoparticles’ surface with 2% SDS with 62.5 mM TRIS hydrochloride and incubated for 5 min at 95 °C. The samples were further centrifuged (20,000 g, 30 min, 4 °C) and the resulting supernatant contained the hard corona proteins, which was analyzed by SDS-PAGE and LC-MS.

**SDS PAGE for hard protein corona analysis.** 1 μg of proteins (in 26 μL of samples volume) were diluted with sample buffer (10 μL) and reducing agent (4 μL), heated up to 70 °C for 10 min and applied on a 10% NuPAGE Bis-Tris Gel. The gel was run for 1 h at 120 V and proteins were further visualized via Pierce Silver Stain Kit according the manufacturers´ instructions.

**Liquid chromatography coupled to mass spectrometry (LC-MS) analysis.** Proteins were digested as previously described. Briefly, proteins were precipitated using ProteoExtract protein precipitation kit according to the manufactures´ instruction. The protein pellet was resuspended with RapiGest SF dissolved in 50 mM ammonium bicarbonate and further reduced with dithiothreitol (5 mM, 56 °C for 45 min) and alkylated with iodoacetamide (15 mM, 1 h). Proteins were digested with trypsin at 37 °C overnight. The reaction was terminated with 2 μL hydrochloric acid.

Resulting peptide samples were dilute with 0.1% formic acid and spiked with 50 fmol/μL Hi3 E. Coli Standard for absolute peptide quantification. LC-MS measurements were carried out on a Synapt G2-Si mass spectrometer coupled to a nanoACQUITY UPLC system. Data-independent acquisition (MS²)
experiments were performed, and peptides were ionized with a NanoLock Spray source in positive ion mode. MassLynx 4.1 and Progenesis QI for proteomics was used for data analysis and peptide identification. A reviewed human data base downloaded from Uniprot and additionally modified with the sequence information of Hi3 *E. coli* standard (chaperone protein ClpB) was used for absolute protein identification. A false discovery rate of 4% and a maximum protein mass of 600 kDa was chosen. Three assigned fragments were needed for peptide identification, for protein identification at least two assigned peptides and five assigned fragments were required. The absolute amount of protein in fmol was determined via TOP3/Hi3 approach.10

Figure S12. Hard protein corona analysis of polystyrene nanoparticles (PS-NP) and dendrimer (PPD3) coated nanoparticles after serum incubation. 1 µg of protein was applied to the SDS-PAGE (reducing conditions). One representative SDS-PAGE is shown. The experiment was repeated three times.
Figure S13. Hard protein corona analysis of polystyrene nanoparticles (PS-NP) and PPD3 coated nanoparticles after plasma incubation. 1 μg of protein was applied to the SDS-PAGE (reducing conditions). One representative SDS-PAGE is shown. The experiment was repeated three times.

Figure S14. Proteomic analysis of the hard protein corona for polystyrene nanoparticles (PS-NP) coated with dendrimers (PPD3) after serum (a) and plasma (b) incubation. The top 20 most abundant corona proteins are visualized in the heat map. The amount of protein is given in % based on all identified proteins. The average of two technical replicates was calculated.
REFERENCES

1. Stangenberg, R.; Saeed, I.; Kuan, S. L.; Baumgarten, M.; Weil, T.; Klapper, M.; Mullen, K., Tuning Polarity of Polyphenylene Dendrimers by Patched Surface Amphiphilicity- Precise Control over Size, Shape, and Polarity. Macromol. Rapid Commun. 2014, 35, 152–160.
2. Stangenberg R.; Wu Y.; Hedrich J.; Kurzbach D.; Wehner D.; Weidinger G.; Kuan S.L.; Jansen M. I.; Jelezko F.; Luhmann H. J.; Hinderberger D.; Weil T.; Müllen K., A Polyphenylene Dendrimer Drug Transporter with Precisely Positioned Amphiphilic Surface Patches. Adv. Healthcare Mater. 2015, 18, 377–384.
3. Kratzer R.F., Kreppel F. Methods in Molecular Biology. In Production, Purification, and Titration of First-Generation Adenovirus Vectors. Kaufmann M., Klinger C., Savelsbergh A., Eds.; Humana Press: New York, 2017; pp. 377–388.
4. Chen, Z.; Wang, Q.; Sun, J.; Gu, A.; Jin, M.; Shen, Z.; Qiu, Z.; Wang, J.; Wang, X.; Zhan, Z.; Li, J. W., Expression of the Coxsackie and Adenovirus Receptor in Human Lung Cancers. Tumor Biol. 2013, 34, 17–24.
5. Musyanovych, A.; Dausend, J.; Dass, M.; Walther, P.; Mailänder, V.; Landfester, K., Criteria Impacting the Cellular Uptake of Nanoparticles: A Study Emphasizing Polymer Type and Surfactant Effects. Acta Biomater. 2011, 7, 4160–4168.
6. Simon, J.; Wolf, T.; Klein, K.; Landfester, K.; Wurm, F. R.; Mailänder, V., Hydrophilicity Regulates the Stealth Properties of Polyporphoester-Coated Nanocarriers. Angew. Chem. Int. Ed. 2018, 57, 5548–5553.
7. Simon, J.; Müller, J.; Ghazaryan, A.; Morsbach, S.; Mailander, V.; Landfester, K., Protein Denaturation Caused by Heat Inactivation Detrimentally Affects Biomolecular Corona Formation and Cellular Uptake. Nanoscale 2018, 10, 21096–21105.
8. Hofmann, D.; Tenzer, S.; Bannwarth, M. B.; Messerschmidt, C.; Glaser, S.-F.; Schild, H.; Landfester, K.; Mailaender, V., Mass Spectrometry and Imaging Analysis of Nanoparticle-Containing Vesicles Provide a Mechanistic Insight into Cellular Trafficking. ACS Nano 2014, 8, 10077–10088.
9. Schöttler, S.; Becker, G.; Winzen, S.; Steinbach, T.; Mohr, K.; Landfester, K.; Mailander, V.; Wurm, F. R., Protein Adsorption Is Required for Stealth Effect of Poly(Ethylene Glycol)- and Poly(Phosphoester)-Coated Nanocarriers. Nat. Nanotech. 2016, 11, 372–377.
10. Silva, J. C.; Gorenstein, M. V.; Li, G. Z.; Vissers, J. P.; Geromanos, S. J., Absolute Quantification of Proteins by LCMSE: A Virtue of Parallel MS Acquisition. Mol. Cell. Proteomics. 2006, 5, 144–156.