Supporting Information

S1. Materials, cell lines and characterization methods

L-Arginine, *p*-toluenesulfonic acid monohydrate, succinyl chloride, adipoyl chloride, sebacoyl chloride, 1**,**2-ethanediol, 1,3-propanediol, Rhodamine-B-Isothiocyanate (RITC), 1, 4-butanediol, triethylamine , *p*-nitrophenol, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *N*-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were all purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. 1,2-distearoylsn-glycero-3-phosphoethanolamine-N-methoxy (polyethylene glycol) (DSPE-PEG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (ammonium salt) (DSPE-PEG Amine) and other lipids/lipid-PEGs were all purchased from Avanti Polar Lipids, Inc. Organic solvents such as methanol, toluene, ethyl acetate, ethyl ether, 2-propanol and dimethyl sulfoxide (DMSO) were purchased from VWR Scientific (West Chester, PA) and were purified by standard methods before use.

Two types of PLGAs with one carboxylic acid end group (PLGA-COOH) are purchased from Lactel absorbable polymers and used without any further purification. High molecular weight PLGA1(50/50, viscosity: 0.55-0.75 dL/g) and low molecular weight PLGA2 (50/50, viscosity: 0.15-0.25 dL/g) have the Mn around 45 kDa and 5.0 kDa respectively, measured by GPC with THF as solvent. Ac2-26 peptide (AMVSEFLKQAWFIENEEQEYVQTVK) was purchased from Tocris Biosciences. BSA, insulin, and ovalbumin are all purchased from Sigma-Aldrich (St. Louis, MO) and used directly. Recombinant human tumor necrosis factor α (TNF-α) and the corresponding ELISA kit were purchased from R&D systems. BSA, ovalbumin, TNF-α (human), insulin, and Ac2-26 peptide were used as model proteins, with MWs around 66.5, 45.0, 17.0, 6.0, and 3.0 kDa, respectively.

Hela and A549 cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). All the cells were grown exactly as the recommended by ATCC protocols. The cell lines were used from passages 5 to 12. Growth media was changed every 2 days. Cells were grown to 70 % confluence before splitting, harvesting or transfection. Dulbecco's modified eagle medium (DMEM), penicillin–streptomycin (PS, 100 U/mL), trypsin–EDTA (TE, 0.5 % trypsin, 5.3 mM EDTA tetra-sodium), fetal bovine serum (FBS) were obtained from Gibco BRL (Rockville, MD). Other cell culture related chemicals, reagents and buffers were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

The prepared materials, nanoparticles and complexes were characterized by various standard methods. For Fourier transform infrared (FTIR) characterization, the samples were ground into a powder and mixed with KBr at a sample/KBr ratio of 1:10 (w/w). FTIR spectra was then obtained with a PerkinElmer (Madison, WI) Nicolet Magana 560 FTIR spectrometer with Omnic software for data acquisition and analysis. ^{1}H-NMR spectra were recorded on a Bruker AVANCED-400 NMR spectrometer. Deuterated water (D_2O-*d*2; Cambridge Isotope Laboratories, Andover, MA) with tetramethylsilane as an internal

standard or deuterated dimethyl sulfoxide (DMSO-*d*6; Cambridge Isotope Laboratories, Andover, MA) was used as the solvent. MestReNova software was used for the data analysis. The molecular weight of polymers was measured by GPC method, using THF or 0.1% (w/v) LiCl in DMAc solution as solvents. The polymers were prepared at a concentration of 1 mg/mL (w/v) in solvent. The sample molecular weights were determined from a standard curve generated from polystyrene standards that were chromatographed under the same conditions as the samples. The solubility of polymers in common organic solvents at room temperature was assessed by using 1.0 mg/mL as a solubility criterion to determine whether a polymer is soluble or not in a solvent. The quantitative solubility of polymers in distilled water at room temperature was measured by adding distilled water drop by drop until a clear solution was obtained. The NP sizes and ζ-potentials were obtained by quasi-electric laser light scattering using a ZetaPALS dynamic light-scattering detector (15mW laser, incident beam 1/4 676 nm; Brookhaven Instruments). Transmission electron microscopy (TEM) was performed at the Harvard Medical School EM facility on a Tecnai G2 Spirit BioTWIN TEM.

S2. Synthesis and characterization of arginine based polycations (PCs)

The arginine based polycations (PCs) were prepared using a modified protocol in this study. They were prepared via polycondensation between the two monomers: di-*p*-nitrophenyl esters of dicarboxylic acid (monomers **I**) and tetra-*p*-toluenesulfonic acid salts of bis (L-arginine), α, ω-alkylene diesters (monomers **II**) (Figure S1).

Di-*p*-nitrophenyl esters of dicarboxylic acids (monomers **I**) were prepared by reacting dicarboxylic acyl chloride varying in methylene length with *p*-nitrophenol[1]. Three monomers **I** were prepared: di-*p*-Nitrophenyl Succinate (**NSu** with x=2); di-*p*-Nitrophenyl Adipate (**NA** with x=4); di-*p*-Nitrophenyl Sebacate (**NS** with x=8). The "x" indicates the numbers of methylene group in the diacid. An example of the diacid monomer synthesis is given below. Di-*p*-nitrophenyl adipate (NA) was by the reaction of the adipoyl chloride (0.15 mol) with *p*-nitrophenol (0.31 mol) in acetone in the presence of triethylamine (0.32 mol). A bath of an ice/water mixture was used to keep the *p*-nitrophenol and triethylamine mixed acetone solution (400 mL) at 0 °C. Adipoyl chloride was diluted in 100 mL of cold acetone before being added dropwise into the above chilled solution with stirring for 2 h at 0 °C and overnight at room temperature. The resulting di-*p*-nitrophenyl adipate was precipitated in distilled water, washed completely, and then dried *in vacuo* at room temperature before final recrystallization in ethyl acetate. This purification process was performed three times. The final product is a brown colored crystal.

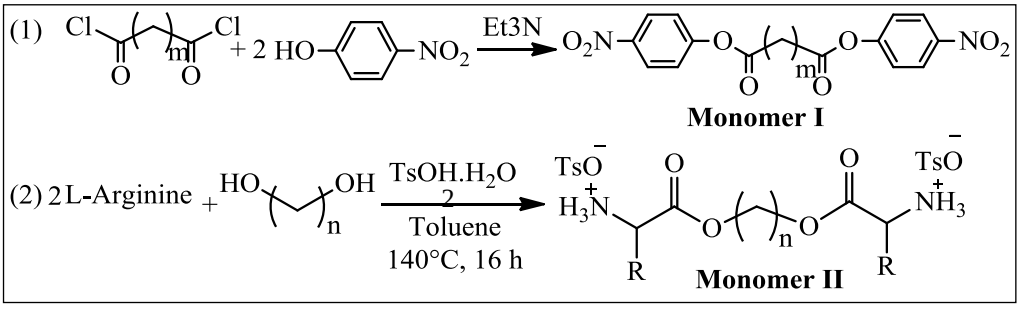

Figure S1. Synthesis scheme of monomers for arginine based polycations

Tetra-*p*-toluenesulfonic acid salts of bis (L-arginine), α, ω-alkylene diesters (monomers **II**) were prepared according to previous reported protocol[2]. The following is an example of synthesis protocol for tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) butane diesters. L-arginine (0.04 mol) and 1, 4-butanediol (0.02 mol) were directly mixed in a three neck round bottom flask with toluene (400 mL, b.p. 110 ºC) with the presence of p-toluenesulfonic acid monohydrate (0.082 mol). The solid-liquid reaction mixture was heated to 130 ºC and reflux with stirring for 24 hr with 2.16 mL (0.12 mol) of water generated. The reaction mixture (viscous solid) was then cooled to room temperature. Toluene was decanted. The resulting product was finally purified by dissolving the product in 2-propanol at 75 ºC with stirring and then precipitating at 4 ºC for three times. The ideal precipitation time is around 12h. 2-propanol was changed each time after precipitating and decanted afterwards, and the white sticky mass was dried *in vacuo*. The final product was a white powder. Three monomers **II** were made: tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) ethane diesters, **Arg-2-S**, y=2; tetra-*p*-toluenesulfonic acid salt of bis (L-arginine) propane diesters, **Arg-3-S**, y=3. tetra-p-toluenesulfonic acid salt of bis (L-arginine) butane diesters, **Arg-4-S**, y=4; S indicated that the arg diester monomer was in the *p*-toluenesulfonic acid salt form. The monomers are a white solid powder. The chemical structures of all the monomers **I** and **II** were confirmed by ^{1}HNMR.

Table S1 The summary of PC library

PC Name	X	Y	Molar ratio (I/II)	Yield (%)	Molecular Weight (Mn, KDa)
PC1	2	2	9/10	88%	7.7
PC2	2	2	3/5	73%	3.8
PC3	2	3	9/10	91%	8.5
PC4	2	3	3/5	77%	5.1
PC5	2	4	9/10	84%	9.4
PC6	2	4	3/5	76%	4.9
PC7	4	2	9/10	88%	8.3

PC8	4	2	3/5	79%	4.2
PC9	4	3	9/10	85%	7.1
PC10	4	3	3/5	72%	3.7
PC11	4	4	9/10	87%	8.6
PC12	4	4	3/5	80%	4.3
PC13	8	2	9/10	90%	9.2
PC14	8	2	3/5	81%	5.0
PC15	8	3	9/10	92%	8.1
PC16	8	3	3/5	78%	4.5
PC17	8	4	9/10	93%	7.7
PC18	8	4	3/5	84%	3.6

The arginine based polycations (PCs) were prepared by solution polycondensation of the monomers **I** (NSu, NA and NS) and monomers **II** (Arg-2-S, Arg-3-S, and Arg-4-S) with a variety of combinations using a modified protocol. One unique property of this PC library is that it offers many variables for fine-tuning the polymer chain structure (e.g., changing the m or n value) to obtain the desired physicochemical properties, such as hydrophobicity and charge density. The MW and end functional groups of PCs can be controlled by adjusting polymerization conditions. The main modification is that the molar ratio of monomers **I** to **II** were changed from 1.0 /1.0 to designed weight ratios so that PCs could be obtained with controllable end groups and molecular weight. In this report, the molar ratio of monomers **I** to **II** is designed to be smaller than 1.0 so that the NH_2 end groups could be obtained. Such combinations of monomers and weight ratios and the resulting PCs are summarized in Table S.1.

An example of the synthesis of the polycation from NS and Arg-2-S with a molar ratio of 9.0 to 10.0 via solution polycondensation is given here. Monomers NS (0.9 mmol) and Arg-2-S (1.0 mmol) in 2.0 mL of dry DMSO were mixed well by vortexing. The mixture solution was heated up to 70 °C with stirring to dissolve the monomers and obtain a uniformed mixture solution. Triethylamine (0.31 mL, 2.2 mmol) was added drop by drop to the mixture at 70 °C with vigorous stirring until the complete dissolution of the monomers. The solution color turned yellow after several minutes. The reaction vial was then kept for 48 hrs at 70 °C in a thermostat oven without stirring. The resulting solution was precipitated in cold ethyl acetate, decanted, dried, re-dissolved in methanol and re-precipitate in cold ethyl acetate for further purification. The purification was repeated 2 times before drying *in vacuo* at room temperature. The final PCs are a yellow or pale yellow solid powder. The chemical structures of all the PCs were confirmed by ^{1}HNMR. All the prepared PCs are also summarized in Table S1. The molecular

weight of PCs with molar ratio(I/II) equal to 9/10 is roughly around 7.0-10.0 kDa, while molecular weight of PCs with molar ratio(I/II) equal to 3/5 is roughly around 3.0-5.0 kDa.

Due to their strong polar nature, all the prepared PCs are soluble in buffers, distilled water (> 1.0mg/mL) and polar organic solvents like DMSO, DMF and methanol. But they are insoluble in non-polar or weak polar organic solvents like ethyl acetate, DCM, chloroform, THF and organic solvents. For the aqueous solubility of PCs developed in this study, the effect of x and y material parameters on PCs water solubility revealed that both x and y had a major impact on the water solubility of PCs. An increase of x or y significantly reduced the water solubility due to the increasing hydrophobicity. For example, PC3 (x=2 and y=3) has a solubility around 10 times of PC15 (x=8 and y=3) (about 200 mg/mL vs 20 mg/mL)

S3. Synthesis and characterization of PLGA-*b*-Polycations (PLGA-*b*-PCs)

PLGA-*b*-polycations were prepared by conjugating NH_2-PC-NH_2 to PLGA-COOH via the NHS/EDC chemistry route. The reaction conditions for the synthesis PLGA-*b*-polycations were optimized in terms of reaction temperature and time, catalyst and its concentration, the molar ratios of PLGA to PC, and concentrations of PLGA and PC. The following is the one example of optimized protocol:

1. Dissolve 500 mg of PLGA-carboxylate (high molecular weight, around 0.01 mmol) in 2.0 mL dichloromethane (DCM).
2. Dissolve NHS (6.0 mg, 0.05 mmol) and EDC (9.6 mg, 0.05 mmol) in 1.0 mL DCM.
3. PLGA-carboxylate is converted into PLGA-NHS by adding the EDC/NHS solution to a PLGA carboxylate solution with gentle stirring for 3 hours.
4. PLGA-NHS is precipitated with 40 mL cold ethyl ether/methanol washing solvent by centrifugation at 2,700×*g* for 10 min to remove residual EDC/NHS.
5. Repeat washing and centrifugation two times.
6. The PLGA-NHS pellet is dried under vacuum for 12 hours to remove residual ether and methanol.
7. After drying under vacuum, PLGA-NHS (200 mg, 0.004 mmol) is dissolved in DMSO (5.0 mL) followed by addition of PC3 with NH_2 end groups (120 mg, around 0.012 mmol) and DIEA (15.0 mg, 0.012 mmol). The mixture solution is incubated for 24 h at room temperature under gentle stirring.
8. Precipitate the resulting PLGA-*b*-PC block copolymer with ether/methanol washing solvent and centrifuge to remove unreacted PC and other impurities.
9. Repeat washing and centrifugation two times.
10. Dry the purified PLGA-*b*-PC polymer under vacuum.

The PCs directly interact with proteins but form polyelectrolyte complexes of very large size and non-uniform amorphous structure. Once PLGA is introduced, a small and condensed solid inner core is formed, and the PC segment forms a loose outer shell via the specific NP formulation method. The final

products of PLGA-*b*-PC copolymers were white or pale yellow powder or viscous solids with good yields (70-90%). The prepared PLGA-*b*-PCs are insoluble in buffers and distilled water and the organic solvents like methanol, ethyl acetate, ACN, ethyl ether, but soluble in polar organic solvents like DMSO and DMF. Some of them have low solubility (a few mg/mL) in DCM, chloroform and THF, depending on the copolymer composition. They should be stored at -20 °C for long term storage. The chemical structures of prepared PLGA-*b*-PC copolymers were confirmed by ^{1}HNMR (Figure S2). Figure S2 shows the ^{1}HNMR spectrum of two PLGA-*b*-PC copolymers with identical PC portions (PC16) but differing PLGA segments (same structure but different MW). The composition of PLGA-*b*-PC was consistent with the HNMR peak integration areas.

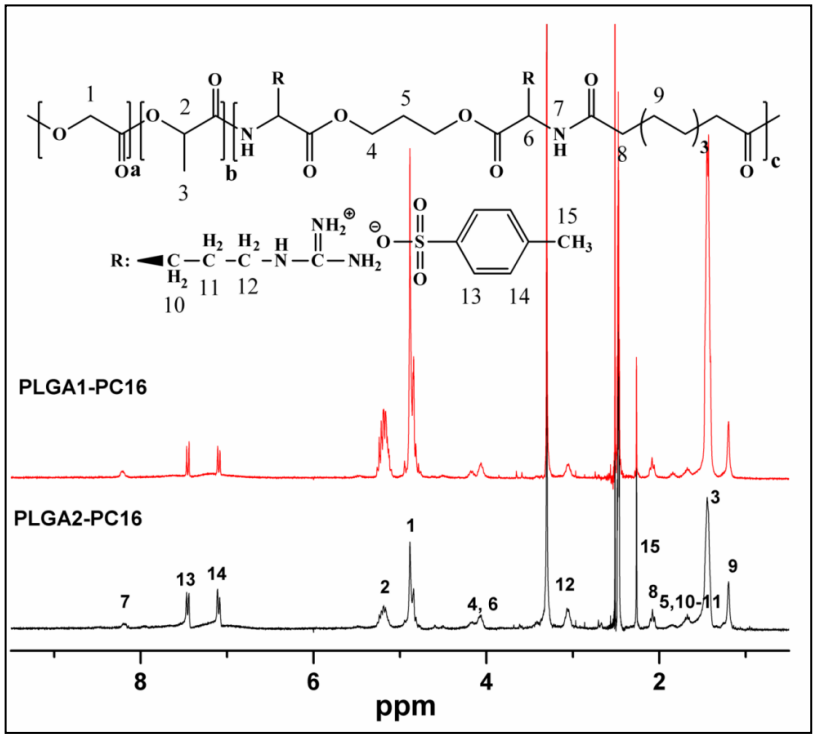

Figure S2, ^{1}HNMR spectrum of PLGA-*b*-PC16 with different PLGA/PC ratios

Table S2 The summary of PLGA-*b*-PC copolymer library

	PLGA1	PLGA2
PC1	PLGA1-PC1	PLGA2-PC1
PC2	PLGA1-PC2	PLGA2-PC2
PC3	PLGA1-PC3	PLGA2-PC3
PC4	PLGA1-PC4	PLGA2-PC4
PC5	PLGA1-PC5	PLGA2-PC5
PC6	PLGA1-PC6	PLGA2-PC6
PC7	PLGA1-PC7	PLGA2-PC7
PC8	PLGA1-PC8	PLGA2-PC8

PC9	PLGA1-PC9	PLGA2-PC9
PC10	PLGA1-PC10	PLGA2-PC10
PC11	PLGA1-PC11	PLGA2-PC11
PC12	PLGA1-PC12	PLGA2-PC12
PC13	PLGA1-PC13	PLGA2-PC13
PC14	PLGA1-PC14	PLGA2-PC14
PC15	PLGA1-PC15	PLGA2-PC15
PC16	PLGA1-PC16	PLGA2-PC16
PC17	PLGA1-PC17	PLGA2-PC17
PC18	PLGA1-PC18	PLGA2-PC18

S4. Synthesis and characterization of nanoparticles of PLGA-polycation, PLGA-PC/protein complex, and PLGA-PC/protein/Lipid-PEG

The nanoprecipitation method was chosen for PLGA-PC NP fabrication because it would produce small, simple PLGA-PC NPs with the desired core (PLGA)-shell (PC) structure that were also fast to manufacture and reproducible. Because of the insolubility of PLGA-PCs in many organic solvents, DMSO or acetonitrile were used as the organic solvents for the nanoprecipitation step. The lipid-PEG coated nanostructure forms were preliminarily investigated in terms of the PEG length and lipid charge type (anionic, neutral, or cationic). It was found that the relatively long PEG chain (MW $\geq$ 2,000) and neutral/positive charged lipid were helpful for the lipid-PEG/lipid coating on the nanostructure forms. The following is the one example of optimized protocol for preparing nanoparticles of PLGA-PC (steps 1-2), PLGA-PC/protein complex (steps 1-3) and PLGA-PC/protein/Lipid-PEG (steps 1-4):

1. Dissolve 20.0 mg of PLGA-*b*-PC in 1.0 mL DMSO or acetonitrile.

2. Precipitate the polymer solution dropwisely to 19 volumes of stirring distilled water (1000 rpm) to obtain a PLGA-*b*-PC NP solution (1.0 mg/mL). DMSO could be removed by dialysis or centrifuge and acetonitrile could be removed by dialysis or evaporation.

3. Dissolve 10.0 mg of BSA in 2.0 mL distilled water. Add the 200 ul BSA solution (5.0 mg/mL) dropwisely to the 8.0 mL stirring (1,000-1,200 rpm) PLGA-*b*-PC NP distilled water solution (0.5 mg/mL) to obtain a PLGA-PC/protein complex nanosphere solution (0.625 mg/mL). To obtain large size complex nanosphere in Fig. 4 for better TEM image quality, the stirring speed should be reduced to 400-600 rpm.

4, DMPC/DSPE-PEG or lecithin/DSPE-PEG (8.5/1.5, molar ratio, MW of PEG is 3,000 Da) aqueous solution was added to the PLGA-PC/protein complex nanosphere to reach a weight ratio of 20 wt% to the PLGA-PC/protein complex nanosphere. The resulting NP solution could be concentrated and the remaining free molecules or organic solvent were removed by washing the NP solution three times using

an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cutoff of 100,000 Da. Then concentrated PBS buffer was added to obtain a final 1X concentration of PBS.

The PLGA-PC NPs were characterized in terms of particle size, surface charge (zeta potential), and particle structure (surface morphology and shape) by a variety of methods. The NP size (diameter, nm) and size distribution are measured by dynamic light scattering at 25 °C, with a scattering angle of 90°, and using a NP concentration of approximately 0.1 to 0.5 mg/mL. The NP surface zeta potential is measured and recorded as the average of three measurements. Transition electron microscopy (TEM) is used to confirm the size and structure of the NPs. A solution of NPs in distilled water (0.1–0.5 mg/mL) is absorbed on grids and negatively stained for 15 seconds. For each sample, 5–6 grids are prepared and viewed. Images were normally taken at 13–49,000× magnification.

For the loading of multiple types of proteins in one NP formulation, protein stock solutions with high concentration were made first and were then mixed with predetermined volume ratio to obtain the desired solution of mixed proteins. All other steps are same as the above.

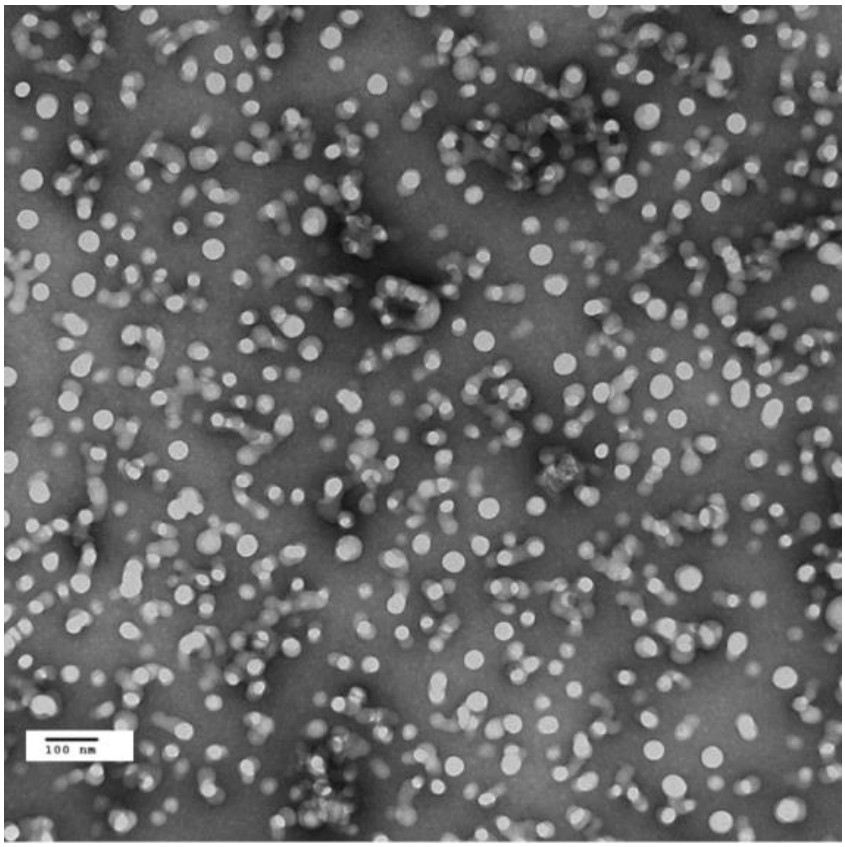

Figure S3. Extra TEM image of PLGA-PC/protein complex nanosphere (PLGA2-PC16 with 2 wt% BSA loading, length bar: 100 nm)

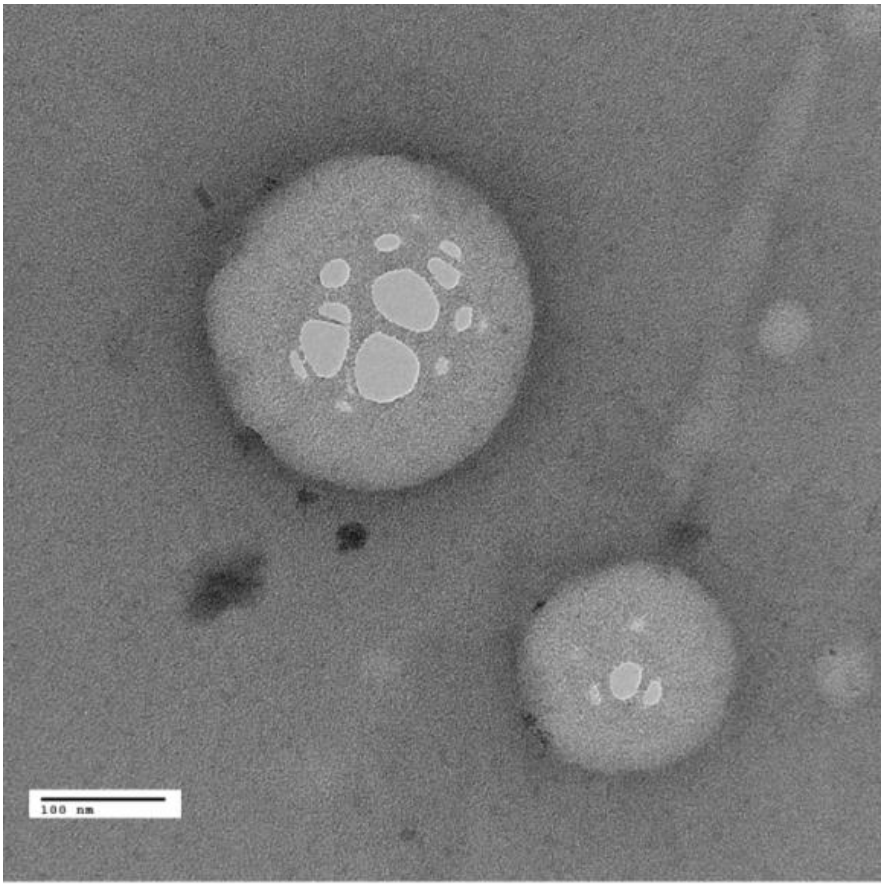

Figure S4. Extra TEM image of PLGA-PC/protein complex nanosphere with interior strucutre (PLGA2-PC16 with 25 wt% BSA loading, length bar: 100 nm).

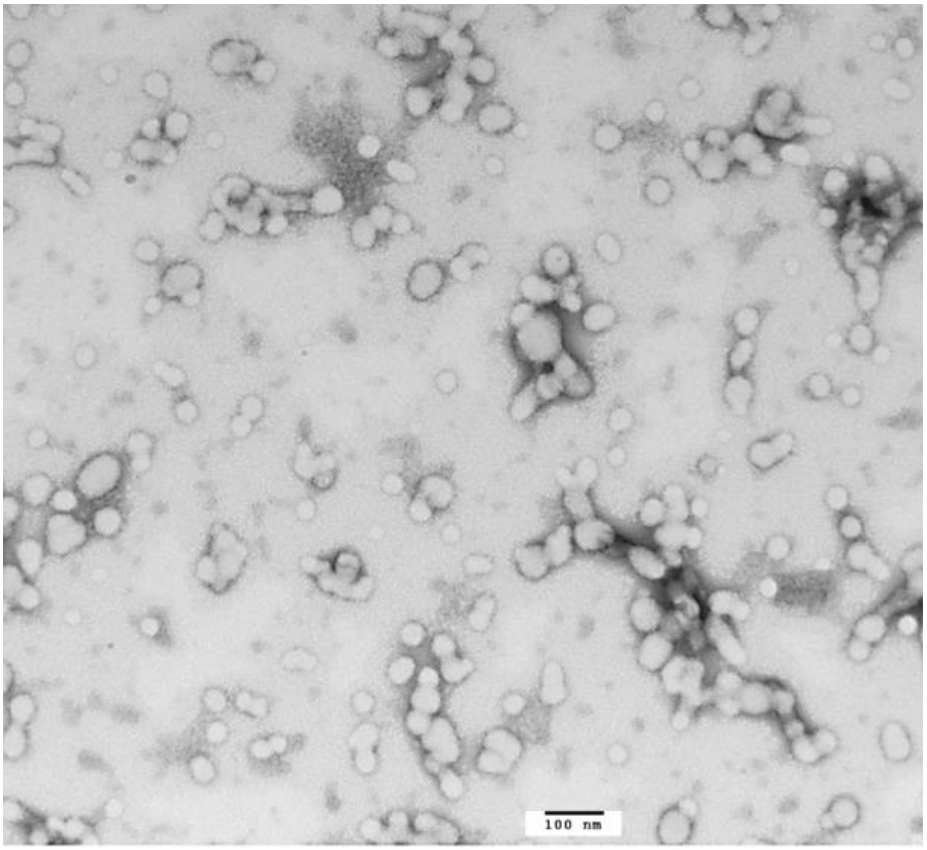

Figure S5. Extra TEM image of PLGA-PC/protein complex nanosphere (PLGA1-PC16 with 0.5 wt% BSA loading, length bar: 100 nm)

Table S3. Particle size and zeta potential of some PLGA-PC NPs

PLGA-PC	Particle size (nm)	Zeta Potential (mv)
PLGA1-PC1	56.2	16.52
PLGA1-PC3	43.4	23.22
PLGA1-PC4	54.9	28.29
PLGA1-PC8	66.1	24.97
PLGA1-PC11	80.5	35.82
PLGA1-PC15	81.7	23.98
PLGA2-PC1	33.5	19.78
PLGA2-PC2	43.7	34.76
PLGA2-PC6	32.5	27.17
PLGA2-PC10	38.1	29.62
PLGA2-PC15	37.2	13.8

Table S4. Protein loading and particle size of some nanosphere complex of PLGA-PC NP and other proteins

PLGA-PC	Protein	Protein loading (wt% NP)	Particle size (nm)
PLGA2-PC16	Ac2-26	25	107.3
PLGA1-PC16	Insulin	2	189.7
PLGA2-PC16	Insulin	25	136.2
PLGA2-PC2	Insulin	25	102.4
PLGA2-PC2	BSA	25	121.2
PLGA2-PC2	BSA	30	127.5
PLGA2-PC15	BSA	15	168.9
PLGA2-PC15	BSA	25	129.6
PLGA2-PC15	BSA	30	148.5
PLGA2-PC16	Ovalbumin	25	129.6
PLGA2-PC16	TNF-α	25	170.4

S5. Measurement of protein loading and release via BCA assay

BCA assay was used to measure the loaded and released protein (BSA, insulin, ovalbumin and Ac2-26) in this report. All the steps are exactly following the manufacturer's protocol. A serial dilution of pure protein solutions with predetermined concentrations were used to make standard curve every time. For the measurement of protein loading and release, since the PC component of PLGA-PC NPs has a lot of amino acids and will affect the BCA results, the supernatant solution was collected and the inside free unloaded proteins were analyzed via BCA assay. Here is one detailed example of the *in vitro* release measurement of proteins: 10.0 ml PLGA-PC/protein/Lipid-PEG nanosphere solution (0.50 mg/mL) was added into an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cutoff of 100,000 Da. The filter was fixed on a shaker at 50 rpm and incubated at 37 °C. At each time point, the centrifugal filter was centrifuged at 3000 rpm for 10-20 mins to have 1-2 ml solution left in the filter. The volume of the centrifuged solution was measured and its protein concentration was measured via BCA assay. The released amount of the protein was then calculated, assuming the released free protein concentration is same in centrifuged solution and the solution remaining in centrifuge tube. The PBS buffer with same volume as the centrifuged solution was then added to the remaining NP solution and the incubation was continued at 37 °C. Repeat the same step in each time point. Protein loading was measured immediately after the NP formulation (time point: 0 h) following the same procedure/method as above.

For the measurement of multiple proteins release in one NP formulation via BCA assay, the MW of encapsulated proteins should have big difference, such as BSA vs insulin or BSA vs Ac2-26. For example, to measure the release of BSA and Ac2-26, first, the released low MW Ac2-26 was collected using a Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cutoff of 10,000 DA, then all other steps are same as the above protocol.

S6. Measurement of *in vitro* and *in vivo* protein bioactivity

The *in vitro* bioactivity of released protein was preliminary evaluated by ELISA assay using TNF-α as a model protein. All the ELISA steps are exactly following the manufacturer's protocol. Freshly prepared PLGA2-PC16/TNF-α/ Lipid-PEG NP PBS solution (100 µg TNF-α and 400 µg PLGA2-PC16) aqueous solution (2 mL) was added into an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cutoff of 100,000 Da. The filter was fixed on a shaker at 50 rpm and incubated at 37 °C for 6 h. Then the filter was centrifuged at 3000 rpm for 10-20 mins to have around 0.5-1 ml solution left in the filter. The protein concentration of the centrifuged solution was measured by BCA assay. Then the untreated TNF-α solution was prepared with same concentration and the two solutions were measured by ELISA assay and compared. The ELISA results (optical density (OD)) indicated the bioactivity of released TNF-α did not show obvious difference from the untreated TNF-α (Figure S6).

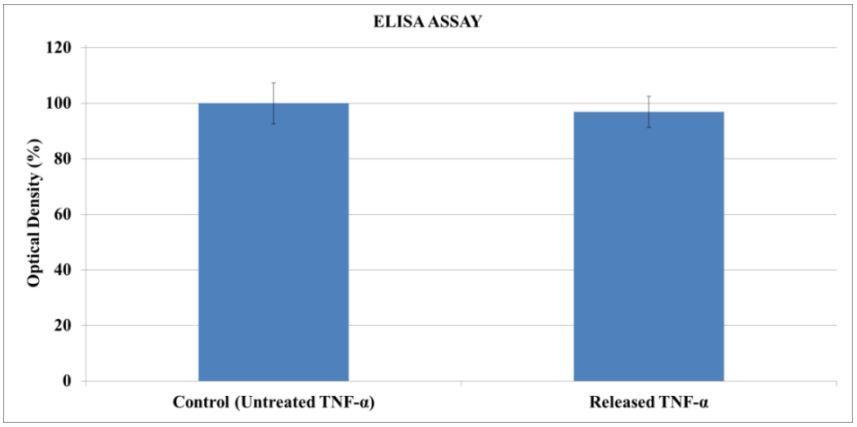

Figure S6. Comparison of bioactivity of untreated TNF-α and released TNF-α

The *in vivo* bioactivity of released protein was preliminary evaluated using insulin as a model. Changes in glucose levels in mouse blood were recorded. All the animal experiments were exactly the same as in our previous report [3]. The animal work has been approved under the Harvard Medical School animal protocol# 05069. The released insulin was collected exactly following the above protocol for TNF-α. The changes in glucose levels indicate that the bioactivity of released insulin was not substantially different from untreated insulin (Figure S7).

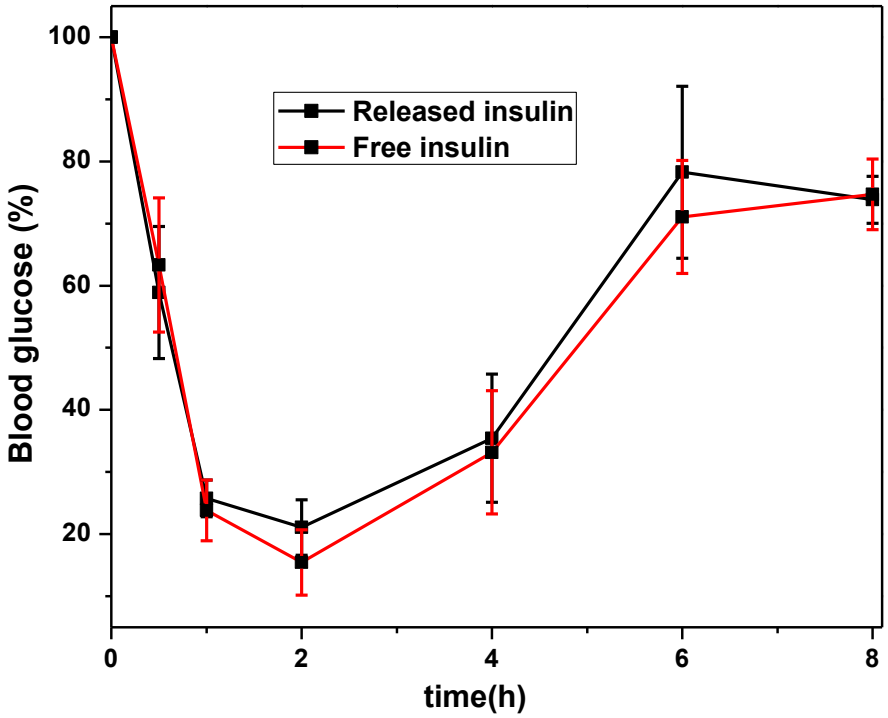

Figure S7. Comparison of bioactivity of untreated insulin and released insulin

S7. Cytotoxicity evaluation of the PLGA-PC and PLGA-PC/Protein/DSPE-PEG complex nanoparticles.

The cytotoxicity of PLGA-PC (PLGA2-PC16) and PLGA-PC/Protein/DSPE-PEG (PLGA2-PC16/BSA/DSPE-PEG3000 (25% BSA)) NPs was evaluated by the MTT assay (Figures S8 and S9 respectively). An increase in cell number (cell proliferation) results in an increase in the amount of MTT formazan production and hence an increase in UV absorbance at the 570 nm wavelength. Two types of cells, Hela and A549, were used for the MTT assay. The cultured cells were seeded at an appropriate cell density concentration (3,000 cells/well) in 96-well plates and incubated overnight in a 5 % CO_2 incubator at 37 ^{o}C. The cells were, then, treated with various NP solutions for 4 h. PLGA-PC NP solutions were stabilized with 1.0 wt % PVA. The mixture of media and NPs was removed and complete DMEM was then added into each well for 44 h incubation (total time: 48 h). The cells treated with normal cell culture media only were used as the negative control (NC). After 48 h incubation (total treatment time) of the treated cells at 37 °C and 5 % CO_2, 15 μL of MTT solution (5 mg/mL) was added to each well, followed by 4 h incubation at 37 °C, 5 % CO_2. Then the cell culture medium was carefully removed and 150 μL of acidic isopropyl alcohol (with 0.1 M HCl) was added to dissolve the formed formazan crystal. OD was measured at 570 nm (subtract background reading at 690 nm) using a VersaMax Tunable Microplate reader. The cell viability (%) was calculated according to the following equation: Viability (%) = (OD_{570}

$_{(sample)}$-$OD_{620\ (sample)}$)/ ($OD_{570\ (control)}$-$OD_{620\ (control)}$) × 100 %; where the $OD_{570\ (control)}$ represents the measurement from the wells treated with medium only, and the $OD_{570\ (sample)}$ from the wells treated with various nanoparticle solutions. Thus, the cell viability was expressed as the percentage of the blank negative control. Triplicates were used in each experiment. The MTT data clearly demonstrated that, after 4 h treatment, all the nanoparticle samples showed the same as or close to the blank control, i.e., very little cytotoxicity to the cells tested even at a large dosage such as 1mg/mL.

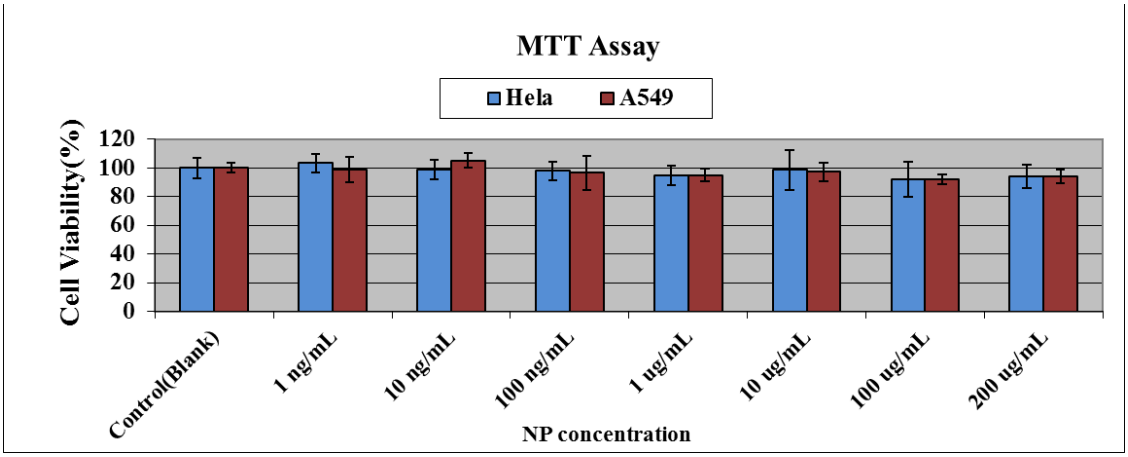

Figure S8. MTT cytotoxicity of PLGA-PC NPs over a wide concentration range. Control (Blank) is cells without any NP treatment. Hela and A549 cells were tested.

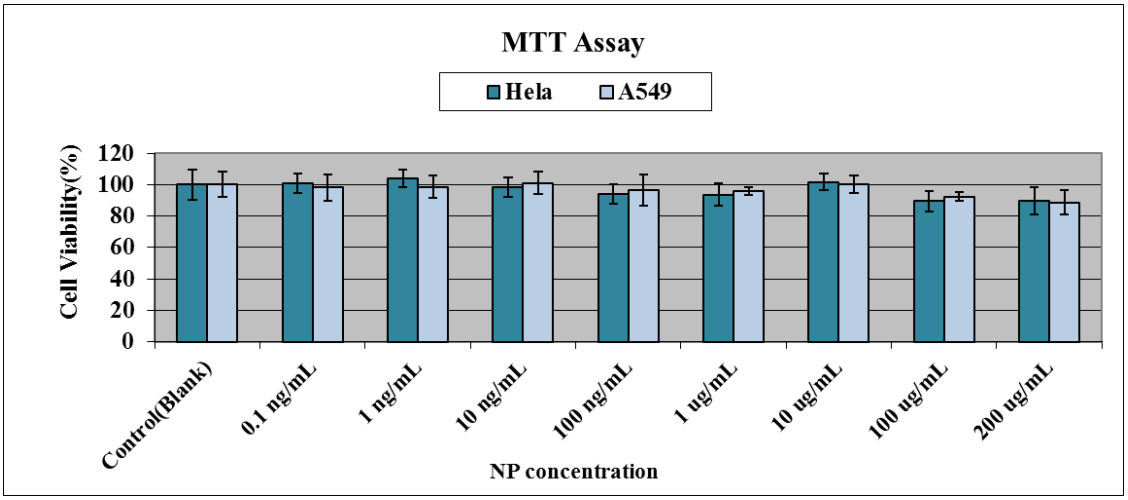

Figure S9. MTT cytotoxicity of PLGA-PC/protein/ DSPE-PEG complex NPs over a wide concentration range. Control (Blank) is cells without any NP treatment. Hela and A549 cells were tested.

S8. Cellular interaction of PLGA-PC/ fluorescent dye labeled BSA /Lipid-PEG.

BSA proteins were labeled with fluorescent dye Rhodamine-B-Isothiocyanate (RITC) and purified according to manufacturer's protocol. The NPs of PLGA-PC/ fluorescence BSA /DSPE-PEG3000 (25

wt% BSA) were fabricated following the above protocol. A549 cells were used for this cellular uptake evaluation. The cultured cells were seeded at an appropriate cell density concentration (3,000 cells/well) in 96-well plates and incubated overnight in a 5 % CO_2 incubator at 37 ^{o}C. The cells were then treated with pure RITC labeled BSA (2.0 ug/mL) or PLGA-PC/ fluorescence BSA /DSPE-PEG NP solution (10.0 ug/mL) for 4 h. A Zeiss Axiovert 200 fluorescence/live cell imaging microscope was used to recorded the images.

S9. PLGA-*b*-poly(L-histidine) and PLGA-*g*-poly(L-lysine): synthesis, characterization, nanoparticle formulation, and interaction with proteins.

Poly(L-histidine)$_{10}$ and Poly(L-histidine)$_{20}$ with NH_2 and COOH end groups were prepared by the Biopolymers & Proteomics Laboratory at MIT. Poly(L-lysine) hydrobromide (MW 4,000-15,000 Da by viscosity) was purchased from Sigma-Aldrich. PLGA-*b*-poly (L-histidine) and PLGA-*g*-poly(L-lysine) were synthesized and characterized using the same protocol described in section S3. In PLGA-*g*-poly(L-lysine), one Poly(L-lysine) hydrobromide chain conjugated with only one PLGA-COOH, and the reacting amine group could be at any site on the poly(L-lysine) chain. However, this small structural difference would not affect the charge property of the polymer. Figure S10 shows an example of the ^{1}HNMR spectrum of PLGA1-g-poly(L-lysine) hydrobromide. The synthesis and characterization of NPs followed the same protocol as S4. Both PLGA-*b*-poly (L-histidine) and PLGA-*g*-poly(L-lysine) polymers formed small NPs around or below 50-60 nm (Figure S11 and S12). However, they could not effectively load large amounts of proteins, and the NP structure did not significantly change (new nanostructures were not detected, Figure S13).

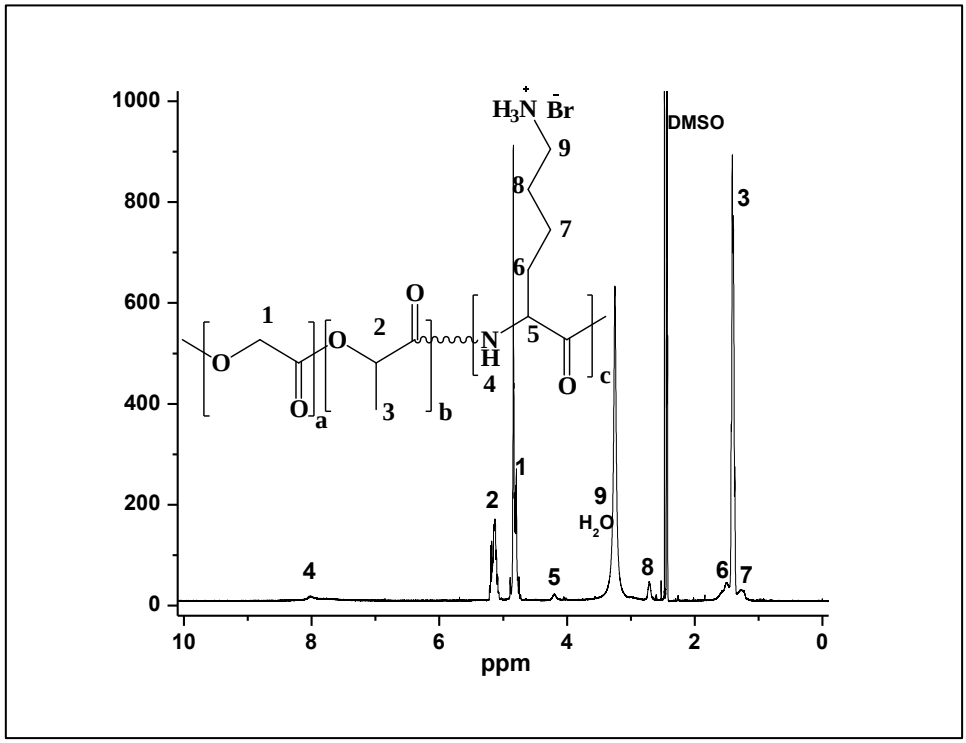

Figure S10, ^{1}HNMR spectrum of PLGA1-*g*-poly(L-lysine).HBr (MW: 4,000-15,000 Da by viscosity)

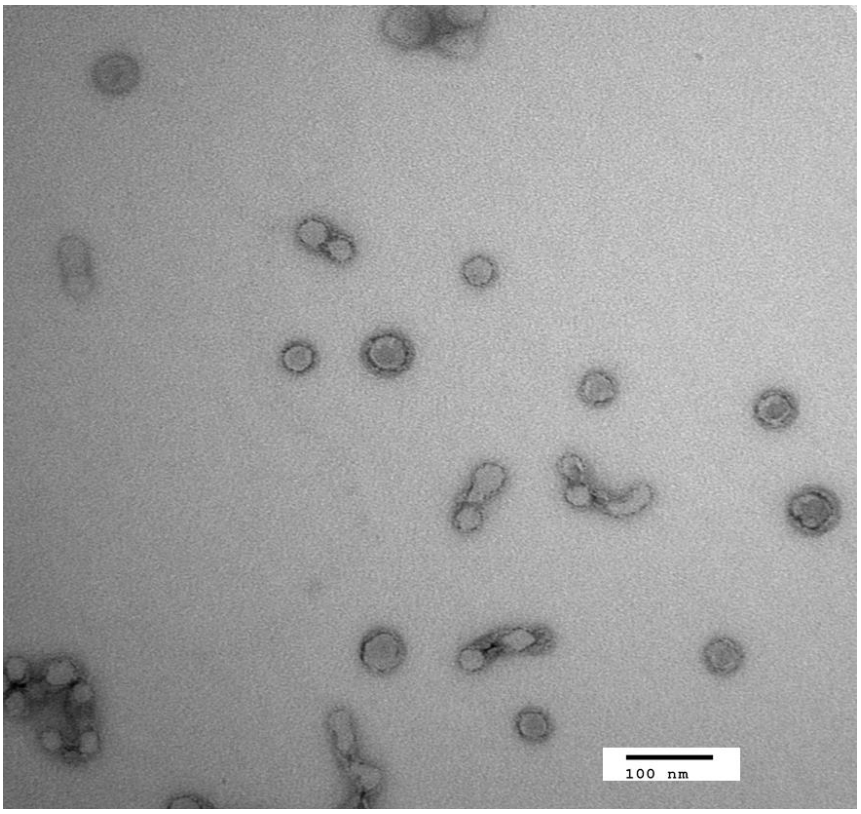

Figure S11, TEM image of PLGA2-poly(L-Histidine)$_{10}$ NPs (length bar: 100 nm)

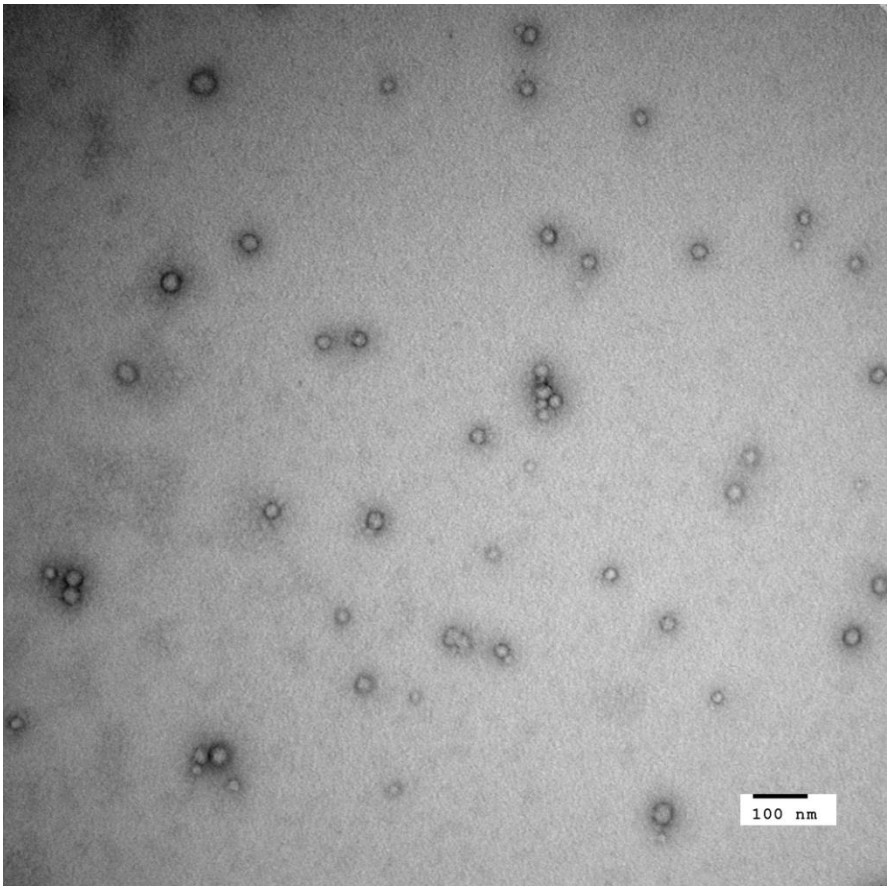

Figure S12, TEM image of PLGA2-poly(L-lysine).HBr (4-15 kDa) NPs (length bar: 100 nm)

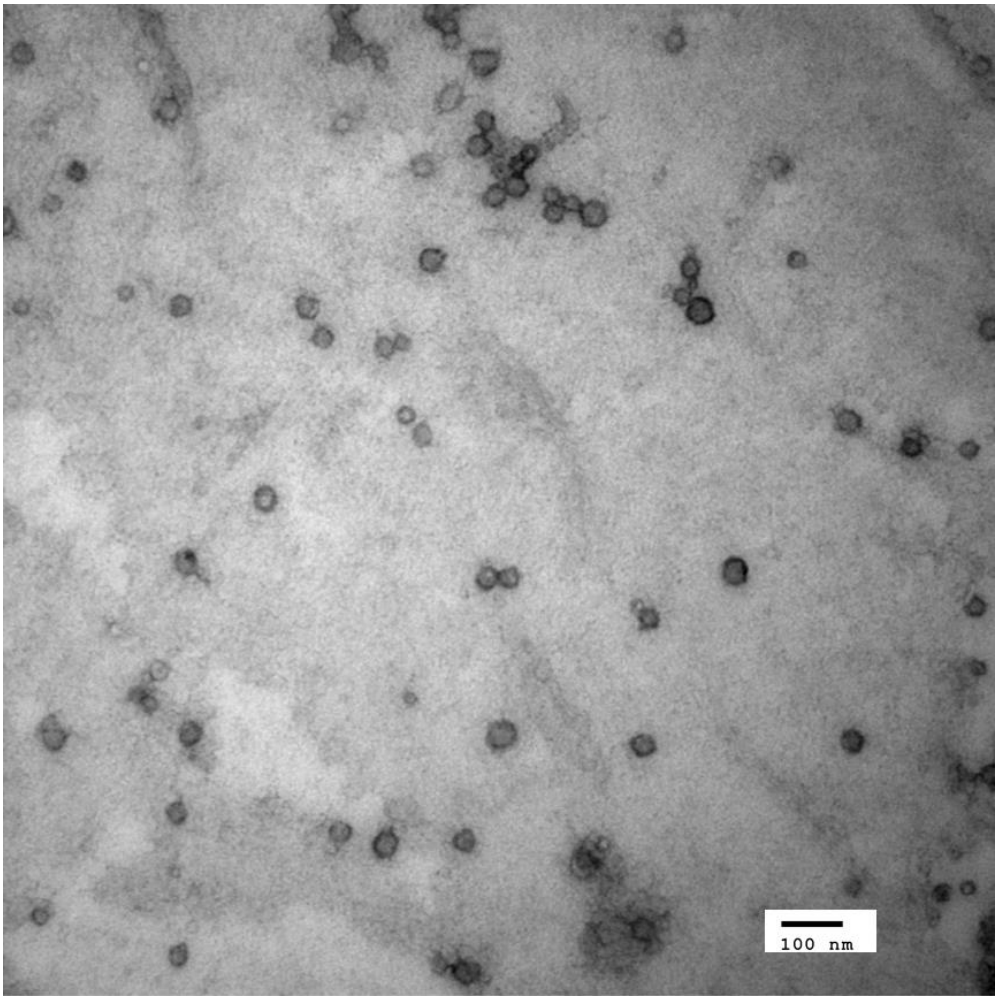

Figure S13, TEM image of PLGA2-poly(L-lysine). HBr (4-15 kDa) NPs/BSA (WR: 4:1) mixture (25 wt% BSA added, length bar: 100 nm)

S10. Statistics

Where appropriate, the data are presented as mean ± standard deviation calculated over at least three data points. JMP software (version 8.0, from SAS Company) was used for data analysis. Significant differences compared to control groups were evaluated by unpaired Student's t-test or Dunnet test at p 0.05, and between more than two groups by Tukey's test with or without one-way ANOVA analysis of variance.

References

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