Supporting information

Characterization of complexes made of polylysine-polyleucine-polylysine and pDNA

Baizhu Chen†*, Lei Yu‡ and Zhibo, Li‡

†Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
‡ Beijing National Laboratory for Molecular Sciences (BNLMS), Laboratory of Polymer Physics and Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

1. Synthesis of polypeptide

The monomers *N*ε-carbobenzyloxy-$_L$-lysine-*N*-carboxyanhydride (Z-Lys NCA) and $_L$-leucine-*N*-carboxyanhydride (Leu NCA) were synthesized according to reported literature.[1-2] The synthesis of polypeptides was performed in dry glove box under N_2. Following is the typical synthetic procedure for sample Lys_{25}-Leu_{15}-Lys_{25} ($K_{25}L_{15}$ K_{25}). To a solution of Z-Lys NCA in THF (0.33 mmol, 2 mL of a 50 mg mL^{-1} solution) was rapidly added a solution of Ni(COD)depe in THF (246 μL of a 20 mg mL^{-1} solution). The reaction was stirred at 20 ^oC overnight. The consumption of Z-Lys NCA was verified by using FTIR. After that an aliquot was sampled for Tandem gel permeation chromatography/Multi-angle light scattering (GPC/MALS) characterization to determine molecular weight and molecular weight distribution. Then a solution of Leu NCA in THF (0.20 mmol, 2 mL of a 50 mg mL^{-1} solution) was rapidly added into the reaction mixture under stirring. The consumption of Leu NCA was also verified by using FTIR. After that a solution of Z-Lys NCA in THF (0.33 mmol, 2 mL of a 50 mg mL^{-1} solution) was added into the vial under stirring. The consumption of Z-Lys NCA

was verified by using FTIR. The deprotection of *N*ε-carbobenzyloxy-$_L$-lysine residues was performed by addition of 33 wt% HBr in acetic acid to a solution of polypeptide in trifluoroacetic acid (TFA) at 0 ^oC for 3 hour. All deprotected copolypeptide were dissolved in DI water and dialyzed against HCl solution (pH = 3). Finally, the samples were dialyzed against DI water for 3 days with the water changed every 12 hours before the solution was lyophilized to obtain the samples as white powders.

A series of triblock copolypeptides ($K_mL_nK_o$) were synthesized by sequential ring-opening polymerization of NCA using Ni (COD)depe initiator in THF, followed by removal of protecting groups and purification. The molecular weight of first K block was determined by GPC/MALS with PDI less than 1.2. The average molecular weight of L and second K block was estimated from feeding ratio given that the ROP is living polymerization.

2. GPC measurement

Polypeptide was synthesized via NCA polymerization, resulting in narrow distribution production. GPC/MALS was performed at 50°C using an SSI pump connected to Wyatt Optilab DSP and Wyatt DAWN EOS light scattering detectors with DMF containing 0.02 mol/L LiBr salt as eluent at a flow rate of 1.0 mL/min, with lysine as template (dn/dc=0.123). Samples were prepared at concentrations of about 5 mg/mL before deprotection.

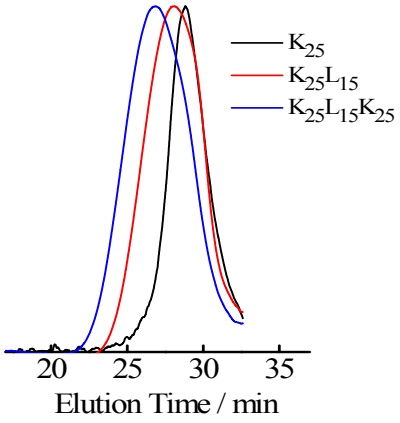

Figure S1. GPC elution profiles recorded K_{25}, $K_{25}L_{15}$, and $K_{25}L_{15}K_{25}$. Samples were measured before deprotection.

Table S1. Molecular weight of Lys_{25}, Lys_{25}-Leu_{15}, and Lys_{25}-Leu_{15}-Lys_{25} (g/mol)

Sample	M_n	M_w	PDI
K_{25}	5122	5428	1.06
$K_{25}L_{15}$	10420	13640	1.309
$K_{25}L_{15}K_{25}$	14690	19890	1.354

3. Circular dichroism spectra

The secondary structure of each synthesized polypeptide was characterized by circular dichroism using a Jasco CD-J715 spectropolarimeter, where the copolypeptides concentration was kept at 0.5 mg/mL in water, the optical path of the quartz cell is 1 mm. In theory, the α-helix structure has two specific absorption peaks at 222 nm and 208 nm.[3] After testing, there is no typical peaks for the tri-block co-polypeptide samples, revealing that there is no α-helix structure in our synthesized samples.

4. Cytotoxicity of polypeptides in HeLa and HepG2 cell lines

HeLa or HepG2 cells were seeded in a 96-well plate at an initial density of 5,000 cells per well. After one day, different free polypeptides or polypeptide/pDNA complexes were respectively added to each well at a series of N:P ratio in DMEM medium with 10% FBS and antibiotic (penicillin at 100 units/mL and streptomycin at 100 μg/mL) and the total volume was 100 μL per well. After treating, cells were further incubated for 48 h at 37°C with 5% CO_2. The MTT reagent (20 μL per well, 5 mg/ml in PBS) was added to each well. The cells were incubated for another 4 h. The culture medium was then replaced by 100 μL DMSO for each well. After shaking for 10 min, the absorbance at 490 nm of each well was recorded by a microplate reader (Bio-rad, USA). The cell viability was calculated as viability = $A_{treated}$ / $A_{control}$, where $A_{treated}$ and $A_{control}$ corresponding to cells treated with samples and those without treated, respectively. Each concentration was performed in quadruplicate and results were shown as the mean with a standard division.

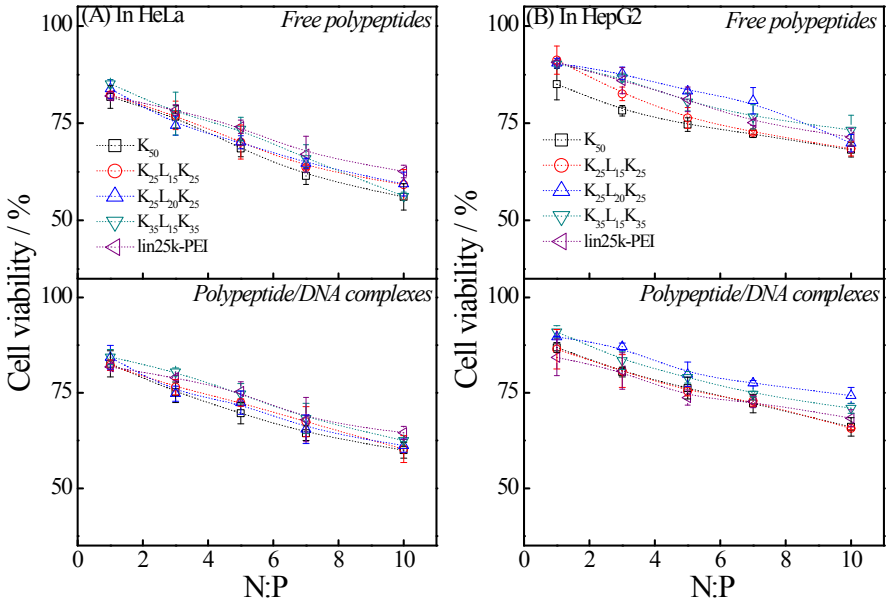

Figure S2. Cell viability of different free polypeptide or polypeptide/pDNA complexes at a series desired working

concentration in HeLa (A) or HepG2 (B) cell line, measured by MTT assay, where linear PEI-25k (linPEI-25k) was used as control.

5. In-vitro gene transfection

In vitro gene transfection assay was performed in Human Chondrocytes. Cells were seeded in a 48-well plates at an initial density of 15,000 cells per well. After one day, polypeptide/pDNA complexes were prepared as described and diluted by using serum-free DMEM to a final amount of 0.4 μg for each well. Linear PEI-25k is used as control. The culture medium was then added to each well. The complete DMEM (containing FBS and antibiotic) was supplied 6 h after transfection. The cells were further incubated for another 48 h before lysis and measured gene transfection efficiency, by using a Luciferase assay system (Promega, USA) and detecting by a Glomax luminometer. The protein concentration was calculated by a Bio-Rad QuickStart Bradford protein assay reagent. Gene transfection efficiency was expressed as a relative luminescence unit per cellular protein. Each experiment condition was performed in quadruple and the data was displayed as the mean value plus a standard deviation (± SD).

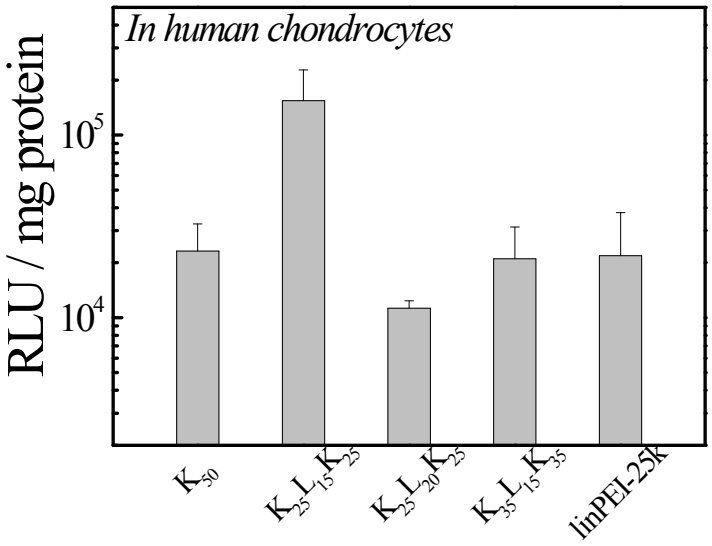

Figure S3. Transfection efficiency of polypeptide/pDNA complexes at N:P=7 in human chondrocytes, where linear PEI-25k was used as control.

6. Flow cytometry

Cellular uptake of polypeptide/pDNA complexes is measured by flow cytometry. PGL3 was labeled with Cy5 using a commercial Label IT kit (Mirus, Madison, WI) according to the manufacturer's instructions. 293 T cells were seeded to a 12 well plate at an initial density of 4×10^5 cells per well one day before gene transfection. Since at N:P=5, all the samples have reached their highest transfection, to keep the sample charge ratio, we use this concentration to prepare the complexes. The polypeptide/pDNA complexes were added to each well in serum free DMEM (500 μL) at a final concentration of 1.6 μg labeled DNA per well. Each sample was performed in triplicates. Linear PEI-25k was used as control (at N:P=5, to keep the same charge ratio). The cells were further incubated at 37 ^{o}C and harvested at different desired time. After rinsed twice by the PBS containing 0.001% SDS and then PBS to remove the extracellularly attached complexes,[4] the harvested cells were detached from each well by 0.05% trypsin-EDTA. Then, the cells were further washed twice by the PBS solution, re-suspended in 4% paraformaldehyde and stored at 4 ^{o}C before the flow cytometry measurements. The fluorescence intensity of the cells was recorded by using a BD FACSVerse flow cytometry system. For each sample, 10^4 gated events were collected. The fluorophore was excited at 640 nm and detected at 660/10 nm. The fluorescence intensity was presented in a logarithmic scale.

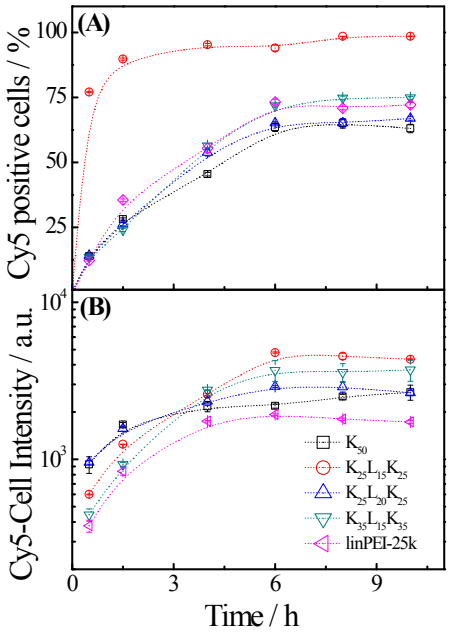

Figure S4. Flow cytometry study on cellular uptake of polypeptide/Cy5-pDNA complexes, where 293 T cells were used, the transfection was respectively carried out at N:P=5 for each sample with linear PEI-25k (linPEI-25k) as control, and cellular uptake extent is expressed as (A) percentage of Cy5-positive cells; (B) average fluorescence intensity of Cy5-positive cell population.

References

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