Electronic Supplementary Information (ESI)

Zn(II) coordination to cyclen-based polycations for enhanced gene delivery

Qing-Ying Yu, Yu Guo, Ji Zhang*, Zheng Huang and Xiao-Qi Yu*

Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University,

Chengdu 610064, P. R. China

*Corresponding authors: jzhang@scu.edu.cn (J. Zhang); xqyu@scu.edu.cn (X.-Q. Yu); Fax: + 86 28 85415886

Table S1 The content of Zn(II) in Zn(II)-polycations aqueous solution (1 mg/ml) obtained by ICP-OES (tv: theoretical value; av: actual value).

Zn(II)-polycations	Zn-cyclen-DG	Zn-cyclen-TG	Zn-cyclen-BD	Zn-cyclen-HD
C (Zn) tv	133.5 μg/mL	122.5 μg/mL	138 μg/mL	130.3 µg/mL
C (Zn) av	133 μg/mL	137.5 μg/mL	140.5 μg/mL	153 μg/mL



Fig. S1 Fluorescence quenching assay of EB/DNA by addition of polycations and Zn(II)-polycations.

0 0.5 1 2 4 8 16 32	0 0.5 1 2 4 8 16 32	0 0.5 1 2 4 8 16 32	0 0.5 1 2 4 8 16 32
Zn-cyclen-HD	cyclen-HD	Zn-cyclen-TG	cyclen-TG

Fig. S2 Release of DNA from polyplxes with the addition of heparin at various heparin/DNA weight ratios. w/w = 0, 0.5, 1, 2, 4, 8, 16, 32; polymers/DNA: w/w = 2.



Fig. S3 Absorption spectra (A-D) and CD spectra (E-F) of DNA/Zn(NO₃)₂·6H₂O, DNA/Zn-cyclen, DNA/**Zn-cyclen-BD** and DNA/PEI polyplexes with different *w/w* ratios, respectively. For $Zn(NO_3)_2$ ·6H₂O, the absorption and CD spectra were measured with same amount of Zn(II) in **Zn-cyclen-HD**.



Fig. S4 Fluorescence microscopy image of pEGFP-transfected HeLa cells in the absence or presence of serum.



Fig. S5 CLSM images of HeLa cells transfected with Cy5-labaled DNA by polymers at optimal weight ratio in the presence of 10% serum. The weight ratios for **cyclen-HD**/DNA and **Zn-cyclen-HD**/DNA polyplexes were 8 and 6, respectively. PEI as control (w/w=1.4). For each row from left to right: Cy5-labeled pDNA (red), cell nuclei stained by Hoechest 33342 (blue), bright field and merged image.



Fig. S6 Relative cellular uptake of polyplexes derived from **cyclen-HD** and **Zn-cyclen-HD** at the optimal transfection w/w ratio with 10% serum in HeLa cells in the presence of various endocytic inhibitors quantified flow cytometry analysis. The weight ratios for **cyclen-HD**/DNA and **Zn-cyclen-HD**/DNA polyplexes were 8 and 6, respectively. Data represent mean \pm SD (n = 3).



Fig. S7 CLSM images of HeLa cells transfected with Cy5-labaled DNA by polymers at optimal weight ratio in the presence of 10% serum. The weight ratios for **cyclen-HD**/DNA and **Zn-cyclen-HD**/DNA polyplexes were 8 and 6, respectively. PEI as control (w/w=1.4). For each row from left to right: Cy5-labeled pDNA (red), lysosome stained by lysotracker green (green), cell nuclei stained by DAPI (blue) and merged image. Pearson's coefficient (R) for colocalization of the Cy5-labeled pDNA with the lysosomes was calculated using Image Pro Plus 6.0.





Fig. S8 ¹H NMR spectra of polycations. For linear (1:1) cyclen-HD, theoretical number of H:a, 4H; b, 2H; c, 4H; d, 4H; e, 4H; f, 4H; cyclen,16H. The ratio of cyclen/HD was (17.8-4)/16:1=0.86, so the polymer was network polymer.