Supporting Information

# Plasmid DNA Mono-Ion Complex Stabilized by Hydrogen Bond for *In Vivo* Diffusive Gene Delivery

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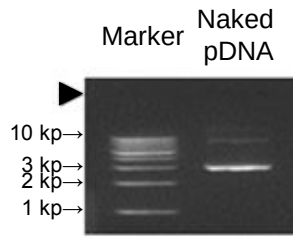


**Figure S1.** The pDNA used in agarose gel retardation assay. The pDNA was electrophoresed with size standard maker. The solid arrowhead indicates the well where the marker and pDNA were loaded.

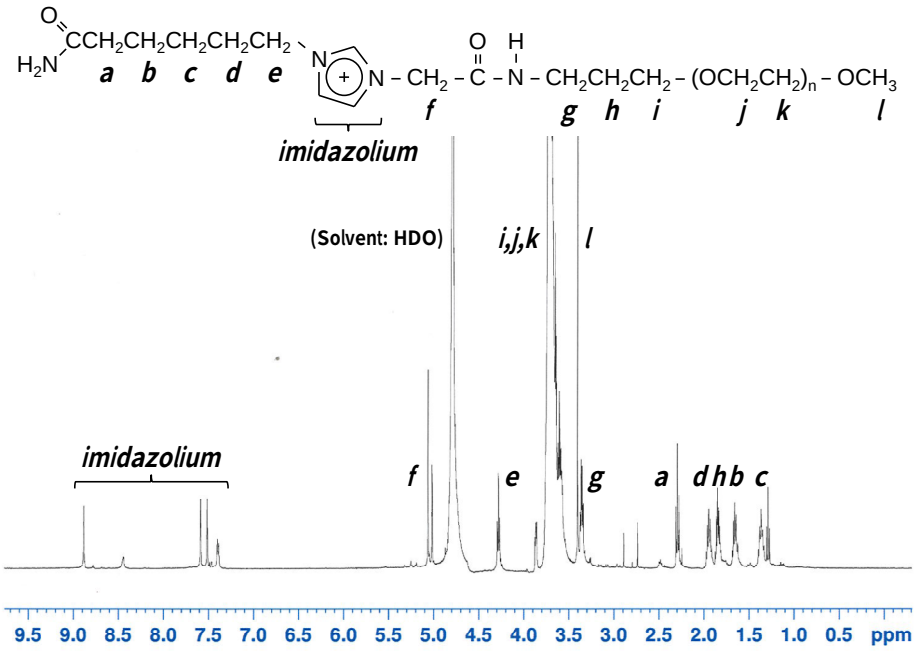


**Figure S2.** $^{1}$H NMR spectrum of APe-Im-PEG in $D_2O$.

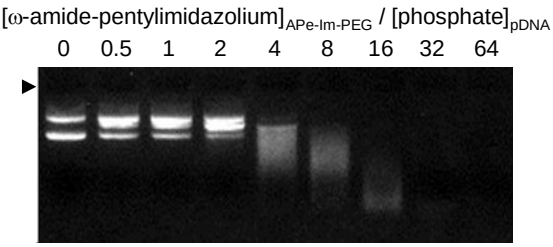


**Figure S3.** Analysis of the MIC formation between pDNA and APe-Im-PEG by agarose gel electrophoresis in the presence of urea. The mixing ratios relative to the ω-amide-pentylimidazolium group of APe-Im-PEG per phosphate group of pDNA ($[\omega\text{-amide-pentylimidazolium}]_{\text{APe-Im-PEG}}/[\text{phosphate}]_{\text{pDNA}}$) are indicated. The solid arrowhead indicates the well where each sample was loaded.

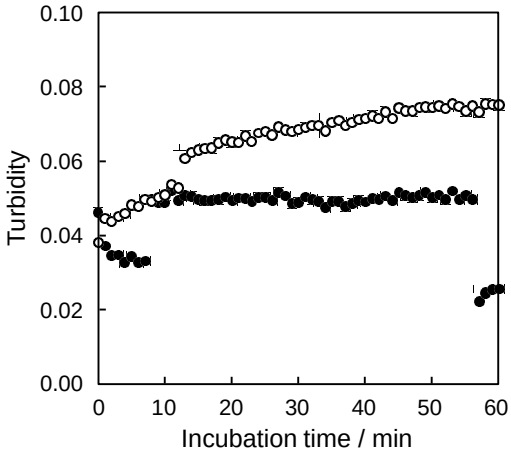


**Figure S4.** Serum stability of the APe-Im-PEG in FBS. The turbidity was measured by monitoring the absorbance at 500 nm of the mixture of the APe-Im-PEG (●) and FBS (50%) during the incubation. As a control, *in vivo* transfection reagent poly(ethyleneimine) (*in vivo*-jetPEI$^{TM}$) (○) was used.

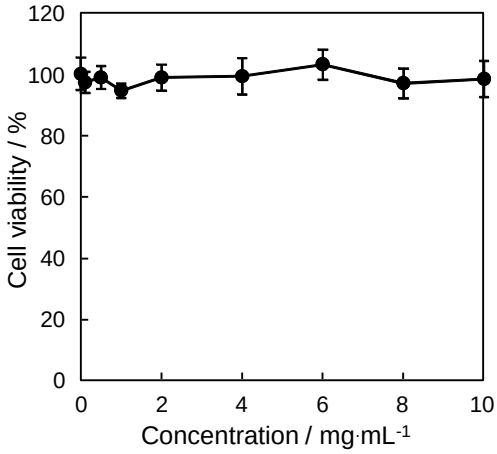


**Figure S5.** Effect of the APe-Im-PEG on the viability of HepG2 cells. Symbols and error bars represent the mean and standard deviation of the measurements made in paired wells (n = 5).

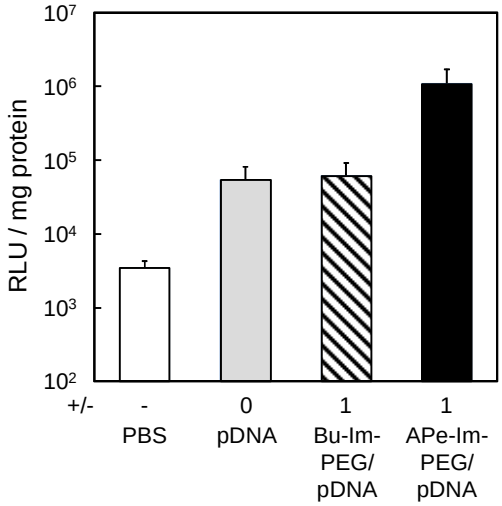


**Figure S6.** Transfection of luciferase gene into the skeletal muscles by APe-Im-PEG/pDNA MIC as well as Bu-Im-PEG/pDNA MIC at a positive/negative charge ratio (+/-) of 1. Gene expression was determined relative light unit (RLU) normalized by the protein concentration. Symbols and error bars represent the mean and standard deviation (n = 2).

**Table S1.** Particle size and zeta potential of APe-Im-PEG/pDNA MICs.

| +/- | Particle diameter / nm | Zeta potential / mV |
|---|---|---|
| 0.5 | (40.7±7.0) | N.D.[a)] |
| 0.8 | (42.6±8.3) | (0.58) |
| 1.0 | (31.1±4.1) | (-3.36) |
| 1.5 | (32.9±5.6) | (-0.46) |

[a)] N.D.: not determined.