Supporting information

Transcellular Delivery of Messenger RNA Payloads by Cationic Supramolecular MOF Platform

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Abbreviation list:

MOF: metal-organic frameworks

PGMA(EA): poly(glycidyl methacrylate)-(ethanolamine)

LDH: lactic Acid dehydrogenase,

UiO66(Zr): Universitetet i Oslo, metal organic framework made up of $[Zr_6O_4(OH)_4]$ clusters with 1,4-benzodicarboxylic acid struts

mRNA: messenger RNA

XRD: X-ray diffraction

TEM: transmission electron microscopy

N/P ratio: molar ratio of the amino groups from the catiomers to the phosphate groups of mRNA

S/P ratio: molar ratio of the sulphur from heparin to the phosphate from mRNA

Zr: zirconium

DLS: dynamic light scattering

ICP-MS: inductively coupled plasma mass spectrometry

EtBr: ethidium bromide

FBS: fetal bovine serum

BiBB: bromoisobutyryl bromide

Materials

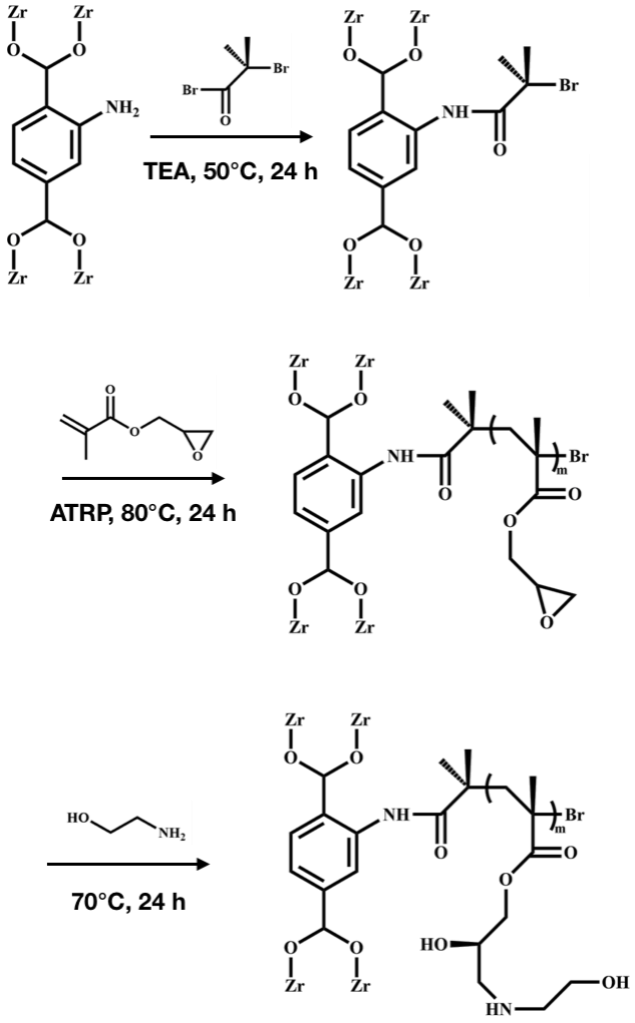
All the chemicals were purchased from Tansoole, Titan Co. Ltd. (Shanghai, China). FUGENE HD was purchased from Promega (Madison, WI). Lipofectamine™ MessengerMAX™ was purchased from Thermo Fisher Scientific (Waltham, MA). jetPEI was purchased from TAMAR laboratory Supplies Ltd (Abu-Gosh, Israel). Cyclofect™ alpha was purchased from Syndean Biotech. Co. Ltd (Dalian, China). mRNA encoding a reporting sequence of luciferase (LUC) was prepared from the template pGL4.13 DNA (Promega, Madison, USA) by *in vitro* transcription using the mMESSAGE mMACHINE T7 Ultra Kit (Ambion, Invitrogen, Carlsbad, CA), followed by polyadenylation with the poly(A) tail kit (Ambion, Invitrogen, Carlsbad, CA). Messenger RNA (mRNA) were labeled with Cy5 using the Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit obtained from Mirus Bio Corp. (Madison, WI) according to the manufacturer's protocol. Dulbecco's modified eagle's medium (DMEM) and Dulbecco's phosphate-

buffered saline (DPBS) were purchased from Sigma-Aldrich Co. (Madison, WI). Fetal bovine serum (FBS) was purchased from GIBCO (Thermo Fisher Scientific, Waltham, MA). Penicillin and streptomycin were from Beyotime Institute of Biotechnology Co. (Shanghai, China). Ethidium Bromide (EtBr), heparin Cell culture lysis buffer and Luciferase Assay System Kit were purchased from Sigma Aldrich China (Shanghai, China). The molecular weight distribution (M_w/M_n) of the yielded product was characterized by gel permeation chromatography (GPC) equipped with TOSOH HLC-8220 calibrated with varied M_w of commercial PEG standards.

Methods

1. Synthetic procedures and characterizations of MOF-PGMA(EA)

The synthetic scheme of MOF-PGMA(EA) was provided in Scheme S1.



Scheme S1. Synthetic scheme in preparation of the dendritic catiomer of MOF-PGMA(EA).

a): Synthesis of NH_2-UiO-66 (MOF)

The synthesis of NH_2-UiO-66 was performed according to a solvothermal approach. In brief, 2-amino-benzenedicarboxylic acid (0.248 g, 1.372 mmol) and $ZrCl_4$ (0.320 g, 1.372 mmol) were dissolved in 80 mL N,N-dimethylformamide (DMF) in Teflon-lined stainless-steel autoclave. Furthermore, 1.236 mL ultrapure water (68.7 mmol, 50 equiv. to $ZrCl_4$) was added to the above DMF solution under stirring. The mixture was sealed for 24 h reaction in oven at 120 °C. After cooling down to room temperature, the precipitate was collected by centrifugation and washed with a mixture of DMF and methanol. The obtained crystal was immersed in methanol for 24 h incubation, rinsed with methanol, and dried under reduced pressure at 80 °C.

b): Synthesis of the Br-functionalized NH_2-UiO-66 (UiO-BiBB) (Br-MOF)

UiO-BiBB was obtained by conjugation of the yielded NH_2-UiO-66 with bromoisobutyryl bromide (BiBB). In brief, 0.30 g NH_2-UIO-66 (containing 1 mmol -NH_2) was dispersed in 20 mL anhydrous tetrahydrofuran (THF) by sonication. Triethyleneamine (TEA, 209 mL, 1.5 mmol) and BiBB (62 mL, 0.5 mmol) were dissolved in 5 mL THF. TEA solution was injected into the NH_2-UiO-66 suspension under stirring, followed by titration of BiBB solution at room temperature under stirring. The reaction was conducted at 50 °C for 24 h. The product was washed with THF and methanol. The resulting UiO-BiBB was collected and incubated in methanol for 24 h, washed with methanol, and dried under reduced pressure for 24 h at 40 °C.

c): Synthesis of UiO-PGMA (MOF-PGMA)

The polymerization of poly(glycidyl methacrylate) (PGMA) from the yielded UiO-BiBB was prepared based on atom transfer radical polymerization (ATRP) approach. In brief, 73.4 mg of UiO-BiBB (calculated to be 0.09 mmol of a-bromoisobutyryl group) (1 equiv.) was dispersed in 35 mL of anhydrous THF by sonication. Bipyridyl (21.08 mg, 0.135 mmol) (1.5 equiv.), glycidyl methacrylate (GMA, 0.833 mL, 6.3 mmol) (70 equiv.), and CuBr (12.9 mg, 0.09 mmol) (1 equiv.) were added to the above THF solution. Note that the reaction mixture was subjected to degassing treatment and kept under a nitrogen atmosphere throughout the reaction. Atom transfer radical polymerization of PGMA segment was conducted at 80 °C for 24 h. After cooling down to room temperature, the yielded solution was centrifuged, and the supernatant was condensed and precipitated in diethyl ether. The final product was dried under reduced pressure for 24 h at 40 °C.

d): Synthesis of UiO-PGMA(EA) [MOF-PMGA(EA)]

The yielded UiO-PGMA was schemed to proceed aminolysis to create hyper-charged UiO-PGMA(EA). In brief, 15 mg UiO-PGMA was dissolved in DMSO (4 mL). Furthermore, 1.5 mL of ethanolamine was added to the above DMSO solution. The reaction was conducted under a nitrogen atmosphere at 70 °C under stirring for 24 h. The crude product was purified by dialysis (Spectra/Por RC, MWCO: 7 kDa) against deionized water for 48 h, followed by lyophilization to obtain UiO-PGMA(EA) as white solid. The resulting product was transferred to ^{1}H-NMR measurement (Figure S2). Note that the protons of benzyl rings in the MOF core of UiO-PGMA(EA) was not visible in ^{1}H-NMR spectrum due to insolubility of MOF core in D_2O. The overall amine groups per MOF supramolecular structure were determined based on the following ICP-MS measurement for quantification of the numbers of Zr-based MOF and fluorescamine assay for the total amine groups in pertinent to the same stock solution of MOF-PGMA(EA).

e): **Synthesis of PGMA(EA)**

The synthesis of the linear control catiomer of PGMA(EA) was conducted to follow the similar procedure according to the previous method.[S1] The chemical descriptions were summarized in Table S1.

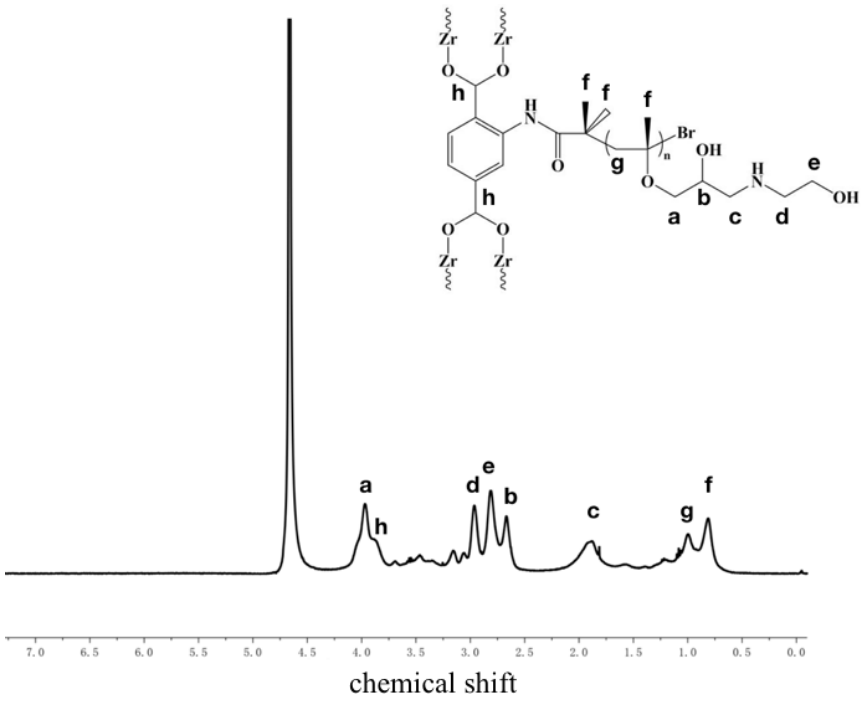


Fig. S1 ^{1}H-NMR spectrum of MOF-PGMA(EA) in D_2O.

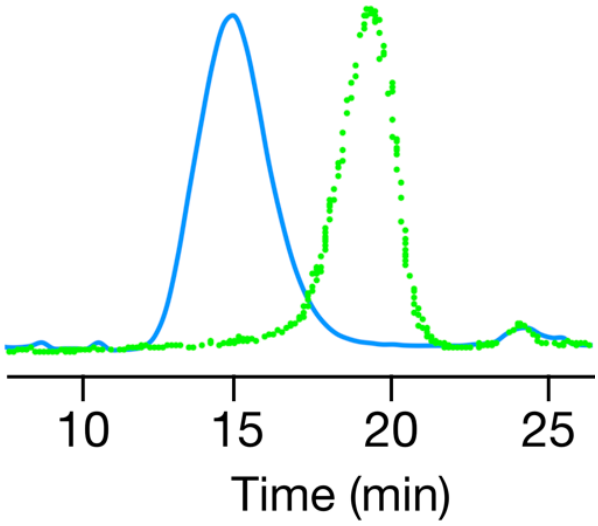


Fig. S2 GPC traces of the linear control catiomer of PGMA(EA) (green) and MOF-PGMA(EA) (blue).

Table S1 Chemical descriptions of MOF-PGMA(EA) and PGMA(EA).

Catiomers	Numbers of PGMA(EA) segments per catiomer	Approximate polymerization degree of PGMA(EA) segment	Total numbers of amino groups per catiomer
PGMA(EA)	1	41	41
MOF-PGMA(EA)	12[a]	35.6[a]	427[b]

[a]Theoretical assumption by considering the number of available ligands for subsequent ATRP.

[b]Experimental calculation based on quantification of total amine groups by fluorescamine assay and quantification of Zr composition by ICP-MS measurement.

2. Preparation of the mRNA complexes

The polycatiomers of PGMA(EA) or MOF-PGMA(EA) and mRNA were separately dissolved in 10 mM HEPES (pH 7.4). The complexes were simply prepared by fast mixing one-unit volume of the polymer solution with two-unit volume of mRNA solution at the N/P ratio of 1.5 (Note that the slight over-stochiometric charge ratio was selected with the aims of eliminating the impact of the uncomplexed cationic polymers). The N/P ratio is defined as the residual molar ratio of amine (N) groups of the catiomers [either PGMA(EA) or MOF-PGMA(EA)] to the phosphate (P) groups of mRNA.

3. XRD

Aliquot 10 mg UiO-BiBB was compressed as a thin layer on a glass plate prior to XRD measurements. XRD data were collected at ambient atmosphere and temperature under SHIMADZU XRD-6000. Diffractometer was conducted at 40 kV, 40 mA for monochromatic Cu Kα (λ = 1.5418 Å) with a scan speed of 5°/min, a step size of 0.02° in 2θ, and a 2θ range of 5-45°.

4. TEM

TEM morphology observation was performed with transmission electron microscopy (TEM) measurement by an H-7000 electron microscope (Hitachi, Tokyo, Japan) operated at 75 kV acceleration voltages for insight on the morphology of complexes containing LUC mRNA at N/P ratio of 1.5. Copper TEM grids with carbon-coated collodion film were glow-discharged for 10 s using an Eiko IB-3 ion coater (Eiko Engineering Co. Ltd., Japan). The grids were dipped into the desired complex solution, which was premixed with uranyl acetate (UA) solution (2% (w/v)) for 30 s to stain the mRNA. The sample grids were blotted by filter paper to remove excess complex solution, followed by air-drying for 30 min and transferred to TEM observation. The obtained TEM image was further analyzed with ImageJ 1.44 (National Institutes of Health) to quantify the diameter in each spherical structure, and 100 individual spherical structures were measured to yield the average diameter of the spherical structures.

5. Dynamic Light Scattering (DLS)

The hydrodynamic diameter and PDI of polymeric formulations were measured by DLS using a Zetasizer Nanoseries instrument (Malvern Instruments). The measurement was performed three times at a detection angle of 173° and a temperature of 25 °C. The rate of decay in the photon correlation function was analyzed according to a cumulant method, and the corresponding diameter was calculated using the Stokes-Einstein equation.

6. *ζ-potential measurement*

The ζ-potential of the complexes was also measured by Zetasizer Nanoseries instrument (ZEN3600, Malvern Instruments, Ltd., UK). The complex solution was injected into folded capillary cells (Malvern Instruments, Ltd.). The ς-potential was determined from the laser-doppler electrophoresis using the Zetasizer nanoseries (Malvern Instruments Ltd., UK). From the obtained electrophoretic mobility, the ς-potential was calculated by using the

Smoluchowski equation: $\varsigma = 4\pi\eta\nu/\varepsilon$ in which η is the electrophoretic mobility, ν is the viscosity of the solvent, and ε is the dielectric constant of the solvent. The results are expressed as the average of three experiments.

Table S2 Characterizations of MOF-PGMA(EA) and PGMA(EA) formulated mRNA polyplexes.

mRNA polyplexes	Diameter by DLS[a]	Diameter by TEM[b]	ζ-potential (mV)
PGMA(EA)	41.7	53.8	+ 32.1
MOF-PGMA(EA)	26.4	31.5	+ 34.2

[a]Diameters of mRNA complexed formations were determined by DLS measurement, and the results were expressed as the number average hydrodynamic diameters.

[b]Diameters of mRNA complexed formations were determined by manually measuring the diameters of the spherical structures in TEM measurements. A total of 100 spherical structures were counted.

7. Quantification of Zr in MOF-PGMA(EA) by ICP-MS

The amount of Zr was determined by ion-coupled plasma mass spectrometry (ICP-MS) by using an Agilent 7700 ICP-MS. Note that the standard Zr solution was diluted to establish the calibration curve.

8. Quantification of amine groups in MOF-PGMA(EA)

The numbers of amine groups in MOF-PGMA(EA) was estimated according to a fluorescamine method.[S2] In brief, the MOF-PGMA(EA) were dissolved in PBS buffer (0.1 mL, 0.5 mg/mL, pH 7.4) and mixed with fluorescamine (10 μL, 5 mg/mL in DMF). Following 10 min reaction at room temperature, the fluorescence emission was recorded by a ND-3300 fluorospectrometer (Nanodrop, Wilmington, DE). The amine groups in the yielded MOF-PGMA(EA) was calculated according to the standard sample of the linear PGMA(EA). Consequently, the amine groups per MOF could be calculated based on the ICP-MS results.

9. EtBr assay

Heparin was dissolved in 10 mM HEPES (pH 7.4) buffer as stock solution (30 mg/mL). Furthermore, the PGMA(EA) or MOF-PGMA(EA) complex solutions (18 μL) were fused with aliquot of heparin stock solution at varying concentrations. The mixtures were allowed for 1 h polyionic exchange reaction at room temperature, followed by supplement with EtBr solution for reaction for 3 h. Note that all the sample solutions have a uniform mRNA and EtBr concentration of 33.33 μg/mL and 16.67 μg/mL, respectively. The fluorescence intensity of EtBr was measured by Nanodrop (ex: 360 nm; em: 590 nm).

10. Quantification of mRNA after serum incubation by quantitative real-time PCR(qRT-PCR)

Solutions of the PGMA(EA) or MOF-PGMA(EA) complexes containing LUC mRNA at an mRNA concentration of 50 μg/mL were subjected to varied incubation period in 10% fetal bovine serum (FBS) or 50% FBS at 37 °C. After sequential treatment with trypsin and dextran sulfate at 4 °C, mRNA purification was conducted with the RNeasy Mini Preparation Kit (QIAGEN). The purified product was transferred for quantitative real-time PCR using an ABI Prism 7500 Detector (Applied Biosystems), where a primer pair for Luc (forward: TGCAAAAGATCCTCAACGTG; reverse: AATGGGAAGTCACGAAGGTG) was used.

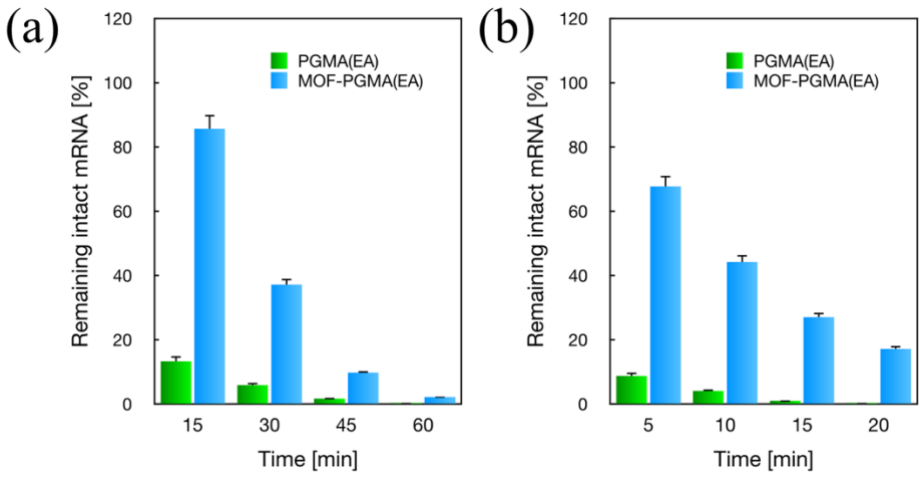


Fig. S3 Quantification of the remain intact mRNA within the polyionic complexes from PGMA(EA) or MOF-PGMA(EA) in 10% FBS (a) or 50% FBS (b) by qRT-PCR.

11. Cellular Uptake

U87 cells were seeded onto 6-well culture plates (50,000 cells/well) with 2 mL of DMEM containing 10% FBS and 1% antibiotics (penicillin and streptomycin) and incubated in a humidified atmosphere supplemented with 5% CO_2 at 37 °C. After 24 h incubation, the medium was replaced with fresh medium. The cells were treated with the complex

solutions (1 μg of Cy5-labeled mRNA/well) and followed by another 24 h incubation. The medium was discarded and the cells were washed three times with PBS to remove extracellular fluorescence. The cells were detached by trypsin-EDTA treatment and harvested from the cell culture plate as suspension in 1 mL ice-cold PBS. The cellular uptake efficiency was measured by a BD LSR II flow cytometer equipped with FACS-Diva software (BD Biosciences). The obtained data were expressed as the mean fluorescence intensity from three independent samples (n = 3).

12. Transfection efficiency

U87 cells were seeded onto 24-well culture plates (20,000 cells/well) with 2 mL DMEM containing 10% FBS and 1% antibiotics (penicillin and streptomycin) and incubated for 24 h in a humidified atmosphere supplemented with 5% CO_2 at 37 °C. After exchanging the medium with a fresh one, 1 μg Luc mRNA containing the complex solutions was added to each well. After 24 h incubation, the medium was exchanged with 400 μL fresh medium, followed by another 24 h incubation. The cells were washed three times with 400 μL ice cold PBS and lysed by 150 μL cell culture lysis buffer at 37 °C for 15 min. Immediately, 20 μL the cell lysate was transferred to a 96-well luminometry plate, followed by the addition of 100 μL Luciferase Assay Reagent (Promega) to each well, and allowed to react for 15 min. The Luc expression was measured for 10 s from the photoluminescence intensity using Mithras LB 940 (Berthold Technologies).

Following the similar manner, the transfection efficiency was also evaluated in a toxic-sensitive healthy human umbilical vein endothelial cells (HUVECs). In brief, HUVECs (20,000 cells/well) were plated on 24-well plates and incubated overnight in 100 μL of MCDB131 containing 10% FBS and 10 ng/mL b-FGF. After overnight incubation, the cell culture medium was exchanged, followed by addition of 1 μg Luc mRNA containing the complex solutions. After 24 h incubation, the medium was exchanged with 400 μL fresh medium, followed by another 24 h incubation. The cells were washed three times with 400 μL ice cold PBS and lysed by 150 μL cell culture lysis buffer at 37 °C for 15 min. Immediately, 20 μL the cell lysate was transferred to a 96-well luminometry plate, followed by the addition of 100 μL Luciferase Assay Reagent (Promega) to each well, and allowed to react for 15 min. The Luc expression was measured for 10 s from the photoluminescence intensity using Mithras LB 940 (Berthold Technologies).

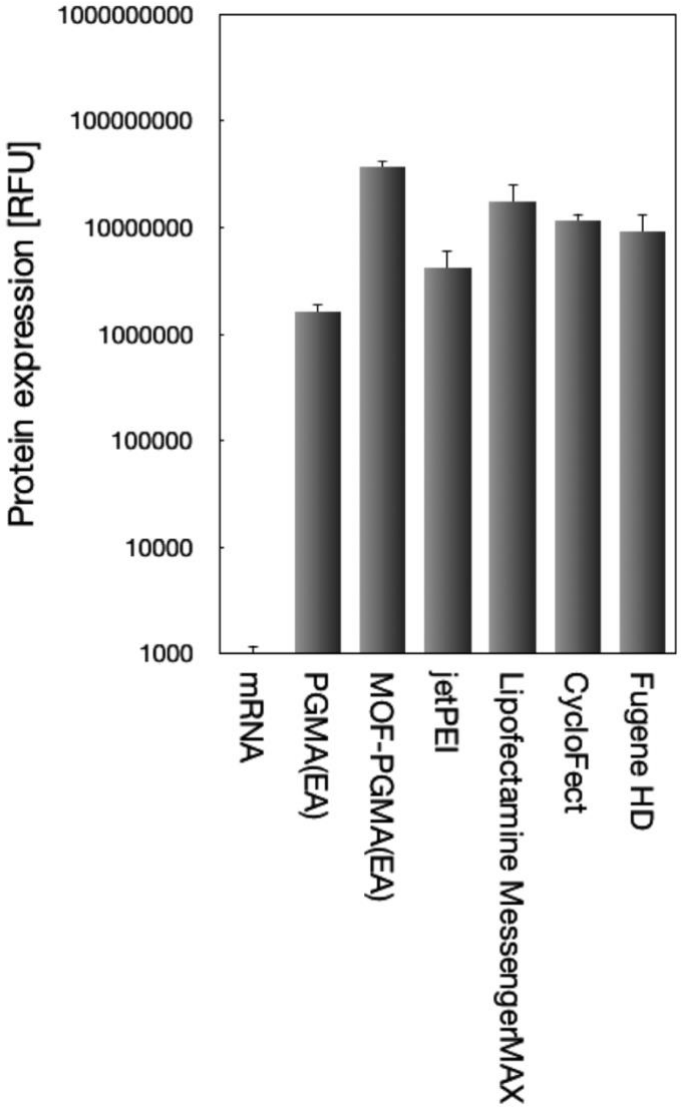


Fig. S4 Gene expression levels of Luc mRNA by a variety of transfection agents in HUVECs.

13. LDH assay

The membrane destabilizing ability of PGMA(EA) and MOF-PGMA(EA) was assessed by measuring lactate dehydrogenase (LDH) activity liberated from the cells in contact with the PGMA(EA) and MOF-PGMA(EA) formulated complex. 5,000 U87 cells were plated on 96-well plates and incubated overnight in 100 μL DMEM containing 10% FBS and 1% antibiotics (penicillin and streptomycin) and incubated for 24 h in a humidified atmosphere supplemented with 5% CO_2 at 37 °C. The cell culture medium was changed to 100 μL of the PGMA(EA) and MOF-PGMA(EA) complex solutions at various concentrations in 20 mM MES buffer (pH 5.5) containing 150 mM NaCl. After incubation at 37 °C for 1 h, 50 μL aliquots in each well were collected for the LDH assay. The LDH activity in these samples was determined utilizing a commercial kit (LDH-Cytotoxic Test Wako, Wako Pure Chemical

Industries, Ltd., Japan) according to the manufacturer's protocol. Percentages of the LDH activity in each well were calculated from a ratio of the obtained value to the control well containing 0.2 wt% Tween20 (the total cell lysis). The results were presented as a mean and standard error of mean obtained from four samples.

14. Cytotoxic profiles

U87 cells were seeded in 24-well culture plates (20,000 cells/well) and incubated overnight in 400 mL DMEM supplemented with 10% FBS. The medium was replaced with 400 mL of fresh medium, followed by addition of 30 mL each complex solution into each well (1 ug mRNA/well). Note that the commercial transfection agents formulated complexes were prepared according to the manufacture's protocol. After 24 h incubation at 37 °C, the medium was changed to 400 mL of fresh medium, followed by another 24 h incubation. The cells were washed with 400 mL, and cell viability was determined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. Each well in 200 mL fresh medium was reacted with 20 mL Cell Counting Kit-8 Agent. After 2 h reaction at 37 °C, the absorbance at 450 nm of the formazan in each well was quantified from a microplate reader (Model 680, Bio-Rad, UK). The cell viability in each well was calculated and presented as a percentage of control wells without any addition.

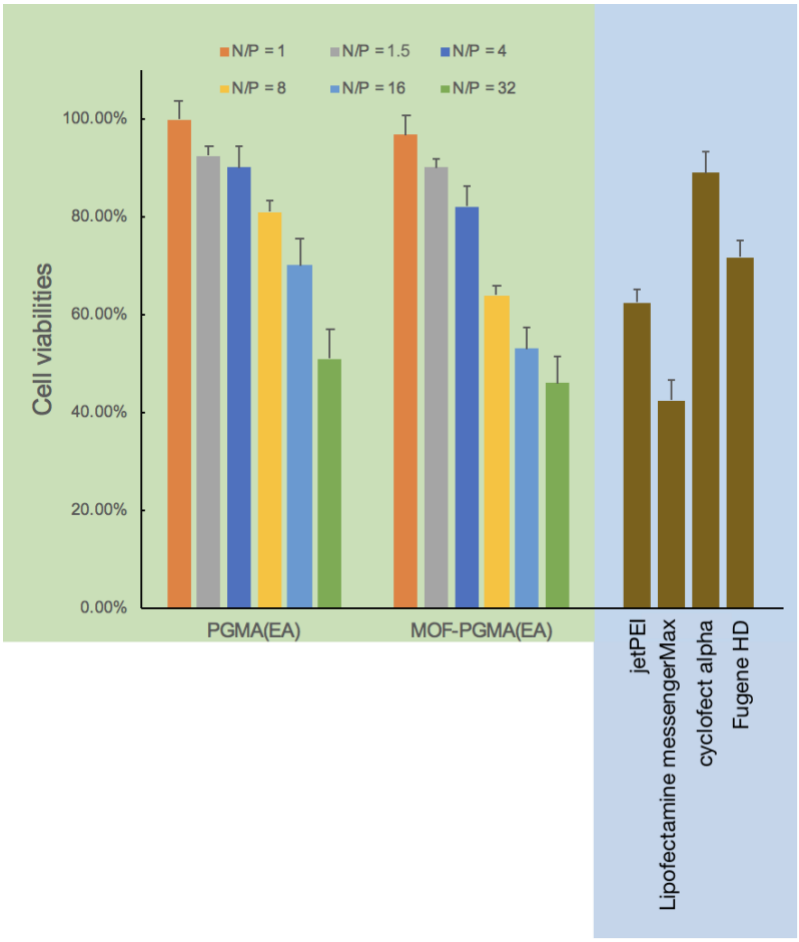


Fig. S5 Cytotoxic profiles of mRNA complexes from PGMA(EA), MOF-PGMA(EA) at varied N/P ratios and commercial mRNA transfection agents.

14. Statistical analysis

The *p* values were determined by the Student's t test using a two-tailed distribution and two-sample un-equal variance with the t test function of Microsoft Excel for statistical analysis for two samples. The *p* values of less than 0.05 were considered statistically significant.

Reference:

[S1] S. Dong, Q. Chen, W. Li, Z. Jiang, J. Ma and H. Gao, *J. Mater. Chem. B,* 2017, **5**, 8322-8328.

[S2] A. Kim, Y. Miura, T. Ishii, O. F. Mutaf, N. Nishiyama, H. Cabral and K. Kataoka, K. *Biomacromolecules* 2016, **17**, 446.