Tunable composition of dynamic non-viral vectors over the DNA polyplex formation mechanism and nucleic acid transfection

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Synthesis of PEGylated squalene

PEGylated squalene was synthesized in four steps as reported in previous papers [1-3]. Briefly, squalene was functionalized to 1,1',2-tris-nor-squalene aldehyde in three steps. In the first step 2-hydroxy-3-bromosqualene was synthesized from squalene with N-bromosuccinimide in tetrahydrofuran at 0 °C for 90 minutes, then, 2,3-oxidosqualene was obtained from 2-hydroxy-3-bromosqualene with potassium carbonate in methanol at room temperature (25 °C) for 2 hours. In the third step, 1,1',2-tris-nor-squalene aldehyde was obtained by reducing the epoxide with periodic acid in water-dioxane solution at room temperature (25 °C) for 2 hours. In the last step, PEGylated squalene was synthesized by mixing 1,1',2-tris-nor-squalene aldehyde (0.206 g, 0.54 mmol, 1 equiv.) with poly-(ethyleneglycol)-bis(3-aminopropyl) (1500 Da) (0.886 g, 0.59 mmol, 1.1 equiv.) in acetonitrile (20 mL) and magnetically stirred for 24h at room temperature (25 °C), under nitrogen atmosphere. The product was obtained in quantitative yield and used further without purification. PEGylated squalene was stored as solution in acetonitrile at 2-4 °C for further experiments. 1H-NMR (400 MHz, CDCl3, TMS) δ (ppm) = 7.64 (1H, t, J=4.8, CH=N), 5.15 - 5.08 (5H, m, CH=C), 3.70 - 3.64 (140 H, m, CH2-CH2-O), 3.19 (2H, t, J=6.4, CH2-NH2), 2.53 - 2.49 (2H, m, CH2), 2.33 - 2.30 (2H, m, CH2), 2.09 - 1.97 (16H, m, CH2-CH2), 1.88 - 1.83 (3H, m, CH3), 1.68 (3H, m, =C(CH3)-CH3), 1.61 (12H, m, =C(CH3)) (Fig 7S). 13C-NMR (101 MHz, CDCl3, TMS) δ (ppm) = 161.67 (C=N), 134.91 (CH3-C=C), 131.25 (C(CH3)=), 124.40 (CH3-C=CH), 124.26 (CH3-C=CH), 72.57 (OCH2-CH2), 70.58 (O-CH2-CH2-O), 70.35 (O-CH2), 70.12 (O-CH2), 69.97 (O-CH2), 61.71(NCH3), 39.73 (CH2-CH2), 39.58 (CH3-NH2), 31.85 (CH3), 28.25 (CH3), 26.77 (CH3), 26.55(CH3), 25.71 (CH3), 17.69 (CH3),16.06 (CH3), 16.01 (CH3).

![PEGylated Squalene](image1)

![PEGylated Squalene-TA](image2)

Figure S1. 1H-NMR spectra of PEGylated Squalene intermediates in CDC13.

Table S1. Compositions for dynamic frameworks libraries synthesis.
| Sample | MW (Da) | Molar ratio (equiv) | m (mg) | n (nmol) | MW (Da) | Molar ratio (equiv) | m (mg) | n (nmol) | MW (Da) | Molar ratio (equiv) | m (mg) | n (nmol) | Final Volume (µL) |
|--------|---------|-------------------|-------|--------|---------|-------------------|-------|--------|---------|-------------------|-------|--------|------------------|
| NV1    | 2008.58 | 1                 | 12.5  | 6.20   | 1500    | 0.1               | 0.93  | 0.62   | 0.1      | 0.93              | 0.62   | 3000   |
| NV2    |         |                   |       |        |         | 0.2               | 1.86  | 1.24   | 0.2      | 1.86              | 1.24   | 3000   |
| NV3    |         |                   |       |        |         | 0.3               | 2.79  | 1.86   | 0.3      | 2.79              | 1.86   | 3000   |
| NV4    |         |                   |       |        |         | 0.4               | 3.72  | 2.48   | 0.4      | 3.72              | 2.48   | 3000   |
| NV5    |         |                   |       |        |         | 0.5               | 4.65  | 3.10   | 0.5      | 4.65              | 3.10   | 3000   |
| NV6    |         |                   |       |        |         | 0.6               | 5.58  | 3.72   | 0.6      | 5.58              | 3.72   | 3000   |
| NV7    |         |                   |       |        |         | 0.7               | 6.51  | 4.34   | 0.7      | 6.51              | 4.34   | 3000   |
| NV8    |         |                   |       |        |         | 0.8               | 7.44  | 4.96   | 0.8      | 7.44              | 4.96   | 3000   |
| NV9    |         |                   |       |        |         | 0.9               | 8.37  | 5.58   | 0.9      | 8.37              | 5.58   | 3000   |
| NV10   |         |                   |       |        |         | 1                 | 9.20  | 6.20   | 1        | 9.20              | 6.20   | 3000   |
| NV11   |         |                   |       |        | 2000    | 0.1               | 1.23  | 0.62   | 0.1      | 1.23              | 0.62   | 2247   |
| NV12   |         |                   |       |        |         | 0.2               | 2.47  | 1.24   | 0.2      | 2.47              | 1.24   | 2247   |
| NV13   |         |                   |       |        |         | 0.3               | 3.70  | 1.86   | 0.3      | 3.70              | 1.86   | 2247   |
| NV14   |         |                   |       |        |         | 0.4               | 4.93  | 2.48   | 0.4      | 4.93              | 2.48   | 2247   |
| NV15   |         |                   |       |        | 3000    | 0.5               | 6.17  | 3.10   | 0.5      | 6.17              | 3.10   | 2247   |
| NV16   |         |                   |       |        |         | 0.6               | 7.40  | 3.72   | 0.6      | 7.40              | 3.72   | 2247   |
| NV17   |         |                   |       |        |         | 0.7               | 8.63  | 4.34   | 0.7      | 8.63              | 4.34   | 2247   |
| NV18   |         |                   |       |        |         | 0.8               | 9.87  | 4.96   | 0.8      | 9.87              | 4.96   | 2247   |
| NV19   |         |                   |       |        |         | 0.9               | 11.10 | 5.58   | 0.9      | 11.10             | 5.58   | 2247   |
| NV20   |         |                   |       |        |         | 1                 | 12.34 | 6.20   | 1        | 12.34             | 6.20   | 2247   |
| NV21   |         |                   |       |        |         | 0.1               | 1.85  | 0.62   | 0.1      | 1.85              | 0.62   | 2247   |
| NV22   |         |                   |       |        |         | 0.2               | 3.70  | 1.24   | 0.2      | 3.70              | 1.24   | 2247   |
| NV23   |         |                   |       |        |         | 0.3               | 5.55  | 1.86   | 0.3      | 5.55              | 1.86   | 2247   |
| NV24   |         |                   |       |        |         | 0.4               | 7.40  | 2.48   | 0.4      | 7.40              | 2.48   | 2247   |
| NV25   |         |                   |       |        |         | 0.5               | 9.25  | 3.10   | 0.5      | 9.25              | 3.10   | 2247   |
| NV26   |         |                   |       |        |         | 0.6               | 11.10 | 3.72   | 0.6      | 11.10             | 3.72   | 2247   |
| NV27   |         |                   |       |        |         | 0.7               | 12.95 | 4.34   | 0.7      | 12.95             | 4.34   | 2247   |
| NV28   |         |                   |       |        |         | 0.8               | 14.80 | 4.96   | 0.8      | 14.80             | 4.96   | 2247   |
| NV29   |         |                   |       |        |         | 0.9               | 16.65 | 5.58   | 0.9      | 16.65             | 5.58   | 2247   |
| NV30   |         |                   |       |        |         | 1                 | 18.50 | 6.20   | 1        | 18.50             | 6.20   | 2247   |
**Figure S2.** Electrophoretic mobility of plasmid DNA in the complexes between dynamic frameworks and pDNA at various N/P ratios. PEG-1500 Da: a.1-a.4, PEG-2000 Da: b.1-b.4 and PEG-3000 Da: c.1-c.4.

**References**

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