Electronic supplementary information (ESI)

Poly(glycoamidoamine) brush nanomaterials for systemic siRNA delivery in vivo

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1. Experimental Details

Materials

siRNAs against factor VII (FVII), firefly luciferase (Fluc) and 1,2-dimyristoyl-sn-glycerol, methoxypolyethylene glycol (DMG-PEG_{2000}) were provided by Alnylam. Dual-Glo® luciferase assay system was purchased from Promega (Madison, WI). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM) with no phenol red, Quant-iT™ RiboGreen RNA Assay Kit, and 3.5 K MWCO Slide-A-Lyzer™ Dialysis Cassettes were purchased from Thermo Fisher Scientific (Waltham, MA). 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) was purchased from Avanti Polar Lipids, Inc (Alabaster, AL). Cholesterol and 1,2-Epoxydecane were purchased from Sigma-Aldrich (St. Louis, MO).

Synthesis of polymer brush materials

Poly(glycoamidoamines) (PGAAs) were synthesized according to the method reported by Reineke.[1-6] PGAAs were reacted with 1,2-Epoxydecane in ethanol at a molar ratio of epoxides/amine 1.5:1 at 140 °C in microwave reactor for 5h. Polymer brush materials were further purified by flash chromatography using a CombiFlash® Rf system (Teledyne Isco, Lincoln, NE) and their structures were confirmed by ^{1}H NMR spectroscopy.

Formulation of nanoparticles

Poly(glycoamidoamine) brush nanoparticles were formulated with newly synthesized polymer brush, DSPC, cholesterol, DMG-PEG_{2000} and siRNA. Briefly, polymer brush, DSPC, cholesterol, and DMG-PEG_{2000} were dissolved in ethanol and mixed at a weight ratio of 0.681:0.0947:0.178:0.0459. siRNA was diluted in 10 mM citric acid buffer (pH=3). The ethanol phase and aqueous phase were combined at a 1:1 volume ratio while keeping the polymer/siRNA weight ratio as 5:1. The mixture was immediately diluted with an equal volume of PBS. Nanoparticles were formed by pipetting for *in vitro* cell studies, or through a microfluidic based mixing device for *in vivo* studies. For *in vivo* studies, formulations were dialyzed in PBS for 1 hour and 20 minutes and kept at 4 °C overnight before use.

Formulation characterization

Small aliquots (30 µl) of polymer brush formulations were diluted with Milli-Q water. A NanoZS Zetasizer (Malvern, Worcestershire, U.K.) was used to determine volume mean particle diameter and particle surface charge. After the first measurement, the particle size was monitored weekly for another one month to evaluate the stability of the formulations. The siRNA encapsulation efficiency was quantified using a Quant-iT™ RiboGreen fluorescent assay. Fluorescence intensity (FI) in the absence and presence of Triton X-100 was determined using a SpectraMax M5 microplate reader (Molecular Devices, LLC., Sunnyvale, CA) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. The entrapment efficiency was calculated using the following equation: Entrapment efficiency (%) = (1 – FI without Triton X-100 / FI with Triton X-100) * 100%.[7]

Cryo-Transmission Electron Microscopy (Cryo-TEM)

The Cryo-TEM sample was prepared using the Vitrobot (FEI, Netherlands) equilibrated at 25 °C and water saturation. A small aliquot (6 µL) of the sample was applied onto a perforated carbon film covered 200 mesh copper grid. The specimen grid was blotted to remove excess liquid and then plunged into liquid ethane for vitrification. The vitrified specimen was kept in liquid nitrogen for storage and transferred in a Tecnai T12 G2 TEM (FEI, Netherlands) using a Gatan 626 cryotransfer holder for imaging. Images were acquired using a Gatan MultiScan 791 camera under low dose conditions.[8-10]

***In vitro* Fluc silencing**

Dual HeLa cells (engineered to express both firefly and renilla luciferase) were cultured in DMEM (no phenol red) supplemented with 10% FBS and maintained at 37 °C with 5% CO_2 under humidified atmosphere. Dual HeLa cells were seeded in 96-well white bottom plates at a density of 1.2 x 10^4 cells/100 µl/well and allowed to attach overnight. FLuc siRNA loaded nanoparticles were added at a siRNA amount of 100 ng or 50 ng. 24 h after transfection, Dual-Glo® Luciferase Reagent was mixed with DMEM (No FBS and Phenol red) at a 1:1 ratio and 100 µl of the mixture was added to each well after medium removal. The luminescence of Fluc was measured immediately after 15 min dark incubation. 50 µl of Dual-Glo® Stop & Glo® Reagent was then added to each well and the luminescence of Renilla luciferase was measured similarly.

***In vivo* FVII silencing**

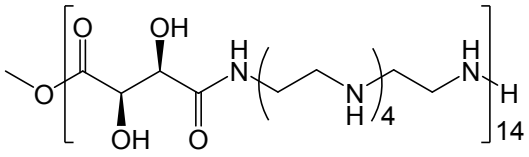
All procedures used in animal studies conducted at MIT were in compliance with Massachusetts laws or guidelines, were approved by the Institutional Animal Care and Use Committee (IACUC) and were also consistent with local, state, and federal regulations as applicable. FVII siRNA loaded nanoparticles were intravenously injected into C57BL/6 mice through tail vein. 24 h after administration, mice were anaesthetized and blood samples were collected in serum separation tubes by tail vein bleeding. A chromogenic assay (Biophen FVII, Aniara Corporation) was performed to quantify protein levels of FVII.

References

1. M. Tranter, Y. Liu, S. He, J. Gulick, X. Ren, J. Robbins, W. K. Jones and T. M. Reineke, *Mol Ther*, 2012, **20**, 601-608.
2. N. P. Ingle, B. Malone and T. M. Reineke, *Trends Biotechnol*, 2011, **29**, 443-453.
3. Y. Liu and T. M. Reineke, *Biomacromolecules*, 2010, **11**, 316-325.
4. P. M. McLendon, K. M. Fichter and T. M. Reineke, *Mol Pharm*, 2010, **7**, 738-750.
5. Y. Liu, L. Wenning, M. Lynch and T. M. Reineke, *ACS Symp. Ser.*, 2006, **923**, 217-227.
6. Y. Liu, L. Wenning, M. Lynch and T. M. Reineke, *J Am Chem Soc*, 2004, **126**, 7422-7423.
7. J. Heyes, L. Palmer, K. Bremner and I. MacLachlan, *J Control Release*, 2005, **107**, 276-287.
8. Y. Dong, J. R. Dorkin, W. Wang, P. H. Chang, M. J. Webber, B. C. Tang, J. Yang, I. Abutbul-Ionita, D. Danino, F. DeRosa, M. Heartlein, R. Langer and D. G. Anderson, *Nano Lett*, 2016, DOI: 10.1021/acs.nanolett.5b02428.
9. D. Danino, A. Bernheim-Groswasser and Y. Talmon, *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 2001, **183**, 113-122.
10. D. Danino, *Current Opinion in Colloid & Interface Science*, 2012, **17**, 316-329.

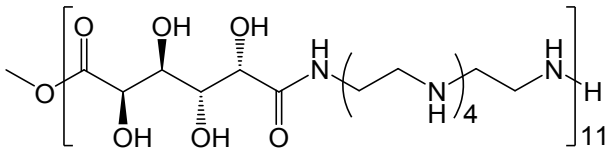
2. ^{1}H NMR Structure Determination

1. Tar-PGAA



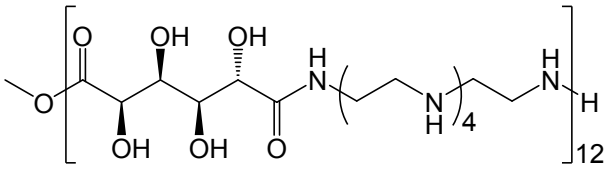
^{1}HNMR (500 MHz, D_2O): δ 4.38 (s, 2H), 3.12-3.39 (m, 4H), 2.47-2.66 (m, 16H).

2. Gal-PGAA



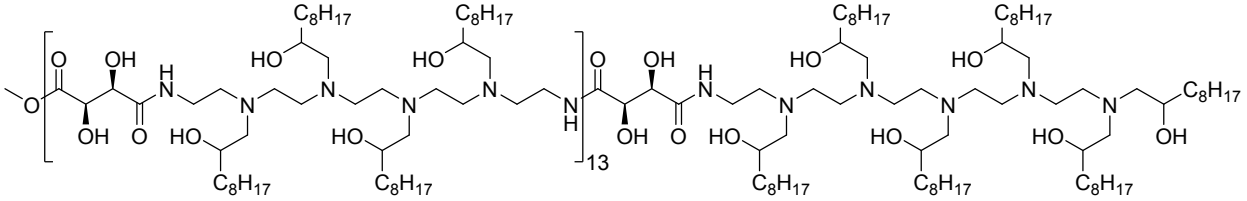
^{1}HNMR (500 MHz, D_2O): δ 4.36-4.44 (s, 2H), 3.93-4.05 (m, 2H), 3.29-3.49 (m, 4H), 2.58-2.79 (m, 16H).

3. Glu-PGAA



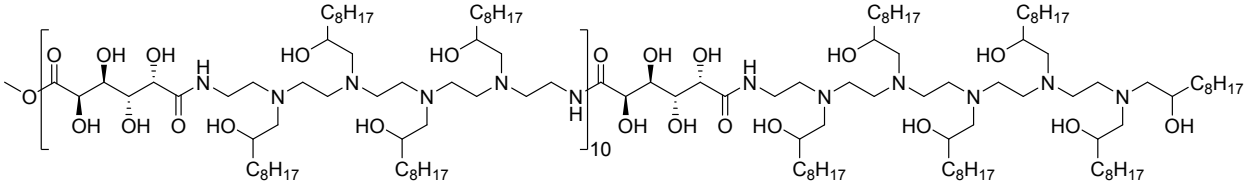
^{1}HNMR (500 MHz, D_2O): δ 4.19-4.37 (m, 2H), 4.05-4.13 (m, 1H), 3.90-3.97 (m, 1H), 3.29-3.49 (m, 4H), 2.59-2.85 (m, 16H).

4. TarN4C10



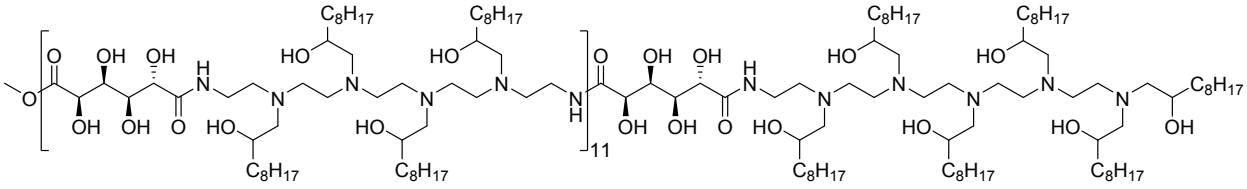
^{1}HNMR (500 MHz, CDCl3): δ 7.92-8.07 (br, 1H), 4.20-4.91 (m, 6H), 3.50-3.75 (m, 4H), 2.01-3.07 (m, 26H), 1.18-1.52 (m, 56H), 0.82-0.98 (t, 12H).

5. GalN4C10



^{1}HNMR (500 MHz, CDCl3): δ 8.03 (s, 1H), 3.45-5.15 (m, 12H), 2.09-2.98 (m, 24H), 1.18-1.53 (m, 56H), 0.81-0.97 (t, 12H).

6. GluN4C10



^{1}HNMR (500 MHz, CDCl3): δ 8.04 (s, 1H), 3.35-4.33 (m, 12H), 1.99-3.10 (m, 24H), 1.18-1.53 (m, 56H), 0.81-0.94 (t, 12H).

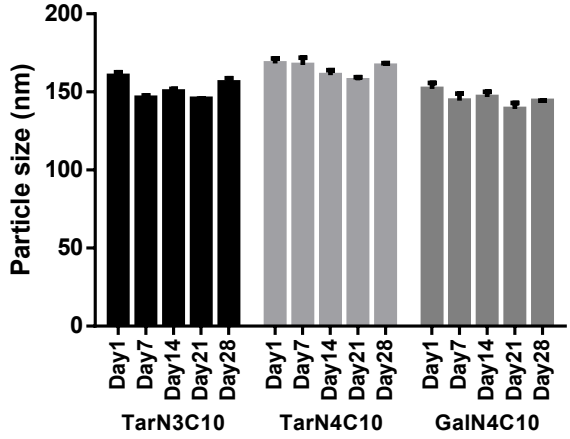


Fig. S1 Stability of TarN3C10, TarN4C10 and GalN4C10 polymer brush nanoparticles.

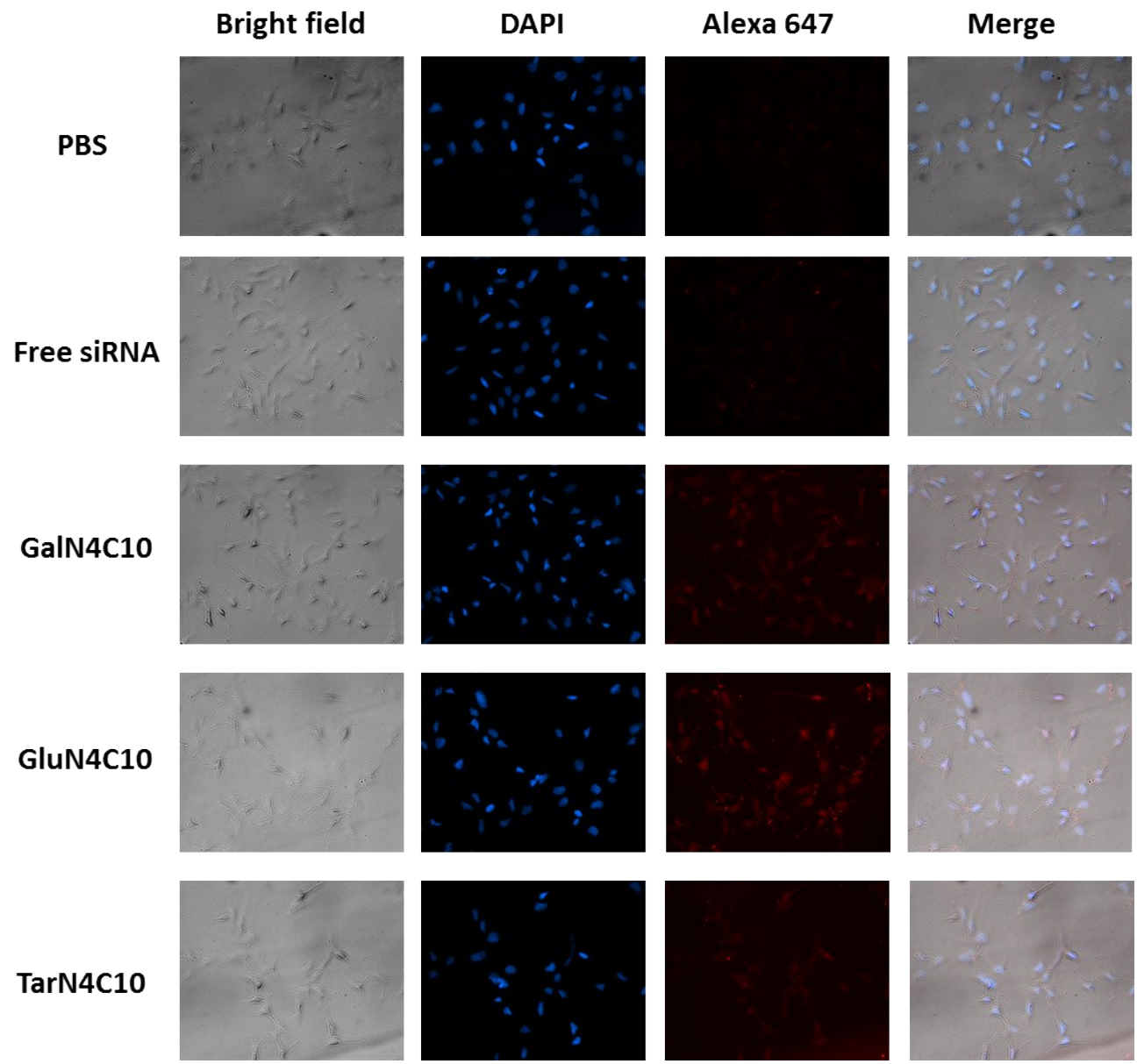


Fig. S2 Cellular uptake of GalN4C10, GluN4C10, TarN4C10 polymer brush nanoparticles. Control groups include PBS and free Alexa 647-labelled siRNA.