Molecular Weight-Dependent Activity of Aminated Poly(α)glutamates as siRNA Nanocarriers

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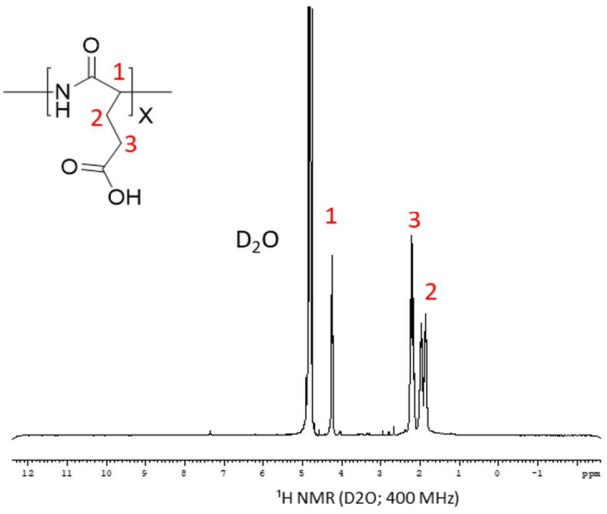
A



^{1}H NMR (D2O; 400 MHz)

B

Polymer	Mn [g/mol]	PDI	DP
Short	11820	1.012	92
Long	21020	1.410	163

Figure S1: Characterization of the PGA precursor. (**A**) ^{1}H-NMR spectrum obtained at 400 MHz. (**B**) a table summarizing the Molecular weight (M_n), polydispersity index (PDI), and calculated degree of polymerization (DP), as obtained by MALS.

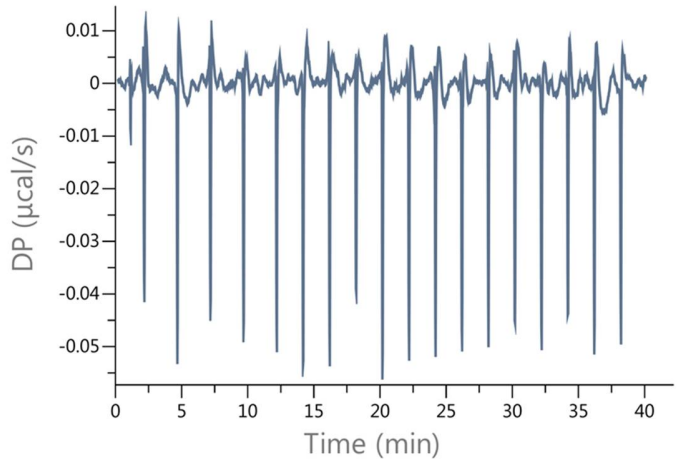


Figure S2: Isothermal titration calorimetry (ITC) of siRNA titrated into buffer. The row data obtained from a titration of 20 μM siRNA solution (760 μM Phosphate groups) into 15 mM HEPES.

A.

PGAamine batch	Mn [g/mol]	PDI	DP	Silencing activity
1	6476	1.358	23	Not active
2	7784	1.376	27	
Short	7762	1.236	27	
3	9210	1.328	32	
4	9847	1.465	35	
5	11060	1.274	39	
Long	15880	1.236	56	Active
6	20200	1.089	71	

B.

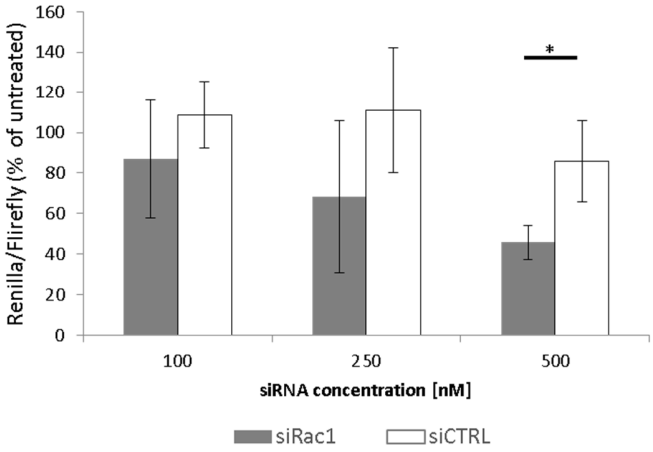


Figure S3: Various PGAamine synthetic batches. (**A**) a table summarizing the molecular weight (M_n) and polydispersity index (PDI) of various PGAamine synthetic batches, as analyzed by MALS, their calculated degree of polymerization (DP), versus the silencing activity, as tested by psiCHECK reporter assay. (**B**) The silencing activity of PGAamine batch #6, as tested by psiCHECK reporter assay. Statistical significance was determined using *t*-test, * $p < 0.05$

A.

Fraction of "Long" polymer	Mn [g/mol]	PDI	DP
Shorter	12180	1.082	43
Longer	15440	1.021	54

B.

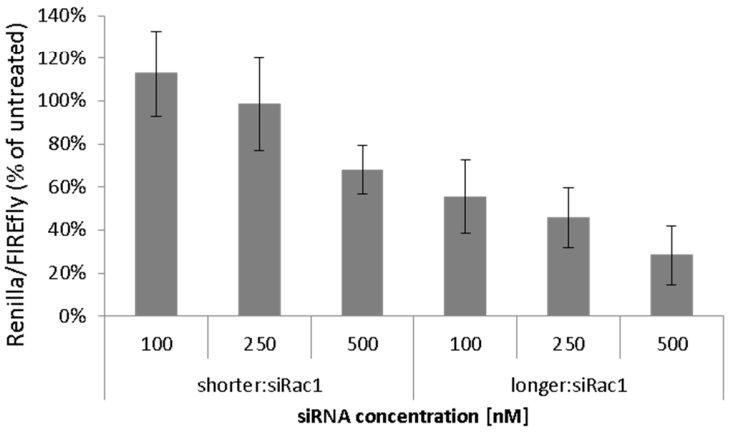


Figure S4: Separation of Long PGAamine into shorter and longer fractions. (**A**) The molecular weight (M_n), polydispersity index (PDI), and calculated degree of polymerization (DP) of the two separate fractions of "Long" PGAamine, as analyzed by MALS. (**B**) Silencing activity of the two separate fractions, as obtained by psiCHECK reporter assay.

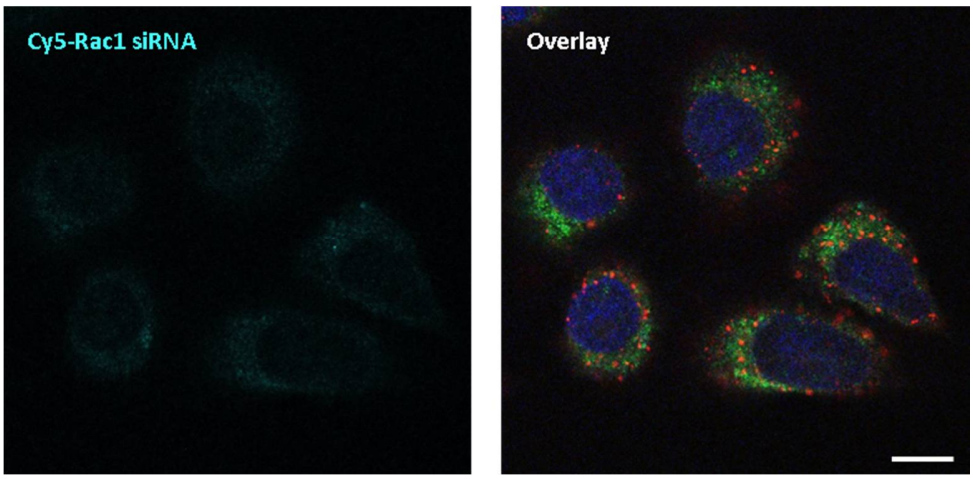


Figure S5: Cy5-Rac1 siRNA hardly internalized into HeLa cells following 24 h of treatment. HeLa cells were incubated with Cy5-Rac1 siRNA at a concentration of 100 nM. Scale bar = 10 µm. Blue-DAPI (nuclei), Red-EEA1 (endosomes), Green- LAMP1 (lysosomes), Cyan- Rac1-Cy5 siRNA.