Supporting Information

Local delivery of siRNA using polyplex-loaded thermosensitive hydrogels

Lies A.L. Fliervoet[1], Heyang Zhang[2], Emma van Groesen[3], Kyra Fortuin[1], Naut J.C.B. Duin[1], Katrien Remaut[2], Raymond M. Schiffelers[4], Wim E. Hennink[1], and Tina Vermonden[1]*

[1] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, PO Box 80082, 3508 TB Utrecht, the Netherlands*
[2]*Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, Ghent 9000, Belgium*
[3] *Biological Chemistry Group, Institute of Biology Leiden, Leiden University, Sylvius Laboratories, 2333 BE Leiden, the Netherlands*
[4]*Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA Utrecht, the Netherlands*

*****Mailing address: Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

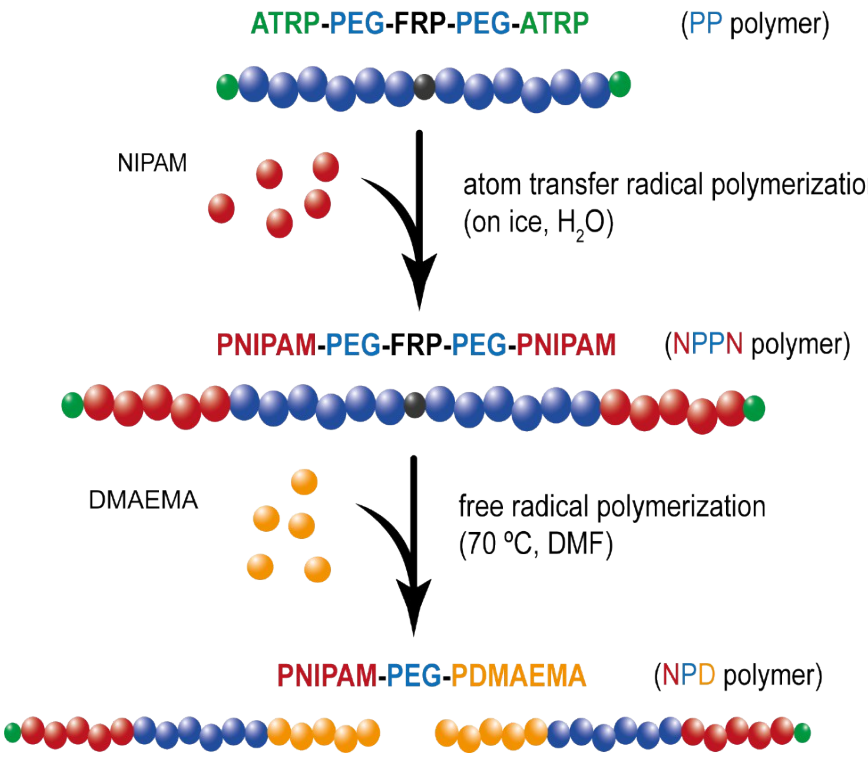
Phone: (+31)620291631

E-mail: T.Vermonden@uu.nl

Materials and Methods for Supporting Figure S5 - Rheological characterization

Rheological analysis of NPN hydrogel was performed on a DHR-2 rheometer (TA Instruments, New Castle, DE) using a 20 mm aluminum cone (1 °) geometry equipped with a solvent trap. Temperature sweep of NPN polymer solution (70 µL of 15% w/w) was performed from 4 to 50 °C at a heating rate of 1 °C per minute with a 30 second equilibration time for each temperature. A fixed frequency of 1 Hz and a strain of 1% were applied.

Supporting Figures



Scheme S1. Schematic drawing of the synthesis route of NPD triblock copolymers using the hetero-functional PEG macroinitiator (PP polymer). Atom transfer radical polymerization (ATRP) is used in the first step to polymerize NIPAM, yielding the intermediate NPPN polymer. Next, DMAEMA is polymerized by free radical polymerization (FRP) using NPPN as macroinitiator to obtain the final NPD polymer.[1]

1. Fliervoet, L. A. L.; Najafi, M.; Hembury, M.; Vermonden, T., Heterofunctional Poly(ethylene glycol) (PEG) Macroinitiator Enabling Controlled Synthesis of ABC Triblock Copolymers. *Macromolecules* **2017,** *50* (21), 8390-8397.

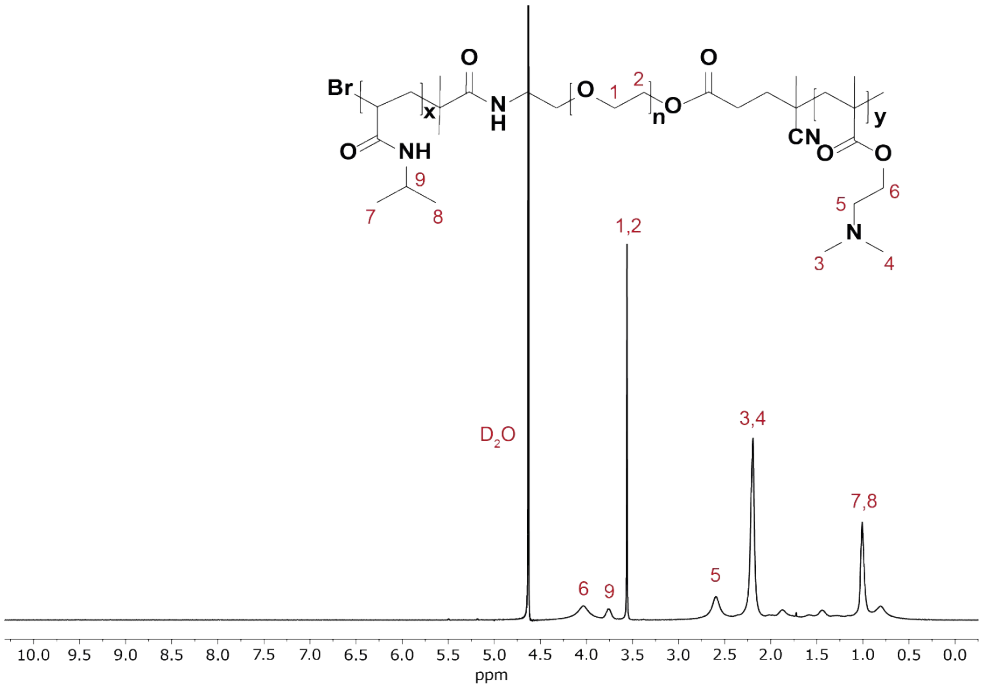


Figure S1. ^{1}H-NMR spectrum of NPD-polymer in D_2O.

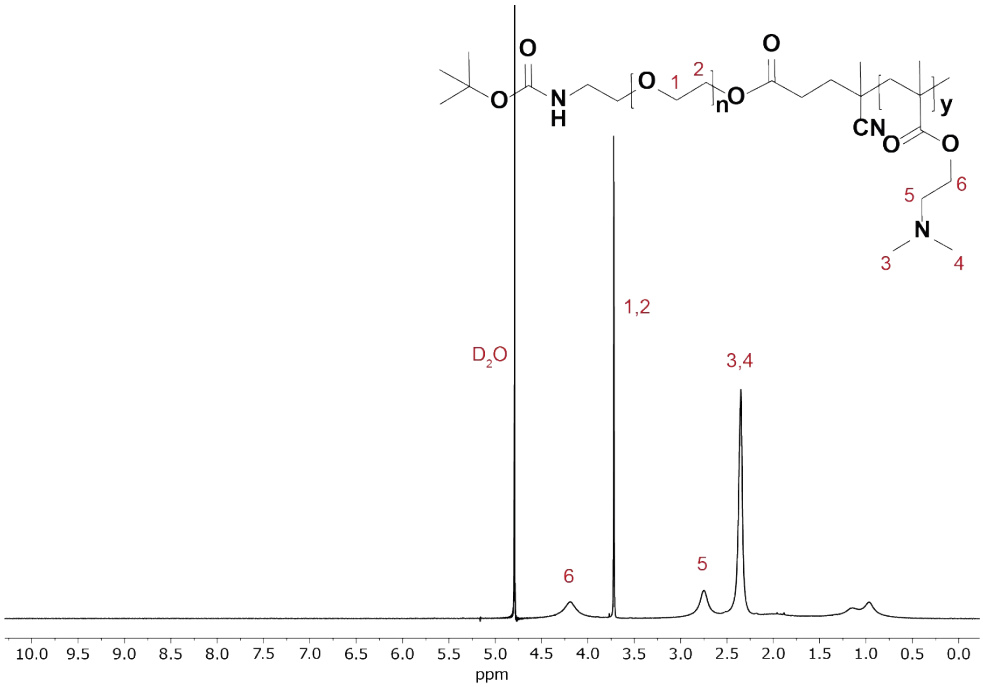


Figure S2. ^{1}H-NMR spectrum of PD-polymer in D_2O.

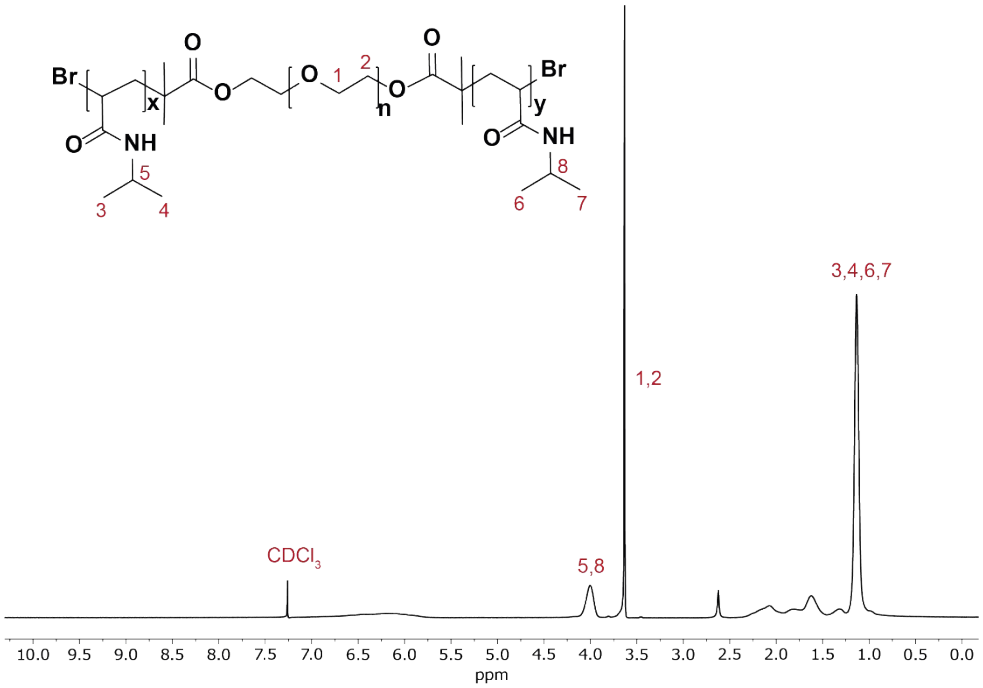


Figure S3. ^{1}H-NMR spectrum of NPN-polymer in $CDCl_3$.

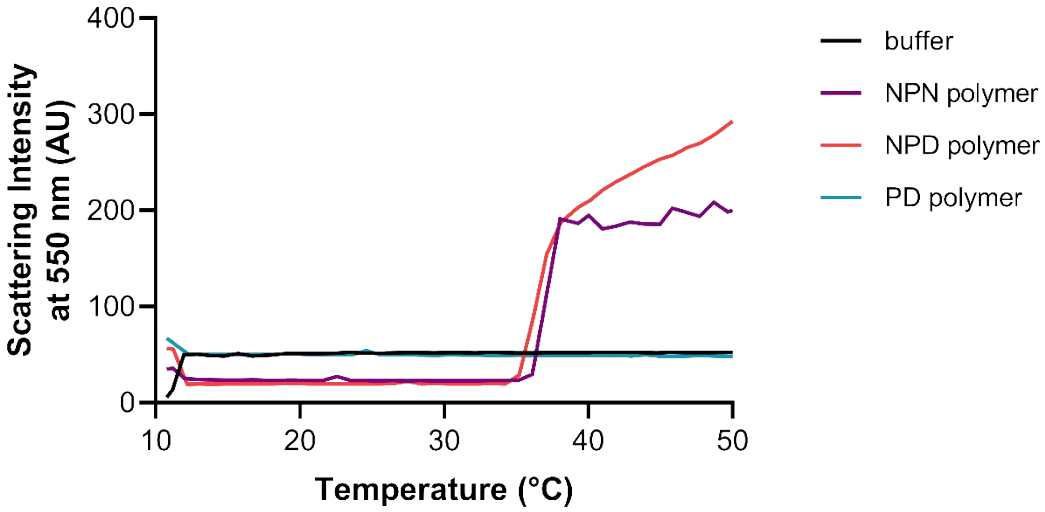


Figure S4. Light scattering intensity at 550 nm as a function of temperature for cloud point determination of thermosensitive polymers in 20 mM HEPES buffer (pH 7.4).

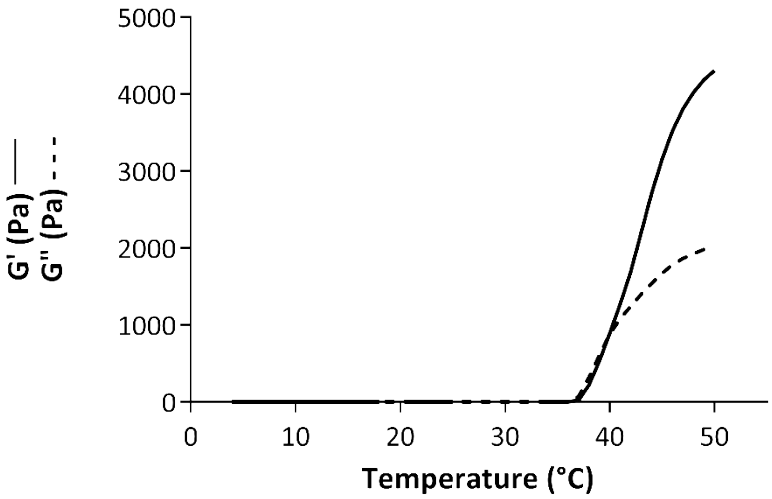


Figure S5. Storage (G') and loss (G") moduli as a function of temperature for NPN polymer at a concentration of 15% (w/w) in 20 mM HEPES buffer (pH 7.4).

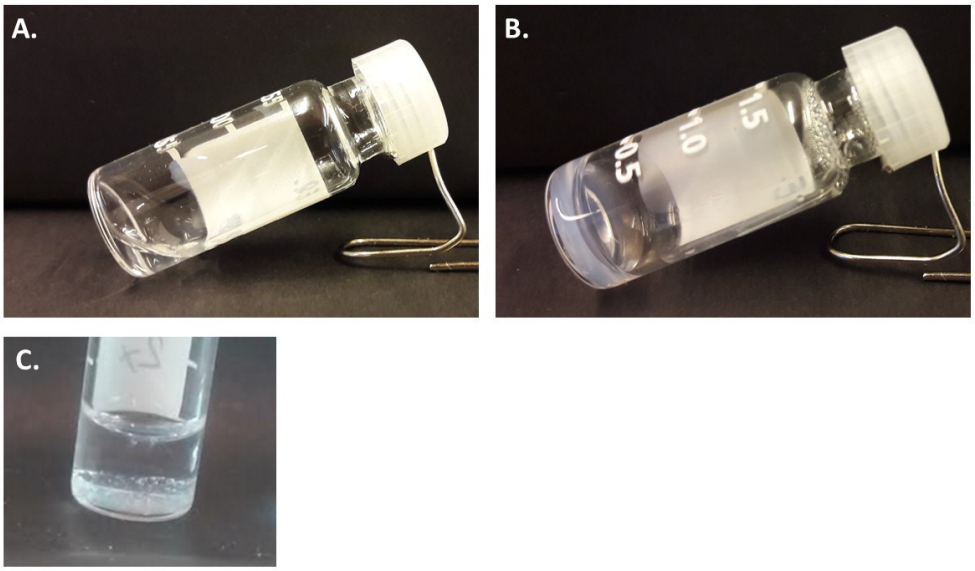


Figure S6. Representative images of the thermosensitive hydrogel based on the PNIPAM-PEG-PNIPAM (NPN) triblock polymer before gelation at room temperature (A), after gelation at 37 °C (B), and after adding release medium at 37 °C (C).

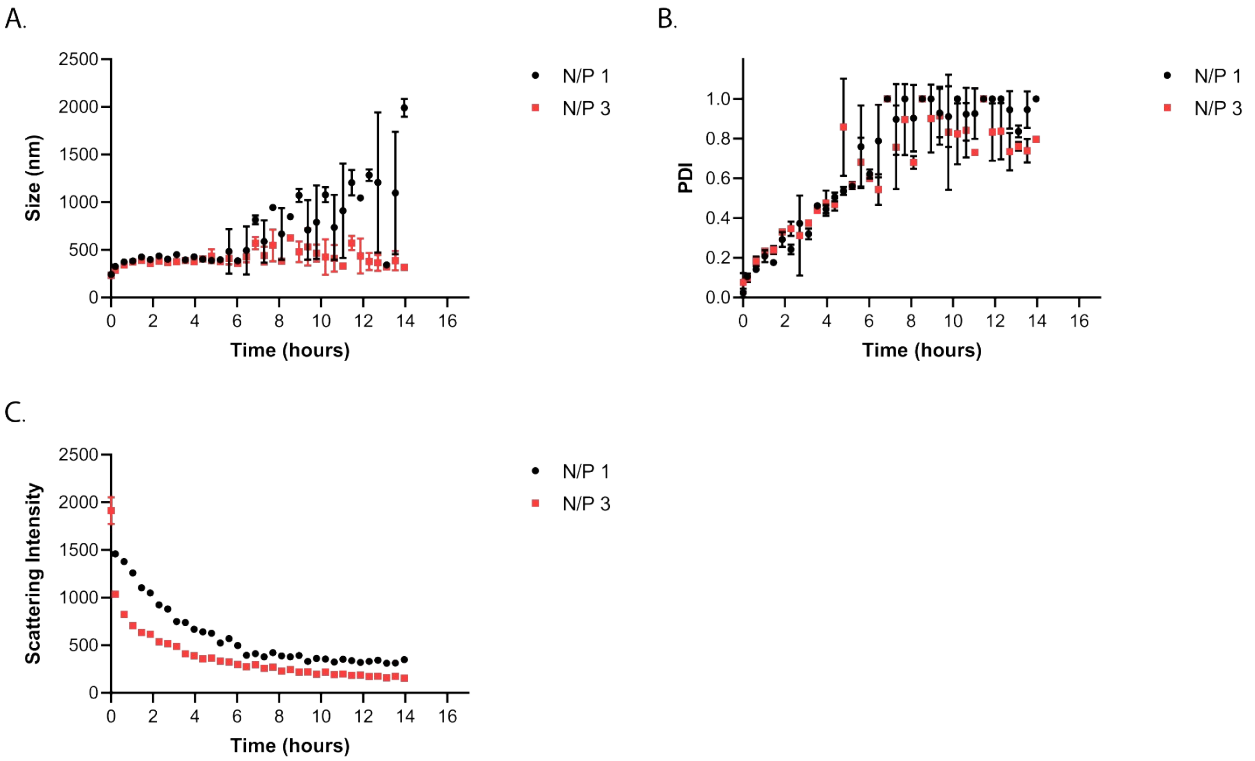


Figure S7. Stability of NPD with N/P 1 and 3 measured by dynamic light scattering (DLS) in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) at 37 °C. Size (A), PDI (B) and scattering intensity (C) are plotted against incubation time.

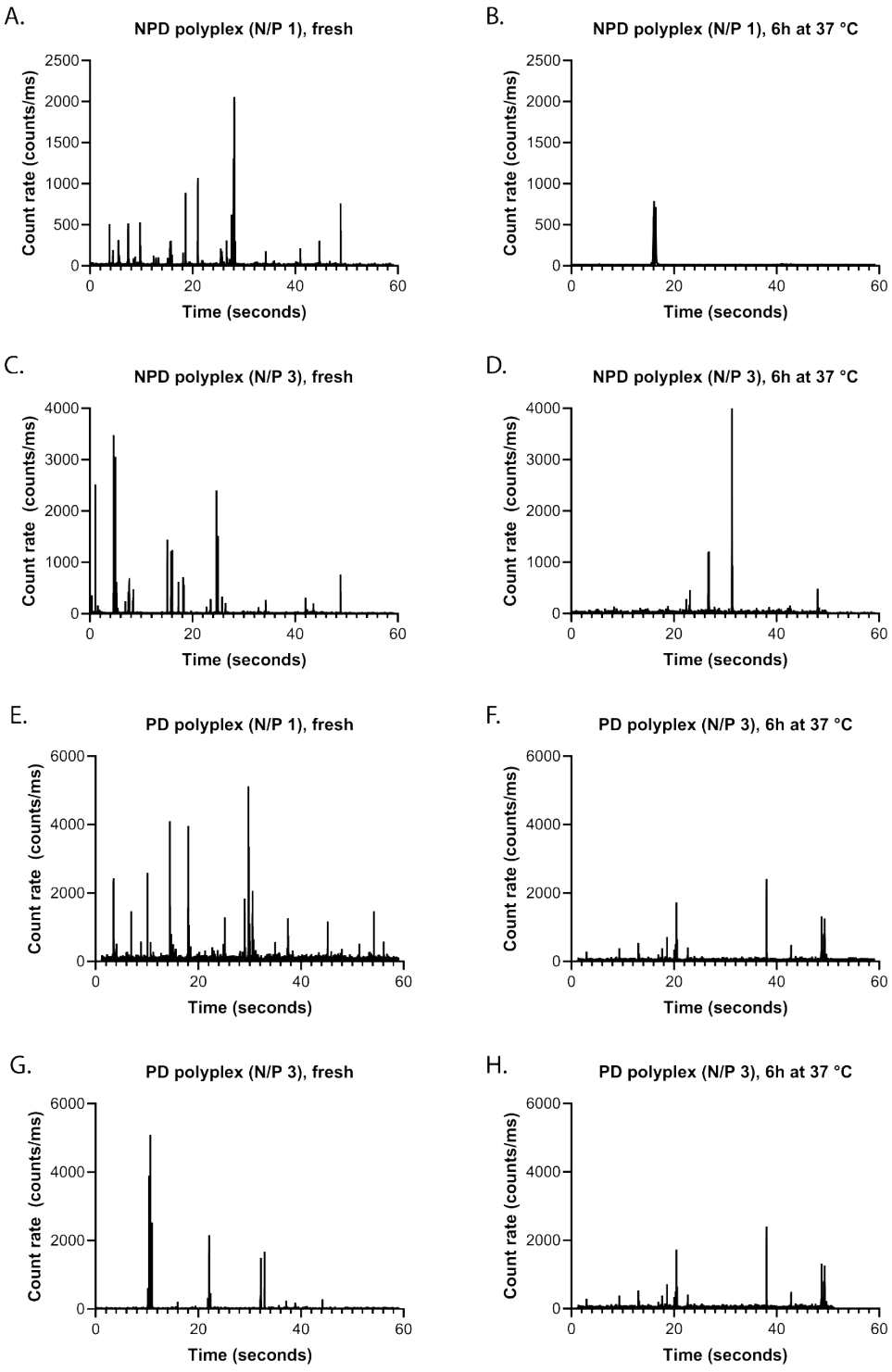


Figure S8. FCS time traces of freshly prepared NPD and PD polyplexes (N/P 1 and 3) and after 6h incubation at 37 °C.

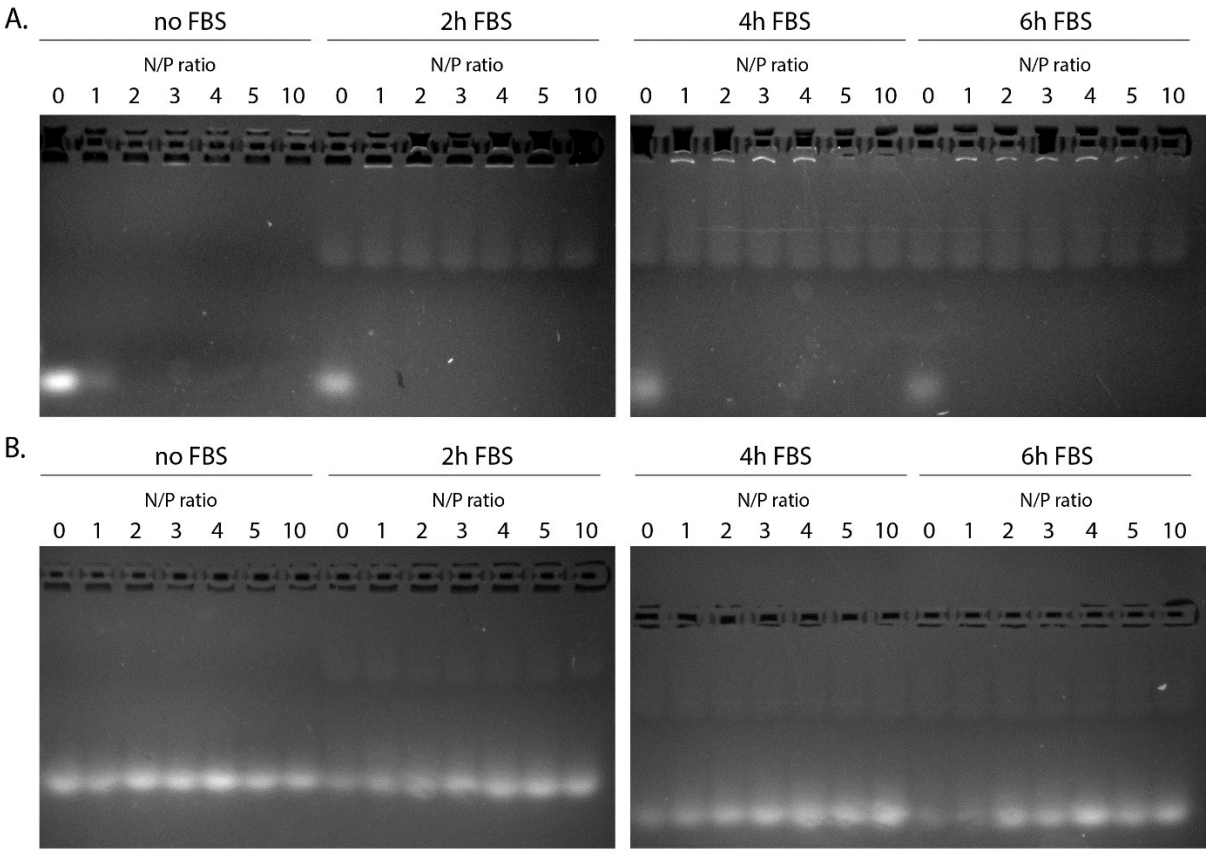


Figure S9. Agarose gel retardation assay of NPD polyplexes prepared with different N/P ratios in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) and incubated for 2, 4 and 6 hours at 37 °C in the presence of FBS (A). In parallel, heparin was added to destabilize the polyplexes and release siRNA from the cationic polymers (B).

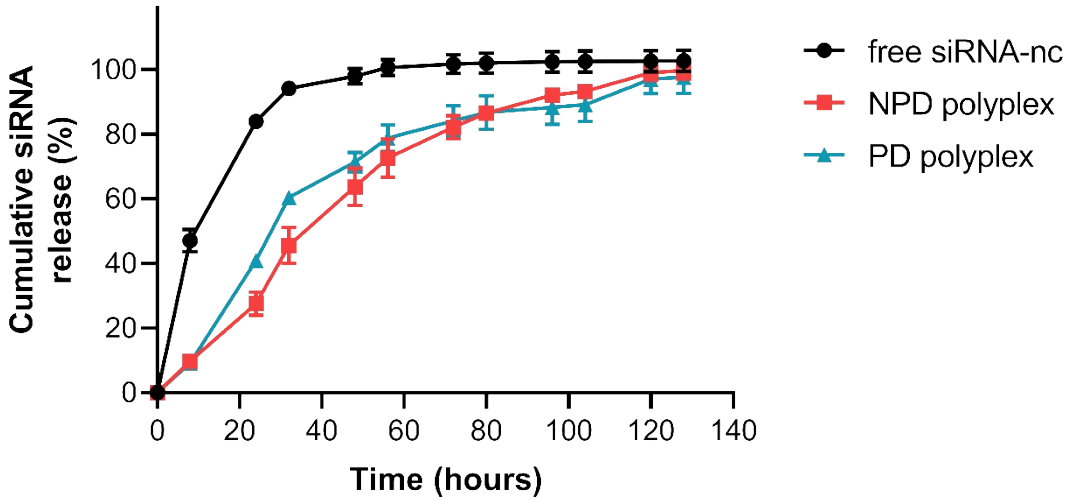


Figure S10. Release of non-coding siRNA (siRNA-nc) from a thermosensitive hydrogel in its free form, or formulated as NPD or PD polyplexes (N/P 5). All values are given as the mean ± SD (n=3).