Aggregation Behavior of Cationic Nanohydrogel Particles with Human Blood Serum

Supporting Information

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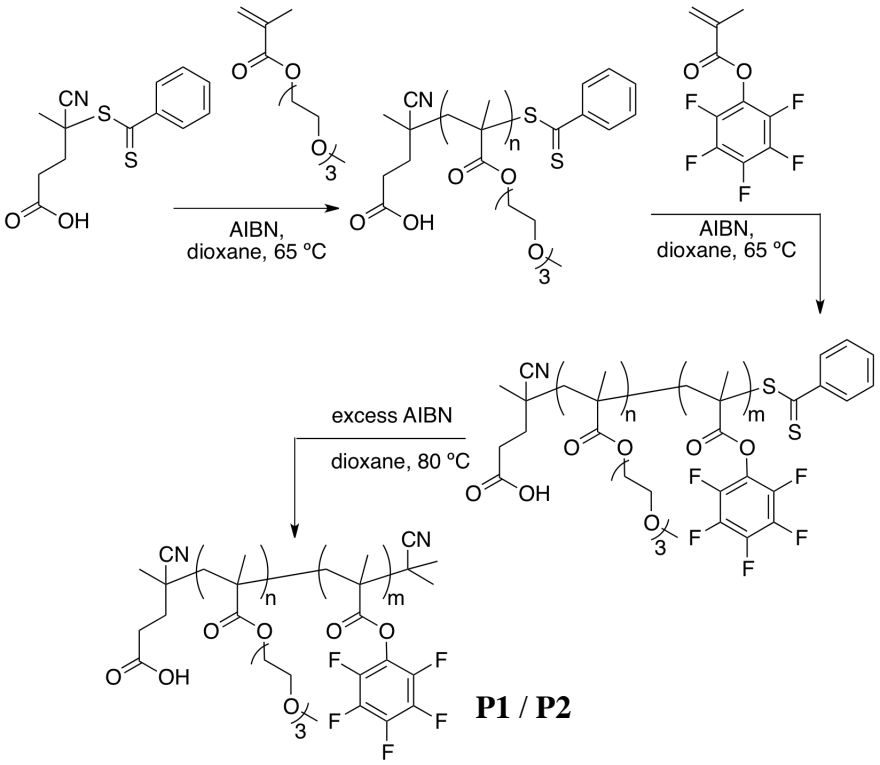
Content

1. Synthesis of P(MEO$_3$MA)-*b*-P(PFPMA) Block Copolymers

The RAFT block copolymerization of P(MEO$_3$MA)-*b*-P(PFPMA) was done according to previously reported[1]:



Scheme S1: RAFT Block Copolymerization of tri(ethylene glycol) methyl ether methacrylate (MEO$_3$MA) and pentafluorophenyl methacrylate (PFPMA).

Analytical data:

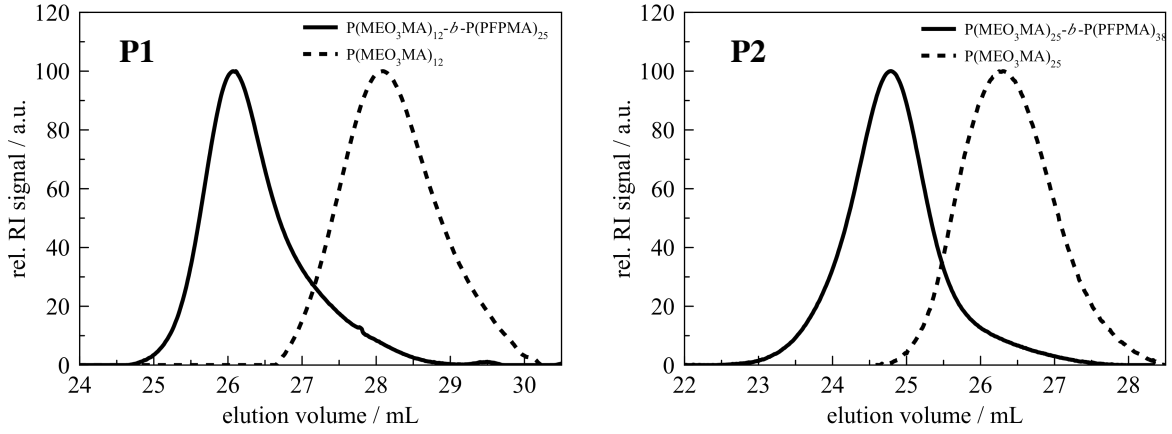


Figure S1: SEC elugram (THF, PS-Std) of P(MEO$_3$MA)-*b*-P(PFPMA) block copolymers P1 and P2 with their corresponding P(MEO$_3$MA) homo polymers as macro chain transfer agents.

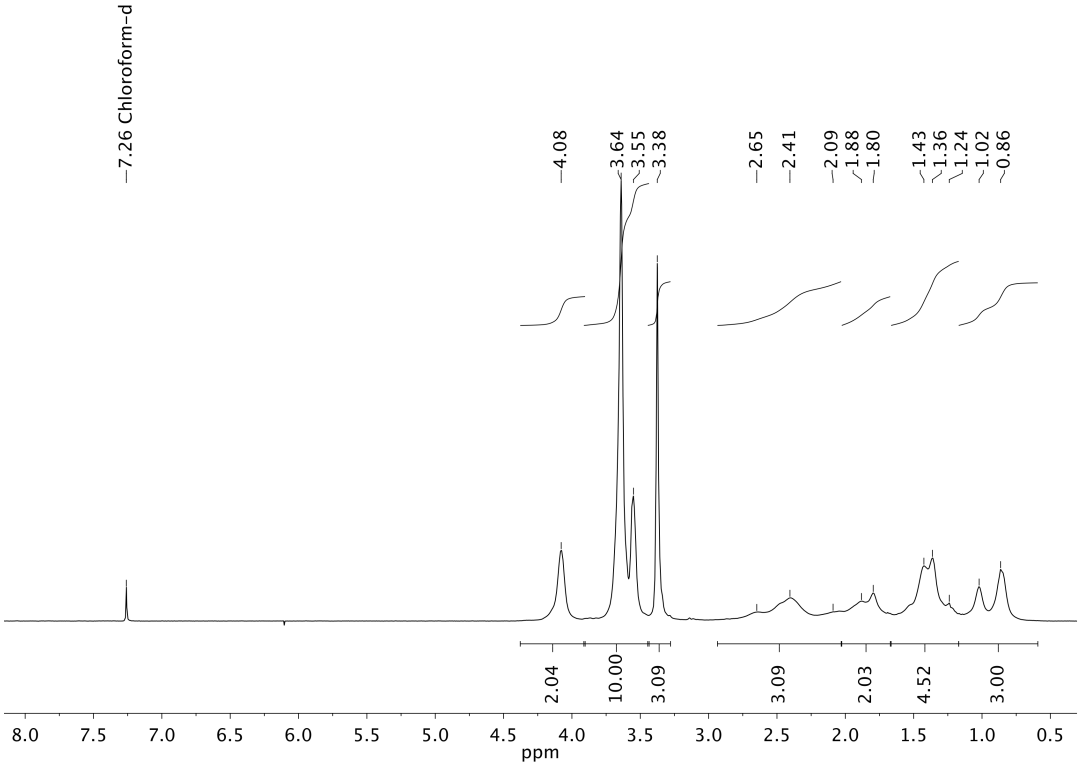


Figure S2: ^{1}H NMR (400 MHz, $CDCl_3$) of P(MEO_3MA)-*b*-P(PFPMA).

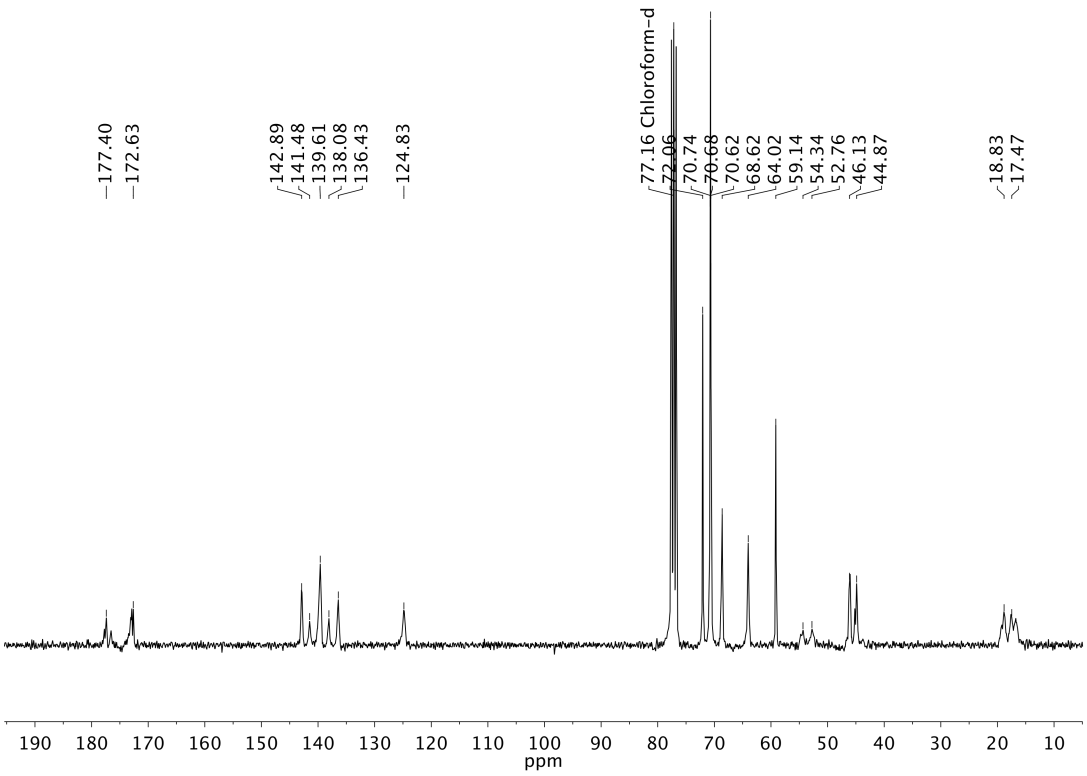


Figure S 3: ^{13}C NMR (100 MHz, $CDCl_3$) of P(MEO_3MA)-*b*-P(PFPMA).

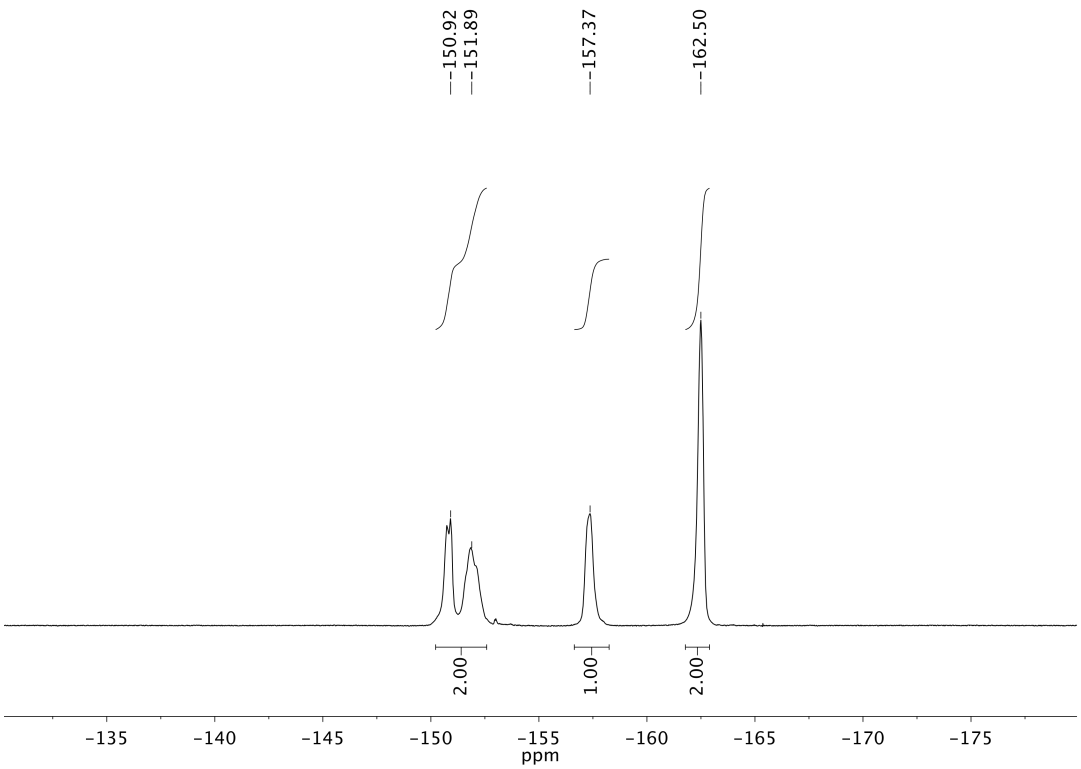


Figure S4: ^{19}F NMR (376 MHz, $CDCl_3$) of P(MEO_3MA)-*b*-P(PFPMA).

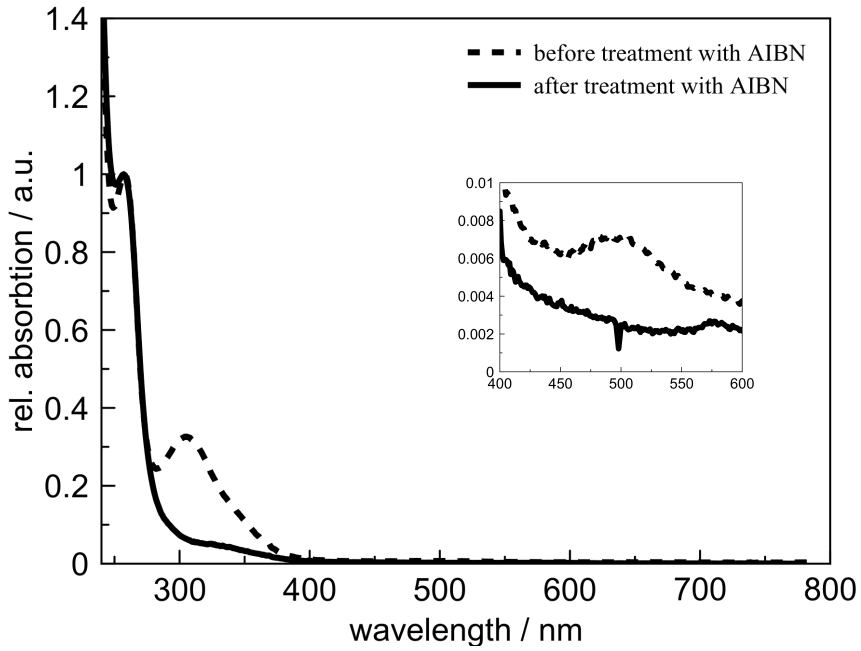


Figure S5: UV-vis spectra of P(MEO_3MA)-*b*-P(PFPMA) in dioxane before and after treatment with excess AIBN to remove the dithiobenzoate end group.

2. Synthesis of Cationic Nanohydrogel Particles

As recently reported,[1] cross-linking of self-assembled micellar P(MEO_3MA)-*b*-P(PFPMA) aggregates with spermine and subsequent nanohydrogel formation can be monitored by ^{19}F NMR:

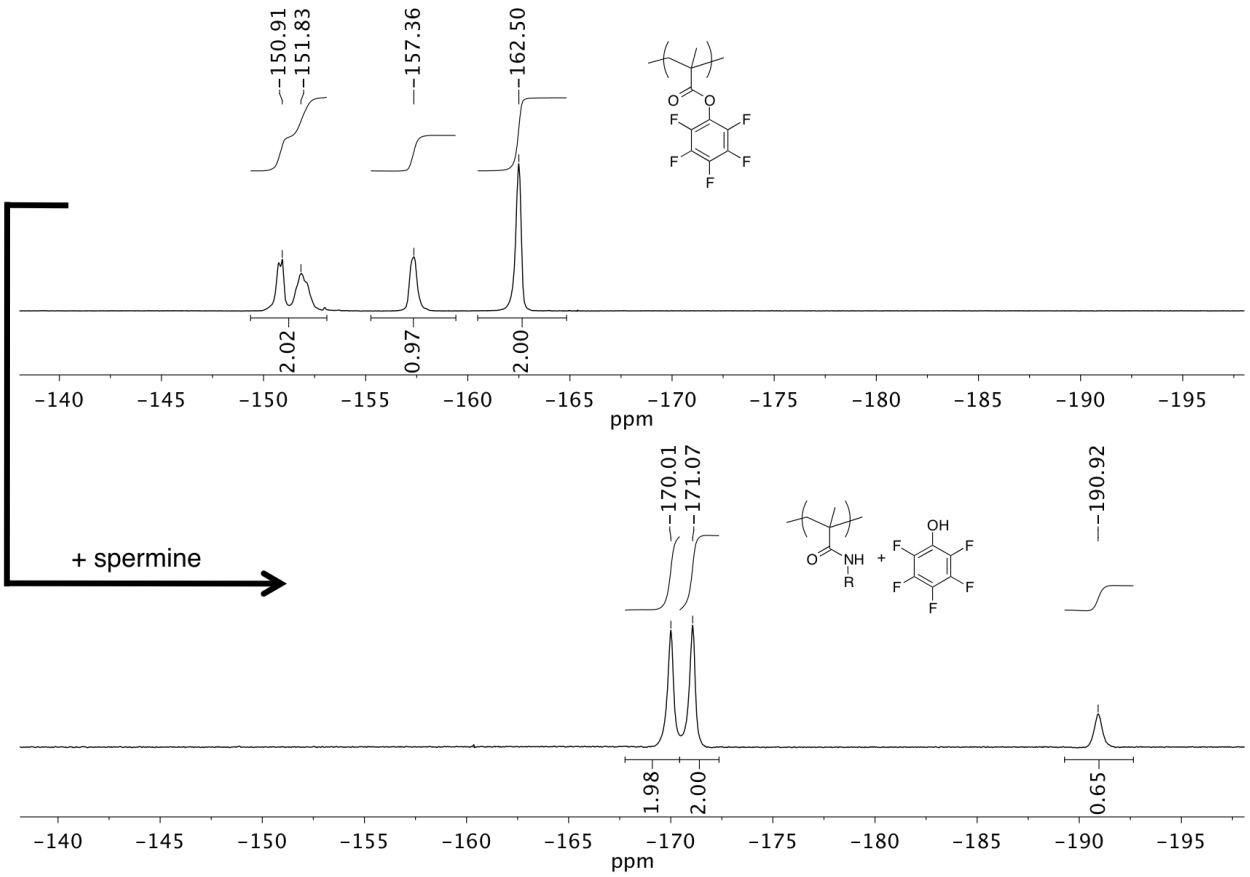


Figure S6: ^{19}F NMR reaction monitoring of nanohydrogel formation by aminolysis of self-assembled micellar P(MEO_3MA)-*b*-P(PFPMA) aggregates in DMSO with spermine.

Via sequential addition of amine-functionalized dyes,[1] the synthesis of dye labeled nanohydrogel particles could be achieved. Moreover, loading with siRNA containing another fluorescent label was done for *intravital* confocal videography studies:

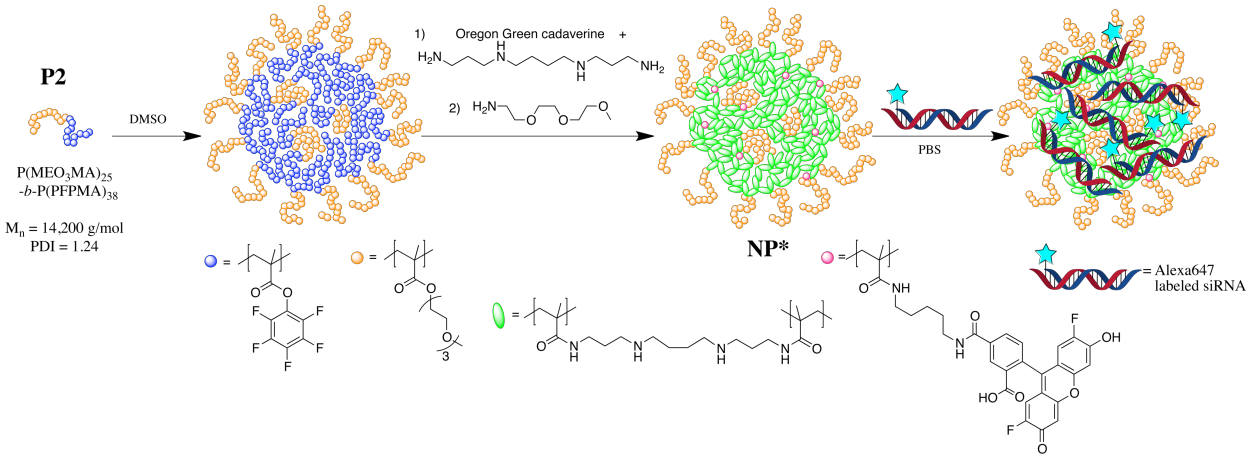


Figure S7: Synthesis of Oregon Green cadaverine labeled nanohydrogel particle NP* and loading with Alexa647 labeled siRNA.

3. Agarose Gel Electrophoresis Experiments of SiRNA Loaded Nanohydrogel Particles in BSA Solution and Human Blood Serum

Sample preparation of siRNA loaded nanohydrogel particles in BSA solution or human blood serum for agarose gel electrophoresis was performed in analogy to the description in the experimental section: Samples were prepared of 70 ng siRNA with nanohydrogel particles at given weight-to-weight ratios in PBS and incubated for 30 min. For the samples in BSA solution, a 250 g/L BSA solution in PBS was used to adjust a concentration of 150 g/L or 50 g/L BSA, respectively. For the samples in human blood serum, 2 vol. equiv. of serum were added (in analogy to the DLS sample preparation). After further incubation at room temperature for 30 min, each sample was mixed with 6x loading puffer and then loaded onto a 0.5% agarose gel in TBE. Electrophoresis was performed at 120 V for 30 min, and upon excitation at 365 nm, Gel Red fluorescence was imaged by a conventional digital camera.

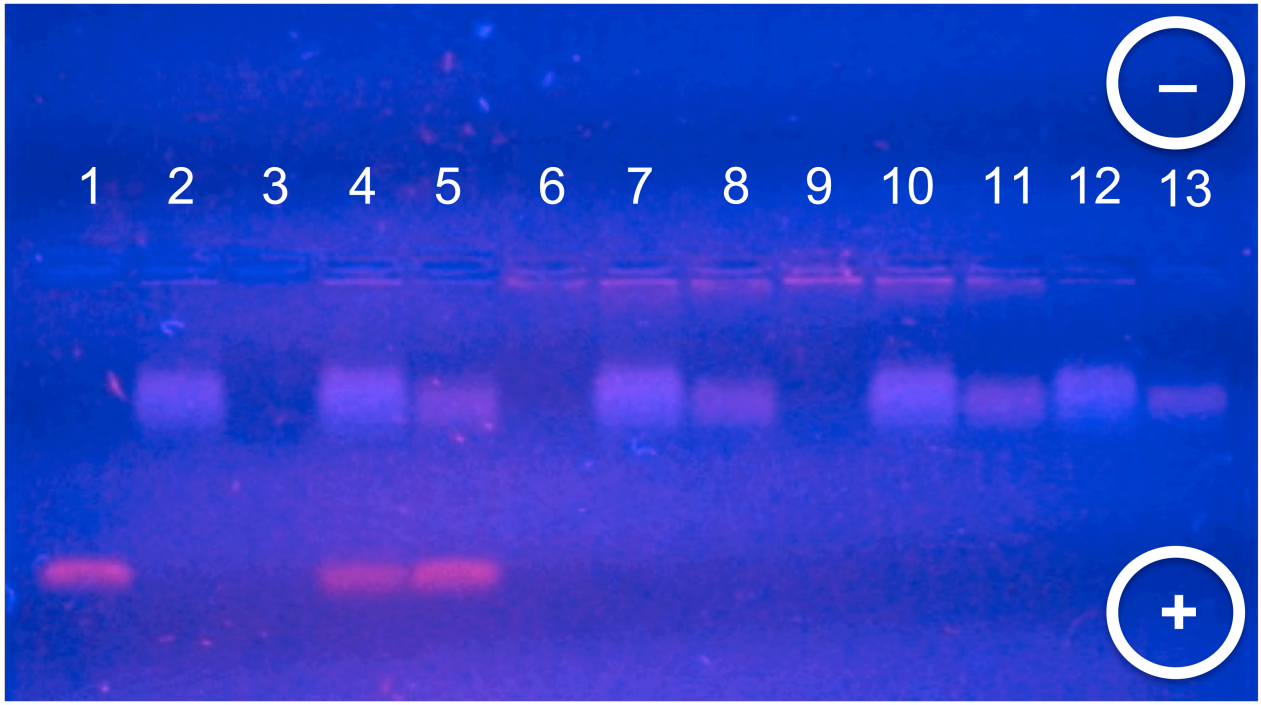


Figure S8: Agarose gel electrophoresis of siRNA loaded nanohydrogel particles in BSA solution – 1 : 70 ng siRNA; 2: 150 g/L BSA; 3: 3.5 μg NP; 4: 70 ng siRNA in 150 g/L BSA; 5: 70 ng in 50 g/L BSA; 6: NP:siRNA=25:1 (1.75 μg NP + 70 ng siRNA); 7: NP:siRNA=25:1 (1.75 μg NP + 70 ng siRNA) in 150 g/L BSA; 8: NP:siRNA=25:1 (1.75 μg NP + 70 ng siRNA) in 50 g/L BSA; 9: NP:siRNA=50:1 (3.5 μg NP + 70 ng siRNA); 10: NP:siRNA=50:1 (3.5 μg NP + 70 ng siRNA) in 150 g/L BSA; 11: NP:siRNA=50:1 (3.5 μg NP + 70 ng siRNA) in 50 g/L BSA; 12: 3.5 μg NP in 150 g/L BSA; 13: 3.5 μg NP in 50 g/L BSA.

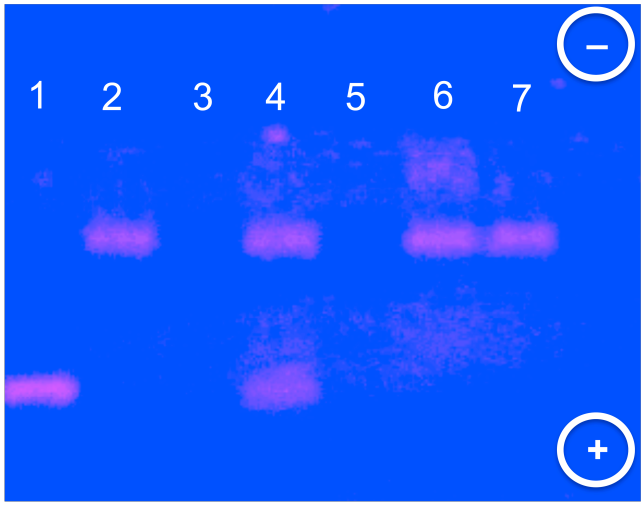


Figure S9: Agarose gel electrophoresis of siRNA loaded nanohydrogel particles in human blood serum – 1: 70 ng siRNA; 2: serum; 3: 3.5 μg NP; 4: 70 ng siRNA in serum; 5: NP:siRNA=25:1 (1.75 μg NP + 70 ng siRNA); 6: NP:siRNA=25:1 (1.75 μg NP + 70 ng siRNA) in serum; 7: 1.75 μg NP in serum.

4. Dynamic Light Scattering of SiRNA Loaded Cationic Nanohydrogel Particles in PBS

To determine the size of siRNA loaded nanohydrogel particles, data evaluation was done according to literature.[2] All recorded correlation functions usually showed monomodal decay and could be fitted by a sum of two exponentials, from which the first cumulant was calculated resulting in an angle dependent diffusion coefficient D or reciprocal hydrodynamic radius R_h^{-1}, respectively, according to formal application of Stokes-Einstein law. By extrapolation of R_h^{-1}/q^2 to $q^2 = 0$ z-average hydrodynamic radius $R_h = 1/\langle R_h^{-1} \rangle_Z$ was obtained (Figure S10).

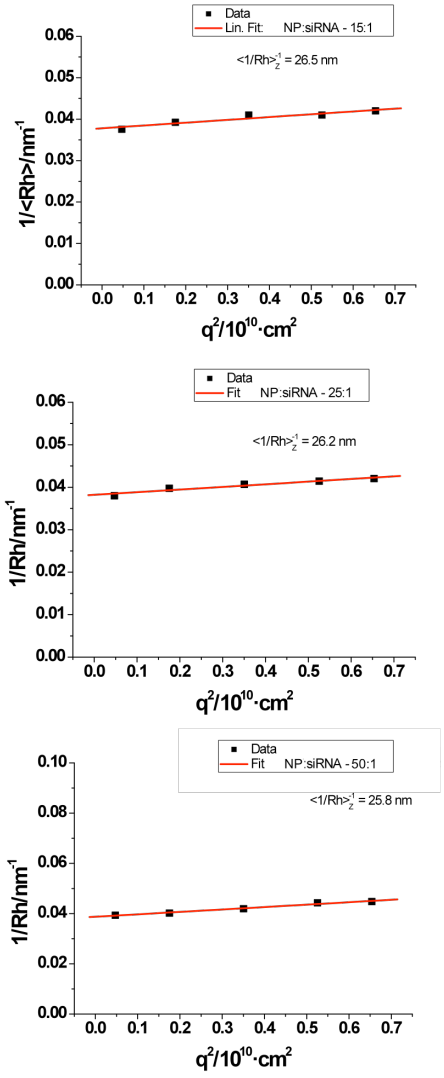


Figure S10: DLS angle dependency of $\langle 1/R_h \rangle$ versus q^2 of siRNA loaded nanohydrogel particle NP at 15:1, 25:1 and 50:1 weight to weight ratio (NP : siRNA) in PBS (0.05 g/L).

5. Dynamic Light Scattering of SiRNA Loaded Cationic Nanohydrogel Particles in BSA Solution

Forced fit method according to Rausch et al.[3] for determining aggregation behavior of siRNA loaded cationic nanohydrogel particles in BSA solution (80 g/L in PBS):

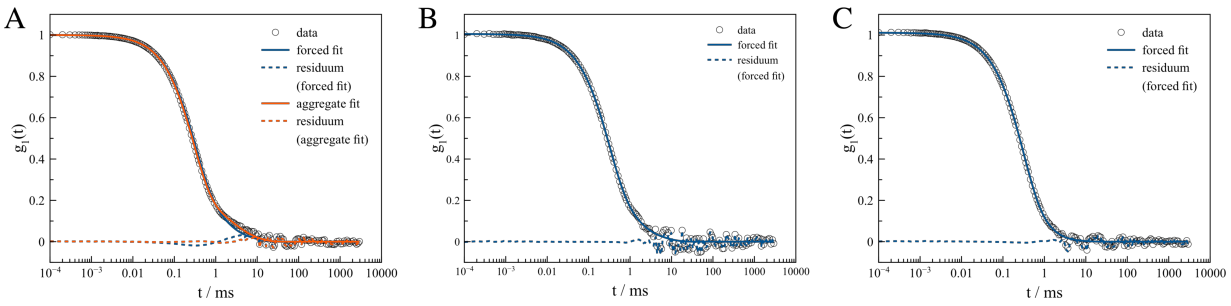


Figure S11: ACF of siRNA loaded nanohydrogel particle NP in BSA solution (80 g/L in PBS) at 50:1 (A), 25:1 (B) and 15:1 (C) weight-to-weight ratio NP:siRNA – scattering angle 30°. For the 50:1 ratio (A) aggregates were found with R_h = 118 nm.

6. Dynamic Light Scattering of SiRNA Only in Human Blood Serum

Sample preparation of siRNA only in human blood serum was done in analogy to the description in the experimental section: First 0.5 mL of a 0.01 g/L siRNA solution (same concentration like the 15:1 ratio of **NP**:siRNA) in PBS was filtered into a light scattering cuvette containing 1.0 mL of filtered PBS buffer. DLS analysis showed no autocorrelation for this sample at this very low concentration ($g_{1,siRNA}(t) = 0$). On the other hand, serum itself (0.5 mL of filtered PBS in 1.0 mL of filtered serum) could be fitted by equation 1. Afterwards, 0.5 mL of a 0.01 g/L siRNA solution in PBS was filtered into a light scattering cuvette containing 1.0 mL of filtered serum and analyzed by DLS. In analogy to equation 3, forced fit method would afford $g_{1,m}(t) = f_s g_{1,s}(t) + f_{siRNA} g_{1,siRNA}(t) = f_s g_{1,s}(t)$, which obviously failed according to Figure S12. Therefore, aggregation function was required as $g_{1,m}(t) = f_s g_{1,s}(t) + f_{siRNA} g_{1,siRNA}(t) + f_{agg} g_{1,agg}(t) = f_s g_{1,s}(t) + f_{agg} g_{1,agg}(t)$ revealing aggregation formation of pure siRNA in human blood serum:

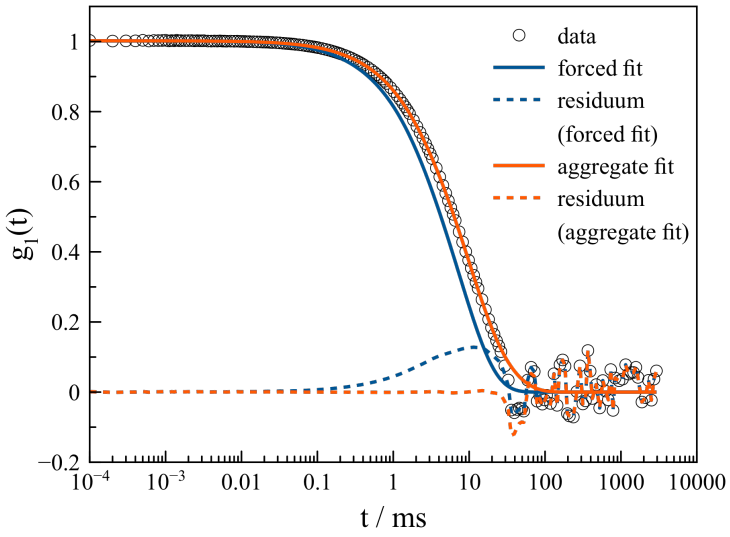
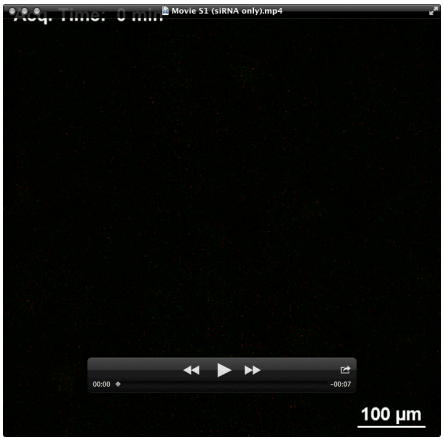


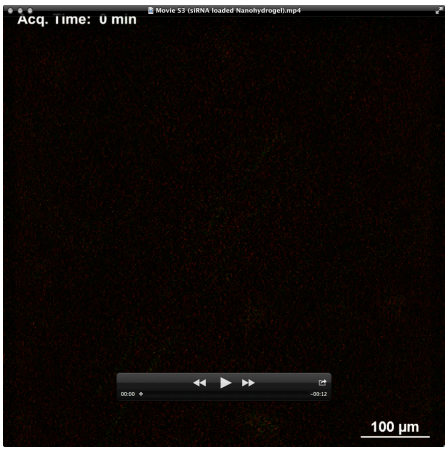
Figure S12: ACF of siRNA only (at c = 3.33 g/mL) in human blood serum – scattering angle 30°. Aggregates were found with R_h = 271 nm.

7. *Intravital* Confocal Videography



Movie S1: *Intravital* confocal viderography of siRNA only – Alexa647 labeled siRNA: red.

Movie S2: *Intravital* confocal viderography of NP* only – Oregon Green labeled NP*: green.



Movie S3: In*travital* confocal viderography of siRNA loaded NP* (weight-to-weight ratio NP*:siRNA = 25:1) – Oregon Green labeled NP*: green; Alexa647 labeled siRNA: red.

8. References

(1) Nuhn, L.; Hirsch, M.; Krieg, B.; Koynov, K.; Fischer, K.; Schmidt, M.; Helm, M.; Zentel, R. *ACS Nano* **2012**, *6*, 2198–2214.

(2) Schmidt, M. In *Dynamic Light Scattering, The Method and Some Applications*; Brown, W., Ed.; Clarendon Press: Oxford, 1993.

(3) Rausch, K.; Reuter, A.; Fischer, K.; Schmidt, M. *Biomacromolecules* **2010**, *11*, 2836–2839.