Electronic Supporting Information

Glyco-copolypeptide Grafted Magnetic Nanoparticles: The interplay between particle dispersion and RNA loading

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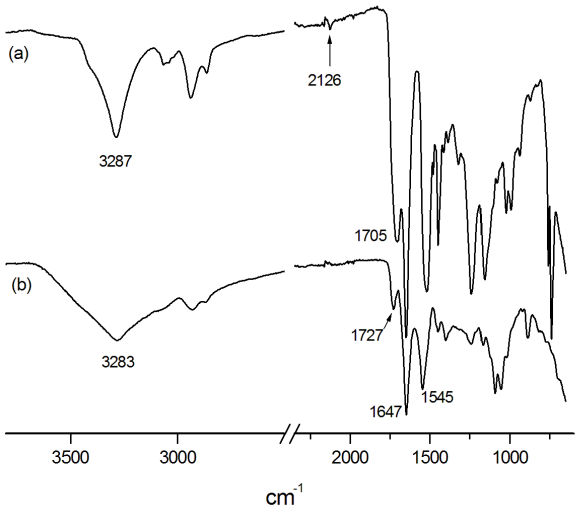

Figure S1. ATR-IR Spectra of (a) MNP-PFLL-*r*-PPLG **3** and (b) *sGal*-MNP **7**.

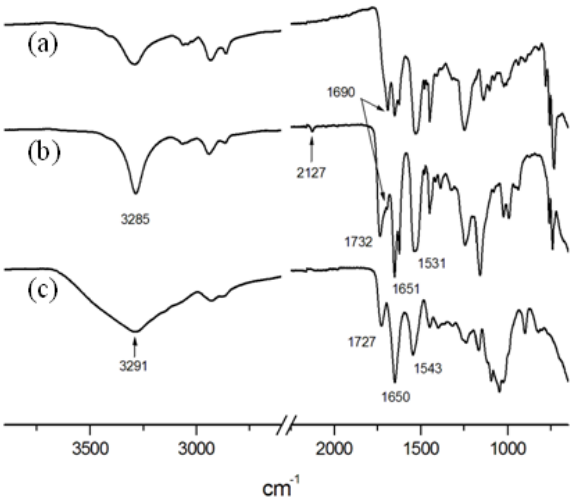

Figure S2. ATR-IR Spectra of (a) MNP-P; (b) MNP-PFLL-*b*-PPLG **2** and (c) *b*Gal-MNP **6**.

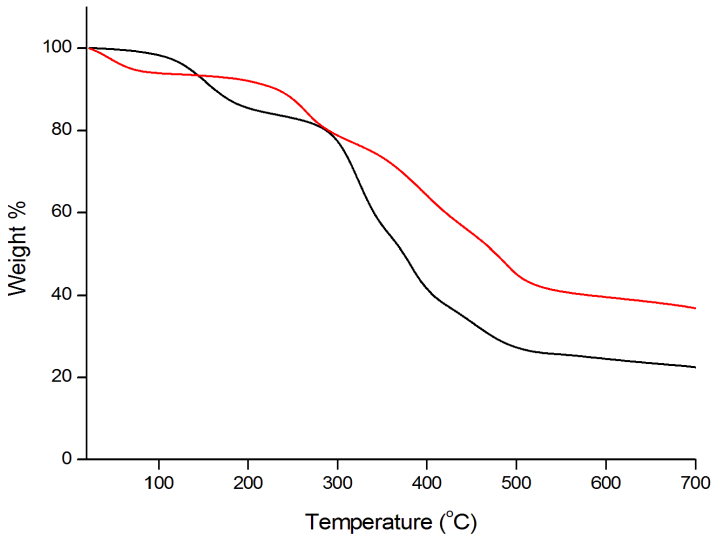

Figure S3. Thermogravimetric analysis of MNP-PFLL-*r*-PPLG **2** (black) and *sGal*-MNP **7** (red).

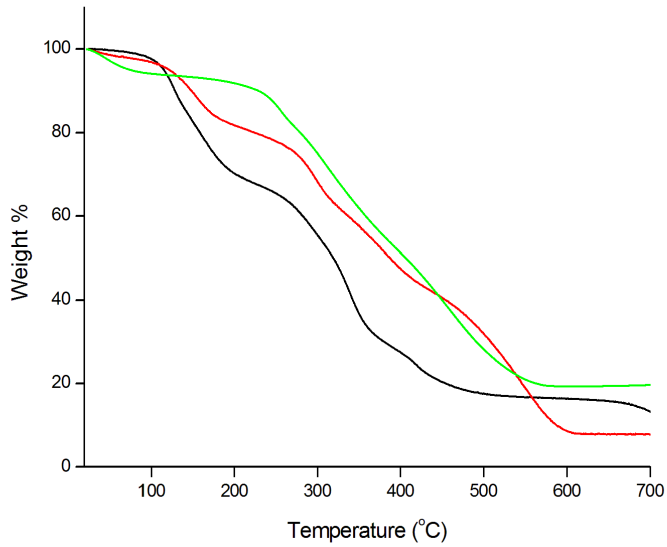

Figure S4. Thermogravimetric analysis of MNP-*g*-PFLL (black), MNP-PFLL-*b*-PPLG **3** (red) and *b*Gal-MNP **6** (green).

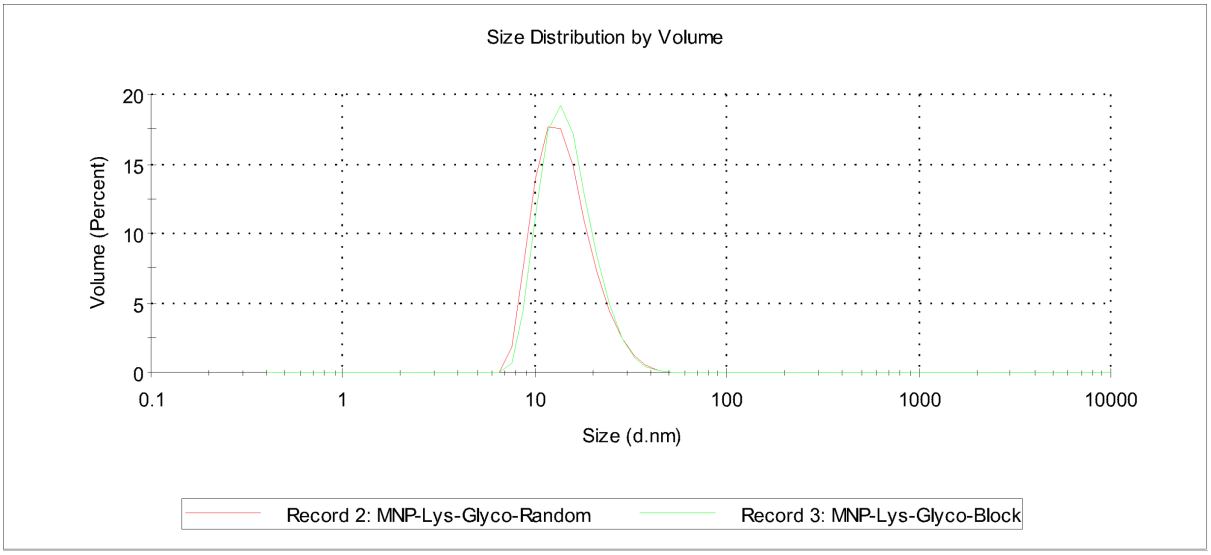

Figure S5. DLS data for hydrodynamic size determination of iron oxide nanoparticles decorated with *b*Gal-MNP **6** (green) and *sGal*-MNP **7** (red)

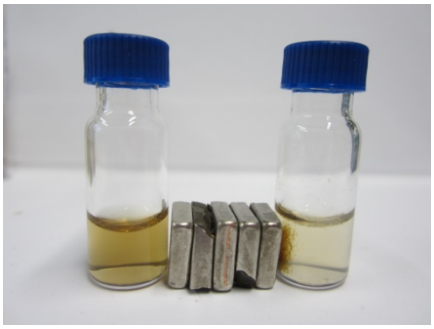

Figure S6. Photographic images showing selective binding of Gal-MNP to lectin RCA_{120}.

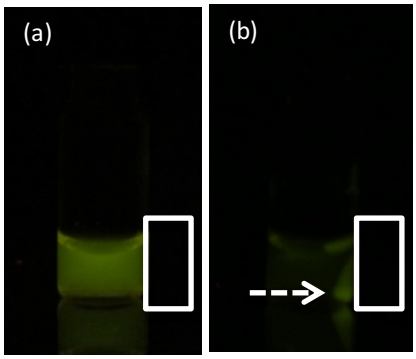

Figure S7. (a) *b*GAL-MNP **6** complexed with fluorescently tagged siRNA. (b) Magnetically captured fluorescent siRNA complexed nanoparticle aggregates (broken arrow) after addition of RCA_{120}.

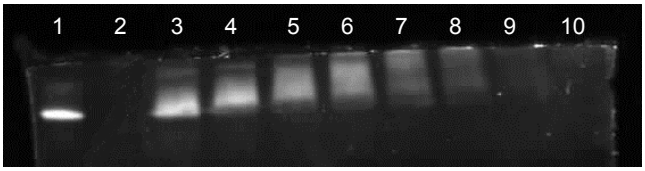

Figure S8. Polyacrylamide gel electrophoresis image of siRNA complexed *b*Gal-MNP with increasing quantity of particles. Lane 1: siRNA reference; lane 2: PEI complexed siRNA; lanes 3-10: siRNA/MNP 575, 287.5, 143.8, 95.8, 71.9, 57.5, 38.3, 28.7 nmol/mg, respectively.

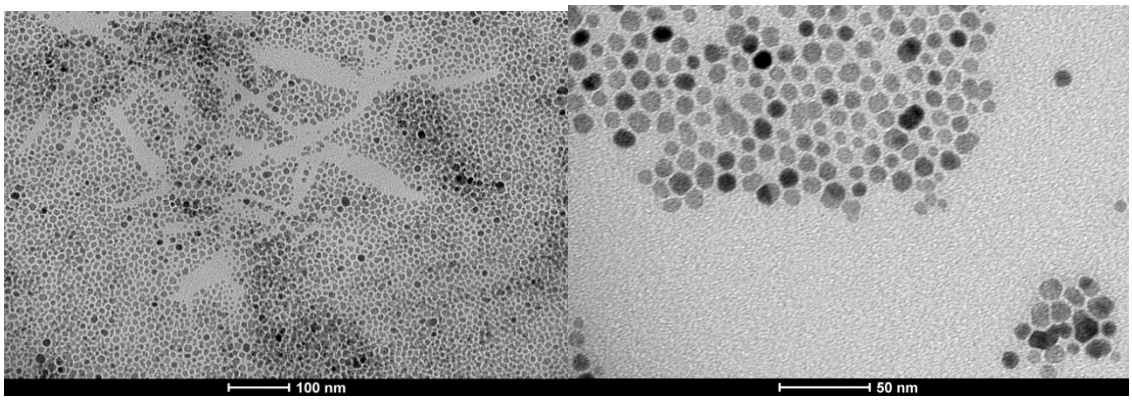

Figure S9. Representative TEM micrograph of NP from surfactant free approach, without silanisation, d_{TEM} = 8.2±1.6 nm.

Table S1: NMRD simulation parameters for MNP dispersions using SPM theory[1]

Sample	M_{NMR} [emu g^{-1}]	D_{NMR} [nm]	τ_0 [ns]	$\Delta E_{ANISOTROPY}$ [GHz]
*b*GAL-MNP, **6**	60.75	9.6	8	0.5
*r*GAL-MNP, **7**	58.5	9.6	12	0.5
PLL-MNP	56	9.6	4	0.5

Table S2. Physical properties of aqueous MNP dispersions.

Sample	d_{hyd} (PDI) [nm]	ζ [mV]	Relaxivity at 16.3 MHz r_1 (r_2)r_2/r_1 [$s^{-1}mM^{-1}$,($s^{-1}mM^{-1}$),-]
PLL-MNP	18.5(0.18)	+56	36.2(98.3)2.7
sGal-MNP, **7**	14.9(0.12)	+47	38.6(80.7)2.1
*b*GAL-MNP, **6**	18.5(0.09)	+31	42.7(81.1)1.9

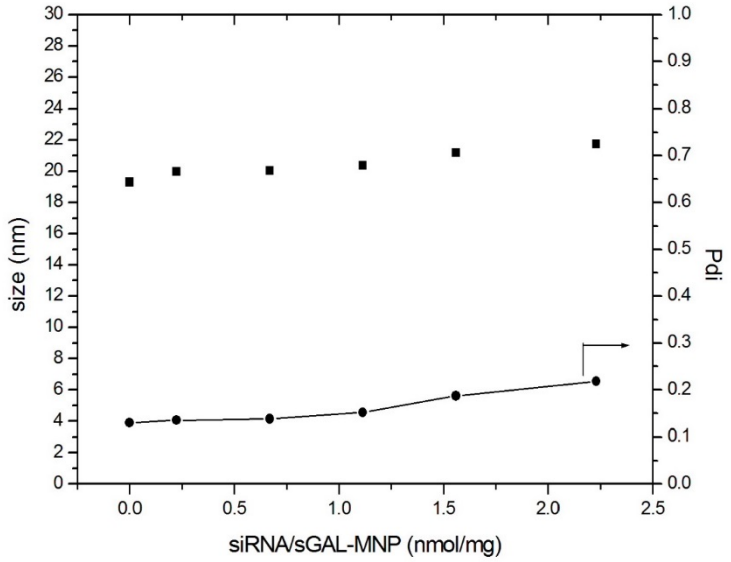

Figure S10. Size (■) and Polydispersity Index (PDI) (●) obtained from DLS measurements of siRNA complexed *s*GAL-MNP with increasing ratio.

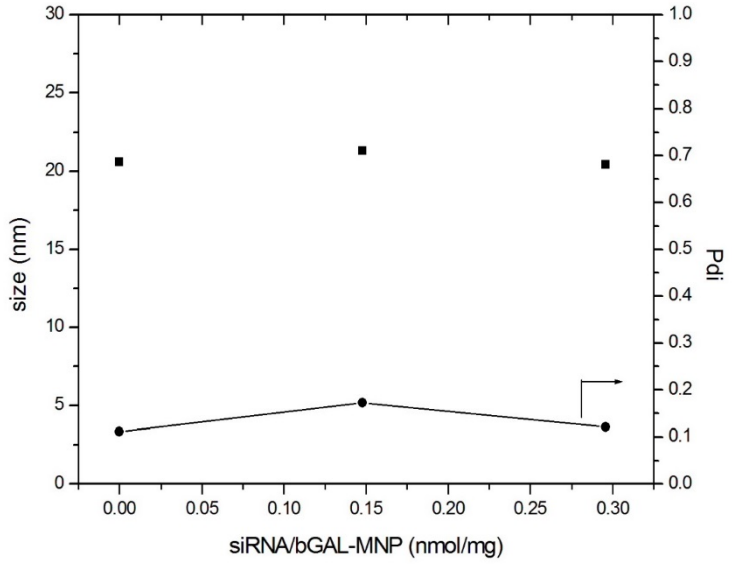

Figure S11. Size (■) and Polydispersity Index (PDI) (●) obtained from DLS measurements of siRNA complexed *b*GAL-MNP with increasing ratio.

Experimental Section

Materials. All materials were obtained from Sigma-Aldrich and used as received unless otherwise noted. All anhydrous solvents mentioned were stored under dry inert atmosphere. Laboratory grade diethyl ether was purchased from VWR. siGENOME non-targeting siRNA #4 (5' AUGAACGUAAUUG-CUAA 3') was obtained from Dharmacon. Aminopropyltriethoxysilane functional Fe_3O_4 nanoparticles (1) and PLG-NCA were synthesized using our previously reported method.[2]

Fmoc-L-Lysine NCA: The NCA of Fmoc-L-Lysine (FLL-NCA) was synthesized using literature procedure[3] with some modifications. In a 250 mL three necks round bottom flask N_ε-fluorenylmethoxycarbonyl-L-lysine (5.00 g, 13.6 mmol) and α-pinene (4.28 g, 31.4 mmol) was added to dry anhydrous ethyl acetate (60 mL). The mixture was stirred under nitrogen atmosphere and heated to reflux. Triphosgene (2.72 g, 9.17 mmol) in ethyl acetate (20 mL) was added drop-wise to this mixture over 30 min. and refluxed overnight until all solid had disappeared. The clear reaction mixture was reduced to 1/3 by evaporating ethyl acetate and immediate precipitation was seen after addition of 40 mL n-heptane. The mixture was heated to clear solution and allowed to cool slowly. The precipitated NCA was filtered, washed with n-heptane, dried under vacuum to yield NCA (4.10 g, 76.6%) as a white powder and stored at -4 ˚C. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.77 (d, 2H, ArH, J = 7.5 Hz), 7.59 (d, 2H, ArH, J = 7.5 Hz), 7.40 (t, 2H, ArH, J = 7.2), 7.31 (t, 2H, ArH, J = 7.2), 6.68 (s, 1H, NH), 4.85 (s, 1H, NH), 4.44 (t, 2H OCH_2CH, J = 6.0), 4.29 (t, 1H, CHC(O), J = 5.4 Hz), 4.21 (t, 1H, $CHCH_2O$, J = 6.5 Hz), 3.18 (m, 2H, CH_2NH), 1.96 (m, 2H,CH_2CH), 1.53 (m, 2H, CH_2), 1.42 (m, 2H, CH_2) ^{13}C-NMR (100 MHz): 169.9 (OC(O)CH), 153.8 (C(O)NH), 152.5 ((CO)NH), 143.8 (Ar), 141.3(Ar), 127.7 (Ar), 127.1 (Ar), 125.0 (Ar), 120.0 (Ar), 66.7 (CH_2O), 57.4 (CH), 47.2 (CH), 40.0 (CH_2), 30.8 (CH_2), 21.3 (CH_2)

MNP-PFLL: To the FLL-NCA (200 mg) in a Schlenk tube, 2 mL suspension of amino functionalised magnetic nanoparticles (1) in anhydrous chloroform was added under nitrogen atmosphere. The reaction mixture was stirred for 24 h at room temperature and completion of polymerization reaction was confirmed by ATR-IR spectroscopy. Poly(Fmoc-L-Lysine) (P) grafted magnetic nanoparticles were precipitated in excess diethyl ether and separated as dry product. To a suspension of above mentioned magnetic particles in anhydrous DMSO (3 mL), piperidine (1 mL) was added drop wise to deprotect poly(Lysine) (PLL) side chain amine groups. The suspension was stirred for another 2 hours followed by precipitation in anhydrous diethyl ether to get PLL grafted magnetic nanoparticles. These nanoparticles were then dispersed in water and the pH of the suspension was adjusted to acidic (~4-5) by adding 1M HCl. The suspension was then dialyzed against water to remove piperidinium salt traces and excess acid. The PLL grafted iron oxide nanoparticles suspension was stored in a fridge.

MNP-PFLL-*b*-PPLG 2: In a clean dry Schlenk tube FLL-NCA (200 mg) was weighed to which MNP-APTS suspension in $CHCl_3$ (2 mL) was added under nitrogen atmosphere at 0 ˚C The reaction was stirred for 24 h at 0 ˚C. An aliquot (0.2 mL) was taken out and the complete consumption of monomer confirmed by ATR-IR. In a sequential step, a solution of PLG-NCA (50 mg) in an anhydrous DMSO (1 mL) was added to the Schlenk tube. The reaction mixture was stirred at 0 ˚C for another 24 h by which the complete conversion of the second monomer had occurred as confirmed by ATR-IR. The material was then recovered as a dry powder by precipitating in diethyl ether.

MNP-PFLL-*s*-PPLG 3: FLL-NCA (200 mg) and PLG-NCA (50 mg) were weighed into a clean dry Schlenk tube to which MNP-APTS suspension in $CHCl_3$ (2 mL) was added under nitrogen atmosphere. The reaction mixture was stirred at room temperature and 1 mL of anhydrous DMSO was added after the initial 15 minutes to improve the dispersability of polypeptide grafted MNP. The reaction was stirred for 24 h and complete monomer conversion was confirmed by ATR-IR. The material was then recovered as a dry powder by precipitating in diethyl ether.

Glycosylation and Fmoc deprotection to form 6 and 7: Copper catalyzed Azide Alkyne Click (CuAAC) was used for the glycosylation of alkyne moieties of the polypeptide shell as reported previously[3] with minor modifications. Similler procedures were followed for both block and statistical co-polypeptide grafted magnetic nanoparticles. In a Schlenk flask, to 5 mL suspension of P-b-PPLG grafted magnetic nanoparticles (0.237 mmol alkyne, considering 100% conversion of monomer to polymer and 100% grafting of polymer on MNP surface) in an anhydrous DMSO, 1-azido-1-deoxy-B-galactopyranoside (97 mg, 0.473 mmol, 2 equiv. to alkyne) and N-N diisopropylethylamine (21 μL, 0.12 mmol, 0.5 equiv. to alkyne) were added. The suspension was degassed by purging with dry nitrogen gas for 30 min. After addition of triphenylphosphine copper(I)bromide ($(PPh_3)_3CuBr$) (44 mg, 0.047 mmol, 0.2 equiv. of alkynes) the reaction mixture was purged again with nitrogen for 30 min. and left stirring for 48 h at room temperature. The glycosylated magnetic nanoparticles were then precipitated in anhydrous THF (50 mL). Because of the good solubility of the $(PPh_3)_3CuBr$ catalyst and almost negligible solubility of the sugar in THF, repeated dispersion of the MNP in DMSO and precipitation in THF was applied to remove all traces of copper catalyst. Therefore, the dry nanoparticles were resupended in DMSO (5 mL) and reprecipitated in THF (50 mL) thrice to remove the copper catalyst. To a catalyst free glycosylated magnetic

particle dispersion in anhydrous DMSO (3 mL), piperidine (1 mL) was added dropwise and stirred for 2 h follwed by precipitation in anhydrous diethyl ether. The final product poly(lysine)-co-glycopeptide grafted magnetic nanoparticles were suspended in water by adjusting the solution pH to acidic (~ 4-5) by addition of 1 M HCl solution. Pipiredinium salt, acid tracess and excess sugar was removed by dialysis against DI water. The nanoparticles were stored in fridge.

Complexation of siRNA with Gal-MNP 6 and 7: siGENOME non-targeting siRNA #4 (5' AUGAACGUAAUUG-CUAA 3') was used in for complexation with iron oxide nanoparticles. The siRNA/Gal-MNP complexes were prepared freshley prior to use. Varying volumes of Gal-MNP solution of known iron content (2 mM in DI water) (1, 2, 4, 6, 8, 10, 15, 20 μL) was used for comlexation with fixed quantity of siRNA (1 ng) in tris HCl buffer (pH 7.4; 25 μL). The incubation was carried out at room temeparture for 30 mins.

Lectin Binding Experiment: In a typical lectin binding experiment, to a 0.5 mL (~1.5 mM) suspension of glycosylated magnetic nanoparticles in DI water, 100 μL (2 mg/mL) lectin from *Ricinus communis* (castor bean) Agglutinin RCA_{120} was added resulting in instantaneous aggregation of nanoparticles. Addition of excess free galactose (5 mg) resuspended the nanoparticle to form homogeneous suspension. A control experiment was performed with addition of 100 μL (2 mg/mL) *Canavalia ensiformis* (jack bean) Concanavalin A to the nanoparticle suspension. Similar sets of experiments were performed with fluorescently labeled Gal-MNP by coupling fluorescein isothiocyanate (FITC) to amines groups of poly(lysine) shell. Traces of unbound FITC was removed by dialysis prior to lectin binding experimet.

Methods. TEM measurements were performed using TECNAI G2 TEM operating at an accelerating voltage of 120 kV. Attenuated total reflectance (ATR) infrared spectra were recorded on a Spectrum GX FT-IR System (Perkin Elmer; Norwalk, CT, USA), under accumulation of 8 scans using a ZnSe trough plate crystal. Dynamic light scattering (DLS) experiments were performed at 25 °C on a Malvern NanoZS (Malvern Instruments, Malvern UK) which uses a detection angle of 173°, and a 3 mW He-Ne laser operating at a wavelength of 633 nm. The hydrodynamic diameter and the polydispersity index (PDI) values were obtained using cumulants analysis. The frequency dependence of the water ^{1}H relaxation, at 25 °C, was recorded over the frequency range 0.01–40 MHz using a Spinmaster Fast Field Cycling NMR Relaxometer (Stelar SRL, Mede, Italy). The system operated at a measurement frequency of 16.3 MHz for ^{1}H, with a 90° pulse of 7 μs. A re-conditioned Bruker WP80 electromagnet was used at 40 and 61 MHz. T_2 was measured using the CPMG pulse sequence. Total iron content was determined by ICP-AES using a Varian Liberty 220ICP. Suspensions were prepared by combining a 0.2 mL aliquot of the sample with 0.5 mL 8.8 N analar grade HCl, and 1 mL deionised water. The mixture was heated until only *c.*1 drop of liquid remained, at which time 10 mL deionised water was added. The solution was heated to boiling and then immediately removed from the heat and allowed to cool to room temperature. The volume was adjusted to 50 mL for spectrometric analysis. Thermogravimetric analyses (TGA) were performed on a TGA Q50 from TA Instrument using a temperature ramp from 20 to 800 °C at 20°C min-1 under nitrogen atmosphere. For Gel Retardatioin Assays Gal-MNP/siRNA complexes were mixed with loading dye (Promega G190A-Blue/orange 6x) and analysed by running 20% non denaturating polyacryl-amide gel

in TBE buffer at 100 V for 1 h. Upon completion, the gel was stained using Gelstar nuclic acid gel stain for 30 min. Visual images were then obtained by UV transillumition (G. Box, Syngene, UK).

(1) Roch, A.; Muller, R. N.; Gillis, P. *J. Chem. Phys.* **1999**, *110*, 5403.
(2) Borase, T.; Ninjbadgar, T.; Kapetanakis, A.; Roche, S.; O'Connor, R.; Kersken, C.; Heise A.; Brougham, D. *Angew. Chem. Int. Ed.* **2013**, *52*, 3164.
(3) Habraken, G. J. M.; Peeters, M.; Dietz, C. H. J. T.; Koning, C. E.; Heise, A. *Polym. Chem.* **2010**, *1*, 514.