Size-controlled clustering of iron oxide nanoparticles within fluorescent nanogels using LCST-driven self-assembly†

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Size-controlled clustering of iron oxide nanoparticles (IONPs) within the fluorescent polymer nanogels was achieved using the lower critical solution temperature (LCST) driven self-assembly and cross-linking of grafted polymer on the IONPs. The grafted polymer was comprised of oligoethyleneglycol methacrylate (OEGMA) and a novel dichloromaleimide functional methacrylate monomer as building blocks. As a result of the temperature responsive behavior of OEGMA, polymer grafted IONPs clustered to form larger nano-sized aggregates when heated above the LCST of the polymer. When these nano-sized aggregates were cross-linked using an amine–dichloromaleimide reaction, well-defined fluorescent hybrid nanogels could be fabricated. Moreover, the size of these hybrid nanogels was effectively controlled by varying the initial concentration of the polymer grafted IONPs in water.

Iron oxide nanoparticles (IONPs) have been under intensive investigation for a variety of biomedical applications, such as magnetic resonance imaging (MRI), hyperthermia therapy, drug delivery, immunoassay as well as cell separation mainly due to their responsiveness to magnetic fields. When the size of IONPs is sufficiently small (around 10–30 nm), they exhibit superparamagnetic behavior, meaning that they rapidly respond to an applied external magnetic field similar to a paramagnet yet exhibit negligible residual magnetism when the external magnetic field is removed. This behavior avoids strong magnetic interactions between the individual IONPs and prevents/inhibits the agglomeration of IONPs in the absence of an applied magnetic field, which makes superparamagnetic IONPs very attractive for biomedical applications. However, the small size of the superparamagnetic IONPs results in a low magnetization per particle, limiting the manipulation of IONP movement using an external magnetic field. To overcome this limitation, increasing attention has been drawn to polymer assisted solution self-assembly of IONPs enabling access to IONPs embedded in colloidal polymer particles with a controlled size and morphology. The vast majority of these systems rely on block copolymer assisted solution self-assembly. As a seminal example, Taton and coworkers formulated polymer micelles loaded with IONPs using the simultaneous self-assembly of amphiphilic copolymers and IONPs. Recently, Khashab and co-workers fabricated nanosized polymer vesicles having tunable layers of densely packed superparamagnetic IONPs using cooperative self-assembly of polymer tethered IONPs and a free block copolymer.

Crosslinking of thermoresponsive amphiphilic random copolymers above their lower critical solution temperature (LCST) provides a facile approach to form stable nanogels in aqueous solutions. As a pioneering example, Thayumanavan and coworkers achieved efficient nanogel synthesis by heating a random copolymer, that contain cross-linkable disulfide units and thermoresponsive oligoethyleneglycol moieties as side-chain functionalities, above its LCST temperature, followed by in situ disulfide crosslinking mediated by the addition of dithiothreitol. Additionally, Sanyal and coworkers demonstrated that a poly(ethylene glycol)-methacrylate-based, maleimide functional random copolymer can yield nanogels through cross-linking of copolymer with a dithiol-based cross-linker above the LCST temperature of the polymer in water. However, to the best of our knowledge, clustering of IONPs into polymer hydrogels using LCST driven self-assembly has never been reported in the open literature so far.

Herein, we report a novel approach for the size-controlled clustering of IONPs into polymer nanogels harnessing the thermoresponsive behavior of an amphiphilic random copolymer tethered on IONPs (Scheme 1). To synthesize IONP encapsulated nanogels, a statistical copolymer of poly(ethylene glycol) monomethyl ether methacrylate (OEGMA mon, M n = 300 g mol−1) and a dichloromaleimide (DCM) functional monomer (DCMMA) was synthesized by reversible addition–fragmentation chain transfer
(RAFT) polymerization using a dopamine functional chain transfer agent (CTA) (Scheme 1A) and the statistical copolymer was tethered on IONPs (Scheme 1B). OEGMA based hydrophilic polymers are known for their biocompatibility, stealth effect as well as exhibiting LCST behavior with a tunable cloud point temperature \( T_{\text{CP}} \) between 20 and 90 °C.\(^{20,21}\) Additionally, surface coating of IONPs using OEGMA based polymers provides an effective method for the stabilization of IONPs in water, thereby avoiding agglomeration.\(^{22}\) Therefore, we used OEGMA\(_{300}\) as the main monomer in the polymer chains, which can stabilize the IONPs in water at room temperature but forms larger nanosized aggregates when heated above its LCST. Fast and efficient crosslinking of the nanosized aggregates is crucial in order to obtain stable nanogels with a monomodal size distribution. It is known that dibromomaleimide (DBM) functional polymers can be modified with primary amine or thiol bearing small molecules in water through single substitution under mild conditions with high efficiency.\(^{22,23}\) Previous work within our group has shown that DCM is even more reactive than DBM.\(^{24}\) Moreover, although DCM and DBM are not fluorescent, the substitution reaction of DBM and DCM with amine or thiol functional molecules yields fluorescent maleimide derivatives.\(^{23,25,26}\) This could provide a facile approach for the synthesis of fluorescent nanogels without any need of post-nanoparticle functionalization with a fluorescent dye. As a consequence, for the crosslinking of the nanogels we chose to use DCM diamine chemistry (Scheme 1C).

The findings on the LCST-driven self-assembly behavior of the statistical copolymer as well as the copolymer tethered IONPs are disclosed herein.

First, a novel dichloromaleimide functional monomer, 3-[3,4-dichloro-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl]propyl methacrylate (DCMMA), was synthesized as demonstrated in Scheme S1 (ESI†).\(^{1}\)\(^{1}\)H and \( ^{13}\)C NMR spectroscopic analysis proved the successful synthesis of the desired monomer (Fig. S1 to S4, ESI†). The initial monomer feed ratio \([\text{OEGMA}_{300}] / [\text{DCMMA}]\) was set to 4 (Table S1, ESI†). A dopamine functional CTA was used in order to attach the synthesized polymer onto IONPs.\(^{27}\) Polymerization was carried out in dioxane at 70 °C with a total monomer concentration of 0.4 mol L\(^{-1}\). The monomer conversions for OEGMA\(_{300}\) and DCMMA were determined as 62% and 68%, respectively, by \( ^{1}\)H NMR using 1,3,5-trioxane as internal standard. The theoretical molar mass \( (M_{n,\text{theo}}) \) of the copolymer was calculated as 19 250 g mol\(^{-1}\) (see the Formula (S1) in the ESI†). \( ^{1}\)H NMR spectroscopy analysis of the isolated polymer clearly revealed the presence of the aromatic dopamine protons of \( \alpha \)-RAFT end groups \((\delta = 6.73, 6.65, \text{and } 6.51 \text{ ppm})\). The characteristic signals that are derived from OEGMA\(_{300}\) \((\delta = 4.0, 3.59 \text{ and } 3.31 \text{ ppm})\) and
DCMMA (δ = 3.90 ppm) (Fig. S11, ESI†). This enables the estimation of the degree of polymerization (DP) for OEGMA300 as 52 and for DCMMA as 14 (see the Formulas (S2) and (S3) in the ESI†) by comparing the integral values of the methine proton peak of the OEGMA300 side chains at δ = 4.00 ppm (peak “2” in Fig. S11, ESI†) and the integral value of the methine proton peak of the DCMMA side chains at δ = 3.90 ppm (peak “3” in Fig. S11, ESI†) and the integral value of aromatic dopamine protons of α-RAFT end groups (δ = 6.73, 6.65, and 6.51 ppm) (peak “1” in Fig. S11, ESI†). The according number average molar mass (Mn) value (20100 g mol⁻¹) calculated by ¹H NMR (see the Formula (S4) in the ESI) is in good agreement with the theoretical value. SEC analysis revealed a molar mass value of 13 100 g mol⁻¹ with a monomodal trace and a relatively low dispersity (Dm = 1.35) (Fig. S12, ESI†). The underestimated molar mass by SEC was attributed to the PS calibration in the SEC system, resulting in a relative molar mass value.

The thermoresponsive behavior of the statistical copolymer in aqueous media was investigated using turbidimetry measurements with a heating and cooling rate of 1 °C min⁻¹. Two heating/cooling cycles were conducted in a temperature range between 20 and 80 °C. Turbidimetry measurements revealed that the polymer was completely soluble in water at low temperatures, but an increase of temperature above 37 °C. After 1.5 min, a calculated amount of 2,2’-(ethyleneoxy)bis(ethylamine) aqueous solution as cross-linker was added into the polymer solution and the solution was stirred at 70 °C for 20 min, followed by cooling down to room temperature. The molar ratio of the dichloromaleimide and the cross-linker was set to 1:1. In order to confirm the reproducibility of nanogel synthesis, this procedure was repeated three times. A schematic illustration of the polymer nanogel preparation is shown in Scheme S3 (ESI†).

Prior to tethering the synthesized polymer on iron oxide nanoparticles, it was important to prove that the synthesized polymer can form nanogels by heating above its LCST and cross-linking via an amine–dichloromaleimide reaction. To this end, the polymer was dissolved in water (2 mg mL⁻¹) and heated at 70 °C for 1.5 min. After 1.5 min, the solution became yellow and it maintained its yellow color as well as its turbidity when cooled to room temperature. The obtained assemblies were characterized using DLS without any filtration. DLS revealed that all batches have very similar intensity average diameters (D_{w,av} = 200 ± 5 nm) with monomodal size distributions and low polydispersity index (PD) values (lower than 0.08) (Fig. S13 and Table S2, ESI†). These results prove the reproducibility of the nanogel synthesis. Then, nanogels synthesized from three different batches were combined and characterized by DLS and TEM. DLS revealed a D_{w, av} of 197 ± 2 nm with a PD value of 0.03 ± 0.02 and zeta potential measurements showed a negative zeta potential value (−21 ± 2 mV) (Fig. 2). TEM analysis revealed

![Figure 1](https://example.com/fig1.png)

**Fig. 1** (A) Schematic depicting of reversible LCST-driven self-assembly behavior of the polymer. (B) Variable temperature turbidimetry analysis of the polymer at 2 mg mL⁻¹ in water (heating and cooling rate = 1 °C min⁻¹). DLS size distributions of the polymer in water at 2 mg mL⁻¹ as a function of the temperature (C, heating; D, cooling).
well-defined spherical morphologies to the synthesized nano-
gels with diameters (from 120 to 170 nm) slightly smaller than
diameters obtained in DLS (Fig. 2). The formation of the nanogels
was attributed to the reaction of diamine cross-linker with the
dichloromaleimide functionalities within the aggregated polymer.

The appearance of a strong absorption band at $\lambda_{\text{max}} = 383$ nm
in the UV-vis spectrum of the nanogels, which is the characteristic
signal of substituted maleimides,\textsuperscript{24} proves this assumption
(Fig. S14, ESI†). Additionally, when excited at 384 nm, nanogels
showed a fluorescence emission with $\lambda_{\text{max}} = 530$ nm (Fig. 2).

To investigate the effect of initial polymer concentration in aqueous
media on the size of the nanogels, the same experiments were
conducted at two additional concentrations (1 and 4 mg mL\textsuperscript{-1}).
DLS measurements showed that 1 mg mL\textsuperscript{-1} yielded smaller
nanogels ($D_{\text{av}} = 178 \pm 2$ nm) and 4 mg mL\textsuperscript{-1} yielded larger
nanogels ($D_{\text{av}} = 334 \pm 6$ nm) compared to 2 mg mL\textsuperscript{-1} ($D_{\text{av}} = 197 \pm 2$

Dopamine terminated polymer was grafted onto the surface of
IONPs using a ligand exchange reaction based on the chelation
of dopamine with the surface of IONPs (Scheme 1B), yielding
P-IONp.\textsuperscript{27} TGA measurements revealed that P-IONp have 27%
iron oxide content (Fig. S18, ESI†). TEM images showed no change in the diameter of the IONPs after polymer grafting
(Fig. S19, ESI†). DLS measurements in water revealed that
P-IONp had a slightly larger number average hydrodynamic
diameter ($D_{n} = 32 \pm 6$ nm), than polymer free IONPs ($D_{n} = 18 \pm 3$

Oleic acid-stabilized IONPs were synthesized using a thermal
decomposition method as reported previously.\textsuperscript{29} DLS measurements
in hexane revealed monomodal size distributions with number
average hydrodynamic diameter ($D_{n}$) of 18 ± 3 nm (Fig. S16, ESI†).
In agreement with DLS, TEM showed spherical IONPs with dia-

Next, to investigate the possibility of clustering IONPs within
the interior of the polymer nanogels, an aqueous suspension of
P-IONp was heated at 70 °C for 30 seconds at three different
concentrations (1, 2 and 3 mg mL\textsuperscript{-1}). After 30 seconds, diamine
was added into the solution to cross-link and the solution was kept
at 70 °C for 5 minutes. After cooling down to room temperature,
the resulting assemblies were characterized by DLS and TEM.
DLS measurements revealed that the hydrodynamic size of the
resulting assemblies was larger than the hydrodynamic diameter
of the nanogels, which is the characteristic signal of substituted maleimides,\textsuperscript{24} proves this assumption
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Fig. 2 (A) DLS size distributions, (B) $\zeta$-potential distribution of nanogels, (C) TEM images of the nanogels synthesized with an initial polymer concentration of 2 mg mL\textsuperscript{-1} (sample was stained using 1 wt% uranyl acetate (UA) solution) and (D) excitation and emission spectra of nanogels in water ($c = 0.165$ mg mL\textsuperscript{-1}), (inset: photograph of solution of nanogels under UV light at 365 nm).
of the P-IONP and the diameter of the resulting assemblies gradually increased with the increasing initial P-IONP concentration in water (Fig. 3). Zeta potential measurements revealed negative zeta potential values for all the fabricated hybrid nanogels around −20 mV (Fig. S21, ESI†). The negative zeta potential values of the nanogels were attributed to the hydrophilic OEGMA_{300} units of the polymers on the surface of the nanogels. Additionally, TEM images of the assemblies showed that IONPs were densely clustered within the well-defined spherical polymer nanogels (Fig. 3). These results indicate that size of the hybrid nanogels could be tuned simply by varying the initial concentration of the polymer grafted IONPs in water. The formation of the hybrid nanogels was attributed to the formation of larger nanosized aggregates as a result of collapse and aggregation of grafted polymer above its LCST and the subsequent in situ intra-/inter-molecular diamine dichloromaleimide crosslinking. In agreement with this, the synthesized hybrid nanogels emit light with \( \lambda_{\text{max}} = 532 \text{ nm} \) when excited at 387 nm (Fig. S22, ESI†).

In summary, a novel dichloromaleimide functional monomer was synthesized and copolymerized with OEGMA_{300} via RAFT polymerization. OEGMA based dichloromaleimide bearing statistical copolymer yielded well-defined fluorescent nanogels in water when the copolymer was cross-linked using an amine–dichloromaleimide reaction above the LCST of the copolymer. This copolymer could be tethered to IONPs and stabilize them in water. The LCST behavior of the grafted polymer provided a facile method for the clustering of IONPs in fluorescent polymer nanogels by heating the polymer tethered IONPs above the LCST of the grafted polymer followed by amine–dichloromaleimide cross-linking. The size of the hybrid nanogels could be tuned by simply varying the initial concentration of the polymer grafted IONPs in water. Consequently, the reported novel approach for the size-controlled synthesis of fluorescent hybrid nanogels may facilitate new opportunities for drug delivery, magnetic resonance imaging, and hyperthermia therapy.

Conflicts of interest

The authors declare no conflict of interest.

References


