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EDGE ARTICLE

Metastable single-chain polymer nanoparticles prepared by dynamic cross-linking with *nor-seco-cucurbit[10]uril*†

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We present the preparation of metastable single-chain polymeric nanoparticles through stimuli-responsive and reversible intramolecular cross-linking of a single polymer chain in water. Functionalisation of poly(*N*-hydroxyethylacrylamide) polymers with viologen, followed by addition of *nor-seco-cucurbit[10]uril* allows for facile preparation of nanoparticles through specific and tightly binding 2 : 1 homo-ternary complexes. Particle sizes can be readily tuned through either polymer molecular weight or degree of cross-linking. By virtue of the supramolecular nature of the intramolecular cross-linking in water, the formation of these metastable nanoparticles represents a step towards the development of synthetic polymers with aqueous phase behaviour analogous to the self folding of biomacromolecules.

Supramolecular cross-linking of polymeric and other macromolecular constructs has led to the formation of dynamic materials with novel properties.^{1–6} In particular, an area of recent interest is the creation of synthetic systems showing self-assembly into well defined three dimensional structures that may mimic those of enzymes. These systems have been demonstrated in organic solvents through the controlled intramolecular cross-linking of single polymer chains to form ‘self-collapsed’ single-chain polymeric entities^{7–13} and novel applications for such systems have been realised including catalysis and *in vivo* imaging.^{14,15} Fabrication of metastable polymeric nanoparticles (NPs) through intramolecular cross-linking of a single polymer chain in water is therefore inherently analogous to the self-folding of peptidic macromolecules, partly driven by specific hydrogen bonding, pi-pi stacking or Cys-Cys interactions.

The supramolecular chemistry of the cucurbit[*n*]uril family (CB[*n*]; *n* = 5–8,10) of macrocyclic host molecules has developed immensely over the past few decades on account of their high selectivity and exceptionally high binding constants in water (K_a up to 10^{15} M^{-1}).^{6,16–20} However, few of the CB[*n*] hosts partake in the formation of ternary complexes, which is necessary in order to form such reversible cross-linking mentioned above. *nor-seco-cucurbit[10]uril* (*ns-CB[10]*) is a recently discovered macrocyclic host of the extended CB[*n*] family and contains two identical cavities

capable of simultaneously binding one guest each to form 2 : 1 ternary complexes,^{21,22} and has recently been used for the preparation of stoichiometrically controlled complexes with multivalent guest molecules.²³ Herein we document the preparation of metastable single-chain polymer nanoparticles using the specific 2 : 1 ternary complex formation of *ns-CB[10]* (Fig. 1). The formation of the NPs is reversible and does not require protection chemistries. Additionally, the sizes of the resulting NPs are highly tunable depending on the cross-link density (*i.e.* the amount of *ns-CB[10]* added) and the precursor polymer molecular weight.

Poly(*N*-hydroxyethylacrylamide) (PHEAm) polymers were synthesised by ATRP²⁴ and the pendant hydroxyl functionality of the polymer allowed for the conjugation of methyl viologen (MV), a good guest for *ns-CB[10]* ternary complexation (Fig. 2). Conjugation of the MV guest moieties was achieved near-quantitatively and in a random fashion through an isocyanate-alcohol coupling reaction catalysed by dibutyl tin dilaurate

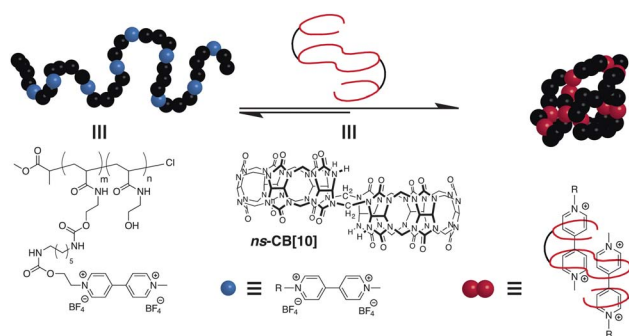


Fig. 1 Schematic representation of the formation of single-chain polymeric nanoparticles through 2 : 1 complexation of MV and *ns-CB[10]*.

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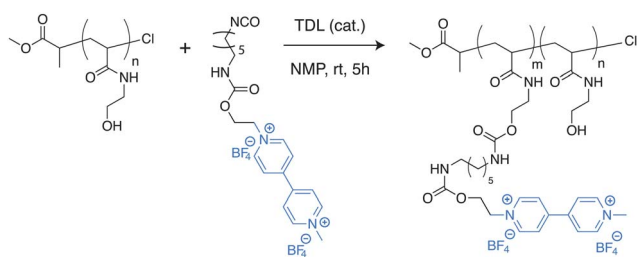


Fig. 2 Synthetic scheme for the preparation of functional water-soluble polymers **P1–4** bearing pendant MV units.

(TDL).²⁵ As demonstrated in Fig. 2, the fluoroborate counterions (BF_4^-) of the MV guest were utilised on account of the broad solubility they impart to the MV moiety in both organic solvents and in water. This feature allowed for each step of this study to be made without the need for further counterion exchange upon moving from organic solvents used for the synthesis of the functional polymers **P1–4** to aqueous media used for the complexation studies with *ns*-CB[10].

In order to have a direct comparison with recent work by Hawker *et al.*⁸ and Meijer *et al.*,⁹ polymers were synthesised with either a 10% or 30% loading of MV. These values were also chosen to provide sufficient cross-linking and significant variation in size and hydrodynamic volume upon polymer chain collapse and to observe the effect of cross-link density on nanoparticle size (*vide infra*). ^1H NMR spectroscopy confirmed the conjugation of the MV moieties (Fig. S1,† Table 1), where signals for the MV moieties are clearly observed in the aromatic region. Furthermore, an overlay of the UV-Vis spectral and refractive index traces from gel permeation chromatography is observed for the functional polymer, indicating that the MV moieties are indeed attached to the polymer chains. The disappearance of the characteristic isocyanate stretching vibration from the MV moiety was clearly observed using FTIR spectroscopy upon conjugation to the polymer. This band is completely absent in the spectra of the final polymers, verifying the complete removal of any unreacted isocyanate-functional MV units (Fig. S2†).

Controlled intramolecular collapse of the functional polymer chains was achieved by stirring a solution of the polymer followed by addition of *ns*-CB[10] as a solid. Successful particle formation was observed at concentrations at or below 0.1 mg ml^{-1} of polymer in solution. Monomodal distributions of polymer entities were observed at these concentrations by dynamic light scattering (DLS), along with a characteristic decrease in

Table 1 Preparation of functional water-soluble polymers to be used for single-chain polymer nanoparticle formation

Polymer ^a	MV _{th} (%)	MV _{ex} (%) ^b	M_n (kDa) ^c	PDI ^c
P1	10	9.8	168	1.15
P2	10	10.1	269	1.15
P3	30	29.5	291	1.19
P4	10	9.9	492	1.38

^a Nanoparticles corresponding to a numbered polymer are denoted **NP1**, *etc.* ^b Determined by $^1\text{H-NMR}$ by comparing integration of aromatic MV protons with the polymer backbone. ^c Determined by H_2O GPC.

particle size upon collapse of the polymer chains (*vide infra*). In contrast, higher polymer concentrations yielded multimodal particle distributions, which are attributed to the formation of multi-chain aggregates. The size of both the polymers and the NPs are dependent on polymer molecular weight (Fig. 3a). Moreover, the polydispersities observed for both the polymers and the NPs decrease upon collapse from the globular and disperse polymeric state to the tightly collapsed nanoparticulate state. These observations provide evidence to suggest that an intramolecular, single-chain folding process is occurring, as aggregation of more than one chain would intuitively yield an increase in size over a single, solvated polymer chain. Furthermore, Fig. 3b clearly demonstrates an inverse linear dependence of the observed hydrodynamic size (R_h) on the cross-link density for **P3**, containing a higher MV content. Higher cross-linking leads to a more highly collapsed state and thus smaller hydrodynamic size, similar to previous observations for other systems.^{9,10}

Control experiments were performed whereby CB[7] was added instead of *ns*-CB[10]. The cavity of CB[7] is only large enough to encapsulate one MV guest, therefore cross-linking and chain collapse cannot occur. In contrast to the addition of *ns*-CB[10], addition of CB[7] led to an increase in the observed particle size, which is consistent with previously reported observations.²⁶ This is likely on account of an increased bulk of the solvated chains with CB[7] complexing with the pendant MV guests.

Atomic force microscopy (AFM) was then utilised to visualise the morphological differences between the polymers and NPs on mica substrates. When highly dilute solutions (100 nM) are drop-cast onto the substrates and dried under vacuum, distinct NPs are observed (Fig. 4). The three-dimensional depiction in Fig. 4c clearly shows the globular NPs on the flat mica substrates. NP size increases as the polymer molecular weight increases, and decreases as the cross-link density increases, corroborating observations from the DLS studies mentioned above. A direct comparison between the NP sizes observed with AFM and DLS is made in Table 2, identifying that the same trends in NP size with polymer precursor molecular weight were observed with both measurement techniques. Furthermore, control experiments demonstrate that no particle formation was detected in the absence of *ns*-CB[10] or in the presence of CB[7] (Fig. S3†).

^1H NMR spectroscopy was also used to confirm that the formation of the NPs was indeed exclusively through the

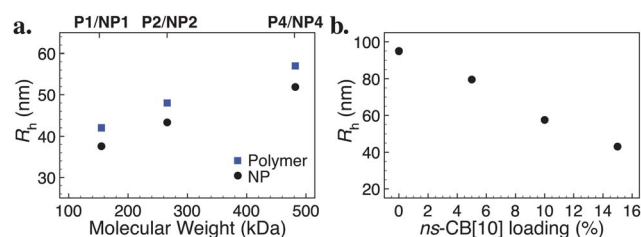


Fig. 3 a) Dynamic light scattering measurements of the functional polymers **P1**, **P2** and **P4** before and after addition of *ns*-CB[10] demonstrate the collapse of a single polymer chain from a well-solvated, globular conformation to a nanoparticle. b) Titration of *ns*-CB[10] to functional polymer **P3** yields a linear decrease in the hydrodynamic radius (R_h) as the nanoparticle becomes more tightly collapsed.

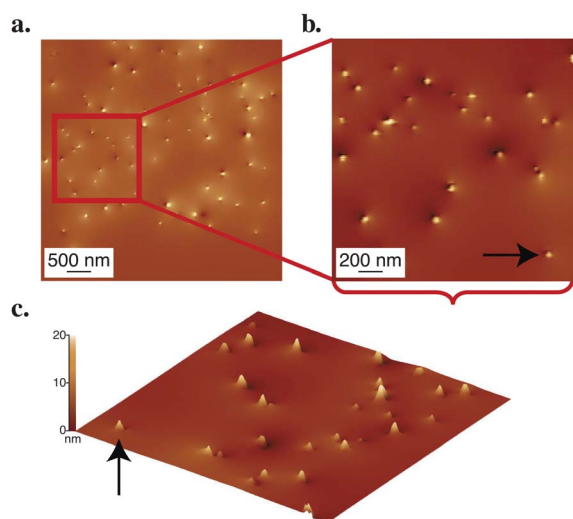


Fig. 4 AFM imaging of single-chain polymeric nanoparticles drop-cast from dilute solutions onto mica substrates: a) large scale image of NP1, b) zoom image of NP1 and c) three-dimensional image showing the spherical nature of the nanoparticles.

Table 2 Characterisation of NP sizes

NP	DLS R_h (nm)	AFM R (nm)
1	37.6 ± 0.8	45 ± 6
2	43.3 ± 0.9	55 ± 5
4	51.9 ± 0.9	64 ± 3

complexation of MV moieties with *ns*-CB[10], as well as controlled reversibility in the presence of competing guests. Signals for the aromatic MV protons on the functional polymer precursor shift upfield and broaden upon addition of *ns*-CB[10] and formation of the NPs (Fig. 5). This trend was expected and consistent with previous literature.²¹ Controlled unfolding of the NPs could be induced through addition of a strongly competing guest, *p*-xylylenediamine, which displaces the MV moieties from the *ns*-CB[10] host-guest complex crosslinks. The sharp and well-resolved signals for the MV moieties are clearly recovered upon

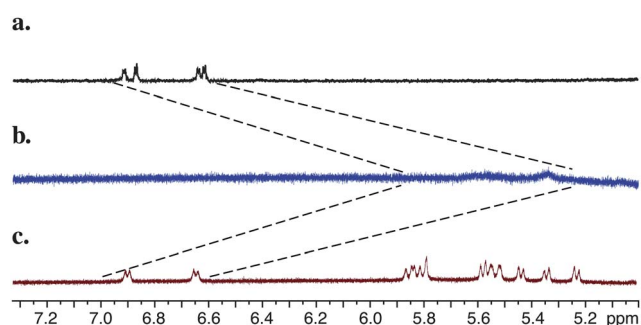


Fig. 5 Proton NMR spectra of the formation of single-chain polymeric nanoparticles and subsequent reversibility upon addition of a competing guest: a) ^1H NMR spectrum of P1, b) ^1H NMR spectrum of NP1 and c) ^1H NMR spectrum after addition of a strongly competing guest, *p*-xylylenediamine, to NP1.

addition of the *p*-xylylenediamine competing guest (Fig. 5c), indicating that the MV moieties are indeed 'liberated' from the host cavities, returning the polymer to the 'native' random-coil state.

In summary, a range of PHEAm polymers were prepared by ATRP and were functionalised using an isocyanate conjugation method to incorporate guest moieties for 2 : 1 ternary complexation with *ns*-CB[10]. Single polymer chains were subsequently 'collapsed' in a reversible, supramolecular fashion utilising an intramolecular *ns*-CB[10]-mediated cross-linking strategy in water. Well controlled and defined structures were obtained, as shown by DLS and AFM measurements. Particle sizes were shown to be easily tailored through the molecular weight of the initial polymers, loading of the guest moieties along the polymer chains, or simply through addition of the cross-linking unit, *ns*-CB[10]. By virtue of the supramolecular nature of the intramolecular cross-linking within a single polymer chain in water, this work represents a step towards the development of synthetic materials with aqueous phase behavior akin to self-folding of peptidic macromolecules.

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