Development of functional amino acid-based star polymers

ARTICLE · NOVEMBER 2011

DOI: 10.1039/C1PY00436K

5 AUTHORS, INCLUDING:

Adrian Sulistio
Monash University (Australia)
20 PUBLICATIONS **215** CITATIONS
SEE PROFILE

Anton Blencowe
University of South Australia
101 PUBLICATIONS **1,030** CITATIONS
SEE PROFILE

Xiaoqing Zhang
Northwest A & F University
26 PUBLICATIONS **634** CITATIONS
SEE PROFILE

Polymer Chemistry

Cite this: DOI: 10.1039/c1py00436k

www.rsc.org/polymers

PAPER

Development of functional amino acid-based star polymers

Adrian Sulistio,[a] Anton Blencowe,[a] Adrian Widjaya,[a] Xiaoqing Zhang[b] and Greg Qiao*[a]

Received 25th September 2011, Accepted 29th October 2011
DOI: 10.1039/c1py00436k

Highly functionalized core cross-linked star (CCS) polymers composed entirely of naturally-occurring amino acids were prepared *via* the sequential ring-opening polymerisation (ROP) of amino acid *N*-carboxyanhydride (NCA) derivatives in a facile one-pot, arm-first strategy. The formation of the star polymers was investigated using side-chain protected poly(ε-Z-L-lysine) (PZLL) and poly(γ-benzyl-L-glutamic acid) (PBGA) macroinitiators with various molecular weights in combination with a cystine NCA cross-linker to afford poly(ε-Z-L-lysine)$_{arms}$poly(L-cystine)$_{core}$ (PZLL$_{arms}$PLC$_{core}$) and poly(γ-benzyl-L-glutamic acid)$_{arms}$poly(L-cystine)$_{core}$ (PBLG$_{arms}$PLC$_{core}$) stars, respectively. As the cross-linker to macroinitiator ratio or macroinitiator molecular weights were increased the molecular weights, average number of arms and core size of the resulting stars also increased. Core-isolated NCA moieties remaining after star formation provided a facile approach to core-functionalization with primary amines bearing different functionalities, including aminomethyl pyrene, propargylamine and hexylamine. UV-vis spectroscopic analysis of PZLL$_{arms}$PLC$_{core}$ and PBLG$_{arms}$PLC$_{core}$ stars core-functionalised with aminomethyl pyrene provided high loadings of 240 and 128 mol/mol stars, respectively. Furthermore, stars with alkyne functionalized cores were capable of undergoing further click reactions with azido derivatives, demonstrating the accessibility of the core-isolated moieties. Deprotection of the PZLL$_{arms}$PLC$_{core}$ and PBLG$_{arms}$PLC$_{core}$ stars yielded water soluble CCS polymers with poly(L-lysine) and poly(L-glutamic acid) arms, respectively, and functionalised cores. In addition, direct hydrazinolysis of the PBLG$_{arms}$PLC$_{core}$ star provided hydrazide functionalities along the arms, which allow for conjugation of drug molecules *via* pH sensitive hydrazone linkers. These results open up exciting opportunities for the development of star polymer drug delivery systems, whereby a lack of functionality, biocompatibility and biodegradability are often limiting factors.

1. Introduction

Since the discovery of amino acid *N*-carboxyanhydrides (NCAs) by Leuchs in 1906[1] and the first account of well-controlled metal-catalysed ring-opening polymerization (ROP) of NCAs by Deming,[2] several controlled ROP systems of NCAs have been developed.[3–7] Consequently, these have provided synthetic pathways to produce well-defined homo/block polypeptides,[4,8] hybrids,[3,9] star-shaped polymers[10,11] and polypeptides with complex architectures.[12] Two excellent reviews by Kricheldorf[13] and Hadjichristidis *et al.*[14] provide comprehensive summaries of the different polymeric architectures that have been synthesized from amino acid NCAs.

Polymers with star-shaped architectures having several linear arms connected to a central core are of particular interest as a result of their structure related properties, including low solution viscosities,[15] encapsulation capabilities[16] as well as enhanced and compartmentalized functionalities.[17–19] The two most commonly employed approaches to synthesize star-shaped polymers involve the use of multifunctional-initiators (core-first approach) or the linking of preformed linear polymers using cross-linkers or multifunctional-linking agents with complementary functionalities (arm-first approach). In the core-first approach, multifunctional compounds which can concurrently initiate the polymerization of monomers to form several arms are used. However, the resulting star polymers are difficult to characterize since the molecular weight characteristics of the arms cannot be measured directly.[14,19] In contrast, the arm-first approach is viewed to be more efficient because it allows for a high degree of control at every stage of the synthetic process and the arms can be characterized easily prior to star formation.[14,19] The preparation of so-called core cross-linked star (CCS) polymers *via* the arm-first approach, involving the reaction of macroinitiators with suitable cross-linkers, has grown in popularity as it is applicable to a wide range of controlled polymerisation techniques and compositionally diverse precursors. Furthermore, it provides access to star polymers with relatively large cores suitable for site-specific isolation of functionalities and large capacity encapsulation capabilities.[19]

[a]*Department of Chemical and Biomolecular Engineering, University of Melbourne, Parkville, Melbourne, VIC, 3010, Australia. E-mail: gregghq@unimelb.edu.au; Fax: +61 3 8344 4153; Tel: +61 3 8344 8665*
[b]*CSIRO Material Science and Engineering, Private Bag 33, Clayton South, MDC 3169, VIC, Australia*

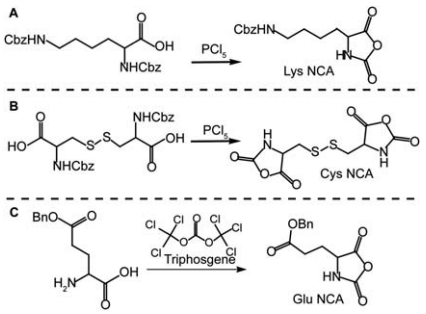
To date, a limited number of star polymers based on amino acid derivatives have been prepared[11,13,14] and until recently no examples were known of star polymers comprised entirely (core and arms) from amino acids.[20,21] Several amino acid-based stars have been prepared *via* the core-first approach. For example, Inoue *et al.* employed the core-first approach to synthesize star polymers containing six PBLG arms attached to a hexakis(4-aminophenoxy) cyclotriphosphazene core. After deprotection of the benzyl groups and functionalisation with a triethylene glycol derivative the stars were used to prepare enantioselective membranes for separation of tryptophan, tyrosine and phenylalanine.[11] Similarly, poly(amido amide) and poly(propylene imine) dendrimers have also been used to prepare star-shaped polymers *via* the core-first approach.[13,14] These amphiphilic stars have shown great potential as drug delivery vehicles as they have the capacity to encapsulate hydrophobic drug molecules within the core and stabilize them in aqueous media.[16]

Recently, we introduced the synthesis of CCS polymers prepared entirely from amino acid building blocks in a one-pot, arm-first approach.[20] These CCS polymers have selectively degradable cores and possess hierarchical functionalities spanning from the peripheral groups, along the arms, as well as on the core itself and have been shown to encapsulate hydrophobic drugs, such as pirarubicin in the core. These star were prepared *via* ROP of protected lysine NCA using hexamethyldisilazane (HMDS) as the initiator to yield poly(ε-Z-L-lysine) (PZLL), which served as the macroinitiator for star formation.[6] The subsequent addition of cystine NCA cross-linker afforded the poly(ε-Z-L-lysine)$_{arms}$poly(L-cystine)$_{core}$ (PZLL$_{arms}$PLC$_{core}$) CCS polymer. Herein we demonstrate the versatility of this approach by preparing amino acid-based CCS polymers composed of different amino acids and possessing various arm and core functionalities. Furthermore, a detailed study investigating the effect of reaction parameters, such as the cross-linker to macroinitiator ratio and macroinitiator molecular weight, on the properties of the resulting star polymers is presented.

2. Results and discussion

2.1 Synthesis of amino acid *N*-carboxyanhydrides (NCAs)

In the current study glutamic acid and lysine were chosen as precursors for star synthesis since their side group functionality would be translated to the arms of the resulting stars, although in theory any amino acid that can be converted to a NCA derivative could be employed. Functional groups such as amines or carboxylic acids along the stars' arms allows for post-polymerization functionalisation with different moieties suited to various applications. In comparison, the choice of cross-linker is limited to amino acids that can be converted to di-NCA monomers, thus the only naturally occurring amino acid which is suitable is L-cystine; a dimer of L-cysteine covalently linked *via* a disulfide bond. The amino acids were converted to their NCA derivatives using either phosphorus pentachloride or triphosgene. For carboxybenzyloxy (Cbz or Z) protected amino acids, di-Z-L-lysine and di-Z-L-cystine, phosphorus pentachloride (PCl_5) was used (Scheme 1A and B, respectively), whereas triphosgene was used for γ-benzyl L-glutamic acid (Scheme 1C).



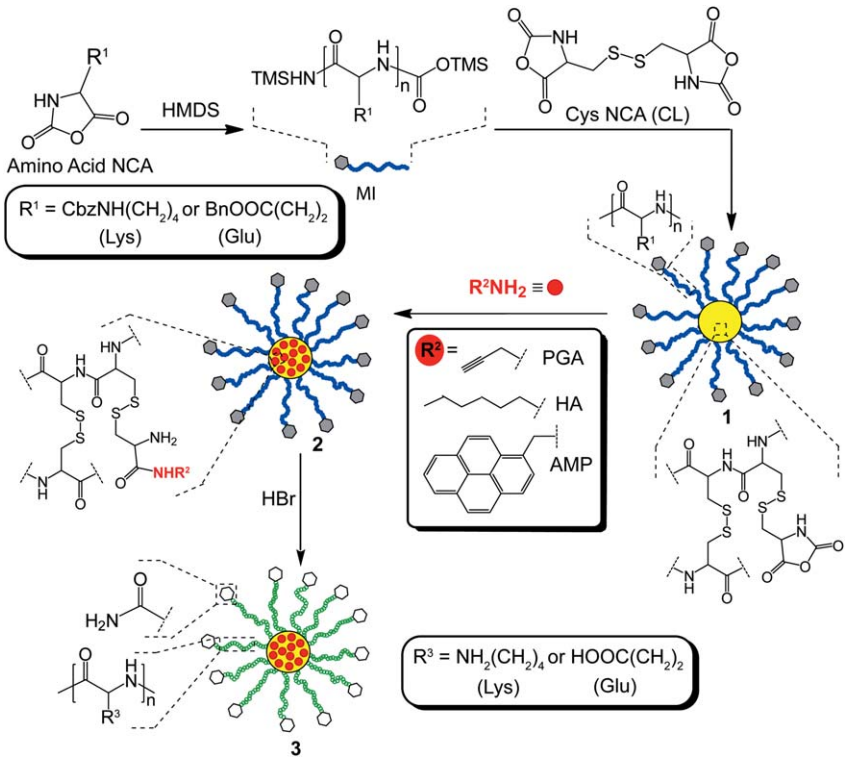
Scheme 1 Synthesis of amino acid *N*-carboxyanhydrides (NCAs): (A) N_{ε}-Z-lysine NCA (Lys NCA) and (B) cystine NCA (Cys NCA) using phosphorus pentachloride and (C) γ-benzyl glutamic acid NCA (Glu NCA) using triphosgene.

2.2 Synthesis of CCS polymers

The formation of the CCS polymers was achieved *via* a one-pot, arm-first strategy using sequential ROP of NCA derivatives. Initially, Lys NCA or Glu NCA were polymerized using the secondary amine initiator, hexamethyldisilazane (HMDS), to afford linear polymer, which served as the macroinitiator (MI) for star formation.[6] Unlike conventional amine initiators, HMDS provides excellent control over the ROP process, yielding polypeptides with expected molecular weights and low polydispersities (PDI).[6] The subsequent addition of the cross-linker (CL), Cys NCA, resulted in the formation of CCS polymers **1**, comprised entirely of amino acid building blocks (Scheme 2).

For synthesis of poly(ε-Z-L-lysine)$_{arms}$poly(L-cystine)$_{core}$ (PZLL$_{arms}$PLC$_{core}$) CCS polymer **1a**, having PZLL arms, Lys NCA was used to prepare the MI. The formation of linear PZLL using HMDS was followed *via* ^{1}H NMR spectroscopy and gel permeation chromatography (GPC) (Fig. 1). As the methine proton (δ_H 4.8 ppm) of the strained Lys NCA (circled in Fig. 1) has a distinctly different chemical shift to its open polymeric form (δ_H 4.2 ppm) it was possible to follow its consumption by monitoring the decrease in the resonance intensity. Thus, the resonance at δ_H 4.8 ppm gradually decreases over time as the Lys NCA is converted to its polymeric form (Fig. 1A), which results in the appearance of a new resonance at 4.2 ppm corresponding to the methine proton on the peptide backbone.

To ensure accurate integration of the resonances the methylene protons of the Cbz protecting group (δ_H 5.3 ppm) were used as a reference for calculation of the monomer (M) conversion with time (Fig. 1B), which reached 83% after 7 h. A plot of ln ($[M_o]/[M]$) against time gave initially a linear relationship, which indicated that the polymerization was well controlled, although at higher conversions ($t > 200$ min) deviation was observed indicating slight loss of control (Fig. 1C). This deviation may result from trace impurities present in the Lys NCA.[22] The conversion of Lys NCA was also confirmed by GPC UV-vis chromatograms (Fig. 1D) recorded at $\lambda = 260$ nm, which revealed a decrease in the peak at high retention time (27 min) and the appearance of a peak at low retention time, corresponding to the Lys NCA and PZLL, respectively. GPC RI chromatograms (Fig. 1E) recorded over the course of reaction revealed a continuous increase in the molecular weight (MW) to afford, after 7.5 h, PZLL with a weight average molecular weight



Scheme 2 Synthesis of amino acid-based CCS polymers having hierarchical functionalities *via* a one-pot, arm-first strategy.

(M_w) of 15.0 kDa (PDI = 1.09). Lower MW PZLL (M_w = 6.6 kDa, PDI = 1.03) was also prepared by variation of the monomer to initiator (M/I) ratio and displayed similar kinetic profiles.

The formation of well-defined PZLL was also indicated by MALDI ToF MS (Fig. 2), which confirmed negligible side reactions. The major series correlates to the expected PZLL product after hydrolysis of the α-trimethylsilyl and ω-trimethylsilyl carbamate end groups resulting from exposure to atmospheric conditions.[6] Interestingly, a minor series was also observed that is tentatively assigned to PZLL after fragmentation, for it has been demonstrated that side chain functionalized lysine residues are susceptible to free-radical induced fragmentation during ionization processes.[23] Most importantly, no series were observed for side products, such as those encountered for the synthesis of polypeptide homopolymers using primary[24] and secondary alkyl amine initiators.[25]

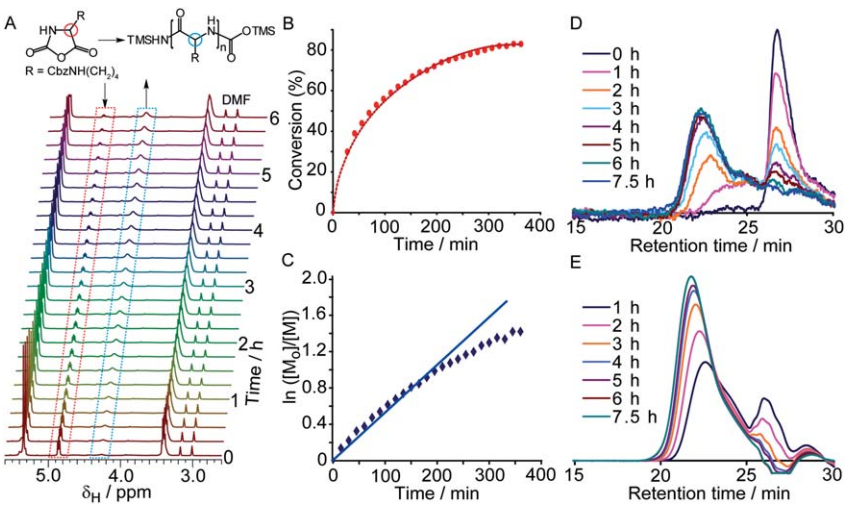


Fig. 1 (A) ^{1}H NMR spectra (d_7-DMF) taken every 15 min over a 6 h period showing decrease in the intensity of the Lys NCA methine proton resonance (δ_H 4.8 ppm) over the course of the polymerization. (B) Monomer conversion *versus* time and (C) plot of ln([M_o]/[M]) *versus* time calculated from NMR signal integration. (D) GPC UV (λ = 260 nm) chromatograms showing the reduction of the Lys NCA absorbance at 27 min and the formation of PZLL at *ca.* 22 min over time. (E) GPC RI chromatograms showing the formation of PZLL over time.

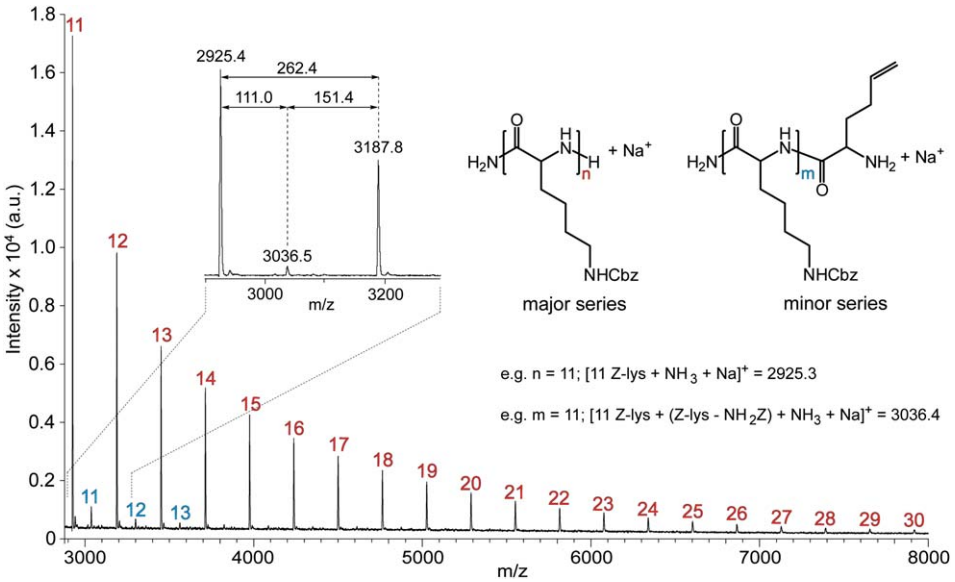


Fig. 2 MALDI ToF MS spectrum of PZLL prepared *via* HMDS-mediated polymerisation recorded in linear/positive mode using α-CHCA/NaI. The numbers on the MS spectrum denote the number of repeating units of PZLL (repeat unit = 262.3 *m*/*z*); *Inset* shows expanded section of MS spectrum complete with peak *m*/*z* values and *m*/*z* differences.

Subsequently, the $PZLL_{arms}PLC_{core}$ CCS polymer **1a** was synthesized by the addition of the Cys NCA CL to the PZLL MI. The optimum CL/MI ratio for star formation was found to be highly dependent on the MI MW, as previously reported for the formation of CCS polymers *via* other controlled polymerisation techniques.[19] For PZLL with M_ws of 6.6 and 15.0 kDa the optimum CL/MI ratios were 15 and 35, and yielded star polymers $\mathbf{1a_L}$ (M_w = 182 kDa; PDI = 1.77; average number of arms (*f*) ≈ 17) and $\mathbf{1a_H}$ (M_w = 523 kDa; PDI = 1.63; *f* ≈ 21), respectively (Table 1). Whereas below the optimum CL/MI ratio lower molecular weight stars were formed, higher ratios led to star-star coupling and gelation. The core size of the stars was also found to increase as the CL/MI ratio increased. This demonstrates that the overall size, *f* and core size of the CCS polymers could be tailored by changing the degree of polymerisation of the arms or the CL/MI ratio (Table 1).

In all cases ^{1}H NMR spectroscopic analysis of the star formation revealed that the Cys NCA CL was completely consumed after 3 h.[20] The disappearance of the CL resonances are a good indication of star formation since the reduced

Table 1 Characterization of CCS polymers **1a** and **1b**

	MI			Star			
Polymer	M_w (kDa)[a]	PDI[a]	CL/MI	M_w (kDa)[a]	PDI[a]	*f*[b]	Core Size (%)[c]
$\mathbf{1a_L}$	6.56	1.03	5	29	1.28	3	24
			15	182	1.77	17	40
			25	3820	1.20	349	59
			35	*Gel*	*Gel*	*Gel*	*Gel*
$\mathbf{1a_H}$	15	1.09	17.5	81	1.43	3	25
			20	89	1.46	4	32
			22.5	114	1.56	5	34
			25	129	1.62	5	32
			30	183	1.83	7	35
			35	523	1.63	21	40
			40	*Gel*	*Gel*	*Gel*	*Gel*
1b	8.7	1.03	20	217	1.36	17	32
			22.5	245	1.40	18	35
			25	297	1.49	22	38
			27.5	515	1.62	36	40
			32.5	2330	1.40	152	44
			50	*Gel*	*Gel*	*Gel*	*Gel*

[a] M_w and PDI determined *via* GPC multiangle laser light scattering (MALLS); dn/dc_{PZLL} = 0.101 mL g^{-1} (25 °C), dn/dc_{PBLG} = 0.085 mL g^{-1} (25 °C). [b] Average number of arms. [c] Percentage core size calculated from the CL/MI with respect to the overall molecular weight of the star polymer. All reactions were conducted at room temperature for 7 h.

segmental mobility of the resulting cross-linked core leads to a reduction and broadening of the cystine signals making them indistinguishable from the baseline;[26] however, it is not possible to determine if complete star formation and growth coincides with CL consumption. Therefore, the reaction was monitored over time *via* GPC (Fig. 3) using a PZLL MI with a M_w of 12.4 kDa (PDI = 1.06) and a CL/MI ratio of 32.5, which revealed that initially the MW increased rapidly up to 6.5 h and then more gradually up to 23 h, after which no further increase was observed. Thus, even after complete CL consumption the star continues to grow, implying that after 3 h the coupling together of preformed low MW stars becomes the dominant process, although the slight decrease in the peak at a retention time of 21.5 min (Fig. 3), which is attributed to MI chain extended with CL, suggests that these prepolymers are also slowly incorporated into the preformed stars.

For synthesis of poly(γ-benzyl-L-glutamic acid)$_{arms}$poly(L-cystine)$_{core}$ (PBLG$_{arms}$PLC$_{core}$) CCS polymer **1b** having PBLG arms, Glu NCA was used to prepare the MI followed by the addition of Cys NCA CL. Optimization of the star **1b** formation was conducted similarly to that described for **1a**, by variation of the CL/MI ratio (Table 1). For a PBLG MI with a M_w of 8.7 kDa (PDI = 1.03) the M_w of the resulting stars was found to increase gradually from 217 to 297 kDa as the CL/MI ratio was increased from 20 to 25. At higher ratios the M_w increased more sharply, with star-star coupling becoming prominent at a ratio of 32.5 and gelation occurring at a ratio of 50. The optimum CL/MI ratio to obtain stars with a large number of arms before significant star-star coupling occurred was found to be 27.5, which resulted in a star with a M_w of 515 kDa (PDI = 1.62) and f of 36.

The GPC RI chromatograms for stars **1a$_L$**, **1a$_H$** and **1b** (Fig. 4A–C, respectively) revealed the presence of unincorporated MI/prepolymers regardless of the CL/MI ratio employed, which can be seen as slight shoulders or small peaks towards higher retention times, similar to those for the MI. This observation is common for CCS polymer preparations, which generally result in incomplete conversion of the MI to star product, regardless of the controlled polymerisation technique employed.[18,19,27] For star **1b** the improved resolution between the star and prepolymer peaks allowed the MI to star conversion to be determined by deconvolution of the RI chromatograms using

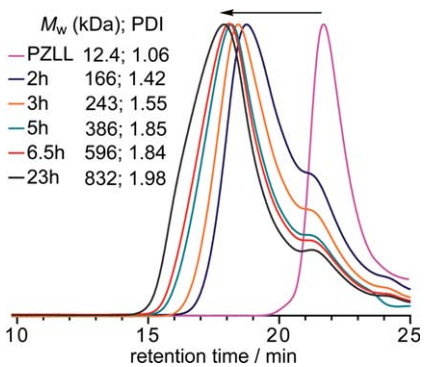


Fig. 3 GPC RI chromatograms following star formation over time, using a PZLL MI with a M_w of 12.4 kDa (PDI = 1.06) and a CL/MI ratio of 32.5.

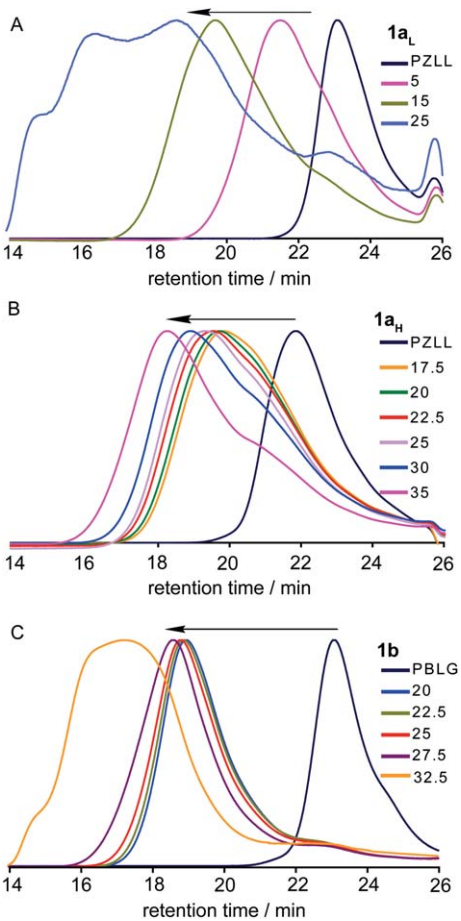


Fig. 4 GPC RI traces for the optimization of CCS polymer (A) **1a$_L$**, (B) **1a$_H$** and (C) **1b** prepared at different CL/MI ratios.

Gaussian functions, which revealed that regardless of the CL/MI ratio employed the conversion was *ca.* 93%.

At this stage, both CCS polymers **1a** and **1b** could be isolated *via* precipitation or core-functionalized through reaction with primary amines. Isolation of star **1a** by precipitation resulted in the formation of a small amount of an insoluble component and led to significant increases in the MW of the soluble component as a result of star-star coupling as described previously.[20] Attempts to directly isolate stars **1a** and **1b** *via* removal of the polymerisation solvent resulted in the formation of insoluble materials.

2.3 Core functionalized CCS polymers

Facile core-functionalization of stars **1a** and **1b** was achieved by addition of primary amines directly to the polymerisation after star formation, which ring-openings the unreacted pendent NCAs in the core, resulting in the release of CO_2 and the formation of cystine-based amide and amine groups (Scheme 1). Although an excess of primary amine is used it is conceivable that the cystine-based amines generated in the early stage of this reaction reinitiate the ROP of the pendent NCAs leading to further intramolecular cross-linking of the core or even intermolecular cross-linking between the cores of adjacent stars. Core-functionalization was conducted by reacting star **1a** (with

either a M_w of 596 kDa (PDI = 1.65) or 832 kDa (PDI = 1.98)) and **1b** (M_w = 503 kDa; PDI = 1.55) with primary amines bearing different functional groups, including propargylamine (PGA), hexylamine (HA) and aminomethyl pyrene (AMP) to yield core-functionalized stars **2a** and **2b**, respectively (Scheme 2). In all cases the star MW and PDI increased after core-functionalization (Fig. 5), although the observed increases were greater than could be accounted for solely through functionalisation of the core. Therefore, it appears likely that the increase in MW probably results from a combination of core-functionalization and star-star coupling caused by intermolecular cross-linking.

Attachment of pyrene to the cores of stars **1a** and **1b** was achieved through reaction of AMP with the unreacted pendent

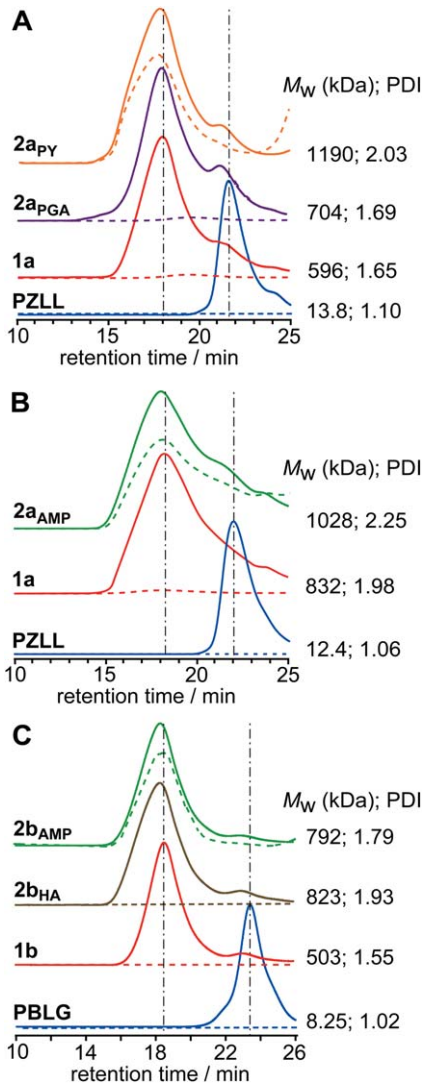


Fig. 5 GPC RI (solid) and UV (dashed) chromatograms of (A) PGA and PY core-functionalized CCS polymers $\mathbf{2a_{PGA}}$ and $\mathbf{2a_{PY}}$, and their precursor PZLL MI and star **1a**, (B) AMP core-functionalized CCS polymer $\mathbf{2a_{AMP}}$, and its precursor PZLL MI and star **1a**, and (C) AMP and HA core-functionalized CCS polymers $\mathbf{2b_{AMP}}$ and $\mathbf{2b_{HA}}$, and their precursor PBLG MI and star **1b**. All polymer concentrations were 15 mg mL^{-1} and UV-Vis spectra were recorded at λ = 340 nm.

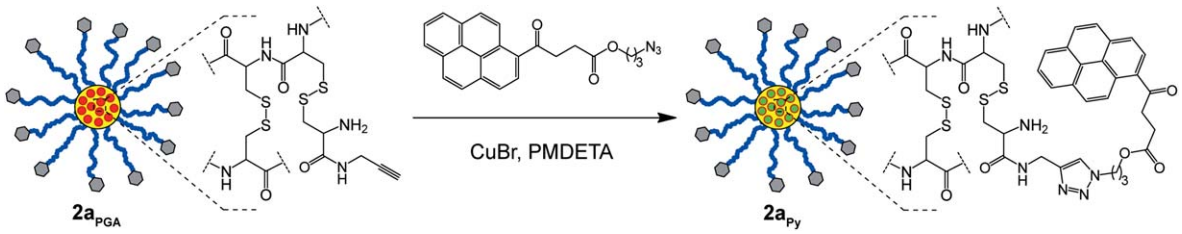
NCAs to afford stars $\mathbf{2a_{AMP}}$ and $\mathbf{2b_{AMP}}$, respectively. GPC UV analysis of $\mathbf{2a_{AMP}}$ and $\mathbf{2b_{AMP}}$ revealed an enhanced absorbance at λ = 340 nm relative to the unfunctionalized precursor stars and provided evidence for the successful incorporation of AMP (Fig. 5B and C, respectively). The extent of this functionalization was quantified using UV-Vis spectroscopic analysis and provided AMP loadings of 240 and 128 mol/mol star for $\mathbf{2a_{AMP}}$ and $\mathbf{2b_{AMP}}$, respectively, which theoretically equates to increases in MW of *ca.* 45 and 23 kDa; however, the increases in MW observed from GPC analysis implies that star-star coupling occurs to a minor extent. Fluorescently labeled amino acid star polymers such as $\mathbf{2a_{AMP}}$ and $\mathbf{2b_{AMP}}$ have huge potential in imaging and sensor applications, as well as for the study of polymer molecular dynamics; for example, the study of polymer-tissue/cell interactions[28] or the detection of metal ions.[29]

Unfortunately, UV-Vis spectroscopy could not be employed to determine the extent of core-functionalization of stars $\mathbf{2a_{PGA}}$ and $\mathbf{2b_{HA}}$ with PGA and HA, respectively (Fig. 5A and C), as these amines do not possess distinctive UV-Vis absorbances. However, the core isolated alkyne moieties of star $\mathbf{2a_{PGA}}$ provided the opportunity to further functionalize the core *via* click chemistry. Therefore, to determine the number of accessible alkyne groups within the core of star $\mathbf{2a_{PGA}}$ it was reacted with an azido pyrene derivative (3-azidopropyl 4-oxo-4-(pyren-4-yl) butanoate) under standard copper catalyzed click conditions to yield star $\mathbf{2a_{PY}}$ (Scheme 3).

GPC UV analysis confirmed the successful incorporation of pyrene into the star (Fig. 5A) and UV-Vis spectroscopic analysis provided a pyrene loading of 117 mol/mol star, implying that the core of star $\mathbf{2a_{PGA}}$ contains at least 117 propargyl groups. Surprisingly, GPC analysis revealed a further increase in M_w from 704 (PDI = 1.69) for $\mathbf{2a_{PGA}}$ to 1190 kDa (PDI = 2.05) for $\mathbf{2a_{PY}}$ after the click reaction (Fig. 5A), suggesting that star-star coupling continues to occurs to a minor extent during reaction or upon isolation. Nevertheless, this experiment demonstrates that the core was successfully functionalized with PGA and that the resulting core-isolated alkynes are accessible for further reactions. These core-isolated alkyne functionalities provide the ability to selectively functionalize the star with azido derivatives and could potentially be employed to attach drug molecules or ligands to the star for biomedical or catalysis applications.

2.4 Arm-functionalized CCS polymers

Arm-functionalized CCS polymers could be prepared by acid-mediated hydrolysis of the protecting groups along the arms, or in the case of $PBLG_{arms}PLC_{core}$ star **2b**, *via* direct conversion of the benzyl esters to their hydrazide derivatives. Thus, hydrazinolysis was performed by addition of hydrazine (2 molar equiv. relative to glutamic acid repeat units) to star $\mathbf{2b_{AMP}}$, which resulted in quantitative replacement of the benzyl protecting groups to afford the hydrazide functionalized star $\mathbf{2b_{AMP\text{-}HYD}}$, as confirmed by ^{1}H NMR spectroscopic analysis (Fig. 6). The ^{1}H NMR spectrum of $\mathbf{2b_{AMP\text{-}HYD}}$ revealed the disappearance of resonances corresponding to the benzyl protecting group protons of $\mathbf{2b_{AMP}}$ at δ_H 5.2 and 7.2 ppm (Fig. 6A) and the formation of new resonances at δ_H 3.6 and 9.0 ppm corresponding to the hydrazide protons (Fig. 6B). The resulting hydrazide-functionalised star $\mathbf{2b_{AMP\text{-}HYD}}$ was no longer soluble in DMF, but



Scheme 3 Copper catalyzed click reaction between PGA functionalized CCS polymer $\mathbf{2a_{PGA}}$ and 3-azidopropyl 4-oxo-4-(pyren-4-yl) butanoate to yield star $\mathbf{2a_{PY}}$.

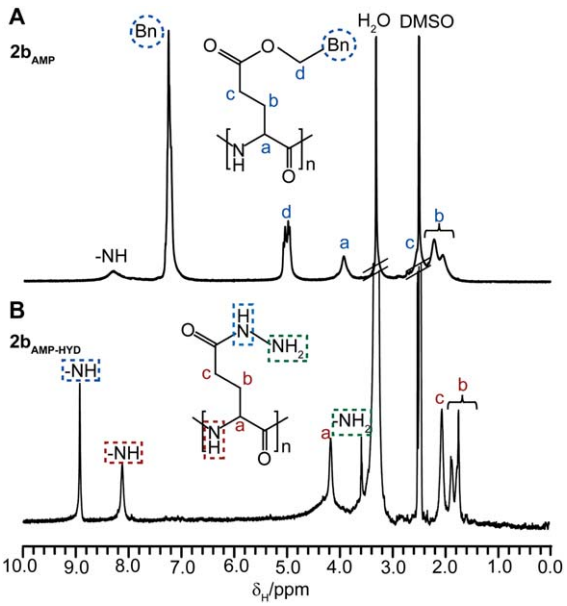


Fig. 6 ^{1}H NMR spectra (d_6-DMSO) and the assigned structures of (A) benzyl protected CCS polymer $\mathbf{2b_{AMP}}$ and (B) hydrazide functionalized CCS polymer $\mathbf{2b_{AMP\text{-}HYD}}$.

became soluble in mildly acidic or basic aqueous solutions. Hydrazinolysis of benzyl esters provides a facile route towards hydrazide functionalized polymers, which can be used to conjugate drugs (*e.g.*, doxorubicin, which is commonly used for the treatment of bladder,[33] breast,[34] and liver[35] cancers) *via* acid-labile hydrazone linkages. These linkages are readily cleaved at acidic pH values, similar to the endosomal/lysosomal environment of cancer cells, which allows for site-specific release of conjugated drugs.[36]

Water soluble amino acid-based stars were prepared from stars **2a** and **2b** *via* deprotection of the side chain groups of the arms (Cbz for **2a** and Bn for **2b**) with 33 wt% HBr in acetic acid.[37] The resulting degradable CCS polymers have amide peripheral groups, poly(L-lysine) (PLL) arms (**3a**) or poly(L-glutamic acid) (PLGA) (**3b**) arms, and functionalized PLC cores. Analysis of the stars **3a** and **3b** *via* ^{1}H NMR spectroscopy confirmed the removal of the majority of the Cbz and Bn protecting groups (~88% removal for both) and displayed similar chemical shifts as their linear PLL and PLGA analogues (Fig. 7A and B, respectively). As before, the core-functionalities were also not visible due to the reduced segmental mobility of the core.[26] In addition, the water soluble star $\mathbf{3a_{AMP}}$ has been shown to encapsulate the water insoluble drug pirarubicin *via* physical interactions such as π–π stacking.[20]

2.5 CCS polymer degradation

Previously it was demonstrated[20] that the disulfide bonds located centrally within the cross-links responsible for forming the core could be successfully cleaved using excess DTT in DMF under argon to afford poly(Z-L-lysine-*b*-L-cysteine) (P(ZLL-*b*-LC) copolymers resulting from the cleavage of the star $\mathbf{1a_H}$ (M_w = 1140 kDa; PDI = 1.33; f = 52) into its constituent components.[30,31] When the star $\mathbf{1a_H}$ degradation solution was exposed to the atmosphere the resulting P(ZLL-*b*-LC) copolymer rapidly formed organogels. This behaviour is not observed for the PZLL MI, which is readily soluble in DMF, and therefore, is attributed to intermolecular thiol/disulfide exchange that occurs in the presence of oxygen between the polycysteine blocks to create a gel network (Fig. 8).[32] This not only demonstrates that the stars are degradable, but also possess the ability to undergo chemically triggered rearrangement to form insoluble macroscopic structures.

In comparison, cleavage of the AMP core-functionalized star $\mathbf{2a_{AMP}}$ did not result in gel formation upon exposure to the atmosphere, although the solution became very viscous after 24 h. GPC analysis of this degradation solution after 1 and 24 h exposure to the atmosphere revealed very high MW species (*ca.* 5 and 12 MDa, respectively) approaching the exclusion limits of the columns. It is proposed that formation of the gel network is disrupted by the AMP-cysteine conjugate products that were also cleaved off from the core when the disulfide bonds were reduced. Comparison of the ^{1}H NMR spectra of the star $\mathbf{2a_{AMP}}$ before and after cleavage (the cleaved products were separated from excess DTT *via* precipitation of the 1 h degradation solution into 2-propanol) confirmed that a mixture of block copolymers and the AMP-cysteine conjugate were formed after degradation of the core with DTT (Fig. 9A and B, respectively).

3. Experimental section

3.1 Materials

Z-L-Lys(Z)-OH (Bachem), H-L-Glu(OBn)-OH (Bachem), L-cystine (Aldrich), hexamethyldisilazane (HMDS) (99.9%, Aldrich), benzyl chloroformate (95%, Aldrich), sodium bicarbonate ($NaHCO_3$) (99%, Ajax Fine Chemical), magnesium sulfate ($MgSO_4$) (>98%, Scharlau)), bis(trichloromethyl) carbonate (triphosgene) (>99%, Aldrich), phosphorus pentachloride (PCl_5) (>99%, Merck), dithiothreitol (DTT) (>99%,

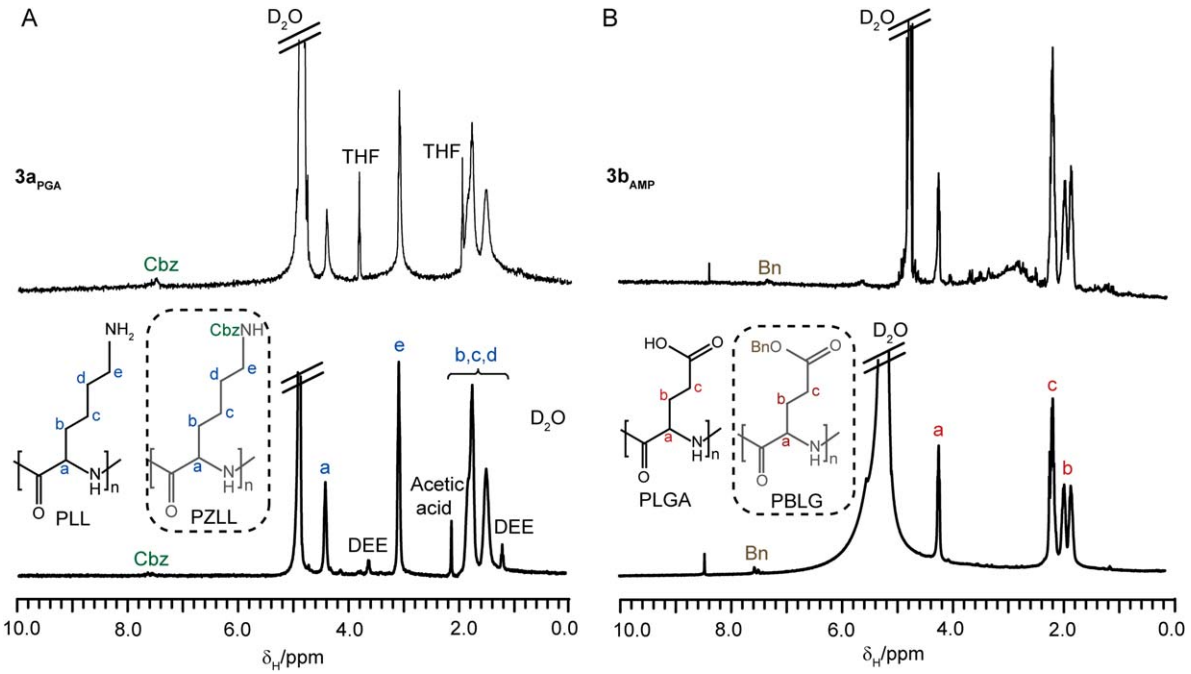


Fig. 7 ^{1}H NMR spectra (D_2O) of (A) water soluble CCS polymer $\mathbf{3a_{PGA}}$ and linear PLL and (B) water soluble CCS polymer $\mathbf{3b_{AMP}}$ and linear PLGA. The structures in the dotted boxes represent the unprotected PZLL (A) and PBLG (B) respectively.

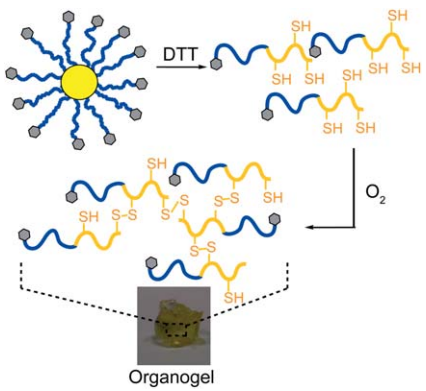


Fig. 8 Organogel formed after DTT cleavage of star $\mathbf{1a_H}$ to form P (ZLL-*b*-LC), followed by oxygen-induced gelation.

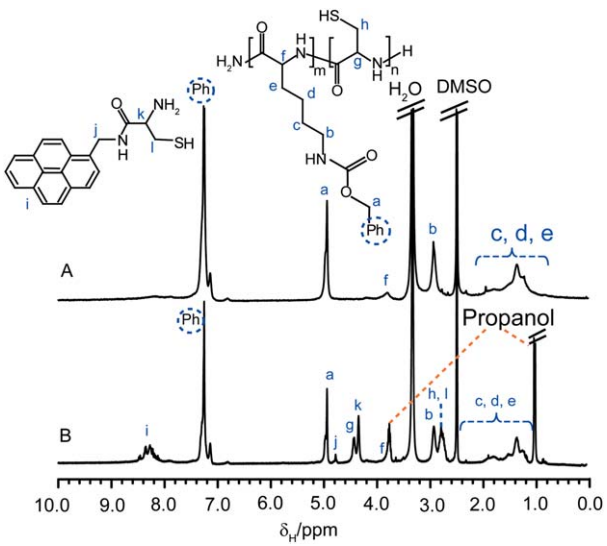


Fig. 9 ^{1}H NMR spectra (d_6-DMSO) of CCS polymer $\mathbf{2a_{AMP}}$ (A) before and (B) after cleavage with DTT and their assigned structures.

A.G. Scientific), 3-bromopropanol (97%, Aldirch), sodium azide (>99.5%, BioUltra), tetrabutylammonium hydrogen sulfate (TBAHS) (>99%, Aldrich), γ-Oxo-1-pyrenebutyric acid (Aldrich), potassium hydroxide (KOH) (> 85%, Biolab), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) (>98%, Fluka), 4-(dimethylamino)pyridine (DMAP) (99%, Aldrich), pirarubicin (>95%, Aldrich), propargylamine (98%, Aldrich), aminomethylpyrene hydrochloride (95%, Aldrich), propylamine (Merck), hexylamine (99%, Aldrich), hydrazine hydrate (99%, BDH), copper(I) bromide (CuBr) (98%, Aldrich), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) (98%, Fluka), hydrobromic acid (33% in acetic acid) (Aldrich), trifluoroacetic acid (TFA) (98%, Aldrich), hydrochloric acid (37%, Scharlau), lithium bromide (99%, Aldrich), tetrahydrofuran (THF) (99.9%, Scharlau) and *N*,*N*-dimethylformamide (DMF) (anhyd., Aldrich) were used as received. n-Hexane, ethyl acetate, dimethylsulfoxide (DMSO), chloroform and methanol were purchased from Chem-Supply and used as received. 1,4-Dioxane (Fluka) and diethyl ether (Chem-Supply) were used to make binary solvent (2 : 3 v/v) and dried for 48 h prior to use over 3 Å molecular sieves. Acetonitrile (HPLC grade, B&J) was stored over 3 Å molecular sieves. Dichloromethane (99.5%, Chem-Supply) was distilled from CaH_2. THF (99%, Lab Scan) was distilled from sodium benzophenone ketal. Milli Q water (18.2 MΩ.cm) was obtained from a Millipore Synergy Water System. Acetone-d_6 (99.9%), DMSO-d_6 (99.9%), DMF-d_7 (99.5%) and D_2O (99.9%) were purchased from Cambridge

Isotope and used as received. MALDI ToF MS matrix α-cyano-4-hydroxycinnamic acid (α-CHCA) (≥99.5%) and cationisation agent NaI (99.999%) were purchased from Fluka and Aldrich, respectively and were used as received. Z-L-Lysine-NCA (Lys NCA), di-Z-L-cystine ((Z-Cys-OH)$_2$), L-cystine NCA (Cys NCA), aminomethylpyrene (AMP), 3-azidopropanol, 3-azidopropyl 4-oxo-4-(pyren-4-yl) butanoate and click functionalized star $\mathbf{2a_{PY}}$ were prepared according to previously published procedures.[20]

3.2 Characterization

GPC analysis was performed on a Shimadzu size exclusion chromatogram fitted with Wyatt DAWN HELEOS LS detector (λ = 658 nm), Shimadzu RID-10 refractometer (λ = 633 nm), and Shimadzu SPD-20A UV-Vis detector using three identical PLgel columns (5 μm, MIXED-C) in series and HPLC grade DMF with 0.05 M LiBr (70 °C, 1 mL min^{-1}) as mobile phase. Astra software (Wyatt Technology Corp.) was used to determine the molecular weight characteristics using known d*n*/d*c* values for PZLL (d*n*/dc_{PZLL} = 0.101 mL g^{-1} (25 °C)) and PBLG (d*n*/dc_{PBLG} = 0.085 mL g^{-1} (25 °C)) in DMF. The d*n*/d*c* values of densely branched CCS polymers and linear polymers of the same monomeric constitution have been reported to be comparable;[31] thus, the d*n*/d*c* values of linear PZLL or PBLG were used to determine the molecular weights of the CCS polymers. ^{1}H NMR spectroscopy was performed using Varian Unity400 (400 MHz) spectrometer using the deuterated solvent as reference and a sample concentration of *ca.* 20 mg mL^{-1}. DLS measurements were performed on a Malvern high performance particle sizer (HPPS) with a He-Ne laser (633 nm) at an angle of 173° and a temperature of 25 ± 0.1 °C, initial sample concentrations of 10 mg mL^{-1} in DMF were used then serial dilutions were performed until stable spectra were obtained. All sample solutions were filtered using 0.45 μm filters. MALDI ToF MS was performed on a Bruker Autoflex III Mass Spectrometer operating in positive/linear mode. The analyte, matrix (α-CHCA) and caternisation agent (NaI) were dissolved in THF at concentration of 10 mg mL^{-1}, 10 mg mL^{-1} and 1 mg mL^{-1}, respectively, and then mixed in a ratio of 10 : 1 : 1. 0.3 μL of this solution was then spotted onto a ground steel target plate and the solvent allowed to evaporate prior to analysis. FlexAnalysis (Bruker) was used to analyses the data. UV-Vis spectrophotometry was performed on a Shimadzu UV-2101PC spectrometer using quartz cuvettes with a 1 cm path length.

3.3 Methods

Synthesis of benzylglutamic acid NCA (Glu NCA). H-L-Glu (OBn)-H (2.00 g, 8.42 mmol) was dissolved in anhydrous THF (50 mL) in a three-necked round bottomed flask under argon. Triphosgene (1.00 g, 3.37 mmol) was added and the mixture was heated at 60 °C for 1 h with continuous stirring. After cooling to room temperature the solvent was removed *in vacuo* and the resulting residue was recrystallised from 1 : 1 ethyl acetate: hexane (20 mL) 2 times to afford Glu NCA (1.73 g, 78%). ^{1}H NMR (400 MHz, d_6-DMSO) δ_H 1.87–2.10 (*m*, 2H, C**H**$_2$), 2.52 (*t*, 2H, *J* = 7.6 Hz, C**H**$_2$), 4.45 (*dd*, 1H, *J* = 5.6 & 8.0 Hz, C**H**N), 5.10 (*s*, 2H, C**H**$_2$O), 7.31–7.40 (*m*, 5H, Ar**H**), 9.09 (*s*, 1H, N**H**) ppm; ^{13}C NMR (100 MHz, d_6-DMSO) δ_C 26.3 (**C**H$_2$), 29.0 (**C**H$_2$), 56.1 (**C**HN), 65.6 (**C**H$_2$), 127.9 (3 Ar**C**H), 128.4 (2 Ar**C**H), 135.9 (Ar**C**C), 151.8 (NH**C**O$_2$), 171.2 (CH**C**O$_2$), 171.6 (CH$_2$**C**O$_2$) ppm.

General procedure for synthesis of CCS polymers 1. The amino acid NCA monomer (Lys NCA or Glu NCA) was dissolved in anhydrous DMF (100 mg mL^{-1}) in a flame-dried, argon purged two-necked flask. HMDS (M/I ratio varied depending on desired MW) was added and the mixture was stirred at room temperature until the NCA conversion reached *ca.* 80% (as determined by ^{1}H NMR) to afford the macroinitiator (MI). Cys NCA (CL/MI ratio varied depending on MI MW) was added and the mixture was stirred for a further 7 h. The resulting CCS polymer could either be isolated *via* precipitation into water:methanol (1 : 1 v/v, 10 times the volume of DMF) or core-functionalised *via* the addition of primary amines.

Synthesis of CCS ***1a$_L$***. Starting from Lys NCA (200 mg, 0.654 mmol) and HMDS (M/I = 20, 6.81 μL, 32.7 μmol) in anhydrous DMF (2 mL), PZLL (M_w = 6.56 kDa, PDI = 1.03) was obtained after 6 h. Addition of Cys NCA (CL/MI = 15, 143 mg, 0.490 mmol) afforded after 7 h PZLL$_{arms}$PLC$_{core}$ CCS polymer $\mathbf{1a_L}$ (M_w = 182 kDa, PDI = 1.77, $f \approx 19$). Precipitation of the polymer solution into water:methanol (1 : 1, 20 mL) followed by isolation *via* centrifugation and drying (0.1 mbar) afford $\mathbf{1a_L}$ (M_w = 500 kDa, PDI = 2.0, $f \approx 52$) as a pale yellow solid, 173 mg (63%).

Synthesis of CCS ***1a$_H$***. Starting from Lys NCA (200 mg, 0.654 mmol) and HMDS (M/I = 40, 3.41 μL, 16.4 μmol) in anhydrous DMF (2 mL), PZLL (M_w = 15.0 kDa, PDI = 1.09, d_h = 10.8 nm) was obtained after 7 h. Addition of Cys NCA (CL/MI = 35, 170 mg, 0.582 mmol) afforded after 7 h PZLL$_{arms}$PLC$_{core}$ CCS polymer $\mathbf{1a_H}$ (M_w = 523 kDa, PDI = 1.63, $f \approx 24$, d_h = 74 nm). Precipitation of the polymer solution into water:methanol (1 : 1, 20 mL) followed by isolation *via* centrifugation and drying (0.1 mbar) afford $\mathbf{1a_H}$ (M_w = 1440 kDa, PDI = 1.33, $f \approx 66$, d_h = 180 nm) as a pale yellow solid, 257 mg (89%).

Synthesis of CCS ***1b***. Starting from Glu NCA (200 mg, 0.760 mmol) and HMDS (M/I = 40, 3.96 μL, 18.9 μmol) in anhydrous DMF (2 mL), PBLG (M_w = 8.70 kDa, PDI = 1.03) was obtained after 7 h. Addition of Cys NCA (CL/MI = 27.5, 153 mg, 0.522 mmol) afforded after 7 h PBLG$_{arms}$PLC$_{core}$ CCS polymer **1b** (M_w = 515 kDa, PDI = 1.65, $f \approx 36$). Precipitation of the polymer solution into water:methanol (1 : 1, 20 mL) followed by isolation *via* centrifugation and drying (0.1 mbar) afford **1b** as a pale yellow solid, 207 mg (70%). GPC analysis was not possible because the isolated product was not soluble in DMF.

Kinetic studies of CCS polymer 1a. Kinetic studies following the consumption of Lys NCA monomers were conducted *via* GPC with an online UV-vis detector and ^{1}H NMR spectroscopic analysis. For GPC kinetic studies aliquots (0.1 mL) were removed from the reaction mixture at hourly intervals, passed through a 0.45 μm syringe filter and injected directly. For ^{1}H NMR spectroscopic kinetic studies reactions were performed in dried and argon purged NMR tubes fitted with septums using DMF-d_7 as the solvent. Initially, a spectrum of the Lys NCA

solution was recorded before the addition of the initiator HMDS. After the addition of HMDS spectra were recorded every 15 min for 6 h. Cys NCA (MI/CL = 35) was then added into the same NMR tubes and vortexed to ensure complete dissolution. The ^{1}H NMR spectra of the CCS polymer **1a** formation were recorded immediately after dissolution of Cys NCA (t = 3 min), and then every 30 min.

General procedure for synthesis of core-functionalized CCS polymers 2. The CCS polymers **1** were directly core-functionalised prior to isolation from their reaction solutions. Thus, primary amines such as propargylamine, hexylamine and aminomethyl pyrene (PGA, HEX, and AMP, respectively) were dissolved in anhydrous DMF and was added directly to the CCS polymer solution to give a final star concentration of *ca.* 10–20 mg mL^{-1}. After a period of time stirring under argon the reaction solution was concentrated *in vacuo* to *ca.* 1.0 mL in volume and precipitated into diethyl ether (30 mL). The residue was isolated *via* centrifugation, redissolved in DMF (2 mL) and precipitated into ethyl acetate (30 mL); this step was repeated 3 times and the residue dried *in vacuo* (0.1 mbar) to afford the core-functionalised CCS polymers **2** as pale brown solids.

Core-functionalisation with PGA to yield ***2a$_{PGA}$***. PGA (20 equiv. relative to Cys NCA used, 0.340 mL, 5.31 mmol) was dissolved in DMF (10 mL) and added to a solution of CCS polymer **1a** (M_w = 596 kDa, PDI = 1.65, 2 mL) and stirred for 3 h to afford, after isolation, CCS polymer **2a$_{PGA}$** (M_w = 704 kDa, PDI = 1.69).

Core-functionalisation with AMP to yield ***2a$_{AMP}$***. AMP (1.3 equiv. relative to Cys NCA used, 1.42 g, 6.15 mmol) was dissolved in DMF (10 mL) and added to a solution of CCS polymer **1a** (M_w = 832 kDa, PDI = 1.98, 2 mL) and stirred for 3 h to afford, after isolation, CCS polymer **2a$_{AMP}$** (M_w = 1028 kDa, PDI = 2.25).

Core-functionalisation with HEX to yield ***2b$_{HEX}$***. HEX (10 equiv. relative to Cys NCA used, 0.312 mL, 2.37 mmol) was dissolved in DMF (10 mL) and added to a solution of CCS polymer **1b** (M_w = 503 kDa, PDI = 1.55, 1 mL) and stirred for 1 h to afford, after isolation, CCS polymer **2b$_{HEX}$** (M_w = 823 kDa, PDI = 1.93).

Core-functionalisation with AMP to yield ***2b$_{AMP}$***. AMP (2.2 equiv. relative to Cys NCA used, 1.20 g, 6.15 mmol) was dissolved in DMF (10 mL) and added to a solution of CCS polymer **1b** (M_w = 503 kDa, PDI = 1.55, 1 mL) and stirred for 3 h to afford, after isolation, CCS polymer **2b$_{AMP}$** (M_w = 792 kDa, PDI = 1.79).

Cleavage of CCS polymers 1a and 2a$_{AMP}$. CCS polymer **1a$_H$** (M_w = 1140 kDa, 100 mg, 87.7 nmol) was dissolved in degassed DMF (1.0 mL) and 10 equiv. of DTT (135 mg, 877 μmol) with respect to the disulfide cross-links was added. After stirring for 2 h under argon the resulting P(ZZL-*b*-LC) copolymer (M_w = 34.4 kDa, PDI = 1.69, d_h = 10.4 nm) was either exposed to the atmosphere and allowed to stand or sonicated. In both cases gelation occurred rapidly within minutes. CCS polymer **2a$_{AMP}$** (M_w = 1028 kDa, 100 mg, 97.2 nmol) was dissolved in DMF (1.0 mL) and excess DTT was added (150 mg, 972 μmol) and stirred for 24 h. For ^{1}H NMR spectroscopic analysis an aliquot of the cleaved polymer was removed after 1 h and isolated by precipitation into 2-propanol, and dried *in vacuo* (0.1 mbar) before being redissolved in d_6-DMSO.

Hydrazinolysis of CCS polymer 2b$_{AMP-HYD}$. CCS polymer **2b$_{AMP}$** (M_w = 792 kDa, 50 mg, 63.1 nmol) was dissolved in DMF (2 mL) and 2 equiv. of hydrazine hydrate (0.30 mmol, 9.6 mg) with respect to glutamic acid repeat units was added. After stirring for 30 min at room temperature the product was precipitated into diethyl ether (20 mL) and dried *in vacuo* (0.1 mbar) to afford CCS polymer **2b$_{AMP-HYD}$**, 21.0 mg (54%).

General procedure for synthesis of water soluble CCS polymers 3. CCS polymers **2** were dissolved in TFA (200 mg mL^{-1}) and 33% HBr in acetic acid was then added (20 mL g^{-1} of star). After stirring for 2 h at room temperature the mixture was precipitated into diethyl ether (10 times the reaction volume). The residue was isolated *via* centrifugation, redissolved in water (2 mL) and precipitated into tetrahydrofuran (30 mL); this step was repeated 3 times and the residue was dried *in vacuo* (0.1 mbar) to afford water soluble CCS polymers **3** as off-white solids. ^{1}H NMR spectroscopic analysis was used to confirm removal of the protecting groups. Regardless of the core-functionality of CCS polymers **2** ^{1}H NMR spectroscopic analysis of the deprotected stars **3** only displayed resonances corresponding to the arms.[26]

Conclusions

Core-functionalized CCS polymers composed entirely from naturally occurring amino acids can be readily prepared in one-pot using sequential reagent addition and the arm-first approach. Two different CCS polymers were synthesized, having either poly(Z-L-lysine) or poly(Bn-L-glutamic acid) arms. Facile core-functionalization followed by deprotection of the star's arms ultimately yields water soluble, biocompatible and biodegradable CCS polymers with a hierarchy of functionalities spanning from the core, along the arms, to the periphery. The core-isolated moieties are accessible for further reaction as demonstrated by the click reaction of alkyne core-functionalized stars with an azido pyrene derivative. Variation of the reaction parameters allows the stars molecular weights, average number of arms and core sizes to be tailored, providing access to stars with selective loading capacities. The degradability of the amino acid-based CCS polymers coupled with the ease of site-specific functionalization and their encapsulation abilities makes this type of star a very promising candidate for the development of biocompatible and biodegradable polymer therapeutics.

Notes and references

1 H. Leuchs, *Ber. Dtsch. Chem. Ges.*, 1906, **39**, 857; H. Leuchs and W. Manasse, *Ber. Dtsch. Chem. Ges.*, 1907, **40**, 3235; H. Leuchs and W. Geiger, *Ber. Dtsch. Chem. Ges.*, 1908, **41**, 1721.
2 T. J. Deming, *Nature*, 1997, **390**, 386.
3 I. Dimitrov and H. Schlaad, *Chem. Commun.*, 2003, 2944.
4 T. Aliferis, H. Iatrou and N. Hadjichristidis, *Biomacromolecules*, 2004, **5**, 1653–1656.

5 W. Vayaboury, O. Giani, H. Cottet, A. Deratani and F. Schué, *Macromol. Rapid Commun.*, 2004, **25**, 1221.
6 H. Lu and J. Cheng, *J. Am. Chem. Soc.*, 2007, **129**, 14114; H. Lu and J. Cheng, *J. Am. Chem. Soc.*, 2008, **130**, 12562.
7 Y. Peng, S. Lai and C. Lin, *Macromolecules*, 2008, **41**, 3455.
8 N. Higashi, T. Koga and M. Niwa, *Langmuir*, 2000, **16**, 3482; P. Papadopoulos, G. Floudas, T. Aliferis, H. Iatrou and N. Hadjichristidis, *Biomacromolecules*, 2005, **6**, 2352; J. R. Hernandez and H.-A. Klok, *J. Polym. Sci., Part A: Polym. Chem.*, 2003, **41**, 1167; J. R. Cha, G. D. Stucky, D. E. Morse and T. J. Deming, *Nature*, 2000, **403**, 289; H. R. Kricheldorf, C. Lossow and G. Schwarz, *Macromol. Chem. Phys.*, 2004, **205**, 918.
9 J. F. Lutz, D. Schütt and S. Kubowicz, *Macromol. Rapid Commun.*, 2005, **26**, 23; T. J. Deming, *Adv. Drug Delivery Rev.*, 2002, **54**, 1145; L. Wang, S. Wang and J. Z. Bei, *Polym. Adv. Technol.*, 2004, **15**, 617; H. R. Kricheldorf and K. Hauser, *Biomacromolecules*, 2001, **2**, 1110; H. Schlaad and M. Antonietti, *Eur. Phys. J. E*, 2003, **10**, 17.
10 H.-A. Klok, J. R. Hernandez, S. Becker and K. Müllen, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 1572.
11 K. Inoue, A. Miyahara and T. Itaya, *J. Am. Chem. Soc.*, 1997, **119**, 6191; K. Inoue, S. Horibe, M. Fukae, T. Muraki, E. Ihara and H. Kayama, *Macromol. Biosci.*, 2003, **3**, 26.
12 J. Li, W. He, N. He, S. Han, X. Sun, L. Li and B. Zhang, *J. Polym. Sci., Part A: Polym. Chem.*, 2009, **47**, 1450; J. Rao, Y. Zhang, J. Zhang and S. Liu, *Biomacromolecules*, 2008, **9**, 2586; H. Lu, J. Wang, Y. Lin and J. Cheng, *J. Am. Chem. Soc.*, 2009, **131**, 13582.
13 H. R. Kricheldorf, *Angew. Chem., Int. Ed.*, 2006, **45**, 5752.
14 N. Hadjichristidis, H. Iatrou, M. Pitsikalis and G. Sakellariou, *Chem. Rev.*, 2009, **109**, 5528.
15 A. K. Ho, P. A. Gurr, M. F. Mills and G. G. Qiao, *Polymer*, 2005, **46**, 6727; T. K. Goh, K. D. Coventry, A. Blencowe and G. G. Qiao, *Polymer*, 2008, **49**, 5095.
16 J. T. Wiltshire and G. G. Qiao, *Aust. J. Chem.*, 2007, **60**, 699; C. Kojima, K. Kono, K. Maruyama and T. Takagishi, *Bioconjugate Chem.*, 2000, **11**, 910.
17 A. Blencowe, T. K. Goh, S. P. Best and G. G. Qiao, *Polymer*, 2008, **49**, 825; J. T. Wiltshire and G. G. Qiao, *Macromolecules*, 2008, **41**, 623; C. T. Adkins and E. Harth, *Macromolecules*, 2008, **41**, 3472; H. Gao and K. Matyjaszewski, *Macromolecules*, 2007, **40**, 399; B. Helms, S. J. Guillaudeu, Y. Xie, M. McMurdo, C. J. Hawker and J. M. Fréchet, *Angew. Chem., Int. Ed.*, 2005, **44**, 6384.
18 A. Spiniello, A. Blencowe and G. G. Qiao, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 2422.
19 A. Blencowe, J. F. Tan, T. K. Goh and G. G. Qiao, *Polymer*, 2009, **50**, 5.
20 A. Sulistio, A. Widjaya, A. Blencowe, X. Zhang and G. G. Qiao, *Chem. Commun.*, 2011, **47**, 1151.
21 A. Sulistio, J. Lowenthal, A. Blencowe, M. N. Bongiovanni, L. Ong, S. L. Gras, X. Zhang and G. G. Qiao, *Biomacromolecules*, 2011, DOI: 10.1021/bm200604h, Article ASAP.
22 D. Ballard and C. Bamford, *J. Am. Chem. Soc.*, 1957, **79**, 2336; P. Doty and R. Lundberg, *J. Am. Chem. Soc.*, 1957, **79**, 2338.
23 H. Yin, A. Chacon, N. A. Porter, H. Yin and D. S. Masterson, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 807.
24 D. L. Pickel, N. Politakos, A. Avgeropoulos and J. M. Messman, *Macromolecules*, 2009, **42**, 7781.
25 W. N. E. van Dijk-Wolthuis, L. van de Water, P. van de Wetering, M. J. van Steenbergen, J. J. Kettenes-van den Bosch, W. J. W. Schuyl and W. E. Hennink, *Macromol. Chem. Phys.*, 1997, **198**, 3893.
26 M. Sawamoto, K. Y. Baek and M. Kamigaito, *Macromolecules*, 2001, **34**, 215; J. T. Wiltshire and G. G. Qiao, *Macromolecules*, 2006, **39**, 4828.
27 N. Hadjichristidis, S. Pispas, M. Pitsikalis, H. Iatrou and C. Vlahos, *Adv. Polym. Chem.*, 1999, **142**, 71.
28 A. J. Limer, A. K. Rullay, V. San Miguel, C. Peinado, S. Keely, E. Fitzpatrick, S. D. Carrington, D. Brayden and D. M. Haddleton, *React. Funct. Polym.*, 2006, **66**, 51; T. K. Georgiou, M. Vamvakaki, L. A. Phylactou and C. S. Patrickios, *Biomacromolecules*, 2005, **6**, 2990.
29 M. A. R. Meier and U. S. Schubert, *Chem. Commun.*, 2005, **36**, 610.
30 E. Setijadi, L. Tao, J. Liu, Z. Jia, C. Boyer and T. P. Davis, *Biomacromolecules*, 2009, **10**, 2699.
31 H. Gao, N. V. Tsarevsky and K. Matyjaszewski, *Macromolecules*, 2005, **38**, 5995.
32 D. L. Rabenstein and P. L. Yeo, *J. Org. Chem.*, 1994, **59**, 4223; D. L. Rabenstein and K. H. Weaver, *J. Org. Chem.*, 1996, **61**, 7391.
33 H. Kawano, S. Komaba, T. Yamasaki, M. Maeda, Y. Kimura, A. Maeda and Y. Kaneda, *Cancer Chemother. Pharmacol.*, 2008, **616**, 973.
34 L. Livi, I. Meattini, C. De Luca Cardillo, V. Scotti, B. Agresti, C. Franzese, L. Sanchez, J. Nori, S. Bertocci, S. Cassani, S. Bianchi, L. Cataliotti and G. Biti, *J. Chemotherapy*, 2011, **23**, 36.
35 J. A. Munoz-Gamez, R. Quiles-Perez, A. Ruiz-Extremera, A. B. Martin-Alvarez, L. Sanjuan-Nunez, A. Carazo, J. Leon, F. J. Oliver and J. Salmeron, *Cancer Lett.*, 2011, **301**, 47.
36 K. Kono, C. Kojima, N. Hayashi, E. Nishisaka, K. Kiura, S. Watarai and A. Harada, *Biomaterials*, 2008, **29**, 1664.
37 J. Lee, S. J. Lee, J. Y. Choi, J. Y. Yoo and C. H. Ahn, *Eur. J. Pharm. Sci.*, 2005, **24**, 441.