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Design of thermoresponsive elastin-like glycopolypeptides for selective lectin binding and sorting

Lourdes Mónica Bravo Anaya,[†] Rosine Petitdemange,[†,‡] Marie Rosselin,[†] Emmanuel Ibarboure,[†] Bertrand Garbay,[†] Elisabeth Garanger,[†] Timothy J. Deming,[‡] and S. Lecommandoux*[†]*

[†] Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, Pessac, F-33600, France.

[‡] Department of Chemistry and Biochemistry, and Department of Bioengineering, University of California, Los Angeles, California 90095, United States.

ABSTRACT

Selective lectin binding and sorting was achieved using thermosensitive glycoconjugates derived from recombinant elastin-like polypeptides (ELPs) in simple centrifugation-precipitation assays. A recombinant ELP, (VPGXG)$_{40}$, containing periodically spaced methionine residues was used to enable chemoselective post-synthetic modification *via* thioether alkylation using alkyne functional epoxide derivatives. The resulting sulfonium groups were selectively demethylated to give alkyne functionalized homocysteine residues, which then reacted with azido-functionalized monosaccharides to obtain ELP glycoconjugates with periodic saccharide

functionality. These modifications were also found to allow modulation of ELP temperature dependent water solubility. The multivalent ELP glycoconjugates were evaluated for specific recognition, binding and separation of the lectin *Ricinus communis agglutinin* (RCA$_{120}$) from a complex protein mixture. RCA$_{120}$ and ELP glycoconjugate interactions were evaluated using laser scanning confocal microscopy and dynamic light scattering. Due to the thermoresponsive nature of the ELP glycoconjugates, it was found that heating a mixture of galactose-functionalized ELP and RCA$_{120}$ in complex media selectively yielded a phase separated pellet of ELP-RCA$_{120}$ complexes. Based on these results, ELP glycoconjugates show promise as designer biopolymers for selective protein binding and sorting.

KEYWORDS

Elastin-like polypeptides (ELPs), saccharides, glycoconjugates, chemoselective post-modifications, click chemistry, lectins.

INTRODUCTION

Lectins are glycan-binding proteins that are widely distributed in nature and have diverse important functions on cells, such as agglutination, redistribution of cell surface components, toxicity, modification of the activity of membrane enzymes, cell aggregation, and immunomodulation.[1-4] Lectins have recently been used to successfully detect cancer biomarkers in tissues and complex fluids.[5] Many lectins have been applied in several medical research areas, such as drug delivery,[6,7] inhibition of cancer cell adhesion,[8] inhibition of cancer cell growth,[9] and biomarkers for disease detection and monitoring,[5] due to their carbohydrate binding specificities. Currently, cancer biomarker discovery is an important field of research, since only a few

biomarkers are available for use in clinical settings.[10] However, identifying the authentic biological ligand(s) for each lectin is still challenging.[11-13] The development of new tools to identify lectin specificity and enable lectin discovery present new opportunities for understanding and using lectin functions.[14]

Related to these goals, C. R. Bertozzi, who was a pioneer in the development of glycopolymers for recognition of specific lectins, described the main applications of glycopolymers,[15] namely, the enrichment of glycoproteins and glycopeptides from complex mixtures, the identification of protein glycosylation sites, for targeted glycoproteomics, and for functional glycoproteomics. All these areas are focused on identifying interactions between glycoproteins and glycan-binding proteins.[15-18] In an important advance using well-defined glycopolymers and Concanavalin A as the model receptor, the group of D. M. Haddleton and C. R. Becer explored the influence of the nature and density of different sugar residues on the inhibitory potency of glycopolymers.[19] The same group demonstrated that a simple glycopolymer can efficiently prevent the interactions between a human dendritic cell associated lectin (DC-SIGN) and the viral envelope glycoprotein gp120.[20] This approach was expected to give rise to novel insights into the mechanisms of HIV infection and provide potential new therapeutics. Similarly, M. I. Gibson et al. developed complex glycopolymers bearing both primary glycan ligands and secondary units to modulate their selectivity toward lectins associated with specific diseases.[21]

Among glycopolymers, synthetic glycopolypeptides are particularly interesting candidates as glycoprotein analogs since their chemical structures give them the potential to closely mimick natural glycoprotein properties.[22-25] Alternative synthetic routes such as solid-phase total synthesis of glycopeptides,[26] native chemical ligations of glycopeptide fragments,[26] and enzymatic synthesis of glycoproteins,[27] have been explored and were successfully used to access

fully synthetic glycoproteins. However, these chemical methods provide limited quantities of pure materials and generally require tedious and expensive syntheses. Overall, limitations of synthetic glycopolymers prevent the establishment of robust structure/activity relationships, either in terms of modest amounts and/or yields of biosynthesized glycoproteins obtained from biosynthetic methods, or in terms of sequence control (primary structure and chain length dispersity) for chemical polymerization methods.

Here, we sought to develop a robust method for producing thermosensitive glycopolypeptides of uniform length and precise carbohydrate spacing based on recombinant elastin-like polypeptides (ELPs) that are readily accessible at the gram scale. Specifically, we sought to utilize the temperature dependent solution properties of ELPs to control the interaction of multivalent galactose-functionalized ELPs with the lectin *Ricinus communis agglutinin* (RCA$_{120}$). Selective binding of these ELP glycoconjugates in complex human serum media was demonstrated through centrifugation/precipitation assays that allowed enrichment and separation of the complexes.

MATERIALS AND METHODS

Materials

Bacto-tryptone and yeast extract were obtained from Biokar Diagnostics (FR). LB medium was purchased from Sigma-Aldrich (FR). Ampicillin was purchased from Eurobio (FR). Glycerol and isopropyl β-D-thiogalactopyranoside (IPTG) were purchased from Euromedex (FR). Glycidyl propargyl ether was obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, FR). Glacial acetic acid, Trizma® and Hexafluoroisopropanol (HFIP) were obtained from Sigma-Aldrich (FR). Deionized water (18 MΩ-cm) was obtained by using a Millipore Milli-Q Biocel
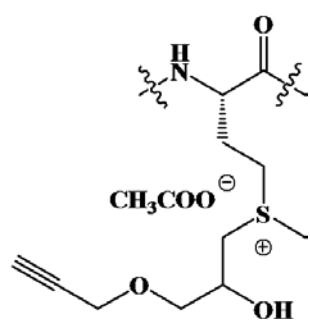
A10 purification unit. Cuprisorb was purchased from Seachem. Ethanol (96.0%, EtOH),

methanol (98.5%, MeOH) and acetonitrile (99.9%, ACN) were obtained from VWR

international. NaCl (99%) was purchased from Alfa Aesar (FR). Azide monosaccharides (β-D-

galactopyranosyl azide, Gal-N$_3$; and β-D-glucopyranosyl azide (Glu-N$_3$) were obtained from

Carbosynth (UK). Ammonium Acetate and Ammonium pyrrolidinedithiocarbamate, APDC,

were purchased from Fisher Scientific (FR). RCA$_{120}$ and RCA$_{120}$-Fluorescein were purchased

from Eurobio (FR). Human serum from human male AB plasma, USA origin, sterile-filtered,

was used as received from Sigma-Aldrich (FR). N,N,N′,N″,N″- pentamethyldiethylenetriamine

(PMDETA) was purchased from Sigma-Aldrich (FR). CuSO$_4$·5H$_2$O was obtained from VWR

(FR). Sodium ascorbate was obtained from Fisher Scientific (FR).

Bioproduction of recombinant ELP

ELP (MW[VPGVGVPGMG(VPGVG)$_2$]$_{10}$) was bio-produced, isolated and purified according to

the procedures established by R. Petitdemange et al.[28] ELP gene sequences and those of the

corresponding protein were reported also by R. Petitdemange et al.[28]
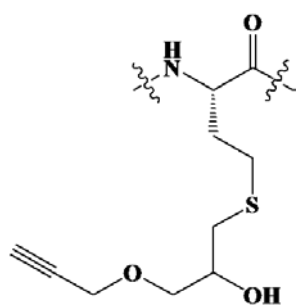
Synthetic procedures

Modification of ELP using epoxides [29]

A 50 mg sample of MW-[(VPGVG) (VPGMG) (VPGVG)$_2$]$_{10}$ was

dissolved in 2.5 mL of an AcOH/HFIP mixture (9/1, v/v) (20 mg/mL).

ELP solution was degassed by bubbling N$_2$ into the solution for 1 h,

which was then stirred under N$_2$. Glycidyl propargyl ether was then

added to the mixture (10 equiv. per methionine residue), which was

stirred for 48 h under N_2 at room temperature. The obtained mixture was transferred into a 3 000

MWCO centrifugal filter tube and washed with 40 mL DI water. The remaining content of the

centrifugal filter tube was lyophilized to obtain the product ELP*(Alkyne)* as a white solid.

Details of ELP(Alkyne): ^{1}H NMR (400 MHz, D_2O, 25 °C): (main peaks) δ 4.5−4.4 (m, 80 H,

αCH VPGXG and αCH VPGXG), 4.3 (s, 22 H, OCH₂CCH), 4.2−4.15 (d, 30 H, αCH VPGVG),

3.06−2.9 (m, 33 H, SCH₃), 1.00−0.75 (br m, 420 H, CH₃ Val). MS-ESI: Theoretical MW =

18279.1 Da, Experimental $[M_{11}]^{11+}$ = 1661.8 Da. Yield= 97 %.

Demethylation of ELP(Alkyne) [29]

A 50 mg sample of ELP*(Alkyne)* was dissolved in 75% EtOH (aq) at a 10 mM concentration and

then treated with ammonium pyrrolidinedithiocarbamate, APDC, (5.0 equiv per methionine

residue). The solution was rapidly flushed with a stream of N_2 and rapidly capped. The reaction

mixture was vortexed until obtaining a homogeneous solution, which was then allowed to stand

for 24 h at room temperature. The obtained mixture was transferred to a 1 kDa MWCO dialysis

bag and dialyzed against 50% MeOH (aq) during 24 h with 3 solvent changes followed by 8 h

dialysis against DI water with 3 changes. The dialysis bag contents were then lyophilized to
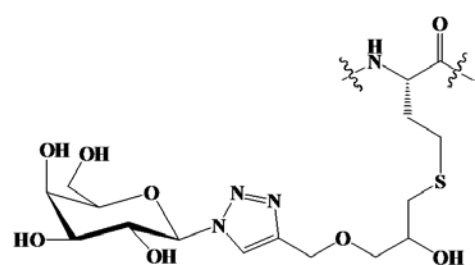
provide the product ELP*(Dem Alkyne)*.

Details of ELP(Dem Alkyne): ^{1}H NMR (400 MHz, D_2O, 25 °C): (main peaks) δ 4.5−4.4 (m, 80 H, αCH VPGXG and αCH VPGXG), 4.25 (s, 22 H, OCH₂CCH), 4.2−4.15 (d, 30 H, αCH VPGVG), 2.8−2.55 (m, 44 H, CH₂SCH₂), 1.00−0.75 (br m, 420 H, CH₃ Val). MS- ESI: Theoretical MW=18113.8 Da, Experimental $[M_{11} + 2H]^{13+}$ = 1394.7 Da; $[M_{11}+$
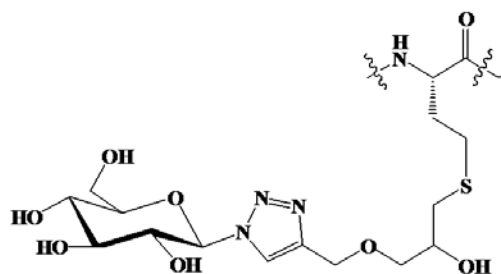
$3Na]^{14+}$ = 1298.2 Da. Yield = 65 %.

Synthesis of ELP glycoconjugates.

A sample of ELP*(Dem Alkyne)* was dissolved in water (5 mg/mL) and the desired azido-
functionalized monosaccharide, either β-D-galactopyranosyl azide or β-D-glucopyranosyl azide
(powder, 1.5 equiv. per alkyne) was added. The solution was degassed by bubbling N_2 for 2 hrs
and then stirred under N_2. Separately, a solution of Cu(I) was prepared by addition of sodium
ascorbate (0.65 equiv. per alkyne) to a degassed solution of Cu(II)SO4 (0.13 equiv. per alkyne)
and pentamethyldiethylenetriamine (0.13 equiv. per alkyne). The Cu(I) solution then was
transferred to the reaction mixture with a syringe. The reaction was stirred under N_2 at room
temperature for 72 hrs. Cuprisorb (100 mg), a powerful absorbent of copper,[30] was added to the
reaction mixture and stirred overnight. Cuprisorb beads were separated by centrifugation and the
supernatant was transferred to a 3000 MWCO centrifugal filter tube, which was washed first
with an aqueous solution of EDTA (0.15 M) and then with 40 mL DI water. The purified
reaction mixture was then lyophilized to provide the ELP glycoconjugate as a white solid.

Details of ELP(Gal): ELP*(Gal)* was prepared from
ELP*(Dem Alkyne)* and β-D-galactopyranosyl azide (Gal-
N_3). Yield = 85 %; ^{1}H NMR (400 MHz, D_2O, 25 °C):
(main peaks) δ 8.4-8.3 (br s, 11 H, triazole-H) 5.85-5.7 (br
s, 11 H, anomeric-H), 4.5-4.4 (m, 80 H, αCH V<u>P</u>GXG and αCH VP<u>G</u>XG), 4.2-4.15 (d, 30 H,
αCH VPG<u>V</u>G), 1.00-0.75 (br m, 420 H, CH$_3$ Val). Theoretical MW = 20 369.7 Da.

Details of ELP(Glc): ELP*(Glc)* was prepared from
ELP*(Dem Alkyne)* and β-D-glucopyranosyl azide (Glc-
N_3). Yield = 88 %; ^{1}H NMR (400 MHz, D_2O, 25 °C):

(main peaks): δ 8.4-8.3 (br s, 11 H, triazole-H) 5.9-5.75 (br s, 11 H, anomeric-H), 4.5-4.4 (m, 80 H, αCH V<u>P</u>GXG and αCH VP<u>G</u>XG), 4.2-4.15 (d, 30 H, αCH VPG<u>V</u>G), 2.7 ppm (s, 9 H, CH_3 Met-ox) 1.00-0.75 (br m, 420 H, CH_3 Val). Theoretical MW = 20 369.7 Da.

^{1}H NMR analyses

^{1}H NMR analyses were performed on a Bruker AVANCE III HD 400 apparatus equipped with a 5 mm Bruker multinuclear z-gradient direct probe operating at 400.2 MHz for ^{1}H and 100.6 MHz for ^{13}C. An amount of 7 mg of material was dissolved in 0.5 mL of deuterated solvent (D_2O) and 128 scans were recorded for ^{1}H experiments. ^{1}H NMR spectra were acquired in D_2O at 25 °C. The solvent signal was used as the reference signal (δ = 4.79 ppm). HSQC analyses were performed on a Bruker AVANCE NEO 400 spectrometer operating at 100.7 MHz, equipped with a 5 mm Bruker multinuclear z-gradient direct cryoprobe-head operating at 25 °C. Data processing was performed using Bruker Topspin Software. Chemical shifts of amino acids are well-known in the literature.[31,32]

Size Exclusion Chromatography (SEC) analyses

SEC analyses of ELP derivatives were performed on a liquid chromatographic system from ThermoScientific equipped with two G4000PWXL and G3000PWXL gel columns (300 x 7.8 mm) (exclusion limits from 200 Da to 300 000 Da) and UV detector at a flow rate of 0.6 mL/min. Column temperatures were held at 25 °C. The system includes a multi-angle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Aqueous solvent composed of acetic acid (AcOH) 0.3 M, ammonium acetate 0.2 M and ACN (6.5/3.5, v/v) was used as the eluent. Ethylene glycol was used as flow marker.

Transition temperature (T_t) measurements of ELP derivatives

Dynamic light scattering (DLS) measurements were performed on a Malvern ZetaSizer Nano ZS instrument equipped with a standard HeNe laser emitting at 632.8 nm (Malvern, U.K.) at a 90° angle at a constant position in the cuvette (constant scattering volume). An amount of 100 μL of sample was introduced in a high precision cell with a light path of 3x3 mm made of quartz. Solutions of ELP, ELP*(Alkyne)*, ELP*(Dem Alkyne)*, ELP*(Gal)* and ELP*(Glc)*, were prepared at a range of concentrations between 0.1 and 2.0 mM in Trizma buffer (50 mM Trizma, 0.15 M NaCl). The selected buffer allowed conserving a constant pH (7.5), which was selected to perform carbohydrate-lectin binding experiments in the appropriate conditions to maintain RCA_{120} conformation and properties.[33] One measurement of four runs (10 s each one) was recorded during a temperature ramp programmed from 15 to 74 °C every 1.8 degrees after a 2 min-temperature equilibration time. The derived count rate (DCR) was defined as the mean scattered intensity normalized by the attenuation factor. The derived count rate was plotted against temperature and the transition temperature (T_t) was determined by taking the onset of aggregation of the ELP, corresponding to the point where the scattered intensity starts increasing on this plot.[29]

Dynamic light scattering (DLS) measurements

Dynamic light scattering measurements were performed on a Malvern ZetaSizer Nano ZS instrument equipped with a standard HeNe laser emitting at 632.8 nm (Malvern, U.K.) at a 90° angle. An amount of 100 μL of sample was introduced in a high precision cell with a light path of 3x3 mm made of quartz. The correlation functions were averaged from three measurements of 10 runs (30 s each one) at a temperature of 25 °C after a 2 min-temperature equilibration time.

Laser Scanning Confocal Microscopy (LSCM)

Laser Scanning Confocal Microscopy images were acquired on an inverted Leica TCS SP5 microscope equipped with an HCX PL APO 63X, NA 1.4 oil immersion objective in fluorescence mode. The laser outputs were controlled *via* the Acousto-Optical Tunable Filter (AOTF) and the two collection windows using the Acousto-Optical Beam Splitter (AOBS) and photomultiplier tubes (PMT) as follows: Fluorescein was excited with an argon laser at 488 nm (12%) and measured with emission settings at 500-550 nm. The helium-neon laser at 633 nm (10%) was only used in transmission mode. Images were collected using the microscope in sequential mode with a line average of 8 and a format of 512*512 pixels or 1024*1024 pixels. Samples (≈30 μL) were injected in μ-slide (chambered coverslip) with uncoated 8 wells from Ibidi GmbH. Processing of fluorescence confocal acquisitions were performed with the ImageJ freeware.

**Centrifugation/precipitation assays**

Solutions of ELP*(Gal)* or ELP*(Glc)* at a concentration of 200 μM were mixed with RCA-Fluorescein (31.2 μM) in Trizma buffer to prepare samples for centrifugation/precipitation assays. After mixture, solutions were incubated under shaking at room temperature for 20 min to allow the carbohydrate-RCA_{120} interaction to take place.[34] Then, temperature was increased to 37 °C and samples were incubated for 20 min. Finally, solutions were centrifuged at 40 °C for 20 minutes at 10,000 g. Supernatant was removed and the pellet was re-suspended in fresh buffer. An UV-Vis lamp at 365 nm was used to illuminate samples to identify presence of the fluorescence signal.

RESULTS AND DISCUSSION

ELPs derived from the hydrophobic domain of tropoelastin consist of repeated pentapeptide sequences of [-Val-Pro-Gly-*Xaa*-Gly-], with the residue *Xaa* being any amino acid except proline.[28,29,35] An interesting property of ELPs comes from their lower critical solution temperature (LCST) phase behavior. When ELPs are dissolved in aqueous media, they hydrophobically collapse above a critical temperature, named T_t, into insoluble aggregates forming a coacervate phase.[36,37] This temperature can be precisely tuned by modifying the initial design of the ELP, namely the amino acid composition and the ELP chain length,[38-40] or by subsequent chemical modification of the ELP backbone.[22,23] Post-synthesis modifications of ELPs reported in the literature often target their chain ends to graft small organic molecules,[41] oligonucleotides,[42] drugs[43] or PEG chains.[44] Modifications at the amino acid side chains within the ELP domain have recently been explored generating a wide variety of chemical functionalizations.[28,29,45,46] Recently, our group reported a method that allows chemical post-synthesis modifications by oxidation or alkylation of selectively introduced methionine residues, allowing both the modulation of T_t as well as the introduction of functional groups.[28,47] This method has improved the versatility of ELP post-synthesis modifications to introduce new functionalities, and modified the ELPs properties such as the thermoresponsiveness. However, up to now, ELPs have not been used to prepare glycopolypeptides, which has become an attractive field of research for a wide range of applications, such as the development of biosensors or bio-responsive drug carriers.

This study is focused on the design and synthesis of thermoresponsive ELP glycoconjugates for selective lectin binding and sorting, featuring galactose (Gal) units grafted onto the methionine

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side chains of ELP[M_1V_3-40] corresponding to the sequence (VPGXG)$_{40}$, where X=V/M (3:1), here simply referred to as ELP (**Scheme 1a**).[29] This ELP was chemoselectively modified so that the T_t at certain concentrations was lower than 40 °C, a temperature at which some proteins start to denature irreversibly and hence critical for temperature dependent sorting of proteins bound to the ELP. All 11 methionine residues of the ELP were chemoselectively alkylated using glycidyl propargyl ether, an alkyne-containing epoxide derivative (**Scheme 1b**).[28] Because the resulting ELP sulfonium derivative, ELP*(Alkyne)*, is too hydrophilic to possess a T_t in the desirable range (< 40°C), the latter was subsequently demethylated (using ammonium pyrrolidinedithiocarbamate, APDC, in 75% ethanol), to give ELP*(Dem Alkyne)* with appropriate T_t. ELP*(Dem Alkyne)* was then reacted with different azido-functionalized monosaccharides, *i.e.* galactose and glucose, via copper-catalyzed azide-alkyne cycloaddition (CuAAC).[48,49] The reaction conditions used (H_2O, Cu(II)SO_4, sodium ascorbate, PMDETA) were similar to those described by Deming *et al.* for the modification of alkyne-functionalized statistical copolymers of Met and Lys.[50] Compared to synthetic polypeptides, a longer reaction time of 72 hrs and excess monosaccharide, *i.e.* 1.5 equiv. azido-sugar per Met residue, was necessary to achieve quantitative functionalization of the ELP.[51]
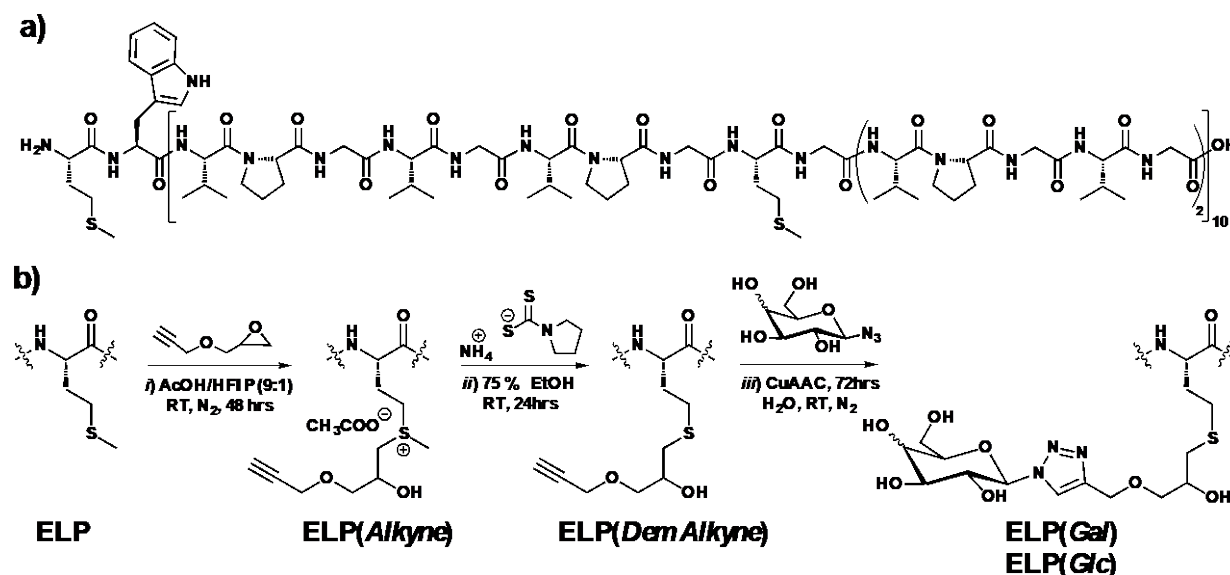
Scheme 1. a) Amino acid sequence of ELP, b) Three step reaction scheme to access ELP*(Gal)* and ELP(Glc)*: i)* alkylation of ELP with glycidyl propargyl ether to introduce alkyne functionality and yield ELP*(Alkyne)*, *ii)* demethylation of ELP*(Alkyne)* to give ELP*(Dem Alkyne),* and *iii)* click chemistry with Gal-N_3 or Glc-N_3 to give ELP*(Gal)* or ELP*(Glc),* respectively*.

The degree of functionalization of ELP*(Alkyne)* after alkylation at the thioether groups was determined by ^{1}H NMR spectroscopy as described previously (**Figure 1a and b**).[29] ^{1}H NMR spectra were calibrated using the resonances centered at 4.45 ppm, which correspond to the αCH protons of the first valine in each (VPGXG) repeat and to the αCH protons of proline, integrating as 80 total protons (**Figures S1 and S2**). Integration of the resonance at ca. 3 ppm, corresponding to the Met sulfonium methyl group of ELP*(Alkyne)* was used to determine the extent of ELP alkylation (full functionalization corresponds to 33 protons for 11 Met sulfonium groups). A degree of functionalization around $92 \pm 2\%$ (n=10) was obtained for the synthesized ELP*(Alkyne)* at this step. A small amount of Met sulfoxide was detected in ELP*(Alkyne)* during the reaction (less than 10%), as evidenced by the appearance of resonances at 2.7 ppm,

corresponding to −S(O)CH_3 protons. Other resonances, such as the singlet at 4.3 ppm,

corresponding to the methylene group in α position to the alkyne function and integrating as 22

protons, were also used to confirm degree of alkylation (**Figures S2** and **S3**). Demethylation of

methionine sulfoniums, leading to a less hydrophilic ELP derivative, ELP*(Dem Alkyne)*, was also

evaluated by ^{1}H and ^{13}C NMR spectroscopy (**Figures 1c, S4, S5** and **S6**). ELP*(Alkyne)* was

found to be efficiently demethylated to give the corresponding S-alkyl-L-homocysteine residues

as confirmed by ^{1}H NMR analysis (**Figure 1c**). Subsequently, complete functionalization with

monosaccharides after click chemistry was assessed by ^{1}H NMR spectroscopy. The resonance

signal of the methylene group in α position to the alkyne group shifted downfield (from 4.3 ppm

to 4.7 ppm) due to the modification of the chemical environment. Simultaneous appearance of

the resonances at 8.2 ppm and at 6.18 ppm is attributed to the proton of the triazole ring and to

the anomeric proton of the monosaccharide, respectively (**Figures 1d, S7, S8** and **S9**). After

click chemistry, functionalization degrees for each glycopolypeptide were found to be 93 ± 2%

for ELP*(Gal)* and 80 ± 3% for ELP*(Glc)*. The molecular weight changes of each ELP derivative

after the chemical modifications were followed by SEC analyses in aqueous solvent (acetic acid

0.3 M, ammonium acetate 0.2 M / ACN; 6.5/3.5, v/v). SEC traces showed expected shifts for

each ELP derivatives (**Figure S11**). SEC also evidenced the presence of a small amount of dimer

species (less than 10 %), the origin of which is not fully understood but considered negligible for
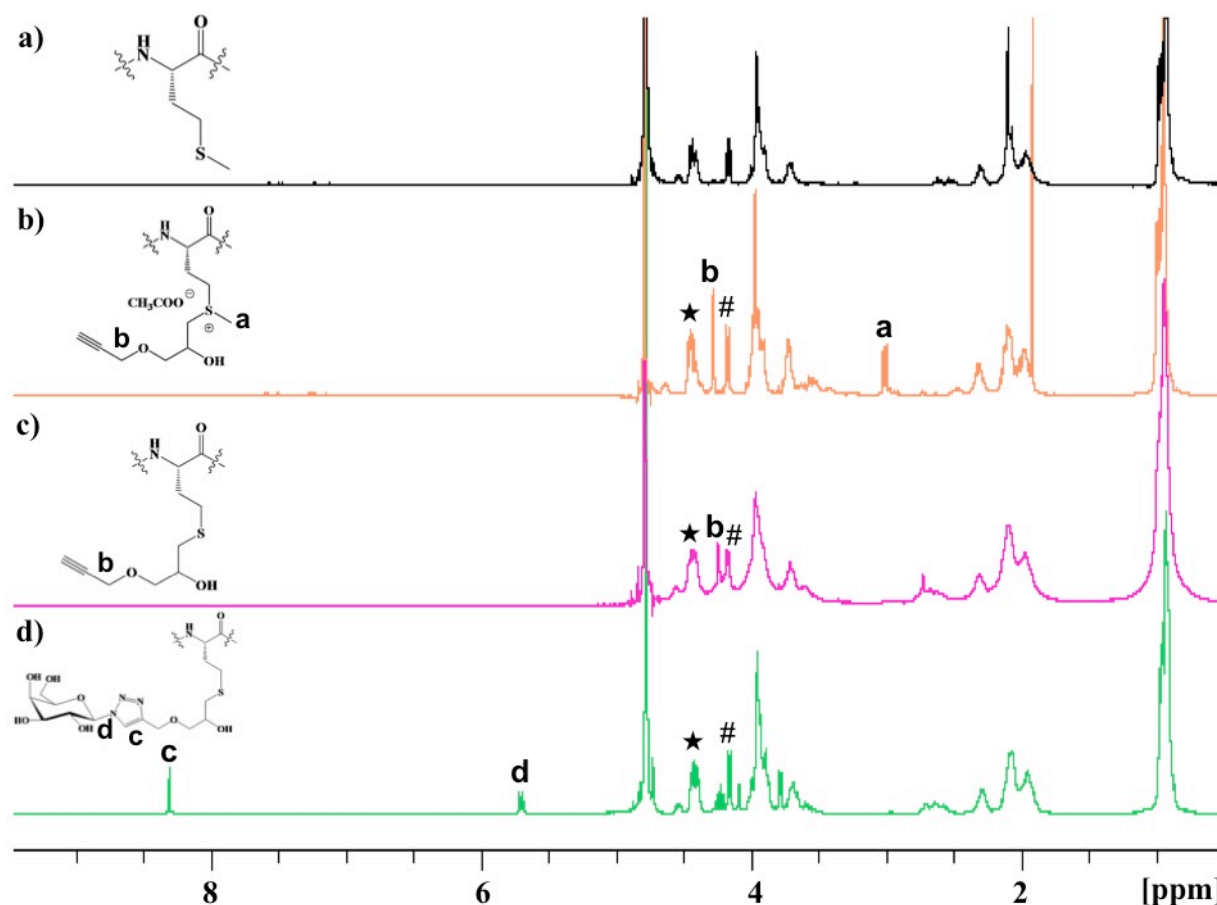
the purpose of this work.

Figure 1. ^{1}H NMR spectra of a) ELP, b) ELP*(Alkyne)*, c) ELP*(Dem Alkyne)* and d) ELP*(Gal)* in D$_2$O at 25 °C. Resonance # corresponds to *Val* αCH of the guest residue in VPG<u>V</u>G repeat units, and resonance ★corresponds to *Val* αCH and *Pro* αCH of <u>VP</u>GXG repeats.

Considering the strong effect of chemical modifications of Met-containing ELPs on their thermoresponsive properties,[28,29,51,52] the temperature-induced aggregation of all ELP derivatives and glycoconjugates described above was evaluated by dynamic light scattering (DLS) at a 90° angle over a wide range of concentrations (**Figure S12**). **Figure 2a** shows the scattered light intensity as a function of temperature for the different ELP derivatives and glycoconjugates (at a concentration of 200 μM), which was measured and plotted versus increasing temperature from

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15 °C to 74 °C. This allowed the determination of the onset temperature of aggregation (T_t). As expected at such concentration, ELP*(Alkyne)* did not present any aggregation in the range of temperatures studied, whereas the pristine ELP did (T_t = 26 °C).[29] This lack of aggregation was readily attributed to the increased hydrophilicity of the thioalkylated ELP resulting mainly from the positively charged sulfonium groups. This hypothesis was confirmed after demethylation, since the T_t of ELP*(Dem Alkyne)* was found around 23 °C, showing that the removal of the multiple positively charged sulfonium groups restored the thermoresponsive properties.[53] Glycopolypeptides, *i.e.* ELP*(Gal)* and ELP*(Glc)*, showed very similar thermoresponsive behavior, presenting T_t values of ca. 36 °C and 38 °C, respectively, at the selected concentration (**Table S1**). At higher temperatures, as well as at higher concentrations (**Figure S11**), a decrease of the scattered intensity was observed due to ELPs precipitation. The increase in T_t observed after ELP glycosylation was expected due to the contributions of the polar hydroxyl groups from the monosaccharides. As shown by Miyajima et al.[54] the relative hydrophobic/hydrophilic character of saccharides however depends on multiple parameters (*i.e.* dimension of hydrophobic/hydrophilic surface area, conformation, hydrogen bonding, etc.) and depending on which parameter is taken into account in the calculation of the hydrophobicity index, the ranking of individual saccharides is subject to variation. Finally, for all samples, except ELP*(Alkyne),* T_t versus concentration was plotted and used to determine accurate estimates of the T_t values for a given concentration (**Figure 2b**).
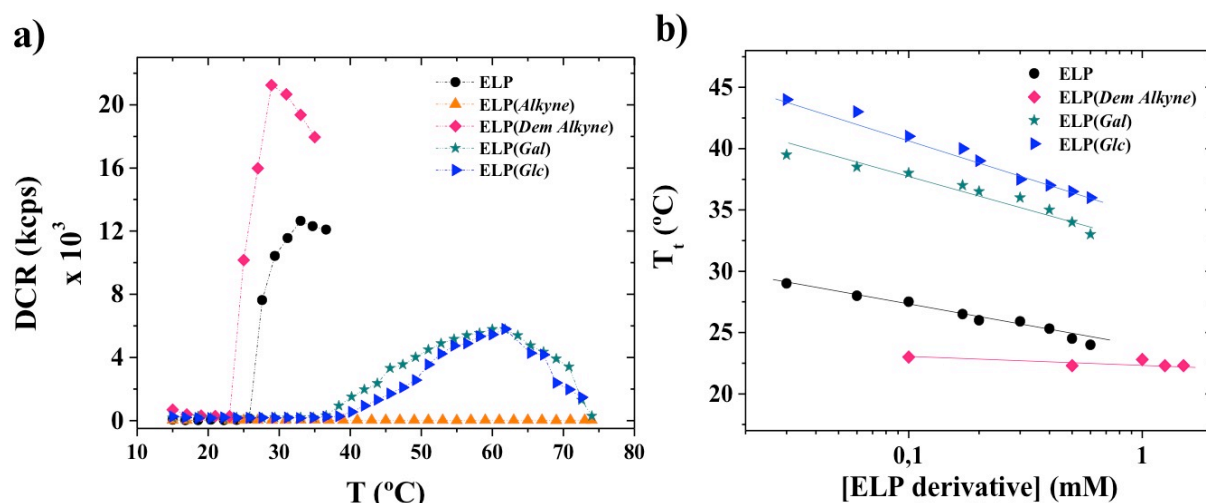
Figure 2. a) Scattered light intensity of ELP derivatives, *i.e.* ELP, ELP*(Alkyne)*, ELP*(Dem Alkyne)*, ELP*(Gal)* and ELP*(Glc)* as a function of temperature at 200 µM in Trizma buffer (50 mM Trizma, 0.15 M NaCl), b) T_t values of ELP (black circles), ELP(*Dem Alkyne*) (pink diamonds), ELP(*Gal*) (green stars) and ELP(*Glc*) (blue triangles) as function of molar concentration.

Dynamic light scattering experiments were then used to determine the binding capacity of ELP(*Gal*) and ELP(*Glc*) to a specific lectin, namely RCA$_{120}$, by monitoring aggregation behavior as depicted by the evolution of the correlation functions of the different species individually or in mixtures. The correlation function measured by DLS is a direct measurement of characteristic time (τ) of objects in solution, related to their Brownian motion: lower τ corresponds to smaller objects/colloids in solution.[55] **Figure 3a** shows that the correlation function for RCA$_{120}$ corresponds to that of small individual colloidal particles,[56] while glycosylated ELPs, either with galactose or with glucose, present nearly the same correlation function with longer decay times

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due to their larger colloidal size. The addition of RCA$_{120}$ into a solution containing ELP*(Gal)*, at

a ratio of 35 sugars per RCA$_{120}$ active site (determined by taking into account 10 sugars per ELP

and 2 active sites per RCA$_{120}$), showed a correlation function depicting strong aggregation of

ELP*(Gal)*, as a consequence of its binding with RCA120.[57] This kind of aggregation phenomena

has also been observed through kinetic studies of light absorbance for complexes of glycopeptide

polymersomes formed from galactose bearing PBLG$_{20}$-*b*-PGG$_{25}$ copolymers with RCA$_{120}$.[34] On

the other hand, no hallmark of aggregation was observed when ELP*(Glc)* was used in a similar

experiment. ELP*(Glc)* was chosen as a negative control because D-glucose is not supposed to

bind to RCA$_{120}$.[57] **Figure 3b** shows the evolution of correlation functions of RCA$_{120}$ solutions at

the same concentration (31.2 µM) after the addition of increasing amounts of ELP*(Gal)*.

Interaction and aggregation of ELP*(Gal)* with RCA$_{120}$ was detected as soon as 2 µM of

ELP*(Gal)* were added to the RCA$_{120}$ solution, corresponding to approximately 3 RCA$_{120}$ active

sites per sugar unit. This technique was also used by Ma et al. to show the interactions between

RCA$_{120}$ and glycopolymers bearing galactose and betulin. They also observed the increase in size

of the aggregates with time upon the addition of the lectin, which was explained by a possible

intermicellar cross-linking due to the two identical binding sites of the RCA$_{120}$.[58]
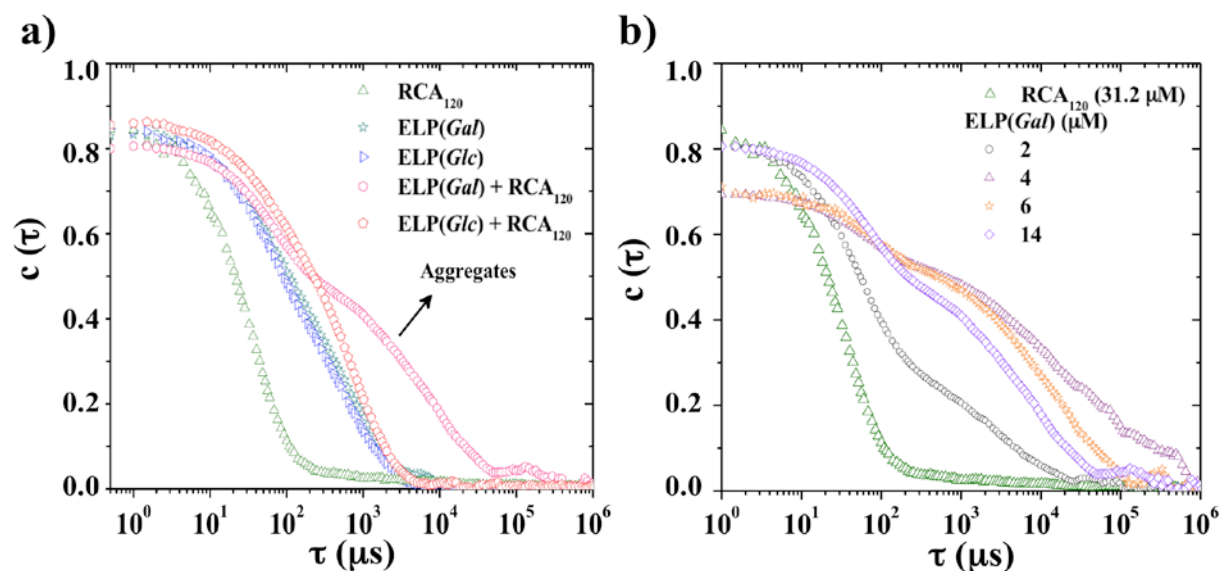
Figure 3. a) Correlation functions of 31.2 μM RCA$_{120}$, 200 μM ELP*(Gal)*, 200 μM ELP*(Glc)*, 31.2 μM RCA$_{120}$/ 200 μM ELP*(Gal)* and 31.2 μM RCA$_{120}$/ 200 μM ELP*(Glc)* at 25 °C, b) Evolution of RCA$_{120}$ (31.2 μM) correlation function during progressive addition of ELP*(Gal)* at 25 °C.

After demonstrating the selective binding of ELP*(Gal)* to RCA$_{120}$ lectin, we sought to take advantage of the thermoresponsive behavior of ELPs which form coacervates above T$_t$, to help precipitate the ELP*(Gal)*/RCA$_{120}$ complexes, and thus achieve sorting and recovery of the lectin from complex media. The specific binding of ELP*(Gal)* to RCA$_{120}$ in buffer and in serum containing medium was then assayed in temperature-triggered aggregation-centrifugation experiments (**Figure 4**). In these experiments, ELP(*Gal*) or ELP(*Glc*) (200 μM) were incubated above T$_t$ at 37 °C with fluorescently labeled RCA$_{120}$ (31.2 μM) and the mixture was subjected to centrifugation at 40 °C. With ELP(*Gal*), a distinct pellet was separated from the supernatant, as

observed under light and fluorescence microscopy (**Figure 4a and 4b, Figure S13**). When the

negative control ELP*(Glc)* was used, no detectable fluorescent pellet was recovered (**Figure 4a**

and 4b, Figure S13) and soluble RCA$_{120}$-fluorescein was recovered in the supernatant. In

contrast, when RCA$_{120}$-fluorescein was incubated with ELP*(Gal)* above T$_t$, only a very weak

fluorescence signal was measured in the supernatant. This can readily be explained by the

specific binding of RCA$_{120}$ to ELP*(Gal)* derivative and the temperature-induced aggregation of

the RCA$_{120}$/ELP*(Gal)* complex upon heating at 40 °C. This was confirmed by the strong

fluorescence signal recovered after re-suspension of the RCA$_{120}$/ELP*(Gal)* pellet in cold buffer,

below the T$_t$ of ELP*(Gal)* (**Figure S13d**). Similar results were obtained when serum was added
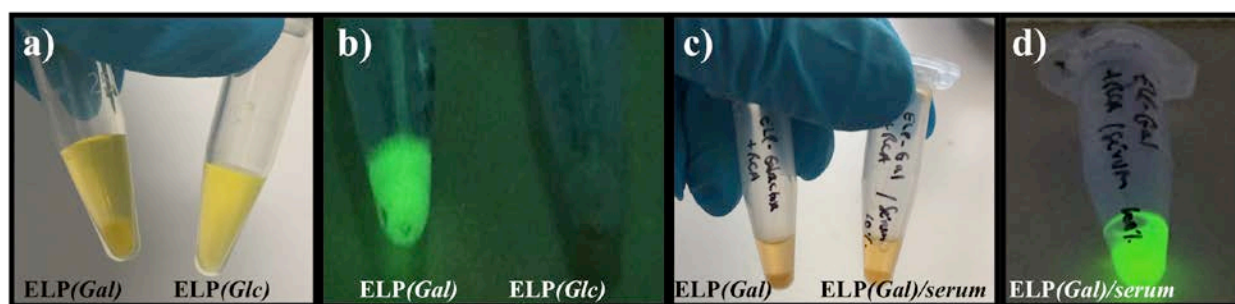
to the medium (**Figures 4c** and **4d**).

Figure 4. a) 31.2 μM RCA$_{120}$-Fluorescein incubated at 37 °C with either 200 μM ELP*(Gal)*

(left) or 200 μM ELP*(Glc)* (right), b) Samples from panel a) were subjected to centrifugation at

40 °C. After supernatant removal, any pellet was re-suspended in cold buffer and the resulting

solution illuminated under UV light at 365 nm, c) 31.2 μM RCA$_{120}$-Fluorescein incubated at 37

°C with either 200 μM ELP*(Gal)* (left) or 200 μM ELP*(Gal)* in the presence of serum (right), d)

31.2 μM RCA$_{120}$-Fluorescein incubated at 37 °C with 200 μM ELP*(Gal)* in the presence of

serum, then subjected to centrifugation at 40 °C. After supernatant removal, the pellet was re-suspended in cold buffer and the resulting solution illuminated under UV light at 365 nm.

Fluorescence microscopy measurements were also performed to visualize the interactions between ELP glycoconjugates and lectins. Samples containing either 31.2 μM RCA_{120}-Fluorescein or 31.2 μM RCA_{120}-Fluorescein/200 μM ELP*(Glc)* were used as negative controls (**Figures 5a** and **5c**). No microscopic aggregate structure formation was observed, suggesting that RCA_{120} maintains its solubility and conformation (it is known to denature above 52 °C) and that RCA_{120}-Fluorescein/ELP*(Glc)* do not bind to each other (**Figure S13**). Interestingly, structures formed at 37 °C in the mixture containing 31.2 μM RCA_{120}-Fluorescein and 200 μM ELP*(Gal)* (**Figure 5b**), appear similar to disordered fractal aggregates.[59,60] Such structures have been recently reported by Roberts et al.[61] for thermally responsive partially ordered polypeptides that combine the stimuli-responsiveness of disordered ELPs with the structural stability of poly(L-alanine) helices. Machado et al. also reported the hierarchical self-assembly of PEO_{43}-Lys_{62}-Leu_{72} to form, through a diffusion-limited aggregation (DLA) process, fractal assemblies at very specific conditions, *i.e.* 0.10% w/w and pH 2 as the solutions dried on a Transmission Electron Microscopy grid. Interestingly, interparticle and particle−surface interactions were shown to have an effect on the structure of PEO_{43}-Lys_{62}-Leu_{72} fractals.[62] In our work, the interaction between ELP*(Gal)* and RCA_{120} allows the formation of such structures, most likely because RCA_{120} possesses two binding sites in its native form. As reported in the literature, the preparation of stimuli responsive glycopolymers that are able to simultaneously enhance and control the lectin-glycopolymer interaction is challenging, but has also been achieved by several research groups.[63-66] Heise et al. reported the synthesis of a glycopolypeptide via ring opening polymerization and click chemistry with combined lectin recognition and thermoresponsive

properties.[67] Additionally, similar aggregation behavior to the one evidenced in this work was also observed for thermoresponsive polymers with pendant glucose functionality, which formed aggregates with bacterial cells and were assembled/disassembled reversibly through temperature cycling.[68]

To study binding specificity in a physiological medium, mixtures of RCA$_{120}$-Fluorescein and ELP*(Gal)* were prepared in presence of 10% or 100% human serum, **Figures 5e** and **5f**, respectively (more details and centrifugation assays in **Figure S14**). RCA$_{120}$-Fluorescein in 10% serum was also analyzed in order to detect possible interactions of the protein with serum components (**Figure 5d**). Only very few microscopic particles of RCA$_{120}$-Fluorescein were observed in 10% serum, in contrast to the abundant fractal aggregates observed when RCA$_{120}$-Fluorescein and ELP*(Gal)* were incubated in the same conditions. When serum concentration was increased to 100%, similar results were obtained although aggregates were more separated from each other, which could be due to the presence of greater amounts of different macromolecules present in serum. However, it is ~~is it~~ important to note that the binding affinity between ELP*(Gal)* and RCA$_{120}$ still occurred in such a complex mixture.

Altogether, our results demonstrate that thermosensitive ELP glycoconjugates can be used to specifically aggregate and pull-down specific lectins by temperature-triggered aggregation and centrifugation. Notably, the entire purification process can be performed within a reasonable temperature range that limits protein denaturation.
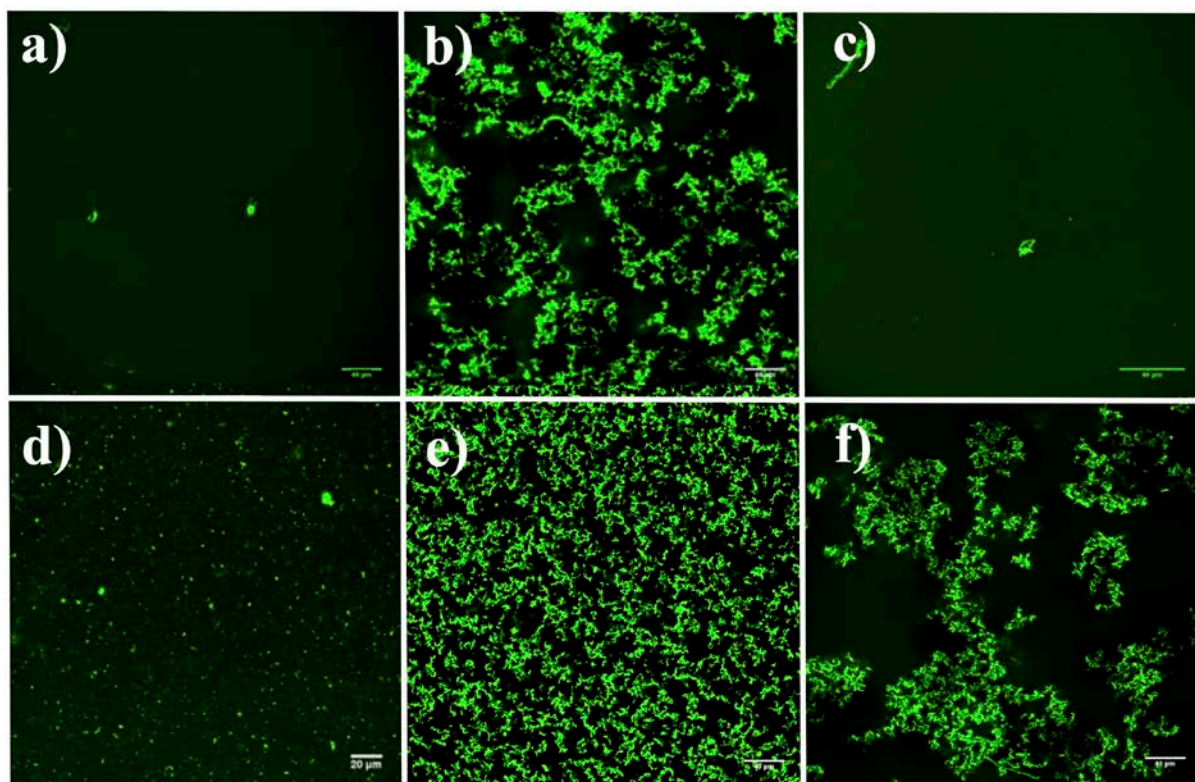
Figure 5. Fluorescence microscopy images (green channel) captured after incubation at 37 °C

for: a) 31.2 μM RCA_{120}-Fluorescein, b) 31.2 μM RCA_{120}-Fluorescein/200 μM ELP*(Gal)*, c) 31.2

μM RCA_{120}-Fluorescein/200 μM ELP*(Glc)*, d) 31.2 μM RCA_{120}-Fluorescein in 10 % serum, e)

31.2 μM RCA_{120}-Fluorescein/200 μM ELP*(Gal)* in 10% serum, f) 31.2 μM RCA_{120}-

Fluorescein/200 μM ELP*(Gal)* in 100 % serum.

CONCLUSIONS

We have reported the design and synthesis of uniform thermosensitive glycopolypeptide-based on recombinant ELP scaffolds for selective lectin-binding and sorting. Thermoresponsive properties of the resulting ELP derivatives were evaluated and showed that alkylation/demethylation of periodic methionine residues in ELPs allows the modulation of T_t and the introduction of reactive functional groups. These were used to prepare multivalent galactose or glucose-bearing ELPs that possessed T_t values in the range of 33-40 °C and 36-44 °C, respectively, at concentrations ranging from 30 to 600 μM. The binding of ELP*(Gal)* towards RCA_{120} lectin was investigated using light and fluorescence microscopy, DLS measurements and centrifugation/precipitation assays, which confirmed the ability of ELP*(Gal)* to specifically bind RCA_{120} lectin. It was found that, aqueous mixtures of thermosensitive ELP*(Gal)* with RCA_{120} lectin above the T_t could be centrifuged to separate and collect the enriched interacting species in the pellet. An ELP glycoconjugate was found to aggregate and pull down a specific lectin by simple temperature-triggered aggregation and centrifugation at reasonable temperature that maintains protein folding and activity. Based on these results, ELP glycoconjugates show promise as designer biopolymers for selective protein binding and sorting.

ASSOCIATED CONTENT

Supporting Information.

Experimental and synthetic procedures, NMR spectra (^{1}H and HSQC), SEC traces, DLS temperature ramps and additional fluorescence and microscopy images are available in the supporting information.

AUTHOR INFORMATION

Corresponding Author

*Mailing address: Department of Bioengineering, 5121 Engineering 5, HS-SEAS, University of California, Los Angeles, CA 90095 USA. E-mail: demingt@seas.ucla.edu. �·SEP�·

*Mailing address: Université de Bordeaux/Bordeaux INP, ENSCBP, 16 avenue Pey-Berland, 33607 Pessac Cedex, France. E-mail: lecommandoux@enscbp.fr.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ABBREVIATIONS

ACN, acetonitrile; CuAAC, Cu^{I}- catalyzed azide/alkyne cycloaddition; DLS, dynamic light scattering; ELPs, Elastin-like polypeptides; Gal, Galactose; Glc, glucose; HIV, Human immunodeficiency virus; LCST , lower critical solution temperature; Met, Methionine; NMR, Nuclear magnetic resonance; PEG, Polyethylene glycol; PMDETA, Pentamethyldiethylenetriamine; RCA_{120}, *Ricinus communis agglutinin*; SEC, Size-exclusion chromatography; T_t, transition temperature.

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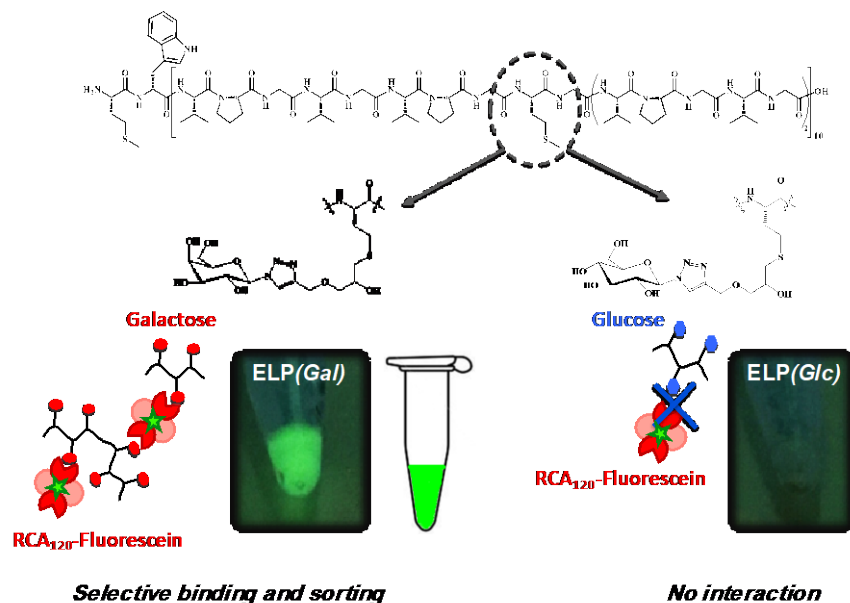
Selective binding and sorting   No interaction

Design of thermoresponsive elastin-like glycopolypeptides for selective lectin binding and sorting

Lourdes Mónica Bravo Anaya, Rosine Petitdemange, Marie Rosselin, Emmanuel Ibarboure, Bertrand Garbay, Elisabeth Garanger, Timothy J. Deming* and Sebastien Lecommandoux*

*Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, Pessac, F-33600, France.
Department of Chemistry and Biochemistry, and Department of Bioengineering, University of California, Los Angeles, California 90095, United States.*

Experimental Procedures

Materials

Bacto-tryptone and yeast extract were obtained from Biokar Diagnostics (FR). LB medium was purchased from Sigma-Aldrich (FR). Ampicillin was purchased from Eurobio (FR). Glycerol and isopropyl β-D-thiogalactopyranoside (IPTG) were purchased from Euromedex (FR). Glycidyl propargyl ether was obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, FR). Glacial acetic acid, Trizma® and Hexafluoroisopropanol (HFIP) were obtained from Sigma-Aldrich (FR). Deionized water (18 MΩ-cm) was obtained by using a Millipore Milli-Q Biocel A10 purification unit. Cuprisorb was purchased from Seachem. Ethanol (96.0%, EtOH), methanol (98.5%, MeOH) and acetonitrile (99.9%, ACN) were obtained from VWR international. NaCl (99%) was purchased from Alfa Aesar (FR). Azide monosaccharides (β-D-galactopyranosyl azide, Gal-N$_3$; and β-D-glucopyranosyl azide (Glu-N$_3$) were obtained from Carbosynth (UK). Ammonium Acetate and Ammonium pyrrolidinedithiocarbamate, APDC, were purchased from Fisher Scientific (FR). RCA$_{120}$ and RCA$_{120}$-Fluorescein were purchased from Eurobio (FR). Human serum from human male AB plasma, USA origin, sterile-filtered, was used as received from Sigma-Aldrich (FR). N,N,N′,N″,N″- pentamethyldiethylenetriamine (PMDETA) was purchased from Sigma-Aldrich (FR). CuSO$_4$·5H$_2$O was obtained from VWR (FR). Sodium ascorbate was obtained from Fisher Scientific (FR).
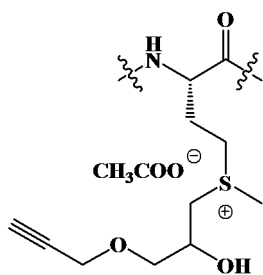
Bioproduction of recombinant ELP

ELP (MW[VPGVGVPGMG(VPGVG)$_2$]$_{10}$) was bio-produced, isolated and purified according to the procedures established by R. Petitdemange et al. 2017 [1]. ELP gene sequences and those of the corresponding protein were reported also by R. Petitdemange et al. 2017 [1].
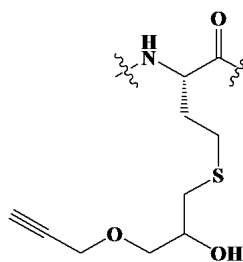
Synthetic procedures

Modification of ELP using epoxides [1]

A 50 mg sample of MW-[(VPGVG) (VPGMG) (VPGVG)$_2$]$_{10}$ was dissolved in 2.5 mL of an AcOH/HFIP mixture (9/1, v/v) (20 mg/mL). ELP solution was degassed by bubbling N$_2$ into the solution for 1 h, which was then stirred under N$_2$. Glycidyl propargyl ether was then added to the mixture (10 equiv. per methionine residue), which was stirred for 48 h under N$_2$ at room temperature. The obtained mixture was transferred into a 3 000 MWCO centrifugal filter tube and washed with 40 mL DI water. The remaining content of the centrifugal filter tube was lyophilized to obtain the product ELP*(Alkyne)* as a white solid.

Details of ELP(Alkyne): ^{1}H NMR (400 MHz, D$_2$O, 25 °C): (main peaks) δ 4.5−4.4 (m, 80 H, αCH VP<u>G</u>XG and αCH V<u>P</u>GXG), 4.3 (s, 22 H, O<u>CH$_2$</u>CCH), 4.2−4.15 (d, 30 H, αCH VPG<u>V</u>G), 3.06−2.9 (m, 33 H, S<u>CH$_3$</u>), 1.00−0.75 (br m, 420 H, CH$_3$ Val). MS-ESI: Theoretical MW = 18279.1 Da, Experimental [M$_{11}$]$^{11+}$ = 1661.8 Da. Yield= 97 %.
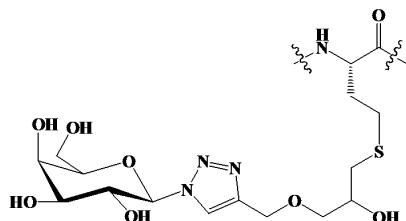
Demethylation of ELP(Alkyne) [1]

A 50 mg sample of ELP*(Alkyne)* was dissolved in 75% EtOH (aq) at a 10 mM concentration and then treated with ammonium pyrrolidinedithiocarbamate, APDC, (5.0 equiv per methionine residue). The solution was rapidly flushed with a stream of N_2 and rapidly capped. The reaction mixture was vortexed until obtaining a homogeneous solution, which was then allowed to stand for 24 h at room temperature. The obtained mixture was transferred to a 1 kDa MWCO dialysis bag and dialyzed against 50% MeOH (aq) during 24 h with 3 solvent changes followed by 8 h dialysis against DI water with 3 changes. The dialysis bag contents were then lyophilized to provide the product ELP*(Dem Alkyne)*.

Details of ELP(Dem Alkyne): ^{1}H NMR (400 MHz, D_2O, 25 °C): (main peaks) δ 4.5−4.4 (m, 80 H, αCH <u>V</u>PGXG and αCH V<u>P</u>GXG), 4.25 (s, 22 H, O<u>C</u>H$_2$CCH), 4.2−4.15 (d, 30 H, αCH VPG<u>V</u>G), 2.8−2.55 (m, 44 H, <u>CH$_2$S</u>CH$_2$), 1.00−0.75 (br m, 420 H, CH$_3$ Val). MS-ESI: Theoretical MW=18113.8 Da, Experimental $[M_{11} + 2H]^{13+}$ = 1394.7 Da; $[M_{11}+ 3Na]^{14+}$ = 1298.2 Da. Yield = 65 %.

Synthesis of ELP glycoconjugates.

A sample of ELP*(Dem Alkyne)* was dissolved in water (5 mg/mL) and the desired azido-functionalized monosaccharide, either β-D-galactopyranosyl azide or β-D-glucopyranosyl azide (powder, 1.5 equiv. per alkyne) was added. The solution was degassed by bubbling N_2 for 2 hrs and then stirred under N_2. Separately, a solution of Cu(I) was prepared by addition of sodium ascorbate (0.65 equiv. per alkyne) to a degassed solution of Cu(II)SO$_4$ (0.13 equiv. per alkyne) and pentamethyldiethylenetriamine (0.13 equiv. per alkyne). The Cu(I) solution then was transferred to the reaction mixture with a syringe. The reaction was stirred under N_2 at room temperature for 72 hrs. Cuprisorb (100 mg), a powerful absorbent of copper [2], was added to the reaction mixture and stirred overnight. Cuprisorb beads were separated by centrifugation and the supernatant was transferred to a 3000 MWCO centrifugal filter tube, which was washed first with an aqueous solution of EDTA (0.15 M) and then with 40 mL DI water. The purified reaction mixture was then lyophilized to provide the ELP glycoconjugate as a white solid.

Details of ELP(Gal): ELP*(Gal)* was prepared from ELP*(Dem Alkyne)* and β-D-galactopyranosyl azide (Gal-N$_3$). Yield = 85 %; ^{1}H NMR (400 MHz, D_2O, 25 °C): (main peaks) δ 8.4-8.3 (br s, 11 H, triazole-H) 5.85-5.7 (br s, 11 H, anomeric-H), 4.5-4.4 (m, 80 H, αCH <u>V</u>PGXG and αCH V<u>P</u>GXG), 4.2-4.15 (d, 30 H, αCH VPG<u>V</u>G), 1.00-0.75 (br m, 420 H, CH$_3$ Val). Theoretical MW = 20 369.7 Da.

Details of ELP(Glc): ELP*(Glc)* was prepared from ELP(*Dem Alkyne*) and β-D-glucopyranosyl azide (Glc-N$_3$). Yield = 88 %; ^{1}H NMR (400 MHz, D_2O, 25 °C): (main peaks): δ 8.4-8.3 (br s, 11 H, triazole-H) 5.9-5.75 (br s, 11 H, anomeric-H), 4.5-4.4 (m, 80 H, αCH <u>V</u>PGXG and αCH V<u>P</u>GXG), 4.2-4.15 (d, 30 H, αCH VPG<u>V</u>G), 2.7 ppm (s, 9 H, CH$_3$ Met-ox) 1.00-0.75 (br m, 420 H, CH$_3$ Val). Theoretical MW = 20 369.7 Da.

^{1}H NMR analyses

^{1}H NMR analyses were performed on a Bruker AVANCE III HD 400 apparatus equipped with a 5 mm Bruker multinuclear z-gradient direct probe operating at 400.2 MHz for ^{1}H and 100.6 MHz for ^{13}C. An amount of 7 mg of material was dissolved in 0.5 mL of deuterated solvent (D_2O) and 128 scans were recorded for ^{1}H experiments. ^{1}H NMR spectra were acquired in D_2O at 25 °C. The solvent signal was used as the reference signal (δ = 4.79 ppm). HSQC analyses were performed on a Bruker AVANCE NEO 400 spectrometer operating at 100.7 MHz, equipped with a 5 mm Bruker multinuclear z-gradient direct cryoprobe-head operating at 25 °C. Data processing was performed using Bruker Topspin Software. Chemical shifts of amino acids are well-known in the literature [3,4].

Size Exclusion Chromatography (SEC) analyses

SEC analyses of ELP derivatives were performed on a liquid chromatographic system from ThermoScientific equipped with two G4000PWXL and G3000PWXL gel columns (300 x 7.8 mm) (exclusion limits from 200 Da to 300 000 Da) and UV detector at a flow rate of 0.6 mL/min. Column temperatures were held at 25 °C. The system includes a multi-angle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Aqueous solvent composed of acetic acid (AcOH) 0.3 M, ammonium acetate 0.2 M and ACN (6.5/3.5, v/v) was used as the eluent. Ethylene glycol was used as flow marker.

Transition temperature (T_t) measurements of ELP derivatives

Dynamic light scattering (DLS) measurements were performed on a Malvern ZetaSizer Nano ZS instrument equipped with a standard HeNe laser emitting at 632.8 nm (Malvern, U.K.) at a 90° angle at a constant position in the cuvette (constant scattering volume). An amount of 100 μL of sample was introduced in a high precision cell with a light path of 3x3 mm made of quartz. Solutions of ELP, ELP*(Alkyne)*, ELP*(Dem Alkyne)*, ELP*(Gal)* and ELP*(Glc)*, were prepared at a range of concentrations between 0.1 and 2.0 mM in

Trizma buffer (50 mM Trizma, 0.15 M NaCl). The selected buffer allowed conserving a constant pH (7.5), which was selected to perform carbohydrate-lectin binding experiments in the appropriate conditions to maintain RCA$_{120}$ conformation and properties [5]. One measurement of four runs (10 s each one) was recorded during a temperature ramp programmed from 15 to 74 °C every 1.8 degrees after a 2 min-temperature equilibration time. The derived count rate (DCR) was defined as the mean scattered intensity normalized by the attenuation factor. The derived count rate was plotted against temperature and the transition temperature (T_t) was determined by taking the onset of aggregation of the ELP, corresponding to the point where the scattered intensity starts increasing on this plot [1].

Dynamic light scattering (DLS) measurements
Dynamic light scattering measurements were performed on a Malvern ZetaSizer Nano ZS instrument equipped with a standard HeNe laser emitting at 632.8 nm (Malvern, U.K.) at a 90° angle. An amount of 100 mL of sample was introduced in a high precision cell with a light path of 3x3 mm made of quartz. The correlation functions were averaged from three measurements of 10 runs (30 s each one) at a temperature of 25 °C after a 2 min-temperature equilibration time.

Laser Scanning Confocal Microscopy (LSCM)
Laser Scanning Confocal Microscopy images were acquired on an inverted Leica TCS SP5 microscope equipped with an HCX PL APO 63X, NA 1.4 oil immersion objective in fluorescence mode. The laser outputs were controlled *via* the Acousto-Optical Tunable Filter (AOTF) and the two collection windows using the Acousto-Optical Beam Splitter (AOBS) and photomultiplier tubes (PMT) as follows: Fluorescein was excited with an argon laser at 488 nm (12%) and measured with emission settings at 500-550 nm. The helium-neon laser at 633 nm (10%) was only used in transmission mode. Images were collected using the microscope in sequential mode with a line average of 8 and a format of 512*512 pixels or 1024*1024 pixels. Samples (≈30 μL) were injected in μ-slide (chambered coverslip) with uncoated 8 wells from Ibidi GmbH. Processing of fluorescence confocal acquisitions were performed with the ImageJ freeware.

Centrifugation/precipitation assays
Solutions of ELP*(Gal)* or ELP*(Glc)* at a concentration of 200 μM were mixed with RCA-Fluorescein (31.2 μM) in Trizma buffer to prepare samples for centrifugation/precipitation assays. After mixture, solutions were incubated under shaking at room temperature for 20 min to allow the carbohydrate-RCA$_{120}$ interaction to take place [6]. Then, temperature was increased to 37 °C and samples were incubated for 20 min. Finally, solutions were centrifuged at 40 °C for 20 minutes at 10,000 g. Supernatant was removed and the pellet was re-suspended in fresh buffer. An UV-Vis lamp at 365 nm was used to illuminate samples to identify presence of the fluorescence signal.
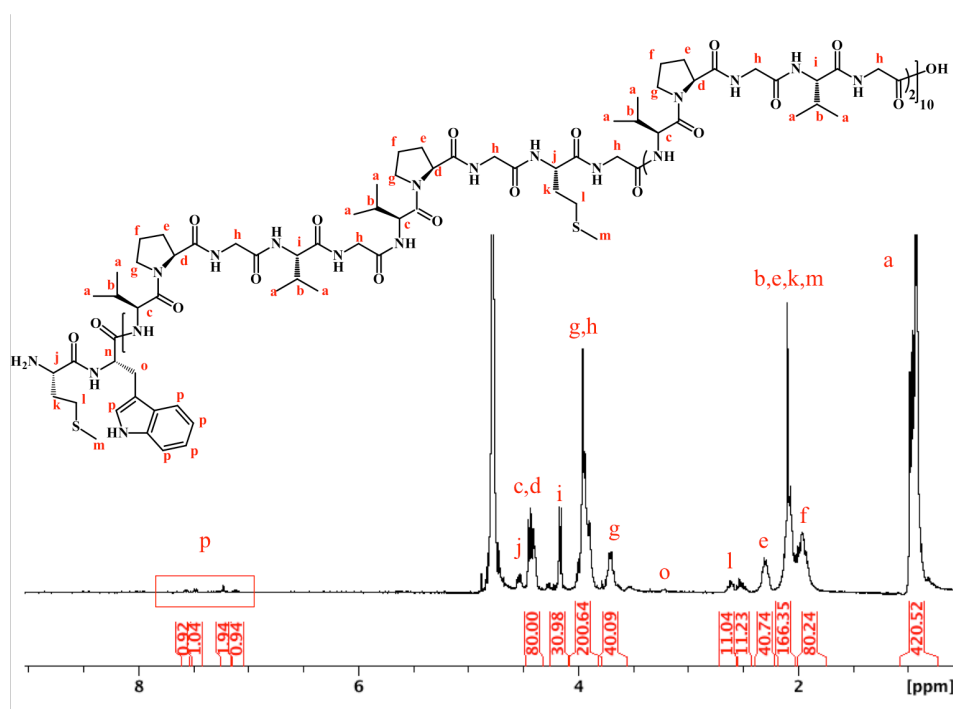
Additional Figures

Figure S1. ^{1}H-NMR spectrum of ELP in D$_2$O at 25 °C.
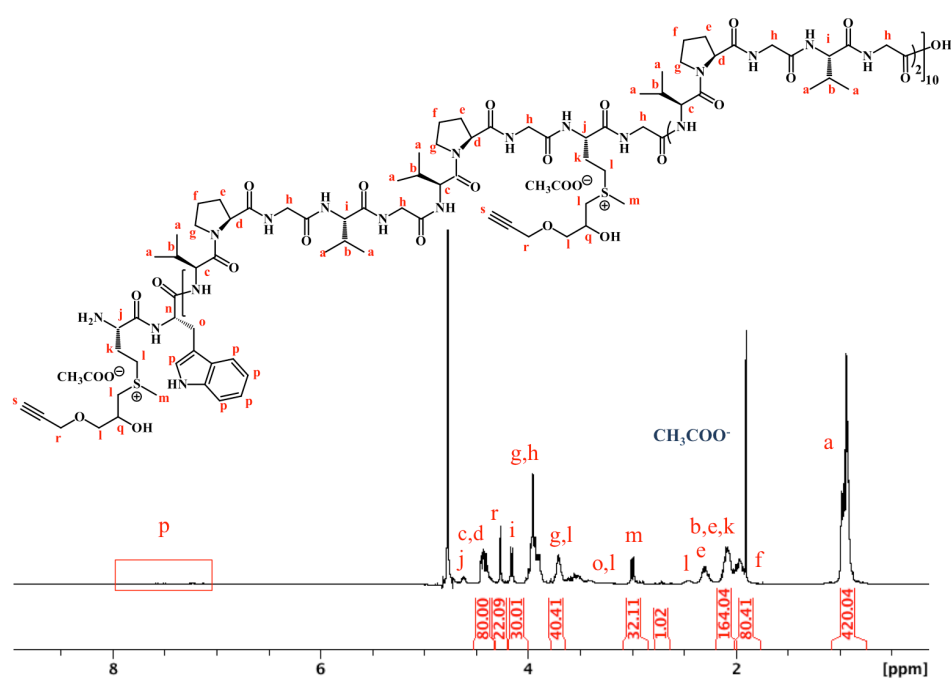
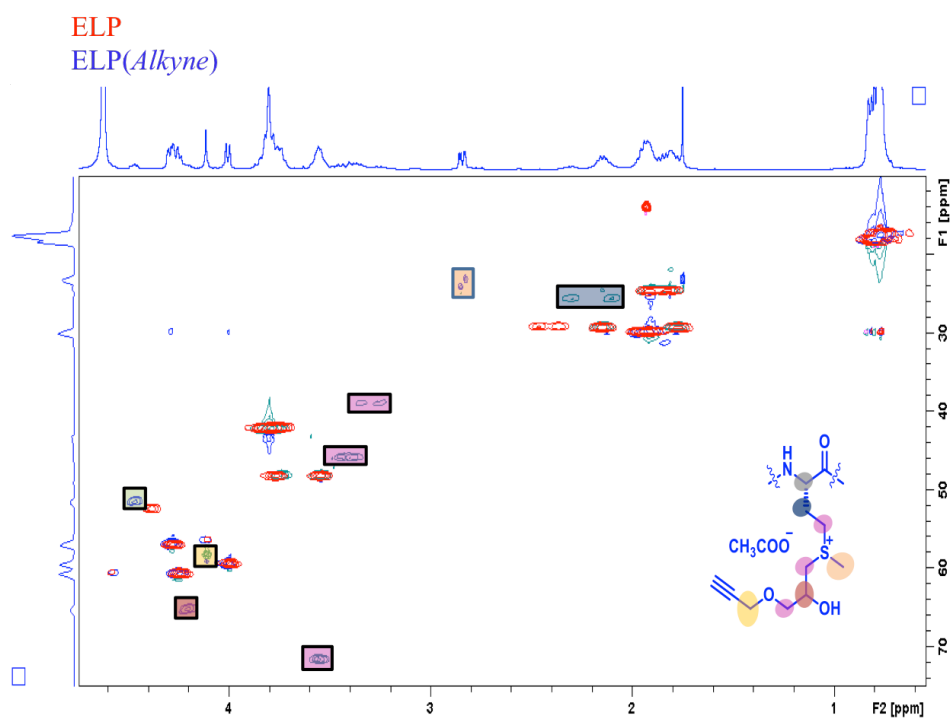**Figure S2.** ^{1}H-NMR spectrum of ELP(*Alkyne*) in D$_2$O at 25 °C.

Figure S3. HSQC spectra of ELP (in red) and ELP(*Alkyne*) (in blue and green) in D$_2$O at 25 °C. Positive peaks are blue (CH, CH$_3$) and negative peaks are green (CH$_2$).
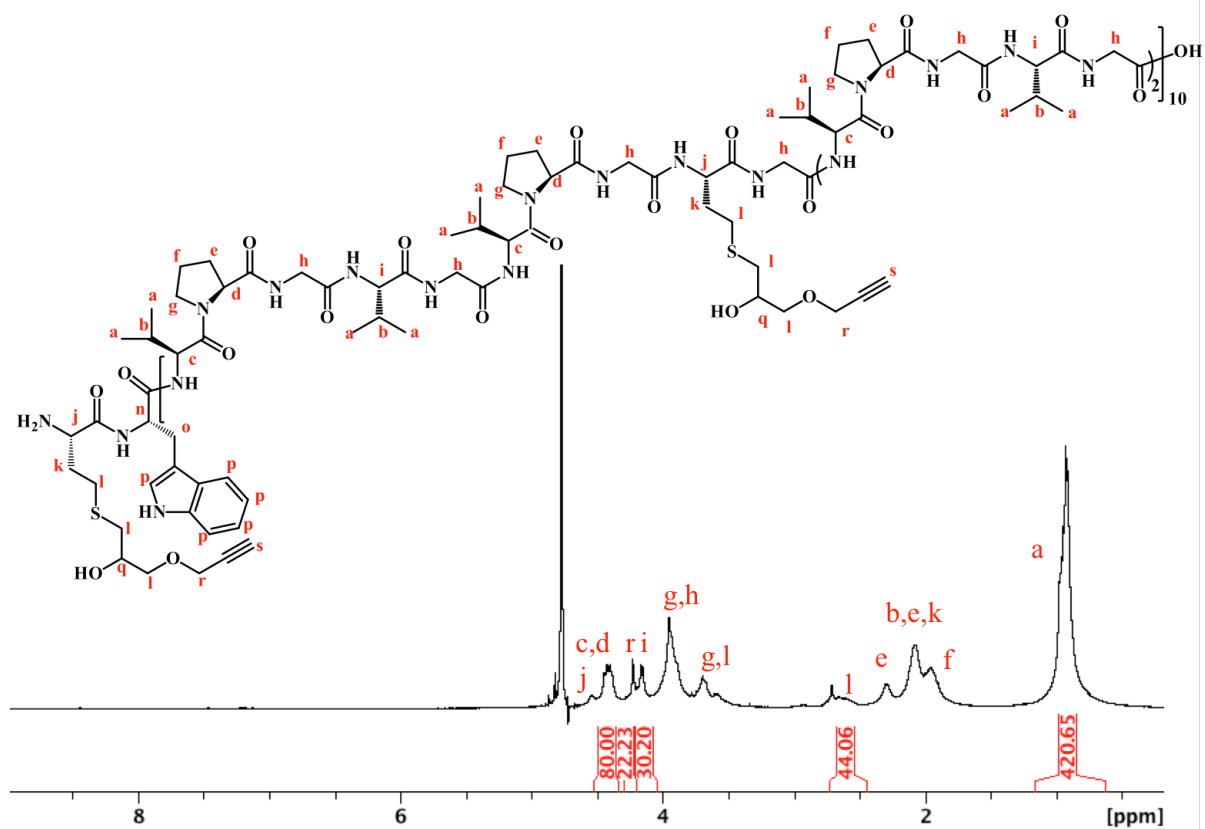
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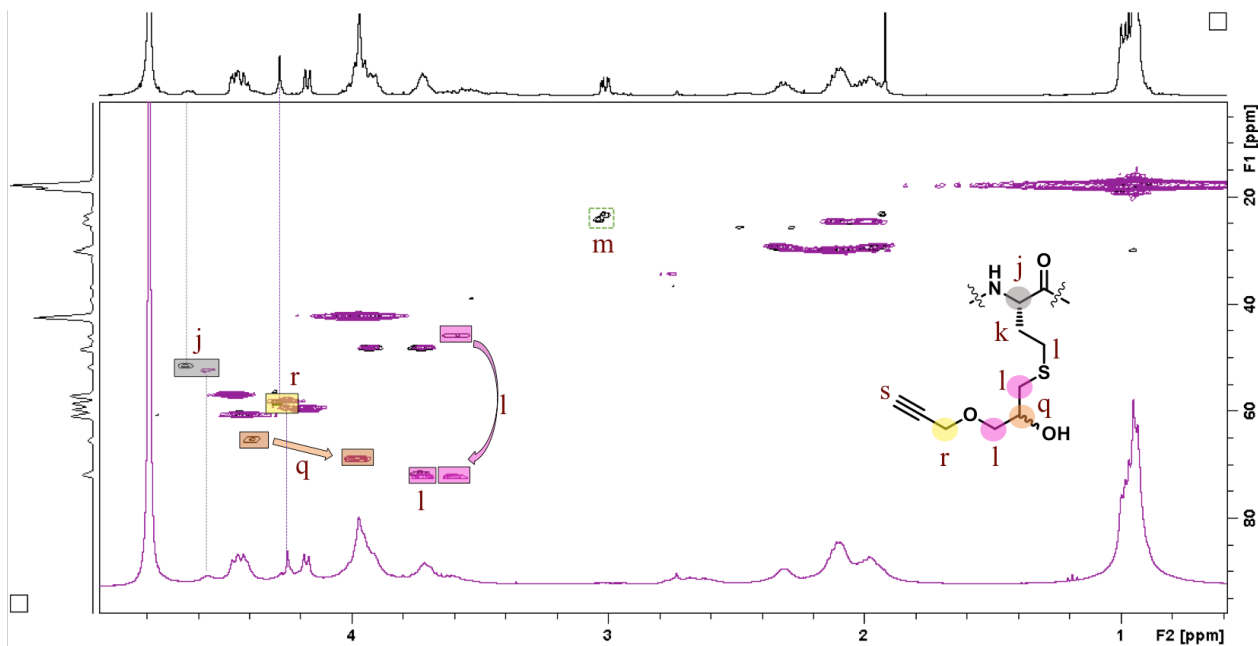**Figure S4.** ^{1}H-NMR spectrum of ELP*(Dem Alkyne)* in D_2O at 25 °C.

Figure S5. HSQC spectra of ELP*(Alkyne) (in black)* and b) ELP*(Dem Alkyne)* (in pink) in D_2O at 25 °C.
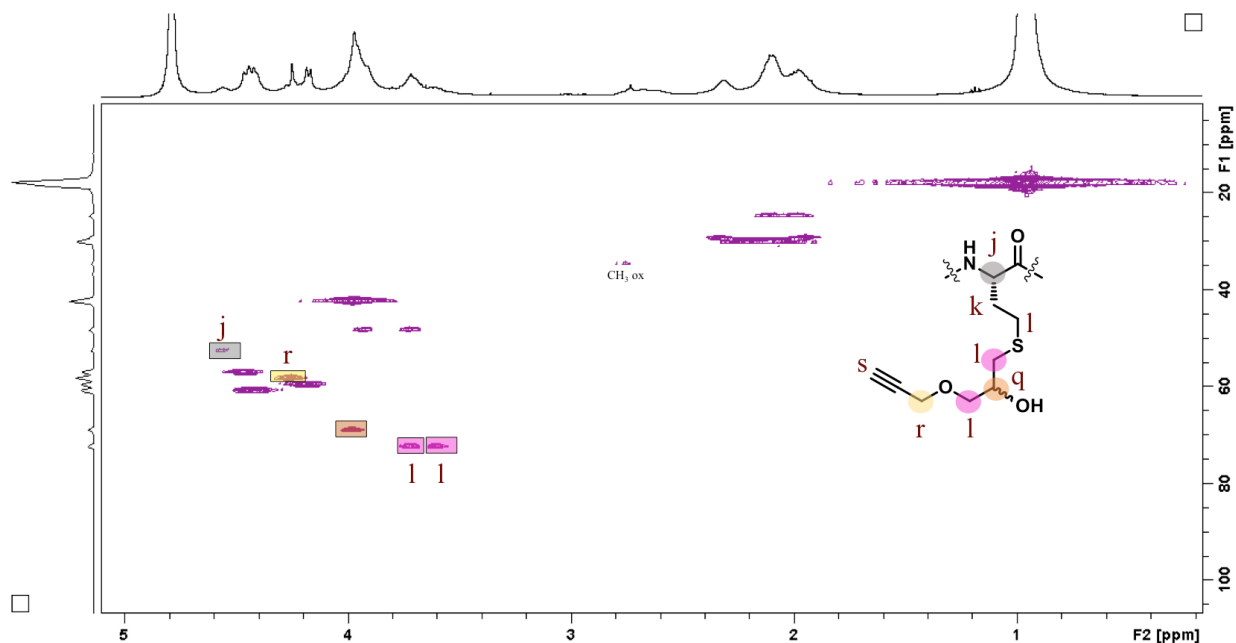
Figure S6. HSQC spectrum of ELP*(Dem Alkyne)* in D$_2$O at 25 °C.
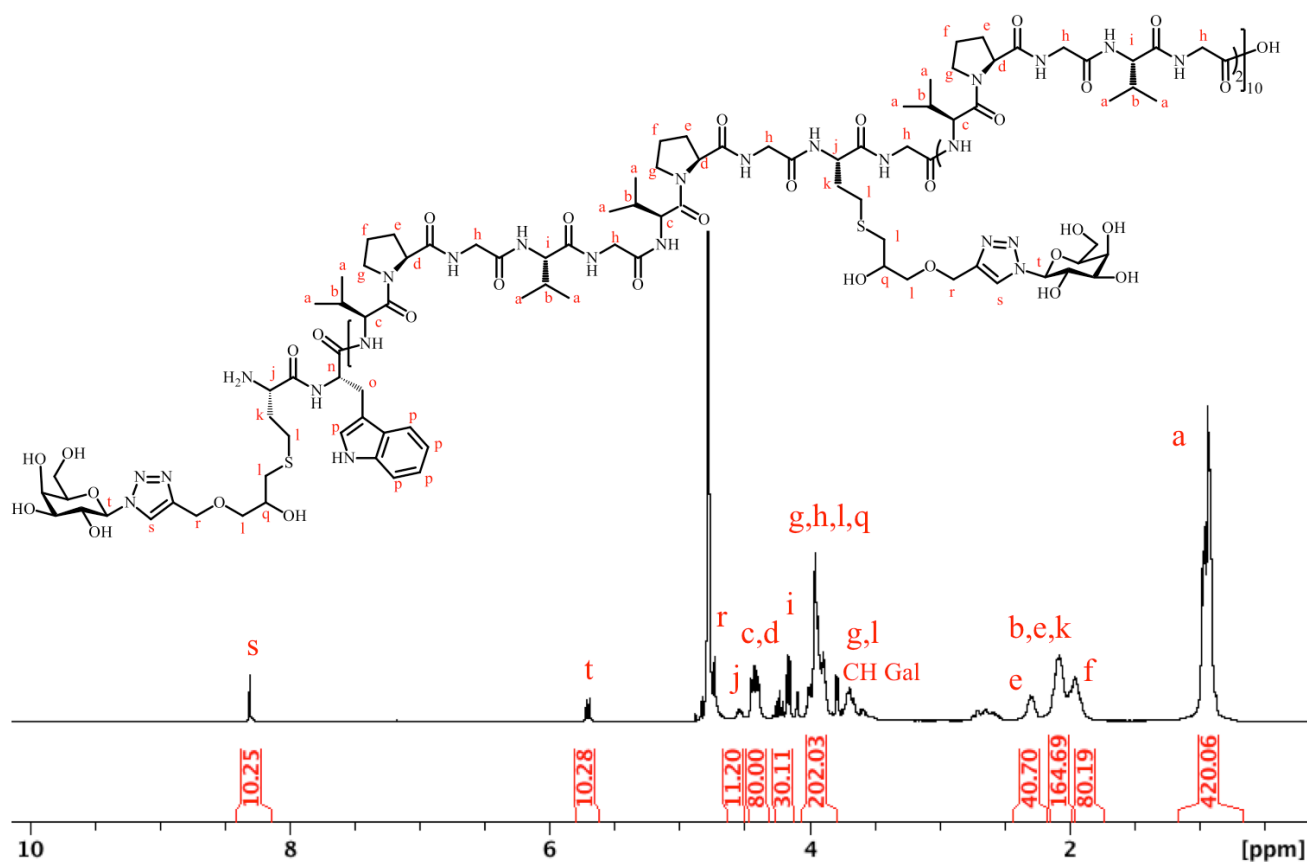
Figure S7. ^{1}H-NMR spectrum of ELP*(Gal)* in D$_2$O at 25 °C.

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a) ELP (*Dem Alkyne*)
ELP (*Gal*)
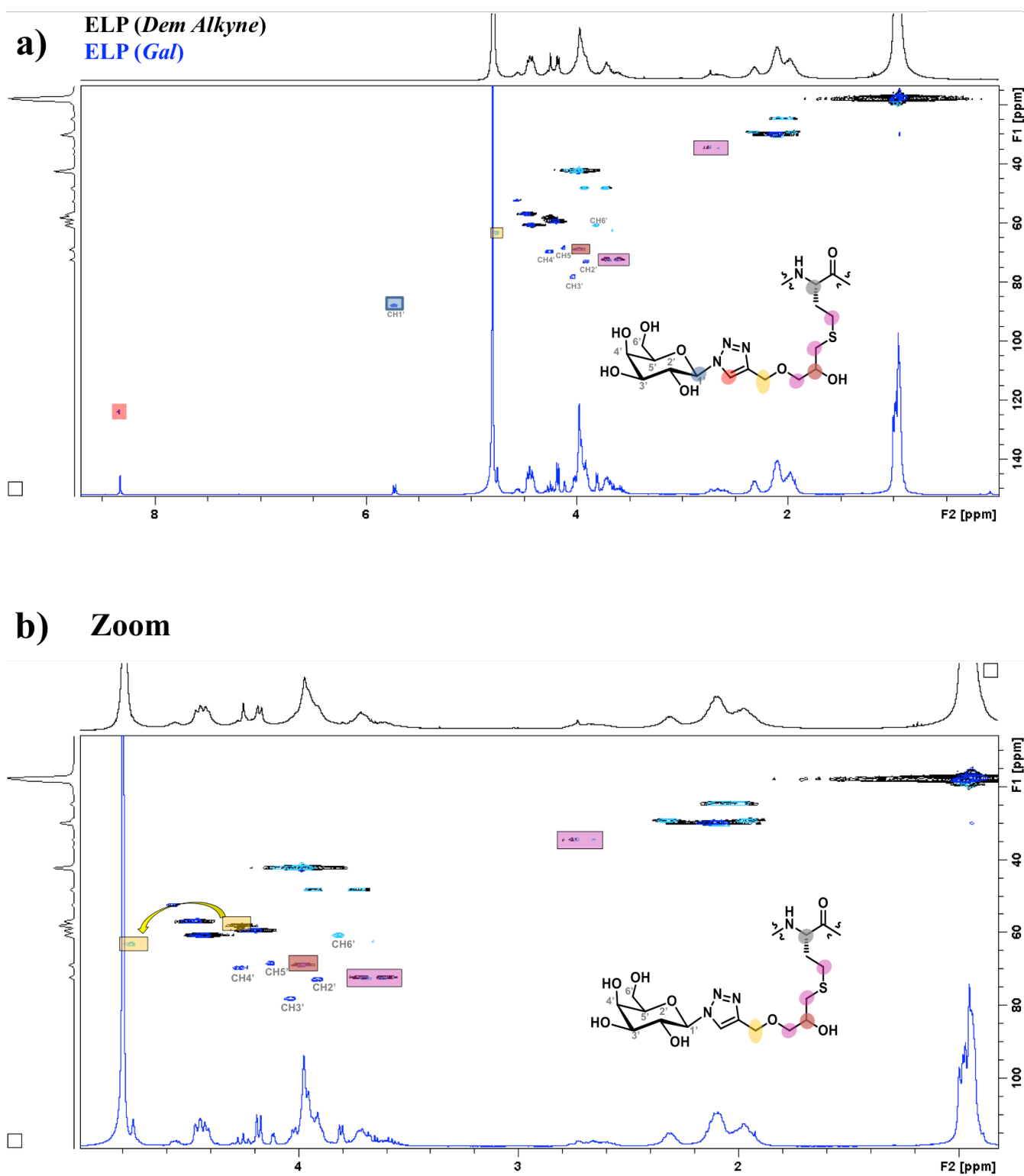
b) Zoom

Figure S8. a) Complete HSQC spectra and b) zoom of ELP*(Dem Alkyne)* (in black) and ELP*(Gal)* (in blue) in D_2O at 25 °C.
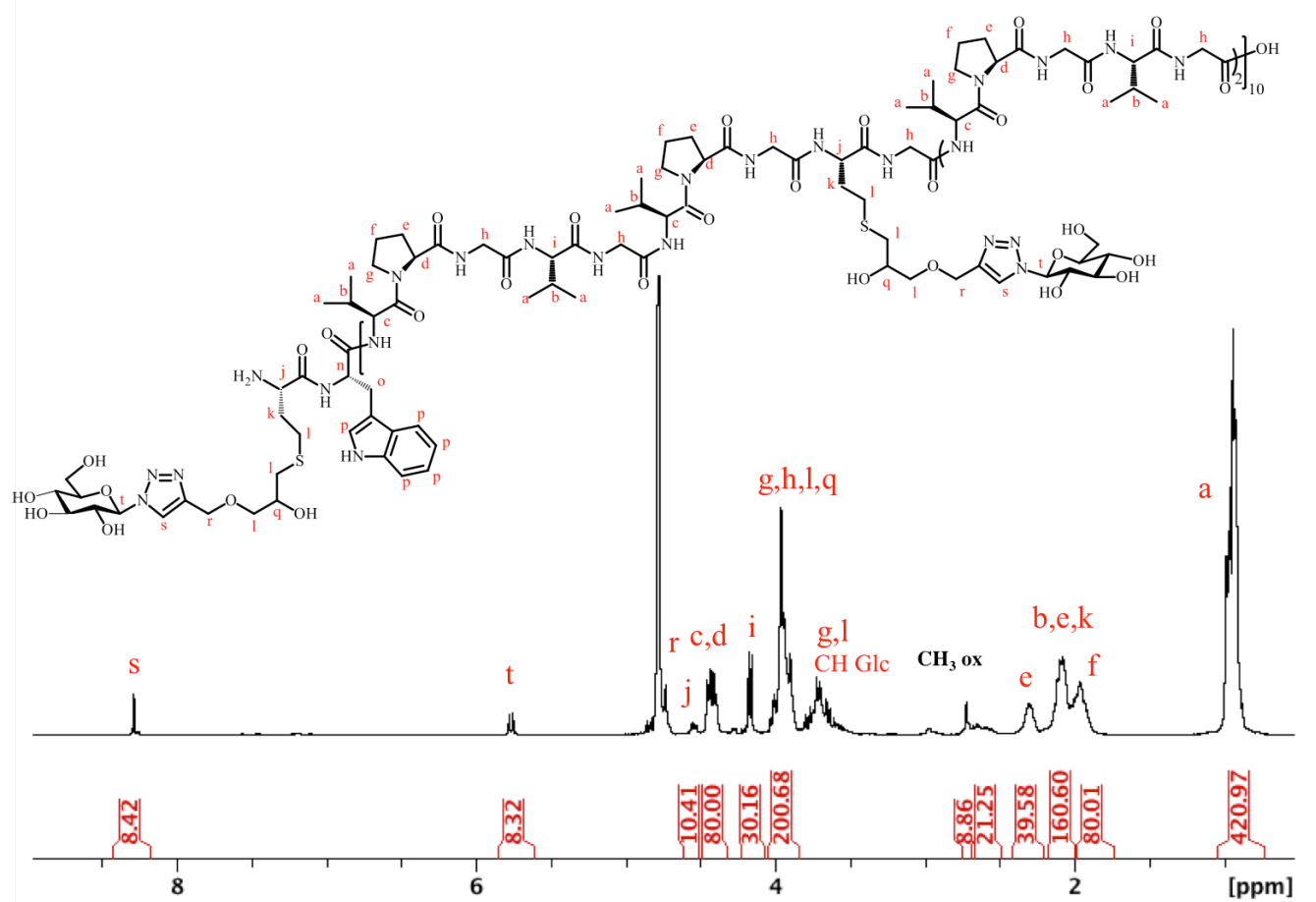
Figure S9. ^{1}H-NMR spectrum of ELP*(Glc)* in D$_2$O at 25 °C.

a) ELP(*Dem Alkyne*)
   ELP(*Glc*)
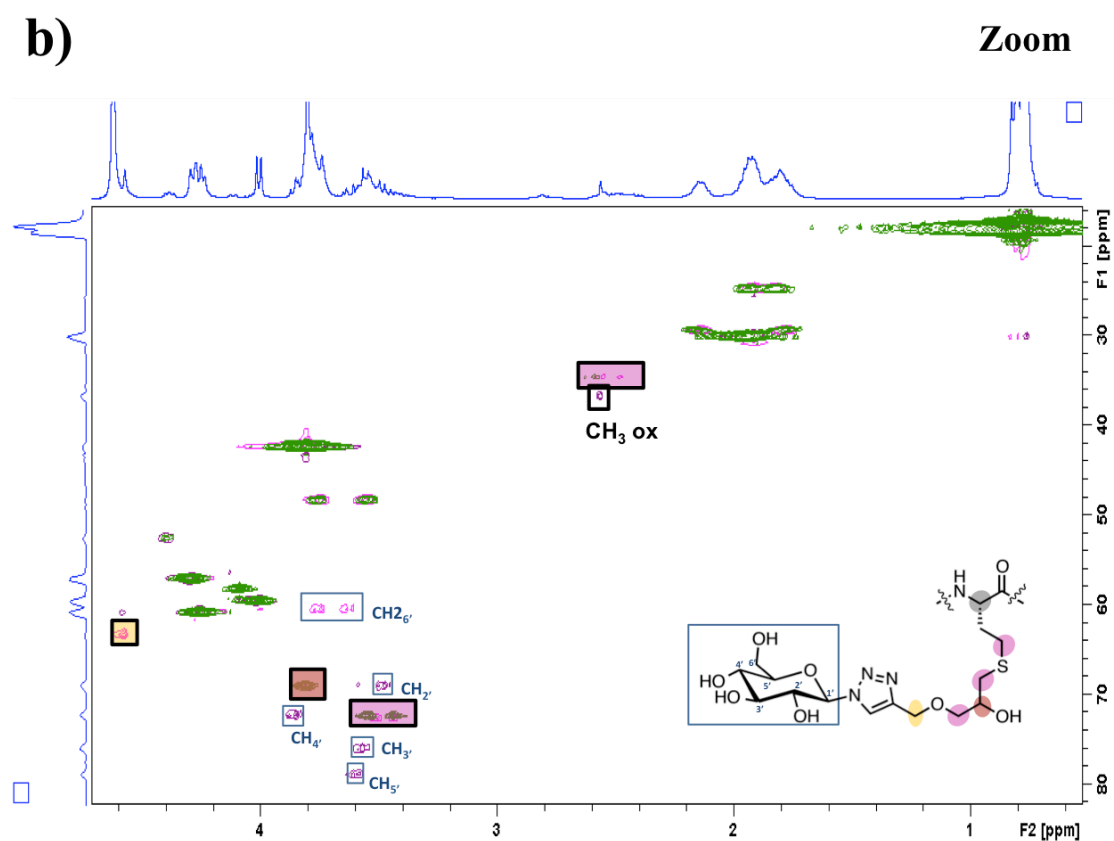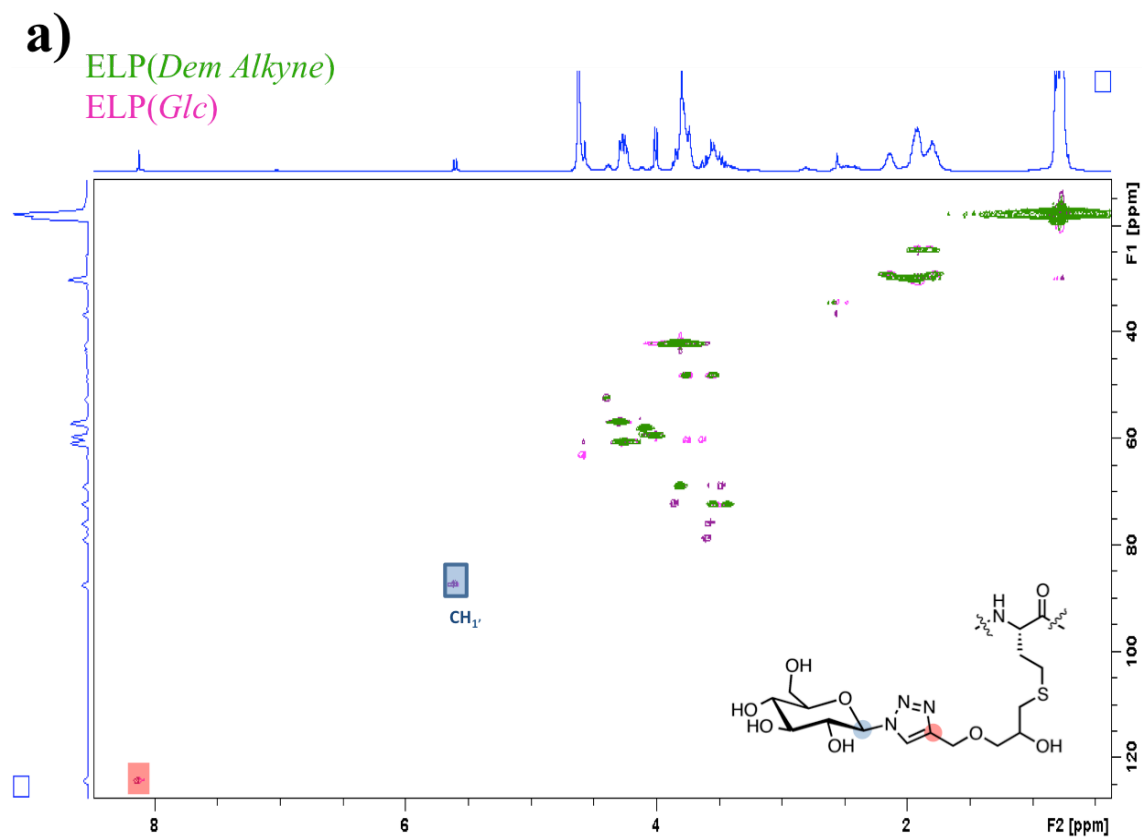
b) **Zoom**

Figure S10. a) Complete HSQC spectra and b) zoom of ELP*(Dem Alkyne)* and ELP*(Glc)* in D$_2$O at 25 °C.

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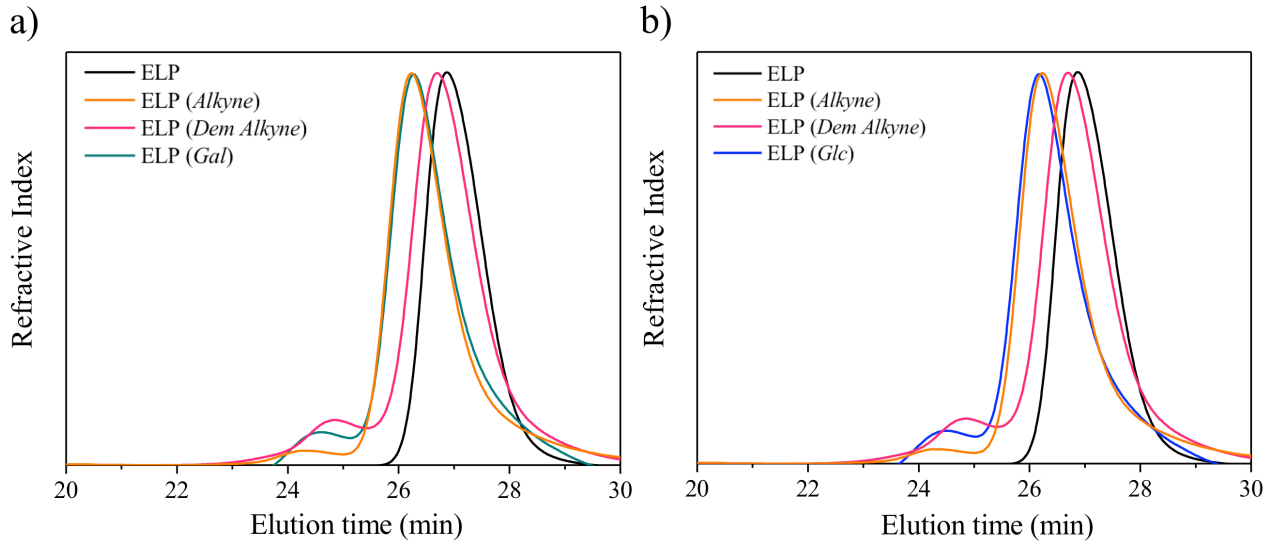
Figure S11. SEC traces in AcOH/ammonium acetate/ACN of ELP (black), ELP*(Alkyne)* (orange), ELP*(Dem Alkyne)* (pink) and (a) ELP*(Gal)* (green) or (b) ELP*(Glc)* (blue) using a RI detector.
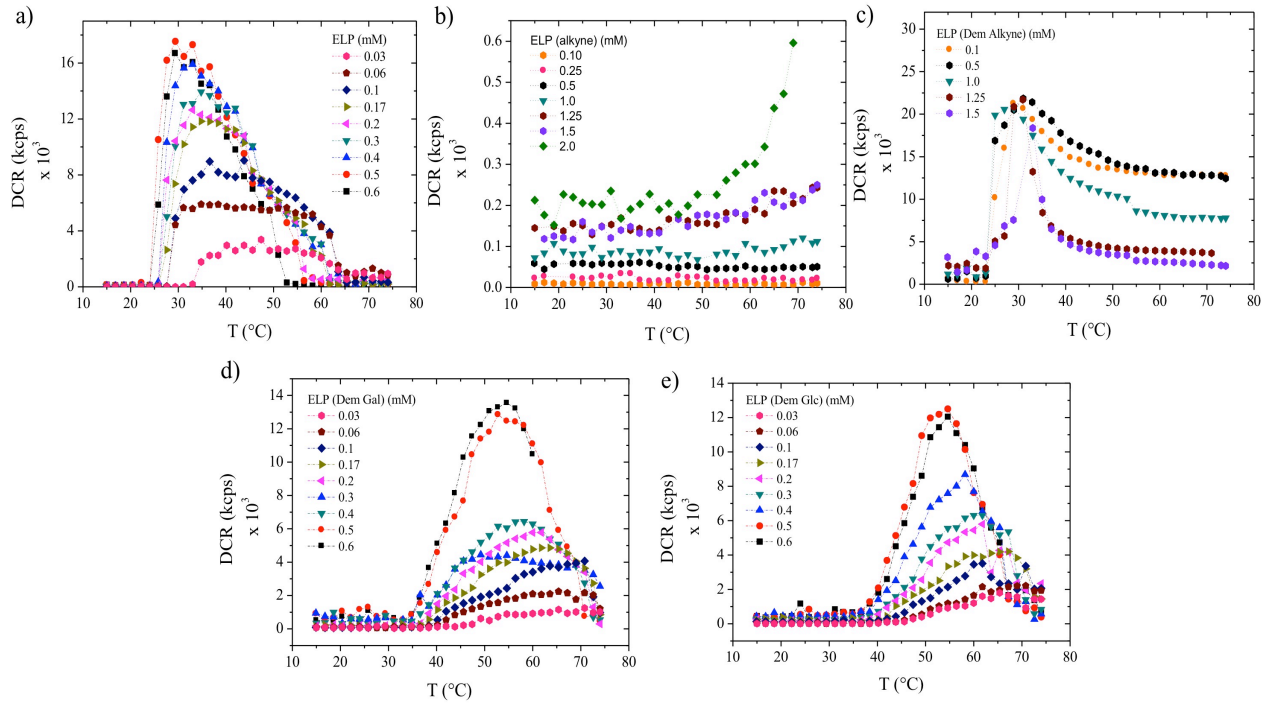

Figure S12. Scattered light intensity as a function of temperature of (a) ELP at concentrations from 0.03 to 0.6 mM, (b) ELP*(Alkyne)* at concentrations from 0.10 to 2.0 mM, (c) ELP*(Dem Alkyne)* at concentrations from 0.1 to 1.5 mM, (d) ELP*(Gal)* at concentrations from 0.03 to 0.6 mM and (e) ELP*(Glc)* at concentrations from 0.03 to 0.6 mM, in Trizma buffer (50 mM Trizma, 0.15 M NaCl).
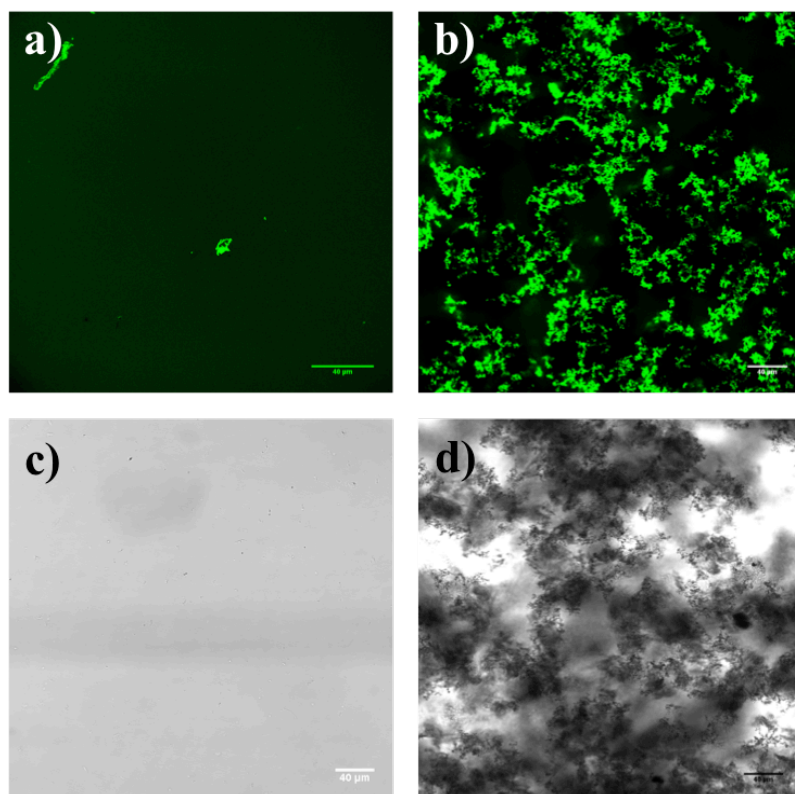
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Figure S13. a) Fluorescence microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/200 μM ELP*(Glc)* (green channel), b) Fluorescence microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/200 μM ELP*(Gal)* (green channel), c) Optical microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/ 200 μM ELP*(Glc)* (transmission), d) Optical microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/ 200 μM ELP*(Gal)* (transmission).
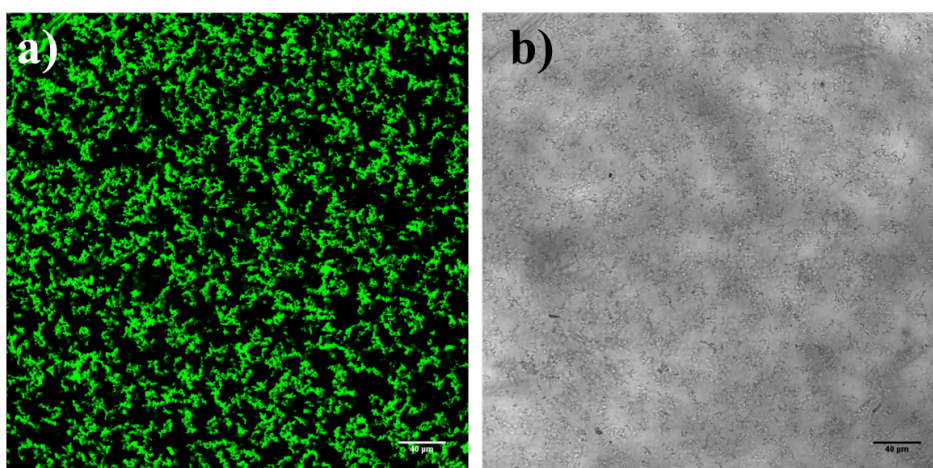

Figure S14. a) Fluorescence microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/ 200 μM ELP*(Gal)* (green channel) in presence of serum, b) optical microscopy image for 31.2 μM RCA$_{120}$-Fluorescein/ 200 μM ELP*(Gal)* (transmission) in presence of serum.

Table S1. T_t values of ELP, ELP(*Dem Alkyne*), ELP(*Gal*) and ELP(*Glc*) as a function of concentration.

ELP derivative	T_t (ºC) at 200 μM
ELP	26
ELP(*Alkyne*)	> 70
ELP(*Dem Alkyne*)	23
ELP(*Gal*)	36
ELP(*Glc*)	38

References

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[6]   Huang, J.; Bonduelle, C.; Thévenot, J.; Lecommandoux, S.; Heise, A. Biologically Active Polymersomes from Amphiphilic Glycopeptides**.** *J. Am. Chem. Soc.* **2012**, *134*, 119−122.

Author Contributions