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Design of Thermoresponsive Elastin-Like Glycopolypeptides for Selective Lectin Binding and Sorting

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Selective lectin binding and sorting using sugar-functionalized elastin-like polypeptides

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Abstract

Selective lectin binding and sorting was achieved using thermosensitive recombinant elastin-like polypeptide (ELP) glycoconjugates in simple centrifugation-precipitation assays. A recombinant ELP, $(VPGXG)_{40}$, was designed to contain periodically spaced methionine residues to enable chemoselective post-synthesis modification *via* thioether alkylation using alkyne functional epoxides. The resulting sulfonium groups were selectively demethylated to give alkyne functionalized homocysteine residues, which were then conjugated 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 measurements. 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 ~~the~~ ELP-RCA_{120} complex~~es~~. Based on these results, ELP glycoconjugates show promise as designer biopolymers for selective protein binding and sorting.

Keywords: Elastin-like polypeptides (ELPs), monosaccharides, chemoselective post-modifications, click chemistry, lectins.

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.[4-7] Lectins have recently been used to successfully detect cancer biomarkers in tissues and complex fluids.[13] Many lectins have been applied in several medical research areas, such as drug delivery[16,17], inhibition of cancer cell adhesion[18], inhibition of cancer cell growth[19], and biomarkers for disease detection and monitoring[13], due to their carbohydrate binding specificities. Currently, cancer biomarker discovery is an important field of research, since only a few ~~of them~~biomarkers are ~~frequently being used~~available for use in ~~the~~ clinical setting~~s~~.[20] However, identifying the authentic biological ligand(s) for each lectin is still challenging.[2,8,9] The development of new tools to ~~show~~ identify lectin specificity and enable lectin discovery present new opportunities for understanding and using lectin functions.[10]

Very interestingly, Bertozzi who pioneered development of glycopolymers to recognize~~d~~ specific lectins, reviewed ~~the developments~~their application in four main areas, *i.e.* in the enrichment of glycoproteins and glycopeptides from complex mixtures, in the identification of ~~sites of~~ protein glycosylation sites; in targeted glycoproteomics; and in functional glycoproteomics, all focus~~ing in~~ed on ~~showing~~ identifying interactions between glycoproteins and glycan-binding proteins.[3] In ~~a~~ pioneer~~ing ed~~ work, ~~by~~ using well-defined glycopolymers and Concanavalin A as the model receptor, the group of Haddleton explored the influence of the nature and densities of different sugar~~s~~ residues on the inhibitory potency of ~~the~~ glycopolymers.[30] 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.[31] This approach was expected to give rise to novel insights into the mechanisms of HIV infection and provide potential new therapeutics. In the same line, Gibson et al. developed complex glycopolymers bearing both primary glycan ligands and secondary units to modulate their selectivity toward lectins associated in disease.[32]

Among glycopolymers, glycopolypeptides are particularly interesting candidates for glycoprotein analogs since their structure gives them the potential for mimicking natural glycoprotein~~s~~ properties.[33-35] Synthetic chemists have developed molecular scaffolds such as glycopeptides, oligonucleotide templates, and cyclodextrin and aromatic scaffolds~~, among others~~.[36,37] Alternative synthetic routes such as ~~total~~ solid-phase total synthesis of glycopeptides[38], native chemical ligations of glycopeptide segments[38] and enzymatic synthesis of glycoproteins[39], have also been studied, and have been successfully used to access fully synthetic glycoproteins. However, these approaches present some limitations that prevent accurate structure/activity relationship correlations, either in terms of yield for the biosynthetic methods, or in terms of chain-length dispersity for chemical polymerization methods.

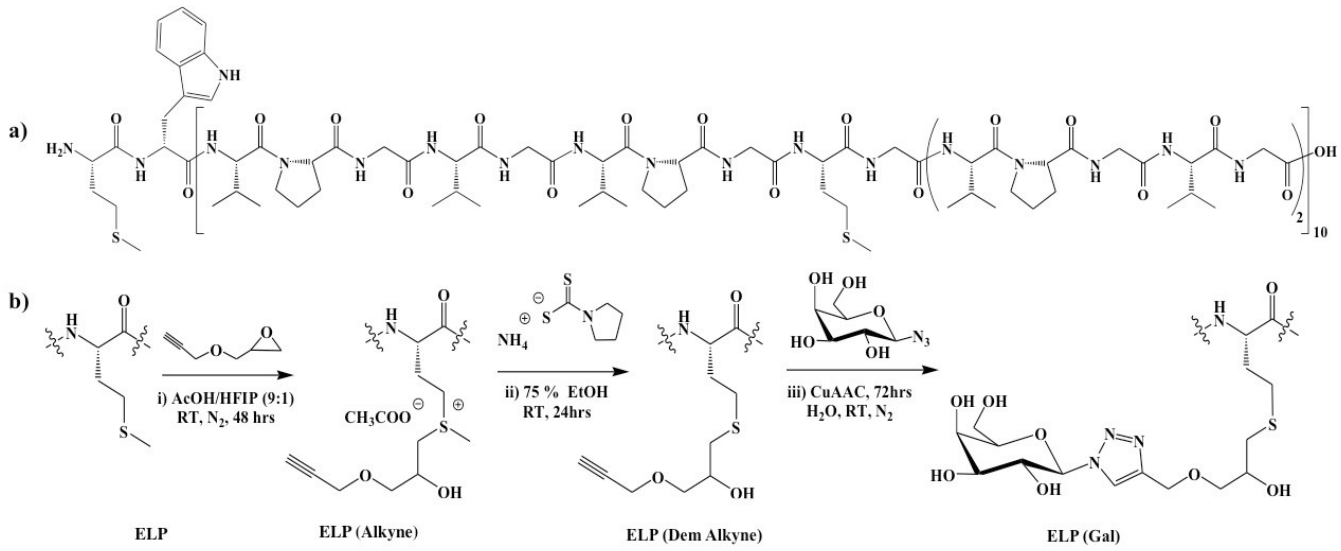
Here we sought to develop a method for producing thermosensitive glycopolypeptides of uniform length with precise carbohydrate spacing based on recombinant elastin-like polypeptides (ELPs). Specifically, we sought to utilize the temperature dependent solution properties of ELPs to control the interaction 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 ~~by taking advantage of the temperature dependent solubility of ELP glycoconjugates~~that allowed enrichment and separation of the complexes.

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.[40-42] 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 Tt, into insoluble aggregates forming a coacervate phase.[43,44] This temperature can be precisely tuned by modifying the initial design of the ELP, namely the amino acid composition and the ELP chain length[45-47], or by the subsequent chemical modification of the ELP backbone.[33,34] Post-synthesis modifications of ELPs reported in the literature often target their chain ends to graft small organic molecules[48], oligonucleotides[49], drugs[50] or PEG chains[51]. Modifications at the amino acid side chains within the ELP domain have recently been explored generating a wide ~~option~~ variety of chemical functionalizations[41,42,52] Recently, our group reported a method that allows chemical post-synthesis modifications by alkylation/oxidation of selectively introduced methionine residues, allowing both the modulation of T_t as well as the introduction of functional groups[42]. This method has improved the versatility of ELP post-synthesis modifications to introduce new functionalities and impact ELP properties such as the tuning of ELP derivative temperature responsiveness. However, up to now, ELPs have ~~never~~ 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 work is thus focused~~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 side chains of $(VPGXG)_{40}$, where X=Val/Met, 3:1, here simply named ELP (**Scheme 1a**)[41]. This ELP was chemoselectively modified so that the Tt at certain concentrations could be tuned to temperatures lower than 40 °C since this is a temperature at which some proteins start to denature and hence would be useful for temperature dependent sorting of proteins bound to the ELP. All 11 methionine residues of the ELP were chemoselectively alkylated using an alkyne-containing epoxide (**Scheme 1b**).[42] Although the initial ELP sulfonium derivative,

ELP*(Alkyne)*~~—~~, is too ~~water soluble~~hydrophilic to possess a useful Tt, a thermoresponsive ELP with an appropriate Tt was obtained through subsequent demethylation ~~(using ammonium pyrrolidinedithiocarbamate, APDC, in 75% ethanol)~~ of the sulfonium groups (using ammonium pyrrolidinedithiocarbamate, APDC, in 75% ethanol), ~~giving~~ to give ELP*(Dem Alkyne)*. ELP*(Dem Alkyne)* was then ~~used to~~ conjugated with different azido-functionalized monosaccharides, *i.e.* galactose and glucose, via copper-catalyzed azide-alkyne cycloaddition (CuAAC).[53,54] The~~se~~ reaction conditions used (H_2O, Cu(II)SO_4, sodium ascorbate, PMDETA) ~~are~~ were similar to those described by *Deming et al.* for the modification of alkyne-functionalized statistical copolymers of Met and Lys[55]. 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.[56]



Scheme 1. a) Amino acid sequence of ELP, b) Three step reaction scheme to access ELP*(Gal):* alkylation with glycidyl propargyl ether to introduce alkyne functionality giving to ELP*(Alkyne)*, demethylation of ELP*(Alkyne)* to give ELP*(Dem Alkyne),* and click chemistry with azido-monosaccharides to give ELP*(Gal)* or ELP*(Glc)*.

The degree of functionalization of ELP*(Alkyne)* after alkylation at the thioether group was determined through ^{1}H NMR spectroscopy as described previously (**Figure 1a and b**).[41] ^{1}H NMR spectra were calibrated using the resonances

centered at 4.45 ppm, which correspond to the αCH protons of the initial 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 of around 92 ± 2% was obtained for synthesized ELP*(Alkyne)* at this step. A small amount of Met oxidation into Met sulfoxides during the reaction (less than 10%) was detected in ELP*(Alkyne)*, as shown 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, functionalization after click chemistry was assessed by ^{1}H NMR spectroscopy by the disappearance of the resonance at 4.3 ppm corresponding to the methylene group in α position to the alkyne group and the appearance of a resonance at 8.2 ppm attributed to the proton of the triazole ring, as well as the appearance of the resonance at 6.18 ppm, attributed to the anomeric proton of the monosaccharide, where both of these integrated as 11 protons indicating 100% functionalization (**Figures 1d, S7, S8** and **S9**). After click chemistry, functionalization yields for each glycopolypeptide were found to be 91 % for ELP*(Gal)* and 82 % for ELP*(Glc)*. ELPs derivative molecular weight shifts during each step of chemical modification were followed by SEC measurements in aqueous solvent (acetic acid 0.3 M, ammonium acetate 0.2 M /ACN; 6.5/3.5, v/v). SEC traces showed accurate shifts for each molecular weight and the appearance of a slight dimerization (less than 10 %), which is negligible for the purposes of this work (**Figure S10**).

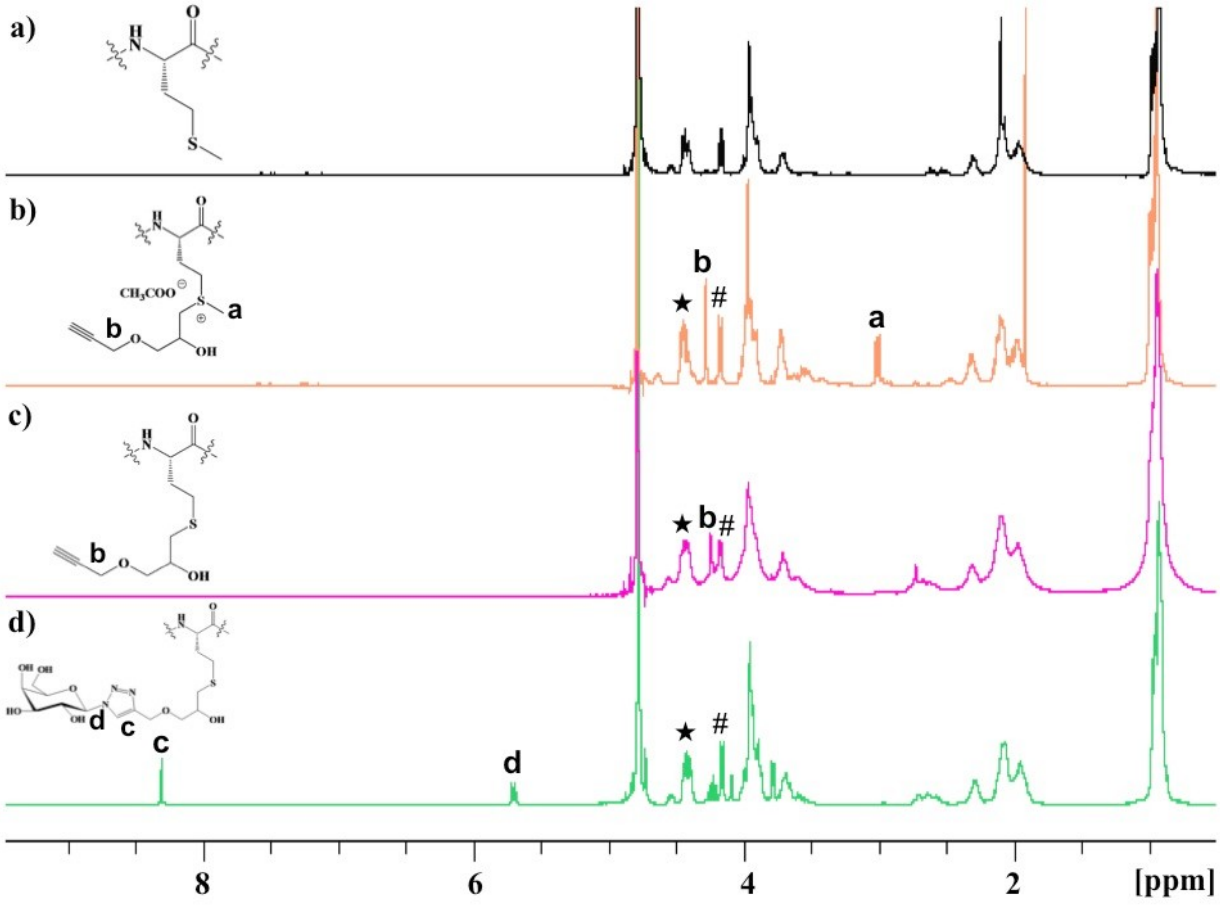


Figure 1. ^{1}H NMR spectra of a) ELP, b) ELP*(Alkyne)*, c) ELP*(Dem Alkyne)* and d) ELP*(Gal)* in D_2O at 298 K. Resonance # corresponds to *Val* αCH of the guest residue in VPGVG repeat units, and resonance ★ corresponds to *Val* αCH and *Pro* αCH of VPGXG repeats.

Considering the relevance of chemical modifications of Met-containing ELPs on their thermoresponsive properties,[41,42,57] 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 S11**). **Figure 2a** shows the scattered light intensity as a function a temperature for 200 μM aqueous solutions of the different ELP derivatives and glycoconjugates, which was measured and plotted versus increasing temperature from 15°C to 74°C, which allowed determination of the onset temperature of aggregation (Tt). It was observed that ELP*(Alkyne)* does not show any aggregation temperature as compared to the thermo-responsive behavior of the parent ELP (Tt = 26 °C). This phenomenon was readily

attributed to the increased hydrophilicity of the thioalkylated ELP resulting from the positively charged sulfonium groups, as well as the hydroxyl groups originating from the epoxide ring opening during the thioalkylation reaction. After demethylation, a much lower Tt was found at around 23 °C for ELP*(Dem Alkyne)*, showing that the removal of the multiple charged sulfonium groups allowed the ELP derivative to recover their thermoresponsive properties.[27] Glycopolypeptides, *i.e.* ELP*(Gal)* and ELP*(Glc)*, showed very similar thermoresponsive behavior, presenting Tt values of ~~around~~ 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 the precipitation of ELPs. The increase in T_t observed after glycosylation of the ELP derivatives was expected due to the contributions of the hydrophilic ~~character of~~ saccharides. As shown by *Miyajima et al.*[58], 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 for ELP*(Alkyne),* which ~~completely~~ lacks thermoresponsive behavior, T_t versus concentration was plotted and can be used to determine accurate estimates of T_t values at a desired concentration (**Figure 2b**).

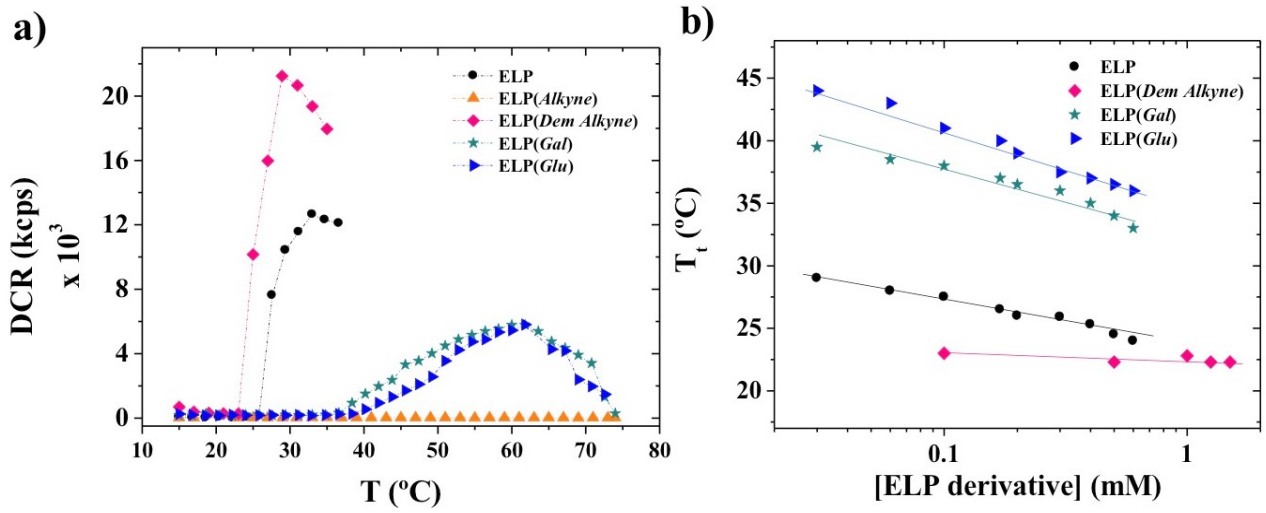


Figure 2. a) Scattered light intensity of ELP derivatives, *i.e.* ELP, ELP*(Alkyne)*, ELP*(Dem Alkyne)*, ELP*(Gal)* and ELP*(Glc)* as a function of temperature at 200 μM in Trizma buffer (50 mM Trizma, 0.15 M NaCl), b) T_t values of ELP(black circles), ELP(*Dem Alkyne*) (pink diamonds), ELP(*Gal*) (green stars) and ELP(*Glc*) (blue triangles) as function of concentration.

Dynamic light scattering experiments were then used to determine the binding capacity of ELP(*Gal*) and ELP(*Glc*) to specific lectins by monitoring aggregation behavior as depicted by the evolution of the correlation functions of different species and 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.[59] **Figure 3a** shows that the correlation function for RCA_{120} corresponds to that of small individual colloidal particles[60], while glycosylated ELPs, either with glucose or with galactose, present nearly the same correlation function with longer decay times due to their larger colloidal size. The solution of RCA_{120} mixed with 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}), displays a correlation function depicting strong aggregation of ELP*(Gal)*, as a consequence of binding affinity between this ~~glycosylated copolymer~~glycoconjugate and RCA_{120}[61]. This kind of aggregation phenomena has also been observed through kinetic studies of light absorbance for complexes of glycopeptide polymersomes formed from $PBLG_{20}$-*b*-PGG_{25} copolymers bearing galactose units with RCA_{120}[62]. On the other hand, ELP*(Glc)*, chosen as a negative control that should not bind to RCA_{120},[61] did not show any hallmarks of aggregation. **Figure 3b** shows the evolution of correlation functions of RCA_{120} solutions at the same concentration (31.2 μM), and after adding increasing amounts of ELP*(Gal)*. Interaction and aggregation ELP*(Gal)* with RCA_{120} were detected as soon as 2 μM of ELP*(Gal)* was added to the RCA_{120} solution, which corresponds to a stoichiometry of around 3 RCA_{120} active sites per sugar unit.

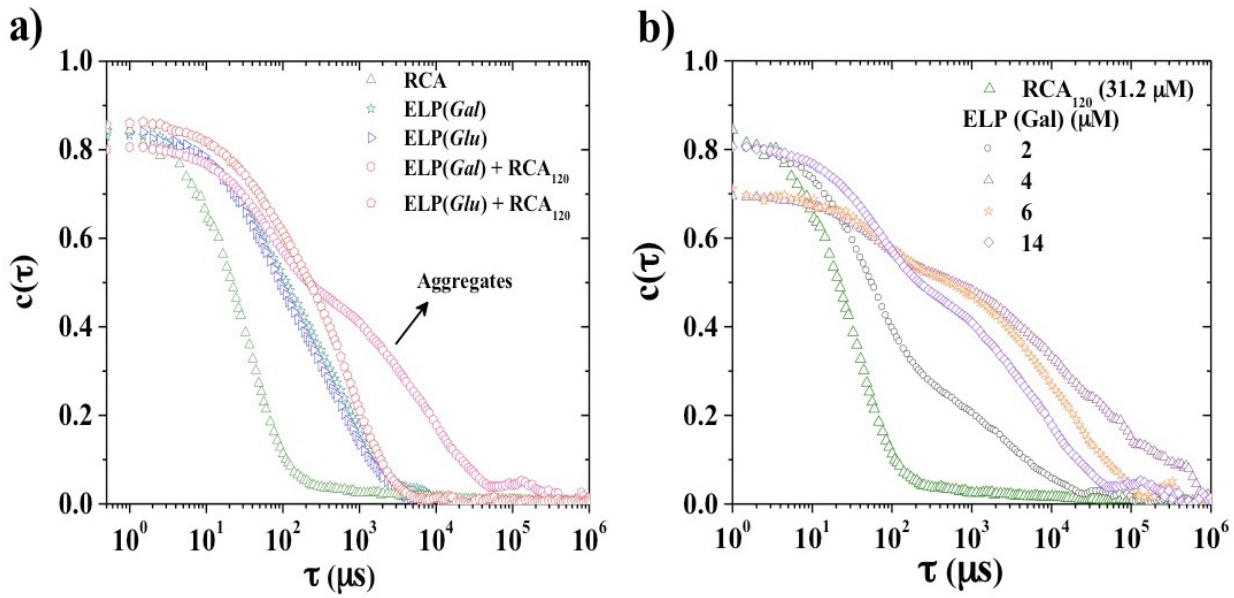


Figure 3. a) Correlation functions of 31.2 μM RCA, 200 μM ELP*(Gal)*, 200 μM ELP*(Glc)*, 31.2 μM RCA/ 200 μM ELP*(Gal)* and 31.2 μM RCA/ 200 μM ELP*(Glc)* at 25 °C, b) Evolution of RCA (31.2 μM) correlation function during progressive addition of ELP*(Gal)* at 25 °C.

After demonstrating the selective binding ability of ELP*(Gal)* to RCA_{120} lectin, we sought to take advantage of the thermoresponsive behavior of ELPs and their

ability to form coacervates 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 serum containing media was then assayed in temperature-triggered aggregation-centrifugation experiments (**Figures 4a to 4d**). 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. AWith ELP(*Gal*), a distinct pellet was separated from the supernatant, as observed under light and fluorescence microscopy (**Figure 4b, Figure S12**). When the negative control ELP*(Glc)* was used, no pellet was formed and soluble RCA_{120}-fluorescein was recovered in the supernatant as observed by fluorescence (**Figure S12**). In contrast, when RCA_{120}-fluorescein was incubated with ELP*(Gal)* above Tt, only a very weak fluorescence signal was recovered in the supernatant. This can readily be explained by the binding of RCA_{120} to ELP*(Gal)* derivative and the temperature-induced aggregation of the ~~of~~ 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 Tt of ELP*(Gal)*, which occurred even in the presence of serum (**Figures 4c and 4d**).

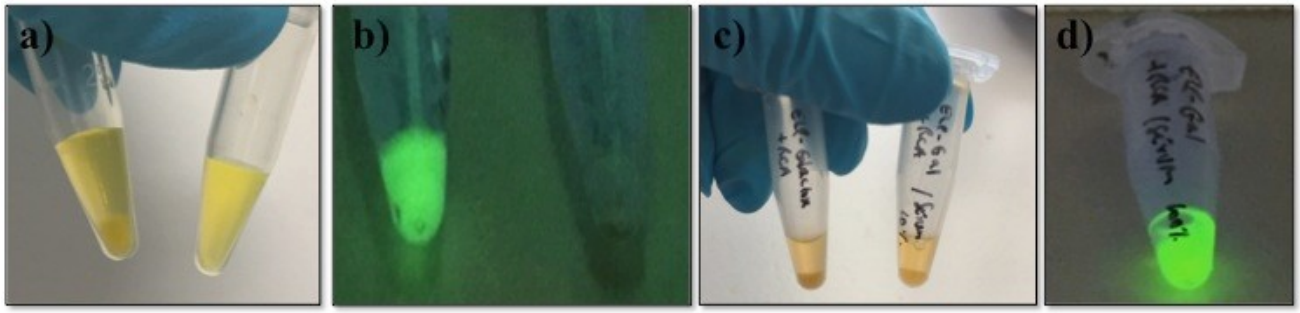


Figure 4. a) 31.2 μM RCA_{120}-Fluorescein/ 200 μM ELP*(Gal)* at left and 31.2 RCA_{120}-Fluorescein/ 200 μM ELP*(Glc)* at right were incubated at 37 °C, b) 31.2 RCA_{120}-Fluorescein/ 200 μM ELP*(Gal)* at left and 31.2 RCA_{120}-Fluorescein/ 200 μM ELP*(Glc)* at right were centrifuged at 40 °C, then, supernatant was removed and any pellet formed was re-suspended, sample was illuminated with an UV-Vis lamp at 365 nm, c) 31.2 μM RCA_{120}-Fluorescein/ 200 μM ELP*(Gal)*, at left, and 31.2 RCA_{120}-Fluorescein/ 200 μM ELP*(Gal)* in the presence of serum, at

right, were incubated at 37 °C, d) 31.2 μM RCA_{120}-Fluorescein/ 200 μM ELP*(Gal)* in presence of serum was centrifuged at 40 °C, then, supernatant was removed and the pellet was re-suspended, sample was illuminated with an UV-Vis lamp at 365 nm.

Fluorescence microscopy measurements were next performed to analyze the resulting mixtures between ELP glycoconjugates and proteins. Samples of 31.2 μM RCA_{120}-Fluorescein and 31.2 μM RCA_{120}-Fluorescein/ 200 μM ELP*(Glc)* were analyzed as negative controls and are presented in **Figures 5a** and **5c**. In those two images, no microscopic aggregate structure formation is 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 S12**). Interestingly, the structures formed at 25 °C from the mixture between 31.2 μM RCA_{120}-Fluorescein and 200 μM ELP*(Gal)* (**Figure 5b**), appear similar to disordered fractal aggregates[63,64]. Such structures have been recently reported by *Roberts et al.*[65] for thermally responsive partially ordered polypeptides that combine stimuli-responsiveness of disordered ELPs and structural stability of poly(alanine) helices. Here, the specific binding of ELP*(Gal)* with RCA_{120} allows the formation of similar structures since RCA_{120} possesses two binding sites in its native state. 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 S13**). 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 ~~are~~ were ~~perceived~~ observed in 10% serum, in contrast to the abundant fractal aggregates observed for RCA_{120}-Fluorescein and ELP*(Gal)* mixtures in presence of different percentages of serum. When serum concentration was increased, aggregates were found to be more separated from each other, which could be due to the presence of a greater amounts of different ~~kind of~~ species present in serum. However, is it

important to note that the binding affinity between ELP*(Gal)* and RCA_{120} still occurs in such a complex mixture.

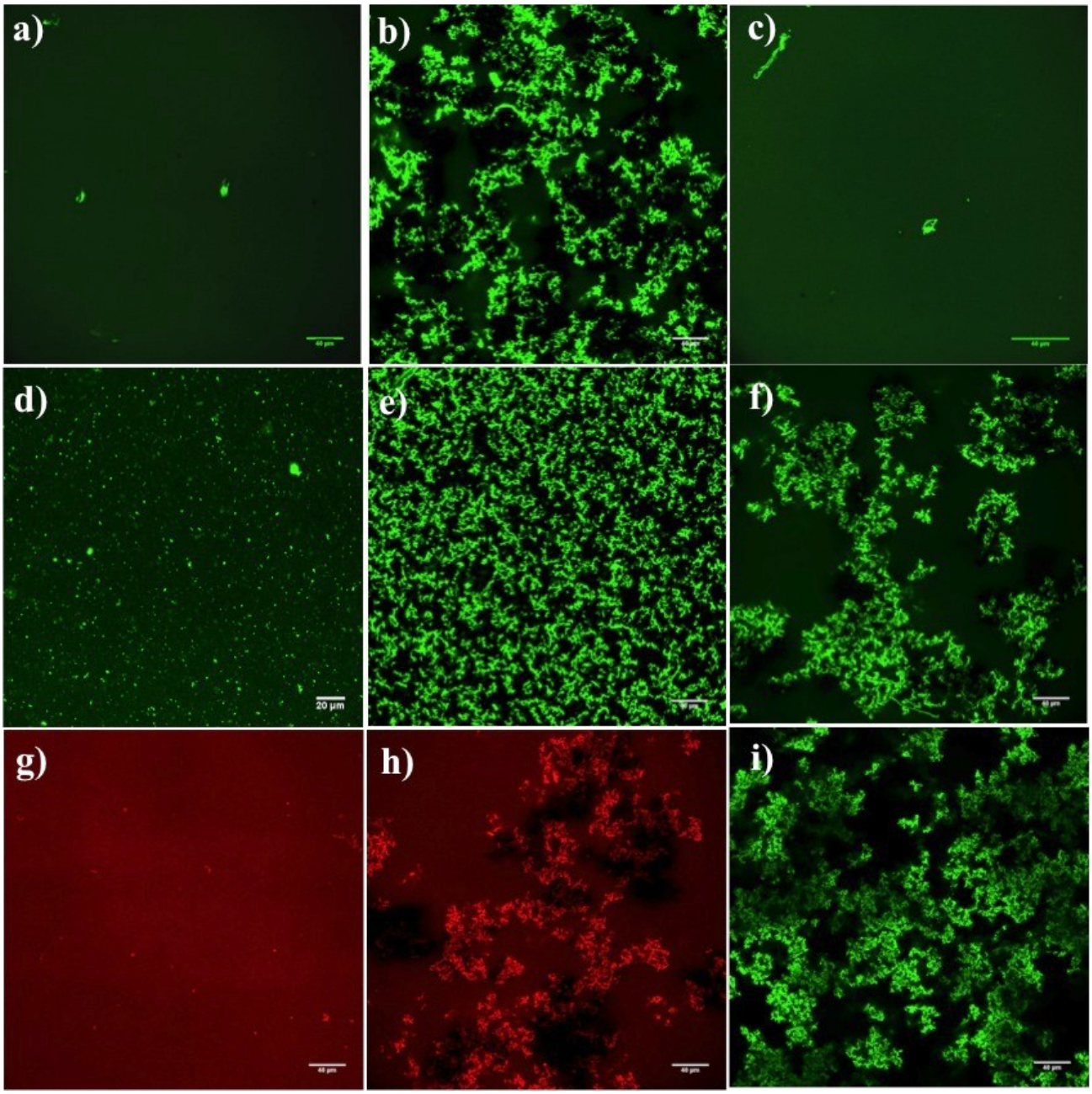


Figure 5. Fluorescence microscopy images for a) 31.2 µM RCA_{120}-Fluorescein (green channel), b) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Gal)* (green channel), c) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Glc)* (green channel), d) 31.2 µM RCA_{120}-Fluorescein in 10 % serum (green channel), e) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Gal)* in 10% serum (green channel), f) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Gal)* in 100 % serum (green channel), g) 31.2 µM BSA-Rhodamine/200 µM ELP*(Gal)* (red channel), h) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Gal)*/ 31.2 µM BSA-Rhodamine (red channel) and i) 31.2 µM RCA_{120}-Fluorescein/ 200 µM ELP*(Gal)*/ 31.2 µM BSA-Rhodamine (green channel).

The results above demonstrate that thermosensitive ELP glycoconjugates can be used to specifically aggregate and pull down specific lectins by temperature-triggered aggregation and centrifugation, at a reasonable temperature that limits protein denaturation. Our next goal was to try to fish out a specific sugar-binding protein from complex protein mixtures. Thus, a mixture of 31.2 μM RCA_{120}-Fluorescein, 200 μM ELP*(Gal)* and 31.2 μM BSA-Rhodamine (as a model abundant serum protein) was studied through centrifugation/precipitation assays and fluorescence microscopy observations. First, a mixture of BSA-Rhodamine and ELP*(Gal)* was analyzed as a negative control (**Figure 5g).** We observed that no aggregation took place in this mixture. Then, a mixture of RCA_{120}-Fluorescein, ELP*(Gal)* and BSA-Rhodamine was prepared, incubated and analyzed. After centrifugation at 40 °C, the collected supernatant did not show a fluorescence signal from the RCA_{120}-fluorescein, but high fluorescence signal was obtained after re-suspension of the corresponding pellet in cold buffer (more details and centrifugation assays in **Figure S14**). Finally, the resulting suspension was analyzed in both red and green fluorescence channels by fluorescence microscopy (**Figures 5h** and **5i**, respectively). We found that ELP*(Gal)* maintains its binding affinity with RCA_{120}, although some BSA might be trapped to some extent in the network formed by the glycopolypeptide/lectin interactions.

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). Complete mini EDTA-free protease inhibitors were purchased from Roche Diagnostics (D). Glycidyl propargyl ether was obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, FR). Glacial acetic acid, Trizma® and Hexafluoroisopropanol (HFIP) were obtained from Sigma-

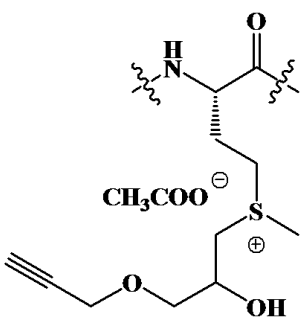
Aldrich (FR). Deionized water (18 MΩ-cm) was obtained by using a Millipore Milli-Q Biocel A10 purification unit. Cuprisorb was purchased from Seachem. Ethanol (96.0%, EtOH), methanol (98.5%, MeOH) and acetonitrile (99.9%, ACN) were obtained from VWR international. NaCl (99%) was purchased from Alfa Aesar (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). Bovine serum albumin (BSA), fraction V, approximately 99%, and human serum from human male AB plasma, USA origin, sterile-filtered, were used as received from Sigma-Aldrich (FR). BSA-rhodamine was synthesized as described previously [1]. N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA) was purchased from Sigma-Aldrich (FR). $CuSO_4.5H_2O$ 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 are the following are reported by R. Petitdemange et al. 2017 [2].

Modification of ELP using epoxides [2]

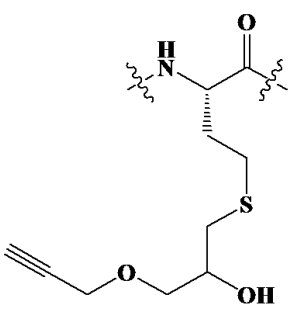
An amount of 50 mg of MW-[(VPGVG) (VPGMG) $(VPGVG)_2]_{10}$ was dissolved in 2.5 mL of an AcOH/HFIP mixture (9/1, v/v) (20 mg/mL). ELP solution was degassed by bubbling N_2 into the solution for 1 h, which was then stirred under N_2. Glycidyl propargyl ether was then added to the mixture (10 equiv. per methionine residue), which was stirred for 48 h under N_2 at room temperature. The obtained mixture was transferred into a 3 000 MWCO ultracentrifugal filter tube and washed with 40 mL DI water. The final content of the container of the ultracentrifugal filter tube was lyophilized to obtain an ELP*(Alkyne)* as a white solid.



Details of ELP(Alkyne): it was prepared from ELP-M-40 and glycidyl propargyl ether. ^{1}H NMR (400 MHz, D_2O, 25°C): (main peaks) δ 4.5−4.4 (m, 80 H, αCH VPGXG and *α*CH VPGXG), 4.3 (s, 22 H, OCH_2CCH), 4.2−4.15 (d, 30 H, αCH VPGVG), 3.06−2.9 (m, 33 H, SCH_3), 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) [2]

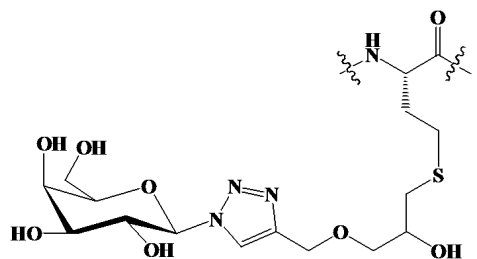
An amount of 50 mg of ELP*(Alkyne)* was dissolved in 75% EtOH (aq) at a 10 mM concentration and then treated with ammonium pyrrolidinedithiocarbamate, APDC, (5.0 equiv per Met residue). The solution was rapidly flushed with a stream of N_2 and rapidly capped. The reaction mixture was vortexed until obtaining 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 demethylated ELPs.



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 VPGVG), 4.25 (s, 22 H, OCH_2CCH), 4.2−4.15 (d, 30 H, αCH VPGVG), 2.8−2.55 (m, 44 H, CH_2SCH_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 %.

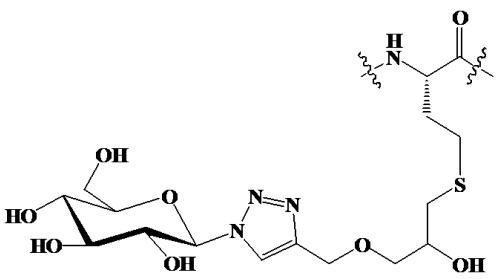
Synthesis of ELP glycoconjugates.

The demethylated alkyne-bearing ELP derivative 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 ultra-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 demethylated alkyne, 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 V̲PGXG and αCH VP̲GXG), 4.2-4.15 (d, 30 H, αCH VPGV̲G), 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 demethylated alkyne (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 V̲PGXG and αCH VP̲GXG), 4.2-4.15 (d, 30 H, αCH VPGV̲G), 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 298 K. 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 298 K. Data processing was performed using Bruker Topspin Software. Chemical shifts of amino acids are well-known in the literature [4,5].

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-M-40, 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 [6]. 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 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, Rhodamine was excited with DPSS laser at 561 nm (15%) using a 575-630 nm window. 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 mixed with RCA-Fluorescein (31.2 μM) in Trizma buffer were prepared to perform 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 [7]. Solutions were centrifuged at 40 °C for 20 minutes at 10,000 g. After separating the pellets and supernatants, the fluorescence spectra were obtained on a Jasco Spectrofluorometer FP- 8500.

Conclusion

We have reported the design and synthesis of uniform thermosensitive glycopolypeptides 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. Multivalent galactose or glucose-bearing ELPs possessed Tt 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 recognize and 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~~s~~ 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.

Supporting information

Experimental procedures, NMR spectra (^1H, ^{13}C and HSQC), SEC traces, DLS temperature ramps, T_t as function of temperature for ELP derivatives, fluorescence measurements and additional microscopy images are available in the supporting information.

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