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Lectin-Glycan-Mediated Nanoparticle Docking as a Step Towards Programmable Membrane Catalysis and Adhesion in Synthetic Protocells

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ABSTRACT: The spontaneous assembly of nanoscale building blocks into continuous semi-permeable membranes is a key requirement for the structuration of synthetic protocells. Engineering the functionality and programmability of these building units provides a step towards more complex cell-like entities with adaptive membrane properties. Inspired by the central role of protein (lectin)-carbohydrate interactions in cellular recognition and adhesion, we fabricate semi-permeable polysaccharide-polymer microcapsules (polysaccharidosomes) with intrinsic lectin-binding properties. We employ amphiphilic polysaccharide-polymer membrane building blocks endowed with intrinsic bio-orthogonal lectin-glycan recognition sites to facilitate the reversible non-covalent docking of functionalized polymer or zeolitic nanoparticles on the polysaccharidosomes. We show that the programmed attachment of enzyme-loaded nanoparticles

gives rise to a membrane-gated spatially localized cascade reaction within the protocells due to the thermoresponsiveness of the polysaccharidosome membrane, and demonstrate that extended closely packed networks are produced *via* reversible lectin-mediated adhesion between the protocells. Our results provide a step towards nanoscale engineering of bioinspired cell-like materials and could have longer term applications in synthetic virology, protobiology and micro-biosensor and micro-bioreactor technologies.

KEYWORDS: polysaccharide-polymer nanoconjugates, interfacial assembly, polysaccharidosomes, glycan-lectin bio-orthogonal interactions, membrane-gated cascade reactions, higher-order reversible assembly.

A diversity of artificial cell-like membrane-bounded architectures based on phospholipid vesicles¹,² inorganic colloidosomes,^{3, 4} fatty acid-,⁵ polyoxometallate-⁶ or copolymer-coated coacervate microdroplets,⁷ polymersomes,^{8, 9} protein-polymer microcapsules (proteinosomes),^{10, 11} dendrimersomes,^{12, 13} polypeptide microcapsules,¹⁴⁻¹⁶ DNAsomes¹⁷ and polysaccharide microcapsules^{18, 19} have been recently reported. As a general rule, membrane assembly is achieved *via* the spontaneous or directed organization of various supramolecular and nanoscale building blocks that are chemically crosslinked to produce protocells with continuous semi-permeable shells and enclosed aqueous-filled interiors. Although the nanoscale building blocks primarily act as structural components of the membrane, increasing the functionality of these units would provide a route to more complex protocells with engineered membranes. Building blocks can exhibit intrinsic functionalities for use for example in membrane-mediated enzyme²⁰ or inorganic catalysis,²¹ membrane-gated permeability¹¹ or isomerization-induced dynamic membrane actuation.²² Alternatively, the nanoscale building blocks can be functionalized post-assembly with grafted polymers to acquire auxiliary membrane properties such as pH-gated reactivity,²³ bio-

orthogonal adhesion,²⁴ biomolecular (streptavidin/biotin) recognition²⁵ and self regulated "breathing".²⁶

In this paper, we develop a strategy that combines both intrinsic and acquired membrane functionalization to generate polysaccharide-based protocells with multiple surface-mediated properties. Our approach is inspired by the central role played by carbohydrates in the mediation of cell-cell surface recognition and intercellular adhesion, as well as their intrinsic biocompatibility, biodegradability and biofunctionality^{27,28}. Although extensive studies have been undertaken on the glycosylation of synthetic amphiphilic polymers and their spontaneous self-assembly into nanoscale objects,²⁹⁻³² there are few examples of the use of such nanoconjugates for the construction of microscale protocells with single-layer semi-permeable membranes. Recently, proteinosomes displaying defined permeability towards the programmed release of biomacromolecules have been fabricated from mixtures of protein-polymer and dextran-polymer nanoconjugates³³ and giant unilamellar vesicles comprising glycosphingolipids assembled and used to induce protocell crosslinking *via* lectin-mediated interactions.³⁴

Herein, we describe the fabrication of cell-like membrane-delimited semi-permeable polysaccharide-polymer microcapsules using constituent nanoscale building blocks with intrinsic lectin-binding properties. We denote the micro-compartments as *polysaccharidosomes* given their resemblance to colloidosomes³⁵ and proteinosomes.¹⁰ To fabricate the polysaccharidosomes, we synthesise two types of thermoresponsive polysaccharide-polymer nanoconjugates and utilise the amphiphilic building blocks to prepare lectin-receptive protocells capable of selective surface binding of concanavalin A or wheat germ agglutinin. By attaching the lectins to polymer or zeolitic nanoparticles, we implement the programmed non-covalent docking of nanoscale units specifically on the outer membrane of the polysaccharidosome, providing a step towards the

targetted functional elaboration of synthetic cell-like objects. Specifically, we demonstrate how lectin-glycan-mediated attachment of enzyme-loaded nanoparticles can be used for the implementation of a membrane-gated spatially localized two-enzyme cascade reaction within the decorated polysaccharidosomes. We also develop procedures for the reversible assembly of tissue-like clusters and networks using lectin-mediated polysaccharidosome adhesion as a step towards the molecular programming of higher-order protocell organizations. Taken together, our results contribute to the advance of bioinspired cell-like materials with potential applications in synthetic virology, cell-protocell science and micro-biosensor and micro-bioreactor technologies.

RESULTS AND DISCUSSION

Fabrication of polysaccharide-polymer nanoconjugates and polysaccharidosomes.

Carboxymethyl dextran (Dex-COOH; M_w , *ca.* 40 kDa) and hyaluronic acid (HA; M_w 80 kDa) were functionalised with at least 106 and 86 primary amino groups per chain, respectively (Figure 1a, Supporting Information, Figures S1-S4 and Tables 1 and 2) and then covalently conjugated with mercaptothiazoline-activated poly(N-isopropylacrylamide (PNIPAAm; M_n = 22,368 g/mol, number of repeat units = 202) (Supporting Information, Figure S5) to produce aqueous solutions of amphiphilic Dex-NH₂/PNIPAAm (Dex-P) and HA-NH₂/PNIPAAm (HA-P) nanoconjugates. Formation of the polysaccharide-PNIPAAm nanoconjugates was confirmed by ¹H NMR spectroscopy (Supporting Information, Figures S6 and S7) and was consistent with changes in the zeta potential from initial values of -40 (Dex-COOH) and -16 mV (HA) to +27 and +21 mV for aminodextran (Dex-NH₂) and aminohyaluronic acid (HA-NH₂), and then to +21 and +15 mV for Dex-P and HA-P (Supporting Information, Figure S8). The number of PNIPAAm grafts per polysaccharide chain was approximately 4.0 and 4.4 for Dex-P and HA-P, respectively (Supporting Information, Figures S9 and S10 and Tables S3 and S4). Both Dex-P and HA-P

exhibited temperature-dependent changes in mean hydrodynamic diameter that were consistent with thermoresponsive behaviour with PNIPAAm lower critical solution temperatures (LCST) of 32 and 35°C, respectively (Supporting Information, Figure S11).

Polysaccharidosomes were prepared by the interfacial assembly of Dex-P or HA-P nanoconjugates at the water droplet/oil interface followed by cross-linking of the polysaccharide-polymer membrane prior to transfer into water (Figure 1a). In both cases, intact spherical microcapsules containing water-soluble guest macromolecules were produced before and after transfer into water at an aqueous/oil volume fraction of 0.06 and conjugate concentration of 10 mg mL⁻¹) (Figures 1b-g). Typically, the mean diameters were ~10 and ~18 µm for polysaccharidosomes prepared from Dex-P and HA-P, respectively, and could be correspondingly increased to ~20 and ~50 µm by decreasing the conjugate concentration (Figures 1h,i and Supporting Information, Figures S12 and S13). The non-crosslinked polysaccharidosomes dispersed in oil remained physically stable with no evidence of coalescence at room temperature over several weeks. Fluorescence labelling of the dextran and HA domains of the different nanoconjugates confirmed the presence of a continuous polysaccharide membrane (Figures 1j-m, Supporting Information, Figures S14 and S15 and Videos S1 and S2), which partially collapsed without loss of structural integrity when air-dried (Figures 1n-q). The polysaccharide-PNIPAAm membrane was sufficiently robust to undergo a reversible swelling/deswelling cycle in water over a temperature range of 25-55°C without degradation of the polysaccharidosome micro-architecture (Supporting Information, Figures S16-S17). Typically, the Dex-P polysaccharidosomes contracted by approximately 20 vol% compared with 14 vol% for the HA-P microcapsules when the temperature was raised up to 55 °C, and expanded to their original swollen state on lowering the temperature

back to 25°C (Supporting Information, Figure S18). The presence of excess nanoconjugates in the polysaccharidosome core did not interfere with their reversible thermoresponsive behaviour.

Polysaccharidosome-mediated carbohydrate-lectin-induced nanoparticle docking.

Given that proteins such as concanavalin A (Con A, M_w 103 kDa, homotetramer, 1 binding site per monomer) and wheat germ agglutinin (WGA, M_n 35 kD, homodimer, 2 binding sites per monomer) non-covalently bind specifically to glucosyl or *N*-acetyl-*D*-glucosamine residues of dextran³⁶ and HA,³⁷ respectively, we utilised the Dex-P and HA-P polysaccharidosomes as dispersible lectin-receptive membrane platforms for the specific recognition and docking of polymer and zeolitic nanoparticles (Figure 2a). To achieve this, we covalently immobilised fluorescein isothiocyanate (FITC)-labelled Con A or Alexa Fluor® 647-labelled WGA onto poly(styrene)-*co*-poly(acrylic acid) (PS-*co*-PAA) spherical nanoparticles with a mean size of 120 nm (Supporting Information, Figure S19). Alternatively, faceted nanoparticles of a zinc-based zeolitic imidazolate framework (ZIF, mean size = 335 nm) were used to physically immobilize Alexa 647-WGA (Supporting Information, Figure S19). The corresponding lectin-presenting nanoparticles were denoted as PS-*co*-PAA@Con A, PS-*co*-PAA@WGA and ZIF@WGA. Successful covalent attachment of the lectins onto the nanoparticles was confirmed by changes in zeta potential values from -39 (PS-*co*-PAA) to -15 or -29 mV for PS-*co*-PAA@Con A and PS-*co*-PAA@WGA respectively. Similarly, physical adsorption of WGA onto the ZIF nanoparticles resulted in a change in the zeta potential from +30.7 to -10.7 mV (Supporting Information, Figure S19f). Incubation of BSA-containing Dex-P polysaccharidosomes with an aqueous dispersion of PS-*co*-PAA@FITC-Con A nanoparticles (1.67 mg mL⁻¹) at 37°C produced intact microcapsules with a green fluorescent outer shell (Figures 2b-d and Videos S3), consistent with binding of the lectin specifically to the aminodextran-PNIPAAm membrane. A similar ring of agglomerated

fluorescent nanoparticles was observed at the periphery of the HA-P polysaccharidosomes after incubation at 25°C with ZIF@Alexa-WGA (1.13 mg mL⁻¹) or PS-*co*-PAA@Alexa-WGA (1.67 mg mL⁻¹) nanoparticles (Figures 2e-g and Supporting Information, Figures S20-S21 and Video S4). Corresponding fluorescence line profiles indicated that the lectin-immobilised nanoparticles were highly localised on the surface of the polysaccharidosomes with no indication of diffusion of free lectins or nanoparticles into the polysaccharidosome lumen (Figures 2b-g (insets)). In contrast, control experiments in which Dex-P and HA-P polysaccharidosomes were incubated with non-bio-orthogonal WGA- or Con A-loaded nanoparticles, respectively, showed no evidence for high affinity surface recognition (Supporting Information, Figure S22), indicating that specific carbohydrate-lectin interactions could be programmed at a rudimentary level into the polysaccharide-protein microcapsules. Similarly, no binding to the outer membrane surface was observed in the presence of BSA-coated PS-*co*-PAA or BSA-coated ZIF nanoparticles (Supporting Information, Figure S23).

Fluorescence-activated cell sorting (FACS) analysis of the Dex-P polysaccharidosome populations before and after binding of PS-*co*-PAA@FITC-Con A nanoparticles showed identical 2D dot plots of side-scattered (SSC) vs forward-scattered (FSC) light (Figures 2h,i), which we attributed to the relatively smooth texture of the nanoparticle-decorated outer surface of the aminodextran-PNIPAAm membrane. Similar results were obtained for binding of PS-*co*-PAA@Alexa-WGA nanoparticles to the HA-P polysaccharidosomes (Figure 2j,k). In contrast, FACS profiles of HA-P polysaccharidosome populations before and after interactions with the ZIF@Alexa-WGA nanoparticles exhibited higher SSC intensities due to the increased surface granularity of the ZIF nanoparticles (Figures 2l,m). Measurements of the mean fluorescence indicated that lectin-mediated binding of the PS-*co*-PAA@FITC-Con A nanoparticles to the Dex-

P polysaccharidosomes increased the green fluorescence intensity within the population by approximately one order of magnitude (Figure 2n), while the blue fluorescence mean signals associated with PS-*co*-PAA@Alexa-WGA and ZIF@Alexa-WGA nanoparticles binding to the HA-P polysaccharidosomes increased by approximately four and two orders of magnitude respectively (Figures 2o,p). These observations were attributed to differences in carbohydrate-lectin binding and were consistent with isothermal titration calorimetry (ITC) measurements obtained from ligand binding studies on the functionalized polysaccharides (Supporting Information, Figures S24 and S25). In this regard, the calorimetric profiles showed a significant decrease in the heat evolved for Con A binding to aminodextran compared with unfunctionalized dextran, whereas minimal changes were observed for WGA binding to HA and amino-HA.

Membrane-gated enzyme cascade reactions in nanoparticle-decorated polysaccharidosomes. We explored the possibility of using the lectin/glycan-mediated docking of functional nanoparticles for the implementation of a two-enzyme cascade reaction within the decorated polysaccharidosomes. The partner enzymes were spatially isolated between the aqueous lumen and membrane by encapsulation of glucose oxidase (GOx) within the polysaccharidosome and by lectin-mediated docking of PS-*co*-PAA nanoparticles functionalized with surface-immobilised WGA and horseradish peroxidase (HRP). High levels of nanoparticle docking and GOx encapsulation were achieved respectively by using HA-P and Dex-P nanoconjugates at a volume ratio of 4.8 : 1.0 to construct hybrid polysaccharidosomes that were subsequently incubated with PS-*co*-PAA@WGA@HRP nanoparticles. Hybrid microcapsules were used in the cascade reactions owing to the fact that Dex-P possess more surface amino-groups for crosslinking (Supporting Information, Tables S1 and S2), as a consequence the hybrid polysaccharidosomes exhibited superior encapsulation efficiency than neat HA-P based polysaccharidosomes. The

nanoparticle-decorated polysaccharidosomes were then exposed to an aqueous solution of glucose and Amplex Red to initiate the spatially coupled enzyme cascade reaction in micro-compartments comprising expanded (25°C) or contracted (45°C) membrane configurations produced below and above the LCST of the polysaccharide-PNIPAAm conjugates (Figure 3a). At 25°C, passive diffusion of glucose across the membrane resulted in GOx-mediated production of hydrogen peroxide (H_2O_2) in the aqueous lumen, followed by transfer to HRP on the outer membrane surface and HRP-mediated reaction with Amplex Red to produce a red fluorescent product (resorufin) that rapidly diffused into the external medium as well as the interior of the polysaccharidosome (Figure 3b and Supporting Information, Figure S26 and Video S5).

In contrast, the rate of resorufin production was attenuated in similar experiments undertaken at 45°C (Figure 3c and Supporting Information, Figure S27 and Video S6). The corresponding concentration profiles obtained from fluorescence intensity measurements indicated an approximately 2-fold decrease in resorufin production after 12 minutes when the reaction was undertaken above the LCST (Figure 3d), suggesting that diffusion of glucose into the GOx-containing lumen was hindered by the increased hydrophobicity of the contracted polysaccharide-PNIPAAm membrane. Mean fluorescence intensity values recorded in different locations indicated that the distribution of resorufin was essentially homogeneously although marginally higher levels of the product were associated with the membrane, consistent with the surface docking of the PS-*co*-PAA@WGA@HRP nanoparticles (Figure 3d). Control experiments involving PS-*co*-PAA@WGA nanoparticles docked onto GOx-containing HA/Dex-P polysaccharidosomes and exposed to glucose and Amplex Red showed no evidence of resorufin production (Figure 3d and Supporting Information, Figure S28).

The above results were in agreement with UV-Vis spectroscopy measurements of the initial rates of the spatially coupled polysaccharidosome-mediated enzyme cascade reaction determined at various temperatures between 25 and 45°C, which showed an approximately 80% decrease in initial rate over this temperature range (Figure 3e). In contrast, the initial rates of the GOx/HRP cascade reaction determined in the absence of the polysaccharidosomes using enzymes in free solution or an aqueous dispersion of PS-*co*-PAA@WGA@HRP nanoparticles with free GOx increased as the temperature was raised from 25 to 45°C (Figure 3e). Taken together, the results indicate that the nanoparticle-decorated polysaccharidosomes can act as temperature-dependent membrane-gated micro-compartments for controlling the reactivity of spatially positioned enzyme cascade reactions.

Higher-order assembly of polysaccharidosomes *via* lectin-mediated crosslinking. The docking of functional nanoparticles onto individual polysaccharidosomes *via* glycan-lectin interfacial recognition was utilised as a potential route to the programmed higher-order reversible assembly of closely packed networks of thermoresponsive materials (Figure 4a). We increased the probability of contact-dependent adhesion between the functionalized polysaccharidosomes by increasing the nanoparticle loading and employing accessible lectin binding sites to establish multiple sites of membrane-membrane non-covalent crosslinking. This was achieved by incubating HA-P polysaccharidosomes in appropriate concentrations of ZIF nanoparticles functionalized with physically adsorbed WGA. In some cases, closely packed aggregates consisting of a few tens of lectin-interconnected polysaccharidosomes were observed along with discrete microcapsules at nanoparticle concentrations of 1.13 mg mL⁻¹ (Figure 4b,c). Formation of the aggregates was not ubiquitous indicating that the higher-order assembly of the polysaccharidosomes was not fully controllable at low nanoparticle concentrations. We attributed

this to the heterogeneous distribution of surface-attached nanoparticles under these conditions. In contrast, extended closely packed networks comprising hundreds of polysaccharidosomes were consistently produced at ZIF@WGA nanoparticle concentrations of 5.7 mg mL^{-1} (Figure 4d-g), indicating that assembly of the higher-order aggregates could be regulated under conditions of high nanoparticle coverage. No aggregated structures were observed when the HA-P polysaccharidosomes were incubated with high concentrations of PS-co-PAA@WGA nanoparticles, suggesting that covalently linking of WGA onto the polymer surface restricted the binding potential possibly through changes in protein configuration.³⁸

The collective thermoresponsive behaviour of the closely packed assemblages was investigated using a confocal fluorescence microscope equipped with a heating chamber. Images of polysaccharidosome aggregates thermally cycled between 25 to 55°C showed reversible contraction and expansion above and below the LCST of the HA-NH₂/PNIPAAm nanoconjugates, respectively, without loss of higher-order structural integrity. (Figure 4h-l and Supporting Information, Figure S29). The collective changes in volume occurred without loss of higher-order structural integrity.

We disassembled the closely packed aggregates of polysaccharidosomes by competitive WGA-hapten binding using *N*-acetyl-*D*-glucosamine, which disrupted the lectin-glycan non-covalent junctions established between the adhered polysaccharidosome membranes. Disaggregation and release of intact polysaccharidosomes occurred slowly over a period of 24 h (Figure 4m,n and Supporting Information, Figure S30), consistent with previous studies on vesicle-vesicle³⁴ and cell-cell agglutination.³⁹ Alternatively, the aggregated structures were deconstructed over 24 h by dissolution of the intercalated ZIF nanoparticles under acidic conditions (pH 4) to release intact polysaccharidosomes and free WGA (Figure 4o,p).

CONCLUSIONS

As a step towards mimicking lectin-carbohydrate interactions in the glycocalyx of living cells responsible for cellular adhesion, recognition and cell-microbe/virus docking,⁴⁰ we constructed lectin-receptive polysaccharidosomes capable of programmable interfacial interactions. We employ amphiphilic polysaccharide-polymer membrane building blocks endowed with intrinsic bio-orthogonal lectin-glycan recognition sites to facilitate the reversible non-covalent docking of functionalized polymer or zeolitic nanoparticles on the outer membrane of the polysaccharidosome. As a consequence, synthetic cell-like objects capable of membrane-gated spatially localized enzyme cascade reactions can be implemented by the onset of interfacial attachment. Moreover, increasing the number of lectin-immobilized nanoparticles on the surface of the polysaccharidosomes induces formation of higher-order extended tissue-like structures *via* reversible non-covalent adhesion between the protocells.

In general, the fabrication of protocells with intrinsic membrane functionality and programmability provides avenues for the exploitation of bio-inspired cell-like materials and addresses the interface between bottom-up synthetic biology and macromolecular engineering with potential applications in cell-protocell research, biosensor and bioreactor microscale technologies and synthetic virology. In the longer term, the ability to prepare micro-compartmentalized cell-like entities with programmable lectin-glycan membrane recognition properties could have value in synthetic biology, especially in the context of engineering tissues, cellular sheets and agglomerates *via* artificial cell-cell adhesion,⁴¹ customizing sensing and response behaviours using synthetic notch receptors,⁴² influencing signalling networks by cadherin cell adhesion⁴³ and inhibiting bacterial adhesion in biofilms.⁴⁴

EXPERIMENTAL SECTION

Materials. N-isopropylacrylamide (NIPAAm, TCI, 98 %) was recrystallised twice in hexane before use. 2,2'-Azobis-(isobutyronitrile) (AIBN, Macklin, 98 %) was recrystallised from methanol. Dextran (Dex, Mw 40 kDa) was purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd, sodium hydroxide (NaOH, $\geq 97\%$), was sourced from Shanghai Lingfeng Chemical Reagent Co., Ltd, chloroacetic acid ($\text{C}_2\text{H}_3\text{ClO}_2$, Macklin, 98%), ethylene diamine (Macklin, 99%), N-(3-dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDAC, 99%) was procured from Shanghai J & K Chemical Technology Co., Ltd, 2-ethyl-1-hexanol ($\text{C}_8\text{H}_{18}\text{O}$, Macklin, 99%), 1-hydroxybenzotriazole (HOBt, Macklin, $\geq 97\%$), 4,4'-azobis(4-cyanovaleric acid) (ACVA, Macklin, 98 %), 2-mercaptothiazoline (Aladdin, 98 %), N'-dicyclohexylcarbodiimide (DCC, Macklin, 99 %), 4-(dimethylamino)pyridine (DMAP, Sigma 99 %), PEG-bis (N-succinimidyl succinate (NHS-PEG-NHS, M_w 2000, Sigma, 98%), 2,4,6-trinitrobenzene sulfonic acid (TNBSA) solution (5 % (w/v) in H_2O , Ark), concanavalin A (Con A, Mw 103 kDa, Aladdin, 98%), wheat germ agglutinin, Alexa Fluor™ 647 Conjugate (WGA, 35 kDa, ThermoFisher® Scientific), fluorescein isothiocyanate (FITC, Macklin, 98%), rhodamine B isothiocyanate (RITC, Aladdin, 98%), glycine (Aladdin, $\geq 99\%$), iodine (I, Macklin, 99.8%) and bovine serum albumin (BSA, Mw 66 kDa, Macklin, 98%) were used without further purification.

Characterization. ^1H NMR and ^{13}C NMR spectra were recorded using an Avance III 400 MHz spectrometer (Bruker, Switzerland) in D_2O ($\delta = 4.79$) or CDCl_3 ($\delta = 7.26$). *UV-vis spectroscopy* was carried out on a Multiskan Sky Microplate reader, ThermoFisher® using a 96 well plate or on a UV-2550 UV-vis spectrophotometry, Shimadzu Japan in quartz cuvettes (path length, 1 cm). Optical and fluorescence microscopy was performed on an Axio Scope.A1 upright fluorescence microscope at $\times 20$, $\times 50$ and $\times 100$ magnifications. Images were analyzed using AxioVision and

Image J software. Confocal microscopy images were captured on a Leica TCS SP8 STED 3X Super-Resolution Multiphoton Confocal Microscope with ‘Adaptive Focus control’ to correct focus drift during time-lapse capture. The microscope was equipped with the following lasers: 65 mW Ar (458, 488 nm lines), 20 mW solid-state yellow (561 nm), 10 mW Red He/Ne (633 nm) and a 50 mW UV 405 nm diode. All measurements were performed in an environmental chamber maintained at 25 °C. Images were analyzed using Las X confocal microscopy software version V3.1.1.

Transmission electron microscopy (TEM) images were recorded using a FEI Tecnai G2 Spirit BioTwin transmission electron microscope (120 kV, FEI, U.S.A.).

Zeta potential and hydrodynamic diameter studies of polysaccharide derivatives, conjugates and nanoparticles were carried out using a ZETASIZER® Nano series instrument (Malvern Instruments, UK) at 25°C, for soluble analyses, measurements were done at concentrations of mg mL⁻¹.

Gel permeation chromatography (GPC) characterisation of mercaptothiazoline-PNIPAAm was carried out using a EcoSEC® GPC (Japan) equipped with a UV–vis detector working at 240 nm, a differential refractometer and a column oven (35 °C). A flow rate of 1.0 mL min⁻¹ using DMF as an eluent and the column was calibrated with polystyrene standards.

Flow cytometry/fluorescence-activated cell sorting (FACS) was undertaken on dispersions (~ 0.5-1 mL) of non-functionalized or nanoparticle-docked polysaccharidosomes. Samples were investigated on a BD LSRFortessa™ equipped with 488 nm, 561 nm and 633 nm lasers and operated at low pressure with a 100 µm sorting nozzle. 2D dot plots of the FSC and SSC light were determined for a total of 10,000 polysaccharidosomes and histograms of the number of counts

against corresponding fluorescence intensity (*e.g.* FITC-A or PE-A) were determined. Data analysis was performed with FlowJo 10.3 software.

Isothermal titration calorimetry (ITC). Dextran, carboxymethyl dextran and aminodextran (ligands, 100 mM) were dissolved in 50 mM Tris buffer pH 7.4 containing 1 mM CaCl_2 , MnCl_2 , MgCl_2 and 150 mM of NaCl, and individually loaded into a 250 μL titration syringe. Con A (1.2 mL, 0.05 mM) was dissolved in the same buffer and loaded into the sample cell of the ITC instrument (NanoITC[®], standard volume, TA instruments). The concentration of Con A was estimated by UV-Vis spectrometry using cuvettes with a 1 cm path length ($E^{1\%} = 11.4$ in 0.1 M NaCl). The ligand was titrated into Con A in the sample cell *via* a computer-controlled syringe in 25 incremental injections of 10 μL with an interval of 400 s per injection; the sample cell was stirred at a speed of 200 rpm and maintained at 37 °C throughout the experiment. Data from the ITC instrument was captured in real-time using ITCrun software version 3.5.0 and processed using NanoAnalyze software version 3.8.0. Similarly, hyaluronic acid and amino-hyaluronic acid (25 mM) were dissolved in 50 mM Tris buffer pH 7.0 containing 50 mM of KCl and individually loaded into a 250 μL titration syringe. WGA (0.1 mM) was dissolved in the same buffer and loaded into the sample cell of the ITC instrument (NanoITC[®], standard TA instruments). The concentration of WGA was estimated by UV-Vis spectrometry using cuvettes with a 1 cm path length ($E^{1\%} = 15$, in 50 mM Tris buffer pH 7.4 with 150 mM NaCl). The experiment parameters were similar to those above, but the sample cell temperature was adjusted and maintained at 26 °C throughout the experiment.

Synthesis of carboxymethyl dextran (Dex-COOH). Carboxymethyl dextran was prepared following a procedure reported in literature with relevant modifications.⁴⁵ Firstly, 2 g (12.3 mmol of anhydroglucose rings, AHG) of dextran was slurried in a 42.5 mL mixture of isopropanol and

water (85:15). 7.5 mL of 7.03 M NaOH was added to obtain a final concentration of 3.8M; the mixture was subjected to vigorous stirring for 1 hour at room temperature. Then, 3g (31.7 mmol) of chloroacetic acid was added and stirred until complete homogeneity. (Molar ratio; chloroacetic acid: dextran AHG rings = 2.5:1), the reaction vessel was transferred to an oil bath preheated at 60 °C and stirred vigorously for a further 90 minutes, after which the pH of the solution was adjusted to 7.0 using glacial acetic acid. The reaction product was precipitated in excess methanol, washed with methanol, dissolved in water and dialyzed (MWCO 14 kDa) against Milli-Q water for 2 days to remove salts and impurities. The final product was obtained by rotary evaporation to dryness and characterized by ^1H NMR spectrometry in D_2O .

Synthesis of aminodextran (Dex-NH₂). Carbodiimide-activated conjugation of 1,2-diaminoethane to the carboxyl groups of carboxymethylated dextran (Dex-COOH) was used to prepare the aminodextran (Dex-NH₂). Typically, 2.22 mL (10-fold excess over the expected number of pendant carboxymethyl groups) of 1,2-diaminoethane was dissolved in 10 mL of 0.1 M MES buffer pH 6.5, the pH adjusted to pH 6.5 using 5M HCl and 1M NaOH, and added dropwise to a stirred solution of Dex-COOH (DS 1.08, 1g in 20 mL of 0.1 M MES buffer pH 6.5). The coupling reaction was initiated by adding *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, 500 mg) immediately, and again (250 mg) after 5 h. The pH was maintained at 6.5 using 1 M HCl and 1 M NaOH; the mixture was stirred for a further 19 h at ambient temperature. The solution was then dialyzed (MWCO 14 kDa) against Milli-Q water for 2 days. The dialysate was concentrated to 20 mL by rotary evaporation, and the above procedure was repeated. The final product was obtained by rotary evaporation to dryness. Dex-NH₂ was characterized by ^1H NMR spectrometry in D_2O .

Synthesis of aminohyaluronic acid (HA-NH₂). 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was used as a coupling agent instead of the conventional EDAC for its superior efficiency and requiring less amine compound for the amidation of hyaluronic acid (HA). 0.4g (1.02 mmol) of HA (Mw 80 kDa) was dissolved in 40 mL of 0.1 M MES buffer pH 5.5 to which 1.13 g (4.08 mmol 4-fold equiv. of pendant COOH groups in HA) of DMTMM was added and stirred for 10 mins to activate the COOH groups, while 133.5 μ L (2.04 mmol 2-fold equiv. of pendant COOH groups in HA) of 1,2-diaminoethane was dissolved 10 mL of 0.1 M MES buffer pH 5.5 and the pH adjusted to 5.5 using 5 M HCl and 1 M NaOH. The amine solution was added dropwise to a stirred HA solution at ambient temperature, the mixture was stirred for 24 hr, and the pH maintained at 5.5 using 1 M NaOH and 1 M HCl. The mixture was then dialysed against Milli-Q water (MWCO 14kDa) for 2 days, concentrated and the amidation reaction was repeated following the procedure described above. The final product was obtained by rotary evaporation to dryness. HA-NH₂ was characterized by ¹H NMR spectrometry in D₂O.

Estimation of the number of primary amino groups in aminodextran and amino hyaluronic acid. 2, 4, 6-Trinitrobenzenesulfonic acid (TNBSA) reacts with primary amines molecules to form a highly chromogenic (orange) product, whose absorbance at 335 to 345 nm can be measured by UV-Vis spectrometry.

Solutions of either Dex-NH₂ or HA-NH₂ (0.05 to 2.00 mg mL⁻¹) were prepared in 0.1 M sodium

bicarbonate buffer (pH 8.5). A 5% TNBSA solution was diluted 250-fold with 0.1 sodium bicarbonate buffer (pH 8.5) and 0.25 mL added to 0.4 mL of the Dex-NH₂ or HA-NH₂ solutions in a glass vial, and the mixture incubated at 37°C for 2 h. Then, 0.65 mL of 0.2 M HCl was added to each vial to stop the reaction. UV-vis spectra of the solutions were recorded (Supporting information, Figure. S3 and S4). To determine the amine concentration, a standard absorbance curve was determined based on the same protocol using glycine.

Synthesis of end-capped mercaptothiazoline-activated PNIPAAm. Mercaptothiazoline activated PNIPAAm was synthesized by RAFT polymerization according to a protocol reported in literature with appropriate modifications.¹⁰ In practice, mercaptothiazoline-activated trithiol-RAFT agent (18.9 mg, 50 μ mol), AIBN (1.7 mg, 10 μ mol), NIPAAm (847 mg, 7.5 mmol) were dissolved in acetonitrile (8 mL) in a 25-mL round-bottom flask. The flask was then tightly sealed, and the solution degassed **via** five freeze-pump-thaw cycles. The polymerization was carried out at 60°C for 24 h, and the polymer was recovered by three precipitation/filtration cycles in chilled diethyl ether/hexane (2:1 volume ratio) The obtained yellowish powdery polymer was characterized by ¹H NMR spectroscopy in CDCl₃ and gel permeation chromatography using DMF as an eluent.

Synthesis of polysaccharide-polymer nanoconjugates. Polysaccharide-polymer nanoconjugates were prepared **via** a catalyst-free amidation coupling reaction between end-capped mercaptothiazoline activated PNIPAAm and amino-polysaccharides. For this, end-capped mercaptothiazoline-activated PNIPAAm (M_n = 17,000 g mol⁻¹ GPC, PDI 1.58, 40 mg in 10 mL of water) was added to a stirred solution of either aminodextran (Dex-NH₂, DS 1.08; 40 mg in 10 mL of 0.2M pH 8.0 PBS) or aminohyaluronic acid (HA-NH₂, DS 0.63; 40 mg in 20 mL of 0.2M pH 8.0 PBS). The mixtures were stirred for 12 or 18 h respectively at ambient temperature, then

purified by dialysis against Milli-Q water (MWCO 25 kDa) to remove any unreacted PNIPAAm and salts. The resulting dialysates were lyophilized to obtain fluffy white solids that were characterized by ^1H NMR spectrometry in D_2O .

Preparation of polysaccharidosomes. Polysaccharidosomes were prepared by mixing aqueous solutions of either Dex/ NH_2 -PNIPAAm or HA- NH_2 -PNIPAAm with the oil phase, 2-ethyl-1-hexanol followed by vigorous shaking by hand for 10s. All samples were prepared at a constant aqueous/oil volume fraction of 0.06. In practice, 60 μL of Dex- NH_2 /PNIPAAm or HA- NH_2 /PNIPAAm (10 mg mL^{-1} in water) were mixed with oil. The polysaccharidosomes were cross-linked by inclusion of PEG-bis(*N*-succinimidyl succinate) in both the encapsulated water phase (25 mg mL^{-1}) and continuous oil medium (5 mg mL^{-1}), or only in the continuous oil phase (5 mg mL^{-1}) prior to assembly. The procedures consolidated the polysaccharidosome structure by reacting with the remnant free primary amine groups in the amino polysaccharides. The cross-linked polysaccharidosomes were then transferred to water by discarding the translucent upper oil layer, then adding 1.0 mL of 65% ethanol followed by moderate shaking. The contents of the vial were dialyzed (MWCO: 1000kDa) against 65% ethanol for 5 hours, 50% ethanol for 2 hours, 35% ethanol for 2 hours and Milli-Q water for 2 days to complete the transfer to water. After transfer to water, the polysaccharidosomes were left to stand for several hours to allow sedimentation, then the excess top aqueous phase was carefully pipetted away and discarded to obtain a final volume of 1 mL. The same concentration of polysaccharidosomes was used in all ensuing experiments. Polysaccharidosomes with hybrid membrane comprising mixtures were also prepared as above by using aqueous mixtures of HA- NH_2 /PNIPAAm and Dex- NH_2 /PNIPAAm, typically at a volume ratio of 4.8 : 1.

Polysaccharidosomes encapsulating fluorescent-labelled BSA or glucose oxidase (2 μL , 5 mg/mL) were prepared following the above procedure with the addition of the desired cargo to the aqueous Dex-NH₂/PNIPAAm or HA-NH₂/PNIPAAm solutions before mixing with the oil phase while maintaining a constant aqueous/oil phase fraction of 0.06.

Fluorescent labelling of BSA and polysaccharides. Bovine serum albumin (BSA), aminodextran and aminohyaluronic acid, were labelled with Rhodamine B isothiocyanate (RITC) or fluorescein isothiocyanate (FITC). 1 mL of 150 mM NaCl was added to 100 mg of BSA pre-dissolved in 20 mL of bicarbonate buffer pH 8.5 followed by addition of 400 μL of RITC or FITC (2 mg mL⁻¹). The mixture was stirred at ambient temperature for 3 hours, then dialysed against Milli-Q water for 2 days, freeze dried and the dry product stored at 4-8°C). Aminodextran and aminohyaluronic acid were labelled following the same protocol.

Synthesis of zeolitic imidazolate framework (ZIF) nanoparticles. First, 0.2 g (0.66 mmol) of Zn(NO₃)₂·6H₂O was dissolved in 8 mL of H₂O. Then, a solution containing 2 g (24.36 mmol) of 2-methylimidazole (2-mim) in 8 mL of H₂O was added dropwise. The reaction mixture was stirred for 45 min at ambient temperature. The precipitate was collected by centrifugation and washed at least three times with deionized water. ZIF nanoparticles were sonicated and characterized by DLS and TEM.

Synthesis of PS-*co*-PAA nanoparticles (NPs). PS-*co*-PAA-NPs were prepared according to a method described in the literature with appropriate modification.⁴⁶ Briefly, 1.6 g of styrene, 0.4 g of AA (acrylic acid) and 100 mL of Milli-Q H₂O were charged into a three-necked flask equipped with a mechanical stirrer and a condenser. The solution was heated to 70 °C and then bubbled with nitrogen to evacuate dissolved oxygen for 30 min, then 0.06 g of potassium persulphate dissolved in 2 mL of H₂O was injected into the reaction vessel **via** a syringe to initiate the polymerization,

and the mixture was stirred and maintained at 70 °C for 8 h. The resulting PS-*co*-PAA-NPs were washed with ultrapure water by centrifugation several times and then re-suspended in water, briefly sonicated and stored at 4-8 °C.

Lectin immobilization on ZIF nanoparticles. 100 µL of Alexa Fluor wheat germ agglutinin (WGA; 2 mg mL⁻¹) was added to 1 mL ZIF nanoparticles (20 mg mL⁻¹) in a 1.5 mL Eppendorf tube, the mixture was vortexed at room temperature for 1 hour, then the lectin-modified ZIF nanoparticles were washed with Milli-Q water several times to remove any un-adsorbed lectin. ZIF@WGA nanoparticles were redispersed in 0.1 M PBS pH 7.3, sonicated briefly on ice and stored at 4-8 °C

Lectin immobilization on activated PS-*co*-PAA nanoparticles. 1 mL of an aqueous suspension of PS-*co*-PAA nanoparticles (20 mg mL⁻¹) were activated by stirring for 2h at ambient temperature in the presence of EDAC (0.5 mL, 0.4 M) and NHS (0.5 mL, 0.8 M). Excess reagents were removed by washing the nanoparticles several times with water. The activated nanoparticles were re-suspended in 1 mL of PBS, 100 µL of Alexa Fluor 647 WGA or 100 µL FITC-Con A (2 mg mL⁻¹) were then added, and the mixture was vortexed at ambient temperature for 18 hrs. The excess coupling sites were blocked by incubating the nanoparticles dispersion with glycine solution (0.5 mL, 200 mg mL⁻¹) for 1h at ambient temperature. The mixture was dialyzed (MWCO 1000 kDa) against Milli-Q water for 2 days to remove any unconjugated lectin and glycine. The resultant PS-*co*-PAA@WGA nanoparticle suspension was stored at 2-8 °C while PS-*co*-PAA@Con A nanoparticles were lyophilised, then re-dispersed in 10 mM HEPES buffer pH 7.4 containing 1 mM Ca²⁺, Mn²⁺ and 150 mM NaCl and stored at 4-8 °C. For the preparation of PS-*co*-PAA@WGA@HRP, the above procedure was followed with simultaneous addition of 100 µL of

Alexa Fluor® 647 WGA (2 mg mL⁻¹) and 100 µL of unlabeled HRP (2 mg mL⁻¹) to the activated nanoparticles.

Nanoparticle docking *via* carbohydrate-lectin recognition in polysaccharidosomes. 100 µL of PS-*co*-PAA@Con A nanoparticles (10 mg mL⁻¹) dispersed in HEPES buffer containing 1 mM Ca²⁺, Mn²⁺ and 150 mM NaCl were added to 0.5 mL of an aqueous dispersion of RITC-BSA-containing Dex-NH₂/PNIPAAm polysaccharidosomes, the vial was inverted several times and incubated at 37 °C for 1 hour. Similarly, 30 µL of ZIF@WGA nanoparticles (20 mg mL⁻¹) or 100 µL of PS-*co*-PAA@WGA nanoparticles (10 mg mL⁻¹) in water were added to 0.5 mL of an aqueous dispersion of FITC-containing HA-NH₂/PNIPAAm polysaccharidosomes and incubated at 25 °C for 1 hour. The polysaccharidosomes were observed by fluorescence and confocal microscopy before being analyzed by FACS.

Membrane-gated enzyme cascade reactions in nanoparticle-decorated polysaccharidosomes.

Enzyme-containing hybrid polysaccharidosomes incorporating HA-NH₂/PNIPAAm and Dex-NH₂/PNIPAAm were prepared following the above procedures except that GOx (2 µL; 5 mg mL⁻¹) was mixed with the nanoconjugate solutions. The resultant GOx-containing polysaccharidosomes were then incubated with PS-*co*-PAA@WGA@HRP nanoparticles at room temperature. A freshly prepared solution of Amplex Red (200 µL, 0.1 mM) was added to the center of a Ø35 mm confocal disk, and 50 µL of an aqueous dispersion of the GOx-containing polysaccharidosomes with surface-attached PS-*co*-PAA@WGA@HRP nanoparticles added at 25 or 45 °C. The Amplex Red/polysaccharidosome dispersion was mounted in a confocal microscope and freshly prepared glucose (10 µL; 5.5 mM) carefully added to the Amplex Red/polysaccharidosome dispersion. Production of the red fluorescent oxidation product

(resorufin) was monitored in real time for 12 or 16 min at 25 and 45 °C respectively, using fluorescence confocal microscopy (λ_{ex} = 530 nm, λ_{em} = 600-670 nm).

UV-vis spectroscopy monitoring of the enzyme cascade reaction was undertaken as follows. GOx-containing polysaccharidosomes (50 μ L) with surface-attached PS-co-PAA@WGA@HRP nanoparticles along with freshly prepared Amplex Red (202 μ L, 0.1 mM) and glucose (2 μ L, 5.5 mM) were added into a well of a 96-well microplate and pipetted up and down to mix thoroughly. Production of resorufin was monitored at a range of temperatures between 25 and 45 °C by UV-vis spectroscopy using a plate reader (Multiskan Sky Microplate Spectrophotometer). Data points were acquired *via* a kinetic loop every 2.0 s over a duration of 600 s. The initial velocity of the enzyme reaction rate was estimated from the slope of the plot of absorbance against time at $t = 0$.

Higher-order reversible assembly of polysaccharidosomes. 200 μ L of ZIF@WGA nanoparticles were mixed with 500 μ L of an aqueous dispersion of RITC-HA-NH₂/PNIPAAm polysaccharidosomes to a final nanoparticle concentration of 5.7 mg mL⁻¹ and incubated at room temperature for 30 min. Formation of tissue-like structures was observed using fluorescence and confocal microscopies. Disassembly of the tissue-like aggregates was undertaken by adding 100 μ L of an aqueous dispersion of the aggregates to 400 μ L of 100 mM *N*-acetyl-*D*-glucosamine in PBS at pH 7.0 or 400 μ L of 0.1 M acetate buffer at pH 4.0 and incubated at room temperature for 2 and 24 or 24 hrs respectively.

ASSOCIATED CONTENT

Conflict of Interest: The authors declare no competing financial interest

Supporting Information: Legends for supporting videos, characterization of functionalized polysaccharides and polysaccharide/polymer nanoconjugates (¹H NMR spectra), size control of polysaccharidosomes, confocal microscopy images of polysaccharidosomes in water, temperature

responsive behaviour of polysaccharidosomes, TEM images and zeta potential measurements of neat and protein-immobilized nanoparticles, additional images depicting glycan-lectin mediated interfacial nanoparticle docking in polysaccharidosomes, Isothermal titration calorimetry plots of functionalised polysaccharides, detailed confocal images showing membrane-gated enzyme cascade reactions in nanoparticle-decorated polysaccharidosomes, higher-order reversible assembly of polysaccharidosomes into closely packed aggregates and permeability studies of polysaccharidosomes toward fluorescent macromolecules of varying molecular weight. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxx

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Figure 1. Fabrication of polysaccharidosomes. **a**, Synthesis of amphiphilic thermoresponsive polysaccharide–polymer nanoconjugates (Dex-NH₂/PNIPAAm; Dex-P) or HA-NH₂/PNIPAAm; HA-P) (top row), and utilisation as building blocks for polysaccharidosome assembly (bottom row). Typically, aqueous solutions of either Dex-P or HA-P were mixed with 2-ethyl-1-hexanol at an aqueous/oil volume fraction of 0.06 followed by shaking by hand for 10s. Crosslinking was undertaken by addition of PEG-bis(*N*-succinimidyl succinate) to the oil and aqueous phases, or to the oil phase alone. **b-g**, Optical micrographs of non-crosslinked Dex-P (**b**) or HA-P (**e**) polysaccharidosomes dispersed in oil, and after transfer to water (**c,f**, respectively) Corresponding

fluorescence microscopy images of **c** and **f** with encapsulated RITC-BSA (**d**) and FITC-BSA (**g**), respectively. Fluorescence is observed throughout the polysaccharidosomes shown in **d** and **g** due to excess amounts of soluble nanoconjugates in the aqueous filled lumen. **h,i**, Plots showing mean diameter (bars) and standard deviations (lines on bars) of non-crosslinked Dex-P (**h**) or HA-P (**i**) polysaccharidosomes dispersed in oil and prepared at different polymer conjugate concentrations (1.0-10.0 mg mL⁻¹) at a water/oil volume fraction; $\phi_w = 0.06$. **j-m**, Confocal fluorescence micrographs of single water-filled polysaccharidosomes prepared from FITC-Dex-P (**j**) or RITC-HA-P conjugates (**k**) and corresponding 3D reconstructions (**l,m**); scale bars: 10 μ m. **n-q**, SEM (**n,o**) and AFM (**p,q**) images of partially collapsed Dex-P (**n,p**) and HA-P (**o,q**) polysaccharidosomes. Scale bars: 5 μ m (**n,o**).

Figure 2. Nanoparticle docking on lectin-receptive polysaccharidosomes. **a**, Scheme showing interfacial carbohydrate-lectin interactions responsible for selective surface binding of nanoparticle (NP)-immobilized concanavalin A (Con A) or wheat germ agglutinin (WGA) on the outer membrane surface of Dex-P or HA-P polysaccharidosomes, respectively. Con A and WGA were covalently coupled to PS-co-PAA and WGA was physically adsorbed onto the ZIF nanoparticles. **b-d**, 3D confocal fluorescence reconstructions of a single Dex-P polysaccharidosome after incubation with PS-co-PAA@Con A nanoparticles showing red fluorescence due to encapsulated RITC-BSA (**b**), and green fluorescence associated with non-covalent binding of the nanoparticles to the outer surface of the polysaccharidosome (**c**); corresponding overlay image of **b** and **c** is shown in (**d**); scale bars in (**b-d**), 5 μm . **e-g**, 3D confocal fluorescence reconstructions of a single HA-P polysaccharidosome after incubation with ZIF@WGA nanoparticles showing green fluorescence due to encapsulated FITC-BSA (**e**), and blue fluorescence associated with non-covalent binding of the nanoparticles to the outer surface of the polysaccharidosome (**f**); corresponding overlay image of **e** and **f** is shown in (**g**); scale bars in (**e-g**), 20 μm . Insets in (**b-g**) show corresponding line profiles of fluorescence intensities across the polysaccharidosomes. **h,i**, FACS-derived 2D dot plots of side-scattered light (SSC) vs forward-scattered light (FSC) of Dex-P polysaccharidosomes before (**h**) and after incubation with PS-co-PAA@FITC-Con A nanoparticles (**i**). The scattering distributions are almost identical ($n = 120,000$). **j,k**, As for (**h,i**) but for HA-P polysaccharidosomes before (**j**) and after (**k**) incubation with PS-co-PAA@Alexa-WGA nanoparticles. The scattering distributions are almost identical. **l,m**, As for (**j,k**) but for HA-P polysaccharidosomes before (**l**) and after (**m**) incubation with ZIF@Alexa-WGA nanoparticles. **n**, Corresponding histograms of the green fluorescence signal (FITC-A) for samples shown in (**h,i**). Low background green fluorescence is observed for the Dex-P polysaccharidosomes before FITC-Con A binding (red plot). Intermediate levels of green

fluorescence are recorded after binding of the PS-*co*-PAA@FITC-Con A nanoparticles (green plot). **o,p**, Corresponding histograms of the blue fluorescence signal (APC-A) for samples shown in **(j,k)** and **(l,m)**, respectively. Low background blue fluorescence is observed for the HA-P polysaccharidosomes before binding of the Alexa-WGA functionalized nanoparticles (light and navy green). Intermediate levels of blue fluorescence are recorded after binding of the PS-*co*-PAA@WGA or ZIF@WGA nanoparticles (dark and light blue, respectively).

Figure 3. Membrane-gated enzyme cascade reactions in nanoparticle-decorated polysaccharidosomes. **a**, Schematic of a spatially coupled GOx/HRP enzyme cascade reaction associated with GOx-containing hybrid HA-/Dex-P polysaccharidosomes after lectin-mediated docking of PS-co-PAA@WGA@HRP nanoparticles (NPs). The tandem reaction is undertaken below (left image; expanded membrane, open gate, V_0) and above (right side; contracted membrane, closed gate, V_1) the LCST of the polysaccharide-PNIPAAm conjugates. A temperature-dependent gated response is observed. (Glc, glucose; GDL, D-(+)-Gluconic acid δ -lactone). **b,c**, Time-lapse confocal fluorescence microscopy images of GOx-containing HA-/Dex-P polysaccharidosomes after PS-co-PAA@WGA@HRP nanoparticle docking and subsequent exposure to glucose and Amplex Red at 25°C (expanded; open gate) (**b**), or 45°C (contracted, closed gate) (**c**). Significant production of resorufin (red fluorescence) is observed only in (**b**) over an initial time period of 720 s. Scale bars; 20 μm . **d**, Time-dependent plots of total red fluorescence intensity (resorufin) recorded from polysaccharidosome-mediated GOx/HRP cascade reactions undertaken at 25 and 45°C . Data sets are derived from the inner aqueous lumen (core) and membrane regions of the polysaccharidosomes as well as from the adjacent external aqueous medium. Data from control (CTL) experiments are also shown. **e**, Plots of temperature-dependent changes of the normalised initial rate of reaction (V_0) for GOx/HRP-mediated cascade reactions undertaken in the presence of glucose (0.21 mM) and Amplex Red (0.1 mM) for enzymes in free solution (control, green), a dispersion of PS-co-PAA@WGA@HRP nanoparticles and free GOx (red), and GOx-containing polysaccharidosomes with surface-attached PS-co-PAA@WGA@HRP nanoparticles (black). Data obtained from UV-Vis spectroscopy using the 570 nm absorption band.

Figure 4. Higher-order assembly of polysaccharidosomes via lectin-mediated crosslinking. **a**, Schematic depicting lectin-mediated reversible assembly of polysaccharidosome-based closely packed networks. Addition of excess ZIF@WGA nanoparticles (NPs) to HA-P polysaccharidosomes gives rise to higher-order assemblages with thermoresponsive properties. (GlcNAc, *N*-acetyl-*D*-glucosamine). **b,c**, Confocal fluorescence microscopy filtered images of HA-P polysaccharidosomes after incubation with excess ZIF@Alexa-WGA nanoparticles (1.1 mg L^{-1}) for 1 hr showing tissue-like clusters of closely packed polysaccharidosomes. Green (**b**) and blue (**c**) fluorescence originate from encapsulated BSA and nanoparticle-immobilized Alexa-WGA, respectively. The lectin is intercalated within the closely packed networks specifically at the adhesion junctions between adjacent protocells (**c**); scale bars, $25 \text{ }\mu\text{m}$. **d,e**, 2D confocal fluorescence filtered micrographs of extended closely packed networks assembled from RITC-labelled HA-P polysaccharidosomes (**d**, red fluorescence) with surface-attached ZIF-@WGA nanoparticles (**e**, blue fluorescence). Scale bars; $100 \text{ }\mu\text{m}$. **f,g**, 3D confocal fluorescence microscopy reconstructions of an extended closely packed networks shown in (**d,e**), respectively. **h-j**, Confocal fluorescence microscopy images of a tissue-like assemblage recorded at 25°C (**h**), 45°C (**i**) and 55°C (**j**). Co-ordinated contraction of the network occurs above 35°C . Red and blue fluorescence correspond to RITC-labelled HA-P polysaccharidosomes and surface-attached ZIF-@WGA nanoparticles, respectively; scale bar; $100 \text{ }\mu\text{m}$. **k,l**, 3D confocal fluorescence microscopy reconstructed images of a polysaccharidosome-based closely packed networks recorded at 25°C (**k**) or 55°C (**l**). Fluorescence labels as in (**h-j**); scale bar, $100 \text{ }\mu\text{m}$. **m,n**, Confocal fluorescence micrographs showing a closely packed cluster before (**m**) and after (**n**) disassembly for 24 h in the presence of an aqueous solution of *N*-acetyl-*D*-glucosamine (GlcNAc). **o,p**, Disassembly of a similar closely packed aggregates in the presence of acetate buffer (pH 4.0) before (**o**) after 24 h (**p**) is also shown; scale bars; $100 \text{ }\mu\text{m}$.

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Programmable glycan/lectin-mediated interfacial docking of nanoparticulate objects onto the semipermeable surface of polysaccharide-polymer microcapsules (polysaccharidosomes) is used to implement a protocell membrane-gated spatially localized cascade reaction and the reversible higher-order assembly into closely packed networks.