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'Sweet as a Nut': Production and Use of Nanocapsules made of Glycopolymer or Polysaccharide shell

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Abstract

Polymeric nanomaterials are deemed to unlock the potential of therapeutics and improve their efficiency, to allow future advances in nanomedicine. Among these, sugar-based nanocapsules prepared from synthetic or natural carbohydrate-based macromolecular chains have raised significant interest in bio-applications. This review exhaustively highlights all described physical or/and chemical techniques to construct both water- and oil-filled glycopolymer/polysaccharide-based nanocapsules. These include usual soft matter processes, such as (double)-emulsification, coacervation or layer-by layer techniques, and most original ones, e.g. PISA or template core removal. Properties and bio-applications of glyconanocapsules (GNCs) are summarized and challenges for future development of this field are finally raised.

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Abbreviation list

AFM	Atomic Force Microscopy
ATRP	Atom Transfer Radical Polymerization
CMC	Critical Micelle Concentration
Con A	Concanavalin A
DA	Degree of acetylation
DCC	N,N'-Dicyclohexylcarbodiimide
DLS	Dynamic light scattering
DMA	Dimethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DS	Degree of Substitution
EDTA	Ethylenediaminetetraacetic acid
FA	Folic acid
FITC	Fluorescein isothiocyanate
GNC	Glyconanocapsule
HF	Hydrofluoric acid
HPMA	N-(2-Hydroxypropyl) methacrylamide
ITC	Isothermal Titration Calorimetry
L-b-L	Layer-by-Layer
LCST	Lower Critical Solution Temperature
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
PBS	Phosphate Buffered Saline
PCL	Polycaprolactone
PEG	Polyethylene glycol
PEI	Polyethylenimine
PEO	Poly(ethylene oxide)
PLL	Poly(L-lysine)
PS	Polystyrene
RAFT	Reversible Addition-Fragmentation chain Transfer
RCA	Ricinus Communis Agglutinin
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
THF	Tetrahydrofuran
TTA	Triplet-triplet annihilation
UCL	Upconversion luminescence
UCST	Upper Critical Solution Temperature
UV	UltraViolet

1 Introduction

In the last decades, remarkable progresses have been made in the synthesis and the modification of sub-micronic carriers (typically, ranging from 10 to 500 nm).[1, 2] Clever design of these colloids led to fruitful achievements in agriculture, food, cosmetic and pharmaceutical domains.[3-6] Among them, polymer-based nanocapsules (including polymer vesicles, so-called 'polymersomes'), are a class of core/shell structures, in which the core is protected by a (preferably solid) polymer shell. The tailored variation of both core and polymeric shell provides capsules with attractive diversity in functionalities and properties: the polymer shell can be obtained from various synthetic or natural polymers, whereas the inner part of the capsules can be filled with aqueous, oily, solid or gaseous phase. The presence of an inner reservoir, in stark contrast to plain nanoparticles, allows high loading capacity and high delivery capability. Polymeric nanocapsules thus represent a powerful platform for encapsulation and delivery of actives (including e.g. genes, proteins, drugs, imaging agents) for pharmaceutical, diagnostic or nanomedicine applications.[7] Polymeric nanocapsules also allow (1) protecting the cargos against degradation and elimination; (2) enhancing the stability of nanocarriers; (3) reducing toxicity of cargos and (4) achieving delivery and release of cargos in a controlled manner.

Together with genes and proteins, carbohydrates (or sugars) moieties are key molecules in living organisms. However, in contrast to vigorous development in "genomics" and "proteomics", research in "glycomics" progresses slowly, surely due to inherent complexity of glycan structures and exclusive biosynthetic pathways. Still, tremendous achievement in interpreting the information of the "glyco-code" and

understanding fundamental functions of carbohydrates have already been made.[8-10] Beyond their essential role in energy storage, carbohydrates are also known to take part in cell communication, biological recognition events,[11] immune function[12] and signal transduction.[13] Since individual interactions of carbohydrates with other bio-molecules are extremely weak, nature found a perfect solution, the so called “glyco-cluster” effect, i.e. multiple simultaneous interactions of carbohydrates on interfaces with e.g. proteins.[14]

Incorporation of carbohydrates on the surface of nano-objects allows mimicking the glycocalyx (a carbohydrate-enriched layer covering the cell membrane). Specific biological recognition and/or interactions of carbohydrates allows both targeting and cumulative uptake by selective cells. Moreover, carbohydrate shells have been shown to confer stealth properties to nanocapsules thus diminishing immune elimination and prolonging their circulation time in the body. Finally, their biocompatible and biodegradable behaviors, together with their immunity, open the way to clinical trials.

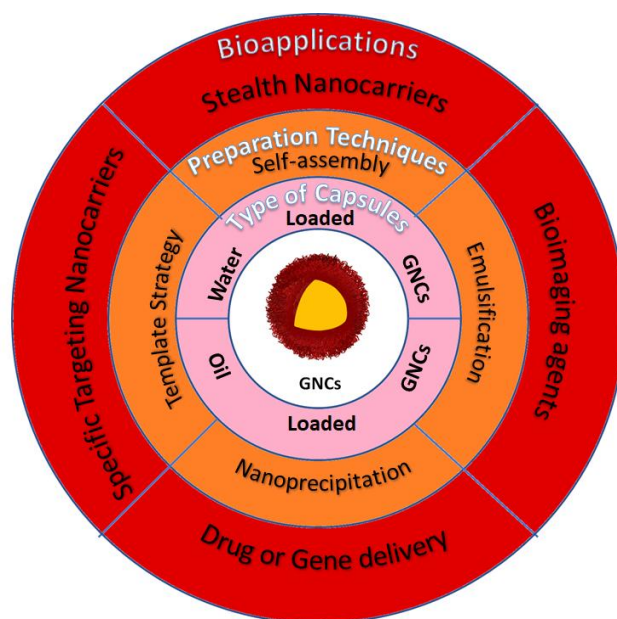
Glycopolymers and polysaccharides with multiple copies of carbohydrates units have been engaged in constructing myriads of nano-objects since the last century. In 2004, P. Couvreur gave a very comprehensive review on preparation and applications of polysaccharide-decorated nanoparticles.[15] From then on, several other reviews have been published,[16-18] all of them describing over the last decade impressive achievements of these sweet nanoparticles as therapeutic agents, drug carriers, bioimaging agents, etc. Thanks to their facile synthesis, carbohydrate-functionalized inorganic nanoparticles (e.g. glyco-magnetic nanoparticles, glyco-quantum dots) have deserved a large part of attention[19, 20], but their usual intrinsic toxicity and

non-degradable character restricted further applications in bio-applications. In contrast, carbohydrate-based organic polymeric nanocapsules (or “glyconanocapsules”) benefit from their functional core/shell nanostructures and biocompatibility but inevitably require more energy and ingenuity for precise construction, which explains that they were hardly mentioned in these reviews.

In this article, recent achievements of **sugar-based nanocapsules** are reviewed (**Scheme 1**). This manuscript first covers all the important strategies for constructing core/shell carbohydrate-based nano-objects, from naturally-occurring polysaccharides to synthetic glycopolymers, and then their use in biomedical applications. To narrow the scope of this review, and since our interest focuses specifically on functional nanocapsules whose polymeric shell is mainly consisting of glycopolymers and/or polysaccharides, approaches relying on post-surface incorporation, functionalization by polysaccharides or cyclodextrin-based colloids will not be described here.[21-23]

All reported chemical or/and physical approaches to **sugar-based nanocapsules** as well as glyco-polymerosomes, are first described, including popular methods such as i) self-assembly of amphiphilic copolymers into polymerosomes or core-degradable micelles, ii) layer-by-layer deposition of polymers on sacrificial template particles, iii) interfacial polymerization under miniemulsion conditions, and iv) new coming technologies. According to the techniques of preparation, the reservoir of capsules can be either filled with water (part 2) or oil (part 3). The final part addresses biomedical applications of these glyconanocapsules, including i) the design of drug delivery systems promoting specific targeting and extended circulation times *in vivo*, ii)

the development of colloids for anti-microbial therapy and iii) the construction of bioimaging tools for visualizing physiological and pathological processes.



Scheme 1: Scheme summarizing the content of this review.

2 Water-filled sugar-based nanocapsules

Nanocapsules with an aqueous core have been long studied for their high potential to encapsulate hydrophilic cargos, that are as numerous as hydrophobic ones. This is particularly difficult to achieve and to keep the payload inside, for obvious pressure gradient effect. This chapter summarizes the most relevant methods to prepare these capsules.

2.1 Formation of hollow nano-objects from amphiphilic copolymers' self-assembly

Water-filled sugar-based nanocapsules can be robustly prepared by three different techniques: direct self-assembly of synthetic amphiphilic copolymers (polymersomes), polymerization induced self-assembly (PISA) and post-core removal of micelles.

2.1.1 Polymersomes

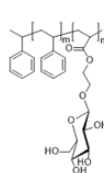
The word *polymersome* was first coined by Discher in 1999 and refers to colloids that mimic the self-assembly of liposomes.[24] Polymersomes are nano- or micro-sized polymeric vesicles or water-filled capsules made of amphiphilic copolymers.[25] The membrane structure of polymersomes is very similar to liposomes, except that liposomes are built from phospholipids with molecular weight typically less than 1 kg/mol, while polymersomes arise from self-assembly of amphiphilic block copolymers of molecular weights of up to several hundred thousands g/mol. This provides polymersomes superior physical and chemical stability over liposomes.

Owing to repulsion effects between the (chemically-tuned) macromolecular segments, amphiphilic block copolymers self-assemble in aqueous solution. The length of the blocks, the degree of repulsion and the nature of the solvent drive the self-assembly of the block copolymers into a myriad of morphologies such as spherical micelles, rods and vesicles or so-called polymersomes. In general, block copolymers containing short hydrophobic segment self-assemble into micelles, whereas polymersomes form in aqueous media when the molar fraction of the hydrophobic segment counts for more than 75%. Solvent displacement, rehydration and injection techniques have been applied to polymersome preparation with diameters ranging from 50 to 800 nm. In the following, we provide an overview on routes to carbohydrate-functionalized polymersomes relying on the self-assembly of amphiphilic carbohydrate-based block copolymers.

2.1.1.1 Glycopolymer-based polymersomes

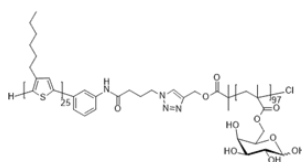
Solvent displacement. The first example of glyco-polymersomes was reported by

Fu-Mian Li's group in 1999.[26, 27] Sequential ATRP polymerizations generated a series of amphiphilic glucose-functionalized diblock copolymers, polystyrene-*b*-poly[(2- β -D-glucopyranosyloxy)ethyl acrylate] (PS₁₀₁-*b*-PGEA₇, PS₈₈-*b*-PGEA₄, PS₇₇-*b*-PGEA₆ and PS₅₅-*b*-PGEA₉, **1**, **Scheme 2**). The self-assembly of the block copolymers was triggered by solvent displacement through addition of water into an organic solution of the polymer (including dioxane, DMF, THF and their mixtures). This resulted in the preparation of morphologies ranging from spheres, vesicles to fibers, depending on the initial composition of the polymer solution (**Figure 1A**).



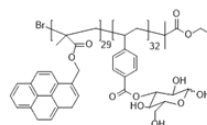
PS-*b*-poly[(2-β-D-glucopyranosyloxy)ethyl acrylate]

1



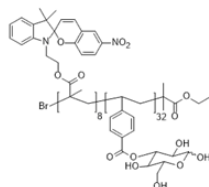
Poly(3-hexylthiophene)-*b*-poly(6-O-methacryloyl-D-galactopyranose)

2



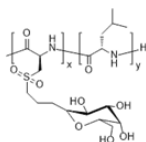
Poly(pyrenylmethyl methacrylate)-*b*-poly(3-O-4-vinylbenzoyl-D-glucopyranose)

3



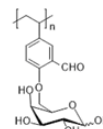
Poly(spiropyran methacrylate)-*b*-poly(3-O-4-vinylbenzoyl-D-glucopyranose)

4



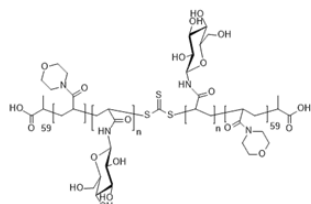
Poly(α-D-galactopyranosyl-L-cysteine)₆₅-*b*-(leucine)₂₀

5



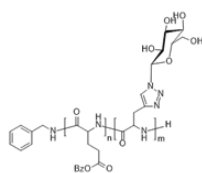
Poly[1,2,3,4-di-isopropylidene-6-O-(2'-formyl-4'-vinylphenyl)-D-galactopyranose]

6



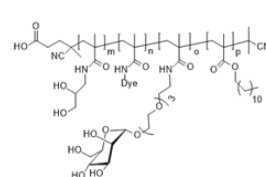
Poly(*N*-acryloylmorpholine)-*b*-poly(*N*-β-D-glucopyranosylacrylamide)-*b*-poly(*N*-acryloylmorpholine)

7



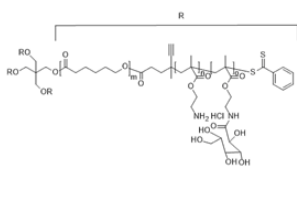
PBLG-*b*-poly(galactosylated propargylglycine)

8



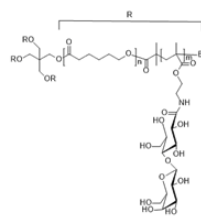
P(DHPMA-*stat*-Mann-*stat*-OG)₃₃-*b*-P(LMA)₂₈

9



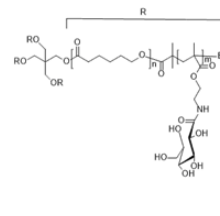
Poly(ε-caprolactone)-*b*-poly(2-aminoethylmethacrylate)-*b*-poly(glycanamidoethylmethacrylate)

10



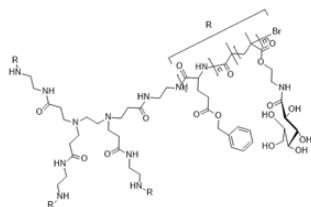
Poly(ε-caprolactone)-*b*-poly(lactobionamidoethylmethacrylate)

11



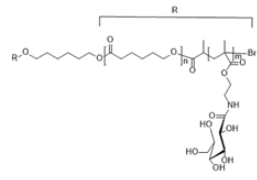
Poly(ε-caprolactone)-*b*-poly(gluconamidoethylmethacrylate)

12



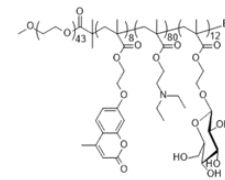
Poly(γ-benzyl L-glutamate)-*b*-poly(D-gluconamidoethyl methacrylate) functionalized G0 PAMAM

13



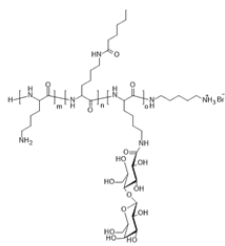
Poly(D-gluconamidoethyl methacrylate)-PPR-poly(D-gluconamidoethyl methacrylate)

14



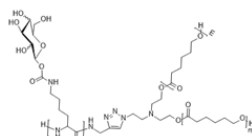
PEO₄₃-*b*-P(CMA₈-*stat*-DEA₈₀-*stat*-GEMA₁₂)

15



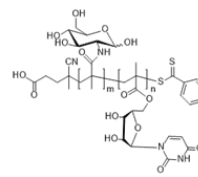
Poly(L-lysine)-*graft*-hexanoyl-lactobionolactone

16



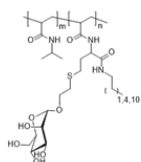
20GP-(PCL₂₅)₂

17



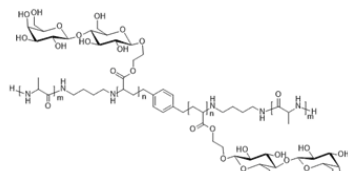
Poly(5'-O-methacryloyl uridine)-*b*-poly(2-methacrylamido glucopyranose)

18



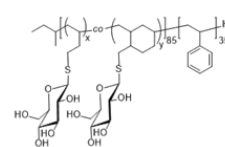
PNIPAAm-co-glycopolymer bearing mannoside and alkylamine

19



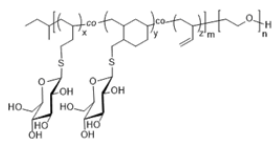
Poly(L-alanine)-*b*-poly(2-acryloyloxyethyl lactoside)-*b*-poly(L-alanine)

20



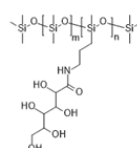
2,3,4,6-tetra-O-acetyl- β -D-1-thioglucofuranose modified 1,2-polybutadiene-*block*-polystyrene

21



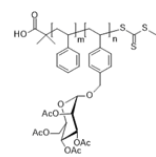
2,3,4,6-tetra-O-acetyl-1-thio- β -D-glucopyranose modified 1,2-polybutadiene-*block*-poly(ethylene oxide)

22



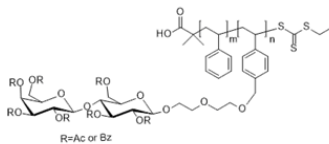
Glucosamine-grafted amphiphilic glycopoly-siloxanes

23



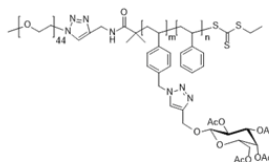
PS-*b*-poly(tetra-acetyl- α -1-mannosyl styrene)

24



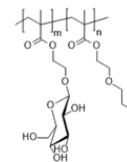
PS-*b*-PLac (Ac or Bz)

25



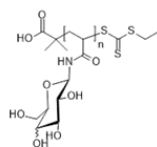
PEG-AcGal_{*m*}-PS_{*n*}

26



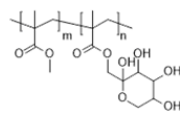
Poly(2-glycosyloxyethyl methacrylate)-*b*-poly(diethyleneglycol methacrylate)

27



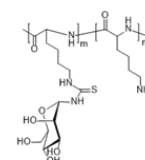
Poly(1-N- β -glucosyl acrylamide) (PGlc)
Poly(1-N- β -galactosyl acrylamide) (PGal)

28



Poly(1-O-methacryloyl- β -D-fructopyranose)-*b*-poly(methyl methacrylate)

29



P(M/Lys₅-co-Lys₂₇)

30

Scheme 2. Structures of glycopolymers used to generate polymersomes by self-assembly.

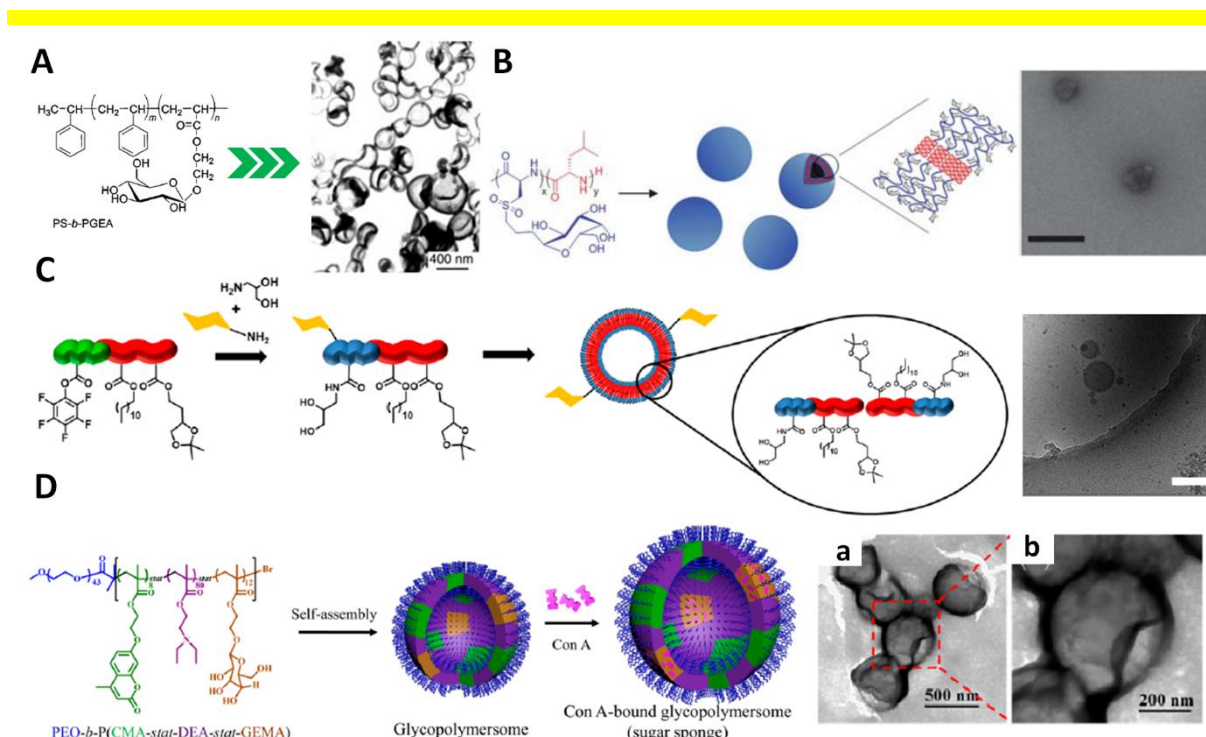


Figure 1. (A) Self-assembly of PS₅₅-b-PEGA₉ into vesicles/polymersomes by adding water in dioxane solution;[26, 27] Copyright 1999 and 2000. Adapted with permission from the Royal Society of Chemistry. (B) Schematic presentation of self-assembly of glycopeptides to polymersomes and TEM image of resulting nanostructures; scale bar is 200 nm;[28] Copyright 2013. Adapted with permission from the Royal Society of Chemistry. (C) Route to carbohydrate-functionalized polymersomes and TEM image of the resulting nano-objects;[29] Copyright 2016. Adapted with permission from American Chemical Society. (D) Self-assembly of PEO₄₃-b-P(CMA₈-stat-DEA₈₀-stat-GEMA₁₂) to generate sugar-embedded glycopolymerosomes, with resulting TEM images of polymersomes.[30] Copyright 2017. Adapted with permission from American Chemical Society.

From then on, solvent displacement has become a popular procedure to trigger self-assembly of amphiphilic glycopolymers. For instance, Aissou *et al.* reported on the preparation of galactose-based nanocapsules from self-assembly of fluorescent copolymer poly(3-hexylthiophene)-*block*-poly(6-*O*-methacryloyl-D-galactopyranose)

(**2, Scheme 2**) by slow addition of a THF/water mixture into 1% w/w polymer/THF solution.[31] Das and coworkers introduced water into DMF solutions of poly(pyrenylmethyl methacrylate)-*b*-poly(3-O-4-vinylbenzoyl-D-glucopyranose) (**3, Scheme 2**)[32] and poly(spiropyran methacrylate)-*b*-poly(3-O-4-vinylbenzoyl-D-glucopyranose) (**4, Scheme 2**),[33] generating glucose-functionalized polymersomes with diameters of 473 and 366 nm, respectively. Deming and coworkers reported the synthesis of glycosylated diblock polypeptides, poly(α -D-galactopyranosyl-L-cysteine)₆₅-*b*-(leucine)₂₀ (**5, Scheme 2**).[28] Addition of water to THF solutions of these polymers resulted in the preparation of vesicles with diameters of 140 nm (**Figure 1B**). Xiao *et al.* prepared glycopolymers by RAFT polymerization of an aldehyde-functionalized glycomonomer, 1,2,3,4-di-isopropylidene-6-O-(2'-formyl-4'-vinylphenyl)-D-galactopyranose (IVDG). After deprotection of the sugar moieties, the resulting homopolymers (**6, Scheme 2**) exhibiting amphiphilic character were transformed into glycopolymersomes, giving diameters ranging between 160 and 410 nm by addition of water into a DMSO solution.[34]

Albertin *et al.* studied the impact of THF/water ratio on the dimensions and the polydispersity of polymersomes. An increase of THF fraction (5-91% v/v) when mixing THF solutions of triblock copolymers poly(*N*-acryloylmorpholine)-*b*-poly(*N*- β -D-glucopyranosylacrylamide)-*b*-poly(*N*-acryloylmorpholine) (**7, Scheme 2**) with water enabled to significantly decrease both diameter and dispersity of resulting nanoparticles, finally giving uniform polymersomes with a diameter of 380 nm in 91% THF.[35] Lecommandoux and coworkers investigated the impact of

the sequence of addition of solvent and non-solvent phases on polymersomes morphologies. Addition of a DMSO solution of PBLG-*b*-poly(galactosylated propargylglycine) (**8**, **Scheme 2**) into water generated spherical nanostructures, rather than worm-like aggregates obtained from the reverse sequence.[36] Recently, Zentel *et al.* used mannose-incorporated multi-block copolymers (**9**, **Scheme 2**) to construct polymersomes (diameter ~275 nm) by gradual infusion of PBS buffer using a syringe pump (**Figure 1C**).[29]

Apart from linear diblock copolymers, the star-shaped glycopolymer poly(ϵ -caprolactone)-*b*-poly(2-aminoethylmethacrylate)-*b*-poly(glyconamidoethylmethacrylate) (**10**, **Scheme 2**) with much more complex microstructure was also able to self-assemble into glyconanocapsules of 59 nm in diameter by solvent shifting process (addition of PBS buffer into DMSO solution).[37] Dong's group reported on the preparation of a series of star-shaped glycopolymers with different core structures, e.g. pentaerythritol (**11** and **12**, **Scheme 2**),[38, 39] G0 PAMAM (**13**, **Scheme 2**)[40] or 1,6-hexanediol (**14**, **Scheme 2**),[41] by combining ring opening polymerization (ROP) of ϵ -caprolactone and subsequent chain-extension ATRP polymerization of gluconamidoethylmethacrylate (GAMA) or lactobionamidoethylmethacrylate (LAMA). Gradual addition of water into DMF/polymer mixture gave rise to self-assembly into vesicles, micelles or worm-like nano-objects, depending on the length of glycopolymer segments.

In contrast to most polymersome structures where sugar moieties protrude at the surface, Du *et al.* carefully constructed glyco-vesicles (diameter ~390 nm) with glucose moieties embedded in the membrane. To do so, they self-assembled

PEO₄₃-*b*-P(CMA₈-*stat*-DEA₈₀-*stat*-GEMA₁₂) (**15**, **Scheme 2**) in a mixture of THF/water (volume ratio, 1/3).[30] Owing to the presence of coumarin groups, the nanocapsules structure could be further “freezed” through photodimerization under UV irradiation ($\lambda \approx 365$ nm). In addition to the covalent cross-linking method, lectin Con A was also introduced to stabilize the membrane by specific carbohydrate-lectin recognition (**Figure 1D**). Interesting “sugar sponge”-like structures were produced, the diameters of which increased with the concentration of Con A from 375 to 537 nm for concentrations of 50 to 200 $\mu\text{g/mL}$, respectively.

Dialysis-triggered self-assembly. Dialysis of an organic solution against water is an efficient, alternative, triggering solvent displacement. Huang *et al.* constructed lactose-based, pH-sensitive polymersomes (diameter of ~ 220 nm at pH 7.4) by dialyzing a solution of methanolic poly[(L-lysine)-*graft*-hexanoyl lactobionolactone] (**16**, **Scheme 2**) against water.[42] Owing to protonation of PLL segments, the resulting nano-objects swelled to reach 260 nm in size after shifting pH to 4.7. Gupta’s group dialyzed a solution of DMSO/ water (1/1, v/v) containing glycopolyptide-*block*-polycaprolactone (20GP-(PCL₂₅)₂) (**17**, **Scheme 2**) against water to produce polymersomes with diameter of 55 nm.[43]

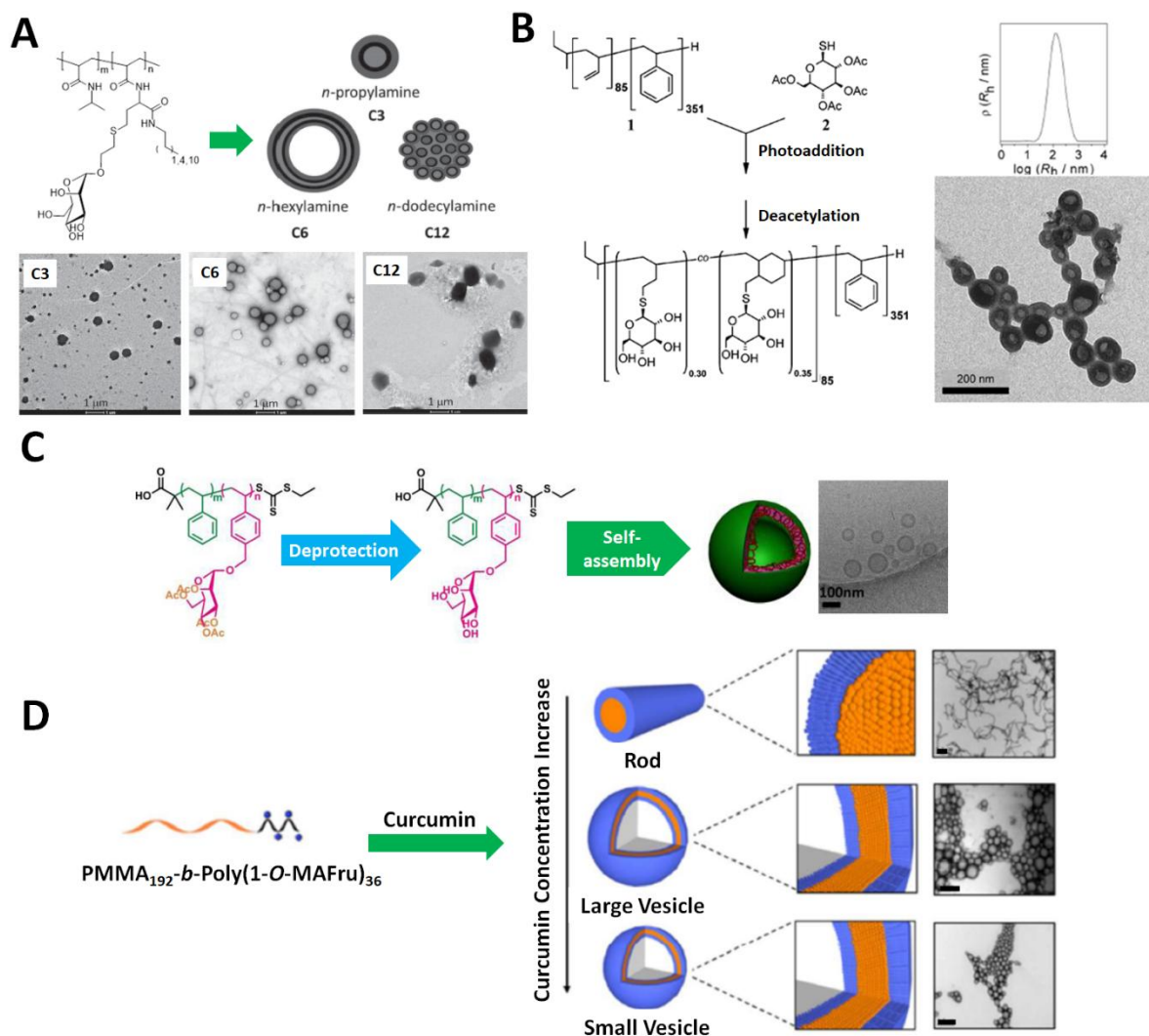


Figure 2. (A) Construction of various glyco-nano-objects through dialysis triggered self-assembly, and TEM images of resulting nanoparticles, C3, C6 and C12 correspond to the length of side alkyl chains;^[44] Copyright 2014. Adapted with permission from American Chemical Society. (B) (Left) Schematic synthesis of block glyco(co)polymer; (Right) TEM image and DLS of Glycovesicles in water;^[45] Copyright 2006. Adapted with permission from American Chemical Society. (C) Schematic representation of deprotection induced self-assembly of glycopolymer to generate glyco-inside nanocapsules and TEM image of resulting nanoparticles;^[46] Copyright 2014. Adapted with permission from American Chemical Society. (D) Self-assembly of Poly(1-O-MAFru)₃₆-*b*-PMMA₁₉₂ (2 mg/ml) in presence of different amounts of curcumin: 0, 0.37

mg/mL and 0.75 mg/mL to produce nanoparticles with various morphologies and TEM image of resulting nanoparticles. Scale bar = 500 nm.[47] Copyright 2018. Adapted with permission from American Chemical Society.

Stenzel's group reported on dialysis of glycopolymers in DMA solution against water to induce self-assembly of 5'-O-methacryloyl uridine (MAU) and 2-methacrylamido glucopyranose pendent block copolymers (**18, Scheme 2**) into nano-objects with morphologies evolving from micelles to worm-like micelles with increasing length of PMAU segments.[48] More recently, the same group prepared random copolymers from *N*-isopropylacrylamide (NIPAAm)/*N*-homocysteine thiolactone acrylamide (TiaAm) by RAFT copolymerization. The pendent thiolactone groups were then opened with various amines (*n*-propylamine, *n*-hexylamine and *n*-dodecylamine) to release thiol moieties which were finally reacted *in situ* with 2-bromoethyl-2',3',4',6'-tetra-*O*-acetyl- α -D-mannopyranoside (**Figure 2A**). Dialysis of a DMF solution of these polymers (**19, Scheme 2**) against water gave birth to micelles (ring opening with *n*-propylamine), large compound micelles (ring opening with *n*-dodecylamine) or vesicles of 100-600 nm in diameter (ring opening with *n*-hexylamine).[44]

Both solvent displacement and dialysis methods make use of a variety of water miscible organic solvents, including THF, dioxane, methanol, DMF and DMSO. The interaction parameters between polymer and solvent and solvent and non-solvent govern the supersaturation and precipitation of polymers into nanoparticles. However, the solvent selection alone cannot be used to predict the morphology of resulting nanoparticles after self-assembly, other factors (i.e. polymer concentration or solid

content, the length of hydrophilic segments and so on) show synergistic effect on the formation of vesicles.

Direct dissolution. When amphiphilic block copolymers display a favorable hydrophilic/hydrophobic balance, self-organization in solution can be obtained from direct dissolution (above the critical micelle concentration). The group of Chaikof introduced triblock copolymers, poly(L-alanine)-*b*-poly(2-acryloyloxyethyl lactoside)-*b*-poly(L-alanine) (AGA) (**20**, **Scheme 2**) of different segment ratios (From $A_{10}G_{10}A_{10}$ to $A_{22}G_{52}A_{22}$) in stirred water to trigger spontaneous self-assembly.[49, 50] The resulting morphologies varied from spheres to worm-like aggregates by varying polymer concentration and pH of the solution. In particular, a vesicular morphology was generated from $A_{22}G_{52}A_{22}$ at 10 mg/L in acidic aqueous solution (pH 2).

Schlaad and coworkers also constructed glucose based nanocapsules by spontaneous self-assembly of a glycopolymer in either pure THF (d~500 nm) or progressive incorporation of water and subsequent evaporation of THF (d~200 nm, **Figure 2B** and polymer **21**, **Scheme 2**).[45, 51] The same group prepared amphiphilic copolymers (**22**, **Scheme 2**) by post-modification of 1,2-polybutadiene-*block*-poly(ethylene oxide) (PB_{68} -*b*- PEO_{34}) with thiol-functionalized glucose (6-9 wt.% per chain).[52] The resulting copolymers readily self-assembled in aqueous solution to form vesicles (d~560 nm). 2D-NOESY-NMR and surface-enhanced Raman proved that the formed membrane displayed an asymmetric structure with sugar moieties on the outer surface and PEO inside. Du *et al.* synthesized glycopolysiloxanes-based large vesicles (**23**, **Scheme 2**) by simple

dissolution in water (d~300-600 nm).[53]

Protection/deprotection. Protection/deprotection is one of the most important strategies in carbohydrate synthesis, offering an opportunity to derive carbohydrate polymers with very complex structures. Chen, Jiang and coworkers applied deacetylation methods to deprotect the hydroxyl groups of sugar moieties, converting a hydrophobic block copolymer polystyrene-*b*-poly(tetra-acetyl- α -1-mannosyl styrene) (PS₇₅-*b*-PManAc₂₅, **24**, **Scheme 2**) into an amphiphilic structure in THF solution, and triggering its spontaneous self-assembly into vesicles with glyco-inside structure (diameter of ~84 nm, **Figure 2C**).[46] The vesicles were decorated with gold nanoparticles by directly adding HAuCl₄ (0.2 mM) to the vesicles solution of PS₇₅-*b*-PMan₂₅ in THF. Importantly, no reducing agent was required here. Further addition of water into THF solution led to the destruction of vesicles and formation of micelles (d~22 nm) with a glycopolymer shell and a PS core.

The kinetics of the deprotection-induced self-assembly process involving different protecting groups was then investigated by DLS, NMR and morphologies evolutions. In contrast to micellar structures generated from removal of acetyl groups, benzoylation of the sugars slowed down the deprotection rate and resulted in thermodynamically-controlled vesicles morphology (glycopolymer, **25**, **Scheme 2**).[54] In 2018, the same group constructed a series of vesicles (d~116-300 nm) by self-assembly of a triblock glycopolymer containing PEG, peracetylated glycopolymer and PS segments (**26**, **Scheme 2**) when adding water into a THF polymer solution.[55] Deprotection by alkali or lipase enzyme triggered an interesting morphology transition to glyco-micelles thanks to the contribution of carbohydrate-carbohydrate interactions.

Temperature induced self-assembly. Thermo-responsive segments of copolymers exhibiting LCST or UCST can lead to macromolecular reorganization with temperature. Pasparakis and Alexander constructed two hydrophilic block copolymers (**27, Scheme 2**) from 2-glucosyloxyethyl methacrylate (PGEMA) and diethyleneglycol methacrylate (PDEGMA) using sequential ATRP (P1, $M_n = 11.2$ kg/mol, $\bar{D} = 1.34$, LCST = 28°C) or RAFT (P2, $M_n = 15.2$ kg/mol, $\bar{D} = 1.11$, LCST = 28°C) polymerizations.[56] Tuning the temperature of the aqueous solution from 15 to 20°C led to self-assembly of copolymers into glycovesicles ($d \sim 250$ nm for P1 and 500 nm for P2). By further increasing the temperature to 37°C (above the LCST), the resulting nanocapsules shrunk to 182 nm (P1) and 300 nm (P2) owing to the collapse of the PDEGMA segments. Jiang and coworkers grafted PNIPAAm segments to two glyco(homo)polymers (PGal or PGlc) through dynamic covalent bonds (**28, Scheme 2**).[57] The PNIPAAm/glycopolymers (2/1) mixtures were shown to self-assemble at 33°C (LCST $\sim 32^\circ\text{C}$) to give birth to uniform vesicles (diameters, 124 and 136 nm, $\bar{D} = 0.09$ and 0.10 for V-PGal and V-PGlc, respectively).

Other processes. Recently, Stenzel's group reported that a model drug, curcumin, is able to participate in the assembly process of poly(1-O-methacryloyl- β -D-fructopyranose)-*b*-poly(methyl methacrylate) (**29, Scheme 2**) after addition of water into a THF solution of polymer and drug.[47] Thanks to the interactions between sugar moieties and drug, the resulting nanoparticles underwent a morphology transition from cylindrical micelles (no drug involved) to vesicles (0.75 mg/mL of drug, diameter ~ 263 nm). Since the curcumin is entrapped in the glycopolymer shell instead of the core, increasing the curcumin content provoked the dehydration of shell and

further shrunk the vesicles (**Figure 2D**).

Besides the above triggering methods, amphiphilic-based polymersomes have been spontaneously formed through pH shifting. In 2010, Wang *et al.* synthesized mannose-modified polylysine copolymers P(M/Lys₅-*co*-Lys₂₇) and P(M/Lys₁₄-*co*-Lys₄₇) by ring-opening polymerization and post-modification (**30**, **Scheme 2**), which both self-assembled into nanoparticles of different morphologies in aqueous solution.[58] At pH 4, both copolymers formed spherical micelles of about 10-15 nm in diameter. At pH > 10, vesicles with diameters of 45-80 nm were generated. The wall thickness of the vesicles were 12 and 20 nm, respectively.

2.1.1.2 Polysaccharide-based polymersomes

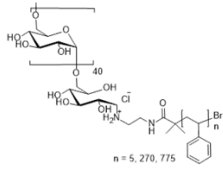
Solvent displacement. To promote self-assembly, natural polysaccharides have been frequently modified by incorporating hydrophobic blocks along the polymer backbone or at their chain-ends. Gnanou, Taton and co-workers converted the anomeric extremity of dextran into an ATRP initiating site by reductive amination.[59, 60] The resulting macroinitiator was then engaged in a controlled polymerization of styrene to afford amphiphilic Dextran₄₀-*b*-PS_n block copolymers (n = 5, 270, 775, **31**, **Scheme 3**). Depending on the length of the PS block, micelles (Dextran₄₀-*b*-PS₅, d = 56 nm) and vesicles (Dextran₄₀-*b*-PS₂₇₀, d = 128 nm or Dextran₄₀-*b*-PS₇₇₅, d = 154 nm) were obtained upon displacement of the organic solvent (THF or DMSO/THF) by water. The size and the shape of the assembled nano-objects were then fixed by cross-linking the dextran shell by reacting hydroxyl groups with divinylsulfone.

Lecommandoux and co-workers also applied the CuAAC reaction to couple natural

dextran and a synthetic polypeptide, poly(γ -benzyl L-glutamate) (**32**, **Scheme 3**). Further preparation of polysaccharide- and polypeptide-based vesicles with thick membranes ($d = 90$ nm, thickness = 20 nm) was also performed by slow addition of water into DMSO solution (**Figure 3A**).^[61] Similar synthesis and preparation methods have also been introduced to construct hyaluronan-based bilayer vesicles ($d = 440$ nm) through addition of warm water to a DMSO solution of PBLG₂₃-*b*-hyaluronan (**33**, **Scheme 3**) set at 55°C.^[62-64] Zhou *et al.* introduced an isocyanate-terminated polypeptide poly(Lys₁₁-*stat*-Phe₁₀) onto a carboxylic acid-functionalized chitosan backbone through an urea covalent bond. Then, half of the remaining chitosan carboxyl groups was further esterified with methanol to obtain amphiphilic copolymers (**34**, **Scheme 3**).^[65] The resulting graft copolymers self-assembled in a water/THF solution (addition of water into water/THF solution) to afford biocompatible nanocapsules ($d \sim 230$ nm) exhibiting antibacterial properties.

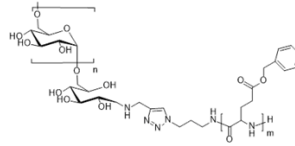
An octadecanol-modified dextran (OMD, **35**, **Scheme 3**) was introduced by Chiang *et al.* at different composition ratios (DS = 0.27, 0.51, 0.84 and 1.08).^[66] Polymer chains with a DS=0.51 self-assembled into vesicles ($d = 192$ nm, 10 vol.% initial water in DMF-water) through addition of water into a polymer/DMF/water solution. The diameter of these polymersomes was tuned from 62 to 192 nm by changing the composition of the copolymers and the initial water content in water-DMF mixture (40 to 10 vol.%). Jayakannan and coworkers developed a series of original vesicular carriers from dextran derivatives (**36**, **Scheme 3**).^[67]^[68] Dextran was initially partially functionalized with a variety of hydrophobic units, i.e. 3-pentadecyl phenol (PDP), cardanol (CAR), or stearic acid (SA). As evidenced by light scattering and

TEM/AFM analysis, the resulting amphiphilic polymers self-assembled in aqueous solution to generate vesicles by solvent displacement (addition of water into a DMSO solution of polymer). The diameter of the vesicles increased along with PDP substitution (from 120 to 300 nm for Dex-PDP-5 and Dex-PDP-19, respectively, where the number corresponds to PDP motifs per chain).



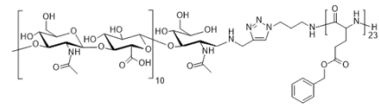
Dextran-*b*-PS

31



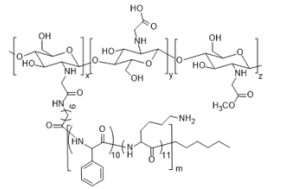
Dextran-*b*-PBLG

32



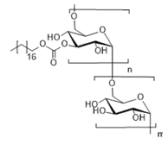
PBLG-*b*-hyaluronan

33



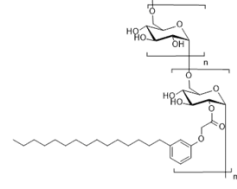
Poly(Lys₁₁-stat-Phe₁₀)-*g*-Cs_x-stat-Cs_{2x}-stat-ECs_{3x}

34



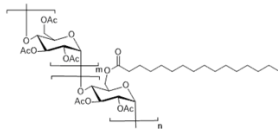
Octadecanol-dextran

35



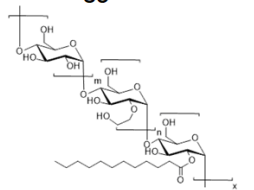
3-pentadecyl phenol modified Dextran

36



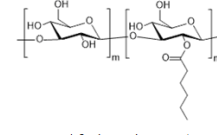
Acetylated starch palmitate

37



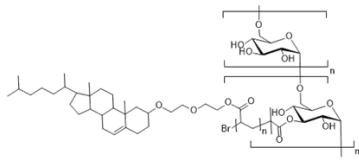
Fatty acid modified hydroxyethyl starch

38



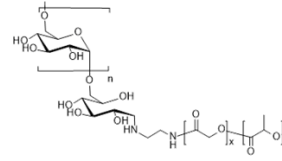
β -1,3-glucan hexanoate

39



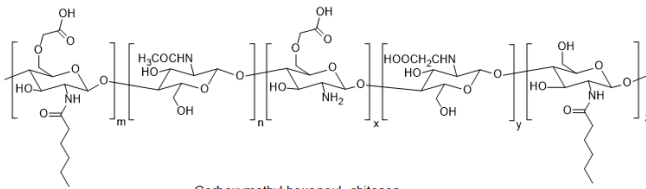
Dex-*g*-PADEGChol

40



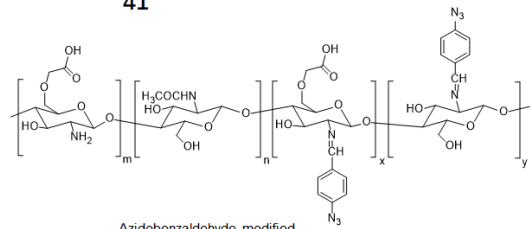
Dextran-*b*-poly(lactide-co-glycolide)

41



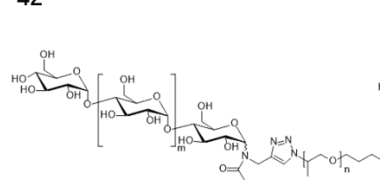
Carboxymethyl-hexanoyl chitosan

42



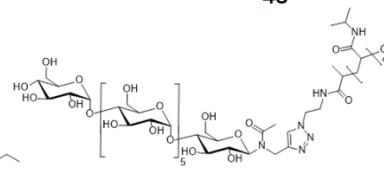
Azidobenzaldehyde modified photocrosslinkable carboxymethyl chitosan

43



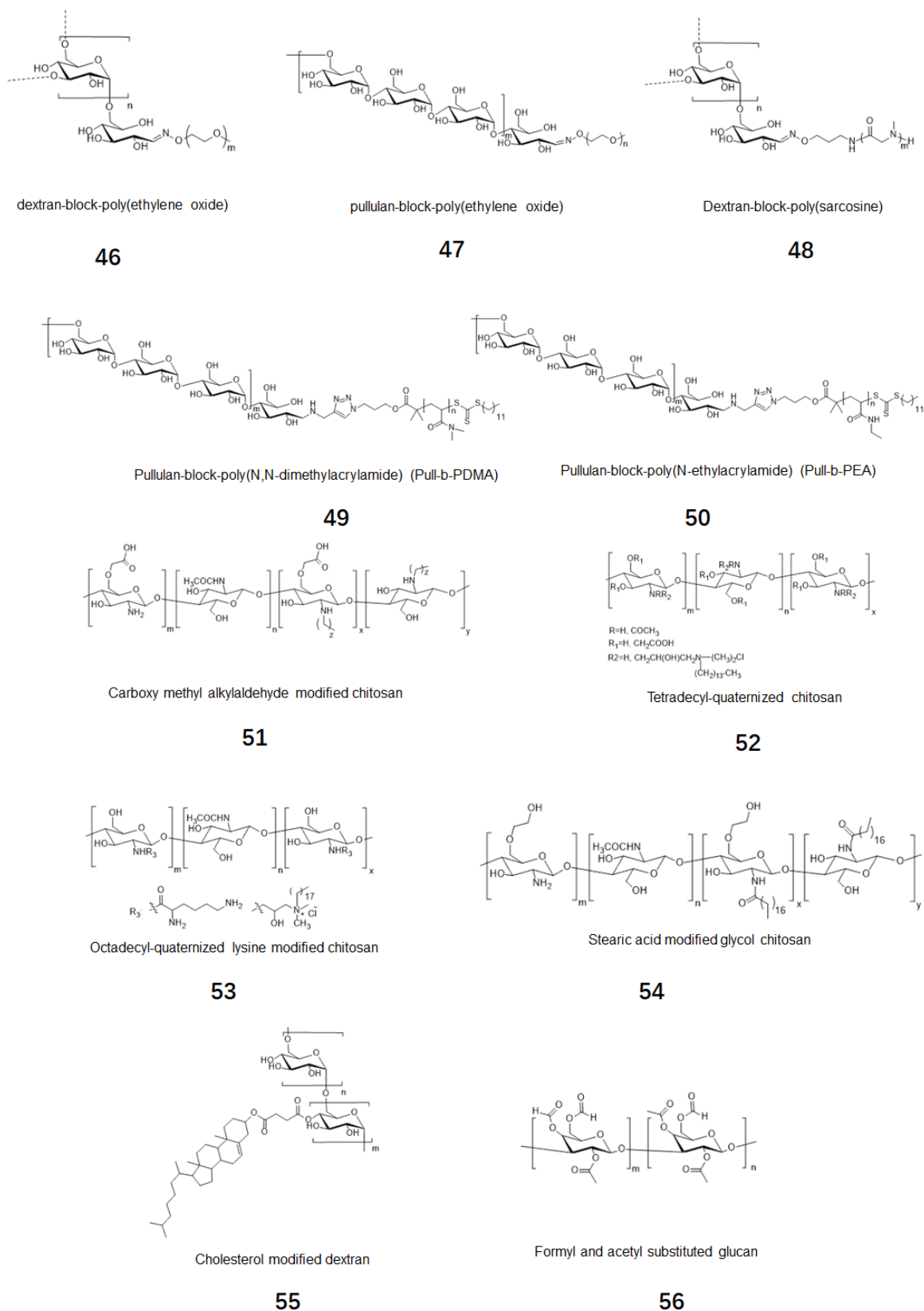
maltoooligosaccharide-*b*-poly(propylene glycol)

44



Maltoheptaose-*b*-PNIPAM

45



Scheme 3. Structures of polysaccharides engaged in polymersomes' self-assembly

Wang and coworkers modified starch with palmitoyl chloride (PA) and acetic anhydride (AC), leading to a series of amphiphilic polymers S-PA-AC of various

degrees of substitution (DS_{PA} ranging from 0.005 to 0.3 and DS_{AC} ranging from 2.51 to 2.78, **37, Scheme 3**).[69] Self-assembly was carried out by addition of water into a THF solution of the polymer. Stable vesicles were generated after evaporation of THF for polymers with high DS_{PA} (0.2). The diameter of the vesicles was easily tuned (from 300 to 1000 nm or more) by varying the initial concentration of polymer in THF (2, 5, 8, 10 and 15 mg/mL). Besheer *et al.* incorporated lauric, palmitic and stearic acids ($DS \sim 0.29, 0.44$ and 0.17) on hydroxyethyl-starch by DCC catalyzed esterification (polymer, **38, Scheme 3**). Addition of water into a water/THF solution (3/1, v/v) finally resulted in micelles ($d \sim 20\text{-}30$ nm) and vesicles ($d \sim 250\text{-}350$ nm) mixtures.[70]

Dialysis triggered self-assembly. Wu *et al.* described the preparation of glucan acetates ($DS = 0.92\text{-}2.46$, BGA) and glucan hexanoates ($DS = 1.87$, BGH). Upon solvent displacement (acetone solution dialyzed against water), the resulting polymer chains self-organized into nanocapsules or solid nanoparticles (with diameters ranging from 132 to 487 nm). Vesicles ($d \sim 490$ nm) were only observed with BGH (**39, Scheme 3**).[71] Six's group synthesized a series of amphiphilic polymers (**40, Scheme 3**) with a dextran backbone and poly(diethylene glycol cholesteryl ether acrylate) (PDEG-CholA) liquid crystal grafts.[72] Vesicular morphologies were generated by dialysis of organic solutions of polymers (in DMSO or THF) against water, with diameters ranging between 94 and 136 nm (DMSO) and 94 and 126 nm (THF).

Solvent-free emulsification. Hadizadeh and Ramezani recently developed dextran-based polymersomes based on amphiphilic copolymers of dextran-*b*-poly(lactide-co-glycolide) (**41, Scheme 3**).[73] A modified direct hydrating

method involving ultrasonication and vigorous stirring in PBS solution gave rise to self-assembly of the copolymers into polymersomes with diameters of 132 and 270 nm, depending on the length of each block. Liu and coworkers designed amphiphilic carboxymethyl-hexanoyl chitosan copolymers (CHCs) of different DSs (0.5 for carboxymethyl groups and 0~0.48 for hexanoyl groups, **42**, **Scheme 3**) to prepare nanocapsules in water.[74] The self-assembly of CHC was impacted by the incorporation of hexanoyl pendent groups. Large nanocapsules, 200 nm in diameter, were formed using CHCs with high hexanoyl content (DS = 0.26~0.48), whereas very small capsules (d ~20 nm) were claimed from CHCs with low hexanoyl content (DS lower than 0.26, no TEM photos available).

Yin and coworkers sequentially functionalized chitosan with chloroacetic acid and azidobenzaldehyde (**43**, **Scheme 3**). Self-assembly of modified chitosan chains in water afforded nanocapsules with a diameter of 150 nm at neutral pH (**Figure 3B**). Owing to the presence of photo-sensitive azidobenzyl moieties, the nano-objects were further crosslinked by UV exposition (15 min, $\lambda = 253$ nm).[75, 76] Akiyoshi's group applied a direct hydration method to self-assemble maltooligosaccharide-*b*-poly(propylene glycol) (**44**, **Scheme 3**) into vesicles (referred in the text as CAPsomes) with diameters of 100-150 nm in PBS solution.[77] The thickness of the bilayered membrane increased with the degree of polymerization of both blocks and the polymersomes also showed molecular-weight dependent permeability property, low molecular weight compounds (≤ 5000 g.mol⁻¹) being able to diffuse into CAPsomes. The nanostructure of polymersomes was further locked without alteration of the dimensions by oxo Michael reaction with divinylsulfone.[78]

Temperature induced self-assembly. Borsali and coworkers connected PNIPAM chains having an azide chain-end (N_3 -PNIPAM_n, n = 28~220) with alkyne-functionalized maltoheptaose through click CuAAC.[79] Above LCST (36 to 51°C), the copolymers Mal₇-*b*-PNIPAM₂₂₀ (**45**, **Scheme 3**) were capable to self-assemble in water to generate oligosaccharide-based vesicles with a diameter of 300 nm at 90°C (**Figure 3C**).

Self-assembly of double hydrophilic block copolymers. In general, self-assembly into vesicle-like morphology implies the use of amphiphilic structures, especially hydrophobic-*b*-hydrophilic copolymers. **The inspiration comes** from water-in-water emulsions where aqueous mixtures of two hydrophilic polymers solutions can phase-separate in two immiscible aqueous phases **when adding salt typically.** Brosnan and co-workers showed that certain combinations of double hydrophilic block copolymers can also self-aggregate into vesicles in a controlled manner. A series of polysaccharide-based copolymers, dextran-*b*-poly(ethylene oxide) (**46**, **Scheme 3**), pullulan-*b*-poly(ethylene oxide) (**47**, **Scheme 3**) and dextran-*b*-poly(sarcosine) (**48**, **Scheme 3**) were synthesized in approximately equal block lengths and molecular weight (40 kg/mol). Hollow-sphere structure were generated by an electroformation process, with diameters ranging between 390 and 578 nm.[80] Schmidt also presented the synthesis of vesicles from two double-hydrophile block copolymers, namely pullulan-*b*-poly(*N,N*-dimethylacrylamide) (Pull-*b*-PDMA) (**49**, **Scheme 3**) and pullulan-*b*-poly(*N*-ethylacrylamide) (Pull-*b*-PEA) (**50**, **Scheme 3**).[81] The diameters of resulting vesicles were around 170 and 220 nm for 0.1 and 1 wt.% of Pull-*b*-PDMA, respectively, and 250 and 360 nm for 0.1 and 1 wt.% of Pull-*b*-PEA, respectively.

Co-self-assembly with surfactants. In addition to the straightforward assembly into polymersomes, some research groups investigated the co-self-assembly of amphiphilic polysaccharide-based macromolecules and surfactants into hybrid nanocapsules. Fan *et al.* prepared vesicles with a diameter of 200 nm through spontaneous self-assembly of amphiphilic carboxymethyl alkylaldehyde-modified chitosan (**51, Scheme 3**) and a cationic surfactant, cetyltrimethylammonium bromide in water.[82] As evidenced by DLS and TEM, the formation of vesicles was favored when long alkyl segments (hexyl or octyl) were incorporated into the chitosan backbone. Li and coworkers mixed tetradecyl-quaternized (carboxymethyl) chitosan (**52, Scheme 3**) and cholesterol (weight ratio of 1/0.81) in chloroform; the mixtures that self-organized through thin-layer evaporation or reverse-phase evaporation were subsequently redispersed in water to afford chitosan-containing liposomes with a hydrodynamic diameter of 184 nm.[83]

Chang and coworkers incorporated methoxy-PEG (mPEG) or folic acid (FA) on octadecyl-quaternized, lysine-modified chitosan (OQLCS) (**53, Scheme 3**) through a carbodiimide-catalyzed coupling reaction to obtain mPEG-OQLCS and FA-OQLCS.[84] All three polymers, i.e. OQLCS, mPEG-OQLCS and FA-OQLCS, were then mixed with cholesterol (weight ratio, 1/1/1/1) in chloroform. This mixture gave rise to FA-functionalized chitosan nanocapsules (diameter of 163 nm) by solvent evaporation. Mishra and coworkers also mixed cholesterol with stearic acid-modified glycol chitosan (**54, Scheme 3**) at a ratio of 4/1 (chitosan/cholesterol) in an ethanol/DMAc mixture by stirring and further vortexing. Addition of this organic phase into an aqueous solution triggered the self-assembly of mixtures into glycovesicles

(d ~ 240 nm).[85]

Apart from small molecules, poly(D,L-lactic acid) (PLA) (from 210 to 48,000 g/mol) were also mixed with cholesterol-modified dextran in DMSO (Chol-Dex, **55, Scheme 3**). Nanoparticles and hollow nanocapsules were obtained through solvent displacement (DMSO-dialyzed against water). Hollow nanocapsules were typically generated using PLA with molar masses above 360 g/mol and high composition of Chol-Dex (Chol-Dex: PLA from 1:1 to 3:1).[86]

Others processes. Recently, Tang's group introduced an interesting self-assembly process of β -glucan by mimicking DNA packing (**Figure 3D**).[87] Thanks to the helix forming capability of β -1,3-glucan, formyl and acetyl-substituted β -1,3-glucan derivatives (**56, Scheme 3**) self-assembled into nanosheets ~30 nm by mixing acetone solution of β -1,3-glucan with water. Sequential deacetylation then loosed two ends of the tiles and activated their packing into mesoporous nanocapsules (d ~ 200 to 500 nm) through end-to-end association.

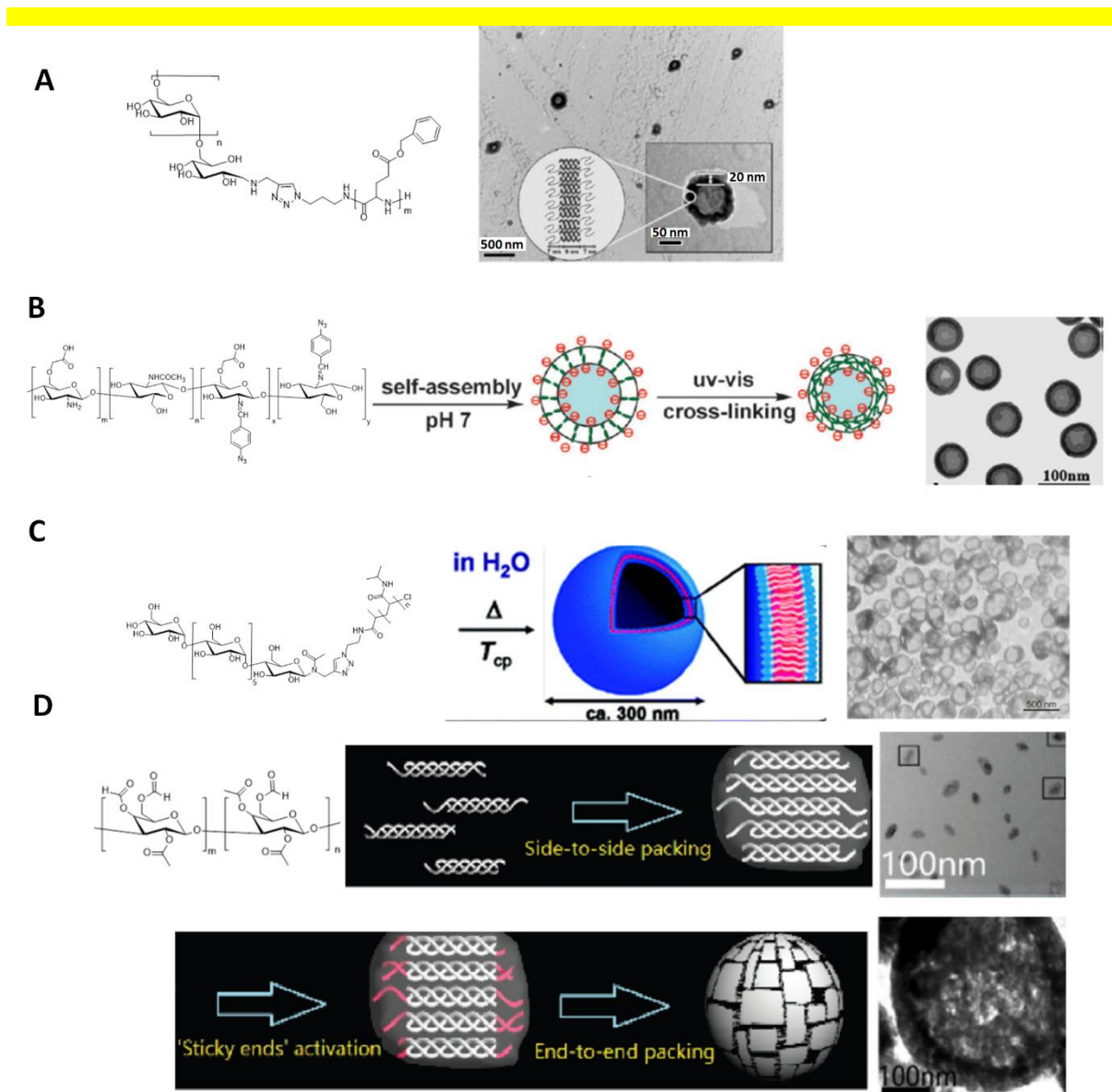
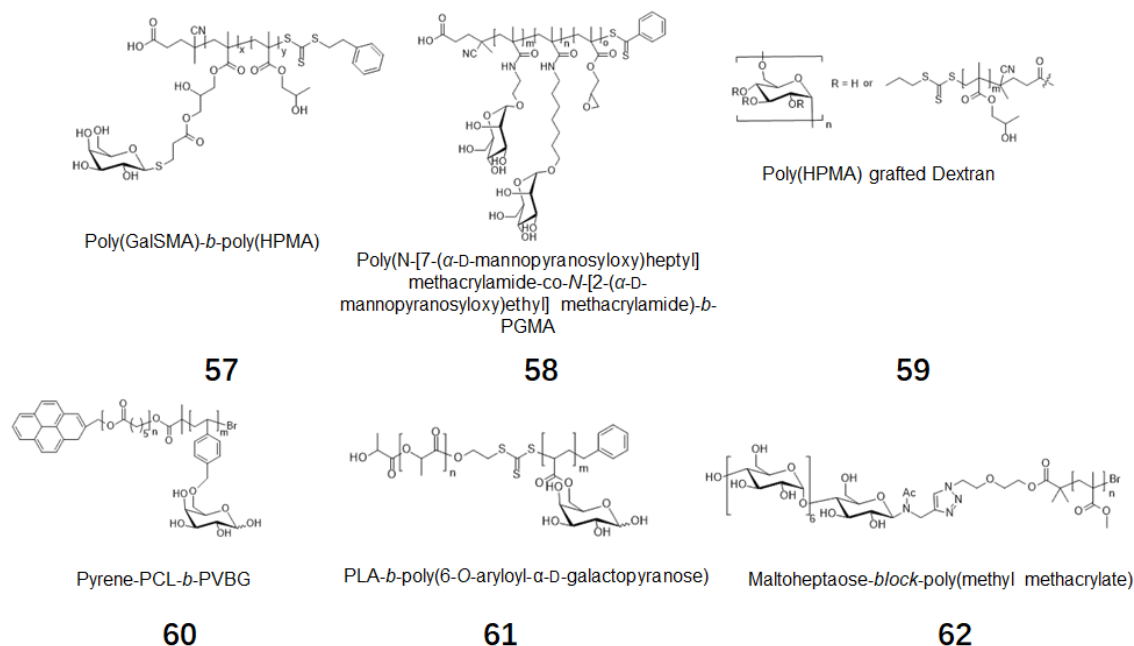


Figure 3. (A) TEM image of dextran-block-PBLG copolymer based nanocapsules.[61] Copyright 2009. Adapted with permission from John Wiley & Sons Inc. (B) Route to photo-crosslinked chitosan water filled nanocapsules and TEM image of resulting nanoparticles .[75, 76] Copyright 2010 and 2014. Adapted with permission from the Royal Society of Chemistry and Elsevier Science Ltd. (C) Preparation process and TEM photo of maltoheptaose-based nanocapsules, scale bar is 500 nm.[79] Copyright 2010. Adapted with permission from American Chemical Society. (D) Schematic and TEM images of the tile-based self-assembly of GFA mesoporous nanocapsules.[87] Copyright 2017. Adapted with permission from the Royal Society of Chemistry.

2.1.2 Polymerization-Induced Self-assembly (PISA)

In comparison with the traditional multi-step strategy that consists in preparing, purifying and self-assembling block copolymers, PISA has emerged as a powerful approach to prepare solutions of well-defined nano-objects with high solid contents in one step. The PISA process can be applied to emulsion or dispersion polymerizations, as first developed by Hawket, Pan and Charleux.[88, 89] It relies on the use of a macromolecular precursor (soluble in the polymerization medium) to grow in a controlled manner a second block, triggering the formation of nano-objects through *in situ* self-organization. By adjusting the composition of block copolymers and the solids content, it is possible to tune the morphology of the resulting nano-objects into spherical and cylindrical micelles or vesicles.[89]



Scheme 4. Structures of carbohydrate polymers used to generate nanocapsules by PISA and micelle core removal strategy.

PISA involving glycopolymers blocks. Armes and co-workers reported the first application of “PISA” to the preparation of sugar-based nanocapsules (**Figure 4A**).[90]

A galactose-based monomer (GalSMA) and glycerol methacrylate (GMA) were

separately homopolymerized by RAFT process to generate two water-soluble Macro-RAFT agents (PGalSMA₃₄, $M_w = 16.3$ kg/mol, $\bar{D} = 1.13$; PGMA₅₁, $M_w = 16.2$ kg/mol, $\bar{D} = 1.15$). These two macro-RAFT agents were mixed at a molar ratio of 9:1 (PGMA₅₁: PGalSMA₃₄) to mediate the polymerization of hydroxypropyl methacrylate (HPMA) in deionized water ($[M]/[CTAs] = 201$, 70°C) and generate amphiphilic block copolymers (**57**, **Scheme 4**). These polymers self-assembled into spherical, cylindrical micellar or vesicular morphologies depending on the polymer concentration and the DP_n of PHPMA (see **Figure 4A** for details).

Yan *et al.* synthesized a series of nanoparticles from poly(N-[7-(α -D-mannopyranosyloxy)heptyl]methacrylamide-co-N-[2-(α -D-mannopyranosyloxy)ethyl]methacrylamide)-*b*-poly(glycidyl methacrylate) (P((HMM-co-EMM)-*b*-GMA), **58**, **Scheme 4**) copolymers by PISA. The chain extension of P(HMM₉-co-EMM₃₄) by GMA was performed in water at 70°C (pH=7).[91] The morphologies transitioned from spheres, rice-shape, worms to large vesicles by increasing the length of GMA segments.

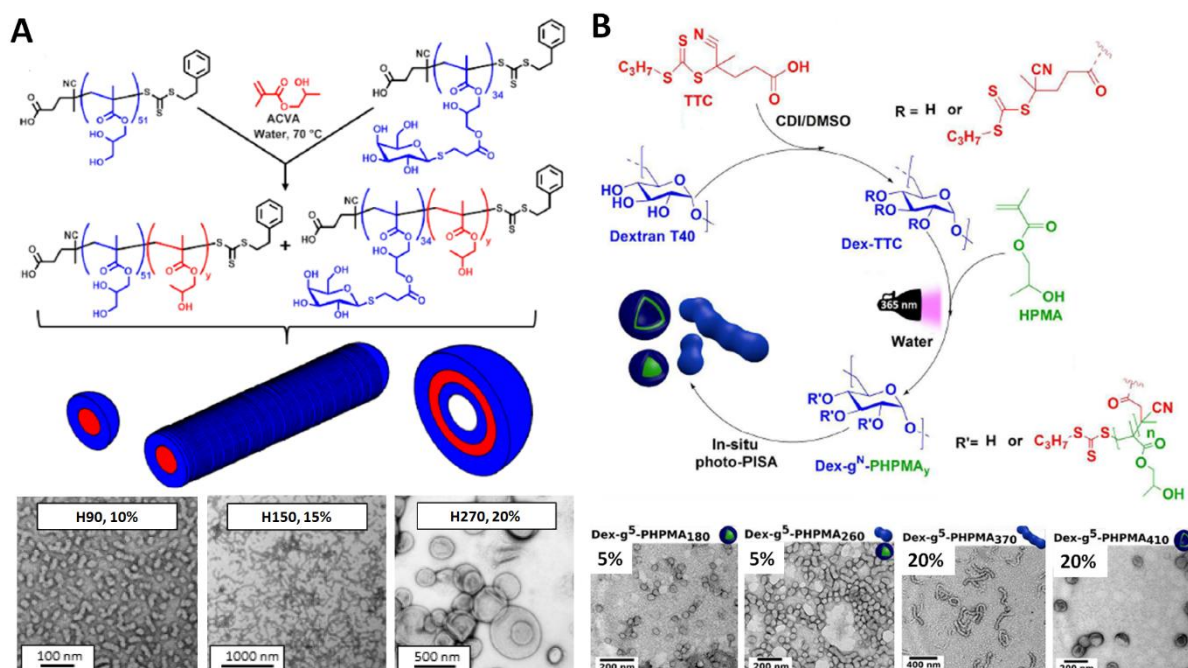


Figure 4. (A) Preparation and TEM images of glyco-nano-objects (spheres, worms or vesicles) by polymerization-induced self-assembly; DPs of PHPMA blocks: 90, 150 and 270, solids contents: 10, 15 and 20%;[90] Copyright 2013. Adapted with permission from American Chemical Society. (B) Synthesis and TEM images of DEX-g-PHPMA based nanostructures using photo-PISA process; DPs of PHPMA blocks are shown in the figure; solids content: 5 and 20%. [92] Copyright 2018. Adapted with permission from the Royal Society of Chemistry..

Table 1. Preparation of water-filled **sugar-based nanocapsules** by self-assembly.

Classification	Glycopolymers or Polysaccharides	Protocol of Self-Assembly	Solvent	Diameter (nm)	Ref.
Polymersomes	Polystyrene- <i>b</i> -poly[(2- β -D-glucopyranosyloxy)ethyl acrylate]	Solvent displacement	Dioxane/DMF/H ₂ O		[26, 27]
Polymersomes	Poly(3-hexylthiophene)- <i>b</i> -poly(6- <i>O</i> -methacryloyl-D-galactopyranose)	Solvent displacement	THF/H ₂ O	178	[31]
Polymersomes	Poly(pyrenylmethyl methacrylate)- <i>b</i> -poly(3- <i>O</i> -4-vinylbenzoyl-D-glucopyranose)	Solvent displacement	DMF/H ₂ O	473	[32]
Polymersomes	Poly(spiropyran methacrylate)- <i>b</i> -poly(3- <i>O</i> -4-vinylbezoyl-D-glucopyranose)	Solvent displacement	DMF/H ₂ O	366	[33]
Polymersomes	Poly(α -D-galactopyranosyl-L-cysteine) ₆₅ - <i>b</i> -(leucine) ₂₀	Solvent displacement	THF/H ₂ O	140	[28]
Polymersomes	Poly[1,2,3,4-di-isopropylidene-6- <i>O</i> -(2'-formyl-4'-vinylphenyl)-D-galactopyranose]	Solvent displacement	DMSO/H ₂ O	160~410	[34]
Polymersomes	Poly(<i>N</i> -acryloylmorpholine)- <i>b</i> -poly(<i>N</i> - β -D-glucopyranosylacrylamide)- <i>b</i> -poly(<i>N</i> -acryloylmorpholine)	Solvent displacement	THF/H ₂ O	380	[35]
Polymersomes	PBLG- <i>b</i> -poly(galactosylated propargylglycine)	Solvent displacement	DMSO/H ₂ O		[36]
Polymersomes	P(DHPMA-stat-Mann-stat-OG) ₃₃ - <i>b</i> -P(LMA) ₂₈	Solvent displacement	DMSO/H ₂ O	274	[29]
Polymersomes	Poly(ϵ -caprolactone)- <i>b</i> -poly(2-aminoethylmethacrylate)- <i>b</i> -poly(glyconamidoethyl methacrylate)	Solvent displacement	DMSO/PBS buffer	59	[37]
Polymersomes	Poly(ϵ -caprolactone)- <i>b</i> -poly(lactobionamidoethylmethacrylate)	Solvent displacement	DMF/H ₂ O	432	[38]
Polymersomes	Poly(ϵ -caprolactone)- <i>b</i> -poly(gluconamidoethylmethacrylate)	Solvent displacement	DMF/H ₂ O	250	[39]
Polymersomes	Poly(γ -benzyl L-glutamate)- <i>b</i> -poly(D-gluconamidoethyl methacrylate) functionalized PAMAM	Solvent displacement	DMF/H ₂ O	274	[40]
Polymersomes	Poly(D-gluconamidoethyl methacrylate)-PPR-poly(D-gluconamidoethyl	Solvent displacement	DMF/H ₂ O	81	[41]

methacrylate)					
Polymersomes	PEO ₄₃ - <i>b</i> -P(CMA ₈ - <i>stat</i> -DEA ₈₀ - <i>stat</i> -GEMA ₁₂)	Solvent displacement	THF/H ₂ O	357-537	[30]
Polymersomes	Poly(L-lysine)- <i>graft</i> -hexanoyl-lactobionolactone	Solvent displacement	MeOH/H ₂ O	220 (pH 7.4)	[42]
Polymersomes	20GP-(PCL ₂₅) ₂	Solvent displacement	DMSO/H ₂ O	55	[43]
Polymersomes	Poly(5'-O-methacryloyl uridine)- <i>b</i> -poly(2-methacrylamido glucopyranose)	Solvent displacement	DMA/H ₂ O	35	[48]
Polymersomes	PNIPAAM- <i>co</i> -glycopolymer bearing mannoside and alkylamine	Solvent displacement	MeOH/H ₂ O	100-600	[44]
Polymersomes	PEG-AcGal _m -PS _n	Solvent displacement	THF/H ₂ O	116	[55]
Polymersomes	Poly(L-alanine)- <i>b</i> -poly(2-acryloyloxyethyl lactoside)- <i>b</i> -poly(L-alanine)	CMC	H ₂ O		[49, 50]
Polymersomes	2,3,4,6-tetra-O-acetyl-β-D-1-thioglucopyranose modified 1,2-polybutadiene- <i>block</i> -polystyrene	CMC	THF	500	[45, 51]
Polymersomes	2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose modified 1,2-polybutadiene- <i>block</i> -poly(ethylene oxide)	CMC	H ₂ O	560	[52]
Polymersomes	Glucosamide-grafted amphiphilic glycopolysiloxanes	CMC	H ₂ O	300~600	[53]
Polymersomes	Polystyrene- <i>b</i> -poly(tetra-acyl-α-1-mannosyl styrene)	Deprotection	THF	84	[46]
Polymersomes	PS- <i>b</i> -PLac (Ac or Bz)	Deprotection	THF	48 (Ac), 116 (Bz)	[54]
Polymersomes	Poly(2-glucosyloxyethyl methacrylate)- <i>b</i> -poly(diethyleneglycol methacrylate)	T	H ₂ O	251, 500 (20°C)	[56]
Polymersomes	Poly(1-N-β-galactosyl acrylamide) (PGal) or Poly(1-N-β-glucosyl acrylamide) (PGlc)	T	H ₂ O	124 (V-PGal) 136 (V-PGlc)	[57]
Polymersomes	Poly(1-O-methacryloyl-β-D-fructopyranose)- <i>b</i> -poly(methyl methacrylate)	Solvent displacement	THF/H ₂ O	263	[47]
Polymersomes	P(M/Lys ₅ - <i>co</i> -Lys ₂₇)	Dissolution	H ₂ O	45-80	[58]

Polymersomes	Dextran- <i>b</i> -PS	Solvent displacement	THF or DMSO-THF/ H ₂ O	128, 154	[59, 60]
Polymersomes	Dextran- <i>b</i> -PBLG	Solvent displacement	DMSO/ H ₂ O	90	[61]
Polymersomes	PBLG- <i>b</i> -hyaluronan	Solvent displacement	DMSO/ H ₂ O	440	[62-64]
Polymersomes	Octadecanol-dextran	Solvent displacement	DMF- H ₂ O / H ₂ O	192	[66]
Polymersomes	3-pentadecyl phenol modified Dextran	Solvent displacement	DMSO/ H ₂ O	120-300	[67] [68]
Polymersomes	Poly(Lys ₁₁ -stat-Phe ₁₀)- <i>g</i> -Cs] _x -stat-Cs _{2x} -stat-ECs _{3x}	Solvent displacement	THF-H ₂ O/H ₂ O	230	[65]
Polymersomes	Acetylated starch palmitate	Solvent displacement	THF/H ₂ O	300-1000	[69]
Polymersomes	Fatty acid modified hydroxyethyl starch	Solvent displacement	THF-H ₂ O/H ₂ O	250-350	[70]
Polymersomes	β -1,3-glucan acetate and hexanoate	Solvent displacement	Acetone/H ₂ O	487	[71]
Polymersomes	Dex- <i>g</i> -PADEGChol	Solvent displacement	DMSO or THF/H ₂ O	47-68 (DMSO), 47-63 (THF)	[72]
Polymersomes	Dextran- <i>b</i> -poly(lactide-co-glycolide)	Sonication and Stirring	PBS buffer	132, 270	[73]
Polymersomes	Carboxymethyl-hexanoyl chitosan	Sonication	H ₂ O	20, 200	[74]
Polymersomes	Azidobenzaldehyde modified photocrosslinkable carboxymethyl chitosan	Sonication	H ₂ O	153	[75, 76]
Polymersomes	maltooligosaccharide- <i>b</i> -poly(propylene glycol)	Hydration	PBS buffer	100-150	[77]
Polymersomes	Maltoheptaose- <i>b</i> -PNIPAM	T	H ₂ O	300	[79]
Polymersomes	dextran-block-poly(ethylene oxide),	Electroformation	H ₂ O	390-578	[80]

	pullulan-block-poly(ethylene oxide), Dextran-block-poly(sarcosine)				
Polymersomes	Pullulan-block-poly(N,N-dimethylacrylamide) (Pull-b-PDMA) Pullulan-block-poly(N-ethylacrylamide) (Pull-b-PEA)	Dissolution	H ₂ O	170-220 (Pull- <i>b</i> -PDMA) 250-360 (Pull- <i>b</i> -PEA)	[81]
Polymersomes	Alkylaldehyde modified chitosan	CMC	H ₂ O	200	[82]
Polymersomes	Tetradecyl-quaternized chitosan	Evaporation	Chloroform	184	[83]
Polymersomes	Octadecyl-quaternized lysine modified chitosan	Evaporation	Chloroform	163	[84]
Polymersomes	Stearic acid modified glycol chitosan	Solvent displacement	EtOH-DMA/H ₂ O	243	[85]
Polymersomes	Cholesterol modified dextran, PLA	Solvent displacement	DMSO/H ₂ O	ND	[86]
Polymersomes	Formyl and acetyl substituted glucan	Solvent displacement	Acetone/H ₂ O	200-500	[87]
PISA	Poly(GalSMA)- <i>b</i> -poly(HPMA)	PISA	H ₂ O	ND	[90]
PISA	Poly(N-[7-(α -D-mannopyranosyloxy)heptyl] methacrylamide-co-N-[2-(α -D-mannopyranosyloxy)ethyl] methacrylamide)- <i>b</i> -poly(glycidyl methacrylate)	PISA	H ₂ O	840	[91]
PISA	Poly(HPMA) grafted Dextran	PISA	H ₂ O	200-220	[92]
Micellar Core Removal	Pyrene-PCL- <i>b</i> -PVBG	Solvent displacement	DMF/ H ₂ O	50-120	[93]
Micellar Core Removal	PLA- <i>b</i> -poly(6- <i>O</i> -aryloyl- α -D-galactopyranose)	Solvent displacement	Formic acid/H ₂ O	250 (micelle)	[94]
Micellar Core	Maltoheptaose- <i>block</i> -poly(methyl methacrylate)	Solvent displacement	Acetone-H ₂ O/H ₂ O	80	[95]

Removal

PISA involving polysaccharidic chains. Recently, Ferji and coworkers showed a successful example of photo-induced PISA starting from a dextran scaffold (**Figure 4B**).^[92] Dextran was partially functionalized by a photosensitive CTA, 4-(propylthiocarbonothioylthio)-4-cyanopentanoic acid, giving Dextran TTC macro-CTA. Aqueous dispersion polymerization of 2-hydroxypropyl methacrylate (Dextran-g-PHPMA, **59**, **Scheme 4**) was further undertaken under UV irradiation (365 nm) while tuning the irradiation time (10 and 60 min) and solid contents (5 and 20 wt%). Depending on the length of PHPMA, (worm-like) micelles were produced at low solids contents (5 wt%), whereas glycovesicles (d ~ 200-220 nm) were only observed at high solids content (20 wt%) and high DP_n (typically superior to 400).

2.1.3 Micellar core removal strategy

Apart from polymersomes, micelles obtained via self-assembly of amphiphilic block copolymers can also be converted to hollow structures through selective removal of the core. Elimination of the core can be triggered by enzymatic degradation, ozonolysis, hydrolysis or aminolysis.

Glycopolymers. The preparation of hollow nanospheres via core degradation method was first introduced by Wooley and co-workers.^[96-98] The first adaptation of Wooley's method to the formation of glyconanocapsules came from Lu *et al.* in 2005.^[93] Amphiphilic block copolymers (Py-PCL₃₂-*b*-PVBG₁₀, **60**, **Scheme 4**) were prepared by ring-opening polymerization (ROP) of ϵ -CL and subsequent ATRP polymerization of a galactose-based monomer, 6-O-(4-vinylbenzyl)-1,2:3,4-di-O-isopropylidene-D-galactose (VBIG). After

deprotection of the pendent galactose units, addition of water into a DMF solution of the polymer induced self-assembly, resulting in the formation of micelles with a PCL core and a glycopolymer shell (diameter between 20 and 90 nm). After cross-linking of the glycopolymer shell with glutaraldehyde, the inner part of the particles was then removed through basic hydrolysis, affording hollow nanocapsules with diameters between 50 to 120 nm (**Figure 5**). Stenzel's group[94] reported the synthesis of poly(lactide)-*b*-poly(6-*O*-acryloyl- α -D-galactopyranose) (PLA₁₇₀-*b*-PAGP₂₁₆, **61**, **Scheme 4**) by poly(lactide) macroCTA mediated RAFT polymerizations. Self-assembly of the copolymers via a solvent displacement process (formic acid solution dialyzed against water) triggered the reorganization of macromolecules into a priori large micelles (diameter of about 250 nm in water). These were further crosslinked via RAFT polymerization of hexanediol diacrylate, and glyconanocapsules were finally obtained after aminolysis of the polyester core by hexylamine (ester bond disruption).

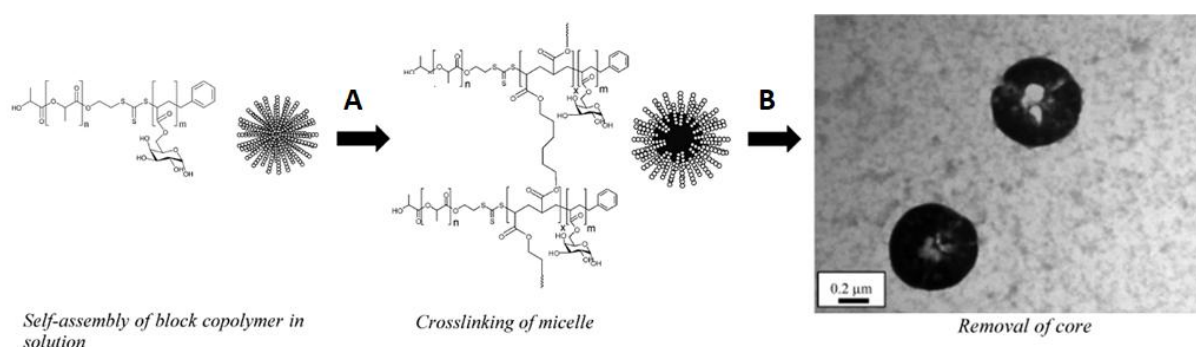


Figure 5. Fabrication of hollow sugar nanocapsules relying on the core removal approach.[94]

Copyright 2009. Adapted with permission from American Chemical Society. (A) Polymerization: Hexanediol diacrylate/water/AIBN/60°C; (B) hexylamine core removal reaction and corresponding

TEM photo.

Polysaccharides. In 2016, Borsali and coworkers prepared an amphiphilic block copolymer, maltoheptaose-*block*-poly(methyl methacrylate) using CuAAC ligation (**62**, **Scheme 4**).[95] A water/acetone solution (0.78/0.22 in weight) of this polymer (3 mg/mL) was slowly added into 3 mL of water while stirring to generate, after acetone evaporation, large micelles (diameter ~140 nm) composed of a PMMA core and a polysaccharide shell. The latter was crosslinked by Michael addition reaction with divinyl sulfone. After 48 h of UV exposure ($\lambda = 254$ nm), the core was photo-degraded, and hollow glyconanoparticles of 80 nm in diameter were finally obtained.

2.2 Template strategy

The template strategy proposes to start from preformed nano-sized templates (colloid, polymer, silica, polymer particles...), on which polymer chains are adsorbed, most likely by electrostatic interactions. **Sugar-based nanocapsules** can be conveniently generated through adsorption of glycopolymers or polysaccharides on the surface of these templates. The number of deposition cycles directly determines the thickness and the composition of the polymer shell. One coating step gives rise to monolayer capsules; multi-layered capsules can be prepared by the so-called Layer-by-layer (L-b-L) deposition. This methodology, first introduced by G. Decher,[99] originally consists in alternate deposition of polycations and polyanions glued together by electrostatic attractions. The main advantage of this strategy is that both the

thickness and morphology of particles can be precisely controlled through careful selection of the starting materials. Nano- or micro-capsules are typically obtained in two steps: 1) sequential assembly of polymer chains at the surface of the template and 2) removal of the sacrificial core.[100] The dimension of nanocapsules is determined both by the template size and the solvent affinity of polymeric shell. The size of nanoparticles progressively grows with the deposition of polymer layers, polymer of high-water affinity leading to a significant increase of the thickness of the shell. In the case of post-core removal, the resulting hollow nanocapsules shrink in some extent through a limited collapse of the polymer shell.

2.2.1 Particle-based strategy

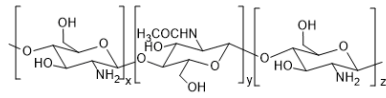
Here, carbohydrate polymers are efficiently deposited on a preformed solid particle. The specific examples are described starting from simple systems (monolayered) to more sophisticated ones (multilayered).

2.2.1.1 Monolayered capsules

Polysaccharides. In 2011, Liu *et al.* deposited a cationic polysaccharide (chitosan, **63**, **Scheme 5**) on carboxylic acid-functionalized P(S-co-MAA) particles (diameter of about 200 nm) prepared by surfactant-free emulsion copolymerization (**Figure 6A**). After cross-linking the shell with glutaraldehyde (GA), the core was dissolved in THF, affording hollow nanocapsules (diameter ranging from 190 to 250 nm). Ibuprofen was also incorporated during the preparation of the P(S-co-MAA) particles.[101, 102] Solid core/mesoporous shell (SC/MS) silica nanoparticles (268, 382 and 574 nm) were coated with low molar mass chitosan.[103, 104] After cross-linking of the chitosan

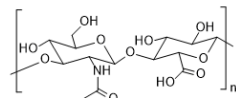
infiltrated into the shell and further degradation of the silica via HF treatment, chitosan nanocapsules with different sizes ($d \sim 220, 270$ and 440 nm), wall thicknesses (28, 45 and 56 nm) and porosities (pore diameter $\sim 2.9, 3.68, 4.85$ nm) were obtained, respectively. These biocompatible nanocapsules were loaded with curcumin, a natural lipophilic anti-cancer drug. The large- and medium-sized nanocapsules displayed 145 and 13 times more loading capacity than the smallest ones, respectively.

Kren *et al.* directly deposited hyaluronan (**64**, **Scheme 5**) on positively-charged DNA-PEI colloidal complexes through a dispersion atomization process to generate hyaluronan-coated gene carriers with a very small diameter of about 20 nm.[105]



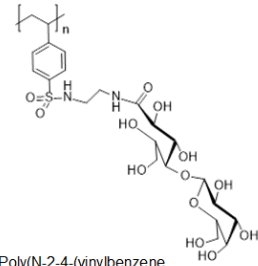
Chitosan

63



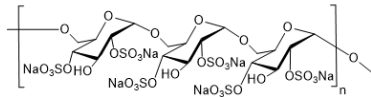
Hyaluronan

64



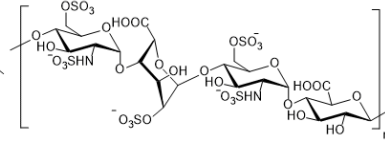
Poly(N-2,4-(vinylbenzene sulfonamido)ethyl lactobionamide)

65



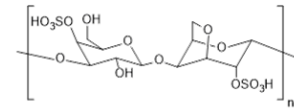
Dextran sulfate

66



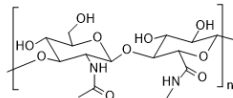
Heparin

67



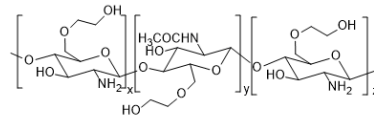
Iota Carrageenan

68



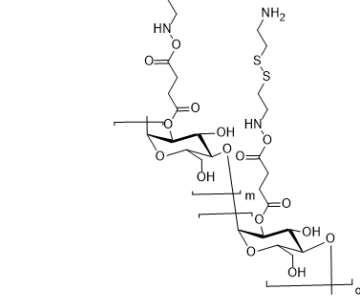
Glycol Chitosan

70



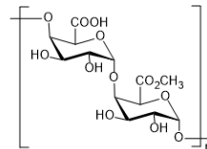
Fucoidan

71



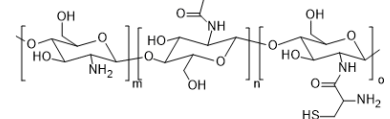
Hyaluronan-g-Starch,

69



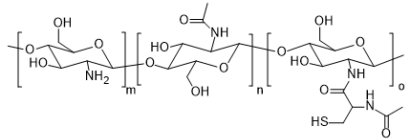
Pectin

72



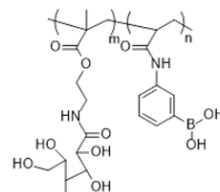
Cysteine-Chitosan

73



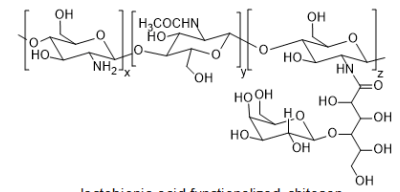
Cystine-Chitosan,

74



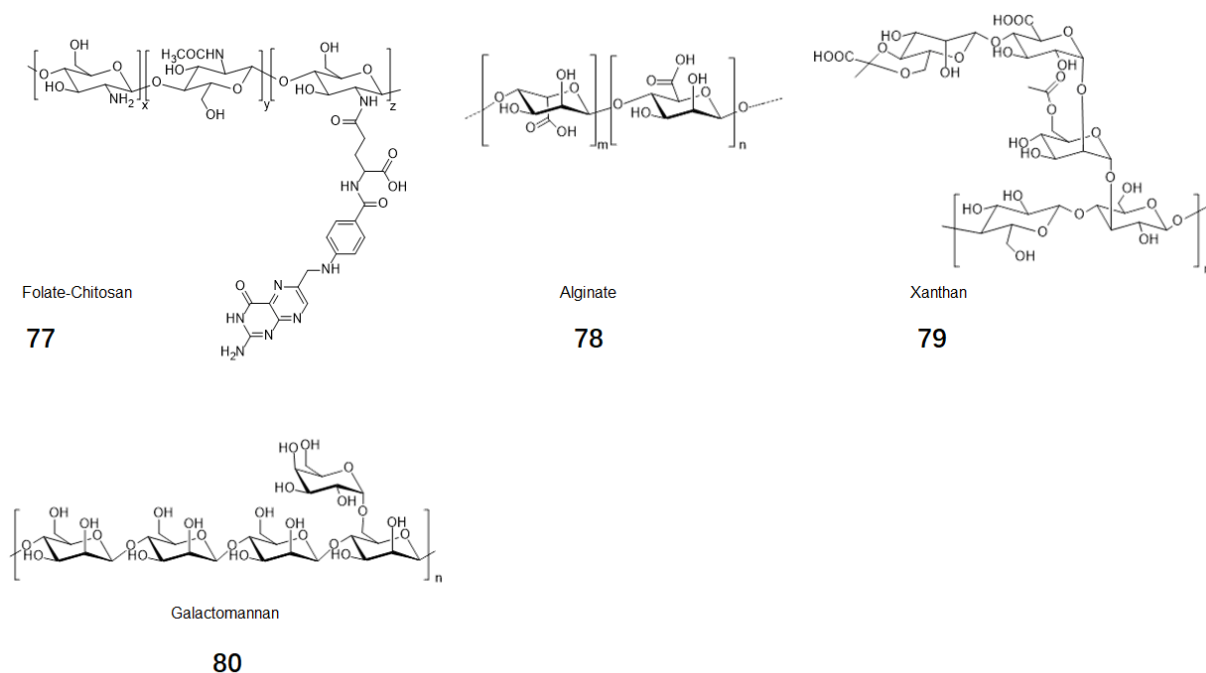
P(GAMA-g-AAPBA)

75



lactobionic acid-functionalized chitosan

76



Scheme 5. Structures of carbohydrate polymers used to generate water-filled nanocapsules by template strategy.

Glycopolymers. Apart from polymer deposition by electrostatic attraction, surface-initiated AGET-ATRP (for 'activator generated by electron transfer for atom transfer radical polymerization') of N-[2-(4-vinylbenzenesulfoneamido) ethyl] lactobione amide (PVBSAELA, **65**, **Scheme 5**) was performed on the surface of PS particles (diameter of 440 nm) to generate lactose-functionalized glycoparticles. Hollow capsules (diameter of 640 nm) were obtained after further dissolution/removal of around 70% of PS chains in THF solution. Core removal step shrunk the size of nanocapsules by 15%. Glyco-microcapsules were also prepared by applying a similar strategy, but using micro-sized PS particles as a template (**Figure 6B**).[106]

2.2.1.2 Multi-layered nanocapsules

In 2004, Akashi and co-workers introduced the L-b-L strategy to construct multilayered hollow **sugar-based nanocapsules** from silica nanoparticles.[107]

Chitosan as first layer and then dextran sulfate (**66**, **Scheme 5**) were sequentially deposited on the surface of nanoparticles (diameter of 330 nm) up to four layers. Polysaccharide multi-layered nanocapsules were finally generated with a diameter of about 300 nm after core removal by HF treatment. The same group also reported pH-responsive hollow nanocapsules using the same template.[108] The multilayered walls of the capsules were constructed from sequential deposition of chitosan and poly(γ -glutamic acid) (γ -PGA) and cross-linking reactions between chitosan and γ -PGA chains (activated by carbodiimide reagent). After core removal, the diameter of the nanocapsules was stable at roughly 250 nm between pH 4 to 10, and significantly increased at pH 1 to about 500 nm, because of electrostatic repulsions between the ammonium groups of chitosan. Modification of the pH then allowed tuning the release behavior of the nanocapsules.

Li and coworkers coated $\text{SiO}_2\text{-NH}_3^+$ nanoparticles with dextran sulfate and chitosan by electrostatic interactions.[109] Hollow nanocapsules were obtained after HF/ NH_4F triggered core removal. Poly(allylamine hydrochloride) and dextran sulfate were sequentially deposited on silica particle templates (diameter of 500 nm) by Raichur and co-workers to form multilayered nanoparticles. These steps were followed by the deposition of silver nanoparticles (formed by reduction of silver nitrate during the deposition).[110] After core removal via HF/ NH_4F treatment, the authors demonstrated that it was possible to encapsulate FITC-dextran in the nanocapsules modified by silver nanoparticles through a permeation process (diameter of about 500 nm, thickness of 30 nm). FITC-dextran was released through sonication owing to

partial destruction of the polymer shell. Silica nanoparticles ($d \sim 220$ nm) were also coated with chitosan and negatively charged heparin (**67**, **Scheme 5**) through L-b-L deposition.[111] Hollow nanocapsules of 200 nm were then generated after elimination of the silica core (0.2 M HF + 0.8 M NH_4F).

Liu *et al.* sequentially deposited chitosan and iota carrageenan (**68**, **Scheme 5**) onto a $\text{SiO}_2\text{-NH}_3^+$ nanoparticles' template; glutaraldehyde was also introduced to further cross-link the polymer shell. Hollow nanocapsules (diameter of 348 nm) were finally obtained after silica core removal.[112] Chakravorty and coworkers alternatively deposited chitosan and dextran sulfate on silica nanoparticles to produce four-layered nanoparticles.[113] The core dissolution led to glyconanocapsules with diameter of 180 nm. Ciprofloxacin and ceftriaxone were loaded in the capsules with an efficiency of 78%, and the release behaviour was again mitigated by changing the pH of the external solution. Lee's group prepared multilayered polysaccharidic vesicles by a three-step process. Specifically, hyaluronate-*g*-starch (**69**, **Scheme 5**) was synthesized by using cystamine as a coupling agent, from which nanoparticles were generated by DMSO evaporation. Hollow vesicle-like structures were built by means of enzymatic degradation (amylase). Finally, glycol chitosan (**70**, **Scheme 5**) or poly-L-lysine-*g*-PEG-*g*-folate was coated on the surface of particles by electrostatic interactions, resulting in biocompatible multilayered nanocapsules with diameter of approximately 150 nm.[114]

Pinheiro *et al.* fabricated multilayered nanoparticles through sequential deposition of chitosan and fucoidan (**71**, **Scheme 5**) on polystyrene nanoparticles

(diameter of 90 nm) through electrostatic interactions.[115] 10-layered hollow nanocapsules with diameter of around 50 nm were finally generated after dissolution of core particles with THF. Recently, Panda et al. sequentially deposited poly-L-arginine and dextran sulfate on preformed Sorafenib (multikinase inhibitor) encapsulated CaCO₃ nanoparticles.[116] Bilayered nanocapsules with diameters ranging from 100 to 300 nm were finally obtained after introducing EDTA to dissolve the core template, with an encapsulation efficiency of up to 61%.

Yao and coworkers alternatively deposited pectin (**72, Scheme 5**) and chitosan on the surface of SiO₂-NH₂ nanoparticles (diameter of about 125 nm), and constructed seven-layered nanocapsules with a diameter of 473 nm after core removal by HF-NH₄F buffer.[117] The resulting nanocapsules showed no cytotoxicity to several cell lines, and high colloidal stability in PBS and BSA solutions. Doxorubicin hydrochloride diffused into the capsule reservoir at pH 6, with an encapsulation efficiency of up to 76.5% and a final loading content of 20.3%. Owing to their polyelectrolyte walls, release of loaded drug from these capsules exhibited a high pH-dependent behavior; cumulative release amount reached 71.2% at pH 6.0 and 85.7% at pH 5.0 over 60 h, for only 8.2% at physiological environment (pH 7.4). In addition to electrostatic attachment between layers stabilizing polymeric shell, thiol moieties introduced on the polymer scaffold further enabled to cross-link the shell via formation of disulfide bonds. Doing so, the resulting glyco-nanomaterials were not only sensitive to pH and enzyme for dissociation and degradation, respectively, but also decomposed (uncrosslinked) in the presence of a reduction agent, *i.e.*

glutathione (GSH).

In 2013, Li and coworkers modified chitosan (**73, Scheme 5**) and poly(L-aspartic acid) with L-cysteine to obtain two thiol-functionalized oppositely charged polymers. During the L-b-L process, the concomitant formation of disulfide bonds from coupling of thiol groups ensured the cross-linking of the polymer shell (diameter of 365 nm and thickness of 40 nm). Hollow nanocapsules were finally obtained after degradation of the silica core.[118]

In the pursuit of increasing the encapsulation rate of protein drug, β -cyclodextrin was conjugated to silica nanoparticles to form inclusion complexes with BSA (diameter of 100 nm), and chitosan was functionalized with cysteine through amidation reaction to afford series of cysteine-conjugated chitosans (CH-SH, targeting modification of 5, 10, 20 and 40%, **74, Scheme 5**).[119] Dextran sulfate and CH-SH were then sequentially deposited on silica templates by L-b-L deposition (**Figure 6C**). Thanks to the presence of thiols along the chitosan backbones, the shell was gradually cross-linked through the formation of disulfide bonds. The disulfide cross-linked nanocapsules (d=151 nm before core removal) were obtained after silica core degradation by HF/NH₄F treatment.

Guo *et al.* sequentially deposited chitosan-*N*-acetyl-L-cysteine (CS-NAC) conjugate (**74, Scheme 5**) and poly(D-gluconamido-ethylmethacrylate-*r*-3-acrylamidophenylboronic acid) [p(GAMA-*r*-AAPBA, **75, Scheme 5**)] on SiO₂-NH₂ particles to form multi-layered nanoparticles (diameter ranging from 250 to 270 nm). After oxidation of the thiols in

the presence of chloramine T and silica core removal, disulfide cross-linked multi-layered hollow nanocapsules were produced (diameter of about 250 nm).[120] In 2016, Akashi and coworkers prepared glucose-responsive capsules by L-b-L assembly based on amine-modified SiO₂ nanoparticle templates.[121] The shell was composed of ten alternating layers of poly[(γ -glutamic acid)-*g*-3-aminophenylboronic acid]] and lactobionic acid-functionalized chitosan oligosaccharides (**76, Scheme 5**). The template was removed by NH₄F/HF treatment to obtain hollow capsules (diameter measured from TEM picture of about 240 nm) with glucose sensitivity, due to competitive interactions between boronic acid/lactobionic acid and boronic acid/glucose.

Apart from traditional template strategies mentioned above, Bui *et al.* reported an original solvent-shifting method to deposit hyaluronan-*b*-poly(*g*-benzyl-L-glutamate (**33, Scheme 3**) on condensed siRNA-PEI complexes.[122] Specifically, positively charged complexes were initially generated by mixing siRNA (23 base pairs) and branched PEI (25 kg/mol) in buffer solution (pH 7.6). The siRNA/PEI complexes were then electrostatically-coated with a layer of diblock whose amphiphilic character was turned off in a DMSO/water mixture (2.5 vol.% of water). Then, a second solvent shifting was performed (aqueous solution at pH = 7.4, 80 vol% of water) to turn the amphiphilicity of the diblock on and promote the formation of a bilayered membrane at the surface of nanoparticles (diameter of 90 nm) thanks to the self-assembly of the excess free chains.

Table 2: Template strategy to prepare water-filled **sugar-based nanocapsules.**

Template	Diameter (nm)	Glycopolymers or Polysaccharides	Deposition process	Layers	Core Removal	Capsule Diameter (nm)	Ref
P(S-co-MAA) particles	200	Chitosan	Electrostatic interactions	Monolayer	THF	190~250	[101, 102]
SC/MC Silica particles	268, 382, 574	Chitosan	Electrostatic interactions	Monolayer	HF	220, 270, 440	[103, 104]
DNA-PEI complexes		Hyaluronan	Electrostatic interactions	Monolayer		20	[105]
P(St-CPEM) particles	440	Poly(N-2-4-(vinylbenzene sulfonamido)ethyl lactobionamide)	Surface polymerization	Monolayer	THF	640	[106]
Silica particles	330	Chitosan, Dextran sulfate	Electrostatic interactions	Multilayers	HF	300	[107]
Silica particles	330	Chitosan	Electrostatic interactions	Multilayers	HF	250	[108]
SiO ₂ -NH ₃ ⁺ particles	166	Chitosan, Dextran sulfate	Electrostatic interactions	Multilayers	HF/NH ₄ F	217 (before core removal)	[109]
Silica particles	500	Dextran sulfate	Electrostatic interactions	Multilayers	HF/NH ₄ F	500	[110]
Silica particles	220	Chitosan, Heparin	Electrostatic interactions	Multilayers	HF/NH ₄ F	200	[111]
SiO ₂ -NH ₃ ⁺ particles	100	Chitosan, Carrageenan	Electrostatic interactions	Multilayers	HF	348	[112]
Silica particles	220	Chitosan, Dextran sulfate	Electrostatic interactions	Multilayers	HF	180	[113]
Polysaccharide particles		Hyaluronan-g-Starch, Glycol Chitosan	Electrostatic interactions	Multilayers	amylase	150	[114]
PS particles	90	Chitosan, Fucoidan	Electrostatic interactions	Multilayers	THF	50	[115]
CaCO ₃ particles		Dextran sulfate	Electrostatic interactions	Multilayers	EDTA	100-300	[116]

SiO ₂ -NH ₂ particles	125	Pectin, Chitosan	Electrostatic interactions	Multilayers	HF/NH ₄ F	473	[117]
Silica particles	260	Cysteine-Chitosan	Electrostatic interactions	Multilayers	HF	365 (before core removal)	[118]
β-cyclodextran-SiO ₂ -NH ₃ ⁺ particles	100	Cystine-Chitosan, Dextran sulfate	Electrostatic interactions	Multilayers	HF/NH ₄ F	151 (before core removal)	[119]
SiO ₂ -NH ₂ particles	152	Chitosan-NAC, P(GAMA-γ-AAPBA)	Electrostatic interactions	Multilayers	HF/NH ₄ F	254	[120]
SiO ₂ -NH ₂ particles	195	Galactosylated chitosan	Electrostatic interactions	Multilayers	HF/NH ₄ F	240	[121]
PEI-siRNA complexes	150~185	Hyaluronan- <i>b</i> -PBLG	Electrostatic interactions	Monolayer		90	[122]
Liposomes		Chitosan	Electrostatic interactions	Monolayer		96	[123]
Liposomes		Folate-Chitosan	Electrostatic interactions	Monolayer		182	[124]
Liposomes		Pectin	Electrostatic interactions	Monolayer		229~418	[125]
Polymersomes	180~414	Chitosan	Electrostatic interactions	Monolayer		183~712	[126]
Liposomes		Alginate, Chitosan	Electrostatic interactions	Multilayers		414	[127]
Cationic Vesicles	220	Alginate, Chitosan	Electrostatic interactions	Multilayers	Triton X-100	500	[128]
Liposomes	80	Alginate, Chitosan	Electrostatic interactions	Multilayers		300	[129, 130]
Liposomes	100	Chitosan, Dextran sulfate	Electrostatic interactions	Multilayers			[131, 132]
Liposomes	108	Chitosan	Electrostatic interactions	Multilayers		215	[133]
Liposomes	62	Xanthan, Galactomannan	Electrostatic interactions	Multilayers		165	[134]

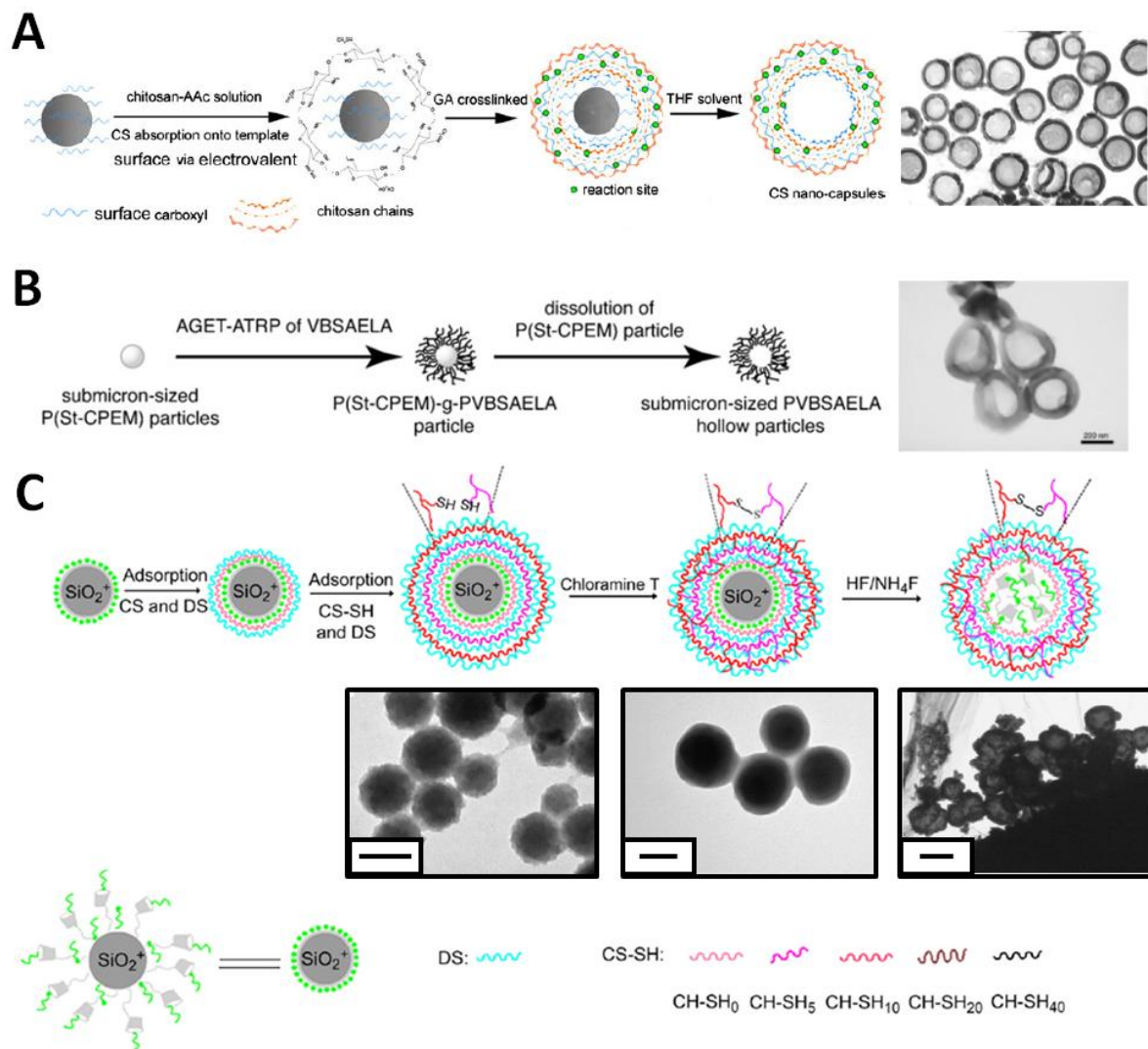


Figure 6. Schemes of the preparation and TEM images of **(A)** chitosan-coated nanocapsules.[101]

Copyright 2011. Adapted with permission from Springer Nature. **(B)** Sugar-based nanocapsules

obtained by template strategy, scale bar is 200 nm.[106] Copyright 2010. Adapted with permission

from Elsevier Science Ltd. **(C)** Dextran sulfate/chitosan crosslinked nanocapsules prepared by

L-b-L strategy, scale bar is 100 nm.[119] Copyright 2010. Adapted with permission from Elsevier

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2.2.2 Liposome-based strategy

The preparation of carbohydrate-functionalized capsules from deposition of polysaccharide chains onto liposomes was first reported by Sunamoto *et al.* in

1980.[135, 136] It is now a very mature research area and numerous papers describe the coating of nano-sized liposomes for drug delivery applications.[137-143] Herein, some of the most recent works are described. In a general procedure, nano-sized liposomes are first prepared, then, charged polysaccharides are sequentially deposited at their surface through electrostatic interactions to produce monolayer or multilayers' carbohydrate-functionalized capsules. Compared to particles template, no core removal step is required for this approach. For this matter also, the cargo (e.g. drugs, fluorescent probes) is pre-encapsulated before polymer deposition, either in the water core or in the bilayer membrane of the liposomes.

2.2.2.1 Monolayered capsules

Park and coworkers deposited chitosan on preset liposomes prepared from self-assembly of phosphatidylcholine/cholesterol mixtures, affording nanocapsules with diameter of 96 nm.[123] In order to increase the bioavailability of the nanocarriers, Yang *et al.* introduced folate groups onto chitosan chains using carbodiimide chemistry. The resulting chitosan conjugates (**77**, **Scheme 5**) were coated on the phosphatidyl choline-based liposome templates, giving nanocapsules of 182 nm.[124]

Klemetsrud and coworkers deposited pectins of different molecular weights (76, 96 and 110 kg/mol for low methoxy (LM), amidated (AM) and high methoxy (HM) pectins) on DPPC/DPTAP liposomes prepared by film hydration method, resulting in series of coated liposomes (0.02% w/w of coated pectin, diameters of 229, 302 and 418 nm for LM, AM and HM pectin-based capsules, respectively).[125] Zhou and coworkers coated thiol-derived chitosan on a preformed liposome to produce chitosan-coated liposomes with a diameter of 406 nm.[144]

Besides liposomes, Borsali and Soldi recently decorated PS-*b*-PAA based polymersomes with chitosan (named 37CS and 5CS, molecular weights of 37 and 5 kg/mol, respectively) to generate a variety of glyconanocapsules.[126] PS₁₃₉-*b*-PAA₁₇ and PS₄₀₄-*b*-PAA₆₃ were first used to construct polymersomes (called C3 and C7, diameters of 414 and 180 nm, respectively) through a co-solvent self-assembly process. The polymer deposition process directly led to a series of chitosan-coated polymersomes (C3/37CS, C3/5CS, C7/37CS and C7/5CS), with diameters of 712, 404, 363 and 183 nm, respectively.

2.2.2.2 Multi-layered nanocapsules

In 2010, Ge and coworkers successively deposited alginate (**78, Scheme 5**) and chitosan on cationic unilamellar DPPC/DDAB liposomes. The resulting nanocapsules (d ~ 414 nm) made of four successive polysaccharide layers were quite stable in SDS aqueous solution and 1/10 in volume water/ethanol solution.[127] Using a similar L-b-L process, Cuomo and coworkers constructed alginate/chitosan based nanocapsules starting from cationic liposomes of DDAB (diameter of 220 nm).[128] After removal of DDAB triggered by the introduction of nonionic surfactant Triton X-100, nanocapsules with a diameter of about 500 nm were obtained. The same group also described the synthesis of multi-layered nanocapsules with a diameter of 300 nm (thickness of 20 nm) through L-b-L sequential deposition of chitosan and alginate on DPPC/DDAB liposome templates.[129, 130] These nanocapsules were able to load FITC-Dextran of different MWs (20, 40 and 70 kg/mol) (efficiency ~ 50%). The release of FITC-Dextran was triggered by the dissolution of the liposome (inner) phase of the nanocapsules.

Fujimoto and coworkers used negatively-charged dilauroyl phosphatidic acid (DLPA)/dimyristoyl phosphatidylcholine (DMPC) based unilamellar liposomes (1/1 molar ratio, $d \sim 100$ nm) as templates for sequential disposition of chitosan (CHI) and dextran sulfate or DNA (**Figure 7**).[131, 132] Contrary to their liposome precursors, the resulting capsules were stable against Triton X-100. Peng and coworkers built double-layered capsules ($d \sim 215$ nm) by sequential deposition of poly(acrylic acid) (PAA) and chitosan on preset liposomes (size of 108 nm) composed of soya lecithin, cholesterol and stearyl amine.[133] Kaminski *et al.* sequentially deposited xanthan (**79, Scheme 5**) and galactomannan (**80, Scheme 5**) polysaccharides on DODAB liposomes (diameter of 62 nm), resulting in bilayered nanocapsules with diameter of 165 nm.[134]

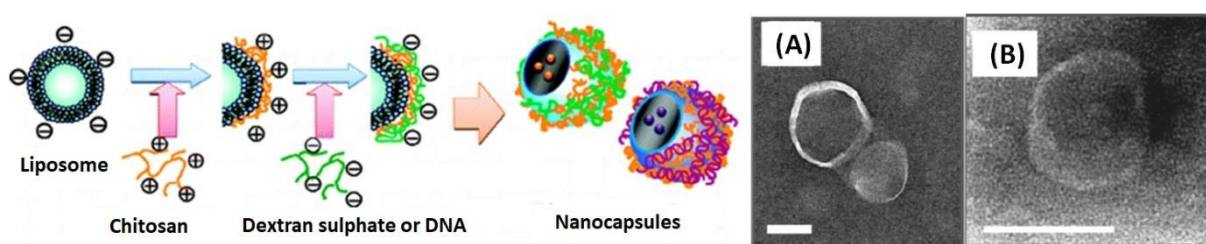


Figure 7. Preparation of multilayered sugar-based nanocapsules through L-b-L deposition on liposomes, TEM images of (A) parent liposomes and (B) resulting carbohydrate nanocapsules.

Scale bars are 100 nm.[131] Copyright 2009. Adapted with permission from American Chemical Society.

2.3 Emulsification routes to carbohydrate-functionalized nanocapsules

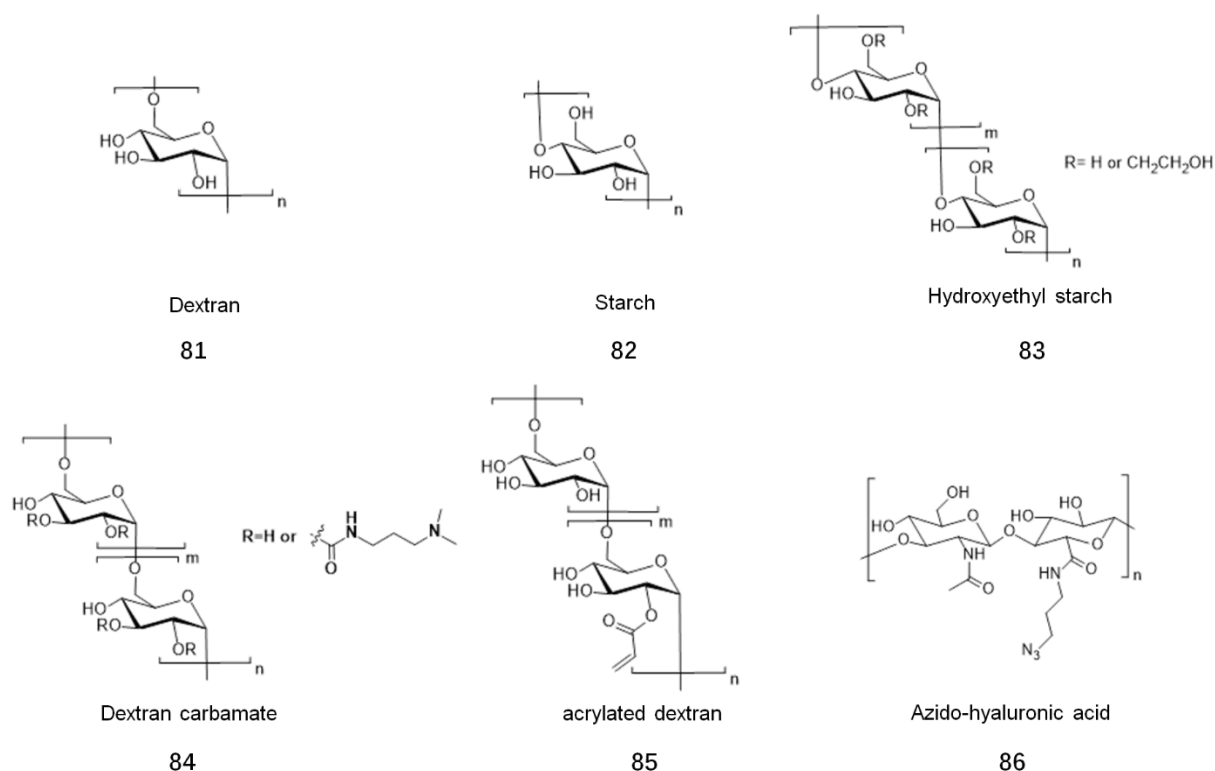
In contrast to oily-cored NCs, water-filled glyconanocapsules through emulsification have been rarely reported. Only two methods could be recorded and are present here, double emulsification technique and miniemulsion.

2.3.1 Double emulsification technique

Double emulsification, also called “emulsion of emulsion”, consists in preparing either a water-in-oil-in-water (w/o/w) or a oil-in-water-in-oil (o/w/o) emulsion. To carry out double emulsification, both hydrophobic and hydrophilic surfactants are necessarily used in a two-steps’ emulsification process. The formation of nanocapsules induced by double emulsification is also associated with nanoprecipitation and emulsion-diffusion methods. One example of w/o/w double emulsification was reported by Wu et al.[145] First an aqueous solution of insulin was emulsified in a phospholipid oily phase; then, this w/o emulsion was gently poured into an alginate aqueous solution to carry out the second emulsification step and generate the double emulsion. After sequentially adding calcium chloride and chitosan (low-molecular weight, 75-85% DA) as a two-step cross-linking process, alginate/chitosan nanocapsules were finally obtained with a diameter of ~ 490 nm.

2.3.2 Miniemulsion technique

Miniemulsions are kinetically metastable oil-in-water dispersions generated by high shear devices such as ultrasonicators. Nanoparticles or nanocapsules can indifferently be generated under miniemulsion conditions. In a typical procedure, water, oil, hydrophobic agents and stabilizers are co-emulsified, resulting in the formation of nanodroplets (either of oil or water, depending whether a direct or inverse miniemulsion is prepared) with sizes ranging from 50 to 500 nm (**Figure 8**).[146] This technique has



Scheme 6. Structures of carbohydrate polymers used to generate water-filled nanocapsules by emulsification.

been successfully applied to radical, ionic, or metal-catalyzed polymerizations, enzymatic or chemical polycondensation and polyaddition reactions. Polymerization normally occurs in the dispersed phase or at the interface of droplets, resulting in the formation of a polymeric membrane. Thanks to its potential applications in pharmaceutical and cosmetic fields, numerous examples of nanocapsules prepared under miniemulsion conditions have been reported over the last twenty years.

Researchers have given many efforts on the construction of oil-loaded sugar-based nanocapsules through the miniemulsion technique, which are presented in details in the next section. Meanwhile, to extend the scope of the technique to the controlled delivery of hydrophilic cargo, Landfester and co-workers developed an inverse miniemulsion process in which the aqueous solution is dispersed in the continuous oil

phase. In a typical process, the emulsification of water/cyclohexane system in the presence of polysaccharide chains gives birth to stable water nanodroplets. Toluene-2,4-diisocyanate cross-linker (TDI) is then added to initiate interfacial polyaddition, affording water-filled nanocapsules.

Table 3: Formation of water-filled **sugar-based nanocapsules** by emulsification.

Process	Polysaccharides or Glycopolymers	Solution	Emulsion method	Cross-linker	Diameter (nm)	Ref.
Double emulsification	Alginate	Water/Phospholipid oil/Water	Stirring	Calcium chloride, chitosan	488	[145]
Miniemulsion	Dextran Starch	Water/Cyclohexane	Sonication	Tolylene diisocyanate	324 (dextran) 524 (starch)	[147]
Miniemulsion	Hydroxyethyl starch	Water/Cyclohexane	Sonication	Tolylene diisocyanate	275	[148]
Miniemulsion	Hyaluronic acid Hydroxyethyl starch	Water/Cyclohexane	Sonication	Tolylene diisocyanate	350	[149]
Miniemulsion	Glycol chitosan	Water/Cyclohexane	Sonication	Tolylene diisocyanate	209	[150]
Miniemulsion	Dextran carbamate	Water/Cyclohexane	Sonication	Tolylene diisocyanate	180	[151]
Miniemulsion	Acrylated dextran	Water/Cyclohexane	Sonication	Phenyl-di(undec-10-en-1-yl) phosphate	280	[152]
Miniemulsion	Azido-hyaluronic acid	Water/Cyclohexane	Sonication	1,6-hexanediol dipropiolate	165	[153]

Using this process, Landfester and co-workers constructed dextran- (**81, Scheme 6**, diameter of 320 nm) and starch- (**82, Scheme 6**, $d = 520$ nm) based nanocapsules.[147, 154, 155] The thickness of the shell was tuned by adjusting the concentration of reactants (ranging from 10 to 20 nm, case of dextran). Dextran-containing capsules were used to encapsulate a hydrophilic contrast agent Magnevist[®], while starch-based nanocapsules were loaded with silver nanoparticles (diameter of 14 nm) or DNA (efficiency of 85%) during the emulsification step. The size of the starch-containing capsules (from 300 to 900 nm) was tuned by introducing different concentrations of surfactant (poly[(ethylene-co-butylene)-*b*-(ethylene oxide)] (P(E/B-*b*-EO)))[156] and of silver particles.[157]

A similar process has been introduced to generate hydroxyethyl starch (HES, **83, Scheme 6**)-based nanocapsules with a diameter of 275 nm (**Figure 8A**).[148] Targeting molecules such as folic acid[148] or mannose[158] were post-attached on the surface of capsules through covalent bonds. After a slight modification of the protocol consisting in incorporating additional oil after the sonication step and further sonicating before the cross-linking procedure, a series of smaller-sized HES nanocapsules ($d \sim 140$ nm) were obtained.[159] Isocyanate-PEG was then introduced into the solution for post-functionalization, offering potent stealth capability to the capsules. This method is also applicable to a polyelectrolyte, i.e. hyaluronic acid, to generate nanocapsules with a diameter of 350 nm.[149] Crespy and coworkers also applied inverse miniemulsion process to generate dextran carbamate- (**84, Scheme 6**) based nanocapsules with a diameter of 180 nm in water/ hexane mixture. After re-dispersion in water, the NCs swell to 264 (pH 4), 295 (pH 7) and 267 nm

(pH 12).[151]

Recently, Landfester and coworkers reported a new strategy combining inverse mini-emulsion, polyelectrolyte complexation and interfacial cross-linking to generate chitosan-based nanocapsules.[150] Specifically, two inverse miniemulsions containing water droplets suspended by poly(γ -glutamic acid) or glycol chitosan (**70**, **Scheme 5**) were robustly and separately prepared. The coalescence of the two colloidal solutions was induced by ultrasonication, resulting in polyelectrolyte complex loaded droplets. Post-introduction of TDI instantly cross-linked the polymers' shell at the interface, leading to the formation of nanocapsules of 209 nm in diameter (**Figure 8B**). Apart from diisocyanate-induced cross-linking, interfacial olefin metathesis was also used to cross-link acrylated dextran with phenyl-di(undec-10-en-1-yl)-phosphate (**85**, **Scheme 6**), generating dextran-based nanocapsules of diameter around 280 nm (**Figure 8C**).[152, 160] Finally, CuAAC was recently performed by post-addition of disulphide 1,6-hexanediol dipropiolate to azide-functional hyaluronic acid-loaded water-in-oil emulsion (**86**, **Scheme 6**), giving negatively charged nanocapsules with a diameter of 165 nm.[153]

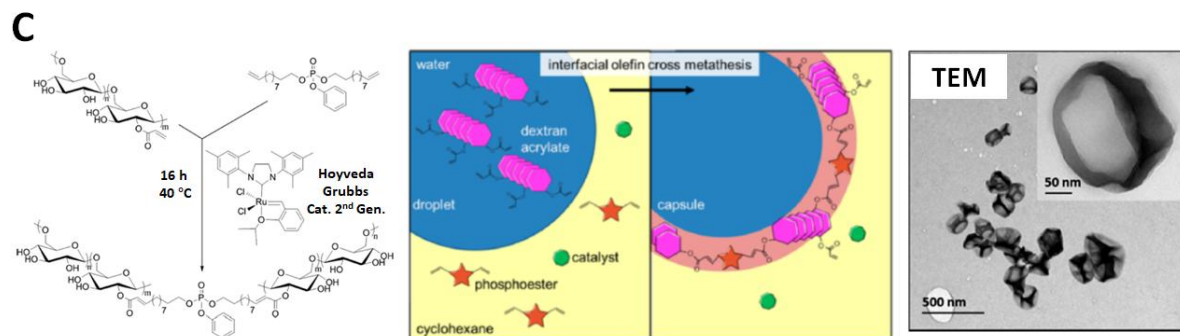
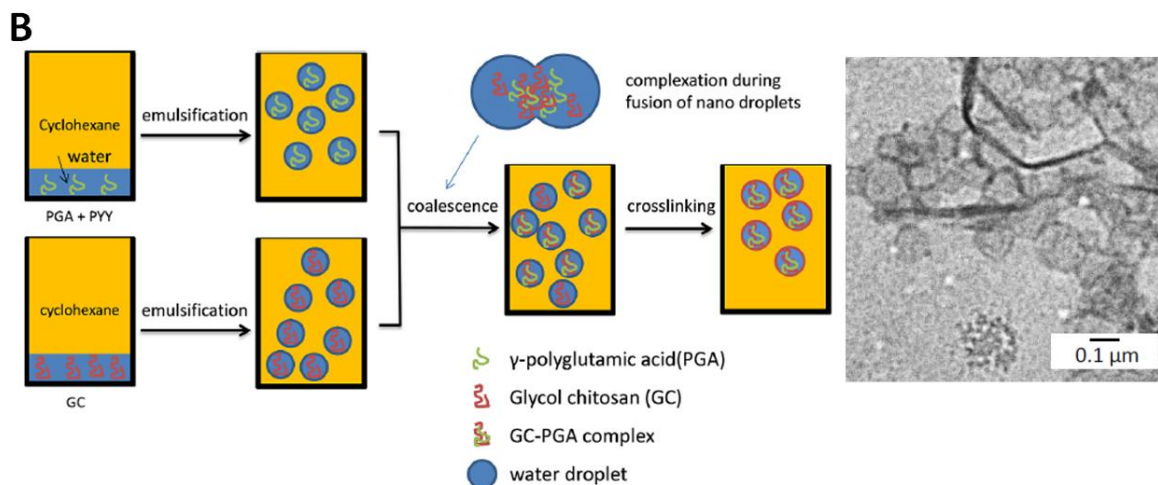
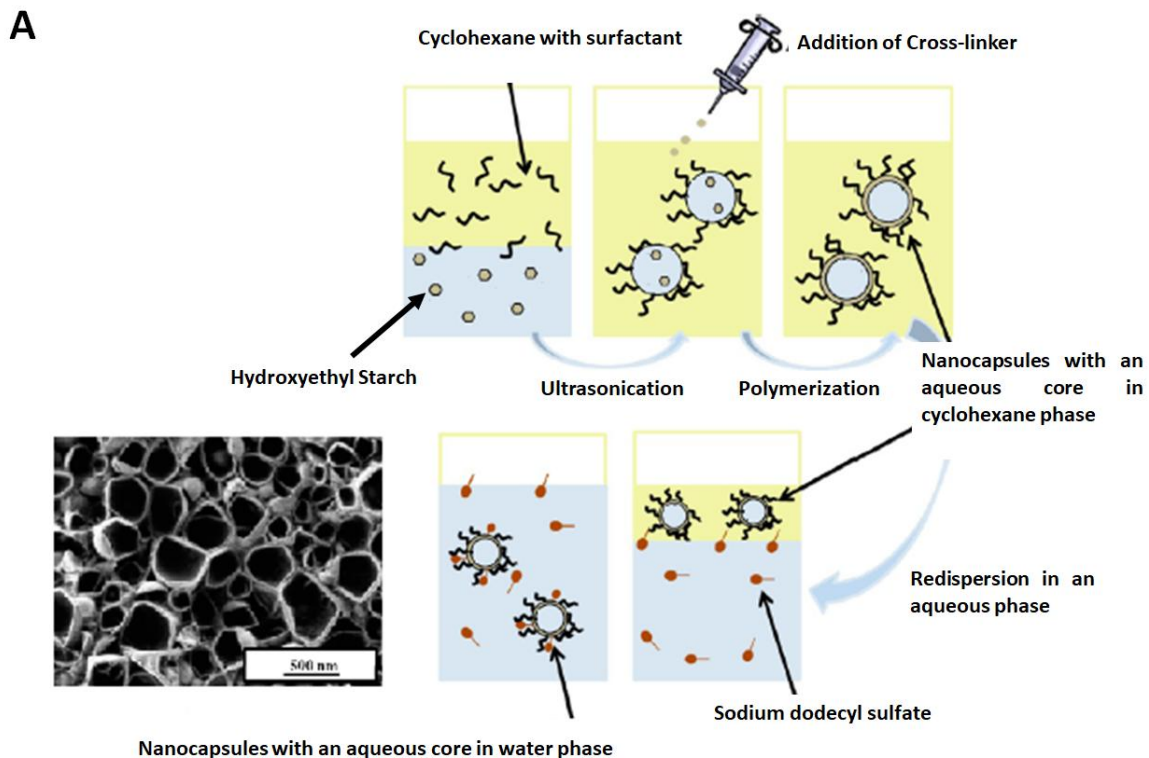


Figure 8. (A) Preparation and SEM image of HES nanocapsules using an inverse miniemulsion process.[148] Copyright 2012. Adapted with permission from American Chemical Society. **(B)** Preparation and TEM image of chitosan nanocapsules by sequential inverse miniemulsion, polyelectrolyte complexation and interfacial cross-linking process.[150] Copyright 2015. Adapted

with permission from American Chemical Society. (C) Schematic representation and TEM image of the interfacial olefin cross metathesis at the water–oil interface for the construction of sugar-based nanocapsules. [152] Copyright 2014. Adapted with permission from American Chemical Society.

3 Oil-filled sugar-based nanocapsules

Considering the medical application of nanocapsules, especially in anti-tumor therapy via controlled release of anti-cancer drug (most of which show poor solubility in water), the construction of oil-filled nanocapsules of synthetic glycopolymers or polysaccharides were reported to facilitate the administration of medicine.

3.1 Emulsification routes to carbohydrate-functionalized nanocapsules

Emulsification routes to oil-filled sugar-based nanocapsules mainly include emulsification/coacervation, polymer coating, double emulsification and miniemulsion.

3.1.1 Emulsion coacervation technique

Typically, an emulsion is first generated through o/w emulsification by mechanical stirring or ultrasound. The polymer is then precipitated from the continuous solution to form a coacervate layer on the emulsion template later converted in nanocapsule walls through formation of cross-linking nodes. The crucial point of this technique is the deposition/precipitation of the coacervate layer triggered by addition of a salt, a non-solvent, an oppositely-charged polymer, or by pH or temperature change. Up to now, sodium alginate and gelatin are the most common polymers used for generation of nanocapsules through the emulsion coacervation technique.

In 2008, Lertsutthiwong's group reported on the preparation of turmeric oil-loaded alginate nanocapsules by emulsion coacervation technique.[161, 162] Practically, a solution of turmeric oil in acetone or ethanol was added into an alginate aqueous

solution. After o/w emulsification by sonication, physically cross-linked nanocapsules were directly generated by addition of calcium chloride, followed by solvent removal. The size of capsules obtained from the ethanol/water system ($d \sim 260$ nm) was smaller than capsules obtained from the acetone/water system ($d \sim 370$ nm). An increase of oil concentration or oil/alginate mass ratio logically caused an increase of nanocapsules' size. Alternatively, the alginate coacervate layer was ionically crosslinked by sequential addition of calcium chloride and chitosan, leading to the formation of larger nanocapsules (above 500 nm). Instead of oil, hydrophobic drugs could also be introduced to constitute the core of the capsules.

Grebinsan *et al.* developed formyl- and acetyl-grafted N-(p-aminobenzoyl)-L-glutamine in a five-step route. The glutamine derivatives were dissolved in a mixture of ethanol, benzyl alcohol and DMSO, and then poured into an alginate aqueous solution. After emulsification by sonication, alginate-based nanocapsules loaded with these derivatives ($d \sim 400$ nm) were obtained after chitosan-induced cross-linking reaction and solvent evaporation.[163] A steroidal male hormone, testosterone, was encapsulated in alginate capsules by emulsification of testosterone/hexane in alginate aqueous solution and calcium-induced cross-linking.[164] Terreno's group coated chitosan on a preset perfluoropentane emulsion,[165] affording liquid-filled chitosan nanocapsules with dimension of around 500 nm. By ultrasonification, the liquid core was vaporized into gas to create chitosan 'nanobubbles' of larger size. Interestingly, the process was reversible, so that the initial size of nanocapsules could be restored. The nanobubbles were capable of encapsulating a drug (prednisolone phosphate) with a high efficiency of 78%.

3.1.2 Polymer coating technique

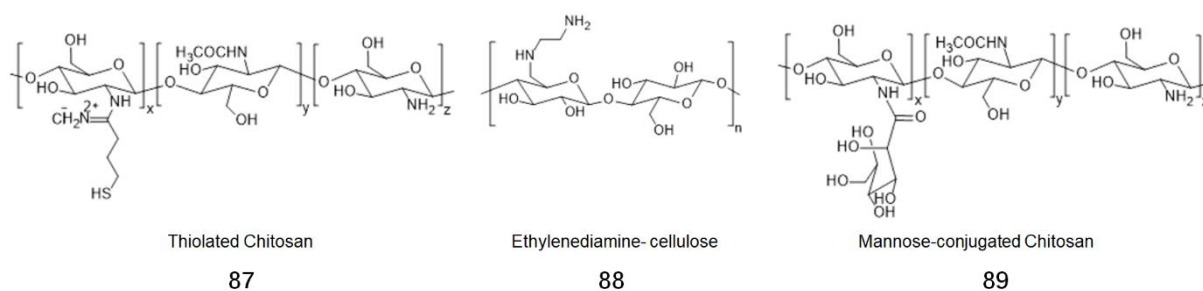
The polymer coating technique consists in a preliminary emulsification step leading to the formation of nanodroplets and a subsequent polymer deposition step at the oil/water interface. In contrast with the emulsion-coacervation technique, the adsorption of polymer chains is generally triggered by solvent evaporation.

In 1997, Alonso's group first used this approach to generate cationically-charged polysaccharide-functionalized nanocapsules. Specifically, miglyol 840 and lecithin (and possibly poly- ϵ -caprolactone) were dissolved in acetone. After pouring the organic solution into an aqueous solution of chitosan, first nanoemulsion (respectively PCL/oil nanocapsules) were generated; then chitosan was coated on the nanocapsules (diameter ranging from 300 to 500 nm) by interfacial deposition after evaporation of acetone (one-stage procedure).[166-168] To promote functionalization of the capsules, MeO-PEG-COOH or biotin-PEG-COOH was grafted onto chitosan chains by EDC-catalyzed coupling amidation.

An analogous procedure was also used to afford PEG-functionalized and biotin-PEG-functionalized chitosan-based nanocapsules (diameters around 250 and 200 nm, respectively). Using a similar strategy, Ortega-Vinuesa and coworkers investigated the influence of molecular weight (MW) and chitosan acetylation degree (DA) on capsules' stability.[169] An acetone/ethanol solution of miglyol 812 and lecithin was added into an aqueous solution of chitosan of either low MW (11 kg/mol, DA ranging from 1.4 to 51%) or high MW (~110 kg/mol, DA ranging from 1.6 to 56%), producing chitosan-based nanocapsules after solvent evaporation (diameters ranging from 150 to 185 nm).[170, 171] Low MW chitosan (viscosity, 20-200 cP) was

deposited at the interface of soybean oil nanoemulsion (generated by solvent evaporation) to afford nanocapsules with a diameter of 145 nm.[172]

Alonso's group then changed the process by post-incubating a preset miglyol nano-emulsion prepared by solvent shifting in an aqueous solution of chitosan for 1 h to build nanocapsules ($d \sim 200$ nm).[173-175] Salmon Calcitonin, a polypeptide hormone, was encapsulated with an efficiency of 60% and released over a period of 6 h. In 2013, the same authors post-modified the nanocapsules with hepatitis B surface antigen (HB) through electrostatic attraction (CS/HB = 1/0.025, weight ratio) resulting in a high antigen association efficiency (70%).[176] The chitosan-based nanocapsules were also modified with MeO-PEG-COOH (~ 5100 g/mol) and biotin-PEG-COOH (~ 4100 g/mol) by EDC-catalyzed coupling amidation, affording PEG-functionalized and biotin-PEG-functionalized chitosan-based nanocapsules (diameters around 250 and 200 nm, respectively). The biotin-PEG-functionalized nanocapsules were further engaged in reaction with monoclonal antibody anti-TMEFF-2 through a convenient two-stepwise avidin-biotin coupling.[173, 177] Using a similar approach, the same group reported on squalene-loaded polyglucosamine nanocapsules (PG, highly deacetylated chitosan).[178] Specifically, squalene (core material) was dissolved with lecithin in 2-propanol. The solution was then poured into an aqueous solution of PG under stirring resulting in PG nanocapsules ($d \sim 240$ nm) after evaporation of 2-propanol.



Scheme 7. Structures of carbohydrate polymers used to generate oil-filled nanocapsules by emulsification.

Tzanov prepared cationic nanocapsules from chitosan or cellulose.[179] Thiolated chitosan (TC, **87**, **Scheme 7**) was prepared by a coupling reaction between 2-iminothiolane and chitosan (~15 kg/mol), whereas, ethylenediamine-functionalized cellulose (AC, **88**, **Scheme 7**) was synthesized by substitution of tosylated cellulose with ethylenediamine. Oil-loaded nanocapsules were obtained without addition of surfactant through a sonication-induced emulsification of aqueous solution of polysaccharide (TC or AC) with sunflower oil. The diameters of nanocapsules prepared from TC or AC were 268 and 247 nm, respectively. The cationic nanocapsules were shown to disrupt bacterial membranes and were not cytotoxic to human cells. Chitosan (150 kg/mol) was also functionalized with mannose by reductive amination (DS=0.53, **89**, **Scheme 7**).[180] A tripolyphosphate (TPP) nanoemulsion with rifampicin (anti-tubercular drug) as core material was generated by first stirring and then pouring the initial DMSO/water solution into an aqueous solution of mannose-conjugated chitosan to afford nanocapsules with a size of 215 nm. The encapsulation efficiency was about 39%, and more than 90% of loaded drug was released after 5h. Besides chitosan, Alonso and coworkers prepared miglyol 812-filled hyaluronic acid (HA, 29 or 160 kg/mol) nanocapsules either through a one-stage procedure (polymer coating during the emulsification) or through polymer coating on

preset nanoemulsion (two-stage procedure, polymer coating after the emulsification).[181] The diameter of the HA nanocapsules prepared by the second process (~250 nm) was bigger than in one step (~180 nm). Nanocapsules made of bovine serum albumin-Dextran (BSA-Dextran) conjugate and soybean oil as core were used to load sensitizer/annihilator (s/a) couples allowing maintaining the mobility of chromophores and avoiding their quenching (**Figure 9**), so as to display an intense photoluminescence emission.[182] The nanocapsules (UCNC) were loaded with several s/a couples [PtTPBP/BDP-G(UCNC-G), PtTPBP/BDP-Y(UCNC-Y) or PdOEP/DPA(UCNC-B)] to afford green, yellow or blue luminescent emissive nanocapsules. UCNC-G and UCNC-Y exhibited up conversion luminescence emission ($\lambda_{\text{ex}} = 635 \text{ nm}$) with quantum efficiencies of 1.7 and 4.8%, respectively.

Table 4: Emulsification routes to oil-filled **sugar-based nanocapsules**.

Process	Polysaccharides or Glycopolymers	Solution	Emulsification Method	Cross-linker	Diameter (nm)	Ref.
Emulsion/coacervation	Alginate	Acetone or ethanol/Water	Sonication	Calcium chloride	263 (ethanol) 373 (acetone)	[161, 162]
Emulsion/coacervation	Alginate	Ethanol, benzyl alcohol or DMSO/Water	Sonication	Chitosan	400	[163]
Emulsion/coacervation	Alginate	Hexane/Water	Stirring, sonication	Calcium chloride	34	[164]
Emulsion/coacervation	Chitosan	Ethanol/Water	Stirring		500	[165]
Polymer coating	Oligochitosan	Acetone/Water	Stirring		200~500	[166]
Polymer coating	Chitosan	Acetone-ethanol/Water	Solvent displacement		148~185	[169]
Polymer coating	Chitosan	Ethanol/Water	Solvent evaporation		145	[172]
Polymer coating	Oligochitosan	Acetone/Water	Solvent displacement		200	[173-175]
Polymer coating	Polyglucosamine	2-propanol/Water	Stirring		242	[178]
Polymer coating	Thiolated Chitosan (TC) Ethylenediamine- cellulose (AC)	Sunflower oil/Water	Sonication		268 (TC) 247 (AC)	[179]

Polymer coating	Mannose-conjugated Chitosan	DMSO/Water	Agitation		215	[180]
Polymer coating	Hyaluronic acid	Ethanol-acetone/Water	Solvent displacement		180~250	[181]
Polymer coating	Dextran	Soybean oil-Toluene/water	High pressure homogenization		93~116	[182]
Double emulsification	Chitosan	Toluene/water/Toluene	Mechanical agitation	Sodium sulfate, glutaraldehyde	100~700	[183]

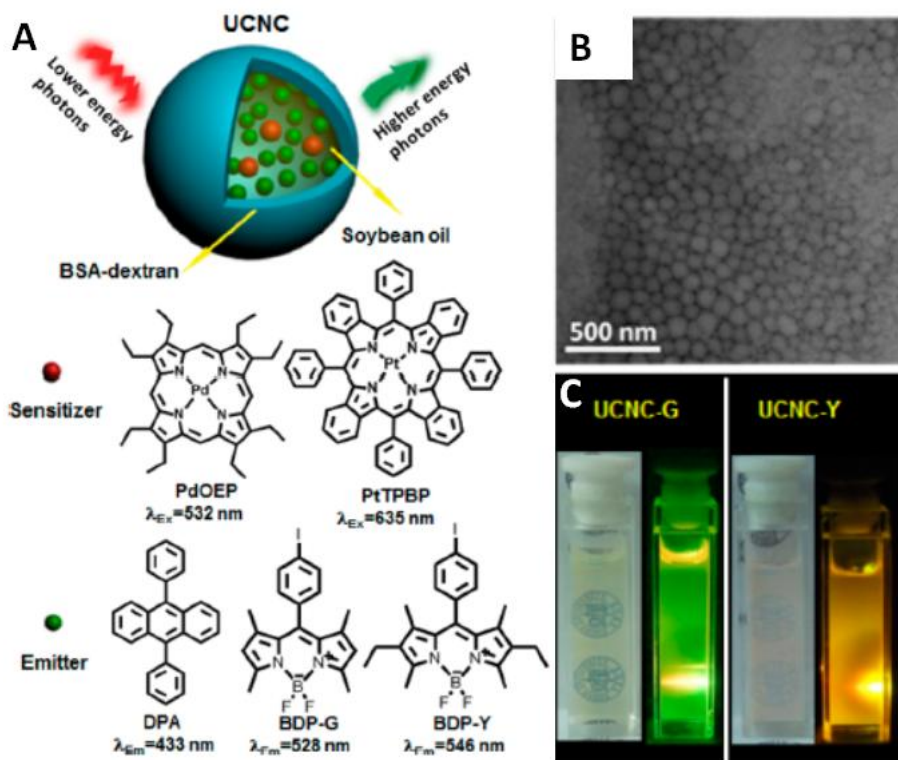


Figure 9. (A) Schematic illustration of the dextran-based UCNC and chemical structures of sensitizers and annihilators;^[182] Copyright 2013. Adapted with permission from American Chemical Society. (B) TEM photo of UCNC-G in pure water; (C) Photos of bright-field and UCL emission of UCNC-G, and UCNC-Y in water (excitation at 635 nm).

3.1.3 Double emulsification technique

Holban and co-workers prepared double BDP cross-linked chitosan nanocapsules by o/w/o emulsification.^[183] A toluene solution of Span80 was first co-emulsified with an aqueous solution of chitosan-gelatin (1/1, weight ratio) containing Tween 80 (low-molecular weight chitosan, 75-85% DA). The resulting emulsion was then added to a second Span 80 toluene solution (final o/w/o conditions, 1/3/17, volume ratios). After a first cross-linking step (induced by addition of sodium sulfite), the solution was emulsified by mechanical agitation. Chitosan containing nanocapsules were finally obtained after cross-linking with glutaraldehyde. The size of nanocapsules (diameters

ranging from 100 to 700 nm) was controlled by the molar ratio between amino groups of chitosan and gelatin and sodium sulfate. Increasing the content of sodium sulfate made the nanocapsules shrinking owing to ionic cross-linking.

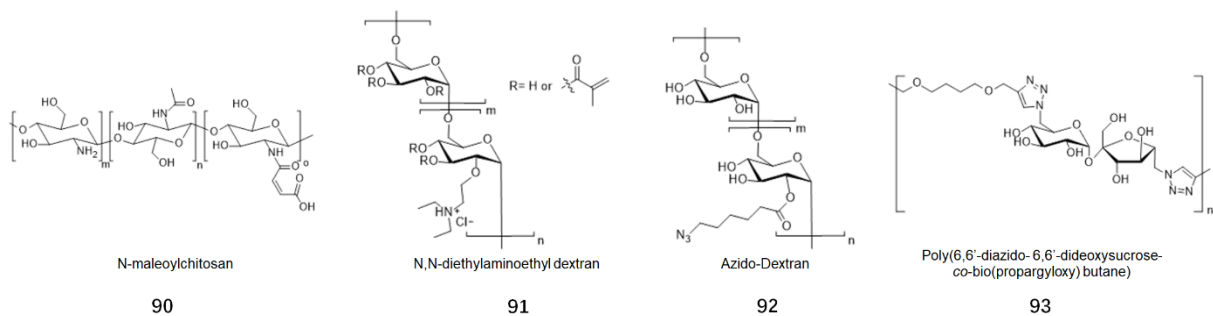
3.1.4 Mini-emulsion technique

Polysaccharides. In 2002, Landfester and coworkers first introduced the miniemulsion technique to generate chitosan-containing nanocapsules.[184] In this seminal work, a solution of toluene containing a diepoxide as cross-linker (Epikote E828), hexadecane, Jeffamine D2000 as surface-active reactant and various hydrophobic diamines (1,12-diamino-dodecane, 4,4'-diaminodicyclohexylmethane, 4,4'-diaminobiphenyl) were dispersed in an acidic aqueous solution of chitosan. Ultrasonication of the emulsion at 0°C afforded a stable mini-emulsion. In situ polyaddition was initiated by increasing the temperature of the medium to 68°C, leading to the formation of oil loaded polymeric nanocapsules with diameter ranging from 130 to 280 nm.

Besides interfacial polyaddition, capsules with a diameter of 100 nm were also built by an interfacial free radical polymerization approach involving *N*-maleoylchitosan (NMCS, **90**, **Scheme 8**) as a macromonomer. An anti-hypertensive drug, felodipine, was spontaneously trapped into the oil core through pre-dispersion of Triton X-100 and a felodipine/chloroform mixture in an aqueous phase containing NMCS.[185] In a similar manner, Chen *et al.* cross-linked *N*-maleoyl-modified chitosan (MCS) with 1,4-butanediol bis(3-mercaptopropionate) via UV-induced thiol-ene reaction at the oil/water interface (**Figure 10A**).[186] Zhang

et al. modified pluronic F127 PEO₁₀₀-PPO₆₅-PEO₁₀₀ by introducing carboxylic acid moieties at both polymer ends as a cross-linking means of chitosan at miniemulsion interfaces. Owing to the thermo-responsive character of Pluronic F127, the diameter of nanocapsules evolved from 37 nm at 37°C to 240 nm at 4°C, thus modifying the permeability of capsules' shell.[187]

Recently, Chirachanchai and coworkers investigated the impact of pH on the morphology of colloids formed in a toluene/water miniemulsion process using ionic polysaccharides.[188] The authors highlighted the key role of pK_a of the polysaccharides on the synthesis of nanoparticles or nanocapsules. When pH > 3, chitosan (pK_a=6.4) cross-linked by glutaraldehyde gave rise to chitosan-based nanospheres. At pH ≤ 3, conditions under which chitosan's amines are fully protonated, electrostatic repulsions led to a decrease in packing structures and favored cross-linking at the interface to produce nanocapsules. The importance of pK_a was further illustrated with alginate (pK_a = 4) with the observation of a morphology transition between nanospheres (pH ≤ 4) and nanocapsules (pH > 4).



Scheme 8. Structures of carbohydrate polymers used to generate oil-filled nanocapsules by mini-emulsion.

Besides chitosan, other polysaccharides have also been used to build

nanocapsules. For instance, Jiang *et al.* reported the preparation of nanocapsules formed either solely from methacrylated N,N-diethylaminoethyl dextran (DexMA, **91**, **Scheme 8**) in aqueous solution or from both DexMA (in water) and methacrylated polylactide (PLAM, in chloroform).[189] After emulsification by mechanical agitation and subsequent addition of initiator, polymerization started at the interface of the nanodroplets to generate capsules. In contrast to DexMA nanocapsules ($d = 200$ nm, thickness = 30 nm), nanocapsules composed of DexMA and PLAM possessed much thicker walls ($d = 233$ nm, thickness = 60 nm). Nouvel and coworkers reported the synthesis of nanocapsules by performing CuAAC reaction between azido-modified dextran (**92**, **Scheme 8**) and α -alkyne polylactide at the interface of miglyol 810 emulsion.[190] After organic solvent evaporation, dextran nanocapsules with a diameter of 160 nm were finally obtained.

Glycopolymers. One example of nanocapsules made of glycopolymers (**93**, **Scheme 8**) was introduced by our group in 2012. Disaccharide derivatives, *i.e.* 6,6'-diazido-6,6'-dideoxy-sucrose (in the aqueous phase) were engaged in an A_2+B_2 interfacial step growth polymerization with bi(propargyloxy)butane (in the miglyol phase) under oil/water miniemulsion conditions, resulting in the formation of stable monodisperse glyconanocapsules with a diameter of 200 nm (**Figure 10B**).[191]

Table 5: Formation of oil filled **sugar-based nanocapsules** using miniemulsion conditions.

Interfacial Reactions	Polysaccharides or Glycopolymers	Solution	Emulsion method	Cross-linker	Diameter (nm)	Ref.
Cross-linking	Chitosan	Toluene/Water	Ultrasonication	diepoxide	130~280	[184]
Cross-linking	N-maleoylchitosan	Chloroform/Water	Mechanical agitation		100	[185]
Cross-linking	N-maleoylchitosan	Chloroform/Water	Ultrasonication	1,4-butanediol bis(3-mercapto-propionate)	57~388	[186]
Cross-linking	Chitosan	1,4-dioxane/Water	Ultrasonication	Pluronic F127 PEO ₁₀₀ -PPO ₆₅ -PEO ₁₀₀	37~240	[187]
Cross-linking	Chitosan	Toluene/Water	Ultrasonication	glutaraldehyde	35	[188]
Cross-linking	N,N-diethylaminoethyl dextran	Chloroform/Water	Mechanical agitation	Poly lactide Macromonomer	233	[189]
Cross-linking	Azido-Dextran	DCM/Water	Sonication	α -alkyne polylactide	160	[190]
Polymerization	Poly(6,6'-diazido-6,6'-dideoxysucrose-co-bio(propargyloxy butane)	Miglyol/water	Sonication		200	[191]

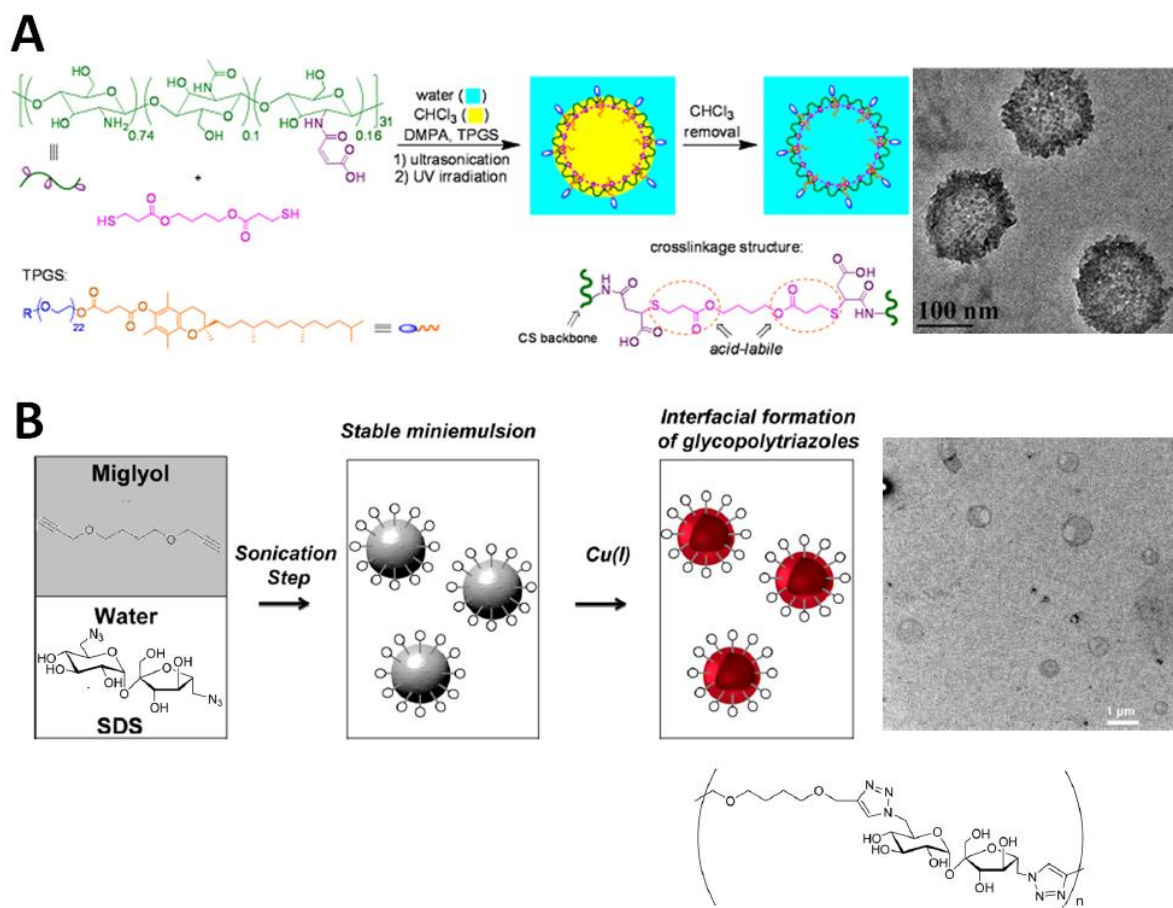
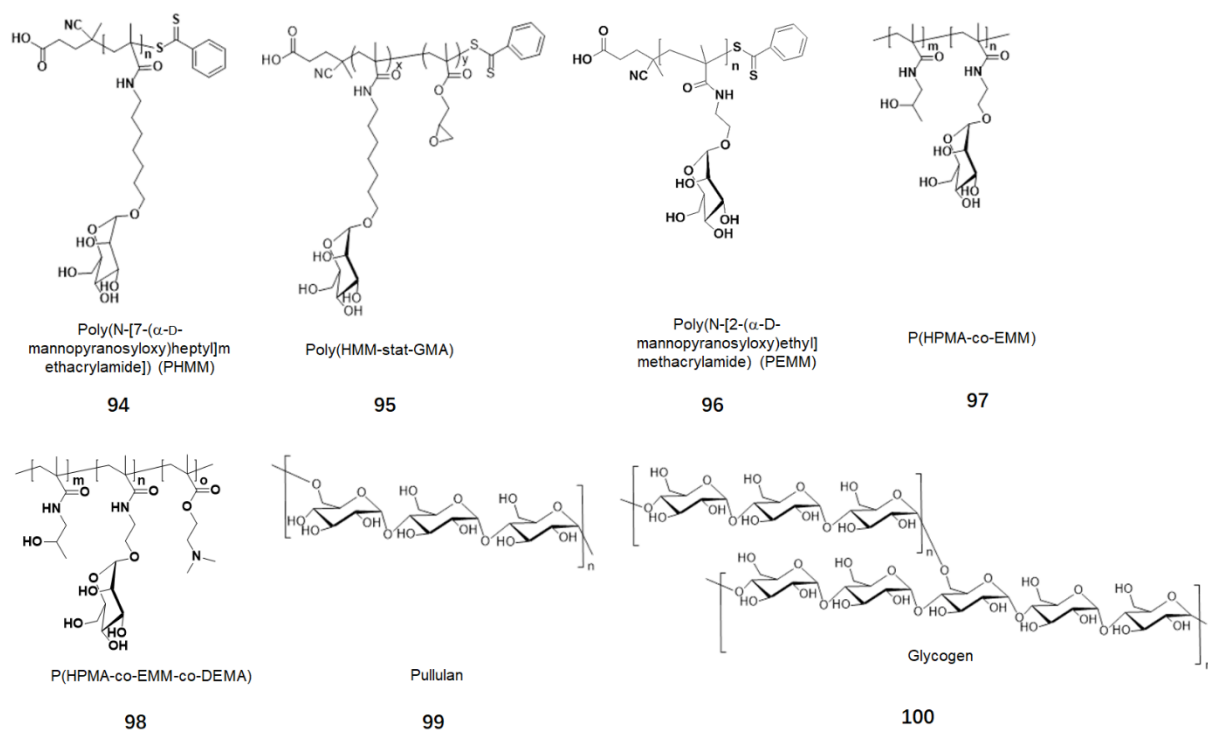


Figure 10. (A) Preparation and TEM picture of chitosan nanocapsules through mini-emulsion and thiol-ene cross-linking strategy.[186] Copyright 2014. Adapted with permission from American Chemical Society. (B) Preparation and TEM image of sugar-based nanocapsules by interfacial CuAAC step growth polymerization, scale bar is 1 μm.[191] Copyright 2012. Adapted with permission from American Chemical Society.

3.2 Nanoprecipitation

Nanoprecipitation[192], also called “Ouzo effect” or “solvent shifting process”, is a simple technique for preparing of colloids with narrow size distribution without recourse to ultra-sonication or other high shearing force techniques, and even addition of surfactants. Since no pre-emulsification is required, this solvent shifting

method is more straightforward than the other approaches described above. In nanoprecipitation, a hydrophobic solute (oil, dye, polymer) is dissolved in an organic solvent fully miscible with water. Upon addition of water, the solution becomes a non-solvent of the solute that comes out of the solution. Under appropriate conditions, this process spontaneously generates metastable dispersions of nanometer-scale droplets or particles without using surfactants. This process is simple, cheap and applicable to numerous solutes and solvents.



Scheme 9. Structures of carbohydrate polymers used to generate nanocapsules by nanoprecipitation.

Successful application of the Ouzo technique requires careful establishment and full understanding of the phase diagrams of solute/solvent/non-solvent ternary systems. Surfactant free microemulsion (SFME), Ouzo and heterogeneous regions of the phase diagram are different zones where the hydrophobic solute aggregates

(Figure 11). Since the precipitation proceeds following different formation mechanisms, the nanodroplets or nanoparticles formed show great distinctions in compositions, dimensions and colloidal stability throughout each region. Besides, hydrophilic polymers first dissolved in the water phase can also come out from the solution upon addition of a non-solvent (typically acetone). This event occurs when crossing the polymer cloud point boundary (see Figure 12). Hydrophobic oil molecules (in the solvent) can then be nanoprecipitated at the same time as hydrophilic polymers (in the water phase), albeit in very specific conditions resolved by phase diagrams' overlap (Figure 11). The polymer that covers the oil droplets is simultaneously cross-linked to produce oily-filled polymer nanocapsules in a one-pot, one-step system.

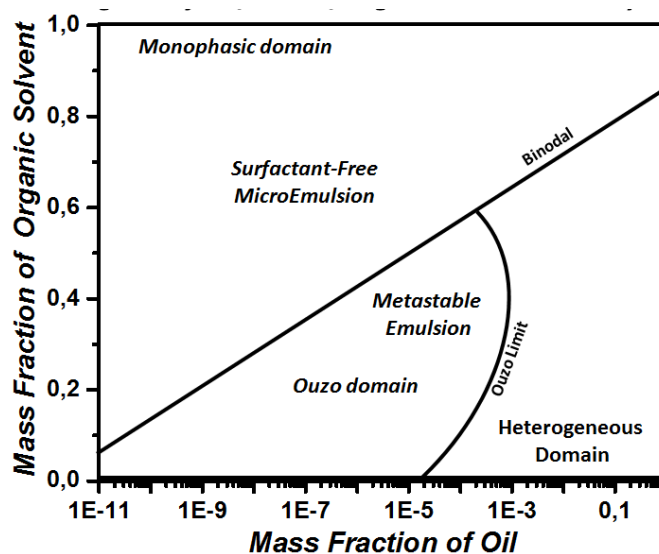


Figure 11. Phase diagram of oil/water/organic solvent ternary system.

Glycopolymers. In 2014, our group introduced nanoprecipitation technique to robustly generate glyconanocapsules in one pot using a synthetic glycopolymer

poly(N-[7-(α -D- mannopyranosyloxy)heptyl]methacrylamide)) (PHMM, **94**, **Scheme 9**) and an oil, hexadecane (HD).[193] We first elaborated and overlapped both phase diagrams of PHMM/HD/water/acetone system. An extremely simple solvent shifting approach was then processed by adding aqueous solution of glycopolymers into acetone solution of hexadecane and of a water-insensitive cross-linker, isophorone diisocyanate (IPDI), leading to co-nanoprecipitation of the two solutes and subsequent crosslinking. Supersaturation of hexadecane generates oily size controllable nano-droplets and in the meantime, the polymer preferentially sticks at the interface to form the polymer shell. Nanoprecipitation in Ouzo and heterogeneous regions resulted in monodisperse nanocapsules with size ranging from 150 to 250 nm and polydisperse microcapsules, respectively (**Figure 12**). We then replaced HD by a biocompatible oil, miglyol 812 (FDA approved), using Poly(HMM-co-GMA) or poly(N-[2-(α -D-mannopyranosyl-oxy)ethyl] methacrylamide)) (PEMM) as the glycopolymer (**95 and 96**, **Scheme 9**) and re-built the phase diagrams. Monodisperse nanocapsules of 110 nm in size were obtained in the Ouzo domain confirming the general character of this nanoprecipitation approach.[194] Using this technique, one can also achieve simultaneous surface functionalization (bio-active molecules, fluorescent probe, and metal nanoparticles) and encapsulation (drug, fluorescent probe) in one-pot.

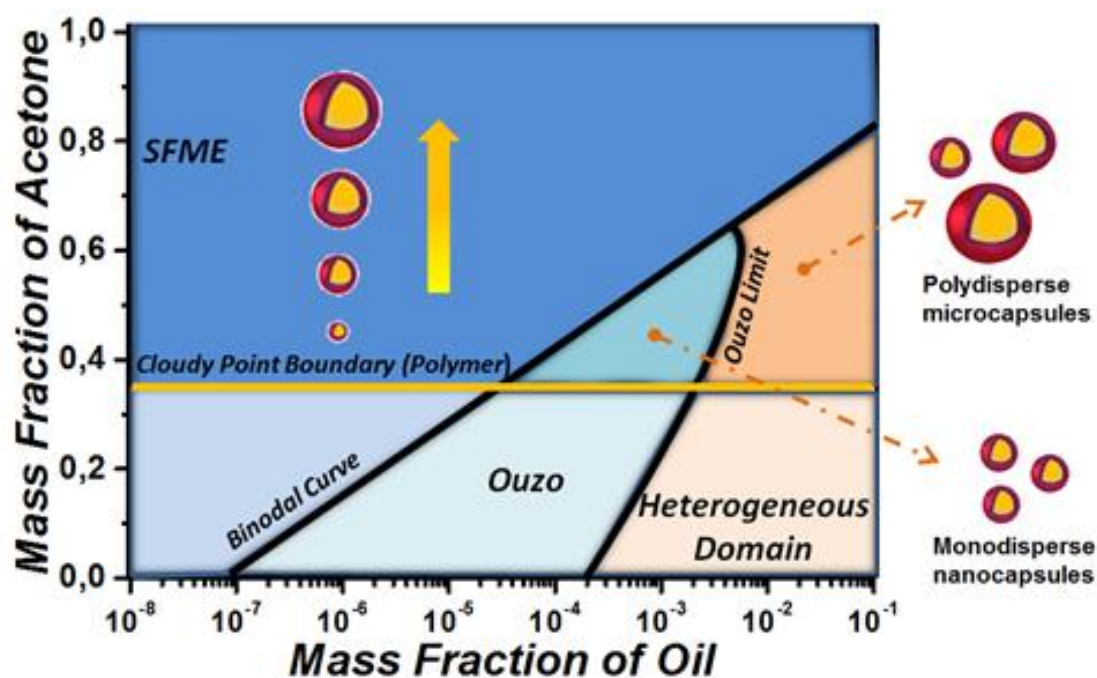


Figure 12. General scheme of hydrophobic oil/hydrophilic polymer/solvent/non-solvent phase diagrams. X-axis presents mass fraction of oil or polymer, y-axis presents mass fraction of organic solvent (solvent for oil and non-solvent for polymer). The concentration of water is deduced from the other two.

Besides Ouzo and heterogeneous regions, we surprisingly observed the presence of nanosized miglyol and HD nanodroplets ($d \sim 20$ to 200 nm) in the previously-believed “monophasic part” of their phase diagram (up to 0.8 acetone mass fraction). In contrast to dimension-solute concentration related behavior in Ouzo and heterogeneous regions, miglyol droplets in the now called Surfactant-Free MicroEmulsion domain (SFME) show sizes independent of solute concentration. In contrast, dimensions grow with acetone/water ratio in the final composition, and the droplets exhibit thermodynamic stability. Thanks to this discovery, we then expanded the nanoprecipitation approach to this new region and selectively produced

size-controllable glyconanocapsules with diameter ranging from 40 to 200 nm using PEMM (96, Scheme 9 and Figure 13).[195]

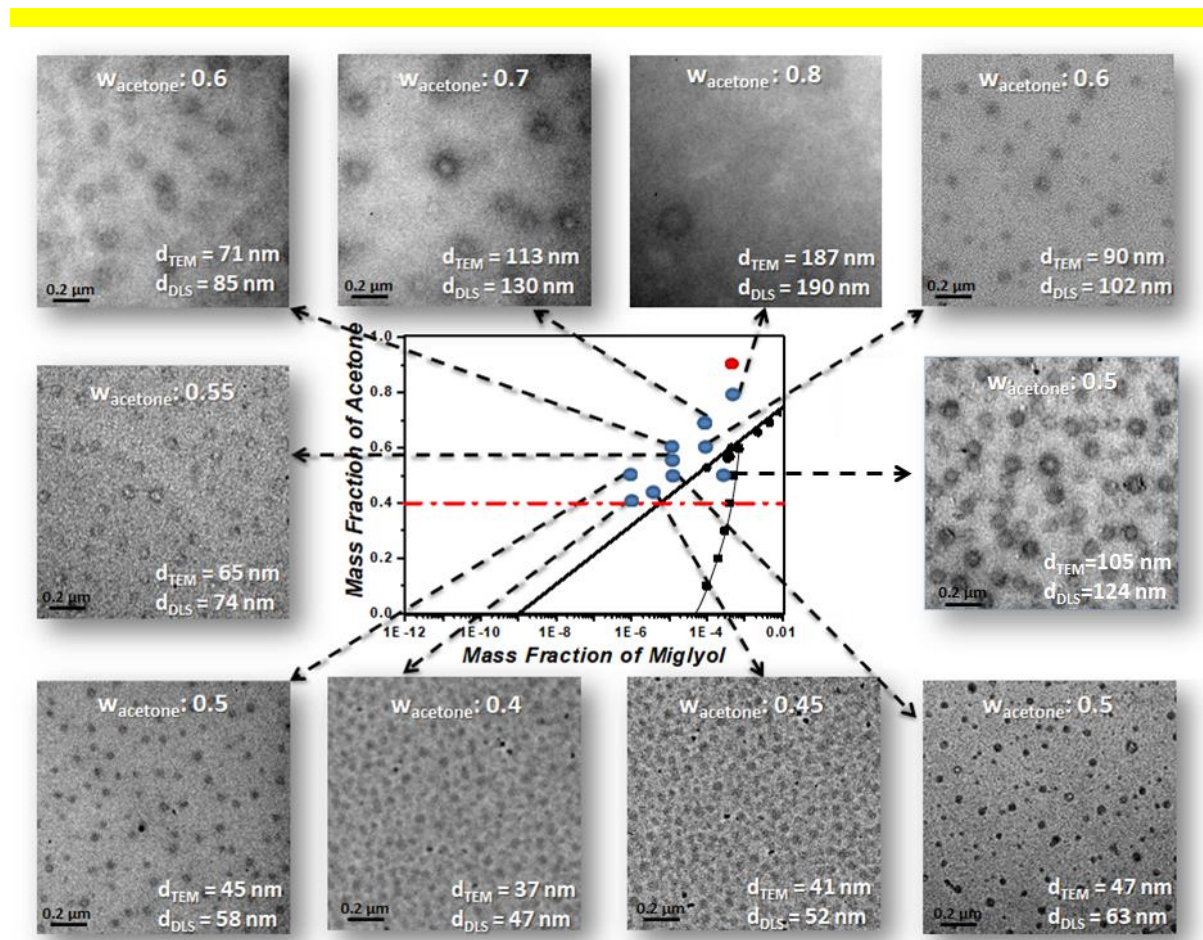


Figure 13. TEM images of glyconanocapsules prepared in the SFME and Ouzo domains (see acetone mass fraction written inside the photos and comparison of average diameters obtained by TEM and DLS).[195] Copyright 2017. Adapted with permission from the Royal Society of Chemistry.

We further transferred this approach to poly(N-(2-Hydroxypropyl) methacrylamide) (PHPMA) and PHPMA based glycopolymers, PHPMA-co-PEMM (97, Scheme 9) and PHPMA-co-PEMM-co-PDEMA (99, Scheme 9, PDEMA: poly(2-(dimethylamino)ethyl methacrylate)). Contrary to PHPMA for which the preparation of nanocapsules was

exclusively envisioned in the SFME domain of the acetone/ water/HD ternary system owing to the strong “acetonophilic” character of the homopolymer, the copolymers were suitable for the construction of nanocapsules in both Ouzo and SFME domains. In the case of PHPMA-co-PEMM-co-PDEMA, the preparation of nanocapsules required to work at pH=10 to eliminate electrostatic repulsions which obstruct the formation of a robust polymer shell. Doing so, versatile pH-sensitive nanocapsules with precisely controlled dimensions ($d \sim 100\text{-}300\text{ nm}$) were finally obtained by selective nanoprecipitation in the SFME or Ouzo domains.[196]

Polysaccharides. Recently, we expanded the application of nanoprecipitation to hydrophilic naturally-occurring polysaccharides and derivatives.[197] In this process, neither a strong amphiphilic character nor a high density of charge is required for the construction of nanocapsules as polymer deposition is actually triggered by the addition of acetone, a non-solvent of this family of polymers (**Figure 14**). We first validated the formation of monocomponent nanocapsules with diverse neutral polymers i.e. dextran, pullulan (**99, Scheme 9**), glycogen (**100, Scheme 9**), and ionic polymers, dextran sulfate and hyaluronic acid by solvent shifting using the miglyol 812/acetone/water ternary system. We further showed that mixed multicomponent oil-filled nanocapsules, e.g. dextran-pullulan, dextran-hyaluronic acid, dextran-dextran sulfate, glycogen-hyaluronic acid, are straightforwardly prepared by performing solvent shifting in Ouzo conditions where several populations of polysaccharides can be simultaneously precipitated.

We also programmed the construction of nanocapsules of more complex

organization by exploiting the dissimilar cloudy point boundaries of polysaccharides as a way to sequentially and selectively precipitate them upon progressive enrichment of the system in acetone. Doing so, we were able to cover the nanocapsules in a controlled order, generating multi-layered **sugar-based nanocapsules**, such as bilayered dextran/pullulan or trilayered glycogen-hyaluronic/dextran/pullulan nanocapsules. Note that purification is not required after each polymer deposition, which differs from the L-b-L technique. The glyconanocapsules can be also prepared in presence of an anti-cancer drug, camptothecin, encapsulated in the oil core with a high efficiency, and the barrier properties of capsules' shell in drug release can be precisely modulated by increasing the number of layers (limited to 3 so far).

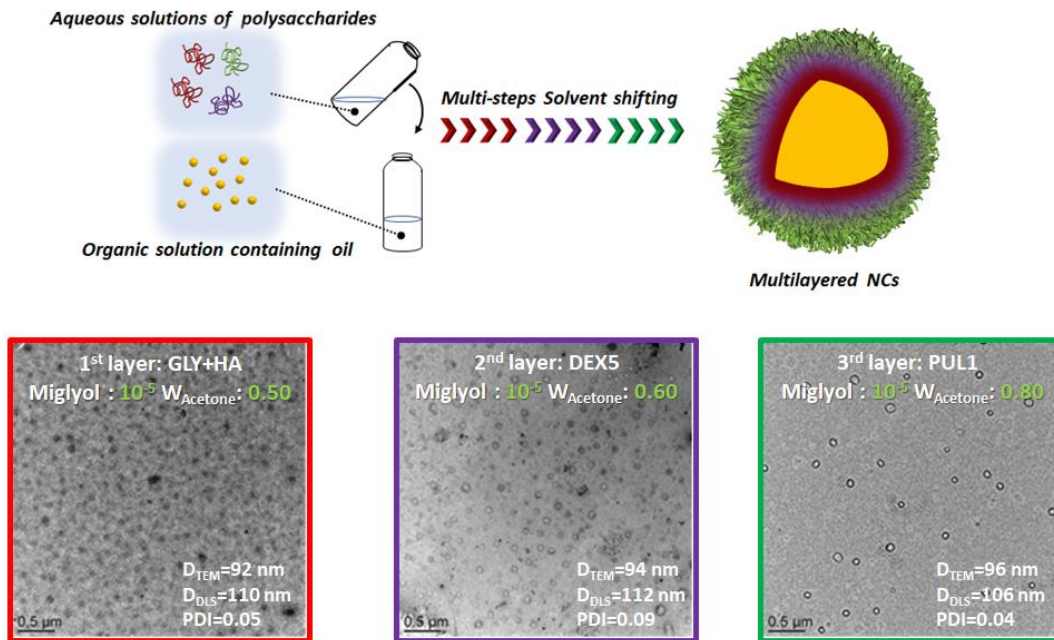
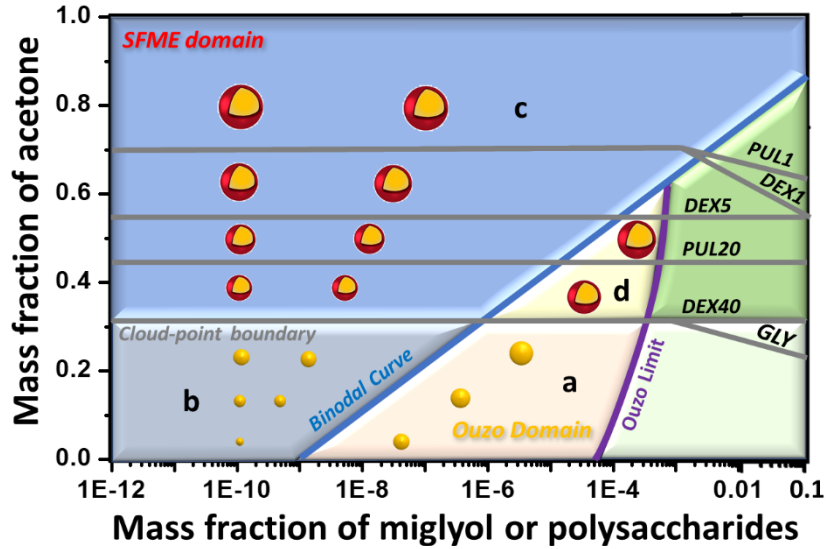


Figure 14. (Up) Overlapped phase diagrams of polysaccharides and miglyol in acetone/water mixtures.[197] Copyright 2019. Adapted with permission from American Chemical Society. Zones **a** and **d** constitute the Ouzo domain, zones **b** and **c** the SFME domain. The cloud-point boundaries in this diagram were established with different polysaccharides; (Down) Illustration of multilayered glyconanocapsules synthesis through sequential nanoprecipitation; TEM images of trilayered glycogen-HA/Dextran(5K)/Pullulan(1K) nanocapsules.

Table 6: Nanoprecipitation technique to prepare oil-filled **sugar-based nanocapsules** (cross-linker: IPDI).

Glycopolymers or Polysaccharides	Oil	Solution	Domain	Diameter (nm)	Ref
poly(N-[7-(α -D-mannopyranosyloxy)heptyl]methacrylamide)	Hexadecane	Acetone/water	Ouzo	150-250	[193]
Poly(HMM-stat-GMA)	Miglyol or Hexadecane	Acetone/water	Ouzo	110 (Miglyol) 118 (HD)	[194]
poly(N-[2-(α -D-mannopyranosyloxy)ethyl] methacrylamide)	Miglyol or Hexadecane	Acetone/water	SFME	40-200	[195]
P(HPMA-co-EMM-co-DEMA)	Hexadecane	Acetone/water	SFME, Ouzo	90, 218, 320	[196]
Dextran	Miglyol	Acetone/water	SFME, Ouzo	74-220	[197]
Pullulan	Miglyol	Acetone/water	Ouzo	386	[197]
Glycogen	Miglyol	Acetone/water	Ouzo	150-229	[197]
Dextran Sulfate	Miglyol	Acetone/water	Ouzo	152-183	[197]
Hyaluronic acid	Miglyol	Acetone/water	Ouzo	187-213	[197]
Dextran, Pullulan	Miglyol	Acetone/water	Ouzo	270	[197]
Dextran, Hyaluronic acid	Miglyol	Acetone/water	Ouzo	100	[197]
Dextran, Dextran Sulfate	Miglyol	Acetone/water	Ouzo	90	[197]
Glycogen, Hyaluronic acid	Miglyol	Acetone/water	Ouzo	98	[197]
Glycogen, Hyaluronic acid, Dextran, Pullulan	Miglyol	Acetone/water	Ouzo	96	[197]

3.3 Template-based strategy

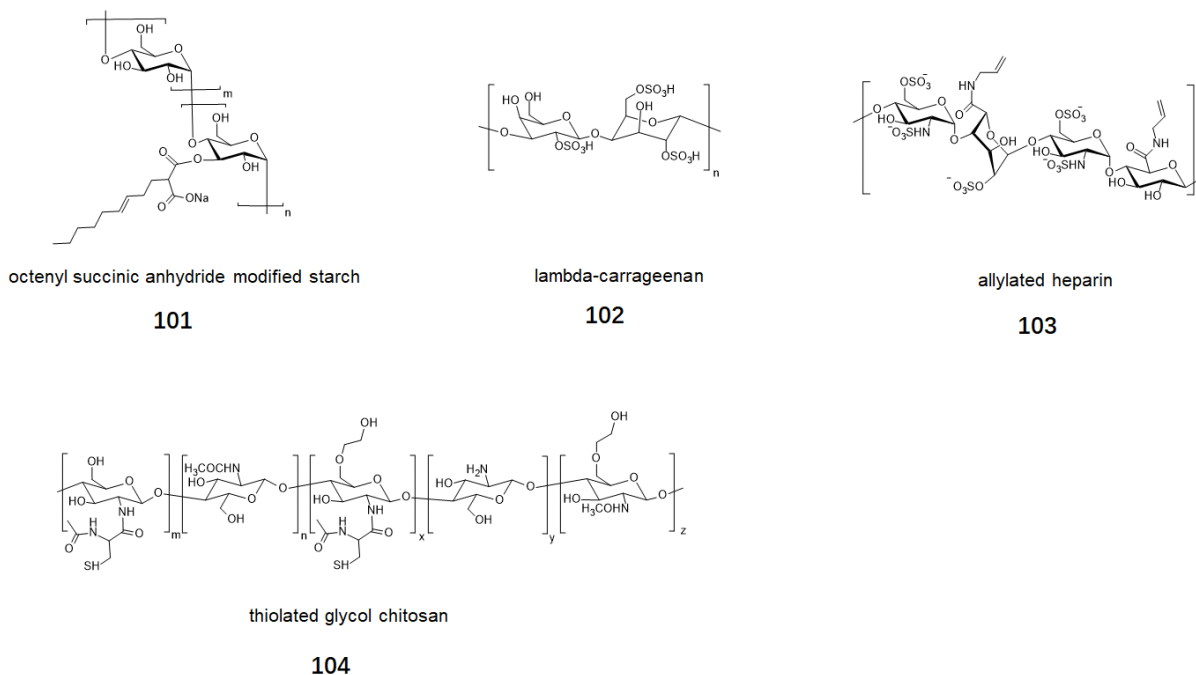
According to the characteristics of the template, the sugar-based nanocapsules can be synthesized by L-b-L deposition of carbohydrate polymers either on preformed oil nanodroplets or on hydrophobic drug nanoparticles.

3.3.1 Starting from oil nanodroplets

Besides solid nanoparticles used in water-filled NCs preparation (see Part 2.2), some groups also introduced the layer-by-layer deposition on hydrophobic colloids obtained by pre-emulsification to generate oil-filled multilayered nanocapsules. For example, Preetz *et al.* reported miglyol-filled trilayered octenyl succinic anhydride modified starch (OSA starch)/chitosan/carrageenan nanocapsules using this strategy.[198, 199] Typically, miglyol 812 was first added into an aqueous solution containing OSA starch (**101**, **scheme 10**). Miglyol-loaded nanocapsules with an OSA starch layer were generated after emulsification through high-pressure homogenization. Chitosan and lambda-carrageenan (**102**, **scheme 10**) were then sequentially deposited via coulombic interactions at the surface of the capsules, generating three-layered nanocapsules of about 130 nm in diameter (miglyol/OSA starch/chitosan/carrageenan = 47.35/47.5/1/ 2.25, mass ratio, $D = 0.23$ from DLS). Thanks to the biocompatibility of all materials, these nanocapsules were generated for future applications in food or pharmaceutical industry.

Kansal and coworkers also applied this strategy to deliver soybean oil-filled protamine/sulfate sodium alginate multilayered nanocapsules ($d \sim 340$ nm).[200, 201] Nano-emulsions were prepared by stirring-induced emulsification using soybean oil in

o/w system. Doxorubicin (DOX) was encapsulated with an efficiency above 80%.



Scheme 10. Structures of carbohydrate polymers used to generate oil-filled nanocapsules by template strategy.

Recently, Vecchione and coworkers reported oil-loaded multilayered chitosan/heparin nanocapsules by a similar strategy.[202] Specifically, allylated heparin (**103**, **scheme 10**) and thiolated glycol chitosan (**104**, **scheme 10**) were synthesized through EDC-catalyzed amidation. The *o/w* soy-bean oil nanoemulsion with diameter of 132 nm was then prepared by ultrasonication. The functionalized chitosan and heparin were sequentially introduced to adsorb on the oil template, resulting in biocompatible oil-loaded trilayered nanocapsules with diameter of 170 nm. Thanks to the presence of thiol and allyl moieties between polyelectrolyte layers, the polymer shell was stabilized by photo-initiated thiol-ene click chemistry. The cross-linked nanocapsules exhibited great stability in PBS, pH 2 and pH 7 buffers.

Bazylińska and coworkers also applied polymer coating and sequential L-b-L

strategy to the preparation of nanocapsules for bioimaging and DNA entrapment.[203] Isopropyl myristate or oleic acid was introduced as an oil phase to be emulsified with water; the resulting nanoemulsions with sizes ranging from 85 to 98 nm were stabilized by different surfactants, namely N,N,N',N'-tetramethyl-N,N'-di(dodecyl)-ethylenediammonium bromide (d(DDA)PBr) or N,N,N',N'-tetramethyl-N,N'-di(dodecyl)-butylenediammonium (d(DDA)BBr). Deoxyribonucleic acid sodium salt (DNA) and chitosan were alternatively deposited on preset nanoemulsions, resulting in 8-layered nanocapsules with diameters ranging from 101 to 119 nm. Beloqui *et al.* recently prepared dextran-protamine-coated nanocapsules.[204] Lipid nanocapsules (d ~ 150 nm) were built using Precirol ATO[®] 5 and miglyol 812 by high-pressure homogenization technique and further addition into a premixed dextran-protamine solution. Resulting nanocapsules (d = 240 nm) were finally obtained after stirring for 30 min.

3.3.2 Starting from hydrophobic drug nanoparticles

Pishko and coworkers directly deposited chitosan and dextran sulfate on paclitaxel particles (antineoplastic drugs), resulting in drug-loaded sugar-based nanocapsules displaying increased encapsulation efficiency without recourse to core removal process.[205]

Table 7: Template strategy to prepare oil-filled **sugar-based nanocapsules** (all samples obtained by L-b-L, i.e. multilayered nanocapsules obtained by electrostatic interactions).

Template	Size (nm)	Emulsification Method	Glycopolymers or Polysaccharides	Capsule diameter (nm)	Ref.
Miglyol droplets		High pressure homogenization	OSA starch, chitosan, lambda-carrageenan	134	[198, 199]
Soybean oil droplets		Agitation	Alginate	342	[200, 201]
Soybean oil droplets	132	Sonification	allylated heparin, thiolated glycol chitosan	170	[202]
Isopropyl myristate or oleic acid	85-98		Chitosan	101-119	[203]
Miglyol loaded Lipid Nanocapsules	150	High pressure homogenization	Dextran	240	[204]
Paclitaxel particles	110		Chitosan, Dextran sulfate	128	[205]

4 Applications of sugar-based nanocapsules

Sugar-based nanocapsules have been extensively studied as nanocarriers for biomedical applications owing to their specific targeting properties, stealth capabilities, high biostability and enhanced cell uptake. Here we summarize the different outputs, independent on the nature of carbohydrate polymers or the technique of capsule preparation.

4.1 Specific targeting nanocarriers

Carbohydrate-protein interactions play a major role in cells' communication. In these physiological processes, glycoconjugates on the cell surface interact with carbohydrate binding proteins (i.e. lectins, antigens, or enzymes) to encode and deliver the biological information. This specific interaction has been known for decades, and many reviews have systematically discussed its mechanism and applications.[206, 207] Therefore, carbohydrate-functionalized capsules are capable of effectively interacting and communicating with cells, both of which significantly enhance their bioavailability.

Numerous research groups have investigated the interactions of sugar-decorated nanocarriers with specific lectins in order to evaluate their targeting capabilities. For instance, the groups of Deming and Lecommandoux both reported that glyco-vesicles made from self-assembly of galactose-pendent copolymers were able to specifically recognize lectin *Ricinus Communis Agglutinin* (RCA₁₂₀) in solution, and caused instantaneous precipitation of lectin at high concentration.[28, 36] Armes and coworkers presented DLS and turbidimetry results giving evidence that PISA-made

glycovesicles containing galactosyl residues could strongly bind to RCA₁₂₀.^[90] They also proved that the lectin assay sensitivity was highly dependent of the morphologies (worms and vesicles>spheres). The presence of galactose moieties on the surface favored the uptake of HDF cells (expressing galectins). Also, the mannose-functionalized capsules were shown to effectively bind lectin Con A, leading to dramatic increase of the dimension of objects (almost 2 times) in aqueous solution.^[44]

Chen's group applied DLS measurements to monitor the interactions between preformed galactose-functionalized capsules and three different lectins, *Arachis hypogaea* (PNA), *Erythrina cristagalli* (ECA), and Con A.^[57] Owing to polyvalent interactions between peripheral galactose residuals and multiple binding sites of proteins, the progressive introduction of galactose-specific lectins (PNA or ECA) into aqueous solution of vesicles gave rise to the formation of aggregates, leading to a significant increase of dimension from 62 nm (capsules) to above 1 μm (**Figure 15**), whereas no size shift was found when adding Con A.

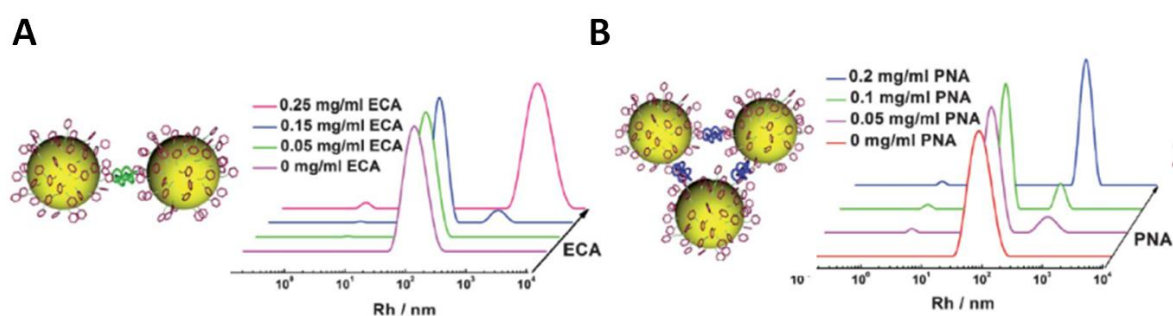


Figure 15. DLS profiles of vesicle aggregation induced by lectins, when (A) ECA and (B) PNA were titrated into the solution of V-PGal, respectively.^[57] Copyright 2012. Adapted with

permission from the Royal Society of Chemistry.

To assess the bioavailability of glucose residues on polymersomes, the group of Alexander investigated interactions of glycopolymer self-assemblies with FITC-labelled Con A. The polymersomes agglutinated the lectin in a more efficient manner than linear glycopolymer chains.[56] The capsules could also selectively interact with FimH-expressing *E. coli* strain (MG1655pGFP), resulting in large bacterial aggregates. In the meantime, no aggregation was observed when incubating with a *E. coli* strain not expressing FimH adhesin (Top 10). Further addition of free glucose into the system enabled to dose-dependently break up the formed bacteria-capsules aggregates.

Using epoxide-amine reactions, Yan *et al.* anchored amine-functionalized fluorescent probes (alexa Fluor 555 cadaverine) and carbon-coated cobalt magnetic nanoparticles onto epoxide-functionalized glyconanocapsules prepared by nanoprecipitation.[194] The resulting nanocapsules induced bacterial agglutination through specific recognition between n-heptyl α -D-mannose moieties that decorate the polymer membrane and FimH adhesins located at the tip of bacteria fimbriae. Thanks to the presence of magnetically-responsive NPs on the capsules, the bacteria were efficiently 'fished' out of solution under magnetic field (**Figure 16**).

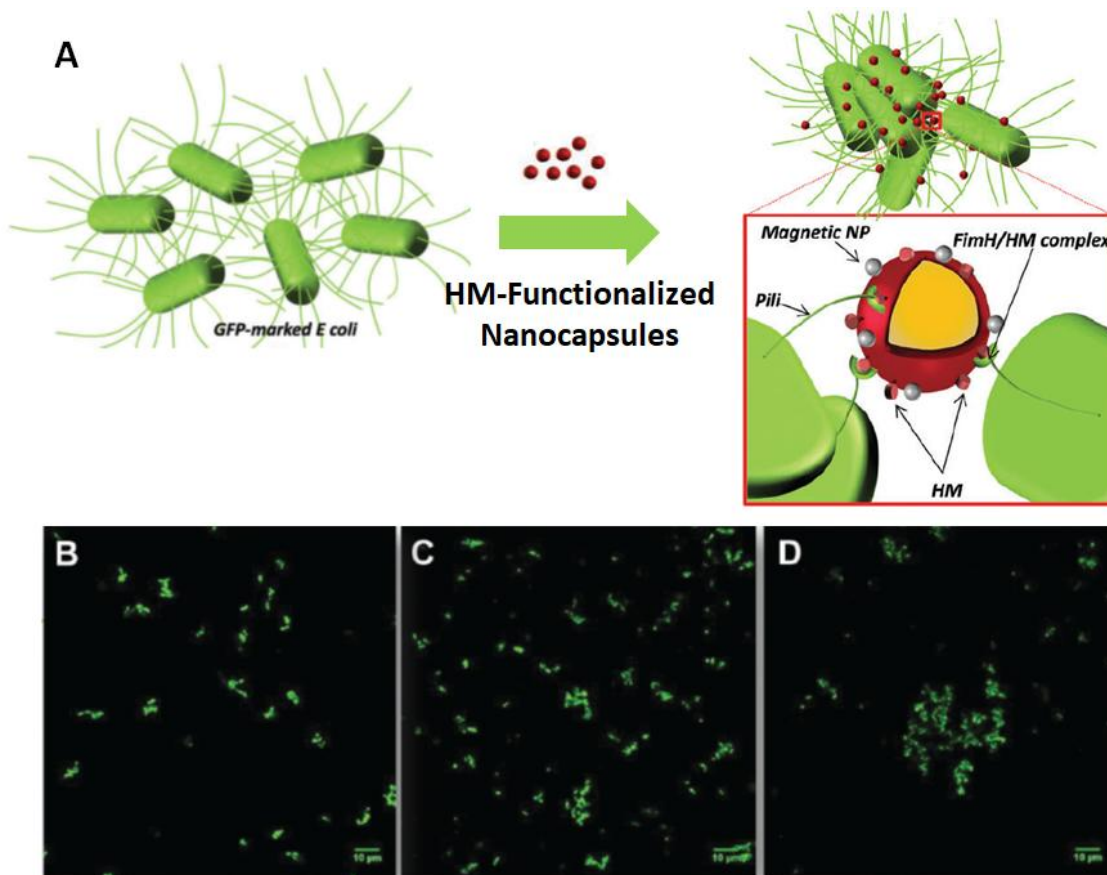


Figure 16. (A) Schematic illustration of bacterial agglutination in the presence of sugar-based nanocapsules. (B to D) Confocal fluorescence microscopy pictures of GFP-marked AIEC LF82 incubated with HM-GC for 30 min (B), 2 h (C) and 3 h (D), scale bar is 10 μm . [194] Copyright 2015. Adapted with permission from the Royal Society of Chemistry.

Mannosylated polymersomes were shown to strongly interact with CD206, a receptor present on the surface of bone marrow-derived dendritic cells inducing a clathrin-dependent endocytosis mechanism.[29] In contrast to non-mannosylated systems, this specific interaction led to an almost 6 times enhancement of particles uptake. Targeting MRC2, a mannose-specific receptor overexpressed on the surface of breast cancer cells MDA-MB-231, mannosylated polymersomes showed enhanced uptake (2-fold increase) in comparison with galactose functionalized ones, thanks to

their receptor-mediated endocytosis capability.[43]

Thanks to strong binding of hyaluronan to CD44, Dox-loaded PBLG₂₃-*b*-hyaluronan based polymersomes were efficiently internalized into C6 glioma tumor cells resulting in cellular accumulation of drugs.[62] Interestingly, encapsulation of the drug delayed the apoptosis without influencing its efficiency. β -glucan-based nanocapsules were shown to strongly interact with RAW264.7 cells (internalization rate of up to 93%) due to the presence of β -glucan receptors such as Dectin-1 on the cells.[87]

4.2 Stealth nanocarriers

In vivo, nanoparticles are rapidly covered by proteins (opsonization) that promote recognition and phagocytosis and *de facto*, alter the efficiency of drug delivery owing to rapid clearance of the nanocarriers in the bloodstream.[208] In general, PEGylation is a common solution for plasma half-life prolongation of nanoparticles' circulation and thus enhancement of delivery efficiency.[209] Recent works reported immunological reactions towards PEG raising concerns on the use of this universal stealth ligand.[210, 211]

Biodegradable polymers such as hydroxyethyl starch (HES) or dextran have been shown to display low protein affinity, to decrease unspecific uptake of cells and to prolong the plasma half-life. Consequently, they can be regarded as relevant and safe alternatives of PEG. Wurm and coworkers investigated the interactions of plasma proteins with HES-based nanocapsules and PEGylated HES-based nanocapsules.[212] ITC results showed that the HES NCs have much lower affinity to

plasma proteins compared to hydrophobic polystyrene nanoparticles (**Figure 17**), as also confirmed by DLS analysis showing no formation of large aggregates. Meanwhile, no difference was traced between HES and PEGylated HES nanocapsules. Proteomic mass spectrometry data also gave a rather similar result for both NCs: while identifying absorbed protein corona, the PEGylated NCs displayed a slightly overall lower protein absorption.

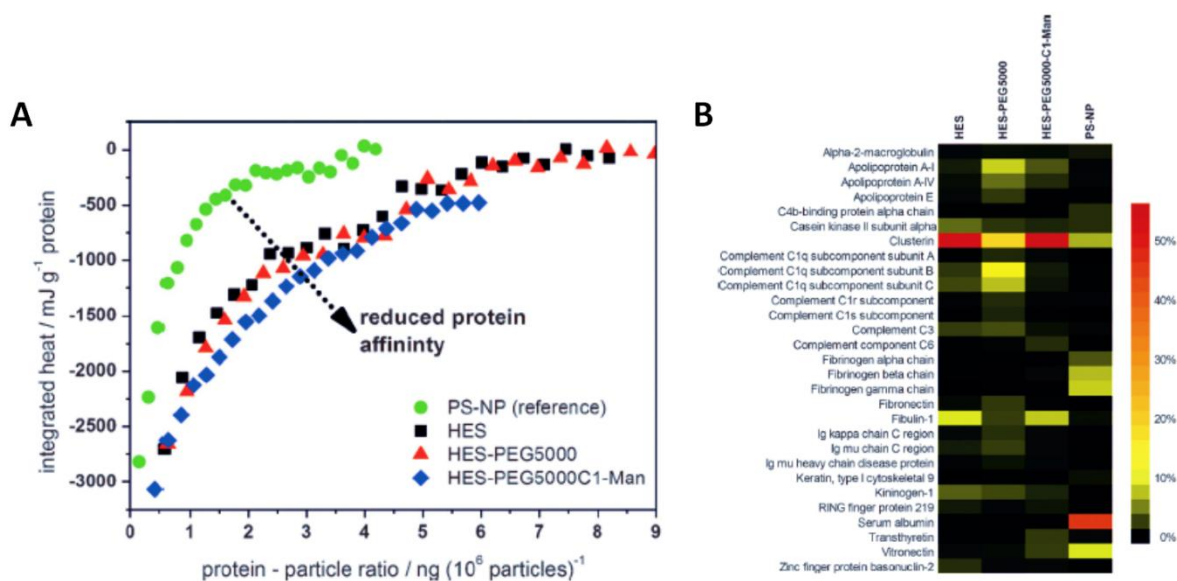


Figure 17. (A) ITC binding isotherms for the adsorption of plasma proteins to different nanocarrier systems, polystyrene nanoparticles as a reference; (B) Heatmap of the most abundant proteins in the corona of nanoparticles determined by mass spectrometry coupled with quantitative liquid chromatography.[212] Copyright 2015. Adapted with permission from John Wiley & Sons Inc.

4.3 Bioimaging agents

Fluorescent probes are practical tools for monitoring biological functions in intracellular compartments, cells and tissues. Flourishing developments in this field have led to new perspectives for the diagnosis and treatment of diseases.

Carbohydrate-based nanocarriers do not generate any fluorescent response, but fluorescent molecules, or organic or inorganic fluorescent particles, can be loaded into the core reservoir of capsules or covalently anchored on the polymer membrane. Owing to the presence of bioavailable sugar residues at their surface, glyconanocarriers can promote selective binding and cellular internalization, affording great improvement of the targeting capabilities of original probes.

Armes' group encapsulated rhodamine B octadecyl ester in galactose-based vesicles by PISA.[90] The dye itself, that cannot enter HDF cells, was efficiently internalized through endocytosis, thanks to its entrapment in glyconanocapsules, and further released to stain the cell membrane, the endomembrane system and the nuclear region as well. Another practical dye, ethidium bromide, was loaded in glucose-functionalized vesicles by polymer self-assembly. Alexander and co-workers incubated these nanocarriers with *E. coli* strain (MG1655pGFP).[56] Owing to the effective communication between the sugar residues located on the membrane and bacteria, the authors hypothesized that this interaction triggers the disruption of membrane and further facilitates the delivery of ethidium bromide into the bacteria. Orange fluorescence was indeed observed in the bacterial cytoplasm after 30 min exposure.

IR-780 was conveniently encapsulated into multilayered chitosan/DNA-based capsules with high efficiency (>90%).[203] The polysaccharide-coated nanocarriers led to an increased cellular uptake of IR-780 on different cell lines. The fluorescent response observed *in vitro* confirmed that the nanocapsules functioned as efficient

fluorescent bioimaging markers. The upconversion of dextran-based nanocapsules carrying several different sensitizer/annihilator (s/a) couples showed high photostability when exposed to atmosphere (**Figure 18**).[182] Owing to the deep penetration of upconversion luminescence, the nanocapsules were applied to *in vivo* imaging on mice showing an excellent signal-to-noise (SNR) ratio, without the need for skin removal.

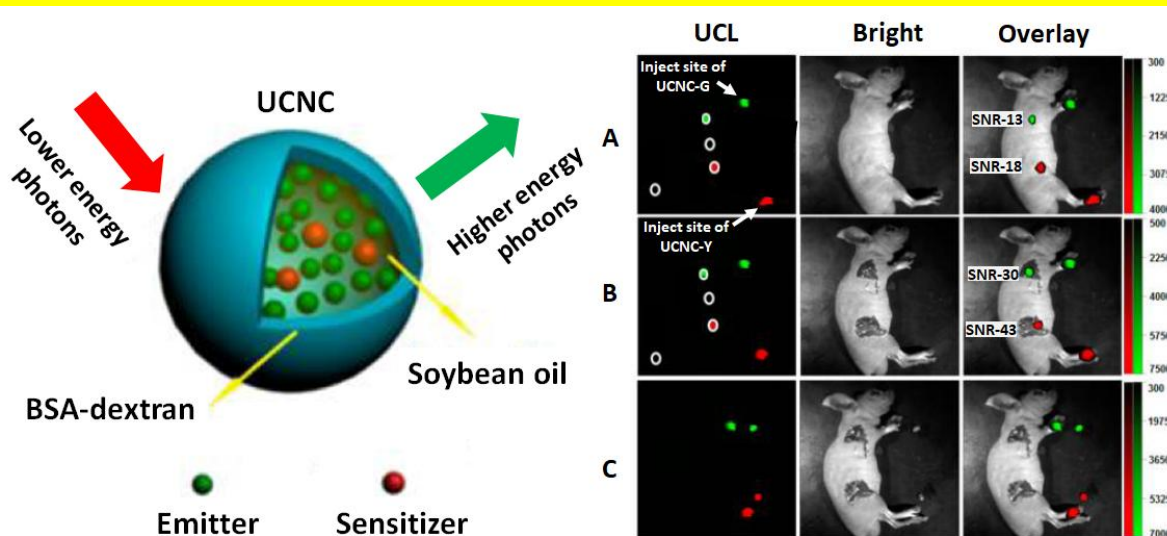


Figure 18. (Left) TTA-UCL Process of Upconversion Nanocapsules, (Right) (A) *In vivo*, (B) *in situ* and (C) *ex vivo* upconversion luminescence lymphatic imaging of the living mouse at post-injection of UCNC-G and UCNC-Y in forepaw and hindpaw, respectively ($\lambda_{ex} = 635 \text{ nm}$, $\lambda_{UCL} = 530 \text{ nm}$).[182] Copyright 2013. Adapted with permission from American Chemical Society.

Inorganic nanoparticles, i.e. quantum dots, were loaded in glyco-vesicles by pre-conjugation with block polymers before self-assembly.[37] The presence of sugar moieties significantly decreased the cytotoxicity of quantum dots and showed specific recognition to Con A. QD-loaded capsules were internalized by HepG2 cells through an endocytosis pathway and emitted green fluorescence in the cells.

4.4 Drug or Gene delivery

With respect to their functionality, biological activity and biocompatibility, carbohydrate-based capsules are promising nanocarriers for applications in drug and gene delivery. For instance, synthetic anti-tumor molecules (L-phenylalanine derivatives) were loaded in chitosan-gelatin based nanocapsules with an efficiency up to 79%.[183] In comparison to the free drug treatment, the encapsulation improved the anti-tumor activity (~10%) against implanted Guerin's carcinoma *in vivo*. Insulin could be locked in alginate-chitosan-coated oil-filled nanocapsules with entrapment ratio of about 47%.[145] *In vivo*, these carriers showed significant hypoglycemic effect to normal and diabetic rats after oral administration (25 and 50 IU/kg of insulin dose), resulting in 60 and 50% of glucose decrease, respectively, which may relieve glycopenia syndrome. Hadizadeh and Ramezani also trapped insulin in dextran-PLGA based nanocapsules.[73] Thanks to the mucoadhesive behavior of dextran, the synthetic nanocapsules effectively enhanced the intestinal absorption of insulin. In contrast to the free drug, the loaded insulin showed obvious hypoglycemic effect for *in vivo* diabetic rats after oral administration, up to 12 hours.

Ovalbumin (OVA) was loaded in peracetylated galactose-functionalized polymersomes.[55] After internalization by dendritic cells, lysosomal lipases were able to deprotect the sugar moieties and trigger a morphology transition to glyco-micelle activating the release of OVA. Compared to free OVA delivery, OVA-loaded polymersomes led a significant increase in IL-2 secretion due to a synergistic effect of antigen and deprotected sugar. Zhou and coworkers entrapped curcumin in

chitosan-coated liposomes with loading efficiency of 94% and drug loading content of 8%.[144] Treatment of MCF-7 cells with capsules showed dose- and time-dependent cytotoxicity, with growth suppression at 200 μ M in 72 h.

Amphotericin B (AmB), a polyene macrolide antibiotic, was encapsulated into glycol chitosan/lipid nanocapsules by a self-assembly process with an efficiency of 26%.[85] The vesicles exhibited high plasma stability and released 66% of AmB within 24 h under sink conditions. The loaded AmB showed an enhanced efficacy against leishmania *in vivo*. Chourasia and coworkers also entrapped AmB in chitosan nanocapsules, showing a good steric stability *in vitro*.[172] As revealed by *in vitro* and *in vivo* studies, the encapsulated drug exhibited an enhanced antileishmanial activity compared to the free drug. Antibiotics, ciprofloxacin and ceftriaxone, were encapsulated in four-layered chitosan-dextran sulfate nanocapsules with 78% of efficiency.[113] The release of drugs was influenced by the pH of the solution (70% after 48h at pH 7.4; 51% after 48h at pH 4.8). *In vitro* and *in vivo* results proved that these nanocarriers were of effective clearance ability for *Salmonella* infection.

An anti-inflammatory drug, indomethacin, was loaded in chitosan-coated PCL nanocapsules with an encapsulation efficiency up to 92%.[167] *In vitro* release study demonstrated that 85% of the drug diffused out of the nanocapsules within 2 h. Besides, *in vivo* investigation confirmed that the presence of positively-charged chitosan on the surface significantly enhanced uptake and corneal penetration of the drug. A pesticide, methomyl, was encapsulated in chitosan-based nanocapsules during the assembly process (efficiency of up to 90%). These were investigated to

fight against armyworm larvae on the foliage of red kidney bean plants.¹⁴³ The cross-linked nanocapsules efficiently restrained the degradation of drug, showing longer effect (5-7 days) compared to free drug and uncross-linked capsules (3 and 4 days, respectively).

Anti-cancer drug doxorubicin hydrochloride was loaded in seven-layered pectin/chitosan nanocapsules by diffusion at pH 6, with encapsulation efficiency of up to 76.5% and high loading content (typically 20.3%).^[117] The presence of galactose moieties on the pectin outer layer of the capsules enhanced uptake by hepatoma HepG2 cells. Since the polymeric shell is made of polyelectrolytes, the release of loaded drug is highly pH-dependent: cumulative release amount reached 71.2% at pH 6 and 85.7% at pH 5 over 60 h but only 8.2% at physiological microenvironment (pH 7.4). These Dox-loaded capsules also showed high selection for killing cancer HepG2 cells. Dox was also loaded in alginate-containing multilayered nanocapsules with an efficiency above 80%.^[200] The external alginate shell electrostatically interacts with the cell membrane favoring phagocytosis by macrophages. In contrast to free drug, alginate-based capsules significantly enhanced the apoptosis efficiency of Dox against *Leishmania* parasites *in vitro* and *in vivo*.

Hydrophilic Rhodamine B (Rh-B) and hydrophobic camptothecin (CPT) were efficiently loaded in dextran vesicles prepared by solvent shifting. Addition of esterase enzyme within the solution (under physiological conditions) resulted in the cleavage of the ester linkages between the dextran backbone and the hydrophobic tails, causing a fast release of CPT or Rh-B. Finally, CPT-loaded vesicles were proven to be effective

in killing fibroblasts.[68] Loading of drug cocktails has been explored for targeting synergistic therapy.[67] For instance, dextran-based nanocarriers were used to load hydrophilic anti-drug DOX and hydrophobic CPT in the core and the layer, respectively, in order to improve breast cancer cells' killing by synergistic effect. Recently, the same group also created a triple antagonist drugs-loaded dextran capsules (cisplatin, CPT and Dox). The system showed much higher internalization and cell uptake compared to the free drugs, and remarkable breast cell killing results (nanogram scale), thus confirming their potential in synergistic cancer therapy.[213]

A natural lipophilic anti-cancer drug, curcumin, was loaded in series of chitosan-based nanocapsules of different dimensions.^[103, 104] The large- (440 nm) and medium-sized (270 nm) nanocapsules displayed 145 and 13 times more loading capacity than the smallest ones (0.16 fg per capsule of 220 nm). In the meantime, the latter showed six times higher efficiency in comparison to equivalent amount of free drug, qualifying it as the potentially most efficient nanocarriers for chemotherapeutic purposes. Docetaxel was loaded in the miglyol core of HA-based nanocapsules.[181] According to specific interactions between HA and CD44 receptor, the application of HA nanocapsules led to improvement in internalization of drug against CD44 over-expressed non-small cell lung cancer NCIH460 cell line, significantly increasing the cytotoxicity (more than 3 times). Noteworthy, the HA nanocapsules were stable at 37°C for 90 days. Sorafenib tosylate (Sraf) was entrapped in multilayered poly-L-arginine/dextran sulfate nanocapsules after dissolution of the CaCO₃ core, with an efficiency reaching 61%. When the carriers were exposed to KB cells, the latter

disrupted cell membrane, disturbing nuclear envelop and causing DNA fragmentation. Compared to the free drug, the loaded Sraf was more potent in inducing antiproliferation and apoptosis of KB cells, and even significantly blocking KB cell migration.[116]

Testosterone was loaded in alginate-based nanocapsules with large drug loading of up to 30%.[164] Either the nanocapsules or the free drug was injected into Female Sprague Dawley rats, for pharmacokinetic investigation. The plasma concentration versus time of loaded testosterone was much higher than for the free drug, showing obvious enhancement in bioavailability. Finasteride, a drug for benign prostatic hyperplasia and male pattern baldness, was loaded in chitosan-coated PS-*b*-PAA polymersomes,[126] which enhanced the skin permeation of the drug in *ex vivo* studies. Due to the presence of positively charged chitosan, the coated polymersomes exhibited strong interactions with skin components compared to uncoated ones. Dextran-protamine coated nanocapsules were capable of loading an antiretroviral drug, saquinavir mesylate (SQV) for enhancing its permeability in cells.[204] These neutral nanocapsules significantly enhanced the permeability of SQV *in vitro* (up to 9 times) across Caco-2 cells membranes, in comparison to uncoated lipid nanocapsules.

siRNA-PEI complexes were loaded in hyaluronan-*b*-PBLG copolymer-based capsules.[122] When the capsules were exposed to HeLa cells, significant amount of siRNA was taken up by cells. The cell uptake of siRNA was highly related to the concentration of copolymers. In the meantime, siRNA-loaded capsules also showed

very high gene-silencing activity, by effectively inhibiting the expression of firefly luciferase in HeLa cells (**Figure 19**). The authors hypothesized that the complexation of PEI and hyaluronan facilitates the dissociation of the core and the release of siRNA into cytoplasm. Steer and coworkers also entrapped *sleeping beauty* transposon in HA-coated nanocapsules. Thanks to the presence of hyaluronan receptors on liver sinusoidal endothelial cells (LSECs), the resulting carriers were proven to stably transfer genes into mice LSECs, showing long-term expression of FVII without formation of antibody, which could lead to therapeutic applications.[105]

Nishimura and co-workers reported the encapsulation of β -galactosidase into maltooligosaccharide-based polymersomes[77] They further added a prodrug, 5-N-(β -D-galactopyranosylbenzyloxy-carbonyl)-doxorubicin (DoxGal), which diffused within the core of the polymersomes to react with the enzyme resulting in the progressive release of Dox in the solution and in a significant antitumor effect *in vitro* and *in vivo*.

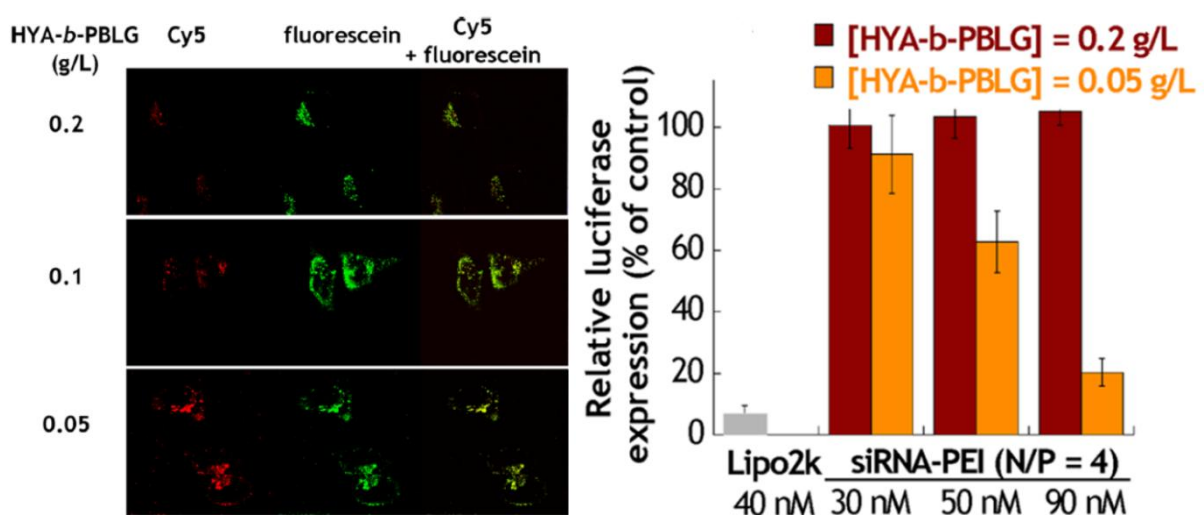


Figure 19. (Left) Uptake of siRNA-PEI complex loaded HYA-*b*-PBLG based nanocapsules in

HeLa cells at different concentrations of copolymers after incubation for 24h. Polymer shell and siRNA are dyed by fluorescein and Cy5, respectively. **(Right)** Relative firefly luciferase expression (Renilla luciferase as internal control) after incubation of HeLa cells with gene carriers for 72h.[122] Copyright 2012. Adapted with permission from American Chemical Society.

4 Discussive Conclusion

This review have presented all the technologies of interest for the construction of carbohydrate-based nanocapsules and recent achievements in the field of nanomedicine. Indeed, thanks to the presence of bioavailable and bioactive sugars on their outer layer, glyconanocapsules are rather unique functional nanocarriers capable i) to extend circulation life time *in vivo*, ii) to mimic glycan signatures, establish specific interactions and promote targeting (to cells, organs), and consequently iii) to deliver drugs or genes with limited unspecific cell uptake. All together, these features make sugar-based nanocapsules appealing candidates for drug-delivery applications.

Table 8 gives an overview of the different techniques of encapsulation, including the size of the objects, the encapsulation efficiency or the achievable solid content. The following discussion complements this table with a rapid description of pros and cons for each technique and their comparison. This should give the reader an estimate of what technique could be more suited for his further work on this field.

Self-assembly of amphiphilic block copolymers (or double hydrophilic block copolymers) is the most popular method to form water-filled nanocapsules. This is surely due to the various triggers available to promote the association of the chains.

We can cite three main families of techniques: 1. Those playing with external conditions (solvent composition, temperature change); 2. Those using the structure and properties of the copolymers (CMC, deprotection); 3. Those playing with the process (PISA, post-core removal). Glycopolymers or polysaccharides can be invariably used, most often directly in water or in presence of a cosolvent (e.g. THF). Though confidential, all techniques but solvent displacement likely generate nanocapsules of good quality, as long as the structure of the glycopolymer is well chosen.

Solvent displacement is by far the most popular trigger to generate water-cored capsules (see the numerous number of references that quote this technique in Table 8). Concerning water-filled capsules, except for PISA, the final solid weight content is rather low (maximum few %) and the encapsulation efficiency is generally not ideal. This is due to the non-specificity of the technique to encapsulate a solute. The range of sizes available span from 30 to 1000 nm, depending on the nature of the polymer (polysaccharide produces smaller capsules) and the technique of emulsification (the use of a solvent generally produces smaller capsules).

Template and emulsification strategies are adaptable to various conditions and compositions to produce again both water- or oil-filled nanocapsules. The former is very popular; it consists in a multistep process where polymers of opposite charges are adsorbed on a template (so-called LbL technology). Sizes obtained here depend on the sacrificial core (generally small, less than 100 nm) and drug loading is high according to the protocol of coatings. This technique presents also the advantage to use only water as the reacting medium. Note that final solid contents were not

reported in the considered articles. Compared to solvent displacement, this technique requires two polysaccharides of different charges, which means generally some backbone modifications. Another disadvantage can be seen in the partial shrinking of the capsules when the core is removed, but this effect is not systematically documented in the papers. Finally, the hydrophilic payload is introduced by post-diffusion into the hollow capsules, which can limit the final encapsulation efficiency. In the case of oil-filled capsules, sizes are lower and encapsulation is much better when starting from an oily template.

Emulsification is a typical soft matter technique to prepare complex colloids with aqueous or oil core. Water-filled capsules are prepared mainly by miniemulsion (only one example for double emulsification). Most often, polysaccharides are involved but glycopolymer surely could do the job as well. Apart from the complexity of the emulsification step and colloidal stability issues during polymer crosslinking/precipitation, capsules thus obtained are of meso size (typically <500nm) and with a high payload for oil cores. As drawbacks, final weight content is typically low and surfactants are needed here for droplet stabilization. For oil-filled capsules, additional techniques of polymer coating or coacervation are available in addition to miniemulsion, but those techniques lead to low solid content dispersions and larger sizes.

Finally, yet importantly, nanoprecipitation affords the straightforward design of oil-filled nanocapsules with a broad range of substrates and precise dimension controllability (typically between 40 and 400 nm). It can be applied to a large range of

amphiphilic polymers, but is adapted only to oil-core capsules (at the moment). Final solid contents are low here even after solvent removal; concentration by freeze drying is nevertheless feasible.

Table 8: Template strategy to prepare oil-filled sugar-based nanocapsules (all samples obtained by L-b-L, i.e. multilayered nanocapsules obtained by electrostatic interactions).

Techniques	Trigger	Nature of Solute	Solvent	Size nm	Solid content wt%	Encap. eff. %	Number of references	remarks
Water-filled glyconanocapsules								
Self-assembly	Solvent displacement	Amphiphilic polymer	H ₂ O/H ₂ O miscible solvent	35-1000	0.05-3	20-63	35	
	CMC	Amphiphilic polymer	H ₂ O or THF	300-600 (glycopol.) 170-360 (polysacch.)	0.02-0.2		4	
		Double hydrophilic polymers	H ₂ O	170-580	0.1-2.5		1	
	Protection/Deprotection	Amphiphilic glycopolymer	H ₂ O/THF	48-116	0.12-1		2	
	Temperature	Temperature-responsive block polymer	H ₂ O	100-300	0.01-0.2		2	
	PISA	Amphiphilic polymer	H ₂ O	200, 840	5, 20		3	
	Post-core removal	Amphiphilic polymer	H ₂ O/H ₂ O miscible solvent	50-250	0.06, 0.8		3	

Template strategy	Electrostatic interaction	Charged polymer	H ₂ O	50-720		40-97	34
Emulsification	Double emulsification	Polysaccharide	Water/Oil/Water	488	0.067	47	1
	Miniemulsion	Amphiphilic polymer	Water/Cyclohexane	165-524	0.5-0.8	68-98	7
Oil-filled glyconanocapsules							
Template strategy	Electrostatic interaction	Polysaccharide	H ₂ O	100-342		82-99	17
Emulsification	Emulsion/Coacervation	Polysaccharide	H ₂ O/hydrophobic solvent	34-500	0.1	78	5
	Polymer coating	Polysaccharide	H ₂ O/hydrophobic solvent	93-500	0.1-3	40-98	8
	Double emulsification	Polysaccharide	Toluene/water/Toluene	100-700		65-79	1
	Miniemulsion	Polysaccharide	H ₂ O/hydrophobic solvent	35-388	0.05-20	80	8
Nanoprecipitation	Solvent shifting	Hydrophilic polymer	H ₂ O/Acetone	40-400	0.0002-0.2	57-65	5

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Notes

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