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Hydrophilic polymers: Current trends and visions for the future ☆

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ABSTRACT

Hydrophilic polymers are a major class of polymers in polymer science. They are found in a broad range of applications from superabsorbers to drug-delivery. In recent years, a plethora of impactful developments in hydrophilic polymers have been reported. The present review gives an overview over these developments with a focus on frequently studied polymer types, aqueous multi-phase systems, hydrophilic block copolymer self-assembly and hydrophilic polymer particles. We cover fundamental work and concepts but also present work with high relevance for application. Finally, we give an outlook towards current challenges and future developments of the field. The further development of hydrophilic polymer is of great importance for a broad range of applications and will have a significant impact on biomedicine and every-day life.

1. Introduction

Hydrophilic polymers belong to the most important classes of polymers that are ubiquitous in research and real-world applications [1,2], e.g. they find uses as grafts on surfaces [3,4], in solution [5,6], as hydrophilic part in amphiphilic block copolymers for assemblies in dispersion [7–9], in food [10], cosmetics [11], pharmaceutics [12] or in hydrogels [13,14]. Over the recent decade, hydrophilic polymers were placed at the forefront of polymer research, which is mainly due to potential high value applications in the biomedical field but also food and cosmetics [15–17].

In particular, applications in drug delivery are in the focus of research, where hydrophilic polymers act as the mediator/interface between biological environment and drug containing compartment [18]. Moreover, hydrophilic scaffolds for tissue engineering are investigated frequently [19,20]. Introduction of hydrophilic polymers into the reaction environment for the enhancement of catalytic processes is also a considerable research direction, e.g. in the development of synthetic cells capable of mimicking biological cell functions [21]. As such, applications in biotechnology are in reach. Other applications of hydrophilic polymers cover sensing, ice crystallization inhibition and cell freeze protection [22] as well as electrolytes for fuel cells and batteries [23,24].

Of course, research and development of hydrophilic polymers faces various challenges. A major point to discuss is the origin of hydrophilic polymer precursors, e.g. monomers, that are often not renewable. One of the few exceptions are polysaccharides that can be obtained from natural resources on a large scale [25] but also several monomers have found synthesis processes from renewable resources, e.g. acrylic acid (AA) or ethylene glycol [26,27]. Another significant challenge is degradability, which is an issue that has to be tackled going forward.

In here, we will highlight a variety of research areas in the field of hydrophilic polymers mostly from the last decade (Scheme 1). At first, the most important hydrophilic homopolymers will be discussed as well as significant applications. Next, the use of hydrophilic polymers in aqueous multi-phase systems will be discussed including an introduction into completely hydrophilic water-in-water (w/w) emulsions. In addition, recent advances in the area of completely hydrophilic block copolymers, mainly double hydrophilic block copolymers (DHBCs) and DHBCs containing a stimuli-responsive block, will be presented. Then, hydrophilic polymer particles will be discussed with a focus on microgels and coacervates. Finally, we will discuss challenges and future trends for the research in the area of hydrophilic polymers.

2. Hydrophilic homopolymers

Hydrophilic polymers have been playing an important role in polymer chemistry for a long time. In this section, we will highlight hydrophilic homopolymers giving an insight to recent developments and properties. We will classify the different polymer

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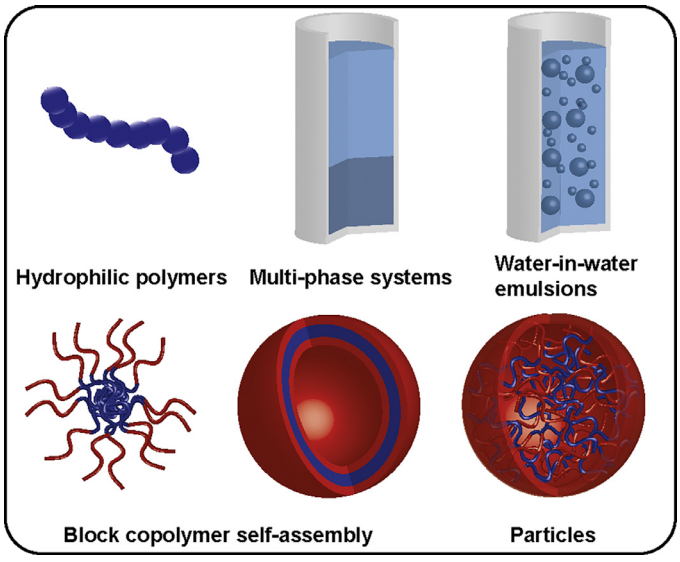
List of acronyms

A3PS	Aqueous three-phase system
α–CD	α–Cyclodextrin
Alg	Sodium alginate
ATP	Adenosine triphosphate
ATPS	Aqueous two-phase system
Azo-PIL	Azobenzene-modified poly(ionic liquid)
BMOD	Bis(2-methacryloyl)oxyethyl disulfide
Bn-PIL	Benzyl-modified poly(ionic liquid)
BSA	Bovine serine albumin
T_{CP}	Cloud point
CLSM	Confocal laser scanning microscopy
CNC	Cellulose nanocrystal
Con A	Concanavalin A
CuAAc	Copper catalyzed azide alkyne cycloaddition
DAAM	Diacetone acrylamide
Dex	Dextran
DHBC	Double hydrophilic block copolymer
DHBG	Double hydrophilic block glycopolymer
DLS	Dynamic light scattering
DOX	Doxorubicin
DP	Degree of polymerization
eGFP	Enhanced green fluorescent protein
EndLys	Endolysin
EV	Extracellular vesicle
FRET	Förster resonance energy transfer
FUS	Fused in sarcoma
g-CN	Graphitic carbon nitride
GD	Grafting degree
GOx	Glucose oxidase
HER2	Human epidermal growth factor receptor 2
HRP	Horseraddish peroxidase
HUVEC	Human umbilical vein endothelial cell
LCI	Antimicrobial peptide liquid-chromatography-peak-I
LCST	Lower critical solution temperature
LLPS	Liquid-liquid phase separation
MBL	Mannose binding lectin
MLO	Membrane-less organelles
MW	Molecular weight
NCA	*N*-Carboxyanhydride
OEGDA	Oligo(ethylene glycol) diacrylate
O/W	oil-in-water
PAA	Poly(acrylic acid)
PAAm	Poly(acrylamide)
PAEA	Poly(aminoethyl acrylamide)
PAGA	Poly(2-acrylamide glycolic acid)
PAM	Poly(4-acryloylmorpholine)
PCB2	Poly(2-((*N*-2-methacryloyloxyethyl-*N*,*N*-dimethyl)ammonio)acetate)
PCBMAA	Poly(carboxybetaine methacrylamide)
PDADMAC	Poly(diallyldimethylammonium chloride)
PDEA	Poly(*N*,*N*-diethylacrylamide)
PDha	Poly(dehydroalanine)
PDIAEMA	Poly(2-(diisopropylamino)ethyl methacrylate)
PDMA	Poly(*N*,*N*-dimethylacrylamide)
PDMAEMA	Poly(2-(dimethylamino)ethyl methacrylate)
PEB	Poly(epoxybutylene)
PEG	Poly(ethylene glycol)
PEI	Poly(ethylene imine)
PEtOx	Poly(2-ethyl-2-oxazoline)
PGEMA	Poly(2-(β-glucosyloxy)-ethyl methacrylate)
PHEMA	Poly(2-hydroxyethyl methacrylate)
PHPMA	Poly(*N*-(2-hydroxypropyl)methacrylamide)
PiPrOx	Poly(2-isopropyl-2-oxazoline)
PLL	Poly(L-lysine)
PLP	Poly(L-proline)
PMAA	Poly(methacrylic acid)
PMeOx	Poly(2-methyl-2-oxazoline)
PMETAC	Poly([2-(methacryloyloxy)ethyl]trimethylammonium chloride)
PMPC	Poly(2-(methacryloyloxy)ethyl phosphoryl-choline)
PNIPAM	Poly(*N*-isopropylacrylamide)
POEGMA	Poly(oligo ethylene glycol methyl ether methacrylate)
PSB4	Poly(4-((*N*-2-methacryloyloxyethyl-*N*,*N*-dimethyl)ammonio)butane-1-sulfonate)
PSBMA	Poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide)
Pull	Pullulan
PVA	Poly(vinyl alcohol)
PVP	Poly(vinylpyrrolidone)
Q-Dex	Quaternized dextran
RAFT	Reversible addition-fragmentation chain transfer
ROP	Ring-opening polymerization
SANS	Small angle neutron scattering
SEM	Scanning electron microscopy
SKM	Solketal methacrylate
ss-oligo	Single stranded oligonucleotide
TEM	Transmission electron microscopy
UCST	Upper critical solution temperature
UO	Urate oxidase
VEGF	Vascular endothelial growth factor
W/O	Water-in-oil
W/W	Water-in-water
ZE	Glutamic acid rich leucine zipper
ZR-ELP	Arginine-rich cationic leucine zipper fused to an elastin-like polypeptide domain

types according to their backbone composition, i.e. the elements present in the backbone (carbon, oxygen, nitrogen or phosphorous) (Scheme 2). A broad range of polymers with C-C backbones is used frequently, for example PAA, poly(vinyl alcohol) (PVA) or poly(acrylamide) (PAAm). More complex monomer structures are used as well leading to polymers with C-C backbone like poly(2-hydroxypropyl methacrylamide) (PHPMA), poly(vinylpyrrolidone) (PVP) or poly(*N*,*N*-dimethylacrylamide) (PDMA). Heteroatom containing backbones can be found in poly(ethylene glycol) (PEG), poly(ethylene imine) (PEI) or poly(2-methyl-2-oxazoline) (PMeOx), for example. Hydrophilic polymers find considerable use in biomedical application and have the potential to be an even more significant material class for these applications in the future, which is a major driver for the development in hydrophilic polymer research. Especially, hydrophilic stimulus-responsive polymers have been investigated often, giving rise to a plethora of properties to exploit in applications [28]. For example, thermoresponsive polymers include poly(*N*-isopropylacrylamide) (PNIPAM) [29], poly(*N*,*N*-diethylacrylamide) (PDEA) [30] and poly(oligo ethylene glycol methyl ether methacrylate) (POEGMA) [31]. Examples for pH responsive polymers are poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) or PAA [32,33]. Needless to say, a broad range of different hydrophilic polymers were developed over the last years and several trends can be observed.

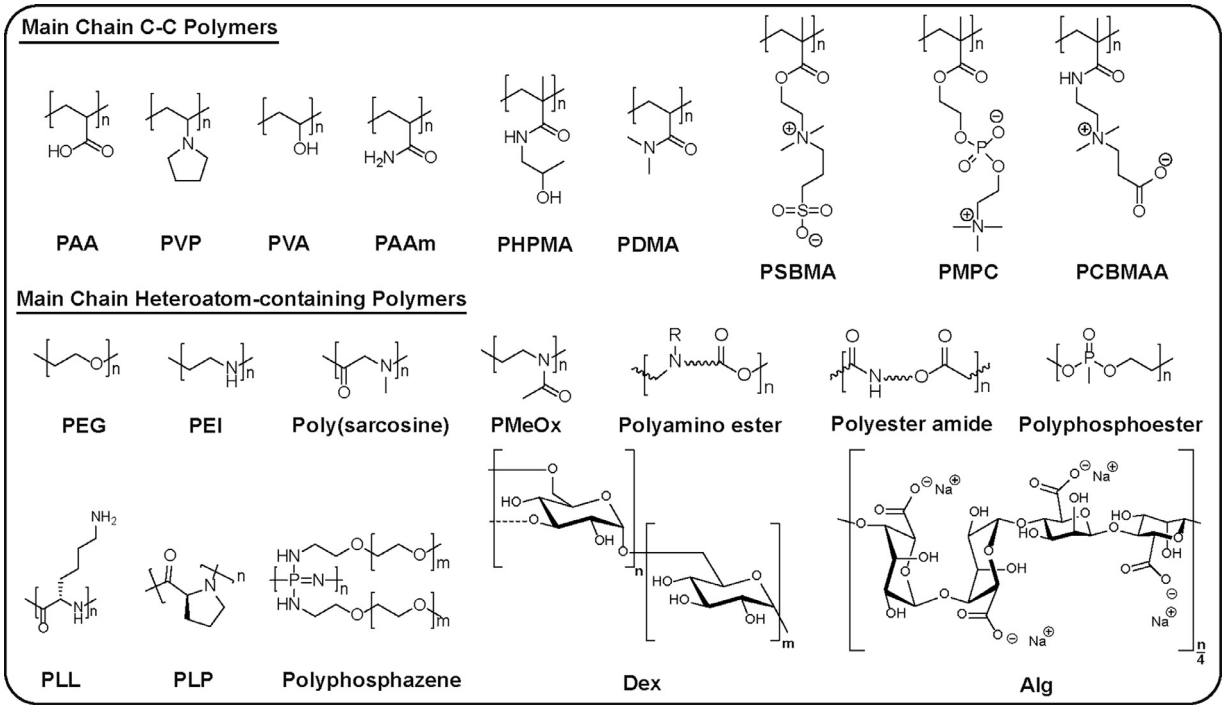
Hydrophilic homopolymers have a significant relevance in industry and real-world applications are widespread [2]. This area is not in the focus of this review, yet a brief introduction is



Scheme 1. Overview over the research areas of hydrophilic polymers covered in this review.

given here including references for the reader to take a further look. A frequent application of hydrophilic polymers is flocculation for wastewater treatment [34]. Commonly, polyelectrolytes are employed as flocculants, e.g. anionic polyelectrolytes like PAA or cationic polyelectrolytes like poly(diallyldimethylammonium chloride) (PDADMAC), but also non-ionic flocculants are used, e.g. PAAm usually with high molecular weight. Polyampholytes, i.e. polyelectrolytes containing positive and negative charges, are employed as well, which can be useful when contaminants with opposing charges are present. A prominent use of hydrophilic polymers is as ingredients in washing and detergent formulations [35]. Plenty of hydrophilic polymers are used for this application introducing various functions into detergent formulations. PAA is frequently used in this application, for example as dispersing agent or anti-redisposition agent, but also PVA is used as film material in liquid detergent capsules. As dye transfer inhibitor, PVP is employed. Of course, hydrophilic polymers are part of surfactants used in detergents, e.g. PEG in PEG-*b*-poly(propylene glycol)-*b*-PEG. A real-world application of crosslinked hydrophilic polymers are superabsorbers that are capable of absorbing significant amounts of water [36]. In most cases, superabsorbers are composed of crosslinked neutralized/partly neutralized PAA, e.g. poly(sodium acrylate).

An area that finds frequent application of hydrophilic polymers is hair care and other cosmetic products [11]. In addition to biopolymers like polysaccharides, synthetic polymers are used as well, e.g. PVP, PEG and PVA. The use of these polymers in cosmetic formulations is often as thickener in order to obtain a product that can be applied to the body conveniently. In addition to thickening, polysaccharides are frequently used as moisturizing ingredients. Similar to cosmetics, hydrophilic polymers are used in food formulations frequently [10]. Often, their purpose is thickening and gelation but also film formation and as coatings. To a broad extent, hydrophilic polymers used in food are polysaccharides like starch, alginate, pectin, guar gum or modified cellulose. Furthermore, hydrophilic polymers are applied frequently in pharmaceutical applications [12]. For example, PEG-drug conjugates as well as nanospheres and nanocapsules are used in drug-delivery . Hydrophilic polymers are also used as hydrophilic components in amphiphiles for micelle or vesicle formation. Another use of hydrophilic polymers is as supporting material, for example PVP as binder in tablet formulations or PVA as emulsion stabilizer. Hydrogel formulations making use of PVA, pectin, dextran or carrageenan are used frequently as well. Overall, these application fields show that the development of new directions in hydrophilic polymers (new types and new properties) will have a considerable impact on industrial application in the future.



Scheme 2. Examples of hydrophilic polymers: Poly(acrylic acid) (PAA), poly(vinylpyrrolidone) (PVP), poly(vinyl alcohol) (PVA), poly(acrylamide) (PAAm), poly(2-hydroxypropyl methacrylamide) (PHPMA), poly(*N,N*-dimethylacrylamide) (PDMA), poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide) (PSBMA), poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC), poly(carboxybetaine methacrylamide) (PCBMAA), poly(ethylene glycol) (PEG), poly(ethylene imine) (PEI), poly(sarcosine), poly(2-methyl-2-oxazoline) (PMeOx), polyamino esters, polyester amides, polyphosphoesters, poly(L-lysine) (PLL), poly(L-proline) (PLP), polyphosphazenes, dextran (Dex) and sodium alginate (Alg).

In the area of C-C backbone-based polymers, glycopolymers, i.e. polymers containing saccharides as sidechain, have been in the focus of research, in particular for biomedical applications [37,38]. These applications stem from the role that glycopolymers play as mimics of natural glycans, which are essential in biological recognition like cell–cell adhesion, new tissue development and infections with bacteria and viruses. As such, glycopolymers are promising materials for targeted drug delivery and biomaterials. Bertozzi and coworkers synthesized poly(vinyl methylketone) and conjugated monosaccharides to the polymer, i.e. aminooxy-*N*-acetylgalactosamine, *via* oxime formation [39]. The end group of the polymer was functionalized with a lipid anchor to study interactions with cell membranes and identify glycopolymers that populate the cell membrane with high stability. As such, the polymers enable tailoring of the glycocalyx. A combination of different saccharides was investigated by Haddleton and coworkers [40]. Polymethacrylates with protected alkyne side groups were synthesized. After deprotection, mannose, galactose, glucose or mixtures of these saccharides were attached *via* copper catalyzed azide alkyne cycloaddition (CuAAc) leading to a library of different saccharide functionalized polymers. These polymers were combined with Concanavalin A (ConA) to study conjugate stoichiometry, rate of binding, rate of clustering, stability of clusters and inhibitory potency. It was found that mannose density is the dominant factor for stoichiometry, binding rate, potency and cluster stability, while galactose content was effective in regulating cluster formation rate. Hartmann and coworkers described a brush-like glycopolymer with a polyacrylamide backbone and peptide grafts containing mannose units [41]. The formation of a polymer scaffold and conjugation of sequence defined side chains with variations of mannose substitution allowed studies on binding with various lectins. The structural parameters of the glycopolymer such as degree of branching, valency per branch as well as overall valency and length of polymeric scaffold were correlated with binding to ConA, DC-SIGN and Mannose Binding Lectin (MBL). Overall, it could be shown that different lectins have different preference in binding to brush glycopolymers. The effect of the sequence of saccharide units along polymer backbones regarding binding with lectins has been in the focus of research. Other options studied were the formation of polymer backbones containing defined monomer sequences and distances of interaction units *via* step growth approaches, e.g. CuAAc or thiol-ene reactions [42,43]. Glycopolymer research has shown a significant potential of this polymer class for biomedical applications and understanding of biological systems. In particular, the precision of polymer structures, i.e. with respect to chain length, architecture and monomer sequence, have been shown to be essential for strong interactions with biological systems. As such, a significant challenge is put on polymer synthesis in order to enable the formation of these well-defined polymers.

Another class of hydrophilic polymer that was studied frequently is zwitterionic polymers containing repeating units with anionic (carboxylate, sulfonate, phosphate) and cationic (amino, quaternary ammonium, pyridine) charge or neutralizing charges along the backbone. These polymers are of particular interest due to their antifouling and stealth properties in biological environment but also in water remediation or sensing as well as additives in bulk construction materials and crude oil [44–46]. Barner-Kowollik and coworkers described the synthesis of poly(carboxybetaine methacrylamide) (PCBMAA) *via* RAFT polymerization [47]. High molecular weights (MWs) could be achieved and also block copolymers with HPMA were synthesized. The hydration behavior of poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide) (PSBMA) was investigated by Chen and coworkers [48]. NMR studies were undertaken to measure the T_2 relaxation time and DSC in order to get an insight into polymer hydration. According to the study, one sulfobetaine repeating unit was bound to approximately eight water molecules, which is a significantly higher quantity than for PEG. Moreover, it was shown that water molecules have a significantly higher freedom beyond the first hydration layer compared to PEG. The hydration behavior of zwitterionic polymers plays a most important role for their antifouling and stealth properties, which has been studied extensively and will pave the way to tailor polymers with these specific functions in mind. A factor that has to be considered with zwitterionic polymers is the anti-polyelectrolyte effect, which covers their specific response to salt addition. In contrast to polyelectrolytes, the addition of salts to solutions of zwitterionic polymers leads to increased solubility and solution viscosity [46]. This feature might be exploited for applications in high ionic strength media.

Zwitterionic polymers are also employed for antifreeze applications, mainly in the form of coatings (see below) [49]. Polymers for antifreeze applications have been of interest recently [50], especially with application in cryopreservation of biological material [51]. Hydrophilic polymers for anti-freeze properties have been investigated thoroughly by Gibson and coworkers [52]. PVA was used to mimic the antifreeze properties of biological antifreeze glycoproteins. RDRP was used to produce PVA and copolymers with controlled MW to study structure-property relationships. It could be shown that the antifreeze properties emerged in the range of degree of polymerization (DP) of 10 to 20. Substitution of vinyl alcohol units led to lower efficiency. The same group developed a cryoprotectant derived from poly(methyl vinyl ether-*alt*-maleic anhydride) that was transformed into a polyampholyte by ring-opening reaction of maleic anhydride with dimethylaminoethanol [53]. These cryoprotectants gave high cell viability and high ratios of cell recovery. This research area is of particular relevance due to the rising interest in cell culture for tissue engineering and has a significant growth potential with respect to the utilization of new polymer types.

A considerable part of research in hydrophilic polymers with heteroatom-containing backbones has been focusing on oxygen containing backbones. PEG has been the main driver in this area. For example, various PEG architectures have been used for coatings [54] and hydrogel formation [55]. Another important point has been the functionalization of polyethers, e.g. in the side chain or at the end groups [56,57]. Side chain functionality can be exploited for crosslinking in hydrogel formation [58], formation of self-assembled structures [59] or interaction with ions [60]. Functionalization of end groups allows the formation of block polymers [56,61], attachment of reactive groups [62,63], tailored electrolyte properties [64] or bioactive moieties [65,66]. In recent years, PEG immunogenicity has been discussed frequently due to the formation of anti-PEG antibodies leading to allergic reactions [67–69]. This is a critical issue due to the ubiquitous presence of PEG in consumer products and medical formulations (see also Section 6), e.g. in PEGylation of drugs [69]. A recent study by Hawker and coworkers showed the synthesis of discrete oligoacrylates with defined oligoEG sidechains with lipid end groups [70]. Compared to commercial linear PEG, reduced anti-PEG antibody binding was observed. Other directions for PEG replacement are polyglycerols [71,72]. A polycycloether was introduced by Prunet, Shaver and coworkers making use of ring-closing metathesis of poly(epoxybutylene) (PEB) and subsequent dihydroxylation of the formed double bond [73], leading to a polymer coined PEGose (Fig. 1). As such, a hydrophilic polymer was obtained that contains a PEG-like backbone with two hydroxyl groups per repeating unit (2 hydroxyl groups per 2 ethylene glycol equivalents). A particular interesting feature of the resulting polymer is the translation of PEB tacticity into polymer conformation. Isotactic PEB leads to the formation of helical polycycloethers, while atactic PEB leads to

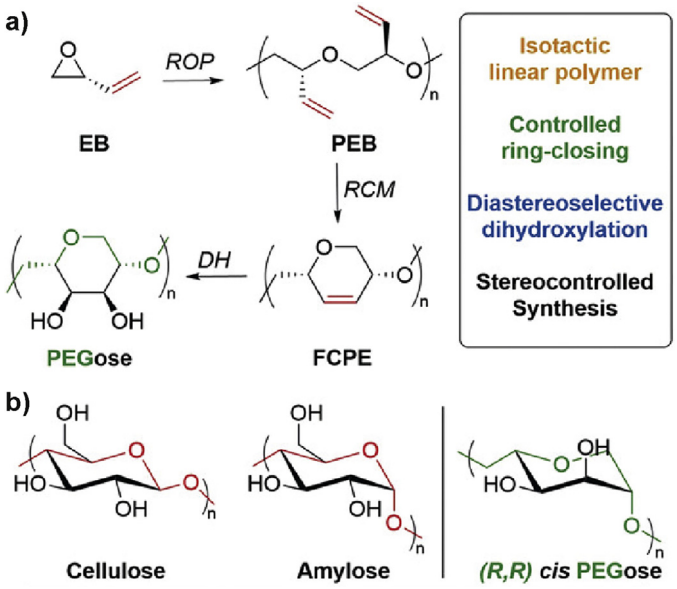


Fig. 1. (a) Synthetic pathway to PEGose starting from epoxy-1-butene (EB) *via* PEB and a polycycloether (FCPE) and (b) comparison with cellulose and amylose (PEG backbone green). Reproduced with permission [73]. Copyright 2018, John Wiley and Sons.

a random conformation. Epoxide-based polymers like PEG and related compounds are found all over polymer technology and in everyday life. In addition to the issue with immunogenicity, the low degradability of PEG is a significant challenge – especially due to the frequent use in consumer products.

A large group of hydrophilic polymers with oxygen in the backbone is polysaccharides that have found more and more use over the last decade, which is mainly due to their renewable sources, biocompatibility and degradability [74,75]. Polysaccharides have all the attributes to bring a significant impact on the field. In particular, polysaccharides have been used frequently in hydrogel formation, nanoparticle formation and as building blocks in polymer self-assemblies. A dextran (Dex)-drug conjugate was described by Kolmar and coworkers [76]. Attachment of a monoclonal antibody facilitated selective binding and transport towards cancer cells, while the Dex scaffold was used for conjugation of multiple molecules, e.g. cytotoxin. The conjugates targeted and killed human epidermal growth factor receptor 2 (HER2)-positive SK-BR-3 cells at subnanomolar concentrations. Zykwinska and coworkers designed glucosaminoglycan mimetics [77]. The authors used a microwave-assisted method for sulfation of low MW exopolysaccharides obtained from the deep-sea hydrothermal vent bacterium *Alteromonas infernus*. As there is a plethora of different hydrophilic polysaccharides available that feature various functional groups and properties, polysaccharides find their way into various applications. Furthermore, polysaccharides can be easily functionalized due to their large density of functional groups. It should be noted though that polysaccharides are rather ill-defined polymers in terms of chain length and depending on the type also monomer sequence. This can be a challenging feature, e.g. in terms of batch-to-batch comparability.

Next to oxygen, nitrogen is another common heteroatom in the backbone of hydrophilic polymers. The simplest polymer with nitrogen in the backbone is PEI, which has been frequently present in research for gene delivery. PEI – being a cationic polyelectrolyte – is capable of complexation with DNA leading to polyplex formation [78,79]. A synthesis approach towards PEI is ring-opening polymerization (ROP) of aziridine [80], which brings the challenge of branched structures and poor control over MW [81]. Very recently, a manganese catalyzed synthesis *via* coupling of ethylene glycol and ethylene imine was described by Kumar and coworkers [82]. This procedure yielded PEI with hydroxyethyl sidegroups and oligoethylene imine branches. A way to obtain linear and well-defined PEI is hydrolysis of *N*-substituted polyaziridines [83] or polyoxazolines [84,85]. The use of *N*-substituted polyaziridines enables formation of PEI [86] but also substituted PEIs [87]. An option for introduction of functionality is either *N*-substitution or addition at a carbon of aziridine. Wurm and coworkers synthesized hydroxy functionalized polyaziridine [88]. A tosyl *N*-substituted aziridine was employed that was further derivatized with an acetal protected alcohol including various spacer lengths between the acetal and the aziridine ring. In such a way, linear polysulfonamides were obtained with protected hydroxyl groups in every repeating unit. The hydroxyl groups could be deprotected as well as the sulfonamides. Indeed, both groups could be deprotected independent from each other by adjusting the deprotection conditions leading to a great flexibility for polymer functionality. Polyaziridines are a highly interesting class of polymers that feature an useful functionality. Regarding the promising application of gene delivery, the rather high toxicity of PEI is an issue that is investigated in various works. For example, polymer architecture but also alternatives to PEI are the subject of research [80]. A way to gain access to specific PEI architectures is *via* polyoxazolines.

Frequently, polyoxazolines have been used as precursors for PEI as polyoxazolines can be transferred into linear PEI *via* hydrolysis under acidic conditions [89]. Hoogenboom and coworkers described the copolymer poly(ethylene imine-*co*-propylene imine), which has a variation in alkyl spacer length along the backbone similar to naturally occurring oligoamines, e.g. spermine and spermidine [90]. In order to produce the copolymer, 2-alkyl-2-oxazolines were copolymerized with 2-alkyl-2-oxazines and the product hydrolyzed. The polymerization process was tuned *via* variation of side chains to achieve a statistical copolymer. The polymers showed a very good transfection efficiency and high stability in serum. Most notably, the use of polyoxazolines as precursors for PEI enables the formation of defined macromolecular architectures. For example, Grayson and coworkers synthesized α-alkyne ω-azide PEtOx to form cyclic polymers *via* CuAAc [81]. In the subsequent step, PEtOx was hydrolyzed to afford cyclic PEI and gene transfection was studied for cyclic and linear PEI. The performance of cyclic PEI was significantly better over a broad range of MWs and N:P ratios. A comparison with the current standard for gene delivery, i.e. branched PEI (MW 25 kg/mol), showed similar performance but less toxicity. Improved transfection efficiencies might be due to higher charge density in more compact cyclic polymers, which underpins the importance of polymer architecture for interactions with biological systems.

In addition to being precursors for PEI, polyoxazolines have found direct use for various applications frequently [91], especially in biomaterials and biomedical applications. Hydrophilicity of polyoxazolines depends strongly on side groups, i.e. PMeOx is water soluble over the whole temperature range [92], while PEtOX, poly(2-propyl-2-oxazoline) [93], poly(2-cyclopropyl-2-oxazoline) [93] and poly(2-isopropyl-2-oxazoline) (P*i*PrOx) [93] feature a lower critical solution temperature (LCST), i.e. the polymer turns insoluble upon heating. An approach to introduce degradable polyoxazolines was described by Schubert and coworkers [94]. PEtOx was first converted into linear PEI *via* hydrolysis and partially oxidized *via* H_2O_2 in methanol. This procedure effectively led to PEI with amide bonds along the backbone. Subsequently, the remaining amino groups along the backbone were acylated with various acyl chlorides reinstalling polyoxazoline repeating units. Hence, polyoxazolines with amide bonds along the backbone were synthesized. Finally, these polymers were subjected to degradation treatment with aqueous HCl (90 °C for two days) leading to complete

degradation into small molecules. Enzymatic treatment with proteinase K at 37 °C for 30 days led to degradation as observed by the presence of free glycine, yet no full degradation was observed over this time span. In another work, acylation of PEI was also used to synthesize a broad range of polyoxazolines, e.g. to introduce oligoethylene glycol side chains [95]. An example for a new class of polyoxazolines was described by Sedlacek, Bera and Hoogenboom, namely poly(2-amino-2-oxazoline)s [96]. Therefore, the new monomers 2-dimethylamino-, 2-ethylmethylamino, 2-diethylamino-, 2-diisopropylamino- and 2-morpholino-2-oxazoline were synthesized. Polymers with a range of water-solubility from extremely soluble to hydrophobic were obtained as well as poly(2-diethylamino-2-oxazoline) featuring LCST behavior. Due to the formation of polyoxazolines *via* cationic ROP, defined polymer structures (MW, dispersity and endgroups) can be obtained. This is of particular importance as biomedical applications are the prime goal for polyoxazoline research, where high definition and functionalizability are often required. Nevertheless, the 2-oxazoline polymerization can be quite challenging, e.g. water-free reagents are needed and side-reactions can occur. The best way to perform the polymerization is *via* microwave, under which side-reactions are suppressed and the polymerization speeds up significantly [97].

Polyamides are another polymer class with nitrogen atoms in the backbone, found frequently in polypeptides/proteins and polypeptoids [98]. Cheng and coworkers synthesized water-soluble peptide polyelectrolytes [99]. At first, γ-(4-allyloxylbenzyl)-L-glutamate-*N*-carboxyanhydride (γ-(4-allyloxylbenzyl)-L-glutamate-NCA) was polymerized *via* ROP. The polymer was subsequently functionalized *via* thiol-ene to introduce carboxylic acid or amino functions. The polypeptides formed helix structures in aqueous solution with DP as low as 10 that were stable even in the case of pH, temperature, salt and urea concentration change. A commonly used polypeptoid is poly(sarcosine) [100], which was used by Haas and coworkers for coating of lipid nanoparticles for mRNA delivery [101] or as ligands for quantum dots by Zentel and coworkers [102]. A convenient synthesis of poly(sarcosine) was described by Schlaad and coworkers [103], who introduced air and moisture stable *N*-phenoxycarbonyl-*N*-methylglycine to be transformed *in-situ* into *N*-methylglycine-NCA during the ROP process. Kramer and coworkers investigated the solution behavior of glycopolypeptide bottlebrush polymers [104]. ROP of an allyloxycarbonyl functionalized lysine-NCA was performed, where the allyl group could be used to conjugate α-*N*-acetylgalactosamine or β-lactose. The grafting density could be controlled by employing non-allyl functional NCA in a copolymerization approach. Furthermore, α-glucose modified lysine-NCA was used, giving rise to three different saccharide units attached to the polypeptoid chain. Bonduelle and coworkers described the ROP of L-proline-NCA leading to poly(L-proline) (PLP) [105]. The polymer showed interesting thermoresponse properties. A significant hysteresis of temperature-induced aggregation in water was observed that spanned between 38 and 57 °C, depending on DP. This behavior was ascribed to the formation of two different secondary structures in solution, i.e. an extended hydrophilic helix and a more compact hydrophobic helix. Copolymerization of both enantiomers led to polymers without thermoresponsive properties. PLP was also studied regarding its ice recrystallization inhibition by Gibson and coworkers recently [106]. Promising properties were shown, e.g. specific ice-face binding and ice recrystallization inhibition down to concentrations of 5 mg mL^{-1}. Sun and coworkers described polypeptoids with oligoethylene glycol (OEG) sidechains to introduce thermoresponse [107]. Poly(*N*-propargylglycine) was synthesized and OEGylated *via* thiol-yne reaction. The polymers showed LCST behavior with a cloud point (T_{CP}) between 20 and 40 °C depending on polymer and graft lengths. Hydrophilic polymers with amide bonds along the backbone belong to the most promising materials in hydrophilic polymers. In particular, these polymers feature degradability and high biocompatibility. Making use of NCA ROP, these polymers can be synthesized with good control over MW, architecture and endgroups as well as block polymers. The formation of polypeptides further enables introduction of defined secondary structures that can be exploited for a specific function. The presence of the amino acid stereocenter is of particular use for the control of polymer structure and properties. In addition to polypeptides and polypeptoids, polyamidoamines containing amino and amido nitrogen in the backbone were investigated frequently [108] as well as polyamino esters and polyester amides containing nitrogen and oxygen in the backbone [109,110]. The main advantage regarding these polymer structures lies on improved degradability.

Water-soluble polymers with phosphorous atoms in the main chain were described frequently as well, e.g. polyphosphoesters [111] or polyphosphazenes (nitrogen and phosphorous in the main chain) [112,113]. Pelosi et al. described protein-polyphosphoester conjugates [114]. A variety of polyphosphoesters were synthesized *via* anionic ROP of cyclic phosphoesters. In particular, the hydrophilicity was varied by introducing methyl, ethyl, ethoxy and ethyl/butyl (*via* comonomers)-side groups. The polyphosphoesters were conjugated to myoglobin and the effect on protein stability tested. Hydrophilic polyphosphoesters had a similar activity to a PEG reference, showing shielding from proteolytic enzymes, low reduction of protein activity and improved thermal stability. The polyphosphoester with methyl sidegroup had the best performance showing promise to become an alternative to PEG. Thus, polyphosphoesters are of particular interest combining the useful properties of PEG with degradability due to their hydrolysable phosphorous-oxygen bonds along the backbone. Here, the polymer structure has to be optimized to be stable enough for the targeted application yet still labile enough to be degraded after the application. As already shown, the properties of polyphosphoesters can be controlled by monomer composition, which is an effective way to optimize polymer properties, i.e. activity, hydrophilicity, stability vs. degradability. An example for polyphosphazenes containing nitrogen and phosphorous in the main chain was described by Teasdale and coworkers [115]. At first, poly(dichlorophosphazene) was synthesized *via* cationic polymerization, which was further reacted with amino-endfunctionalized grafts (i.e. Jeffamine M-1000 with various spacers) substituting chloro side groups. The products showed high water-solubility, biocompatibility and degradability in aqueous environment. As such, polyphosphazenes are a promising hydrophilic polymer class despite the rather challenging synthesis *via* cationic polymerization.

Hydrophilic polymers are also used frequently as grafts or coatings on surfaces [116]. Some applications that are addressed with hydrophilic polymers are ice and fogging inhibition, or in the biomedical field for example antibiofouling surfaces [117–119] or lubricant surfaces [120,121]. Wang et al. reported a layer-by-layer coating of PEI and PAA/hyaluronic acid blend to form anti-fogging/frost-resistant surfaces [49]. The effect is due to uniform dispersion of the adsorbed water molecules *via* hydrogen bonding interactions with the coating. A grafting-from polymerization approach was described by Pester and coworkers [122]. Surface-initiated photo-induced electron/energy transfer RAFT polymerization was used to produce superhydrophilic surface brushes, for example from poly(2-(methacryloyloxy)-ethyl) trimethylammonium chloride) (PMTAC). The polymer films showed significant optical clarity upon deposition and sustained functionality over multiple uses for anti-fogging applications. Superhydrophilic surfaces were described by Takahara and coworkers as well, who studied polyelectrolyte brush surfaces [123]. These brushes repelled air bubbles, hexadecane and silicone oil. Lubricant properties of surfaces were studied by the same group [124]. Therefore, DMAEMA, MPC, MTAC and 3-sulfopropyl methacrylate potassium salt (SPMK) were

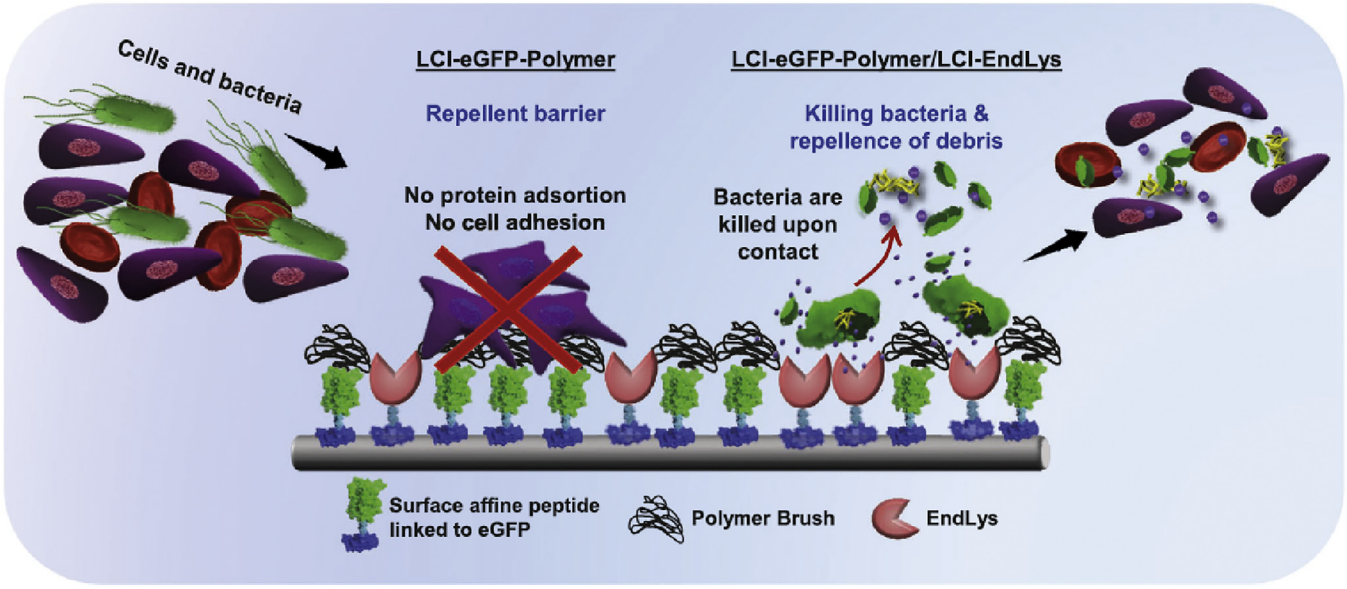


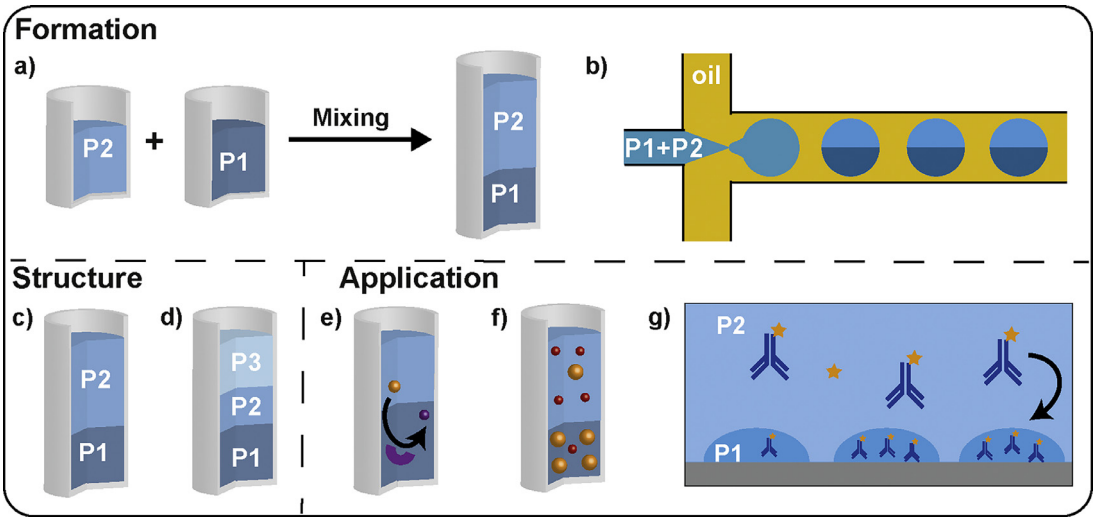
Fig. 2. Schematic of a wound dressing coating to kill and repel bacteria: 1. LCI-eGFP-Polymer suppresses adhesion of proteins, skin cells, and bacteria to the dressing. 2. LCI-EndLys hydrolyzes the peptidoglycan of bacteria upon contact causing their death in case of passage through polymer layer. 3. LCI-eGFP-Polymer repels debris of killed bacteria from the surface. Reproduced with permission [129]. Copyright 2021, John Wiley and Sons.

grafted on silicon wafers. Ball-on-plate tribology was used to measure friction coefficients of water swollen brushes. It could be shown that SPMK brushes had the lowest friction and crosslinking of brushes increased durability of the coating. The grafting of hydrophilic polymers on surfaces enables the introduction of various useful properties, e.g. anti-fogging or lubrication properties. In particular, the type of polymer can be used to tailor the surface properties. Furthermore, the grafting of hydrophilic polymers on surfaces can be utilized to mitigate interactions with biological systems and biological media.

Anti-biofouling properties were investigated by Riedelová et al. [125]. In particular hemocompatibility of a variety of different hydrophilic polymer brushes was studied, e.g. PSBMA, PHPMA, PHEMA, poly(carboxybetaine acrylamide) and PCBMAA. Fouling from undiluted blood plasma was reduced up to 99 % by minimizing adhesion of platelets and leukocytes as well as preventing thrombus formation. In a similar way, cell adhesion can be prevented *via* coating of surfaces with hydrophilic polymers [126,127]. Antimicrobial surfaces were described by Salentinig and coworkers based on MTAC [128]. UV initiated photopolymerization was used to form the polymer on nitrile, cotton, glass and silicon surfaces. Microbiological studies *in vitro* revealed rapid elimination of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains within 5 min of contact. The contact killing effect was ascribed to electrostatic interactions between positively charged surface and negatively charged bacterial membrane leading to modification of membrane curvature, loss of barrier function and death.

A combination of anti-biofouling and antimicrobial properties was studied by Rodriguez-Emmenegger and coworkers (Fig. 2) [129]. Therefore, a brush of hydrophilic coating was formed by the surface adhering antimicrobial peptide liquid-chromatography-peak-I (LCI) that was combined with the bactericidal enzyme endolysin (EndLys) as well as the enhanced green fluorescent protein (eGFP) and PHPMA-*b*-PCBMAA. Wound dressings were fabricated, where the polymer coating prevents proteins, skin cells, and bacteria from adhering to the dressing. Furthermore, endolysin in the coating hydrolyzes the peptidoglycan of the bacteria that surpass the polymer barrier upon contact, causing their death. Any debris from killed bacteria is further repelled from the dressing by the polymer coating, which in turn reinstates the integrity of the coating and minimizes debris accumulation that would lead to a decrease of efficiency. As mentioned earlier, antimicrobial activity is an important application of hydrophilic polymers, particularly polyelectrolytes containing amino groups are in the focus of research [130]. For example, polypeptides containing lysine monomer units are used frequently [131,132]. A C-C main chain-based polymer with antimicrobial properties was described by Perrier and coworkers [133]. A copolymer of NIPAM and a protected guanidinium containing monomer or aminoethyl acrylamide was synthesized. The placement of the monomers along the chain was varied to study effects of monomer sequence on activity. It was shown that toxicity towards epithelial cells was associated with an improved hemocompatibility by a reduced hemagglutination activity as well as potency against *Staphylococcus aureus* that depended on polymer structure. The use of hydrophilic polymer brushes to introduce antifouling and antimicrobial properties is an area with considerable impact. One can appreciate the need for these kinds of surfaces in order to improve the clinical practice, e.g. less contamination with pathogens or spread of pathogens as well as longer life-time of medical implants due to lowered fouling. In particular, the polymer composition and polymer functionality play an important role for these applications, for example, when polymer sidegroups have a direct interaction with bacterial cell membranes.

The topology and molecular properties of hydrophilic polymers play an important role for surface properties, which opens up significant options for tailored surfaces [134]. Bennetti and coworkers studied the effect of polymer topology on brush properties [135]. In particular, linear polymer brushes were compared with cyclic polymer grafts based on poly(2-carboxypropyl-2-oxazoline) or poly(2-carboxyethyl-2-oxazoline). Cyclic polyelectrolyte brushes showed enhanced repulsive interactions between charged polymer segments upon deprotonation, which led to an increased expansion and a stronger swelling in comparison to their linear analogues of similar molar mass. Dispersity of polymer chain lengths in the side chains of grafted polymers also has a considerable effect on brush properties [136], e.g. in the case of POEGMA. Polydisperse OEG side chains prevented the presence of hydrophobic van der Waals interactions and thus supports association with water molecules. In turn, interfacial physicochemical properties are modulated, which



Scheme 3. Formation: (a) ATPS *via* mixing of two homopolymer solutions and (b) microfluidic approach for ATPS droplet formation in oil; Structure: (c) Illustration of an aqueous two-phase system (ATPS) (two polymer components and water) and d) an aqueous three-phase system (A3PS) (with additional third polymer component); Application: (e) Catalysis, (f) molecule separation and (g) biosensing.

is highly relevant for technological applications and for the design of nanobiointerfaces. This includes the aforementioned hydration of brushes, adhesion, lubrication, and anti-biofouling. In general, dispersity within brushes is ascribed as a considerable factor for brush properties when swollen in aqueous media, in addition to the common features of composition, MW, and surface coverage.

Hydrophilic homopolymers find a broad range of uses. Just using homopolymers gives rise to a plethora of properties and applications. Furthermore, molecular design on homopolymer chains itself enable designed properties for specific applications. Mixtures of homopolymers give rise to even more applications, especially in aqueous multi-phase systems.

3. Hydrophilic polymers in multi-phase systems

In recent years, completely hydrophilic multi-phase systems have been in the focus of research in the area of hydrophilic polymers [137]. Their ability to partition biomacromolecules in selected aqueous phases is of particular use for applications in molecular purification [138], sensing [139,140], tissue engineering [141], cell culture [142] or cargo loading [143]. Completely aqueous multi-phase systems feature various advantages over common water-oil-based systems, e.g. high permeability between the phases, no requirement for use of oil as well as easy demulsification. Nevertheless, these advantages are connected to disadvantages as well, i.e. lower specificity of partitioning of molecules in the different phases, high polymer concentrations are needed to form the multi-phase system and less stability against dilution. Like water-oil-based systems, aqueous multi-phase systems can be present in macroscopic phase separation (Section 3.1) or dispersed water-in-water (w/w) emulsions (Section 3.2).

3.1. Aqueous two-phase systems

Aqueous two-phase systems (ATPS) are formed from two incompatible polymers dissolved in water (Scheme 3a and c) [144], such as PEG and Dex. ATPS containing water soluble non-polymeric components, like salts, have been described as well [145]. In general, phase separation in polymeric systems containing two polymer types and a solvent are very common [146,147]. Specifically in aqueous solution, a significant number of polymer combinations show incompatibility and repulsive interations. Needless to say that ATPS formation does not occur in the case of attractive interaction between the polymers. ATPS formation resolves in the formation of two individual phases that are enriched with one or the other polymer and in equilibrium with each other. Thermodynamically the phenomenon of ATPS formation is driven by the enthalpy associated with the interactions of the components (polymer-polymer and polymer-water interactions) that is opposed by entropy loss due to phase segregation [148,149]. Due to the aqueous nature of both phases, ATPS feature very low interfacial tensions below 10^{-2} mN m^{-1} leading to rather dynamic properties [150]. In addition, the interface in ATPS has a large width in the range of tens of nm [151], which is larger than the correlation length of the polymer solutions and enables small molecules to move through the interface easily [150]. Due to the high number of incompatible polymer combinations also more than two distinct phases can be formed in aqueous multi-phase systems, e.g. aqueous three-phase systems (A3PS) (Scheme 3d) or even more phases [147].

Liquid-liquid phase separation (LLPS) can closely mimic phase separation which occurs in cells leading to the presence of membrane-less compartments without hydrophobic barrier [152,153]. LLPS is also exploited by viruses to evade antiviral immune responses and replication [154]. A membrane-less compartment allows for ease of small molecules, proteins, biomolecules and enzymes to pass through the phase interfaces [155]. The multi-phases are also a means to separate enzymes in the biological setting. In terms of technology, these multi-phase systems can be used to purify biomolecules, which has been one of the major applications of ATPS in the past [156].

Macromolecular crowding is a key feature in cell biology, which influences the LLPS process [157]. Furthermore, crowding appears to affect the efficiency of the enzymatic activity, however the mechanism behind the change in reactivity is poorly understood with some cases showing the enzymatic activity decreasing and others showing increasing activity. Mukherjee and coworkers investigated the effects of macromolecular crowding on enzymatic cascade reactions with horseradish peroxidase (HRP) and glucose oxidase [158]. Using PEG, Dex and Ficoll as inert crowders to form LLPS systems, the model produced an increase in enzymatic activity inside the phase separated droplets whilst the enzymatic activity prior to phase separation was severely hindered. The investigation of phase selectivity of supramolecules was investigated by Obayashi and coworkers [159]. Using the common PEG – Dex sys-

tem the localization of supramolecules formed by cyanuric acid (Cya-PAs) and melamine modified nitrobenzofuran (Mel-NBD) was studied. It was found that in a 5 wt% PEG and Dex system, where there are Dex droplets in a PEG continuous phase, fibrous assemblies of Cya-PA3/Mel-NBD formed and separated into the Dex rich droplets. As such, these Dex/PEG ATPS droplets could be used to adsorb proteins, which was further exploited in subsequent studies. As the synthesized supramolecular polymers were shown to adsorb proteins into the Dex droplets, HRP and glucose oxidase (GOx) enzymes were adsorbed as well. The localization of these enzymes caused an increase in cascade reaction rate. Pavlovic et al. also investigated LLPS as a method for enhancing enzymatic catalysis (Scheme 3e) [160]. Inspired by LLPS in living cells causing the partitioning of biological components the enzymatic efficiency of HRP and urate oxidase (UO) was investigated with partitioning of the enzymes across PEG/Dex ATPS. Using high molar mass Dex and low MW PEG along with fine tuning the phase separation by altering pH the most effective partitioning of enzymes could be constructed, i.e. by altering the pH to a value where the HRP and UO have opposite net charges. An ATPS composed of 10 w/v% PEG (3 kg mol^{-1}) and 15 w/v% Dex (500 kg mol^{-1}) and 50 mM NaCl concentration with the addition of a phosphate buffer to set the pH to 7.8 was formed along with 100 mg mL^{-1} of UO and 5 mg mL^{-1} HRP. The resulting activity of enzyme in the separate phases showed higher enzymatic activity for the Dex-phase and lower activity for the PEG phase in the cascade reaction of Guaiacol or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) oxidation, this could be due to interactions between PEG and the substrates. The difference in activity was also dependent upon the substrate used which indicates varying translational diffusion coefficients of different substrates. These examples show that enzyme catalysis is one of the promising applications for ATPS. Especially the enrichment of enzymes in different phases provides access to more complex enzyme cascades, e.g. from incompatible enzymes. In order to make this a viable application, the partitioning behavior has to be investigated further.

Further applications of ATPS are within biomolecule separation and extraction (Scheme 3g). For example, Seo et al. developed a method for model extracellular vesicle (EV) extraction based upon ATPS [161]. Triple emulsion droplets were used to achieve isolation of the model EV, with an oil phase around droplets of an inner ATPS consisting of Dex and PEG and an outer continuous aqueous phase, forming $w_1/w_2/o/w_3$ emulsions. Introduction of poly(butadiene)-*b*-PEG in a mixture of chloroform and cyclohexane as the oil phase allowed poly(butadiene)-*b*-PEG polymersomes to be formed on evaporation of chloroform, with the ATPS in the core. The ATPS droplets were synthesized using a glass capillary microfluidic device consisting of two capillaries, one for injection and one for collection, with the inner surface of injection and collection capillary treated to be hydrophilic and the outer surface of injection capillary treated to be hydrophobic. Another additional small capillary was then inserted to the outer capillary for the formation of ATPS by injecting both PEG and Dex simultaneously. The resulting droplet had a Dex inner core and PEG outer core with an oil shell, where the polymersome membrane was formed. The use of microfluidics allowed formation of monodisperse droplets with the required well defined compartments. Through these droplets containing an ATPS, the EV was removed by selective rupture of polymersomes around the ATPS. Due to the water-based nature of ATPS and the intrinsic difference of the individual phases, biomolecule separation and purification have been the major application of ATPS. Biomolecules have a stronger or weaker partitioning into the different phases and can be separated this way without requiring potentially harmful organic solvents. As shown by the aforementioned EV-extraction, the use of organic solvents can be useful for more complex extraction tasks.

Cheng et al. developed ATPS-based particles, i.e. in the form of Janus particles where there are two distinct sides to the composition of the particle [162]. In this case the particle was formed from PEG and Dex, mixing PEG (20 kg mol^{-1} at 10 wt%) and Dex (450–650 kg mol^{-1} at 15 wt%) using a superhydrophobic surface to support the ATPS droplets along with a humidity controller to ensure volume was unchanged. Using different ratios of the hydrophilic polymers could also result in different morphologies of droplets due to differences in concentration of the polymers and the changing of the proportions of separated phases. Altering the Dex length was also found to lower the Janus balance of the resulting particles due to its increased interaction with water. Changes in concentration and MW of polymers was shown to alter the interfacial curvature, resulting in easily synthesized and tunable Janus particles. More investigations into ATPS were conducted by Plucinski et al., studying ATPS beyond traditional PEG/Dex systems [163]. By using Ultra-high MW polyacrylamides, ATPS could be produced in lower concentrations. The ATPS's were formed with PDMA, PAAm, and poly(4-acryloylmorpholine) (PAM) synthesized *via* reversible addition-fragmentation chain transfer (RAFT) polymerization. Phase diagrams produced of these polymer systems showed that the lowest concentration for an observed ATPS was present in the PDMA and PAAm system with an overall polymer concentration of 0.6 wt%, which also had the best separation of polymers. These ATPS were then transformed into w/w emulsions stabilized using Mg/Al-CO_3-layered double Hydroxide nanoparticles with diameter of around 100 nm. The emulsions formed were stable in the lower phase for at least 4 weeks. A solution of all three of the polymers was also produced, causing an A3PS to form (Scheme 3c), with the emulsion of this system forming water droplets in a polymer-enriched aqueous continuous phase, the resulting systems might be useful for catalysis or for biomolecule separation in the future.

ATPS formed from the thermo-responsive polymer Pluronic®F127 and methacrylate homopolymers based on PEG were investigated by Georgiou and coworkers, where the phase separation occurred via thermal stimulus [164]. The methacrylate polymers tested had a range of 2 to 9 ethylene glycol groups in the side chains, and varying MW. The phase separation was observed in both homopolymer methacrylate solutions and in mixtures with Pluronic®F127. The temperature at which phase separation occurs was found to be dependent on the length of the PEG side chain with the phase change temperature lowering from 65 °C to 20 °C with $OEGMA300_x$ and di(ethylene glycol) methyl ether $methacrylate_{60}$, where $OEGMA300_x$ has ~4.5 PEG units and di(ethylene glycol) methyl ether $methacrylate_{60}$ with 2. The thermal phase separation of $OEGMA300_x$ was further controlled by MW, with increasing MW causing a decrease in phase separation temperature. The study and variation of phase transition temperatures is of particular use for ATPS formation. On one hand, it shows at which temperature an ATPS will form. On the other hand, it can be exploited in application for example to mix compounds at a specific temperature in one phase and then change the temperature to induce a partitioning of molecules and thus separation.

Yan and coworkers investigated the formation of fibrillar structures through LLPS with a solute rich phase and solute poor phase (Fig. 3a/b) [165]. The solute rich phase acted as a nucleation site for the self-assembly of amino acids to supramolecular nanofibrils. The mechanism worked by a lowered nucleation barrier towards the nanofibrils due to a metastable liquid phase in the solute rich droplet. LLPS was the fundamental first step in the process to allow for supramolecular self-assembly, which then formed solvated nanoclusters acting as building blocks for supramolecular structures. The use of LLPS to form these supramolecular polymers enabled further insight into construction of biomimetic systems. Another method for mimicking biological systems was the de-

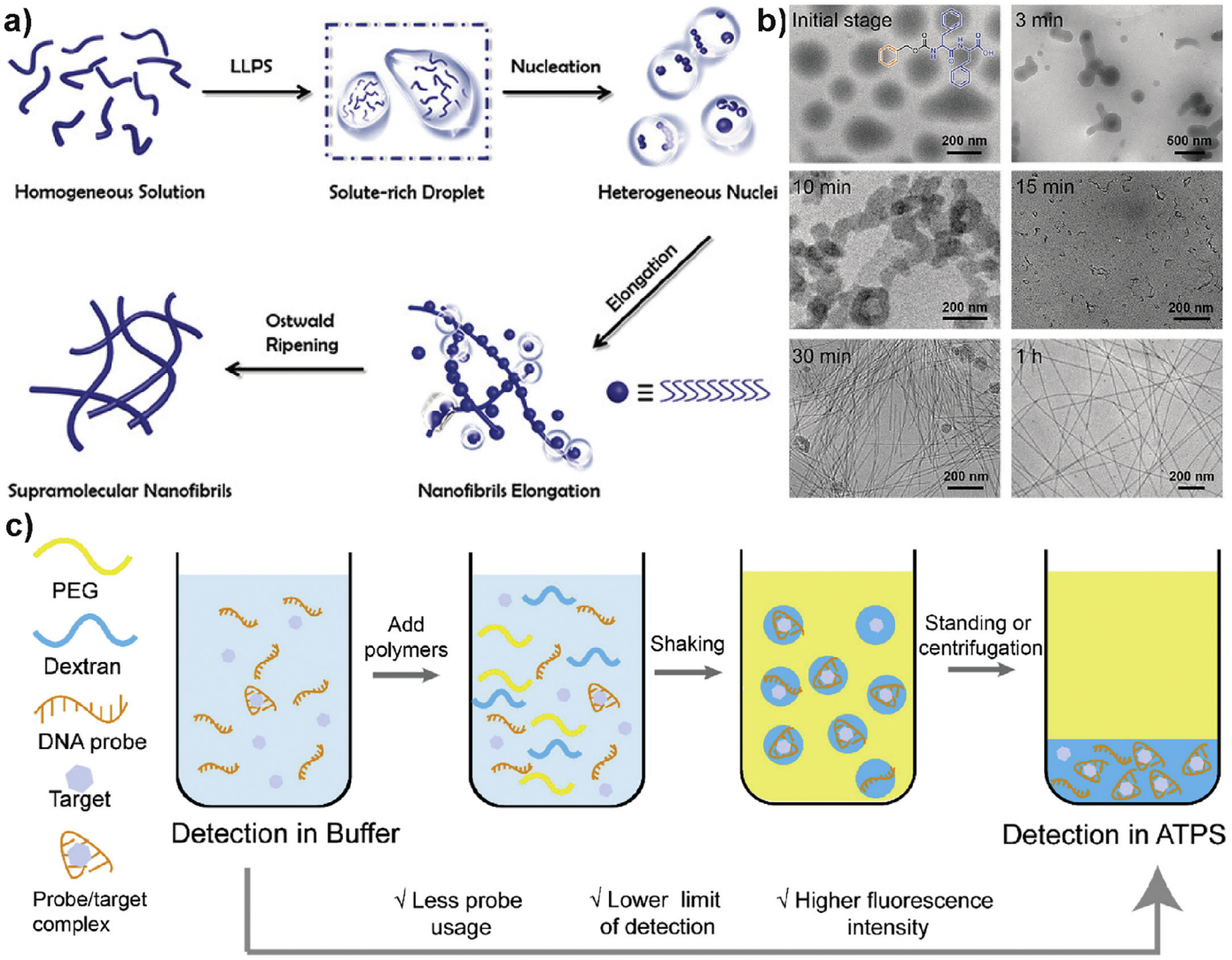


Fig. 3. (a) Schematic illustration of the formation of self-assembling supramolecular nanofibrils by amino acids or short peptides *via* liquid–liquid phase separation (LLPS) into solute-rich and solute-poor liquid phases prior to the nucleation of supramolecular nanofibrils based on amphiphilic amino acid or short peptide self-assembly and (b) Cryo-TEM images for the dynamic evolution process of carboxybenzyl (Z)-protected diphenylalanine dipeptide self-assembly over time, demonstrating the structural evolution from the metastable liquid droplets to the thermodynamically favorable nanofibrils. Reproduced with permission [165]. Copyright 2019, John Wiley and Sons. (c) Application of a PEG and Dex ATPS for the partition of DNA probes: Dex-enriched droplets formed in the PEG-enriched phase, DNA probes and analytes partition in the Dex-enriched droplets. After settling of the Dex-enriched phase, probe DNA and analytes accumulate at the bottom improving the sensitivity of detection. Reproduced with permission. [167] Copyright 2021, American Chemical Society.

velopment of a biomolecular condensate by Cai and coworkers [166]. By RAFT photocopolymerization of ion-pair monomers an asymmetric charge sequence of zwitterionic segments was achieved. With the polymerization performed on PHPMA$_{45}$-*b*-poly(cystamine methacrylamide hydrochloride)$_{70}$/poly(2-acrylamido-2-methylpropanesulfonic acid)$_{100}$ seed vesicles interface, a condensation driven self-assembly due to restricted space took place. The hierarchical self-assembly went from vesicles-to-lamellae transition to lamellae-to-sheets transition, layer-by-layer sheet self-assembly, and redispersing into a fibril network as the polymerization occurred. These nanostructured networks were formed by the asymmetric sequencing of charge of the zwitterions and might be used for emulating biomolecular condensates such as proteins. These results show the property of ATPS to not only enrich (bio)molecules in specific phases but also to act as a template for the formation condensates or self-assemblies.

An application for ATPS is improving biosensing processes by concentration of analytes in one of the two phases (Scheme 3h). For example, DNA-based probes in biosensing were studied for detection of DNA, Hg^{2+} or adenosine triphosphate (ATP). Liu and coworkers focused on the classic ATPS of PEG and Dex as an aqueous environment for investigating DNA probe sensitivity (Fig. 3c) [167]. By tuning the ATPS, the partitioning of DNA could be modified. Between pH 6 to 10 the DNA was mainly present in the Dex phase, whereas at pH 12 the DNA was partitioning into the PEG phase. As the concentration of PEG increased, so did the concentration of DNA in the Dex phase, similarly longer PEG chains also caused an increased concentration of DNA in the Dex phase. The probe sensitivity in an ATPS of Dex (MW of 100 kg mol^{-1}, 16 wt%) and PEG (MW of 80 kg mol^{-1} 20 wt%) was compared against that in common buffer. The target and the probe both concentrated in the Dex phase, which led to a higher fluorescence, i.e. 3.8 times more in the ATPS than that of the plain buffer system. Furthermore, the limit of detection improved by 3.6 times by employing the ATPS. The system was tested for detection of $Hg^{2+}$and ATP as well, where there was no preference of target analyte to the different phases. The enriched DNA probe in the Dex phase however

still accounted for improved sensitivity in detection of both Hg^{2+} and ATP. Shum and coworkers described an avenue for ATPS-based isolation of analytes followed by analysis [168]. A monoclonal antibody was used as the analyte in a Dex/PEG ATPS. Due to partitioning of the analyte in the Dex phase, isolation of the analyte took place together with a separation from a deliberately added interfering analyte. As such, an overall improvement of analysis was achieved. In recent years, ATPSs have been used more and more for analytical tasks. Here the partitioning of analytes into one phase leads to an increase in concentration. Improved sensitivity is achieved by the increased concentration analyte. The partitioning of biomacromolecules could be also used for DNA purification as presented by Takinoue and coworkers [169]. A PEG/Dex ATPS was used to purify DNA origami, DNA hydrogels and DNA microtubes from excess smaller constituents, e.g. small DNA fragments remaining from origami synthesis like short single-stranded DNA staples that tend form unintended aggregates. The method could be used for a variety of structures. It was found that remaining polymers did not hamper the further use of the purified DNA origami.

A tunable multi-phase system was developed by Tang et al., which might have applications for separation and purification systems [170]. The tunable ATPS was synthesized using azobenzene and benzyl modified poly ionic liquids (Azo- or Bn-PILs). The grafting degree (GD) of the polymers impacted the separation behavior of the system, with GD of Bn-PIL at 30 % and the GD of Azo-PIL at 10 % the lower phase was enriched with the Bn-PIL and the upper phase enriched with Azo-PIL. With the GD of Bn-PIL at percentages lower than 30 % the enrichment of the phases swapped. The enrichment of the phases also swapped with temperature increasing to about 65 °C, which then reversed when the temperature lowered. Additionally, when the system was exposed to UV light the separation of phases disappeared and the system became a single-phase environment, this could also be reversed by exposing the system to visible light, resulting in tunable light-sensitive ATPS. Magnetic-stimuli were introduced as well, e.g. ATPS of PEG and Dex have been employed with superparamagnetic sodium citrate stabilized maghemite nanoparticles to form ferrofluidic ATPS which exhibit lower interfacial tension compared to that of traditional ferrofluids. The connection of ATPS and ferrofluids was achieved by Timonen and coworkers [171]. With the nanoparticles preferring the Dex-rich phase, the resulting structures produced by the ATPS were square like patterns with smaller periodicity than that of traditional ferrofluidic systems. These systems could be used for purification of biomolecules based on partitioning in the ATPS and sorting with applied magnetic field. The use of low magnetic fields to cause strong deformations might lead to applications in magnetically responsive materials, with having magnetically induced compartments which can be used as microreactors.

An ideal way to form well-defined ATPS droplet structures in oil environments is *via* glass membranes [172] or microfluidics (Scheme 3b) [172–174]. For example, Pavlovic et al. formed ATPS and A3PS in water droplets in an oil phase with the help of a surfactant *via* a microfluidic approach [175]. Water-based droplets were formed in the oil phase in the microfluidic channel containing the required polymer mixture for aqueous phase separation. Dex, PEG and Ficoll were combined leading to water droplets with tunable inner structure as the geometry of aqueous droplets could be controlled by the hydrophilicity and the volume ratios of the polymer-rich phases. Droplet morphologies could be tuned by polymer MW, ratio and surfactant hydrophilic-lipophilic balance. Finally, the change of droplet morphology in the case of Dex/PEG ATPS containing dextranase was studied. Dextranase caused degradation of Dex, which in turn led to lower MW and a change in droplet morphology. Another A3PS was investigated by Cai et al. [176]. PEG, Na_2CO_3 solution and a fluorosurfactant were used to form three phase containing droplets in vegetable oil. Emulsification and demulsification were studied with respect to the ratio of the components. A completely water-based droplet system with ATPS core was described by Shum and coworkers [177]. Therefore, a PEtOx and Dex containing phase was injected into a continuous PEG containing phase. As such, droplets were formed that contained PEtOx-rich and Dex-rich phases in a Janus fashion after LLPS.

Compartmentalization of coacervate micro droplets *via* ATPS has been described by Mann and coworkers [178]. At first coacervate droplets were formed from PDADMAC and ATP, subsequently tetraethylene glycol was added leading to formation of a vacuole in the droplets. Dex was added leading to ATPS formation with a tetraethylene glycol and a Dex phase inside of the coacervate droplet. Lecommandoux and coworkers described multi-phase droplets based on Dex/PEG and polymer-protein conjugates (Fig. 4) [179]. A microfluidic approach was used to form water droplets in an oil. The water droplets consisted of a PEG/Dex ATPS. In addition, elastin-like polypeptides (ELP) conjugated to Dex or PEG were added. This way the partitioning of ELP into the PEG or Dex phase could be controlled. Furthermore, the thermoresponse of the ELP block was exploited to induce micellization upon heating, leading to a well-defined aggregation of the polymer-polypeptide conjugates in the droplets at the droplet surface. Addition of free ELP to both polymer-ELP conjugates induced formation of a mixture of two micelle types with ELP coacervate core in the droplets upon heating, i.e. a defined multi-compartment systems was obtained.

Lu and Spruijt mixed different coacervates to form multi-phases [180]. One-phase and three-phase coacervates were formed by using 2, 4 or 6 polymers. Depending on interfacial tension and density, different droplet morphologies were obtained. Interestingly, high selectivity of condensate formation was observed even if the same attractive interactions between opposite charges were present. The coacervates did not mix if the individual components have a sufficient length. Another example of spatial organization of liquid condensates was described by Huang and coworkers [181]. PEG, Dex and quaternized Dex were encapsulated into proteinosomes leading to multiple phases inside of the capsules. The phase morphology inside of the droplets could be adjusted by the PEG MW, e.g. control over the number of spherical domains up to an onion-like morphology. The inner morphology could be further shifted reversibly, e.g. by salt addition, water addition, water addition or PEG addition. Finally, the droplets were employed as environment for enzymatic catalysis.

An ATPS as base for a viscoelastic network was synthesized by Cui et al. [182]. The ATPS was composed of biocompatible polymers PNIPAM and Dex. The thermal transition of PNIPAM from hydrophilic coils to hydrophobic globules was implemented to form the porous networks with aqueous LLPS and liquid-solid phase separation. The hydrophobic interactions from the PNIPAM due to temperature change formed a reversible porous network due to transitioning from liquid to solid, with aqueous Dex containing droplets in the network. The network was also able to dissolve fused in sarcoma (FUS) proteins. By mixing FUS proteins into the PNIPAM/Dex system at 25 °C, FUS proteins separated into the Dex phase and formed condensates, when the system was then heated to 35 °C the FUS condensates dissolved in the PNIPAM network. The resulting network is a new approach to viscoelastic networks which can be assembled and disassembled through an external temperature trigger. Overall, stimulus-responsive ATPSs as shown here with temperature-, salt-, light- and magnetic-field-response are a promising area in ATPS research. The introduction of stimulus-response will bring significant opportunities for applications, e.g. purification tasks, formation of dynamic structures and catalysis, but more research is needed in this area.

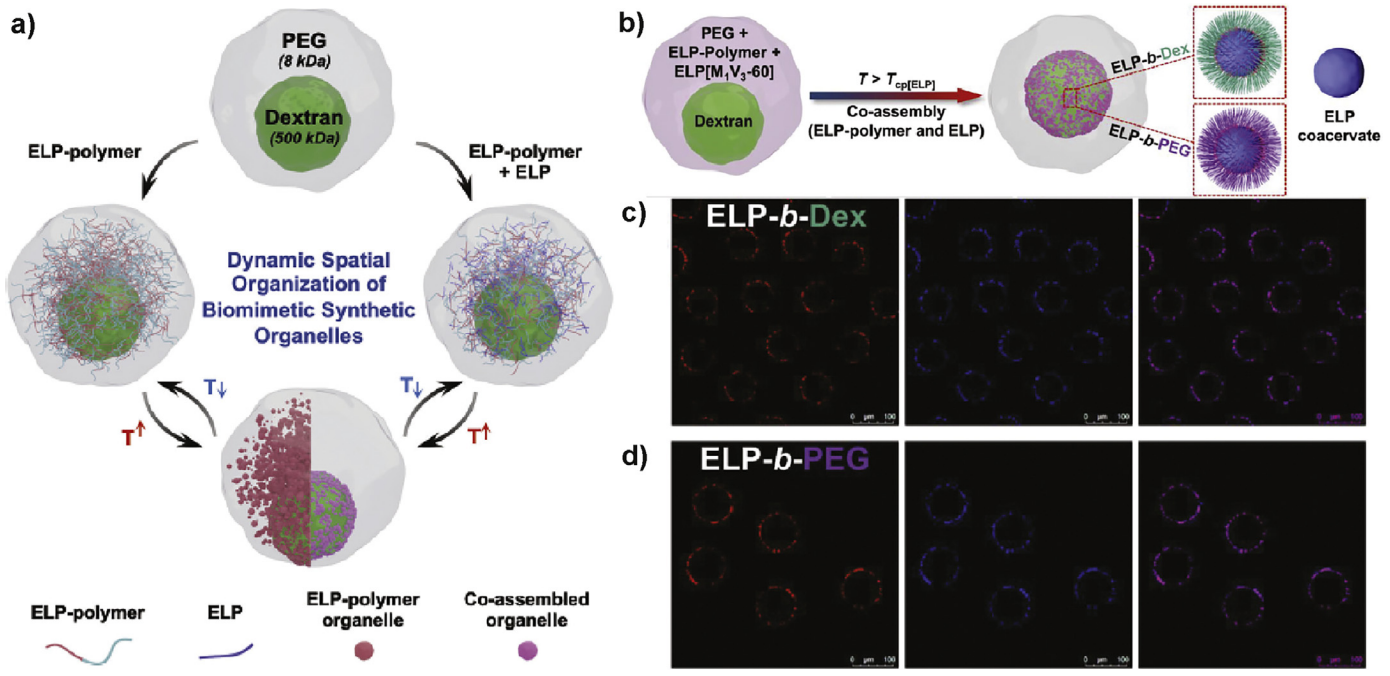


Fig. 4. (a) Schematic overview illustrating the formation of a crowded (Dex 500 kg mol^{-1} / PEG 8 kg mol^{-1}) ATPS droplet containing elastin-like polypeptide (ELP)-polymer conjugates (left just ELP-polymer conjugates; right ELP-polymer conjugates and free ELP); (b) detailed scheme for ELP-coacervate formation after heating in the ATPS droplets; (c) fluorescence imaging showing the formation of ELP coacervates at 35 °C (Red: ELP-*b*-Dex; blue: free ELP) and (d) fluorescence imaging showing the formation of ELP coacervates at 35 °C (Red: ELP-*b*-PEG; blue: free ELP). Licensed under CC-BY [179].

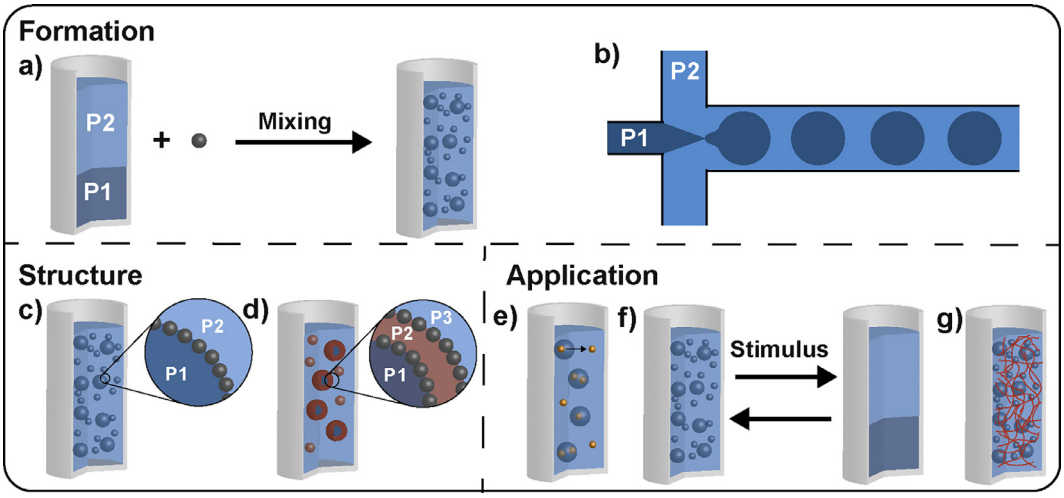
Becker et al. used ATPS for 3D printing [183]. In particular, the ATPS approach offered low viscosity bioprinting. Therefore, an ink containing one polymer type, e.g. PEG, was injected into a bath containing another polymer type, e.g. Dex, and crosslinked. Due to the lower viscosity, faster printing speed and higher cell viability was observed. Another ATPS-based printing technology was described by Kong et al. [184]. A continuous phase of polymer solution was used as bath and a second polymer solution injected with spatial control. Various polymers were used, e.g. PEG, PAAm, Dex and PAA. Careful control of the rheological characteristics of the system was employed to obtain all-liquid all-aqueous architectures with defined inner features including the option of spatial cell deposition. Overall, the 3D printing of ATPS-based hydrogels enables the formation of multicompartment materials moving away from the liquid to the solid state. As such, a broad range of additional applications get in reach, e.g. cell culture up to tissue-engineering or wound dressings.

3.2. Water-in-water emulsions

ATPS can be used to form w/w emulsions (Scheme 4a/c) [150,185] or even w/w/w emulsions (Scheme 4d) [186]. The use of these emulsions might be in the replication of cell environments [187] but also for applications in biomedicine, food and cosmetics [185,188,189]. Cell interiors contain membrane-less organelles (MLO), with the organelle formation occurring due to LLPS, the formation of MLOs is also known as compartmentalization and is a fundamental property of living cells. As 40 % of the cell volume are taken up by biomolecules, with ATPS typically being constructed with high concentrations of polymers, ATPS are good candidates to mimic these environments [190]. With the use of ATPS to form w/w emulsions, harsh organic solvents are no longer needed, which improves biocompatibility. W/w emulsions can also be used as a method for encapsulation and controlled release techniques with the ability to encapsulate delicate and water-soluble substrates [191,192]. In particular, w/w emulsions are a good method to encapsulate fragile solutes as they have an all-aqueous environment, however this also means that the formation of w/w droplets is fragile with surface tensions of ~0.1–100 μN m^{-1} meaning stabilization is required.

Stabilization of w/w emulsions requires finding suitable stabilizers for the specific ATPS. As the water/water interface spans several nanometers and does not have any hydrophobic handle to associate hydrophobic or amphiphilic molecules, common surfactants cannot be employed as stabilizer [150]. Hence, w/w emulsions are stabilized *via* nanoparticles, i.e. a Pickering emulsion approach (Scheme 4c), or *via* multi-layer droplet coverage. W/w Pickering emulsions have been studied frequently, for example making use of silica Janus nanosheets [193], gelatin microgels [194] and cellulose nanocrystals [195]. Another option of w/w emulsion stabilization reported by Buzza and coworkers is the utilization of an amphiphilic triblock terpolymer [196]. A requirement for the formation of w/w emulsions is segregative phase separation of the parent polymers. Similar to oil-in-water (o/w) emulsions, the immiscible phases can be dispersed and stabilized, albeit not with surfactants as mentioned before[150]. For the formation of w/w emulsions similar techniques as for o/w emulsions can be used, e.g. ultrasound, shaking or homogenizing (Scheme 4a). In particular, completely water-based microfluidics are useful for w/w emulsion formation with a significant control over droplet size and loading (Scheme 4b) [197]. For example, two streams of aqueous polymer solution can be designed, each containing one of a couple of incompatible polymers. In the microfluidic device these streams are brought together to form droplets containing one polymer solution in a continuous phase of the other solution. Due to the immiscibility of both phases and with proper stabilization, stable w/w droplets are formed.

The use of xanthan gum as an appropriate stabilizer for PEG and Dex ATPS was investigated by Meng et al. [198]. Indeed, xanthan gum could stabilize emulsions where one phase is dispersed as droplets into the other phase for up to one week at a concentration of 0.15 wt% and vice versa. With equal contents of both PEG



Scheme 4. Formation: (a) Water-in-water (w/w) emulsion by mixing (e.g. ultrasound, shaking, homogenizing) and (b) microfluidic approach for w/w droplet formation; Structure: (c) W/w emulsion stabilized by Pickering approach (nano particles, two polymer components and water) and (d) water-in-water-in-water emulsion; Application; (e) Cargo transport/release, (f) stimulus-response and (g) compartmentalized hydrogels.

and Dex, the domains of each of the phases were penetrated by the other phase, which could also be stabilized for up to a week at 0.3 wt% stabilizer concentration. When a strong linear shear was applied to these w/w emulsions thin strands were formed that broke up into small droplets. The droplet formation from thin strands could be slowed down to a couple hours with addition of 0.5 wt% stabilizer. A nanocellulose stabilized emulsion with liquid crystal features was described by Bai et al. [199]. The use of rod-like cellulose nanocrystals (CNC) led to spontaneous self-assembly into a helical arrangement. Stable w/w emulsions were formed featuring micrometric CNC/PEG dispersed droplets and a continuous CNC/Dex phase. After some time, the w/w emulsion demixed into an upper, droplet-lean isotropic phase and a bottom, droplet-rich cholesteric phase. Another example of w/w emulsions templated liquid crystals was described by Chu and coworkers [200]. PEG and Dex were combined with CNC to form a bicontinuous liquid crystalline phase. The emulsion phase could be switched from bicontinuous to aspherical droplets by changing the ratio of polymers.

In order to gain an understanding of how MLOs are formed in cellular environments, the LLPS of bovine serine albumin (BSA) in biphasic coacervate droplets of PLL, quaternized dextran (Q-Dex) and single stranded oligonucleotides (ss-oligo) was monitored by Bai and coworkers (Fig. 5a) [201]. Without any BSA present Q-Dex, ss-oligo and PLL formed biphasic coacervate droplets with PLL/ss-oligo core and Q-Dex/ss-oligo surrounding phase. Addition of BSA led to significantly altered droplet morphologies, with 12.5 mg/mL BSA the Q-Dex/ss-oligo phase became the core and the PLL/ss-oligo phase became smaller and scattered on the surface. Introduction of the BSA caused a phase inversion and hindrance of PLL/ss-oligo phase growth as the BSA could form complexes with PLL and Q-Dex. At increasing BSA concentration the growth of particles continued to be hindered and at a concentration of 50 mg/mL the PLL/ss-oligo and Q-Dex/ss-oligo phases connected to form worm-like structures. Thus, increasing concentration of BSA induced the structure change from inverted droplets to worm-like chains and then to a branched network structure. This is thought to be similar to the formation of cellular environments, i.e. MLOs, as the biomolecular crowders might also form complexes with target molecules, which can in turn effect the LLPS. One technique to mimic the compartmentalization of cellular environments was developed by Yuan et al. [202]. Inspired by microbially induced calcium carbonate precipitation in uratolytic bacteria an ATPS of PEG and Dex was constructed. With a Dex dispersed phase containing urease and a PEG continuous phase containing calcium chloride and urea, calcium carbonate particles then formed at the w/w interface. The resulting robust aqueous compartments differed from that of Pickering emulsions as the random adsorption of particles was eliminated. The $CaCO_3$ layer could also be tuned by the quantities of urea, urease and $CaCl_2$ added to the initial ATPS. Furthemrore, the capsules obtained could also be used for drug encapsulation and delivery as they had enhanced loading of BSA model proteins and dissolved in acidic conditions leading to release of BSA. W/w emulsions are particularly useful to mimic cellular compartments being closely related to MLOs. As research in synthetic cells is a rapidly growing field, w/w emulsions will certainly be utilized in that regard more frequently. Nevertheless, the traditional polymer system for w/w emulsion formation, i.e. PEG/Dex, has to be expanded towards other polymers to obtain results closer to actual natural processes.

The use of w/w emulsions as templates for capsule formation has been investigated frequently [204,205]. An investigation into Pickering emulsions in w/w systems for capsule formation was conducted by Zhang et al., wherein a w/w emulsion was stabilized using polydopamine nanoparticles (PDP) [206]. The resulting PEG-in-Dex emulsions stabilized with PDP were stable for up to 16 weeks. Upon 50 % dilution the formed emulsions with PDP became unstable and no droplets were observed with microscopy. To further stabilize the emulsion, the PDP particles were crosslinked with PAA and carbodiimide forming colloidosome-like structures. The resulting emulsion did not break upon dilution, yet the droplets in the emulsion swelled which indicated a success in crosslinking. Ravaine and coworkers investigated microgel stabilization of coacervate droplets and crosslinking [207]. Polyampholyte (based on *N,N*'-dimethylethylenediamine grafted PAA) coacervate droplets were formed by adjustment of the pH and stabilized by PNIPAM microgels that were grafted with free methacrylates. After droplet stabilization, the methacrylates were crosslinked *via* photoinitiation to yield crosslinked capsules. After removal of the coacervate template by pH switch, colloidosomes were obtained. The colloidosomes could then be used to encapsulate various molecules.

Huang et al. formed coacervate based capsules *via* water-in-water droplets [208]. Therefore, a solution of negatively charged sodium alginate (Alg) was added dropwise to a solution of positively charged PLL. During the penetration of the added droplets

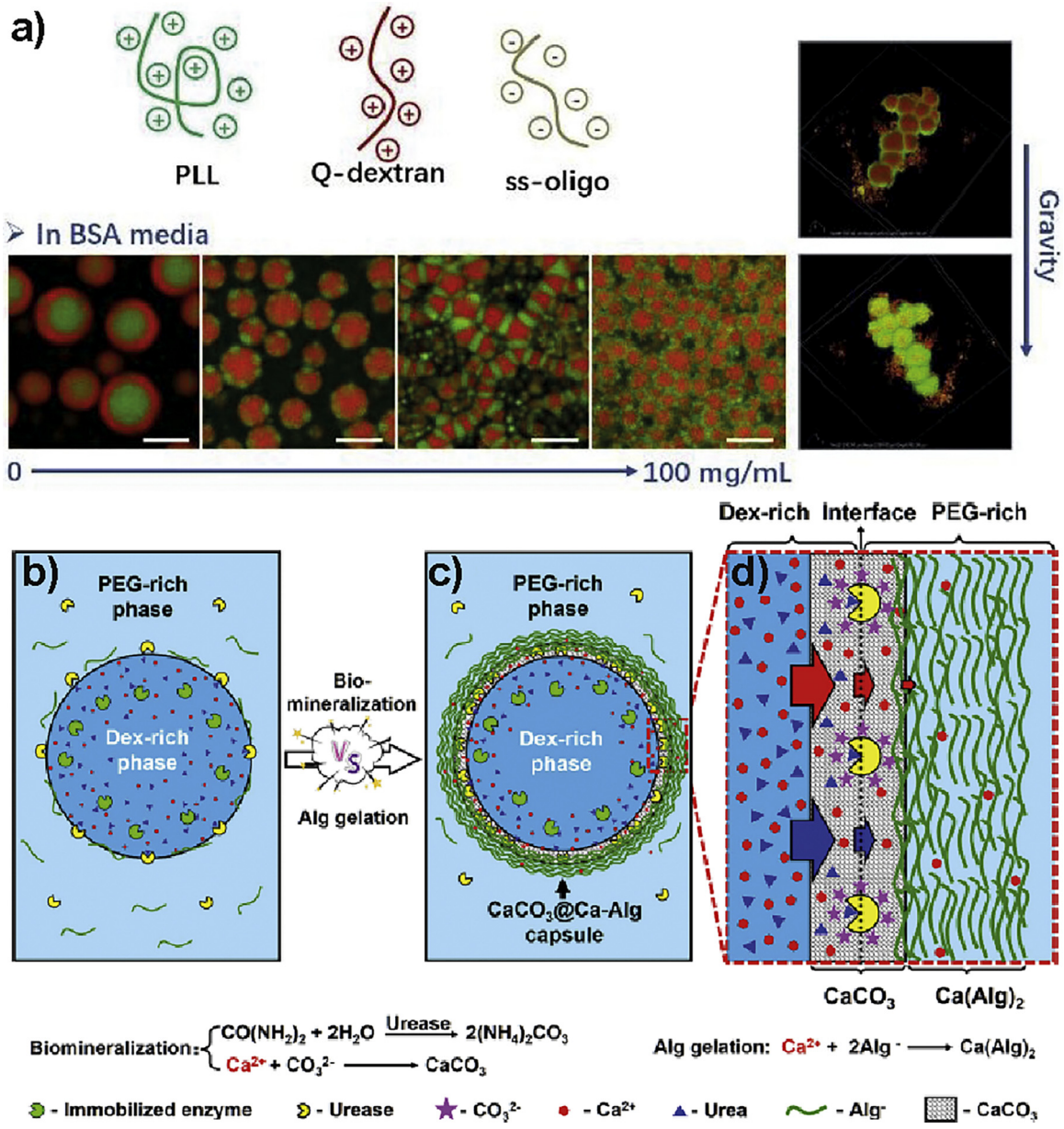


Fig. 5. Top: (a) Mimicking membrane-less organelle formation by variation of concentration of bovine serine albumin (BSA) in biphasic coacervate droplets of poly(l-lysine) (PLL; green), quaternized dextran (Q-Dex; red) and single stranded oligonucleotides (ss-oligo) (Scale bar: 15 μm.): Increasing BSA concentration leads to structure change from inverted droplets to worm-like chains and then to a branched network structure. Reproduced with permission [201]. Copyright 2022, American Chemical Society. Bottom: Preparation of double-walled $CaCO_3/Ca^{2+}$-alginate (Alg) capsules for enzyme immobilization from a w/w PEG/Dex emulsion. (b) Emulsion droplets, PEG-rich phase including urease and Alg, and Dex-rich phase containing laccase, urea, and calcium ions, (c) double-walled $CaCO_3/Ca^{2+}$-Alg capsules, and (d) competitive reaction between $CaCO_3$ mineralization and Alg gelation at the w/w emulsion droplet interface. Reproduced with permission [203]. Copyright 2023, American Chemical Society.

through the interface of the continuous phase, complex coacervation took place leading to the formation of a shell around the added droplets. The mechanical properties of droplets could be modified *via* addition of Ca^{2+} that interacted with remaining Alg inside of the droplets forming a hydrogel, which could be reversed by addition of competing monovalent ions or chelates, e.g. EDTA. Finally, the encapsulation of a lipophilic compound was probed as well as investigations of antibacterial properties. Tang et al. formed capsules with inner hard wall and outer soft wall (Fig. 5b–d) [203]. A w/w emulsion of Dex droplets containing Ca^{2+} and urea in a PEG continuous phase containing Alg was formed. Furthermore, urease was added to the system that converted urea to carbonate. At the same time gelation *via* complexation of $Ca^{2+}$and Alg took place as well as $CaCO_3$ formation. Due to the placement of the individual reactants, the inner part of the interface was mostly covered by hard $CaCO_3$, while the outer part of the interface was covered by soft calcium-alginate gel. The thickness of the capsule walls and mechanical properties could be adjusted by the ratio of reactants. Further, laccase enzymes were encapsulated and enzyme activity probed with respect to the capsule wall thickness and composition. The combination of hard and soft shell revealed an improved enzyme activity and capsule stability over soft shell alone. The formation of capsules *via* w/w emulsion templating offers several advantages over o/w or w/o emulsions, e.g. the organic solvent does not have to be removed, the absence of organic solvent enables the use of sensitive molecule loadings or the interface between continuous and dispersed phase is rather permeable. Nevertheless, there are also disadvantages of w/w templated capsules like missing access to hydrophobic polymer capsules or a more challenging control over molecular movement.

A method for the stabilization of w/w emulsions that does not make use of nanoparticles was developed by Coudon and coworkers [192], where the self-assembly of sodium oleate/1-decanol bilayers occurred on Dex rich droplets in a continuous PEG phase. It was found that these lipids assemble as multi-lamellar structures, and the resulting lipid membrane was impenetrable to proteins, oligonucleotides and also for low MW dyes. The synthesis of these stabilized emulsions just required addition of sodium oleate and 1-decanol to Dex-in-PEG droplets and vigorous shaking. The technique allowed for a simple synthesis and is capable of encapsulating biomolecules in an all-aqueous environment. Using DNA as a method to stabilize PEG/Dex w/w emulsions was investigated by Wang and coworkers [209]. The resulting emulsion presented multi-layered stabilized droplets in the presence of DNA strands as the linear DNA could pack at the w/w interfaces. In comparison to double stranded DNA, single stranded DNA is more flexible and has a stronger hydrogen bonding interaction with the Dex phase and so the stabilization is more effective. Higher concentrations of DNA led to the formation of onion-like microdroplets on glass slides. The formation of this droplet morphology is thought to be due to "vacancy condensation mechanism" when a droplet of the formed emulsion is placed on a solid slide, Dex droplets containing DNA strands form a nanofluid film against the glass slide. The DNA strands then diffuse to the meniscus of the droplet which causes vacancies where the DNA was originally. The fluid in these vacancies is then depleted and the film is gradually thinned, and the onion-like structures are formed. As shown here, new stabilization mechanisms for w/w emulsions beyond nanoparticles is an area of particular interest. This is relevant in order to modify interface properties like permeability but also stability of w/w emulsions. Furthermore, other ways to stabilize w/w emulsions might give rise to smaller droplet sizes.

Stimulus-sensitive w/w emulsions have been of interest lately, giving rise to emulsification and demulsification on demand (Scheme 4f). A light sensitive emulsion was described by Zhang et al. [210]. In this work, for stabilization of emulsions using Pickering emulsions, a stable emulsion of PEG and Dex of up to 16 weeks was formed using graphitic carbon nitride (g-CN). g-CN is a metal free photocatalyst, with its sheet like composition making it viable for use in stabilizing w/w emulsions. The resulting emulsions could be broken deliberately on dilution, surfactant addition and with light irradiation. The presence of g-CN might also open up the system to photocatalytic properties. Stimulus-sensitive stabilization of emulsions was also achieved by using a pH responsive block copolymer as investigated by Plucinski et al. [211]. Producing ultra-high MW PDMA through RAFT polymerization and combining the polymer with commercial pullulan (Pull) an ATPS was developed. This ATPS was then transformed into a stable emulsion through use of poly(styrene) (PS) nanoparticles or poly(2-(dimethylamino)ethyl methacrylate-*b*-POEGMA (PDMAEMA-*b*-POEGMA). Emulsions were able to be formed with the PS nanoparticles in both basic and acidic conditions. PDMAEMA-*b*-POEGMA block copolymer however was only able to form stable emulsions in basic conditions, which is due to the aggregation of the PDMAEMA block at basic pH. As such, the emulsion state could be turned on or off by pH switch. The result of this pH sensitive stabilized emulsion might lead to applications for biomolecule encapsulation or drug delivery.

A microgel stabilized pH-sensitive w/w emulsion was described by Zhang et al. [212]. Hydroxypropyl methylcellulose and Dex were used as polymers for the emulsion formation employing β-lactoglobulin microgels as stabilizers. Emulsions were stable between pH 3 and 5, while demulsification occurred above pH 5 due to partitioning of the microgels from the interface into the Dex phase. Another method for controlled stabilization of w/w emulsions has been developed by Pavlovic et al. [213], this time for temperature-control through the use of block copolymer PDMA-*b*-PDEA. At T_{CP} (ranging from 32 °C to 42 °C), the double hydrophilic block copolymer becomes an amphiphilic block copolymer and forms micelles and other aggregates. Once above T_{CP} the block copolymer was used to stabilize PEG 35 kg mol^{-1} – Dex 40 kg mol^{-1} emulsions. Upon cooling the emulsions rapidly became unstable and turned into ATPS eventually. Thermosensitive w/w emulsions were investigated by Ravaine and coworkers as well [214]. Microgels consisting of PNIPAM and Dex were synthesized to stabilize PEG/Dex w/w emulsions. Due to the temperature-sensitivity of PNIPAM, w/w emulsions could be formed or demulsified depending on the temperature. Moreover, it could be shown that the incorporation of Dex in the PNIPAM microgel allowed for a tailoring of the emulsion *via* the affinity of the stabilizer for one or the other phase. Similar to ATPS being investigated regarding stimulus-response, also w/w emulsions are studied in that regard, which is mainly driven by stimulus-responsive stabilizers. The recent examples of stimuli-responsive w/w emulsions allow access to another level of control. Stimulus-responsive w/w emulsions are of particular interest for delivery applications and catalysis.

The formation of compartmentalized hydrogels is a timely topic in soft materials [20]. W/w emulsions present an efficient way to form multicompartment hydrogels without hydrophobic barriers (Scheme 4g). In this regard, multicompartment hydrogels in a stabilized emulsion have been achieved in a development by Zhang et al. (Fig. 6) [215]. Using the basic PEG and Dex ATPS, the emulsion was stabilized using PDP. With addition of α–cyclodextrin (α–CD) to the system and heating to 65 °C complexes between the α–CD and the PEG continuous phase formed the compartmentalized hydrogel. With addition of competitive guests to the emulsion (such as anthranilic acid) the hydrogel network was destroyed however the emulsion was still stable. Demulsification could then be achieved by dilution. As such, the system could have four different states: w/w emulsion, hydrogel containing droplets, w/w emulsion and solution. These compartmentalized hydrogels might have applications as biomimetic hydrogel catalysts, or as a framework for hydrogel-based tissues. Nicol and coworkers described w/w emulsion-based hydrogels [216]. The ATPS of PEG/gelatin was used, where the PEG block was endfunctionalized with methacrylate groups. As such, the ATPS could be photocrosslinked after mixing with or without xanthan as stabilizer. Finally, the hydrogels were used for cryopreservation of hepatocytes with promising results. Yin et al. investigated the interface of Dex in PEG droplets stabilized by a coacervate [217]. The coacervate was formed *via* CNC and PDADMAC. The whole system could be used to 3D print Dex in PEG structures. In a similar way, Gonçalves et al. printed liquid-liquid structures based in Dex in PEG [218]. Stabilization of the printed structures was achieved by gelation of Alg and PLL. The formation of microporous polymers *via* w/w emulsions templating was described by Zhou et al. [219]. Dex in PEG droplets were formed by CNC stabilization. Furthermore, monomer and crosslinker were added. After polymerization, the template polymers were removed to reveal a highly porous hydrophilic polymer foam. Just like hydrogels from ATPS, compartmentalized hydrogels from w/w emulsions enable the translation from the liquid to the solid state, giving rise to a variety of further applications, again in tissue-engineering or cell storage but also in catalysis.

Aqueous multi-phase systems and w/w emulsions have been in the focus of research recently. These systems are completely oil-free, feature enhanced permeability and biocompatibility. Hence, application in the biomedical field, cosmetics and food are discussed frequently. Although hydrophilic polymers encompass a broad number of polymer types, the Dex/PEG system is used and studied in the literature predominantly. Certainly, there is a lot of room for new developments in the future.

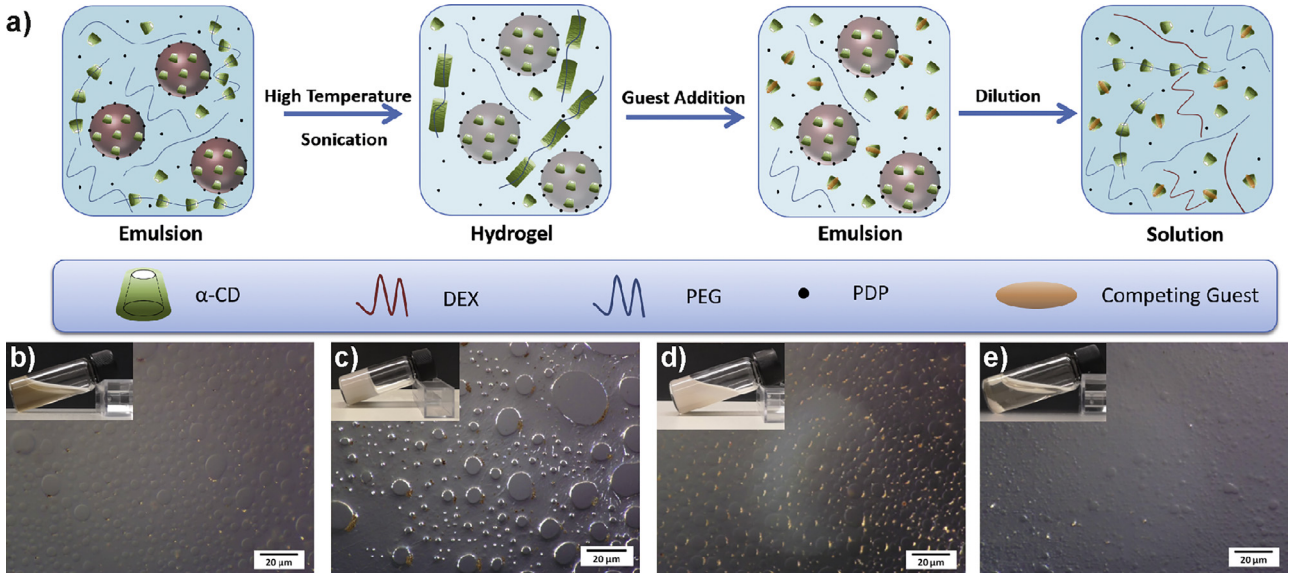


Fig. 6. Overview over compartmentalized supramolecular hydrogels from w/w emulsions: (a) Schematic depiction of the various states that can be addressed showing emulsion droplets, (b) poly(dopamine) nanoparticle (PDP) stabilized w/w emulsion of PEG (7 wt%)/Dex (3 wt%) in water, (c) compartmentalized hydrogel after addition of 140 mg mL^{-1} of α-CD, heating to 65 °C and cooling to ambient temperature, (d) reformation of emulsion state after addition of competing guests, and (e) solution state after dilution. Licensed under CC-BY [215].

4. Hydrophilic polymer self-assembly

The self-assembly of hydrophilic polymers is an important mechanism in the research area of hydrophilic polymers. In particular, self-assembly is studied with block copolymers. We will discuss the self-assembly of completely hydrophilic block copolymers (Section 4.1). Furthermore, we will give a short introduction into block copolymer self-assembly that is based on stimulus-responsive polymers (Section 4.2). Here, the self-assembled state is due to stimulus-induced amphiphilicity of the block copolymers.

4.1. Completely hydrophilic block copolymer self-assembly

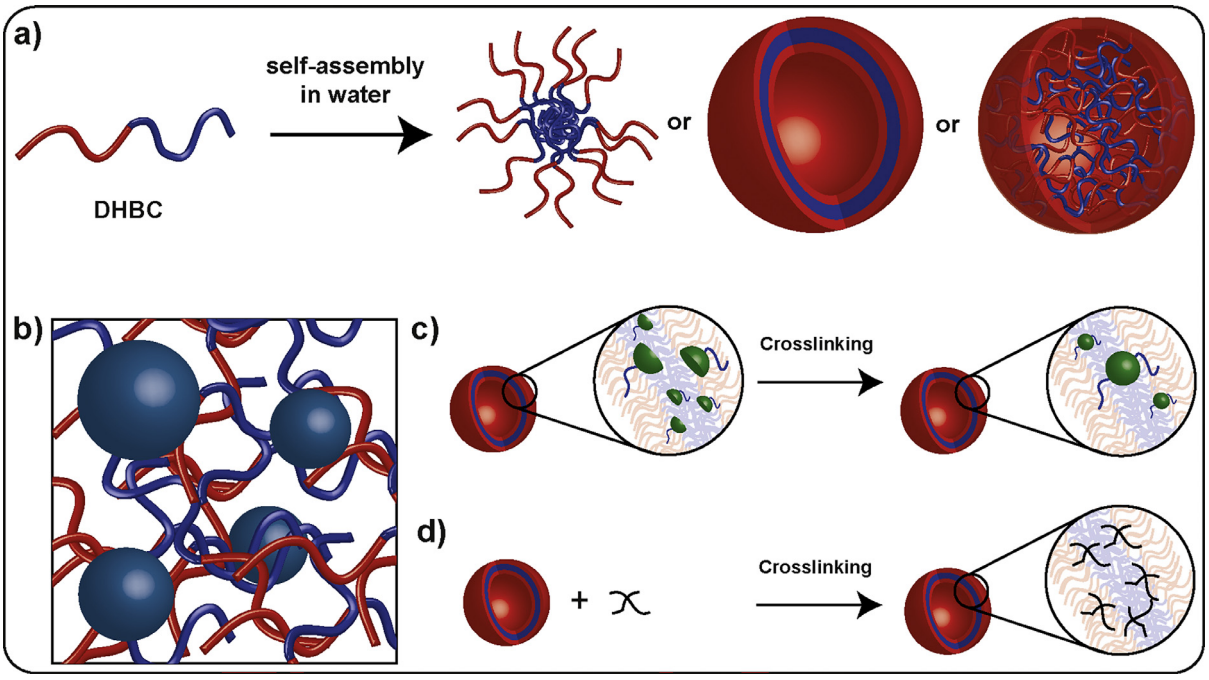
DHBCs (block polymers which consist of only hydrophilic blocks) have been gathering increasing attention (Scheme 5a). The ability of DHBCs to form self-assemblies in aqueous environments is related to that of their counterpart amphiphilic block copolymers [220,221]. While amphiphilic block copolymer self-assembly is dependent on the hydrophobic effect, DHBC self-assembly is dependent on the hydrophilic effect, i.e. the difference in hydration of each block [220]. To allow for self-assemblies of DHBCs, the hydrophilicities of the chosen blocks need to be significantly different [220,222]. Other factors, in DHBC self-assembly are polymer architectures [223,224] and block length ratios [225] as well as polymer concentration [226–228]. This way, a variety of structures was observed, e.g. micelles [229], capsules [230], droplets [227] or particles [231].

From a theoretical point of view, DHBC self-assembly can be understood by starting from ATPS [220]. As a separation of hydrophilic homopolymers occurs on the macroscopic scale, separation should proceed on the microscopic scale if both homopolymers are connected, i.e. in a DHBC, and suitable blocks and concentrations are chosen. In comparison to traditional amphiphilic block copolymer self-assembly, where aggregation is due to water being a selective solvent, the aggregation of DHBCs is not proceeding *via* the hydrophobic effect. For DHBC self-assembly this would be rather the hydrophilic effect as water performs as a neutral solvent for both blocks [222]. Also, theoretical calculations showed that the difference in water affinity is the origin of DHBC self-assembly [221]. Demixing of the hydrophilic blocks is driven *via* differing osmotic pressure in the polymer domains [226]. Due to differences in water affinity for the individual blocks, each polymer domain features a different osmotic pressure, which is compensated by demixing on the microscopic scale.

The use of fully hydrophilic polymers in biomedical applications, such as drug delivery systems or as nanoreactors, brings the advantage of increased biocompatibility and biodegradability compared to that of amphiphilic self-assemblies [232]. The water-soluble nature of DHBCs also relates to increased permeability of aggregates formed, which could indicate future use for cellular mimics [227].

A way to drive DHBC self-assemblies is *via* metal complexation [233]. This feature was used to turn DHBCs into nanoreactors as investigated by Schacher and coworkers [234]. The DHBC P*i*PrOx-*b*-poly(2-acrylamide glycolic acid) (P*i*PrOx-*b*-PAGA) was used to form inorganic-organic micelles with CdS nanoparticles. CdS is an attractive photocatalyst as it requires low energy for production of H_2 (2.4 eV) however it decomposes in case of prolonged irradiation and can aggregate. The formation of DHBC micellar templates which contain ultra-small CdS nanoparticles could help eliminate these limitations. The P*i*PrOx-*b*-PAGA block copolymer formed micelles with Cd^{2+} due to chelation of Cd^{2+} with the PAGA block forming the core of the micelle. Further reaction with Na_2S led to CdS nanoparticle formation in the micelle core. The photocatalytic ability of the resulting micelles was far greater than that of free CdS particles with hydrogen production increasing to 1.27 mmol g^{-1} h^{-1} for the micelles from 0.07 mmol g^{-1} h^{-1} of the free CdS. The increased activity could be due to the micelle core protecting the CdS from aggregation and decomposition due to photo corrosion. The authors presented a facile method for synthesis of nanoreactors and the resulting capsules might be used for photocatalytic hydrogen production for solar energy.

The DHBC Pull-*b*-PDMA showed formation of self-assembled vesicular aggregates of 200 to 300 nm at concentrations of 2 wt% to 10 wt% as indicated by light scattering studies and cryo scanning electron microscopy (SEM) by Willersinn et al. [230]. The synthesized DHBC formed these self-assemblies in absence of any external factor and might have interesting applications in the biomedical field. In contrast, aqueous solutions of this DHBC at increased concentration of around 20 wt% de-mixed and formed a contin-



Scheme 5. (a) Double hydrophilic block copolymer self-assembly into micelles, capsules or particles (reprinted with permission. Copyright 2018, John Wiley and Sons), (b) formation of DHBC coacervate phase with polymer-depleted droplets, (c) internal crosslinking and (d) external crosslinking.

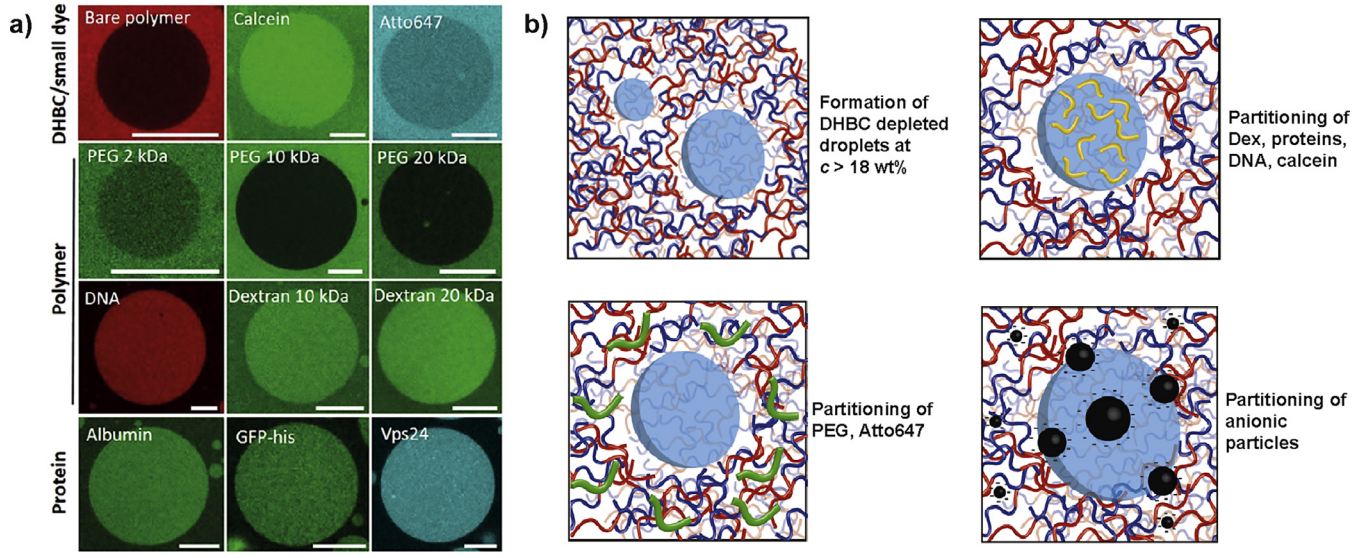


Fig. 7. (a) Confocal laser scanning microscopy images of labeled molecules enriched in either continuous DHBC-enriched phase (Pull-*b*-PDMA at 18 wt%) or DHBC depleted droplets (scale bars: 20 μm) and (b) schematic illustration of DHBC depleted water droplets in DHBC matrix and partitioning of different compounds. Adapted from literature. Licensed under CC-BY [235].

uous polymer-rich phase with water-rich droplets, which was investigated by Lira et al. (Scheme 5b, Fig. 7) [235]. The phase behavior of the DHBC is thought to be driven by interactions of the different blocks, in this case with Pull acting as a hydrogen bond donor and PDMA acting as a hydrogen bond acceptor. The resulting phase separated system was tested for partitioning of small molecules as well as large polymers and biomacromolecules. Small molecules had little preference over the polymer-depleted phase or polymer-rich phase. However, the tested guest polymers PEG and Dex showed preference for specific phases with PEG preferring the polymer-rich phase and Dex preferring the polymer-depleted phase. Tests with proteins (Vps24, GFP-his, and albumin) showed preference for the polymer-depleted phase. DNA also partitioned to the polymer-depleted phase with no influence of different sizes of DNA. The resulting DHBC system was shown to partition GFP-his protein 10 times better than that of the classic PEG-Dex ATPS and has also shown to strongly concentrate Vps24 protein in the polymer-depleted phases even at overall low protein concentrations. The phase separation occurring with just one polymer-enriched phase compared to that of ATPS formed from two polymer-enriched phases also reduces product contamination if the system is used for protein purification. As protein enrichment occurs in the polymer-depleted phase in the DHBC system, polymer contamination of the purified protein can be further reduced, indicating this system might have many uses in protein purification. Further applications such as in enzyme complex formation, nanoparticle synthesis and protein aggregation might also be applied to this system.

Subsequently, crosslinking was investigated for this system (Scheme 5c). Therefore, DHBC aggregation of the block copolymer Pull-*b*-poly(DMA-*co*-(diacetone acrylamide)) (Pull-*b*-P(DMA-*co*-DAAM)) was conducted by Plucinski et al. [236]. The copolymer was produced *via* RAFT polymerization in a ratio of 1:4 DAAM:DMA and the block copolymer was synthesized through CuAAc. The resulting block copolymer showed mesoscale phase separation at high concentrations of 20 wt%, similar to the DHBC with DMA. For lower concentrations of 0.1 wt% –1 wt% aggregates were formed of sizes around 160 nm and 450 nm. To stabilize the formed aggregates, the block copolymer was crosslinked *via* oxime formation, which produced aggregates of sizes between 350 nm and 1.3 μm that were stable at lower concentrations. These aggregates were shown to have higher stability then that of the non-crosslinked block copolymers and might have future applications in the biomedical field.

Crosslinking is an avenue to stabilize DHBC aggregates in order to shift the equilibrium from unimers to aggregates [237]. An investigation into the stabilization of the aggregates formed by the linear-brush DHBC PVP-*b*-POEGMA *via* crosslinking with α-CD was conducted by Al Nakeeb et al. (Scheme 5d) [238]. Brush DHBCs have enhanced self-assembly compared to that of linear-linear DHBCs however the aggregates still disassemble upon dilution. To prevent this disassembly, crosslinking with α-CD was attempted. The crosslinking step led to self-assemblies being partially reversible with dilution due to the supramolecular interaction of OEGMA and α-CD. The results indicated that the crosslinking did not fully stabilize formed assemblies but instead introduced an additional equilibrium which enhanced the abundance of self-assembled aggregates of the DHBC system. Addition of anthranilic acid was also used for release of the DHBC crosslinking by introducing a competitive guest molecule for the supramolecular host/guest complex, which could be induced without changing the concentration. A similar chemistry was used in the formation of thermogels that were previously synthesized from this PVP-*b*--POEGMA DHBC and investigated by Li et al. through crosslinking with α-CD[239]. By adding a 12 wt% α-CD solution to a 25 wt% solution of PVP-*b*-POEGMA and mixing, a hydrogel formed due to host/guest complexation and crystal formation between PEG and α-CD units. The formed hydrogels were then heated to 65 °C which resulted in a viscous sol and a flowing gel when cooled. By heating to 85 °C, the hydrogel turned into a clear solution and then formed a hydrogel on cooling again. The hydrogel properties depended strongly on the previous thermal history. The formed flowing gels also showed shear thinning behavior which indicated the hydrogel might be used as an injectable gel. The thermo-adaptive properties presented in this system might also be useful for sensing applications.

Another supramolecular route for crosslinking was also pursued, i.e. *via* hydrogen bonding. Crosslinking PVP-*b*-POEGMA with tannic acid was performed by Al Nakeeb et al. where tannic acid acted as non-covalent crosslinker with the PVP block *via* hydrogen bonding [240]. After addition of tannic acid, dynamic light scattering (DLS) showed decreased abundance of unimers in the system and the formation of small aggregates with a size around 15 nm. The crosslinking showed stabilization with dilution as the crosslinked aggregates showed no free polymer present with DLS at 0.1 wt% while the absence of crosslinker showed 84 % free polymer at 0.1 wt%. With addition of base to the system the hydrogen bonding between tannic acid and PVP blocks was interrupted. Thus, with addition of NaOH more unimers were present in the system and the breakdown of the small aggregates occurred. As shown here, in the area of low concentration DHBC assemblies/aggregation, crosslinking is an essential part of the systems. Crosslinking can be used to stabilize aggregates that are formed only in low abundance by shifting the equilibrium. Aggregates can also be formed at high concentration, crosslinked and then diluted. In such a way, completely hydrophilic block copolymer nanoparticles are formed that are stable in dilution and organic solvents. Another type of hydrophilic copolymer containing a brush-like block was described by Glaive et al. [241]. A statistical graft copolymer containing MeOx in the main chain as well as PEtOx grafts was synthesized by a combination of cationic ROP for the synthesis of the backbone and CuAAc for the attachment of PEtOx grafts. The individual building blocks PMeOx (containing azido pentyl oxazoline) and PEtOx precursors formed an ATPS in water. Directly related was the self-assembly of the graft copolymer that formed spherical aggregates with sizes between 200 and 700 nm in aqueous solution at high concentration as shown by DLS and cryo TEM.

Glycoproteins and glycolipids are important macromolecules for cellular function and being able to develop mimics of these macromolecules could be a way forward for developing synthetic cellular mimics, e.g. through synthesis of glycopolymers. Hence, gerecopolymer-based DHBCs appear to be a perfect target for self-assembly and have been studied as well. Loos and coworkers synthesized both hydrophilic and amphiphilic block glycopolymers for self-assembly without external triggers [242]. The double hydrophilic block glycopolymer (DHBG) consisted of a poly(2-hydroxyethyl methacrylate) (PHEMA) block and a poly(2-(β-glucosyloxy)-ethyl methacrylate) (PGEMA) block synthesized through RAFT polymerization. The DHBG formed micellar self-assemblies in aqueous environments with a PHEMA core and PGEMA corona and had low micelle self-assembly concentration of only 0.30 mg mL^{-1} which is similar to that of the formed amphiphilic block glycopolymer. The formed micelle diameter of the DHBG was 8.5–9.9 nm. These formed micelles might have applications for protein drug delivery or as biosensors through interactions with the PGEMA block. A glycopeptoid was described by Okuno et al. [243]. Maltopentaose was conjugated to poly(sarcosine) *via* CuAAc, where the polypeptoid block had a DP of 86. DLS and cryo TEM showed the formation of particles with sizes in the range of 150 nm. These results are a logical development following the discussion about glycopolymers in Section 2 as glycopolymers are gaining considerable attention due to their relevant interactions with biological systems. Thus, glycopolymers are of significant interest for DHBC assemblies, too. In addition to potential applications, glycopolymers have several useful properties, e.g. high functionality with a high number of hydroxyl groups. Furthermore, glycopolymers give access to mimicking proteoglycans, which are natural structures that form aggregates like DHBCs.

Self-assemblies of PEG_{45}-poly(mannose)$_n$ $P_{45}M_n$ DHBG were investigated by Miura and coworkers [244]. The synthesized DHBG formed well defined spherical structures in aqueous HEPES buffer containing Ca^{2+} and Mg^{2+} with particle sizes of 188, 94 and 134 nm with differing poly(mannose) chain lengths of $P_{45}M_{33}$, $P_{45}M_{60}$ and $P_{45}M_{107}$, respectively. The self-assemblies were present at a concentration of 3 g L^{-1} however at low concentrations there were no self-assemblies present. An investigation on the methods of self-assembly was conducted and found that for the $P_{45}M_{107}$ DHBG the aggregates in the presence of Ca^{2+} were around 148 nm and in the absence of Ca^{2+} the aggregates became a lot smaller at around 7 nm. When Ca^{2+} was removed through addition of EDTA the aggregates dissociated, this indicates that the DHBG requires the presence of divalent metal cations to form self-assemblies. The effect of hydrogen bonding being responsible for aggregate formation was also investigated. The addition of guanidine hydrochloride which cleaves hydrogen bonds led to decreased particle sizes of 14 nm. The aggregates were also disassembled at temperatures above 35 °C, which further indicated that hydrogen bonding is required for DHBG self-assembly as the increased temperature weakens the bonding. The investigation concurred that the self-assembly process was a balance of hydrogen and coordinate

bonding. The resulting system might have applications in mimicking the properties and functions of cell walls in plant cells. As plant cell walls are mainly composed of polysaccharides, glycopolymers might act as mimics to form similar structures. Quan et al. described galactose functionalized DHBCs for self-assembly [245]. Poly(di(ethylene glycol)methyl ether methacrylate)-*b*-poly(6-*O*-vinyladipoyl-D-galactose) was synthesized featuring a LCST in the poly(di(ethylene glycol)methyl ether methacrylate) block. Nevertheless, completely hydrophilic micellar aggregates were observed below T_{CP} at high concentration.

Higaki and coworkers investigated the lyotropic morphology of double zwitterionic diblock copolymers poly(2-((*N*-2-methacryloyloxyethyl-*N*,*N*-dimethyl)ammonio)acetate) (PCB2) and poly(4-((*N*-2-methacryloyloxyethyl-*N*,*N*-dimethyl)ammonio)butane-1-sulfonate) (PSB4) [246]. The resulting DHBC $PCB2_{45}$-*b*-$PSB4_{166}$ formed ordered structures in salt-free aqueous solutions. Upon dilution the lyotropic morphology changed. At 60 wt% it was indicated *via* SAXS that there was a disordered structure, at 50 wt% however the polymer formed hexagonally packed ordered cylinders, with the assumption that PCB2 produced cylindrical domains for conformational entropy. At 40 wt% the hexagonal morphology became more ordered with the volume fractions of the PCB2 increasing. At further dilution to 30 wt% the morphology changed from hexagonal to lamellar with PCB2 and PSB4 thickness being identical. At 20 wt% the lamellar thickness expanded and became more distorted, with dilution to 10 wt% the system became disordered and the DHBC formed micellar aggregates.

Further investigation on the effect of DP on the lyotropic morphology was conducted by the same group, where more DHBCs with varying DP were tested focusing on the block copolymer $PCB2_{112}$-*b*-$PSB4_{106}$ (Fig. 8) [247]. The concentration dependent morphologies differed to the previously investigated $PCB2_{45}$-*b*-$PSB4_{166}$, wherein at 66 wt% the $PCB2_{112}$-*b*-$PSB4_{106}$ polymer formed a periodic lamellar structure, and at a concentration of 45 wt% the lamellar thickness became even. The lamellar thickness then changed again at 40 wt%. At 26 wt% the morphology changed from lamellar to columnar, with the cylinder radius decreasing with decreasing concentration to 17 wt%. With a final dilution to 8 wt% a micellar solution formed. The results showed that polymer concentration changed the water selectivity, while above a concentration of 55 wt% there was neutral selectivity proven by the unchanged lamellar structure. Below the threshold concentration water was selective towards the PCB2 block. The further understanding of zwitterionic DHBCs might lead to applications in aqueous functional materials and in biomedical applications. The interaction of small molecule zwitterions with zwitterionic DHBCs was investigated by Higaki as well [248]. The addition of small molecule zwitterions had an impact on DHBC self-assembly depending on concentration and properties of the small molecule, e.g. partial charge density, dipole moment, and hydrophobic interactions. Micellar DHBC aggregates agglomerated with increasing concentration and at the highest concentration only unimers were present. A different system for hydrophilic polymer aggregation was described by Ghosh and coworkers. In contrast to the previously mentioned DHBC examples, random copolymers containing zwitterionic units were investigated [249]. Therefore, OEGMA was combined with glycidyl methacrylate. The epoxide side chains were ring opened with cysteine to form a hydrophilic zwitterionic repeating unit. DLS and TEM showed the formation of aggregates.

Over the past years, more and more examples of DHBC self-assembly have been reported. In particular, variation of concentration has been investigated as well as crosslinking techniques. Of course, various polymer types have been investigated regarding DHBC self-assembly, e.g. zwitterionic polymers and glycopolymers. The self-assembly systems still need further improvement in order to shift the equilibrium from unimers to self-assembled structures.

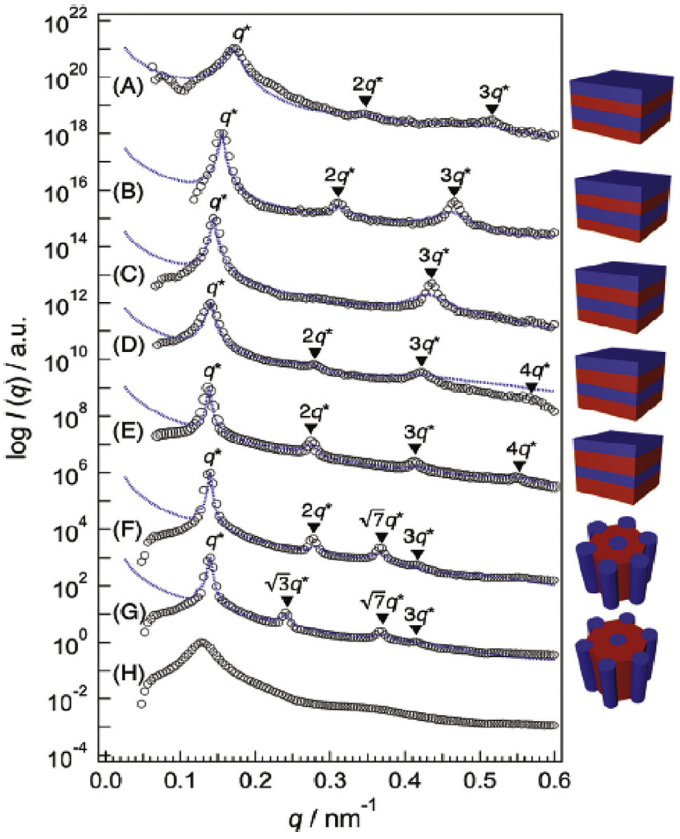
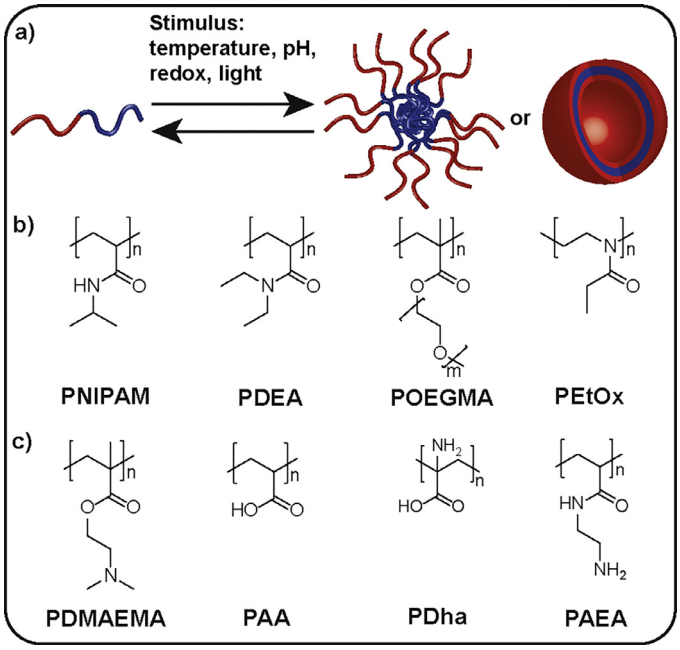


Fig. 8. SAXS profiles for the self-assembly of $PCB2_{112}$-*b*-$PSB4_{106}$ in aqueous solution (experimental shown as open circles and calculated shown as blue dotted lines) with polymer concentrations of (A) 0.66, (B) 0.55, (C) 0.45, (D) 0.40, (E) 0.35, (F) 0.26, (G) 0.17 and (H) 0.08 wt% at 25 °C and corresponding morphologies. Reproduced with permission [246]. Copyright 2022, John Wiley and Sons.

Also the self-assembly mechanism needs further investigation. On the contrary for stimulus-responsive DHBCs, the equilibrium between unimers and self-assemblies is not an issue as these block copolymers literally transform into amphiphilic block copolymers *via* external triggers, which is the focus of the next section.

4.2. Stimulus-responsive (double) hydrophilic block copolymers

A more frequent research area is the use of DHBCs that contain a stimulus-responsive block to switch solubility between hydrophilic and hydrophobic (Scheme 6). In this state the polymers are not completely hydrophilic anymore, yet we want to give a basic account on stimulus-responsive block copolymers as well. For further information the reader shall be referred to more specialized articles [250–252]. The switch between DHBC state and amphiphilic block copolymer state turns water into a selective solvent for the hydrophilic block and non-solvent for the newly formed hydrophobic block. Compared to pure DHBCs, this approach involves a strong driving force for self-assembly in the amphiphilic state, which can be exploited to form well-defined self-assemblies, like micelles or vesicles. In addition, the responsive behavior enables targeted dissolution of the assemblies or tailored permeability after crosslinking. As such, these responsive DHBCs are placed between traditional amphiphilic block copolymers and pure DHBCs. Various types of stimuli are employed frequently, including temperature [253,254], pH [255–257], light irradiation [258,259] or redox reactions [260,261]. Depending on stimulus and polymer type, a variety of potential applications is discussed for these polymers, e.g. drug-delivery, tissue engineering or electronics. The stimulus



Scheme 6. (a) Transition of a DHBC containing a stimulus-responsive block to micelles or vesicles, (b) examples of thermoresponsive polymers (poly(*N*-isopropylacrylamide) (PNIPAM), poly(*N,N*-diethylacrylamide) (PDEA), poly(oligo ethylene glycol methyl ether methacrylate) (POEGMA), poly(2-ethyl-2-oxazoline) (PEtOx)) and (c) examples of pH responsive polymers (poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), poly(acrylic acid) (PAA), poly(dehydroalanine) (PDha), poly(aminoethyl acrylamide) (PAEA)).

can be used to perform specific tasks like triggered cargo release or dissolution of a polymer template. The arguably most frequently employed stimulus is temperature making use of either an LCST or upper critical solution temperature (UCST, i.e. the polymer turns insoluble upon cooling), property in one of the blocks [262–264].

A thermoresponsive PNIPAM-*b*-PAA assigned to be used for wastewater treatment was described by Kafetzi and coworkers [265]. Therefore, the block copolymer was dissolved in a $CuSO_4$ solution, where the AA units complex with Cu^{2+} while the NIPAM units complex Cu^{2+}, HSO_4^- and SO_4^{2-}. The thermoresponsive property of the PNIPAM block was exploited to easily remove the complexed ions *via* precipitation. The formation of mixed polyplex micelles were described by Kanto ert al. [266]. They synthesized PNIPAM-*b*-poly(vinylamine) and poly(*N*-acryloyl-L-lysine)-*b*-poly(vinylamine). Next, polyplex micelles were formed *via* complexation of the cationic poly(vinylamine) block with DNA, where the zwitterionic poly(*N*-acryloyl-L-lysine) block acted as stabilizer for the micelles. The PNIPAM block introduced temperature responsivity, complexation induced change in the chiroptical nature, and temperature induced formation of assembled structures and aggregation.

Müller-Buschbaum and coworkers studied block copolymers based on PNIPAM-*b*-POEGMA [267]. Morphologies and hydration of aggregates were studied with respect to the PNIPAM block length. In particular, small angle neutron scattering (SANS) was used to study the nanostructure, which showed a shift from hierarchical structures to more defined core-shell morphologies on the way from the dissolved to the assembled state. A detailed study about the thermoresponsive behavior of polyoxazoline-based block copolymers was described by Trinh Che et al. [268]. Block copolymers containing a PEtOx block and a random copolymer block of EtOx and 2-*n*-propyl-2-oxazoline were synthesized that feature an LCST. The thermoresponsive properties of the polymers was studied by DLS and turbidimetry, revealing a complex aggregation behavior showing up to four different species where three might be present at the same time. Although LCST featuring block copolymers have been investigated frequently in the past, new developments are being introduced. Still the fundamentals of assembly formation and assembly mechanisms are investigated. Of course, also new polymers or new combinations of polymer blocks are introduced giving rise to new properties.

Lim et al. described a temperature responsive block copolymer based on sulfobetaine monomers [269]. In particular, poly(2-((2-(methacryloyloxy)ethyl)dimethylammonio)acetate)-*b*-poly(3-(*N*-(2-methacryloylethyl)-*N,N*-dimethylammonio)-propanesulfonate) was synthesized featuring a sulfobetaine and a carboxy betaine-based block. The block containing the sulfobetaine moieties showed UCST behavior, leading to micelle formation upon cooling. The polymers showed three different types of structures in solution that changed depending on temperature: unimers at high temperature, intermediates with highly swollen association coexisting with unimers and polymeric micelles. Furthermore, particle sizes and temperature response could be tuned *via* block ratios and MW. Another UCST-type polymer was explored by Oh et al. [270]. A PEG block was combined with a glycopolymer block based on mannose (D-mannose-1H-1,2,3-triazole-4-yl)methyl methacrylate). The glycopolymer showed an UCST effect in the presence of sodium chloride, albeit in the presence of guanidine hydrochloride, which is known to cleave hydrogen bonds, no T_{CP} was observed. Depending on block ratio as well as overall MW, different types of assemblies and various assembly sizes were observed. Block copolymers of polysaccharides, i.e. Dex, hyaluronic acid or laminarihexaose, and an elastin-like peptide were described by Lecommandoux and coworkers [271]. Both blocks were combined *via* CuAAc and their solution behavior studied. A particular focus was set on thermoresponsive properties as the elastin-like peptide featured a phase transition upon heating. In this way, stable nano-objects were formed with sizes in the range of several hundred nm. In comparison to block copolymers that contain an LCST block, UCST block containing block copolymers are reported less frequently. This is due to various reasons, e.g. the more complex conditions required for the UCST effect, especially salt content, and the types of polymer blocks available (challenging synthesis and analysis) [272]. Nevertheless, UCST blocks add a very useful feature to self-assemblies. Release of molecules upon heating rather than cooling seems to be more useful in the case of fever in a patient, for example. Hence, having self-assembled structures in the normal ambient temperature state and being able to break them instead of forming them under conditions out of the ambient state is intriguing in terms of application, e.g. for drug delivery.

Another frequently studied stimulus is pH, which is of particular interest due the different pH in the various environments in the human body allowing to trigger disassembly at specific locations. Battaglia and coworkers investigated the formation of vesicles from poly(2-(methacryloyloxy)ethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-*b*-PDIAEMA) [273]. The block copolymer was completely water-soluble in acidic environment and formed assembled structures at elevated pH. Aggregate morphology showed variations due to block lengths as well as formation temperature. The differences in aggregate formation with temperature variation were explained with the effect of temperature on the pKa of PDIAEMA leading to a change in protonation degree connected with a change in swelling and molecular packing parameter. Shin et al. described giant polymersomes formed from PEG-*b*-PAA [274]. Due to the carboxylic acid functions in the PAA block, solubility could be switched from soluble at pH > 4.5 to insoluble at pH < 4.5. In this work polymersomes were formed at pH 2.3 *via* gel-assisted rehydration method and were visualized by confocal laser scanning microscopy (CLSM) showing vesicle diameters above 5 µm. Interestingly, vesicles were obtained

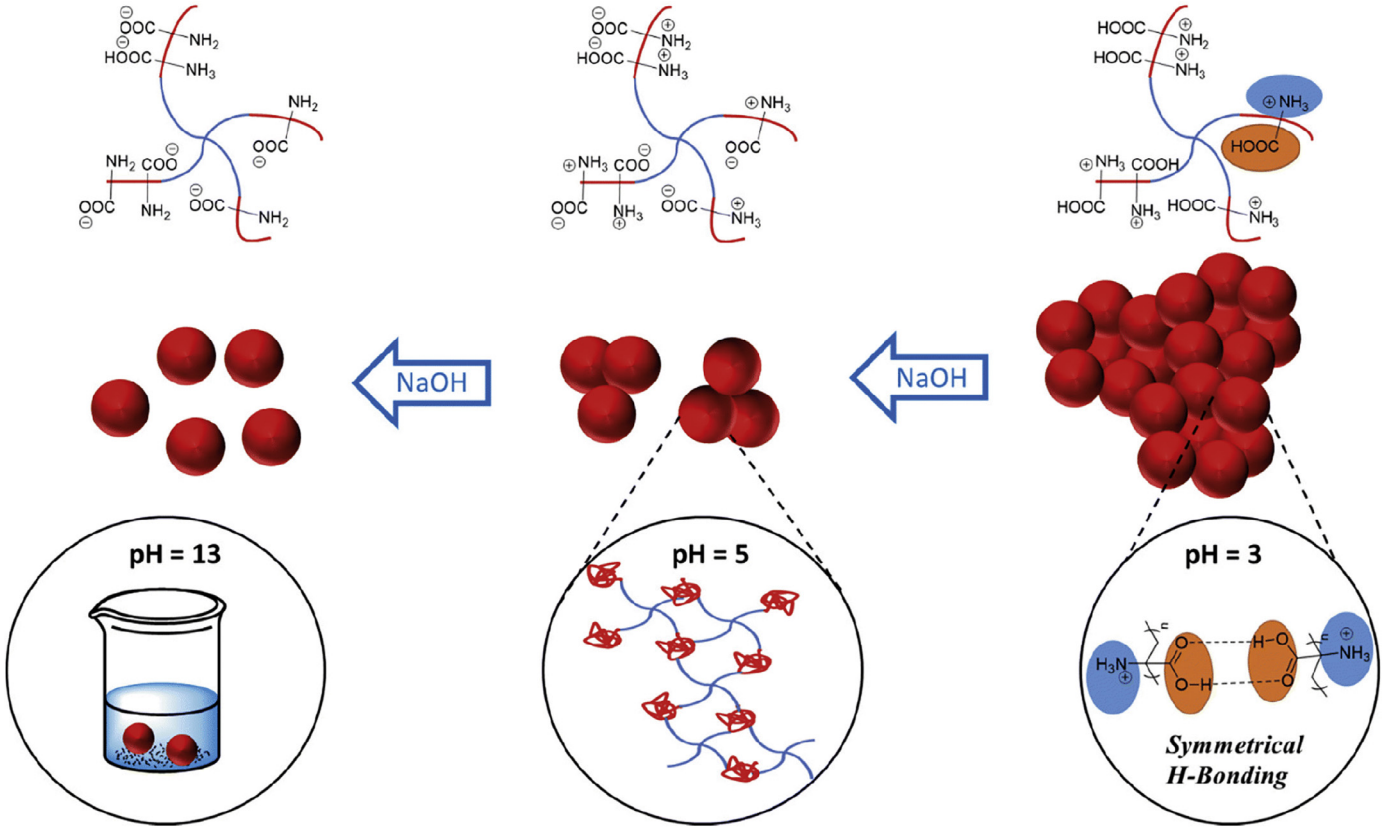


Fig. 9. Overview of the aggregation behavior of DHBC star polymer [PEG_{27}-*b*-poly(dehydroalanine)]$_4$ with different pH. Reproduced with permission [277]. Copyright 2022, Royal Society of Chemistry.

for a wide range of block ratios, which might be due to additional hydrogen bonding interactions between the polymer blocks.

The encapsulation of cargo and pH responsive release was studied as well leading to efficient release and complete dissolution of the polymer material. The disruption of a giant vesicle *via* pH was studied by Sincari et al. [275]. The block copolymer PEG-*b*-PDIAEMA was chosen to form vesicles due to the very small window of the solubility switch of the PDIAEMA block between pH 6.30 and 6.95. Giant unilamellar vesicles were formed *via* microfluidics at pH 7.4 together with PEG-*b*-poly(1,2-butadiene) and their response to pH changes studied by CLSM. In particular, the disruption of vesicles was studied with respect to pH value, kinetics of disruption and the change of vesicle shape during the process. A pH responsive triblock copolymer forming micelles were described by Luo et al. [276]. Therefore, the triblock copolymer poly(methacrylic acid)-*b*-PEG-*b*-poly(methacrylic acid) (PMAA-*b*-PEG-*b*-PMAA) was synthesized in various MWs between 6 kg mol^{-1} and 80 kg mol^{-1} that formed micelles with sizes between 18 and 89 nm depending on block lengths. Furthermore, cytotoxicity of the triblock copolymers was probed showing low toxicity below micelle concentrations of 400 mg L^{-1}. Encapsulation and pH dependent release of the corticosteroid drug Prednisone revealed sustained release at pH 7.4. These examples show similar to thermoresponsive block copolymers that pH response introduces the option of cargo encapsulation and release as well, which is of particular use due to the different environments in the body with different pH.

A double hydrophilic star polymer was described by Schacher and coworkers (Fig. 9) [277]. At first, a four arm PEG star polymer was synthesized, then the arms were chain extended with *tert*-butoxycarbonylaminomethyl acrylate leading to pH responsive ampholytic poly(dehydroalanine) (PDha) blocks after deprotection. Due to the ampholytic PDha block an interesting pH responsive self-assembly behavior was observed. At pH 13, small particles were formed, stabilized by an excess of negative charges due to deprotonation of carboxylic acid groups. At pH 5, slightly larger particles were observed where the PDha block is in a zwitterionic state. At pH below 3, large aggregates with sizes in the micrometer range were prevalent, which is probably due to hydrogen bonding of the individual chains. PDha was also employed by the Schacher group in graft copolymers in order to synthesize templates for pH-controlled formation of metal nanoparticles [278]. In particular, PDha-*graft*-PEG was synthesized featuring a PEG sidechain at every repeating unit. Tuning of solution pH was used to tailor net charges and charge density of the grafted polymers to control complexation with Ag^+ and $[AuCl_4]^-$ salts. Nanoparticles were formed by reduction, where nanoalloy composition could be controlled easily *via* pH adjustment. Overall, block copolymers featuring a pH responsive block that can act as acid or base, i.e. an ampholytic block, enable access to a broader range of self-assembled structures by using one polymer only. This is of considerable interest in order to switch the activity of self-assembled systems, e.g. as environment in catalysis or for complex release tasks.

Multiple stimulus-responsive blocks can be introduced into one polymer as well, which allows solubility switching of individual blocks [279–281]. As such, self-assembled structures can be inverted [254,282], morphologies switched [283] or solubility/permeability of specific domains in self-assemblies controlled precisely [284]. Recently, Leer and coworkers presented core-crosslinked micelles that were designed to be temperature and pH responsive [285]. A block copolymer comprised of thermoresponsive PDEA and pH responsive poly(aminoethyl acrylamide) (PAEA) was synthesized including the monomer 2-(pyridin-2-yldisulfanyl)ethyl acrylate acting as disulfide containing crosslinker.

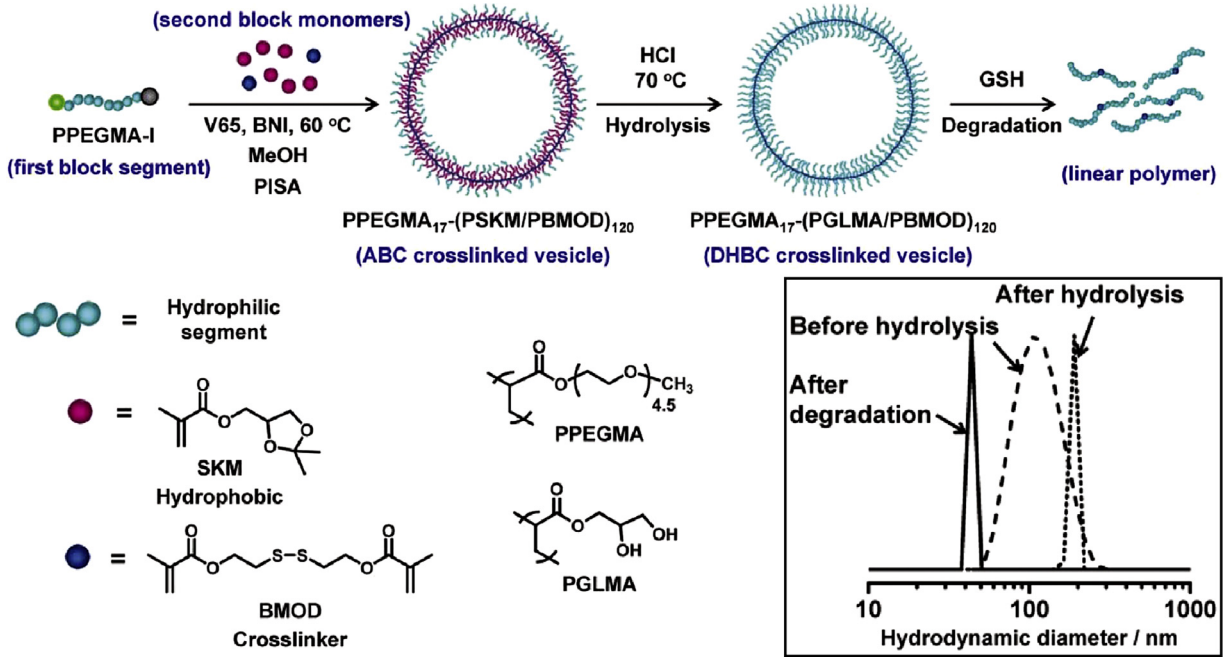


Fig. 10. Synthesis and degradation of reduction-responsive DHBC-based crosslinked vesicle. Inset: Intensity-weighted particle size distributions at 0.1 wt% concentration of $PPEGMA_{17}$-*b*-(PSKM-*co*-PBMOD)$_{120}$ vesicles in water before (dashed line), after HCl treatment (dotted line) and after GSH treatment (solid line) obtained *via* DLS. Adapted with permission [288]. Copyright 2021, Royal Society of Chemistry.

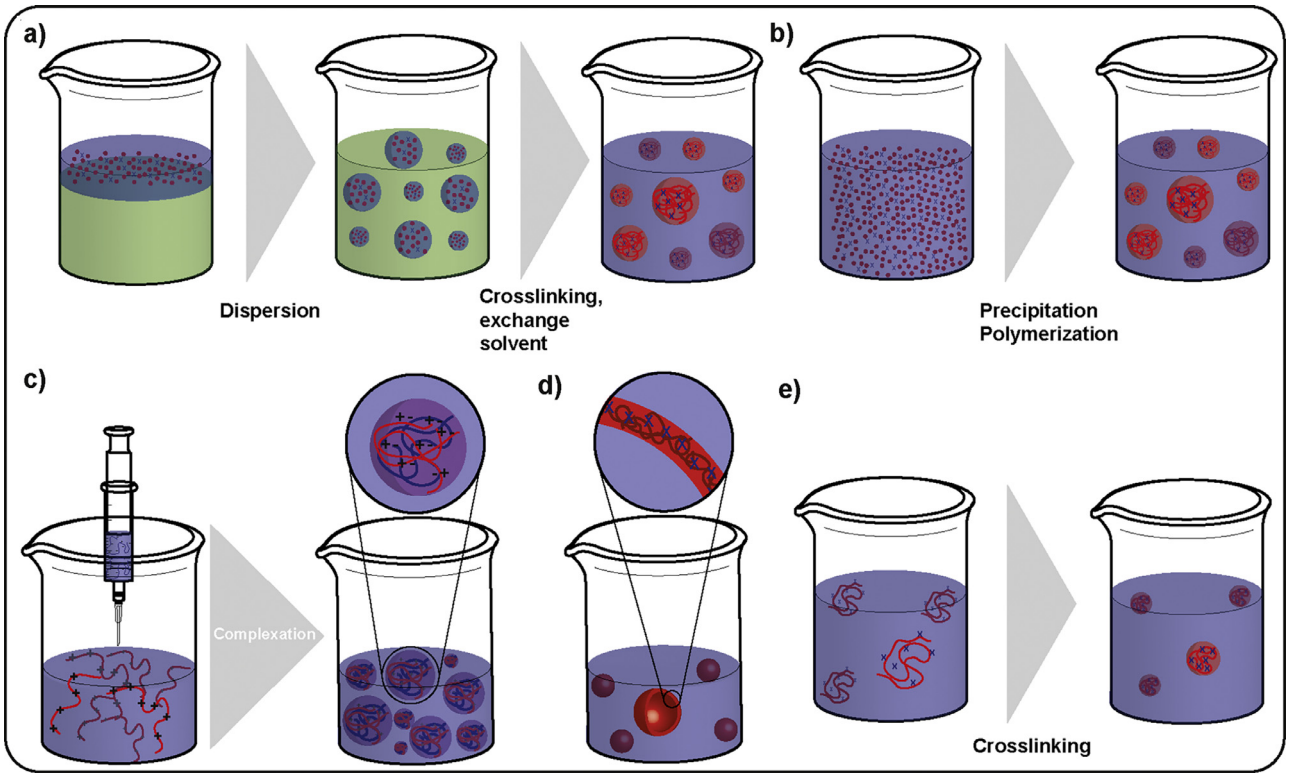
After heating above the T_{CP} of the PDEA block, micelles formed that were crosslinked *via* a thiol exchange reaction with 1,6-hexane dithiol. In this way, core-crosslinked micelles were obtained that swelled after cooling below T_{CP} of the PDEA-based core. Finally, the micelles were used for plasmid DNA delivery to HEK183T cells showing a 13-fold higher transfection efficiency compared to free PAEA. A reduction responsive and pH cleavable block copolymer was described by Maruya-Li et al. [286]. A PEG block was combined with a POEGMA block copolymerized with a disulfide containing monomer. Between both blocks an acetal linkage was introduced to insert a pH sensitive cleavage point between both blocks. After heating above the T_{CP} of the POEGMA block, crosslinking was performed *via* reduction of the disulfide with a catalytic amount of reducing agent followed by disulfide thiol exchange. Core-crosslinked micelles were formed that were further studied for the delivery of small drug molecules like Doxorubicin (DOX). A combination of light and temperature trigger was published by Wu et al. [287]. Two block copolymers were synthesized consisting of a PEG block and a PNIPAM block. The PNIPAM blocks further contained benzoxadiazole- or Rhodamine B-based monomers acting as Förster resonance energy transfer (FRET) donor and acceptor, respectively. Furthermore, the PNIPAM block contained 5-(2-(dimethylamino)ethoxy)-2-nitrobenzyl acrylate, i.e. a monomer that can be cleaved upon UV irradiation leading to an acrylic acid repeating unit. Both block copolymer types were mixed in aqueous solution leading to weak FRET. Upon heating above T_{CP} mixed micelles were formed as observed by a significant increase in FRET. Application of UV-irradiation was followed by disassembly of the micelles due to a shift in T_{CP} of the PNIPAM block to lower temperatures as hydrophobic nitrobenzyl sidechains were converted into polar carboxylic acids. These examples show the additional value obtained, when multiple stimulus-responsive blocks are introduced into the same block copolymer. As such, more intricate self-assembly pathways and self-assembled structures are in reach.

A redox responsive vesicle system was described by Goto and coworkers, who used polymerization induced self-assembly to form crosslinked vesicles (Fig. 10) [288]. In order to obtain vesicles, a POEGMA starting block was extended with the hydrophobic monomer solketal methacrylate (SKM) and the crosslinker bis(2-methacryloyl)oxyethyl disulfide (BMOD). In the next step the SKM block was hydrolyzed to leave a completely hydrophilic vesicle behind. Degradation of the disulfide crosslinker *via* reduction led to disruption of the vesicles and formation of completely water-soluble DHBCs. Brendel and coworkers described a redox responsive system as well [289]. The monomer *N*-acryloyl thiomorpholine was employed to synthesize amphiphilic block copolymers *via* polymerization induced self-assembly starting from a PAM block that led to micelles with poly(*N*-acryloyl thiomorpholine) core. Oxidation of the thioether functionality in *N*-acryloyl thiomorpholine led to sulfoxide formation and changed the solubility of the polymer block from hydrophobic to hydrophilic. The utilization of stimulus-responsive blocks to form defined self-assemblies followed by crosslinking and switch to hydrophilic again, either permanently or just by the stimulus, is a way to obtain completely hydrophilic structures. As such, the disadvantage of pure double hydrophilic block copolymer self-assembly, i.e. a weak driving-force for self-assembly, can be circumvented.

DHBCs including a stimulus-responsive block allow the formation of a plethora of aggregate structures introducing various interesting properties. In contrast to traditional amphiphilic block copolymers, DHBCs with stimulus-responsive blocks enable triggered disassembly allowing for release of cargo on demand but also triggered permeability. As such, applications in the biomedical field, cosmetics or food are discussed frequently. In addition to self-assemblies from DHBCs, complete hydrophilic polymers have found significant attention as basis for hydrophilic polymer particles as well, which will be discussed in the following section.

5. Hydrophilic polymer particles

As mentioned in previous chapters, hydrophilic polymer particles belong to the most relevant structures derived from hydrophilic polymers [290–292]. In contrast to structures formed from amphiphilic polymers, we will focus on completely hy-



Scheme 7. (a) Formation of hydrophilic polymer particles *via* dispersion polymerization, (b) formation of hydrophilic polymer particles *via* precipitation polymerization, (c) formation of hydrophilic polymer particles *via* complex coacervation (Adapted from the literature [290], licensed under CC-BY), (d) hydrophilic polymer capsules and (e) formation of hydrophilic single chain nanoparticles.

drophilic particles in the following discussion. Similar to particles formed from amphiphilic polymers, hydrophilic polymer particles play an important role for a variety of applications, e.g. in drug-delivery, as food additive or in emulsion stabilization. Completely hydrophilic particles feature a considerably different behavior in aqueous environment compared to amphiphilic particles mainly due to the absence of hydrophobic barriers and hydrophobic phases.

All sorts of hydrophilic polymers can be used for particle formation, yet the formation mechanism introduces some restrictions or requirements. Hydrophilic polymer particles can be formed by coacervate formation or various means of crosslinking (Scheme 7). Coacervate particles are based on liquid-liquid phase separation, as mentioned above, driven by supramolecular interactions. For example, ionic interactions are exploited in the so-called complex coacervates or hydrogen bonding. In particular, coacervate particles have been in the focus of research in recent years [293,294]. Hydrogel particles are based on crosslinking, either *via* supramolecular [295] or covalent pathways [296], e.g. *via* crosslinking during free radical polymerization in suspension [297,298], in mini emulsion [299–301] or in precipitation polymerization [302,303].

5.1. Covalently crosslinked hydrophilic polymer particles

Covalently crosslinked particles feature a high stability against dilution and nonaqueous solvents, which can be a significant factor in processing. Particle sizes can be controlled to great extent *via* the preparation method (Scheme 7). A covalent crosslinking approach and polymer functionalization of the polysaccharide Pull was described by Fundueanu et al. [304]. Therefore, hydroxyl functionalities in Pull were exploited to react with epichlorohydrin in a water-in-oil emulsion leading to microspheres. In the next step, Ce(III) was used to initiate NIPAM/AAm copolymerization from the Pull backbone. In addition, Pull hydroxyl groups were used to ring-open succinic anhydride leading to carboxyl functionalities. As such, polysaccharide microspheres with thermo-and pH-responsive grafts were obtained. Dex-based beads were described by Lima et al. [305]. The introduction of crosslinkable groups was performed *via* ring-opening of glycidyl methacrylate, which leaves methacrylate groups behind. Finally, methacrylate-grafted Dex was combined with PNIPAM containing crosslinker and polymerized under UV light in microdroplets on a superhydrophobic surface leading to thermoresponsive polymer beads. Ning and coworkers formed hydrophilic polymer particles *via* dispersion polymerization in methanol [306]. These particles were then included in calcite synthesis leading to composites with ordered inner structure. Interestingly, the particles deformed from spherical to ellipsoid during the crystallization process depending on crosslinking degree.

A frequent application of hydrophilic polymer particles is the encapsulation of enzymes for biotechnology. Anionic nanogels for enzyme encapsulation were described by Ni et al. (Fig. 11) [307]. Here, the inclusion of negatively charged polymers brought an increase in enzyme encapsulation for improved catalytic effect. To form the nanogels, a cationic-neutral DHBC was used as template, i.e. PEG-*b*-PDMAEMA, and then AA as well as a crosslinker were photopolymerized in aqueous solution in the presence of the template. Due to the electrostatic interactions between DHBC and AA, a micelle with PEG shell and polycation/crosslinked AA was formed. Subsequent washing with NaCl solution led to the removal of the template and pure PAA nanogels. In the next step, enzymes, namely cytochrome C or lysozyme, were introduced into the particles and enzyme kinetics studied showing enhanced enzyme activity.

An approach towards single-enzyme nanogels was described by Delaittre and coworkers [308]. Various types of enzymes were encapsulated into a PAAm/bisacrylamide nanogel without pre-

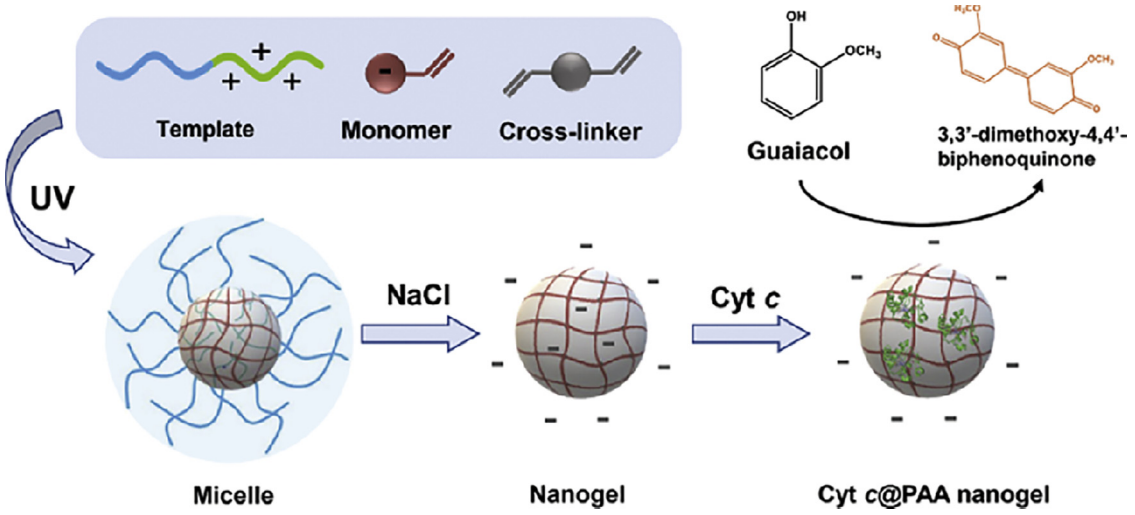


Fig. 11. Synthesis of PAA nanogels *via* PEG-*b*-PDMAEMA template for cytochrome C encapsulation and conversion of guaiacol to 3,3′-dimethoxy-4,4′-biphenoquinone. Reproduced with permission [307]. Copyright 2022, American Chemical Society.

modification of the used enzymes due to the presence of sucrose in the polymerization mixture. The gel shell thickness could be controlled by the polymerization conditions, which enabled an improved control over enzyme stability and activity. This is of particular importance in multi-enzyme processes, where the interaction between different enzymes often has to be suppressed. Pich and coworkers synthesized Janus microgels by precipitation polymerization [309]. NIPAM was copolymerized with 1-vinylimidazole (leading to cationic charge) or itaconic acid (leading to anionic charge) together with a bisacrylamide crosslinker. Both copolymerizations were started individually and the solutions of the growing precursors were mixed after predetermined times. A careful adjustment of start of mixing, mixing time and polymerization time after mixing allowed the formation of Janus microgels composed of a binary complex of polycationic and polyanionic microgels. Furthermore, the microgels were temperature and pH sensitive. Li et al. described the formation of polysaccharide-based nanogels and their encapsulation of lysozyme [310]. Starch was oxidized and crosslinked with sodium trimetaphosphate under basic condition leading to an anionic hydrogel material. In the next step, the hydrogel was dried, grinded and filtered to obtain microgels. The uptake and release of lysozyme was studied with respect to crosslinking density and charge density of the microgels. It could be shown that an intermediate crosslinking density is ideal for efficient uptake. As these examples show, the formation of hydrophilic polymer particles for encapsulation of proteins/enzymes has various advantages. First of all, stability of proteins/enzymes is increased this way, which is of particular use for biotechnological applications. Furthermore, stimulus-response can be introduced in order to be able to control access to the biomacromolecules remotely and being hydrophilic, access to the protein/enzyme is not hindered by hydrophobic barriers.

An avenue for the formation of microgels *via* microfluidics was presented by Thiele and coworkers [311]. Two directions were studied to form PNIPAM-based microgels, namely gelation *via* free radical chain growth and gelation *via* polymer-analogous crosslinking. Both approaches were initiated by light. The authors showed that polymer-analogous crosslinking led to improved microgel homogeneity *via* phase-contrast microscopy, optical diffraction tomography, and confocal Brillouin microscopy. Finally, the microgels were employed as temperature sensors in optical stretchers that are used to measure mechanical properties of living cells. For the measurement laser light is employed, which might introduce heat into the cell environment. The measurement of temperature during the stretching experiment is required to obtain a reliable calibration of the optical stretcher setup taking temperature effects into account. Sinclair et al. described zwitterionic microgels as injectable cell culture scaffold [312]. Therefore, a macroscopic hydrogel formed from zwitterionic monomers was converted into microgels by extrusion through a steel mesh. The microgels could be lyophilized and hydrated in a reversible fashion. Furthermore, the microgels were malleable due to inter-microgel interactions leading to shear-thinning hydrogel materials. These materials were then used to prepare injectable formulations with therapeutic cells or drug-loaded microspheres as well as a stem cell culture scaffold.

5.2. *Hydrophilic polymer particles* via *supramolecular interactions*

Supramolecular crosslinking to form hydrophilic particles has been in the focus of researchers, mainly due to the reversible nature of the crosslinking process enabling degradation, adaptability and response to stimuli as well as their similarity to membraneless organelles. Especially, coacervate-based particles formed by electrostatic interactions are utilized frequently. Nanoparticle formation *via* polyelectrolyte complexation of a cationic-neutral block copolymer was described by Tirrell and coworkers [313]. In particular, poly(L-lysine) (PLL), poly((vinylbenzyl)trimethylammonium chloride), PLL-*b*-PEG and poly((vinylbenzyl)trimethylammonium chloride)-*b*-PEG were synthesized and their complexation with single stranded as well as double stranded DNA investigated. Block copolymer and single stranded DNA led to the formation of spherical micelles, while combination with double stranded DNA led to cylindrical micelles. The corresponding cationic homopolymers formed coacervates with single stranded DNA and precipitates with double stranded DNA. The studies showed that the polyelectrolyte complex micelle morphology depended strongly on the hybridization state of the DNA, while the sizes of formed particles correlated with the length of the cationic block. Photoactive coacervate droplets were described by Liu et al. [314]. For coacervate formation a sulfonated anionic porphyrin was combined with cationic diethylamino Dex leading to droplets with sizes in the micrometer range. The porphyrin incorporation enabled photocatalysis *via* J-aggregates. This could be used for oxidation of water-soluble iodide *via* singlet oxygen or the oxidation of hydrophobic 1,3-diphenylisobenzofuran *via* singlet oxygen. Hence, both hydrophilic and hydrophobic substrates could be reacted within the membrane-less coacervates.

A proteoglycan-mimicking coacervate was described by Zandi et al. [315]. PLL was combined with either the polysaccharide dermatan sulfate sodium salt or carboxylic acid containing gum tragacanth. As such, polyelectrolyte complex nanoparticles were obtained that could be used to bind to positively charged drugs or growth factor vascular endothelial growth factor (VEGF). The combination of the coacervate nanoparticles and VEGF led to enhanced and prolonged mitogenic as well as metabolic activity of the growth factor in human umbilical vein endothelial cells (HUVEC). An aggrecan mimetic was described by Kipper and coworkers [316]. Chitosan or *N,N,N*-trimethyl chitosan were combined with heparin or chondroitin sulfate to obtain glucosaminoglycan-rich polyelectrolyte nanoparticles with a similar structure to aggrecan. The particles showed binding and stabilization effect on fibroblast growth factor-2 leading to enhanced mitogenic activity and the metabolic activity of ovine marrow stromal cells in low serum media. These polyelectrolyte nanoparticles are a promising route for use in growth factor delivery. Proteoglycans are highly interesting structures with significant biological value, i.e. in the organization of tissues. Therefore, mimicking these structures with synthetic polymers allows a better understanding of the biological system but also enables exploitation in tissue-engineering.

Reineke and coworkers studied the interaction of DNA and block copolymer micelles toward application in DNA delivery [317]. In particular, the complexation between plasmid DNA and various polymers were investigated, i.e. PDMAEMA homopolymers, PDMAEMA-*b*-PEG, PDMAEMA-*b*-poly(*n*-butyl methacrylate) and PEG-*b*-PDMAEMA-*b*-poly(*n*-butyl methacrylate). On one hand, PDMAEMA homopolymers and PDMAEMA-*b*-PEG complexed with pDNA to form polyplexes, which led to large aggregates or globular structures over time. These aggregates had pDNA condensed in the core leading to decreased availability for gene transfection. On the other hand, PDMAEMA-*b*-poly(*n*-butyl methacrylate) as well as PEG-*b*-PDMAEMA-*b*-poly(*n*-butyl methacrylate) formed micelles first due to the hydrophobic poly(*n*-butyl methacrylate) and complexed pDNA in the form of micelleplexes. These micelleplexes had pDNA strands wrapped around individual micelles and pDNA strands bridging micelles. This structure formation increased transfection efficiency due to improved internalization, maintaining the native helical B form of DNA due to the packing motif in the micelleplex that mimics DNA wrapping of histones in chromatin and a higher number of amine groups per micelleplex aiding in endosomal escape.

Recently, dynamics of coacervate droplets have been of considerable interest [318], as also discussed in Section 3, in the formation of coacervates from poly(sarcosine) and maltopentaose [243]. Boekhoven and coworkers described the formation of complex coacervates from a cationic peptide, poly(uridylic acid potassium salt) and poly(styrene sulfonate) [319]. A mixture of three components revealed the formation of various coacervate morphologies depending on peptide concentration, e.g. formation of one phase, two phases or three phases. In order to introduce chemical reaction induced dynamics, a peptide with an aspartic acid group at the C-terminus was introduced that could be converted into an anhydride upon addition of a chemical fuel, i.e. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. The peptide initialized droplet formation only in the state with anhydride at the C-terminus, which had a short half-life of 45 s due to hydrolysis. Therefore, the presence of droplets was relying on the presence of fuel and droplets dissolved after depletion of fuel. Mann and coworkers described coacervate droplets containing bacterial cultures [320]. Hence, coacervates from PDADMAC and adenosine triphosphate were formed to capture *E. coli* and *Pseudomonas aeruginosa*. The membrane-free coacervates could capture the bacteria within minutes (*E. coli* inside and *P. aeruginosa* at the surface). Lysis of the bacterial cells led to coacervate droplets covered with *P. aeruginosa* derived lipids and *E. coli* cytoplasmic components in the interior, the so called bacteriogenic protocells. Further tests revealed that these coacervates were able to perform simple cyctomimetic tasks, e.g. enzymatic catalysis or gene expression. Addition of carboxymethyl Dex (leading to a cytoplasm mimic) as well as histone and DNA (leading to a nucleus mimic) allowed ATPS formation inside of the coacervate. A combination of these bacteriogenic protocells with *E. coli* could be exploited to introduce ongoing ATP production and sustained activity of the bacteriogenic protocells, e.g. in gene expression or filament formation. Prolonged experiments led to non-spherical amoeba-like bacteriogenic protocells. Overall, recent developments in coacervate dynamics and functions move ever close to synthetic cells that behave similarly to living cells or are able to fulfil tasks that living cells do. Furthermore, these systems enable the investigation of cellular processes on a simple system, e.g. the effect of diffusion and molecular transport [321].

A coacervate-to-vesicle/capsule transformation was described by Jang et al. (Fig. 12) [322]. Recombinant fusion proteins were used to form coacervates, namely a globular mCherry protein fused to a glutamic acid rich leucine zipper (mCherry-Z_E), a globular enhanced Green Fluorescent Protein fused to a glutamic acid rich leucine zipper (eGFP-Z_E) and arginine-rich cationic leucine zipper fused to an elastin-like polypeptide domain (Z_R-ELP). The combination of Z_E and Z_R led to coacervates at elevated temperature that transitioned into vesicles due the formation of an amphiphilic structure. Exploiting the synthesis of mCherry and enhanced Green Fluorescent Protein fused Z_E, the pathways of coacervate to vesicle transition were studied. Mixture of both types of fluorescent proteins with Z_R-ELP at low temperature led to a random accumulation of proteins in the vesicle membrane. Combination of the proteins after coacervate formation led to vesicle membranes with individual domains composed of one or the other of the fluorescent proteins. Mixing at the stage of formed vesicles led to a simple mixture of vesicles composed of either mCherry or eGFP-Z_E and Z_R-ELP. The authors found out that solution temperature and aging-time can be exploited to control the size of formed coacervates. Furthermore, the size and composition of coacervates correlated with the size and membrane organization of the final vesicles.

Especially the development of coacervate or hydrogel capsules has been in the focus of research recently [191]. An approach of capsule formation by suspension polymerization was presented by Omura et al. [323]. Anionic 2-acrylamido-2-methyl-1-propanesulfonic acid, cationic 1-vinylimidazole and oligo(ethylene glycol) diacrylate (OEGDA) were copolymerized in an inverse suspension in the presence of Dex. The structure of the particles and shell permeability could be tuned *via* crosslinker and Dex concentration. Furthermore, the permeability could be adjusted by salt addition. A similar structure was described by Hann et al., yet in a w/w emulsion system [324]. At first, Dex and cationic PDADMAC droplets were formed in a PEG enriched continuous phase *via* electrospray. In order to form a crosslinked shell, the PEG phase contained silica nanoparticles that interacted with the PDADMAC component in the droplets. Due to osmotic stress imbalance, diffusion of water out of the capsules and PEG droplets from the continuous phase into the capsules took place, leading to double emulsions.

A multicompartment core-shell particle for enzyme catalysis was described by Qu et al. [325]. The authors used spray ejection to form core particles that could be designed to consist of various compartments from Alg (crosslinked by Ca^{2+}), chitosan (crosslinked *via* tripolyphosphate), poly(acrylonitrile) or cellulose acetate with various loadings e.g. iron oxide nanoparticles or dyes. In the next step, the core particles were decorated with calcium carbonate particles that were coated with poly(dopamine) for improved adhesion. Finally, the core particles were incubated with Alg and D-(+)-gluconic acid-δ-lactone. During the incubation D-

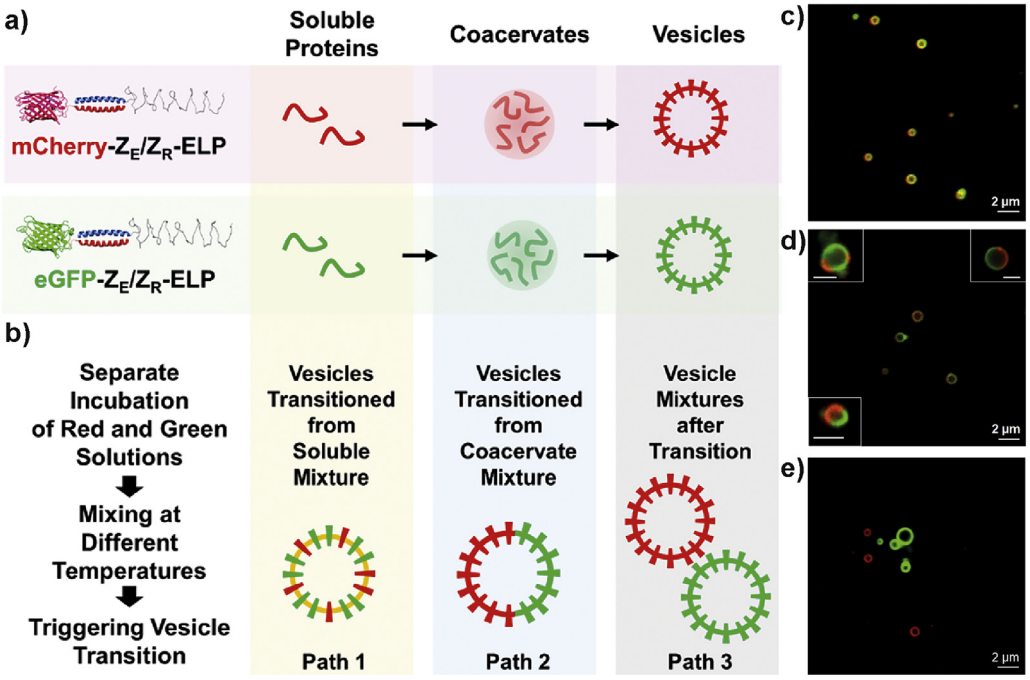


Fig. 12. Schematic of coacervate-to-vesicle transition and CLSM imaging: (a) Soluble proteins of either mCherry-ZE/ZR-ELP or eGFP-ZE/ZR-ELP fusion protein complexes transition into red or green fluorescent vesicles through coacervates with increasing temperature, (b) three types of vesicle samples controlled by incubation pathway (Path 1 vesicles directly transitioned from soluble protein mixtures, Path 2 vesicles from mixtures of coacervates and Path 3 vesicles mixed after coacervate formation), (c) homogeneous red and green protein "yellow" vesicles made from soluble protein mixtures according to Path 1, (d) partially segregated, heterogeneous protein vesicles transitioned from red and green coacervate mixtures according to Path 2 and (e) single color red and green vesicles mixed after transition according to Path 3. Adapted with permission [322]. Copyright 2019, American Chemical Society.

(+)-gluconic acid-δ-lactone acidified the solution slowly triggering the release of Ca^{2+} from calcium carbonate nanoparticles, which initiated subsequent crosslinking of Alg around the core particle. As such, core-shell particles with multiple compartments were obtained that were used for enzyme cascade reactions with a two-compartment core. As cascade reaction xanthine oxidation by xanthine oxidase was employed leading to the production of H_2O_2 and the consumption of H_2O_2 in the oxidation of o-phenylenediamine *via* peroxidase. Rodriguez-Emmenegger and coworkers described a supramolecular vesicle formation [326]. A copolymer consisting of a zwitterionic and a cationic monomer was synthesized. The copolymer was combined with negatively charged didodecylhydrogen phosphate to form an amphiphilic structure. Interestingly, vesicular structures were obtained that closely resembled low MW phospholipids. Co-assembly with structure-directing glycolipids, incorporation of transmembrane proteins and fusion with the cell periphery of *E. coli* was possible. Overall, capsule and core/shell particle formation plays an important role in hydrophilic particles. Commonly, methods like suspension polymerization or microfluidics making use of organic or w/w emulsions templates are used for the formation of these structures. In particular supramolecular crosslinking allows a dynamic shell formation, for example for interactions with the environment but also for degradability.

5.3. Hydrophilic single-chain nanoparticles

In recent years, single-chain nanoparticles and single-chain folding have been in the focus of research [327–329]. Of course, hydrophilic single-chain nanoparticles and folding of hydrophilic polymers have been investigated as well, for example with supramolecular, dynamic covalent or covalent folding/crosslinking points [330–332]. Single-chain nanoparticles are formed from single polymer chains and have been in discussion regarding a way to mimic the folding behavior of proteins. In particular, the aim is to obtain structural protein mimics that also feature protein like functions, e.g. catalytic properties [333–335], as well as biomedical applications in drug-delivery or biosensing [335]. In order to form single-chain nanoparticles, crosslinking has to be performed at high dilution to avoid the connection of multiple polymer chains. As such, individual particles are obtained formed from a single polymer chain (Scheme 7e). Depending on the crosslinking mechanism, the particles can be concentrated afterwards, which is the case for covalent and dynamic covalent crosslinking. In contrast, for supramolecular crosslinking particle-particle connections might happen during concentration.

Supramolecular hydrophilic single-chain nanoparticles were described by Becer and coworkers [336]. Therefore, a multiblock copolymer of DMA was synthesized that contained three distinct blocks with comonomers, i.e. a block with DMA and an adamantly functional monomer, a block with DMA and a mannose-based glycomonomer and a block with DMA and β-cyclodextrin functional repeating units. Due to the supramolecular host/guest interaction between adamantane and β-cyclodextrin the polymer folded into single chain nanoparticles that exposed mannose. Next, the interaction between nanoparticles in the folded and non-folded state with lectins were studied. It could be shown that the folded state had a stronger interaction with lectins probably due to a better accessibility of the sugar units. Thus, the effect of secondary structure in polymers on biological targets was indicated. Paulusse and coworkers described the formation of glycopolymer-based single-chain nanoparticles for cell uptake [337]. At first, a copolymer a glycomonomer based on glucose (C1 or C6 connected, reducing or non-reducing) and a xanthate containing monomer was synthesized. Subsequently, the xanthate was hydrolyzed to reveal thiol groups that could be used to crosslink the single-chain with a diacrylate *via* thiol-Michael reaction. Binding assays with ConA showed the strongest binding with C6-bound glucose particles. The

cell uptake was similar with respect to reducing or non-reducing properties of the sugar but differed depending on glucose connection to the polymer. Biomimetic β-lens crystallins based on single-chain nanoparticles were described by Liang et al. [338]. A copolymer of AAm, AA and phenyl acrylamide was synthesized that was crosslinked under high dilution *via* amide formation between the carboxylic acids and added hexamethylenediamine. The refractive index and viscosity of the single chain nanoparticle solution matched that of β-lens crystallins. Furthermore, high biocompatibility was observed making these particles a promising replacement of β-lens crystallins for treatment of presbyopia and cataract. Hydrophilic single-chain nanoparticles add several new features to the field of hydrophilic particles. These particles are rather small, especially compared to microgels, and very defined as only single chains are used to form the particle, which can be analyzed *via* common methods of polymer chemistry. As such, hydrophilic single-chain nanoparticles are ideal candidates for the investigation of structure-property relationships, where a lot of novel directions can be expected in the future.

Completely hydrophilic polymer particles feature a variety of properties and can be designed according to the targeted application. Similar to other hydrophilic polymer structures, applications in biomedicine are the main target, although other directions are of interest as well, e.g. in catalysis, food and cosmetics.

6. Visions for the future

As described in the preceding sections, hydrophilic polymers encompass a broad range of polymer types and properties. Of course, there are various challenges, where hydrophilic polymers can be an important factor for future applications, and new challenges are arising frequently. Although researchers have introduced a broad range of hydrophilic polymers in the past, there is still room for the development of completely new polymer types.

A serious challenge in biomedical applications is the development of anti-PEG antibodies in the general population and patients, leading to severe side effects of drugs and vaccines [69] including loss of therapeutic efficacy and an increase in adverse effects. Hence, hydrophilic polymers as substitute of PEG are highly sought after [68,339]. Materials like polyoxazolines, polyphosphoesters, polypept(o)ides and PHPMA are discussed frequently in that regard with some of them being subject to clinical trials at the moment or in the past [111,340–342]. The potential replacements require similar or better properties than PEG, e.g. high water-solubility, stability and stealth behavior in the body. The development of new hydrophilic polymers is also a useful endeavor to improve efficiency of therapeutics, as the development of therapeutics is ongoing and requires continuous development of supporting technology, e.g. for drug delivery. In particular, new elaborate drugs like proteins, enzymes or DNA need well-designed delivery mechanisms, where hydrophilic polymers play a prime role. As applications in the biomedical field are a major target for hydrophilic polymers, biocompatibility has to be taken into account and tested thoroughly. Research has shown that changes in polymer architectures and composition can have a significant effect on biological properties and here biocompatibility should be considered a prime property to be investigated. A particular use for hydrophilic polymers, mainly for PEG so far, is introduction as stabilizer in liposome-based carriers, e.g. in the Pfizer/Biontech COVID-19 vaccine, which is highly relevant in current formulation development and is applied in addition to current basic research of polymersome-based carriers. Due to the issue with side effects of PEG-based formulations, a significant potential for real world applications lies in PEG replacements and this will be a growing concern in the coming years. Hence, the introduction of new PEG-based polymers and polymer architectures will play a significant role in the application of hydrophilic polymers for pharmaceutical applications.

A research area that received more and more attention in the last years is aqueous multi-phase systems and w/w emulsions. Currently, these aqueous polymer systems are utilized for separation tasks, which has been a longstanding area of application [343]. In polymer and colloids research a strong focus is placed on the structuring of ATPS and w/w emulsions. Also, the loading of ATPS and w/w emulsions with various compounds is investigated frequently, in particular with respect to bio(macro)molecules [344]. Furthermore, the stabilization of w/w emulsions with various stabilizers and the search for new stabilizing mechanisms has been of interest over the last years. This field has significant growth potential, in particular as only a limited number of polymer types has been investigated so far. Looking at the applications discussed for these systems in sensing, drug delivery, catalysis, cosmetics, food but also templating for inorganic structures, the drive towards completely aqueous multi-phase systems seems a logical direction. The application of ATPS and w/w emulsions in 3D printing and bioprinting will have a profound effect on the capability of these methods [345,346]. One can expect a large variety of innovative technologies resulting from this combination. A feature of polymers that gives rise to a plethora of new opportunities but has been only marginally introduced into ATPS and w/w emulsions, is stimulus response [182,347]. As stimulus-responsive polymers have been a significant area of research in the last decades, introduction into ATPS and w/w emulsions will definitely be an area to look out for. Aqueous multi-phase systems are also prone to be utilized as medium for chemical reactions [348]. Especially, partitioning of catalysts and substrate molecules can be exploited in order to control reaction outcomes and kinetics. Applications in the life sciences and biotechnology appear to be a prime target for further research. Here, permeability of multi-phase systems is a key property that allows completely new release and interaction profiles.

Permeability is also a main driver for the development of coacervate systems and completely hydrophilic block copolymer self-assemblies. It should be noted though that coacervates and aqueous multi-phase systems are significantly higher developed than completely hydrophilic block copolymer self-assemblies/aggregates. The similarity to natural MLOs is indicatory of possible applications for permeable hydrophilic polymer assemblies, i.e. in synthetic cells [349]. Mimicking natural systems is a significant target for polymer chemists and - as biological systems work in aqueous environment – hydrophilic polymers play an important role here. In many cases, these systems are designed to mimic nature for an improved understanding of biological processes. We can expect that more and more sophisticated systems will be developed in this direction in the coming years [350]. Also the combination of aqueous multi-phase systems with classical compartmentalized systems, e.g. liposomes, will open up new possibilities to study biological processes [351]. Certainly, permeability plays a considerable role for catalysis and hence biotechnology as well. Starting from the current achievements in coacervates and aqueous multi-phase systems, one can imagine further developments of more complex and tunable catalyst environments. Another area that is starting to receive attention is the formation of multicompartment hydrogels based on permeable compartments, which allows the formation of a macroscopic material that has microscopic permeable compartments included [20]. These materials will be of particular use in tissue-engineering but also applications in hydrogel patches for wound dressings. For completely hydrophilic block copolymers, research focused on assembly/aggregate structures and investigations of the underlying mechanisms so far. Development towards functional systems similar to coacervates and multi-phase systems will be the next signif-

icant step for completely hydrophilic block copolymer-based systems.

Stimulus-responsive polymers have found significant interest over the last years as well. Also here, permeability is a main property but also the formation of assemblies/particles in relevant sizes for biomedical applications [251,352]. In the area of hydrophilic stimulus-responsive polymers and block copolymers, developments over the last decade have moved ever closer to application with a main target in drug-delivery. Selective release based on external stimuli is of particular interest for this application, e.g. by increasing permeability of capsules or vesicles as well as slow dissolution of the carrier *via* external stimuli. Hybrid materials are another area, where stimulus-responsive polymers and block copolymers find use, for example in combination inorganic materials. In particular, the area of (bio)sensing is a growing field that benefits from stimulus-responsive polymers. The utilization of stimulus-responsive hydrophilic polymer-based vesicles as nanoreactors is also expanding, as in this case stimuli can be used to control and tailor catalytic tasks. Besides these rather obvious directions, stimulus-responsive hydrophilic polymers can be also used for surface modifications, e.g. as a self-cleaning surface against (bio)fouling [119,353].

Hydrophilic nanoparticle research has been on the rise in the last couple of years as well [290]. An advantage of hydrophilic polymer particles is their rather easy and scalable synthesis, it should be noted that single-chain nanoparticles are more challenging to scale up due to high dilution required. Hydrophilic polymer particles will play a significant role in hydrophilic polymer materials in the coming years. In particular, uses in pharmaceuticals for drug delivery, in food or in cosmetics will be a focus of applied research. Here, features like high and adjustable stability as well as predictable loading/release profiles are crucial. Furthermore, formation of Pickering emulsions employing hydrophilic particles is a growing area in conjunction with the more frequent use of Pickering emulsions in order to avoid small molecule surfactants that might be harmful for end-users. Hydrophilic polymer particles based on polysaccharides are especially useful due to their renewable sources and degradability [290].

As mentioned before, one driver for the development of new hydrophilic polymers is the depletion of fossil resources, which requires new avenues to existing hydrophilic polymers but also development of hydrophilic polymers from renewable resources, e.g. polysaccharides [354], poly(itaconic acid) [355], lignin [356] or other water-soluble building blocks like keto glutaric acid [357]. As sustainable chemistry and sustainable products receive more and more attention in the consumer base, a real world application of sustainable alternatives for hydrophilic polymers is a likely development over the coming years. This is especially relevant for products that are purchased by the end consumer directly, where personal attitude might influence the buyer decision process. Most promising seem materials based on polysaccharides with a focus on (modified) chitosan and cellulose as they provide sustainable resources but also degradability without competing with food.

A major concern with all polymers is their degradability [358,359]. This also counts for hydrophilic polymers [35,360,361]. Although hydrophilic polymers are used to a great extent for low volume and high value applications, i.e. in the biomedical field, still a broad range of hydrophilic polymers is found on large markets, e.g. superabsorbers, food ingredients and cosmetic formulations (refer to Chapter 2). In particular, hydrophilic polymers are water-soluble and will be transported in aqueous solution around the whole planet. Unlike hydrophobic polymers, hydrophilic polymers cannot be filtered or collected from the environment – and the task is already a serious challenge for hydrophobic polymers. Due to solubility and mobility, various hydrophilic polymers are persisting in the environment [361]. Recent studies showed the negative ecological impact of acryl-based hydrophilic polymers and their low biodegradability [362]. Degradability concerns mostly C-C backbone containing polymers as well as polyethers but also a variety of other polymers. The degradability issue has found some developments recently, e.g. incorporation of comonomers in C-C main chains [363–365]. The incorporation of cyclic ketene acetals as comonomers is a promising avenue to introduce degradable ester bonds along the backbone of C-C main chain polymers. In particular, more focus was put on cyclic ketene acetals as comonomers in hydrophilic polymers recently, showing a direction to improve degradation [364,365]. Biological pathways are of particular interest for degradation especially in seawater environment [35,366,367]. Also degradation *via* acoustic cavitation was investigated [368]. As PEG is one the most frequently used hydrophilic polymer, biological degradation pathways for PEG [369,370] as well as chemical pathways towards degradation were studied [371]. Despite the efforts in the research of degradation of hydrophilic polymers, the field is far from technology ready solutions. As discussed above, several strategies for hydrophilic polymer degradation are in development. Especially, the use of biobased polymers like polysaccharides and in part polyamides is a way to degradable hydrophilic polymer materials. Nevertheless, the goal of degradable hydrophilic polymers is very challenging if C-C main chain polymers are considered. One of the issues is the plethora of hydrophilic polymers used and is brought into the environment that all have different characteristics regarding their degradation. In contrast to hydrophobic polymers, hydrophilic polymers are much harder to remove from aqueous environment but also from soil. The use of water as a way to dissolve hydrophilic polymers in order to separate them from other materials generates significant amounts of wastewater. Therefore, degradation mechanisms that take place in aqueous environment and soil are most relevant. Of course, the potential toxicity of degradation products has to be considered as well. As such, degradability is a serious issue associated with hydrophilic polymers and a fundamental change of focus is needed as well as significant amount work has to be done to bring research in the right direction. In addition to new degradation pathways, emission of hydrophilic polymers into the environment has to be mitigated as well.

In the realm of technology and large-scale applications, various developments have to be considered. In particular, PAAm is used on a large scale for flocculation applications [35] but issues with toxic and carcinogenic acrylamide require a closer look at alternatives, e.g. copolymers from methyl acrylate and [2-(acryloyloxy)ethyl]-trimethyl ammonium chloride have been discussed [372] as well as polysaccharides. One industry that requires water-soluble polymers as flocculants is hydrometallurgy and mineral processing [373]. Due to the rising demand of metals for all sorts of every-day applications, also the demand for water-soluble polymers in the processing is increasing significantly. In a similar way, modern oil and gas processing, e.g. chemical enhanced oil recovery, requires hydrophilic polymers [374,375] even more so the harder it gets to extract the crude oil from the reservoir, where questions about degradability and sources are relevant as well. In detergents, new developments target to introduce alternatives for PAA in order to improve degradability. Here polyNCAs with carboxylic acids in the side group are discussed that feature a peptide backbone and hence biodegradability. Other options include poly(epoxysuccinic acid) derivatives that can be synthesized by anionic ROP [376]. This example shows clearly that the transition to heteroatom containing backbones is a viable option to improve degradability. A new application for superabsorbers in agriculture has been discussed frequently [36]. These could be either used for sustained hydration of plants but also for controlled release of fertilizers. Besides agriculture and the common use in hygiene products, superabsorbers are also a promising material for drug-

delivery. Looking at the large scale applications of hydrophilic polymers mentioned in here and the connected markets (pharmaceuticals, home care (detergents), water treatment (flocculants), mineral treatment (flocculants), and oil/gas industry), a significant growth for the use of hydrophilic polymers can be expected.

7. Conclusions

Overall, hydrophilic polymers belong to the most important types of polymers with a broad range of applications and prospective applications on the horizon. There are high volume applications in technology but also very sophisticated directions in biomedicine and catalysis. While the portfolio of hydrophilic polymers is significant already, new polymers are developed as well in order to give rise to new properties and applications but also to improve known systems. A quickly developing research area are LLPS systems and the whole field of artificial cells that nicely connects to synthetic biology/chemical biology. The traditional area of amphiphilic block copolymer self-assembly has been expanded with stimulus-responsive hydrophilic block copolymers as well as completely hydrophilic block copolymers over the last decades bringing new properties like tailored permeability. In a similar way, completely hydrophilic polymer particles have been investigated mainly regarding encapsulation and release applications making use of improved permeability properties. A plethora of nano- and micro-structures formed from hydrophilic polymers have been investigated and many more innovations can be expected. Nevertheless, there are major challenges to overcome for a sustainable future of this class of polymers that will be a focus of future research. In particular, degradability and the development of sustainable alternatives to common hydrophilic polymers will be a major research goal. Looking at the progress of research in the area of hydrophilic polymers it is obvious that hydrophilic polymers have a bright future ahead and a broad range of developments are going to emerge in the years to come.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Niamh Bayliss: Writing – original draft, Writing – review & editing. **Bernhard V.K.J. Schmidt:** Writing – review & editing.

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