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Polymer bioconjugates: Modern design concepts toward precision hybrid materials

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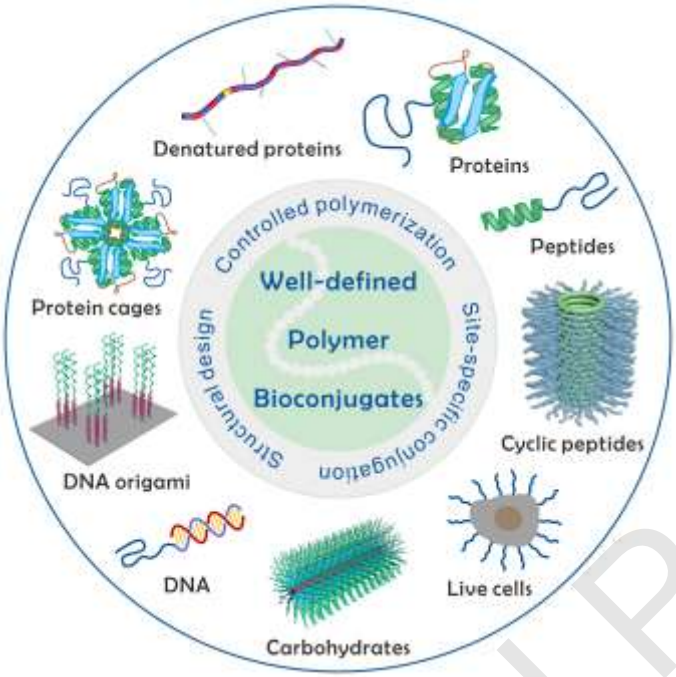
Graphical abstract



ABSTRACT

The conjugation of synthetic polymers with various biomolecules provides an easy access to biohybrid materials which combine advantages from both the synthetic world and Nature. Due to the rapid development of synthetic tools and deepening understanding of biomolecule structure and function, these polymer bioconjugates are not only important for biomedical applications, but also can serve as innovative constructs in materials science. This review summarizes a selection of structurally defined polymer bioconjugates and their application as building blocks for preparing hierarchical biohybrid materials. From this perspective, we discuss and illustrate recent breakthroughs, which portray how the field may potentially develop. We first introduce the general synthetic approaches that have been employed for the construction of precision polymer bioconjugates. Various chemistries for site-specific conjugation, different approaches to control the size, distribution, topology, and function of polymers, as well as the versatile manipulation of bioconjugate architecture are presented. Subsequently, recent advances of polymer bioconjugates based on different biological entities including proteins/peptides, nucleic acids, carbohydrates, lipids and even live cells are discussed individually. In particular, we focus on various forms of well-defined constructs at different length scales ranging from precision polymers and nanostructures templated by biomolecules to highly

ordered assemblies of polymer bioconjugates in solution, in bulk and on surfaces. Some representative applications of these biohybrids resulting from their high degree of structural precision are also highlighted.

Keywords:

Polymer bioconjugates, peptide–polymer conjugates, protein–polymer conjugates, DNA–polymer conjugates, controlled radical polymerization, site-specific modification, polymer biohybrids

Abbreviations

2D	two-dimensional
3D	three-dimensional
6SL	6'-sialyllactose
Ad5	adenovirus 5
AEMA	2-aminoethyl methacrylate
AFM	atomic force microscopy
AIBN	azobisisobutyronitrile
ARGET	activators regenerated by electron transfer
ATRP	atom transfer radical polymerization
bFGF	basic fibroblast growth factor
BSA	bovine serum albumin
BTA	1,3,5-benzenetricarboxamide
β-CD	β-cyclodextrin
μCP	microcontact printing
cryo-TEM	cryogenic transmission electron microscopy
CTA	chain-transfer agent
CuAAC	copper-catalyzed azide–alkyne cycloaddition
DABCYL	4-(dimethylaminoazo)benzene-4-carboxylic acid
DMA	dialkyl maleic anhydride
DNA	deoxyribonucleic acid
DNL	dip-pen nanodisplacement lithography
DTT	dithiothreitol
eATRP	electrochemically mediated ATRP
EBL	electron-beam lithography
ELP	elastin-like polypeptide
EQE	external quantum efficiency
FDA	Food and Drug Administration
FITC	fluorescein isothiocyanate
FND	fluorescent nanodiamond
FRET	Förster resonance energy transfer
Gd-DTPA	Gd-diethylene triamine pentaacetic acid
GFP	green fluorescent protein
GOx	glucose oxidase
HPG	hyperbranched polyglycerol
HPMA	2-hydroxypropyl methacrylate
HSA	human serum albumin
HSP	heat shock protein
ICAR	initiators for continuous activator regeneration
IFN	interferon-α
LCST	lower critical solution temperature
βLG A	β-lactoglobulin A
mPEG	methoxy PEG
MRI	magnetic resonance imaging
NHS	*N*-hydroxysuccinimide
NIPAM	*N*-isopropyl acrylamide
NMP	nitroxide-mediated polymerization
NQMP	3-(hydroxymethyl)naphthalene-2-ol
NTA	nitrilotriacetic acid

OPG	osteoprotegerin
PAA	poly(acrylic acid)
PAMAM	polyamidoamine
PB	phosphate buffer
PBA	poly(*n*-butyl acrylate)
PCB	poly(carboxybetaine)
PCR	polymerase chain reaction
PDL	α-poly(D-lysine)
PE545	phycoerythrin 545
PEG	poly(ethylene glycol)
PEGASYS	PEGylated interferon-α
PEGA-1k	methoxy-PEG acrylamide-1k
PG1	poly[3,5-bis(3-aminopropoxy)benzyl methacrylate]
PGMA	poly(glycidyl methacrylate)
photo-ATRP	photoinitiated ATRP
PHPMA	poly(2-hydroxypropyl methacrylate)
PISA	polymerization-induced self-assembly
PNA	peptide nucleic acid
PNB	polynorbornene
PNIPAM	poly(*N*-isopropylacrylamide)
POEGMA	poly[oligo(ethylene glycol) methyl ether methacrylate]
PPEGA	poly(PEG acrylate)
PS	polystyrene
PSS	polystyrene sulfonate
p(SS-*co*-PEGMA)	poly[sodium 4-styrenesulfonate-*co*-poly(ethylene glycol) methyl ether methacrylate]
QD	quantum dot
RAFT	radical addition–fragmentation chain transfer
RBC	red blood cell
RNA	ribonucleic acid
ROMP	ring-opening metathesis polymerization
ROP	ring-opening polymerization
ROS	reactive oxygen species
SEM	scanning electron microscopy
siRNA	small interfering ribonucleic acid
SMA	sodium methacrylate
SNA	spherical nucleic acid
SPL	scanning probe lithography
ssDNA	single-stranded deoxyribonucleic acid
St	styrene
*t*BA	*tert*-butyl acrylate
TCEP	tris(2-carboxyethyl) phosphine
TEM	transmission electron microscopy
VBA	vinylbenzyl adenine
VBT	vinylbenzyl thymine

1. Introduction

Since the seminal work of Hermann Staudinger published in 1920 [1], polymer science has arguably created significant impact on society in various areas with around 400 million tons of plastics produced annually worldwide since 2015 [2]. The emphasis within the field has also significantly evolved over the past 100 years: starting from the creation of these now ubiquitous plastic materials, their tunable properties in improving the standards of living to the present global concern of plastic contamination in the environment. Objectively, these paradigm shifts have brought scientists back to the drawing board to achieve greater understanding towards these materials and rethink strategies aided by modern synthesis technologies unavailable in the past. As the knowledge within polymer science deepens, the molecular consequences how each individual monomer is arranged along the chain, which also has an impact on their spatial organization, become much more apparent and crucial for the design of macromolecules that exhibit complex programmable behavior. Here, the first connection between synthetic polymer chemistry and Nature's macromolecules was made in order to bridge their differences and to find potential synergistic properties.

Molecular precision is the central hallmark among biomacromolecules, i.e. proteins and nucleic acids, where their sequence is coded elegantly serving both as their unique identity and function. This unique feature, alone, accounts for the vast disparity between synthetic polymers and biomolecules in most of their macromolecular properties. Each biomacromolecule has a defined surface contour within a rigid architecture, where each amino acid residue (for proteins) or nucleotide [for deoxyribonucleic acids (DNAs)] has a precise three-dimensional (3D) coordinate within the folded structure, which is a prerequisite to their biological function. In contrast, the position of monomers within a synthetic polymer is largely governed by a statistical distribution, which can be tailored, only to a limited extent, by controlled polymerization techniques [3-5]. Therefore, the inter- and intramolecular interactions within each polymer chain vary from one to the other, producing irregular nanostructures. As a result, on a molecular level, there is a limit in resolution to accurately determine structure–activity relationships for an observed outcome.

Although biomolecules are often perfect in their molecular construction, they do not possess the breadth in chemical design that polymer science allows. The flexibility in monomer synthesis and the repertoire of polymerization technologies available to synthesize novel materials is unquestionable and has demonstrated its solid potential throughout the decades. From this perspective, the community intuitively realized that the properties of polymer chemistry naturally complement the capabilities of biomolecules and vice versa, leading to the first inception of polymer bioconjugates in the 1970s [6, 7]. In 1977, Davis et al. reported the first example of poly(ethylene glycol) (PEG) conjugation to a protein [8]. Since the late 1980s, Hoffman and Stayton et al. have intensively studied the conjugation of temperature-responsive polymers to random and specific sites of protein surfaces [9-13]. After that,

functional polymer bioconjugates have developed rapidly for broad disciplines, ranging from therapeutics, nanotechnology, biophysics and materials science (Fig. 1). In this regard, several comprehensive reviews have been consolidated summarizing the progress in each theme [14-16].

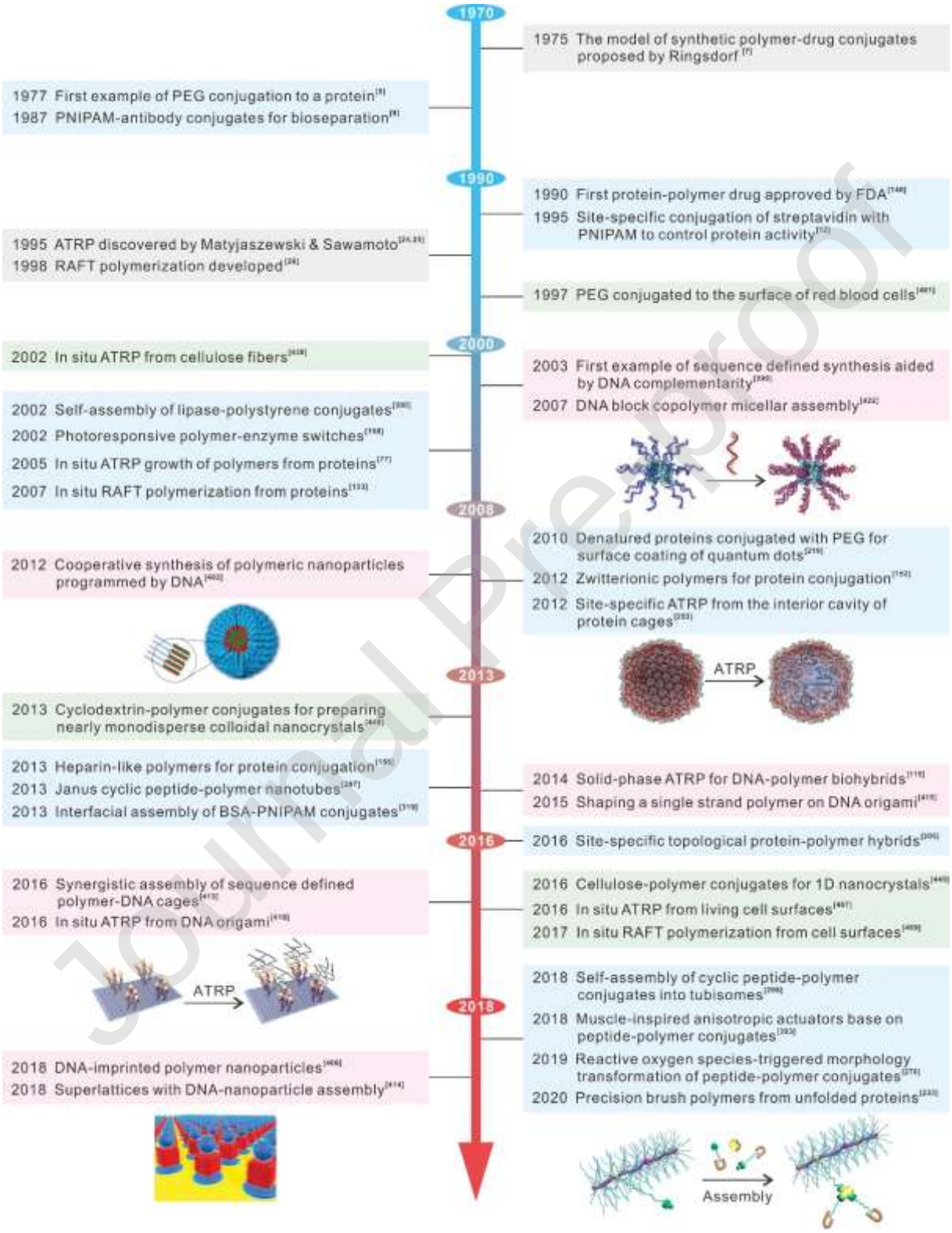


Fig. 1. Timeline of major milestones in the development of polymer bioconjugates.
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While the benefits of these conjugates towards application driven areas are unambiguous, there has been a focus in recent years to investigate how biomolecules and synthetic polymers can influence each other on a fundamental level. Some of the raised questions include the possibility of using sequence information of biomolecules to guide the precise arrangement of monomers along a synthetic polymer chain, which could not be achieved by state-of-the-art polymer chemistry [17]. Correspondingly, by appending a synthetic polymer onto a biomolecule using modern bioconjugation methods, the stability, bioactivity profile, and self-assembly behavior can be modified and controlled to a large extent by the polymer chain [18]. Within each major class of biomolecules (nucleic acids, proteins/peptides, carbohydrates and lipids), the synthesis strategies to achieve bioconjugates and the impact of the attached synthetic polymer differs greatly as they have different molecular constituents as well as intrinsic 3D structure.

At the molecular level, nucleotides, amino acids, and monosaccharides have their characteristic features that translate separately into the diverse architectures found in Nature. For nucleotides and amino acids, the transformation of these molecules into a defined 3D nano-object is dictated by a set of specific interactions that is predefined among the library of building blocks. Here, the machinations of biology are typically involved in the synthesis, orientation and folding process in a way that the system is funneled and guided through the energy landscape, eventually reaching a precisely defined nano-object. Therefore, it is intriguing for the community whether biomimetic strategies or even biomolecules themselves can be programmed to create the next generation polymeric materials with higher structural definition. Hence, this review provides only a brief background of the synthesis as well as each category of biomolecules while mainly focusing on research highlights that would possibly inspire the development of polymer-bioconjugates in the future.

2. Synthetic approaches for well-defined polymer bioconjugates

The conjugation of synthetic polymers to various biomolecules such as proteins, peptides, and nucleic acids can be realized using one of three synthetic strategies: *grafting to*, *grafting from* and *grafting through* [19, 20]. Briefly,

grafting to is the coupling of a pre-synthesized polymer with a biomolecule, while *grafting from* refers to in situ growth of a polymer from a biomolecule or alternatively the synthesis of a biomacromolecule using a preformed polymer as the initiator. These two strategies are more frequently used than *grafting through*, which is a strategy to polymerize biomolecule-containing monomers yielding bioconjugates with multiple biofunctional groups along the polymer backbone.

Conventional conjugation of polymers to biomolecules using these strategies may encounter some limitations. For example, the preparation of protein–polymer conjugates through coupling to abundantly presented amines on protein surfaces generates a heterogeneous product mixture with random numbers of polymer chains introduced at arbitrary positions causing significantly reduced biological activity [19]. The isolation and purification of the resultant mixture, including positional isomers, would be daunting and extremely difficult to achieve [21]. In addition, polymers synthesized by traditional polymerization techniques may lack of control over their structure and distribution. Therefore, it is highly desirable to synthesize well-defined polymer bioconjugates, which possess at least the following two characteristics: First, a determined number of polymers are conjugated to specific sites of biomolecules, and second, the polymer chain should have a narrow distribution as well as defined length and architecture.

This chapter aims to summarize the various attempts to meet these two requirements. First, current chemical and biological techniques, such as chemoselective ligations and genetic engineering facilitate the preparation of site-specific and stoichiometric polymer bioconjugates [22, 23]. The second requirement has been largely addressed by the rapid development of polymerization techniques including atom transfer radical polymerization (ATRP) [24, 25], radical addition–fragmentation chain transfer (RAFT) polymerization [26], nitroxide-mediated polymerization (NMP) [27, 28], iniferter radical polymerization [29], ring-opening polymerization (ROP) [30], ring-opening metathesis polymerization (ROMP) [31], and living anionic/cationic polymerization [32, 33]. The two most popularly used techniques, ATRP and RAFT polymerization, are discussed in detail in the second section of this chapter. The architecture of polymer bioconjugates is very important for their features and consequent applications. Therefore, an overview of the structural regulation of polymer bioconjugates at the monomer, polymer and conjugate levels is provided in the third section.

2.1. Site-specific polymer conjugation of biomolecules

Due to the large number of lysine residues on the surface of biomolecules, the first-generation methods of polymer conjugation based on the coupling to amines are nonspecific. This type of modification has allowed to reduce the immunogenicity of protein therapeutics as well as increase the stability and circulation time [19]. However, the benefits of preparing site-specific and stoichiometric polymer bioconjugates are obvious, i.e. to purify the product, to provide precise and reproducible control over many properties, particularly their bioactivity [34]. Moreover, well-defined polymer bioconjugates can further be used as precision templates and building blocks for preparing advanced materials with controlled structures.

In order to prepare site-specific polymer bioconjugates, polymers can be directly conjugated to desired locations of biomolecules using various chemoselective interactions. Nevertheless, this strategy often results in low efficiency and conversion due to slow reaction kinetics and the steric effect to connect these high-molecular-weight and sterically demanding macromolecules. Therefore, introduction of functional small molecules in a site-specific manner has been an alternative approach. These small molecules include chemical handles that enable high-efficiency coupling using bioorthogonal chemistries and initiating groups which allow *in situ* polymer growth with controlled polymerization techniques. The site-specific conjugation of polymers and functional small molecules to biomolecules can be achieved through rapidly expanded chemical and bioengineering techniques [35, 36].

An effective approach to prepare site-specific polymer bioconjugates is to target specific functional groups at the surface of biomolecule which are less common [19]. For instance, cysteine residues often form disulfide bonds inside the protein structure, and only a limited number of cysteines are accessible providing free thiols on the surface of polypeptides. Therefore, many chemistries, such as disulfide exchange with a pyridyl disulfide and addition reactions with alkenes, alkynes, maleimides or vinyl sulfones to form thioethers, have been employed to target free thiol groups [37]. Among these reactions, the thiol-maleimide interaction under acidic or neutral conditions is one of the most widely used chemistries for preparing site-specific polymer bioconjugates. In addition, disulfide bridges exposed on the surface have also been used as specific sites for the incorporation of polymers [38-42]. Brocchini and Shaunak et al. reported site-specific PEGylation of native disulfide bonds using a bis-thiol alkylating reagent to form a three-carbon bridge [43, 44]. Inspired by this work, our group has reported a versatile toolbox of bis-alkylation reagents that re-bridge disulfide bonds of peptides and proteins [45-47]. Tyrosine, which is present in many peptides and proteins represents another possible conjugation site. It reacts with diazonium salts [48] and allows functionalization through a three-component Mannich-type reaction [49]. Due to the lower pK_a than that of amines from lysine, the N-terminal amine is more reactive and can therefore also be used for site-specific attachment of

polymers and functional small molecules [50-52]. Chilkoti et al. reported the conjugation of an ATRP initiator to myoglobin via N-terminal selective transamination, which was further applied for in situ ATRP growth of polymers [53]. More examples of the above-mentioned and other natural amino acids for site-specific polymer conjugation are summarized in Fig. 2A and can also be found in other excellent reviews [19, 23, 54].

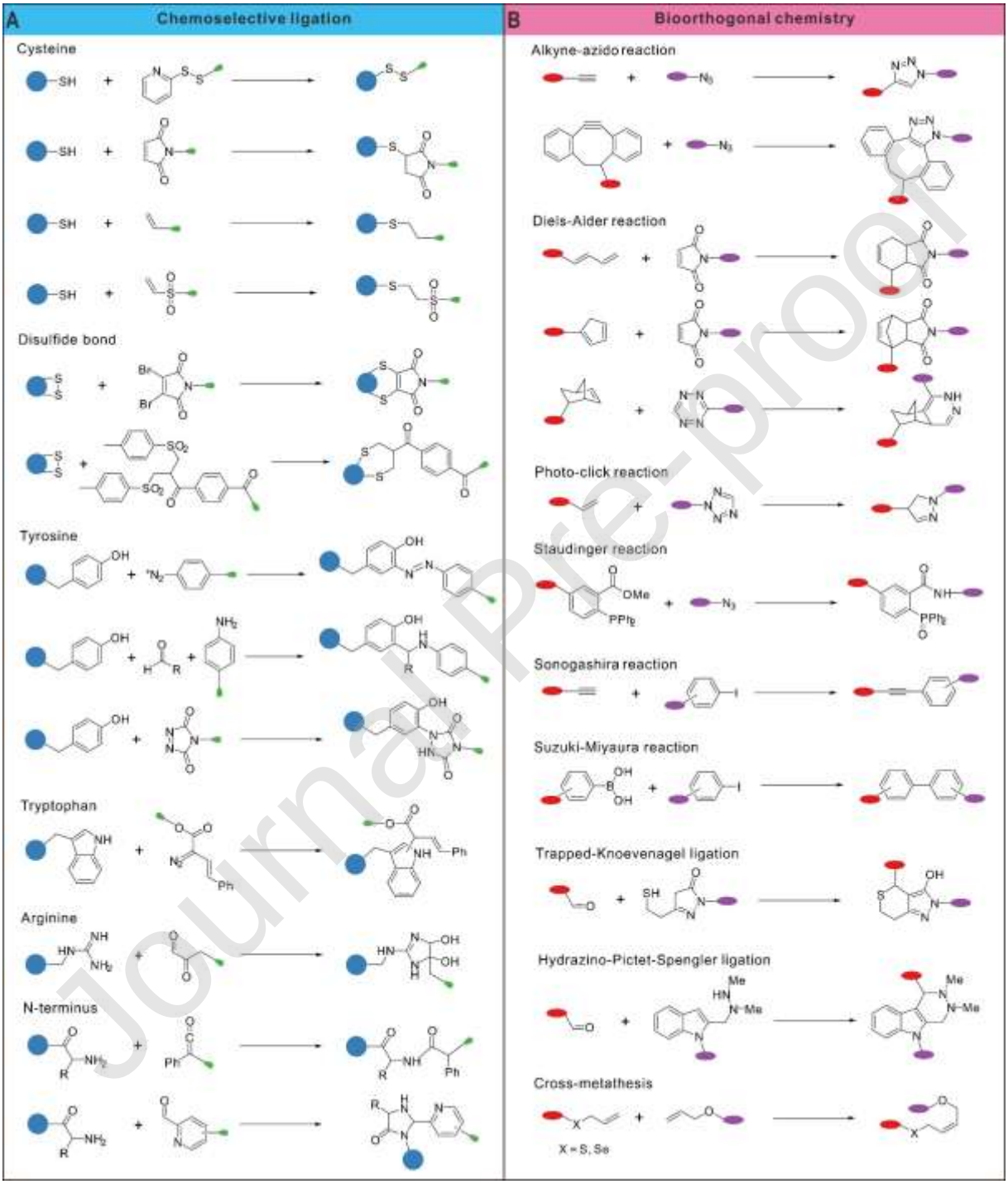

Fig. 2. Representative reactions for site-specific conjugation of biomolecules. (A) Chemoselective ligation with canonical amino acids. (B) Biorthogonal chemistries available for polymer bioconjugation. The blue circle represents biomolecules and the green pear-shaped symbol indicates polymers or functional small molecules. The red and purple ovals refer to either biomolecules or functional small molecules/polymers, and they are interchangeable.$

In addition to intrinsic reactive groups of native biomolecules, both canonical and non-canonical amino acids can be incorporated at the desired location through bioengineering techniques that provide a platform for site-specific conjugation using chemoselective ligations and a wide range of bioorthogonal chemistries (Fig. 2B). As an example of natural amino acids, cysteine has been genetically introduced into interferon α-2 for site-specific PEGylation, generating well-defined mono-PEGylated proteins with enhanced circulation half-lives and antitumor properties [55, 56]. An oligo-histidine tag, which binds to a Ni^{2+} complex of nitrilotriacetic acid can be genetically tagged on the C- and N-termini of proteins [57]. Lee et al. demonstrated the site-specific PEGylation of a protein based on a hexahistidine tag, and the polymer bioconjugate provided excellent stability without compromising bioactivity [58]. Non-canonical amino acids with orthogonal chemical reactivity to the 20 canonical amino acids represent a huge toolbox for the preparation of well-defined polymer bioconjugates [59]. For instance, *p*-azidophenylalanine was site-specifically incorporated into proteins enabling a copper-medicated Huisgen [3+2] cycloaddition with alkyne end-capped PEG [60]. Matyjaszewski and coworkers incorporated two azide-containing non-canonical amino acids to amino acid residues 134 and 150 on the surface of green fluorescent protein (GFP) by site-directed mutagenesis [61]. These modified proteins were then linked into linear oligomeric strands by PEG with two alkyne ends. A ketone-containing amino acid, *p*-acetylphenylalanine, was also developed for site-specific conjugation of PEG and an aminooxy-derivatized cationic block copolymer to human growth hormone [62] and antibodies [63], respectively. Some reviews have summarized the advances of non-natural amino acids that enable various orthogonal chemistries for site-specific polymer bioconjugation [59, 64-66].

Small-molecule initiating groups, which allow in situ growth of polymers have also been introduced site-specifically to biomolecules by various techniques. For example, Chilkoti et al. reported two genetic engineering approaches, intein-medicated initiator installation [67] and sortase-catalyzed initiator attachment [68], to introduce an ATRP initiator solely at the C-terminus of proteins and peptides. The sortase-catalyzed initiator attachment was further employed by Gao and coworkers to prepare site-specific protein conjugates with improved stability for cancer therapy [69, 70]. Mehl et al. designed the non-canonical amino acid 4-(2'-bromoisobutyramido)phenylalanine, which was used as an initiator for ATRP [71]. It can be genetically engineered at desired sites and therefore represents a general approach to quantitatively encode ATRP initiators to the protein backbone.

Most reported polymer bioconjugates are based on irreversible covalent interactions. However, the conjugation of synthetic polymers and biomolecules with cleavable linkers may provide additional advantages such

as more spatiotemporal control over the conjugates and on-demand release of biomolecules [72-75]. By combining enzymatic and chemical bioorthogonal coupling strategies, Meinel et al. demonstrated the site-specific PEGylation of insulin-like growth factor I with a protease-sensitive peptide linker [76]. The growth factor could be released after exposure of the PEGylated conjugate to activated matrix metalloproteinases in inflamed tissues, resulting in the recovery of its bioactivities. In addition, reversible non-covalent interactions such as biotin-streptavidin recognition [77] and host–guest interactions [78, 79], have also been used for site-specific polymer conjugation. Anderson and coworkers reported the supramolecular PEGylation of insulin through strong non-covalent binding of cucurbit[7]uril to its N-terminal phenylalanine residue [80]. In comparison to covalent conjugation, this supramolecular approach holds a unique advantage that the authentic therapeutic entity remains unmodified.

Above, we have introduced various strategies for the site-specific polymer conjugation of proteins and peptides. Although many chemical and bioengineering techniques have been established for the site-specific labeling of DNA and ribonucleic acid (RNA) with functional small molecules [81-85], current approaches for polymer conjugation to nucleic acids mainly proceed at the terminus of the oligonucleotide sequence, which results in nucleic acid-containing block copolymers [86, 87]. Generally, these methods can be categorized into solution conjugation chemistry and solid-phase synthesis. As amino- and thiol-terminated oligonucleotides are commercially available, functional small molecules and hydrophilic polymers can be easily introduced via the formation of an amide or disulfide bond in solution [88-90]. For example, Weil et al. prepared two RAFT agent-terminated single-stranded DNA (ssDNA) sequences via *N*-hydroxysuccinimide (NHS) or pentafluorophenyl ester coupling, which were used for photoinduced RAFT polymerization to synthesize well-defined DNA–polymer conjugates [91]. In addition, Michael addition [92] and the copper-catalyzed azide–alkyne cycloaddition (CuAAC) [93] are also popular reactions for the highly efficient conjugation of polymers to nucleic acids. Due to their different solubilities, the coupling efficiencies of hydrophobic polymers with nucleic acids in solution are often much lower. Therefore, solid-phase synthesis approaches were developed. In this regard, the use of 2-cyanoethyl-*N*,*N*-di*iso*propylphosphoramidite groups is a commonly applied method to introduce functional groups to the 5'-end of oligonucleotides via solid-phase synthesis [94]. Particularly, fully automated solid-phase synthesis of DNA conjugates based on hydrophobic polymers such as poly(propyleneoxide) in DNA synthesizers is now available [86]. Recently, Matyjaszewski, Das and coworkers reported the automated synthesis of DNA–polymer conjugates by photomediated ATRP using a DNA synthesizer [95]. In addition, molecular biology techniques such as polymerase chain reactions (PCR) have also been

successfully used for polymer conjugation to nucleic acids [96]. Previously, two excellent reviews have been published that deliver a comprehensive overview on DNA-containing amphiphilic block copolymers[86, 87].

Other small biomolecules such as lipids, monosaccharides, and oligosaccharides could be also connected to polymers in a site-specific fashion. The obtained biohybrids can serve as precision building blocks for the construction of hierarchical structures. For instance, Akiyoshi et al. synthesized amphiphilic carbohydrate-conjugated poly(2-oxzoline)s using a small molecule maltotriose-containing initiator enabling the preparation of polymer vesicles with molecular permeability [97]. Additional examples will be discussed within each class of polymer bioconjugates in chapter 5.

2.2. Controlled radical polymerizations for polymer bioconjugation

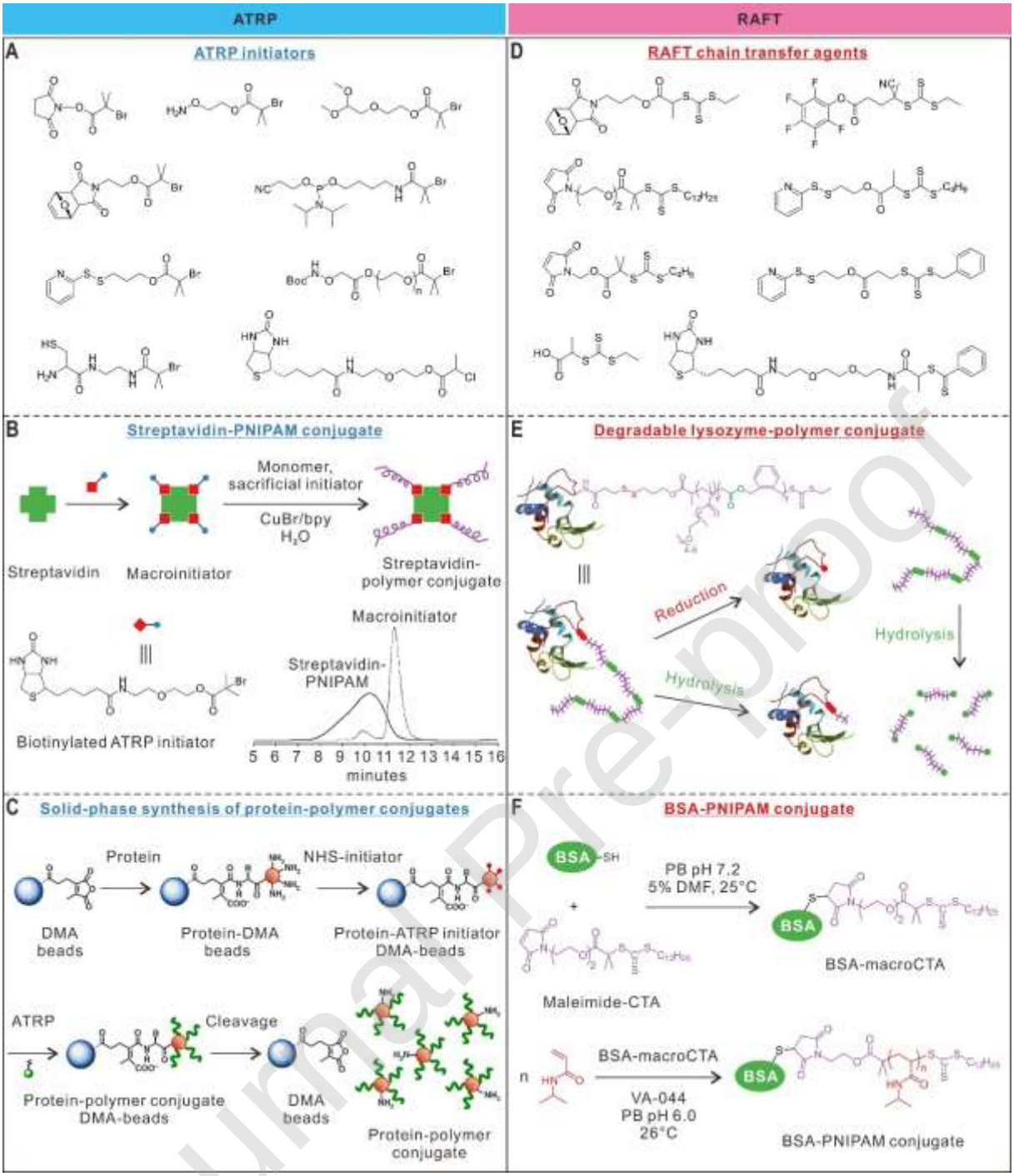

Fig. 3. ATRP and RAFT polymerization for polymer bioconjugation; (A) Selected examples of ATRP initiators reported for polymer bioconjugation; (B) Synthesis of streptavidin-PNIPAM conjugates by in situ ATRP; (C) Solid-phase synthesis of protein–polymer conjugates via ATRP from protein macroinitiators reversibly immobilized on dialkyl maleic anhydride (DMA)-modified agarose beads; (D) Selected examples of RAFT CTAs reported for polymer bioconjugation; (E) Degradable lysozyme–polymer conjugate synthesized by RAFT polymerization using the *grafting to* approach; (F) Site-specific and in situ RAFT polymerization of *N*-isopropylacrylamide (NIPAM) for the synthesis of BSA–PNIPAM conjugate.
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(E) [130], Copyright 2015. Reproduced with permission from Elsevier Ltd.
(F)[135], Copyright 2008. Reproduced with permission from the American Chemical Society.

2.2.1. Atom transfer radical polymerization

ATRP is a powerful controlled radical polymerization technique, which enables precise synthesis of functional polymers with determined molecular weight and narrow molecular weight distribution [98]. Due to its applicability to various monomers, solvents, catalysts, and reaction conditions, ATRP has been employed for the preparation of a broad range of advanced polymeric materials with controlled architecture and functionality [5, 99]. Because it can be carried out at room temperature in aqueous solution, ATRP is particularly useful for the conjugation of polymer chains to biological entities such as proteins, peptides, nucleic acids, viruses, and even live cells. Fig. 3A shows representative ATRP initiators reported in the literature for the synthesis of polymer bioconjugates by ATRP using either *grafting to* or *grafting from* approach.

Maynard et al. reported the preparation of thiol-reactive polymers by ATRP using an initiator functionalized with a pyridyl disulfide group, which were then selectively grafted to the single surface-exposed cysteine group of bovine serum albumin (BSA) [100]. However, the *grafting to* approach often encounters low coupling efficiency especially for high molecular weight polymers due to their steric demand and the challenging removal of unreacted polymers and biomacromolecules. To avoid these limitations, the *grafting from* strategy has become a more popular procedure because ATRP initiators can be easily attached to biomolecules using both chemical means and genetic engineering. As illustrated in Fig. 3B, Maynard et al. reported the first example of in situ ATRP synthesis of protein–polymer conjugates using modified streptavidin as a macroinitiator in 2005 [77]. Streptavidin is an intensively studied protein that binds four biotin ligands. Poly(*N*-isopropylacrylamide) (PNIPAM) chains were quantitatively conjugated to the protein at the biotin binding sites only, and the bioactivity of streptavidin remained unaffected. This straightforward approach was also extended by the same group to other proteins including BSA and the enzyme lysozyme [101]. Similarly, chymotrypsin modified with 2-bromoisobutyramide was also used to initiate ATRP of nonionic, cationic, and anionic monomers for the synthesis of near-uniform protein–polymer conjugates while retaining 50–86% of the original enzyme activity [102]. Chilkoti et al. demonstrated the in situ ATRP growth of a brush-like polymer, poly[oligo(ethylene glycol) methyl ether methacrylate] (POEGMA), with narrow distribution and high yield, solely from the N-terminus of myoglobin or C-terminus of GFP [53, 67]. The resulted site-specific and stoichiometric bioconjugates showed significantly improved pharmacological profiles such as increased blood exposure compared to those unmodified proteins.

With the rapid expansion of different monomers and biomolecules, ATRP remained as a versatile tool to prepare polymer bioconjugates has also greatly evolved especially under biologically relevant conditions [5]. For example, new ATRP techniques such as activators regenerated by electron transfer (ARGET) ATRP [103-105],

initiators for continuous activator regeneration (ICAR) ATRP [106, 107], electrochemically mediated ATRP (eATRP) [108-110], and photoinitiated ATRP (photo-ATRP) [111-113] have been developed by continuous regeneration of active catalysts with various external stimuli, which allow the preparation of polymer conjugates with low catalyst loading under biologically benign polymerization conditions. Russell et al. demonstrated the solid-phase synthesis of protein–polymer conjugates by ATRP from protein macroinitiators reversibly immobilized on modified agarose beads (Fig. 3C) [114]. This effective and simple method is readily automated and therefore could dramatically reduce the time for the synthesis and purification of protein–polymer conjugates. Matyjaszewski, Das and coworkers also reported a straightforward method for the solid-phase incorporation of an ATRP initiator onto a DNA strand, allowing the direct preparation of DNA–polymer conjugates on the solid support [115]. Although ATRP has been successfully employed to grow polymers from biomolecules under aqueous conditions, its oxygen sensitivity is still a vexing challenge. Inspired by aerobic respiration of cells, Matyjaszewski et al. recently demonstrated a fully oxygen tolerant well-controlled ATRP, which used glucose oxidase (GOx) to continuously catalyze the conversion of oxygen to carbon dioxide in the presence of glucose and sodium pyruvate [116]. This "green" ATRP procedure could be conducted under air exposure and it was successfully used for the synthesis of well-defined protein–polymer conjugates. Based on the exciting new development, they further reported an "oxygen-fueled" ATRP using a biocatalytic system composed of GOx and horseradish peroxidase with ppm level of Cu catalyst [117]. This enzymatic cascade polymerization, which requires continuous oxygen supply to generate radicals, was used to prepare BSA–POEGMA and DNA–POEGMA bioconjugates.

2.2.2. Reversible addition–fragmentation chain transfer polymerization

RAFT polymerization is another controlled radical polymerization, which has been popularly used for the preparation of well-defined polymer bioconjugates [118, 119]. It tolerates various chemical groups and is applicable for a broad range of solvents and monomers [120]. Similar to ATRP, RAFT polymerization has been employed to synthesize functional polymers of determined molecular weight, low polydispersity, as well as precisely designed architecture and functionality [121]. One distinct advantage of the RAFT approach is that metal catalysts are not needed. Instead, chain-transfer agents (CTAs) such as dithioesters, dithiocarbamates, trithiocarbonates, and xanthates are required because polymers are generated via equilibrium between a growing radical and the RAFT CTA [122]. Therefore, the structure of the CTA is of great significance for the controlled growth of polymers. Fig. 3D displays selected RAFT CTAs for the synthesis of polymer bioconjugates.

Similar to ATRP-based systems, polymer bioconjugates can also be prepared by RAFT polymerization using both *grafting from* and *grafting to* approaches [123-128]. For instance, α-chymotrypsin, an enzyme that digests other proteins, was conjugated to well-defined polymers made by RAFT polymerization [129]. These conjugates were able to significantly improve the stability of the protease without affecting its bioactivity. Maynard et al. conducted RAFT copolymerization of cyclic ketene acetal monomer with poly(ethylene glycol methyl ether methacrylate) yielding functional polymers, which were subsequently conjugated to lysozyme through a reducible disulfide linkage [130]. As illustrated in Fig. 3E, the polymer is backbone degradable and also could be easily cleaved off from the lysozyme–polymer conjugate in a reducing environment. For the *grafting from* approach, Börner et al. demonstrated the RAFT polymerization for the synthesis of bioactive oligopeptide–polymer conjugates using a trithiocarbonate-based peptide–CTA [131]. DNA–polymer conjugates on a planar solid support were prepared by covalently attaching CTAs to ends of surface-immobilized oligonucleotides and then initiating RAFT polymerization [132]. The first example of RAFT-mediated in situ formation of protein–polymer conjugates was reported by Bulmus, Davis, and coworkers [133]. They synthesized site-specific BSA–poly(PEG acrylate) (PPEGA) conjugates via gamma-radiation-initiated RAFT polymerization using a mixture of water and *N*,*N*-dimethylformamide as the solvent. However, the gamma radiation source may cause structural damage on some biological molecules. To avoid this detrimental effect and also the usage of organic solvents, a room temperature azo-initiator and a new water-soluble RAFT CTA were used for the in situ generation of well-defined BSA–PNIPAM and BSA–poly(hydroxyethyl acrylate) conjugates in completely aqueous solutions [134]. Importantly, the structural integrity and esterase-like activity of BSA were retained under the polymerization conditions, showing the general applicability of this RAFT approach for the preparation of bioactive protein–polymer conjugates. In these two systems, both RAFT CTAs [general formula Z–C(=S)S–R] were attached to BSA through the "Z-group". As shown in Fig. 3F, the Sumerlin group synthesized a new type of macroCTA by conjugating BSA to the "R-group" of the CTA with thiol–maleimide coupling, which was subsequently applied for room temperature RAFT polymerization of NIPAM in aqueous media [135]. This design provides better polymerization control due to reduced steric hindrance and the labile thiocarbonylthio moiety at the free chain end could be potentially used for further functionalization. In addition, they also prepared well-defined block copolymer conjugates of BSA–PNIPAM-*b*-poly(*N*,*N*-dimethylacrylamide) by two consecutive *grafting from* RAFT polymerizations using this macroCTA [136]. Apart from these two conventional strategies, Thang et al. have recently reported the *grafting through* RAFT polymerization of a methacrylamide monomer containing a pending RGD peptide to afford well-defined peptide–polymer conjugates that were used for enhanced cell adhesion [137].

2.3. Structural design of polymer bioconjugates

2.3.1. Variation of the polymer chain

The conjugation of PEG to peptides and proteins, known as PEGylation, has been widely used in therapeutic fields to improve the stability and biopharmaceutical performance [138]. PEG is regarded as safe and there are many PEGylated protein drugs which have been approved by US Food and Drug Administration (FDA) in the market [139]. However, PEG can also impose a negative impact on the biomolecule such as reduced bioactivity, non-degradability, and immunological responses [140]. Therefore, a variety of alternative functional polymers have been developed for the conjugation of different biomolecules. For example, poly(quaternary ammonium) was grafted from the chymotrypsin surface to afford a dense cationic shell for the modulation of substrate specificity and inhibitor binding [141]. A series of polymers of varying functionality and length was conjugated to lysozyme to investigate the impact of the respective polymer on enzyme stability and activity [142]. Russell, Whitehead and coworkers prepared BSA–polymer conjugates with a phenylpiperazine-containing polymer, which selectively facilitated transepithelial protein transport [143]. Gao et al. have grafted poly(*N*,*N'*-dimethylamino-2-ethyl methacrylate) site-specifically from the N-terminus of GOx to modulate H_2O_2 generation for cancer starvation and H_2O_2 therapy [144]. Reactive water-soluble, azlactone-containing copolymers synthesized by RAFT polymerization were conjugated to holo-transferrin and ovotransferrin forming protein bioconjugates that were internalized by cells via receptor-mediated endocytosis [145].

Biomimetic polymers inspired by biological components found in Nature have also been designed for bioconjugation. Biocompatible, zwitterionic polymers with cell membrane-mimicking characteristics were employed to construct biomaterials minimizing the interactions with proteins and cells [146-151]. Jiang et al. reported the conjugation of zwitterionic poly(carboxybetaine) (PCB) (Fig. 4A) using α-chymotrypsin as a model protein and PCB was found to protect proteins from chemical and thermal denaturation [152]. Remarkably, the PCB conjugates demonstrated superior stability in comparison to the corresponding PEG conjugates of similar molecular weights (Fig. 4B) and similar hydrodynamic size (Fig. 4C). More importantly, enhanced binding affinity with a peptide-based substrate was observed for PCB conjugates which could be attributed to differences on how PEG and PCB affected substrate binding affinities: PEG reduces enzyme–substrate hydrophobic–hydrophobic interactions due to its amphiphilic features while super-hydrophilic PCB promotes these interactions and the binding affinity through strong ionic structuring of water molecules (Fig. 4D). Recently, PCB was also conjugated to insulin via amine–NHS ester conjugation and the conjugate showed increased ability to lower in vivo glucose compared with native insulin [153].

Gao et al. presented the conjugation of zwitterionic poly(2-methacryloyloxyethyl phosphorylcholine) to the C-terminus of interferon-α, and the resulting polymer bioconjugates showed significantly improved in vitro antiproliferative bioactivity and in vivo antitumor efficacy compared to those of PEGylated interferon-α [154]. Inspired by the natural disaccharide trehalose, which protects proteins and cells in many plants and animals, well-defined glycolpolymers with pendant trehalose side chains were prepared for stabilization of protein bioconjugates to environmental stressors [155]. Similarly, a heparin-mimicking polymer consisting of styrene sulfonate units and PEG methyl methacrylate units was covalently conjugated to basic fibroblast growth factor (bFGF) [156]. As shown in Fig. 4E, the obtained bioconjugate exhibited significantly improved stability against heat, mild and harsh acidic conditions, storage and proteolytic degradation compared to native and PEGylated bFGFs.

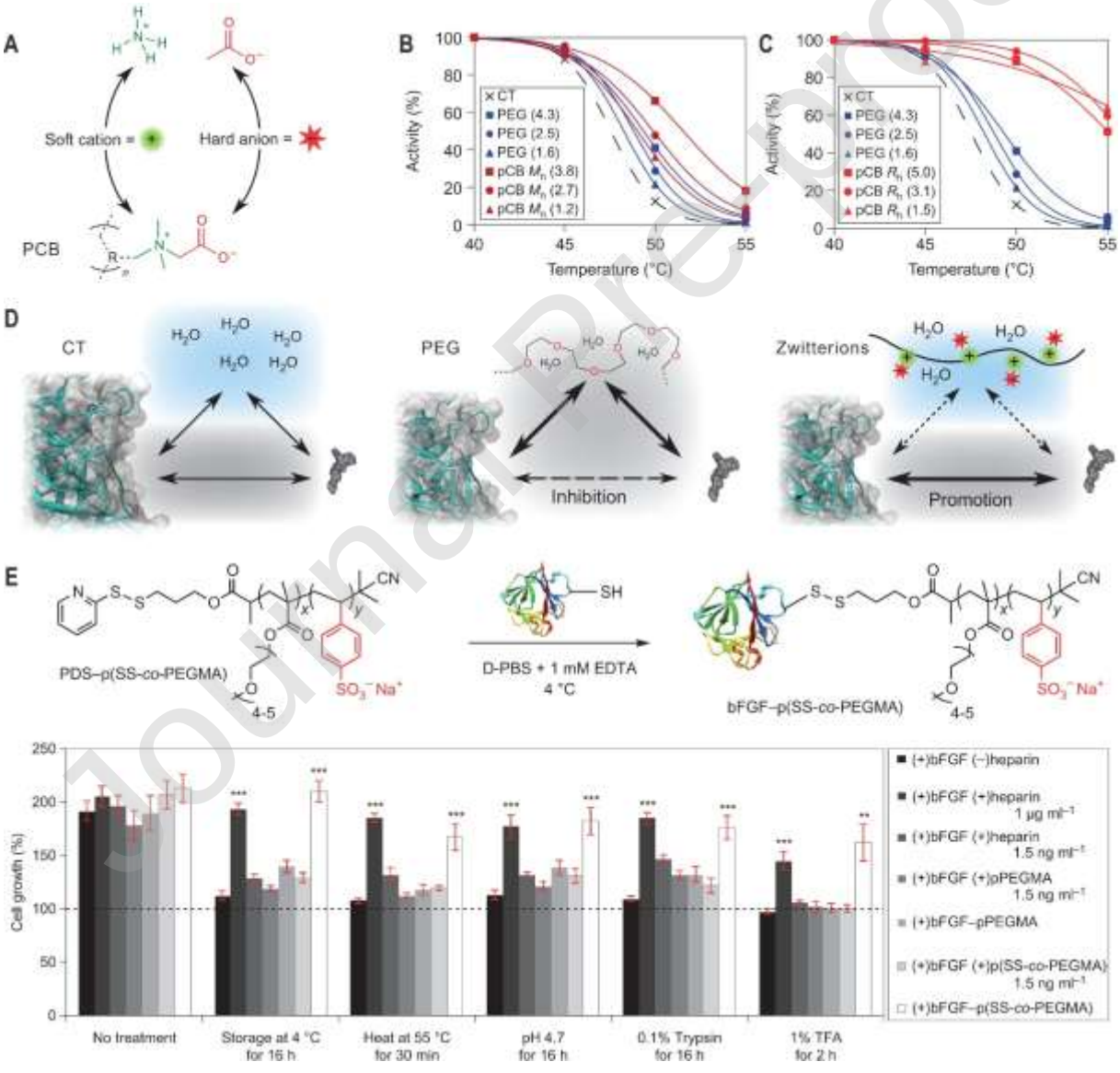

Fig. 4. Biomimetic polymers for protein conjugation. (A) The structure of PCB and its relationship with ammonium acetate. The R group represents a methacrylate backbone. (B and C) Relative activity of PEG and PCB conjugates of similar molecular weight (M_n) and similar hydrodynamic size (R_h). (D) Mechanism of how PEG and PCB polymers affect binding affinity. (E) Structure of a heparin-mimicking polymer, poly[sodium 4-styrenesulfonate-*co*-poly(ethylene glycol) methyl ether methacrylate] [p(SS-*co*-PEGMA)] and its conjugation to protein bFGF. The bottom shows the stability of the resulting polymer bioconjugate bFGF-p(SS-*co*-PEGMA) and its impact on cell growth compared to control samples after different treatments. It is obvious that the bioactivity of the conjugate was comparable to the positive control which had a 700-fold molar excess of heparin, and significantly higher than other control samples, under all environmental stresses. [152, 156], Copyright 2012 and 2013, respectively. Reproduced with permission from Springer Nature.

The conjugation of smart polymers, which can respond to various stimuli such as pH, temperature, and light to biomolecules, may allow on-demand regulation of solubility, stability and bioactivity of the resulting conjugate [157]. For instance, light was successfully used to tune enzyme catalytic activity when an azobenzene-containing copolymer was conjugated to a distinct location near the catalytically active site [158]. Thermo-responsive PNIPAM is one of the most famous smart polymers, which has been attached to various biomolecules such as proteins, peptides, nucleic acids, and polysaccharides through different conjugation strategies [159]. Haddleton et al. reported the conjugation of PNIPAM to BSA, lysozyme, bovine hemoglobin, salmon calcitonin, and insulin by aqueous single electron-transfer living radical polymerization [160]. PNIPAM–DNA conjugates were also synthesized and used for preparation of pH and temperature dual-responsive hydrogels, which could find potential applications for sensing and smart drug release [161]. For more examples on the conjugation of stimuli-responsive polymers to biomolecules, the reader can refer to other reviews [157, 159].

Because nondegradable polymers may accumulate in biological systems or persist in the environment, the design and synthesis of degradable polymers has received great significance especially for therapeutic applications. For instance, acid-degradable PEG chains were synthesized by introducing a cleavable acetaldehyde acetal into the backbone, which were employed for BSA conjugation [162]. Well-defined and water-soluble polyphosphoesters prepared by living anionic polymerization with chain-end functionalization have also been used for protein conjugation [163]. The resulting bioconjugates exhibited comparable bioactivities compared to PEGylated proteins, and the polymer shell degradation at physiological conditions was proved by online triple detection size exclusion chromatography and gel electrophoresis. Recently, the Maynard group has developed a powerful strategy to prepare a series of degradable polycaprolactones with different side groups including trehalose, lactose, glucose, carboxybetaine, and oligo(ethylene glycol), by combining ROP and thiol–ene post-modification [164]. These degradable polymers were conjugated to protein granulocyte colony-stimulating factor offering enhanced stability against storage and heat stressors.

2.3.2. Alteration of the polymer topology

In addition to functionality and degradability, the polymer topology is also an important factor, which could have a profound influence on the biomolecule and the unique properties of the resulting polymer bioconjugates [139]. This part highlights representative examples on the bioconjugation of synthetic polymers with various controlled topologies such as block copolymers, hyperbranched polymers and dendrimers. Although biomolecule-conjugated polymer networks, particularly hydrogels, have broad applications in the biomedical fields [165, 166], these works have not been included because their resulting structures are not clearly defined.

Beside linear homopolymers, functional random and block copolymers have been extensively used for bioconjugation [167, 168]. For example, Stayton et al. modulated the activity and aggregation properties of the conjugate of streptavidin with a dual stimuli-responsive block copolymer PNIPAM-*b*-poly(acrylic acid) (PNIPAM-*b*-PAA) [169]. Through two consecutive *grafting from* reactions via RAFT polymerization, Sumerlin et al. prepared block copolymer conjugates of BSA [136] and lysozyme [170]. Moreover, block copolymer conjugates of lysozyme were also prepared by combining the *grafting to* and *grafting from* strategies [171].

Synthetic polymers of brush-like, hyperbranched, and dendritic topologies have been widely reported for biomedical applications demonstrating some unique features in comparison to their linear counterparts [172-178]. The conjugation of branched polymers to biomolecules has therefore emerged as an exciting new area to achieve bioconjugates with improved stability and prolonged circulation times *in vivo* [140, 179-183]. In order to investigate the impact of the polymer architecture on bioconjugate activity, three polymers with similar molecular weights but different topologies ranging from linear, loosely branched, to densely branched were conjugated to osteoprotegerin (OPG), a protein that can be used for inhibition of bone resorption [184]. The obtained bioconjugates were nontoxic, and *in vivo* studies indicated an increase in the bone mineral density of rats treated by the loosely branched polymer–OPG bioconjugate. Klok et al. reported squaric acid mediated synthesis of functional polymers with varying architectures including linear, midfunctional, hyperbranched, and linear-*block*-hyperbranched polyglycerol copolymers, which yielded a broad range of BSA and lysozyme polymer bioconjugates [185]. Bioactivity of conjugates made from high molecular weight midfunctional polyglycerol copolymers was obviously higher than that of linear polymers of similar molecular weights. Brush-like polymer POEGMA has been demonstrated to significantly improve the circulation time and antitumor effect of myoglobin and GFP [53, 67]. Exendin-4, a peptide drug for type 2 diabetes mellitus, was also conjugated by POEGMA site-specifically at the C-terminus, and the resulting bioconjugate demonstrated reduced blood glucose for up to 120 h in fed mice with one single subcutaneous

injection [186]. Importantly, the reactivity to anti-PEG antibodies could be completely eliminated by optimizing the length of PEG side chains, showing distinct advantages of these novel bioconjugates compared to those based on linear PEG polymers.

Dendrimers and dendrons are highly branched molecules, which allow the preparation of precisely defined polymer bioconjugates [187, 188]. For example, our group demonstrated the dynamic covalent attachment of a positively charged polyamidoamine (PAMAM) dendron to different enzymes including trypsin, papain, and DNase I via the pH-responsive interaction between salicyl hydroxamate and boronic acid (Fig. 5A) [189]. The formation of dendronized enzyme constructs was first confirmed by a fluorescence assay, which demonstrated the stoichiometric substitution of fluorogenic Alizarin Red S by the salicyl hydroxamate containing PAMAM dendron (Fig. 5B). At pH 7.4, the functional dendron formed a protective shell on the surface of active enzymes blocking the catalytic sites. Due to the positive charges of the conjugated PAMAM dendrons, these enzyme–dendron conjugates could be efficiently internalized by A549 cells and colocalized in the acidic intracellular compartments (Fig. 5C). The enzyme activity was then recovered causing cytotoxicity and these smart conjugates can therefore serve as structurally defined biotherapeutics. Leroux et al. reported a polycationic dendronized polymer poly[3,5-bis(3-aminopropoxy)benzyl methacrylate] (PG1) for the stabilization of orally administered enzymes in the gastrointestinal tract through covalent conjugation [190]. Specifically, they compared the retention and stabilizing effect of four polymers with different architectures and functional groups (Fig. 5D). Enzymes conjugated to the positively charged dendronized polymer PG1 showed prolonged retention due to the strong mucoadhesive interactions with mucin on the stomach wall (Fig. 5E). In addition, this dendronized polymer could also stabilize the enzyme for over three hours in the stomach of rats while the other three polymers, including α-poly(D-lysine) (PDL), methoxy PEG (mPEG) and PAA, provided little or no retention/protection.

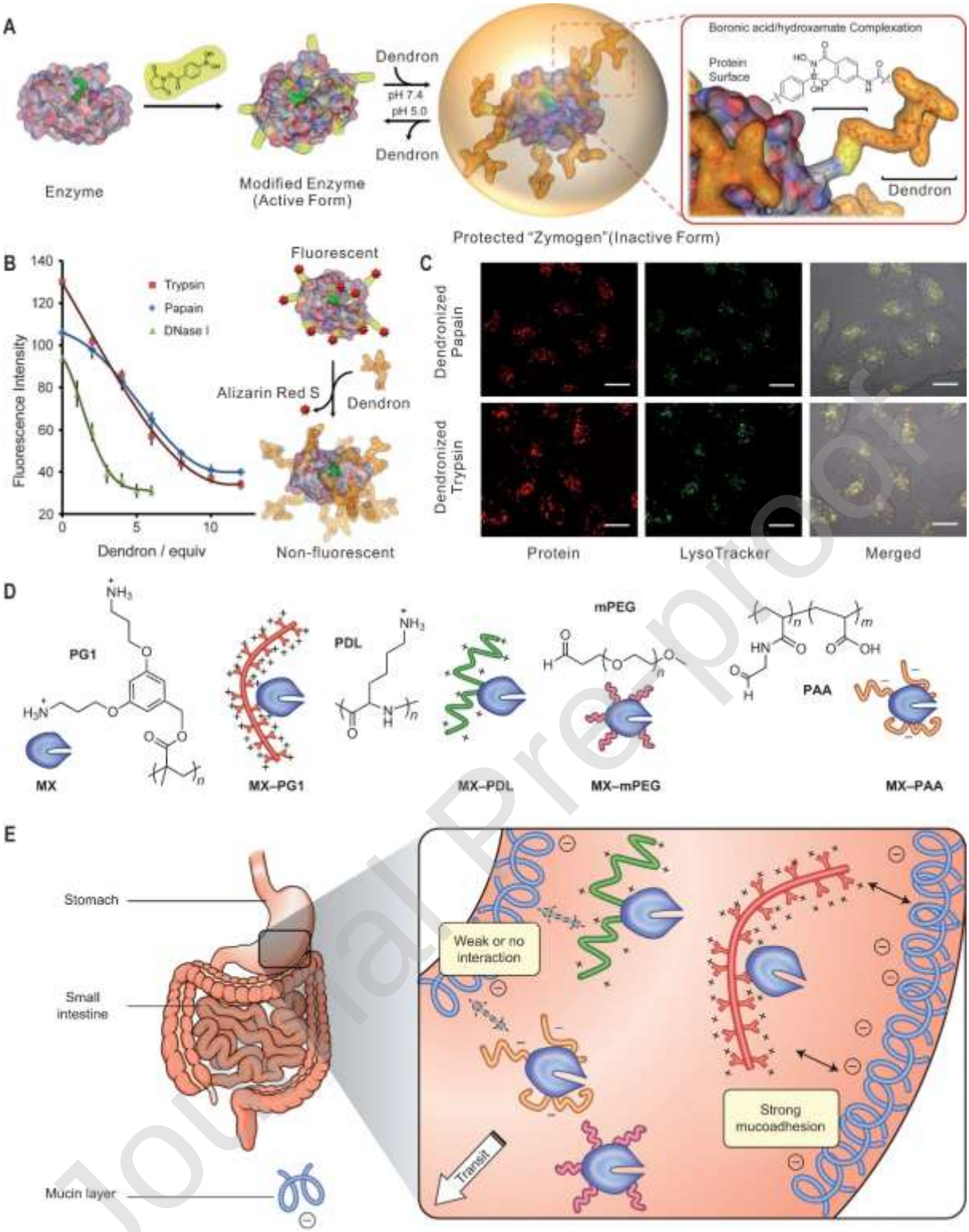

Fig. 5. Branched polymers for bioconjugation. (A) Preparation of supramolecular protein–dendron conjugates based on the pH-controlled interaction between boronic acid and hydroxamate. The residues highlighted in green represent catalytic sites. (B) The quantitative replacement of Alizarin Red S by the PAMAM dendron on protein surfaces revealed by a fluorescence assay. (C) Confocal microscopy images showing the dendron-mediated uptake by A549 cells and colocalization of these dendronized proteins within acidic cellular compartments. Scale bars: 20 µm. (D) Chemical structures of the four polymers used for enzyme conjugation and gastric stabilization. (E) The behavior of enzyme–polymer conjugates in the gastrointestinal tract.

2.3.3. Manipulation of the conjugate architecture

The structural control of polymer bioconjugates is not only focused on the polymer part. Due to the flexibility of using various synthetic tools, the conjugate architecture can also be programmed yielding innovative constructs with superior properties for specific applications [191]. To mimic protein dimerization occurring in Nature, well-defined linear PNIPAM produced by RAFT polymerization was functionalized with protein-reactive maleimide groups at both ends to synthesize homodimeric protein–polymer conjugates using a V131C mutant T4 lysozyme as the model protein [192]. The maleimide–thiol coupling was able to prepare the homodimers in 21% yield after 16 h. To increase the conjugation efficiency, the rapid tetrazine–*trans*-cyclooctene ligation was applied to afford the respective dimers in 38% yield within 1 h [193]. Recently, Bode et al. reported that potassium 2-pyridyl acyltrifluoroborates can be used to construct homodimeric protein–polymer conjugates under near equimolar conditions with a good yield of 82% [194]. Apart from these examples, heterodimeric protein–polymer conjugates have also been prepared by linking two different proteins with heterotelechelic polymers [195-198].

The conjugation of one polymer with multiple biomolecules, particularly functional peptides, forming multivalent systems is proven to be a successful strategy for enhancing specific molecular recognition in biological systems [199]. As an example, Klok et al. synthesized a series of multivalent side chain peptide–polymer conjugates to inhibit HIV-1 entry into a host cell and improved antiviral activity was achieved by midsized polymer conjugates [200]. To enhance targeting of integrin-expressing cells, a new type of "polymultivalent" polymer–cyclic RGD peptide cluster conjugates with two levels of multivalency were introduced and up to ~2 orders of magnitude potency enhancement was observed in a competitive cell adhesion assay [201].

The architecture of bioconjugate has significant effects on its properties and applications [202-204]. Based on a one-pot, two-step polymerization process, Lu et al. reported the easy synthesis of heterotelechelic poly(amino acid)s offering rapid access to protein–poly(amino acid) conjugates with various topologies (knot-like, dumbbell-like, and circular) under mild conditions [205]. This approach was based on two orthogonal chemical handles, including a thioester for native chemical ligation and a polyglycine for sortase A-mediated ligation, which were in situ installed at the C- and N-termini of substrate poly(amino acid)s. Notably, the head-to-tail cyclic conjugates using therapeutic interferon-α as a model protein exhibited dramatically improved protease resistance and thermostability. In a recent study, they further investigated the antitumor pharmacological activity of the cyclic conjugate in

comparison to its linear counterparts [206]. In vitro and in vivo experiments revealed distinct advantages of the cyclic conjugate in antiproliferative activity, circulation time, tumor retention and penetration, as well as antitumor efficacy.

3. Protein/peptide–polymer conjugates

Peptides and proteins are oligomers and polymers composed of amino acids, which often possess hierarchical structures and specific biological functions. Through conjugation of synthetic polymers, a novel class of soft hybrid materials, namely "protein/peptide–polymer conjugates" can be obtained combing the unique advantages of both natural and synthetic polymers [18, 19, 191]. One of the most attractive features of natural building blocks is their structure precision in view of sequence, molecular weight, 3D structure and supramolecular complex formation based on precisely defined intra- and intermolecular interactions. In chapter 2, we have discussed the site-specific polymer conjugation at the surface of individual native proteins. The main focus of this chapter is to discuss important advances for the preparation of peptide/protein–polymer conjugates, which have, to some extent, well-defined architectures.

In the first section, we introduce the conjugation of synthetic polymers to precision templates derived from native proteins, focusing on denatured proteins and protein cages. Thereafter, self-assembly of protein/peptide–polymer conjugates into defined architectures such as spherical nanoparticles, fibers, vesicles, and nanotubes are summarized. Moreover, the formation of well-defined structures on surfaces including the covalent immobilization of biomolecules by polymer brushes to gain spatial control over the respective biological activities are also discussed. This wide spectrum of well-defined structures based on protein/peptide–polymer conjugates enables various applications in both biomedical and non-biological areas, ranging from cancer treatment, antibacterial, and antivirus to artificial membrane channels, enzymatic catalysis, and soft actuators. In the last section of this chapter, we highlight selected examples of the most exciting applications, in which structural precision and well-defined structure formation play critical roles for enabling the specific application.

3.1. Proteins as precision templates for polymer conjugation

Proteins are the main components in most biological processes enabling, for example, structure formation, catalysis and transport. These unique features are based on their defined monomer sequence and precise 3D structures. In addition, some proteins are able to form well-defined higher order superstructures under specific conditions [207]. Therefore, proteins represent ideal building blocks to construct well-defined nanomaterials by providing precise

structure information at different levels. Here, we highlight recent advances on the construction of well-defined nano-architectures based on protein-derived templates such as the monodisperse polypeptide backbone of denatured proteins as well as highly symmetrical and ordered protein cages.

3.1.1. Precision nanomaterials based on denatured proteins

Globular proteins can be denatured by external stress such as solvents, inorganic salts, exposure to acids or bases, and by heat, which alters their secondary and tertiary structures but retains the peptide bonds of the primary structure between the amino acids [208]. Since all structural levels of the protein determine its function, the protein is usually no longer bioactive once it has been denatured. However, unfolded proteins could be regarded as monodispersed biopolymers providing well-defined contour length and various functional groups at determined positions along the main chain. In 2003, Whitesides et al. pioneered an approach for preparing linear polymers with determined chain lengths and functional groups at defined locations along the chain by acylation of denatured proteins [209]. In the past decade, our group has explored denatured proteins as a unique polymer platform for the construction of defined nano-architectures and nanomaterials for various applications [210]. For protein denaturation, protein aggregation during the denaturation process needs to be strictly avoided as it is very challenging to disaggregate the protein agglomerates once they have precipitated, which reduces yields and makes purification more difficult. Typically, chaotropic agents such as urea to break hydrogen bonds and other supramolecular forces and mild reducing agents such as tris(2-carboxyethyl) phosphine (TCEP) are added. Stabilizing hydrophilic polymer chains can be attached to the polypeptide backbone before or after the denaturation step to prevent aggregation of the denatured polypeptide chains [210]. In our design, PEG chains of different molecular weights (2000–5000 Da) have been covalently linked through either thiol–maleimide chemistry or amine–NHS ester chemistry. PEG chains provide sufficient stability under the denaturing conditions as well as biocompatibility and they alter the hydrophilic–hydrophobic balance of the denatured polypeptide chain consisting of hydrophilic and lipophilic sequence patterns preventing undesirable supramolecular interactions within the chains also due to the steric effect [211]. Figure 6A shows a typical procedure for PEG conjugation followed by unfolding of the blood plasma protein human serum albumin (HSA, 66 kDa) by 5 M urea–phosphate buffer (PB) in the presence of TCEP. Thiol groups of the unpaired cysteines and reduced disulfide bonds are typically exposed during the denaturation step and they can be capped by different maleimides such as PEG–maleimide and *N*-(2-aminoethyl)maleimide to avoid reformation of disulfide bonds. Noteworthy, the optimal denaturing conditions need to be carefully identified as each protein has a different

inherent stability based on its folding as well as the number and location of the disulfide bridges. In this way, hen egg white lysozyme with a molecular weight of 14 kDa requires more drastic denaturation conditions, i.e., 8 M guanidine and excess of the reducing agent dithiothreitol (DTT) for denaturation compared to HSA [212]. By reacting single accessible thiol groups of BSA with PEG–bismaleimide to synthesize a protein-dimer precursor, a giant polypeptide–PEG–polypeptide triblock copolymer of defined structure, composition and a very high molecular weight of about 400 kDa has also been reported via the PEGylation and denaturation strategy [213].

The denatured protein–PEG conjugates synthesized by the convenient approach provide several attractive characteristics: (1) biocompatibility; (2) biodegradability by proteases; (3) defined peptide sequence; (4) the final polymers offer narrow molecular weight distributions that can be characterized by mass spectrometry ensuring the quality control of products; (5) various functionalities in specific positions which allow the realization of complex tasks such as cellular uptake and intracellular delivery; and (6) tunable transition between globular, collapsed and extended architectures. In addition, the PEG side chains could reduce protein binding and provide "stealth properties" by shielding the immunogenic recognition sites (epitopes) [214]. Therefore, polypeptide–PEG conjugates based on denatured proteins provide various attractive features for biomedical applications and as precision substrates for templated synthesis of well-defined nanomaterials (Fig. 6).

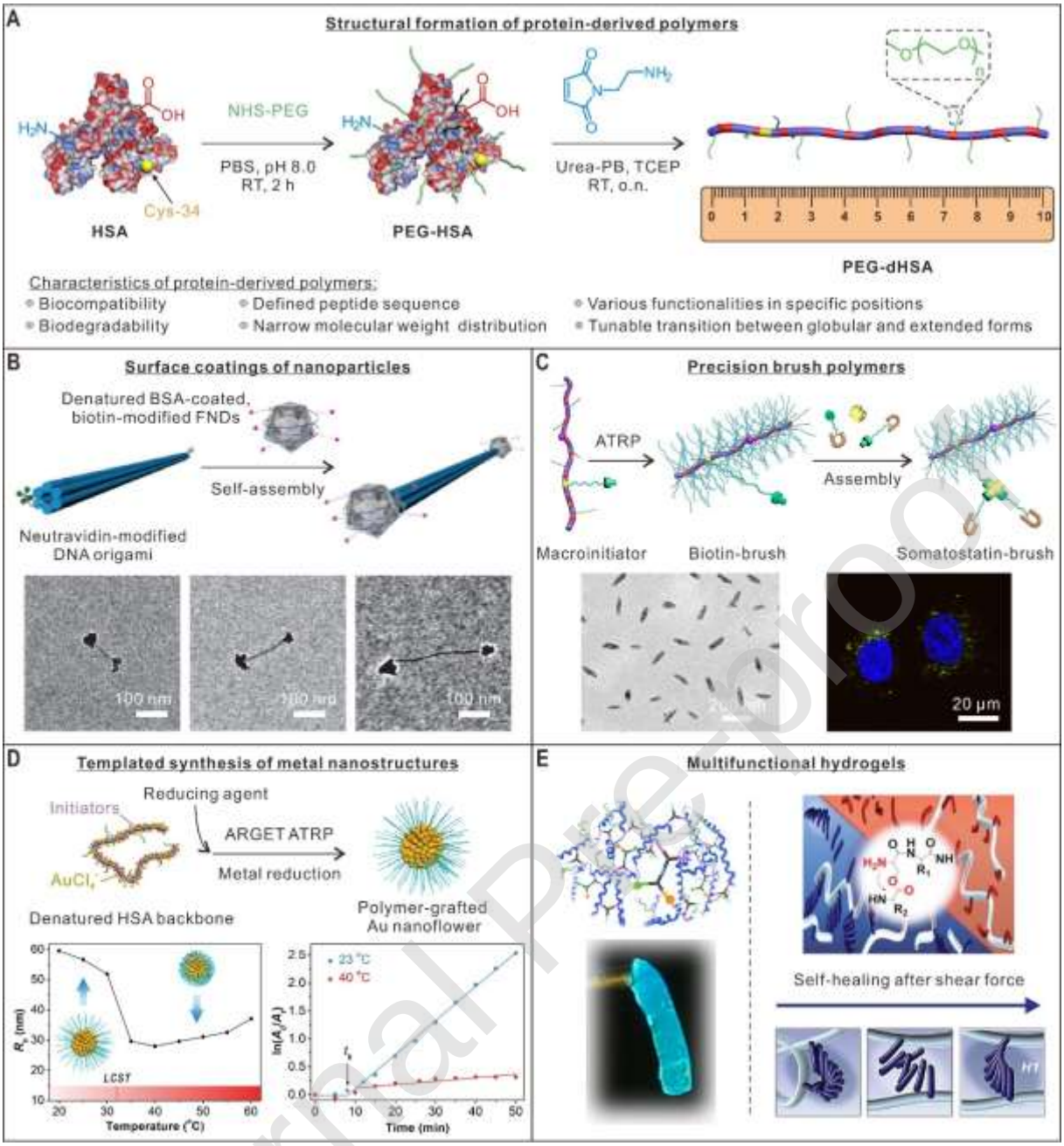

Fig. 6. Synthesis and applications of denatured protein–PEG conjugates. (A) A typical procedure to synthesize protein-derived polymers by PEG conjugation and denaturation of HAS; (B) Surface modification with denatured protein–PEG conjugates and precise assembly of nanodiamonds by DNA origami; (C) Denatured proteins as a precision backbone for the synthesis of anisotropic brush polymers, which allow site-specific functionalization of the main chain and assembly; (D) Templated synthesis of PNIPAM-grafted gold nanoflowers in one pot for temperature-controlled catalysis; E) Denatured protein–PEG conjugates as multifunctional and degradable backbones to prepare functional hybrid hydrogels. Left: DNA-induced crosslinking of the denatured protein–PEG backbone affording protein–DNA hybrid hydrogels. Right: self-healing hydrogels with inner fibrillar structures by crosslinking of the copolymers with self-assembling peptides as pH-responsive gelators for cell cultivation.;

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Because of their unique optical properties, quantum dots (QDs) and fluorescent nanodiamonds (FNDs) are two highly promising probes for tracking biological processes i.e. with super-resolution microscopy and drug delivery applications [215, 216]. However, applications of the "bare" nanoparticles are severely limited by their poor solubility in various biological environments. In addition, other challenges include the toxicity of QDs [217] and the surface modification of FNDs that provide undefined surface functionalities with high batch-to-batch variations [218]. Denatured protein–PEG conjugates serve as attractive nanoparticle coatings due to the availabilities of many reactive amino-, carboxylic acid and thiol groups that could interact with various nanoparticle surfaces though electrostatic interactions or hydrogen bonds as well as the presence of hydrophobic amino acids that bind hydrophobic surfaces by van der Waals interactions. For example, denatured HSA–PEG conjugates functionalized with multivalent thioctic acid groups stabilize the surface of CdSe–CdZnS QDs [219]. The coated QDs gain improved water-solubility and unique pH-responsiveness, which was attributed to conformational rearrangements of the polypeptide coating at different pH. This could alter the capacity of the polymer to efficiently passivate and protect the nanoparticle surface. Based on this strategy, a polycationic polypeptide–PEG conjugate based on denatured BSA was achieved that encapsulated QDs and enabled their cellular uptake and allowed DNA complexation [220]. In these systems, the QD core served as an *in situ* reporter for pH changes, DNA complexation and ultimately even DNA transfection because its photoluminescence dropped significantly with increasing quantities of complexed DNA. Similarly, the cationized and denatured protein–PEG conjugates could also offer excellent colloidal stability to FNDs so that they remained stable even in the presence of high ionic strength buffers required for DNA origami folding (Fig. 6B). In this way, the first DNA origami-assembled FND nanostructures were formed, which is a critical step to study the coherent coupling of ordered spin arrays [221]. Moreover, the biopolymer-coated FNDs remained stable even after encapsulating high amounts of hydrophobic doxorubicin drug molecules and revealed high uptake into human lung adenocarcinoma A549 cells and in vivo efficacy attractive for cancer therapy [222].

In comparison to synthetic polymers, the most prominent advantages of denatured proteins are their monodisperse lengths and defined amino acid sequences. Therefore, the denatured protein–PEG conjugates can be used as precision templates for the preparation of various structurally defined nanomaterials. Very recently, Weil et al. have reported the construction of precision brush polymers using denatured proteins as a monodisperse macromolecular backbone (Fig. 6C) [223]. By introducing ATRP initiators to denatured HSA–PEG conjugates, anisotropic brush polymers with monodisperse contour lengths and narrow distributions were obtained by grafting polymer side chain from the backbone. The size and anisotropy of the brush polymers were tuned by varying

polymerization conditions and the initiator density on the polypeptide backbone. Particularly, a distinct functionality can be introduced onto an absolute position located asymmetrically along the polypeptide backbone of these brush polymers. By combining this site-specific functionalization strategy with biotin—streptavidin interactions, various functional entities such as a single fluorescent dye, a gold nanoparticle, the hormone somatostatin, and a model antibody were introduced via site-specific assembly to fabricate novel higher ordered constructs, which may find potential applications in both biomedicine and nanoscience [223]. As shown in the confocal laser scanning microscopy image of Fig. 6C, biotin-containing brush polymers self-assemble with biotin-functionalized somatostatin in the presence of streptavidin and the formed construct revealed somatostatin-mediated uptake into cancer cells.

Due to the presence of abundant amino groups in the backbone, denatured protein–PEG conjugates possess strong capability to bind metal ions. Therefore, the biopolymer providing high water solubility was used as an ideal substrate for templated synthesis of metal nanoparticles. For instance, our group has reported a denatured HSA–PEG conjugate functionalized with TAT peptide, and mitochondria targeting triphenyl-phosphonium groups for the synthesis of ultrasmall gold nanoparticles with good biocompatibility and high stability [224]. Recently, the denatured HSA–PEG conjugate has been employed as a precision template for the preparation of polymer-grafted gold nanoflowers by combining ARGET ATRP and metal reduction in a one-pot fashion [225]. The cationized biopolymer with immobilized ATRP initiators serves both as a platform to bind chloroauric anions and as a macroinitiator for ARGET ATRP. Ascorbic acid was then added continuously into the system to activate ATRP catalyst precursors and to reduce gold ions in parallel (Fig. 6D). PNIPAM-grafted gold nanoflowers of controllable sizes, shapes and thermo-responsiveness have been achieved and applied as smart nanoparticle catalysts for the hydrogenation of *p*-nitrophenol to *p*-aminophenol. This convenient approach based on protein-derived templates could be expanded to other functional polymers and noble metal nanoparticles, providing access to various polymer-coated metal nanostructures for broad applications in catalysis, sensing, and biomedicine [225].

The architecture of denatured protein–PEG conjugates responds to changes of the balance of hydrophilic and groups along the polypeptide backbone. These changes could either be lipophilic functionalities that are covalently attached or the presence of hydrophobic guest moieties that interact with the lipophilic amino acid side chains via supramolecular interactions. In this way, well-defined core–shell nanostructures were formed suitable for catalysis and delivery of lipophilic molecules into cells. When the cationized and denatured BSA–PEG conjugate was modified with just a few hydrophobic groups such as alkynes, stable nano-sized micelles were formed

spontaneously [226]. Complexation with the hydrophobic chromophore perylenetetracarboxydiimide, a denatured HSA–PEG conjugate functionalized with folic acid groups, has been shown to form globular micelles, which were uptaken into cells via receptor-mediated endocytosis [227]. The lipophilic drug doxorubicin has also been encapsulated into these micelles by complexation [228] or covalent conjugation [226] and efficient delivery into various cancer cells has been shown. To achieve selectivity and better control over the drug release profile, a pH-responsive hydrazone linker has been introduced to conjugate doxorubicin to the denatured protein backbone that potentially allows release in the acidic microenvironments of tumor tissue as well as in acidic endosomal vesicle [229]. The sophisticated core–shell delivery system composed of a polypeptide core with doxorubicin drug molecules and a PEG shell adopts a two-step drug release based on proteolytic degradation of the backbone and acid-induced drug release. In vitro test of the drug-loaded micelles revealed very high cytotoxicities against Hela cells and leukemia cell lines. More importantly, 100% survival rates of mice that received ex vivo transplantation of engrafted leukemic tumor cells after 12 weeks were demonstrated [229].

In combination with various crosslinking chemistries, the denatured protein–PEG conjugate served as biocompatible and biodegradable high molecular weight scaffold to prepare injectable hybrid hydrogels. As crosslinkers, multi-arm DNA [230] as well as self-assembling peptide sequences [231] have been applied. Denatured HSA–PEG conjugates were functionalized with ssDNA sequences that could hybridize with complementary Y-shaped DNA [230]. The formed hydrogel was used to immobilize active proteins including GFP and YFP, which were released by proteases as well as nucleases independently (Fig. 6E). Furthermore, conjugation of a recombinant Rho-inhibiting C3 toxin that inhibits growth and migration of bone degrading osteoclast cells to the multi-arm DNA linker allows the toxin-loaded hydrogel to reduce osteoclast formation and bone resorption without affecting differentiation and mineralization of bone forming osteoblast cells [232]. In another example, self-assembling peptides that spontaneously form cross β-sheet fibrillary structures were grafted to the backbone of denatured HSA–PEG conjugate. To control fibril formation of the peptides, they were masked as depsi-precursor peptides. The depsi peptide sequences do not aggregate at acidic pH until an intramolecular O,N-acyl shift occurs at higher pH values affording the formation of peptide nanofibers, which served as pH-responsive gelators (Fig. 6E). The obtained hydrogels are cytocompatible, biodegradable, reveal rapid self-healing abilities and cells migrated into this porous matrix, rendering them attractive for 3D tissue engineering [231].

3.1.2. Protein cages for grafting synthetic polymers

Protein denaturation destroys the 3D structure of native proteins so that nanostructures are mainly formed within the polymer chain by external guests or stimuli. In another class of nanostructures, the self-organizing features of proteins are retained so that distinct and large protein nanostructures are formed. Protein cages of different sizes are widely formed in Nature, such as mammalian ferritins with a diameter of 12 nm and virus-derived icosahedral protein cages with diameters from approximately 28 nm (brome mosaic virus and cowpea chlorotic mottle virus) to 95 nm (human adenovirus) and more than 500 nm (megavirus chilensis) (Fig. 7A). These well-defined 3D hollow architectures with symmetric shapes and uniform sizes are formed via the self-assembly of individual protein subunits [233]. They have received rapidly growing interests of the materials science community due to their broad applications as nanoscale reactors, as scaffolds for nanomaterial synthesis, and as versatile vehicles to deliver a broad range of drugs, genes, and imaging agents [233, 234]. In addition, the subunits of protein cages can be chemically or genetically modified at specific locations, allowing the conjugation of functional moieties within the interior cavity and/or on the exterior surface in a site-selective manner [235]. Polymer conjugation of protein cages give them entirely new properties and expand the range of applications. For instance, PEGylation of protein cages is a very popular and effective strategy to reduce the immunogenic response facilitating their usage for biomedicine [236-238]. In addition, surface engineering with functional polymers offers stimuli-responsiveness [239], increased stability [240, 241], and solubility in organic solvents [242, 243].

Based on the well-established chemistries to prepare peptide–polymer conjugates, the *grafting to* approach using reactive groups at the surface of these cages is a relatively straightforward modification strategy to decorate protein cages with synthetic polymers. For example, Finn et al. have attached poly(2-oxazoline)s to the exterior surface of bacteriophage Qβ via the CuAAC click reaction [240]. They used a multiple-point conjugation strategy and the polymer-conjugated protein cages showed significantly enhanced thermal stability, surviving at temperatures higher than 100 °C. Thermo-responsive smart polymer PNIPAM has also been conjugated to the surface of vault, a recombinant protein cage with a size of 41 × 41 × 72.5 nm, by coupling the thiol group at N-terminus of the major vault protein [239]. The obtained vault nanoparticles exhibited reversible aggregation behaviors that can be controlled by temperature. Pokorski et al. have attached water-soluble polynorbornene (PNB) chains, which were synthesized by ROMP, to the outer surface of bacteriophage Qβ [244]. Significantly, PNB with brush-like architectures demonstrated better shielding effect from antibody recognition than PEG for the protein cages [245]. In general, the direct conjugation of polymer chains to the interior surface of protein cages is considered more challenging to achieve due to the steric effect. In this regard, dendritic PAMAM has been conjugated into the protein cage thermosome, a

group II chaperonin that possesses a large pore size of 7 nm [246]. The thermosome–PAMAM conjugate was successfully used for small interfering ribonucleic acid (siRNA) delivery [246] and templated synthesis of gold nanoparticles inside of the protein cage [247].

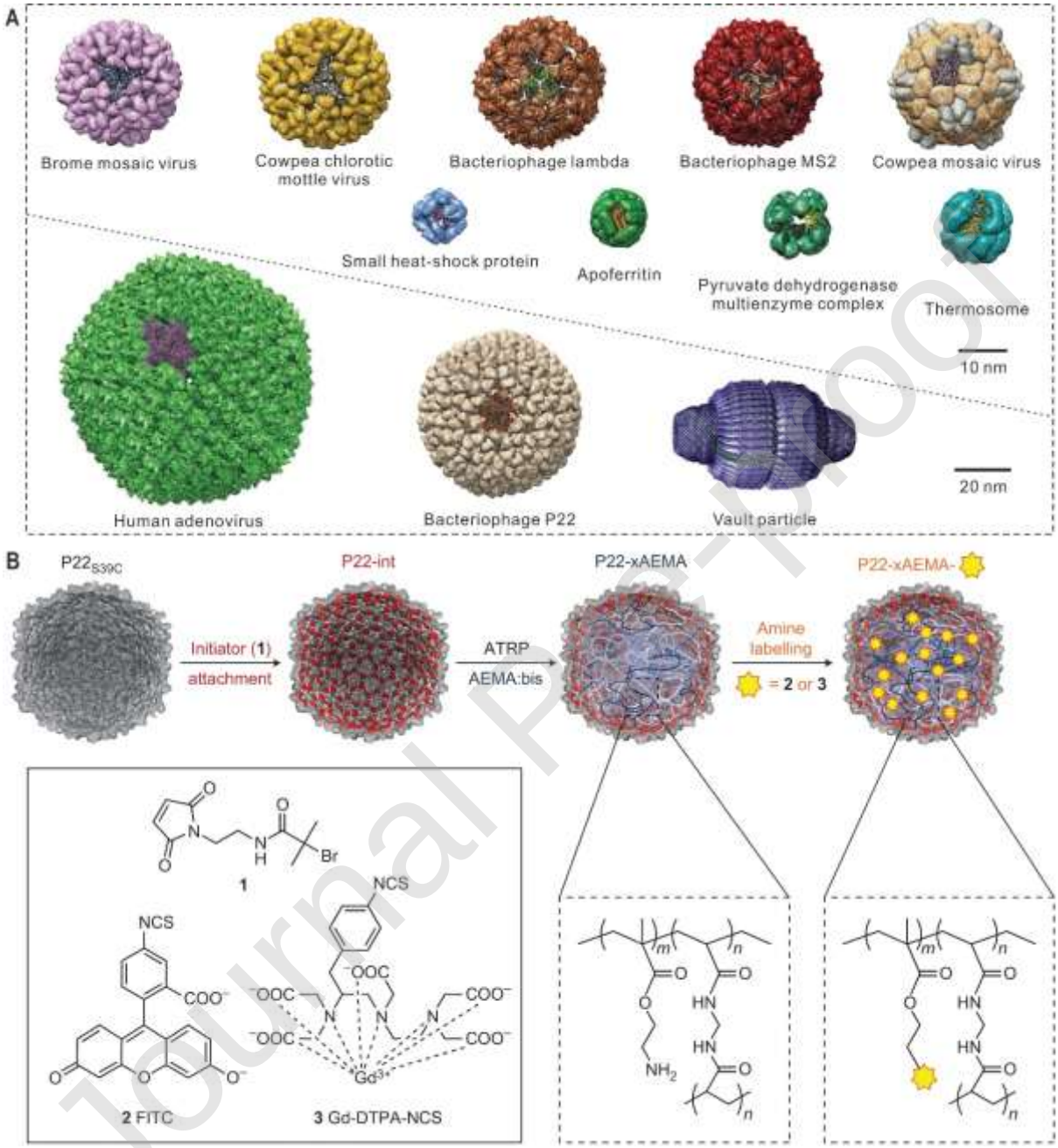

Fig. 7. Protein cages for polymer conjugation. (A) Structures of representative protein cages; (B) Site-specific ATRP growth of functional polymers from the interior cavity of the bacteriophage P22 virus-like particle. The internal functional polymer was subsequently labeled with a fluorophore or a paramagnetic MRI contrast agent.
(A) [233], Copyright 2016. Reproduced with permission from the Royal Society of Chemistry.
(B) [253], Copyright 2012. Reproduced with permission from Springer Nature.

In contrast to the *grafting to* approach, it has become a recent trend to conjugate polymers to protein cages via the *grafting from* approach [248], which should generate polymer conjugates with better-defined structures. By modifying the exterior surface of horse spleen ferritin nanocage with an ATRP initiator, the polymerization of 2-methacryloyloxyethyl phosphorylcholine and PEG methacrylate has been realized by Russell, Emrick and coworkers [249]. Antibody recognition experiments revealed the "stealth" properties of these hydrophilic coatings. Böker et al. have reported the copolymerization of NIPAM and photo-crosslinkable 2-(dimethyl maleinimido)-*N*-ethyl-acrylamide from the surface of the same protein cage [250]. These bioconjugates have been shown to stabilize emulsions, which allows the formation of thermo-responsive capsules for controlled drug delivery after cross-linking. Finn et al. have polymerized an azido-functionalized oligo(ethylene glycol) methacrylate from the exterior surface of the bacteriophage Qβ by ATRP [251]. This monomer even facilitated post-functionalization of the protein cage via the CuAAC reaction, which was demonstrated by conjugation with an alkyne-substituted Alexa Fluor 488 dye, a gadolinium complex (Gd–DOTA) contrast agent for magnetic resonance imaging (MRI), as well as a pH-sensitive and clickable doxorubicin derivative for anticancer therapy.

In addition to the functionalization of the outer surface, Douglas and coworkers pioneered the site-specific growth of both branched [252] and linear [253] polymers from the interior surface of protein cages. They showed stepwise synthesis of a dendritic polymer from heat shock protein (HSP), whose interior cavity has a diameter of 6.5 nm [252]. Here, cysteine reactive sites genetically introduced to the inner side of the protein cage served as the initiation sites. By sequential conjugation of azide and alkyne monomers using click chemistry, the polymer grew to generation 2.5, which formed fully cross-linked network across the protein subunits, rendering the protein cage thermally stable even at 120 °C. In addition, a large number of free amines has been incorporated into the branched polymer chains, which further offers addressable sites to load additional functional components. In their following contributions, the Gd-diethylene triamine pentaacetic acid (Gd-DTPA) contrast agent was attached to the reactive amines of the polymer network [254]. Each protein cage was shown to incorporate up to a maximum of 159 Gd, and the functionalized protein cages demonstrated a per particle relaxivity of 4200 mM^{-1} s^{-1} which was among the highest reported values for protein cage–Gd MRI contrast agents. This strategy was further extended to construct a branched iron–phenanthroline based coordination polymer within the protein cage of HSP [255]. However, the stepwise growth method involves tedious reaction steps, and it is very challenging to achieve polymers with high molecular weights and high densities within the protein cage. To address this issue, the same group has reported the first example of site-specific ATRP growth from the inside cavity of a mutant of the bacteriophage P22 capsid [253]. This P22 protein

cage consists of 420 subunits with an interior diameter of 54 nm. ATRP initiators were attached to the only addressable cysteine of each protein subunit, which was mutated to be exposed within the inner cavity, in a near-quantitative manner (Fig. 7B). By copolymerization of 2-aminoethyl methacrylate (AEMA) and bisacrylamide using standard ATRP conditions, cross-linked polymer networks were formed in the interior of the protein cage. Importantly, the reactive primary amines of the polymer chains were still accessible, as confirmed by post-functionalization with small molecules such as fluorescein isothiocyanate (FITC), Gd-DTPA, a photosensitizer (Eosin-Y), and a cobaloxime catalyst [253, 256]. Notably, the obtained polymer-conjugated protein cages revealed a high loading capacity of 9100 ± 800 Gd per cage, affording an ultrahigh per particle relaxivity of 200,000 mM^{-1} s^{-1}. In order to obtain nanoreactors for photocatalytic applications, AEMA was also copolymerized with [ruthenium(5-methacrylamido-phenanthroline)$_3$]$^{2+}$ from the inner surface of the P22 capsid [257]. A similar approach have also been demonstrated by Finn and coworkers to polymerize a positively charged monomer *N*,*N*-dimethylaminoethyl methacrylate from the interior surface of Qβ for the delivery of siRNA [258].

Collectively, protein cages constitute well-defined templates for grafting synthetic polymers with defined inner holes and outer surfaces and they receive emerging interest for drug delivery and bioimaging. It should be noted that protein cages can also be combined with synthetic polymers by many other interactions such as electrostatic complexation or non-covalent encapsulation of synthetic polymers into protein cages [233]. For example, the protein corona on adenovirus 5 (Ad5), one of the main vectors in gene therapy, has been mimicked by polyphenylene dendrimers with a distinct amphiphilic surface pattern [259]. These dendrimers coated the surface of Ad5 by distinct non-covalent interactions, which abolished binding of blood coagulation factor X, facilitated uptake into receptor negative cells, which was not possible for Ad5 alone. The dendrimer corona had a significant impact on Ad5 in vivo trafficking and the Ad5–dendrimer complexes revealed a new bioactivity profile, which could be attractive to broaden the therapeutic applications of Ad5. In addition, some attention has been paid to the self-assembly of protein–polymer conjugates into protein cages and protein cage-mimicking nanostructures, which are discussed in greater details in section 3.2.2.

3.2. Assemblies of protein/peptide–polymer conjugates

3.2.1. Polymer conjugates based on self-assembling peptides

Polypeptides have been frequently used as building blocks for the preparation of amphiphilic block copolymers. In contrast to proteins, peptides provide shorter chain lengths, lower molecular weights and less complex

tertiary structures. In the past two decades, self-assembly of polypeptide-based block copolymers into micelles and vesicles has been intensively explored, particularly for applications in catalysis and drug delivery [260-264]. In contrast to conventional synthetic polymers, peptide sequences can interact with each other via different supramolecular interactions such as hydrogen bonding, π-π stacking, and metal ion coordination to form well-defined secondary structures and superstructures. This attractive characteristic offers additional opportunities to control the self-assembly of polypeptide-based copolymers and many unique structures have been achieved [265, 266]. For example, Hawker, Knight and coworkers reported the self-assembly of peptide–polymer conjugates based on metal ion coordination of peptides [267]. As shown in Fig. 8A, the amphiphile oSt$(His)_6$ consists a hydrophobic polystyrene block and a hydrophilic block, hexahistidine, which can coordinate with divalent transition metal ions to form dimers. In the absence of metal ions, oSt$(His)_6$ spontaneously self-assembled into vesicles in a noncoordinating buffer (HEPES, 100 mM, pH 7). When different metal ions [Mn(II), Co(II), Ni(II), Cu(II), Zn(II), and Cd(II)] were added during the self-assembly process, the conjugate formed a wide range of new structures including micelles [Ni(II), Cd(II)], aggregated micelles [Zn(II), Co(II), Cu(II)], and multilamellar vesicles [Mn(II)].

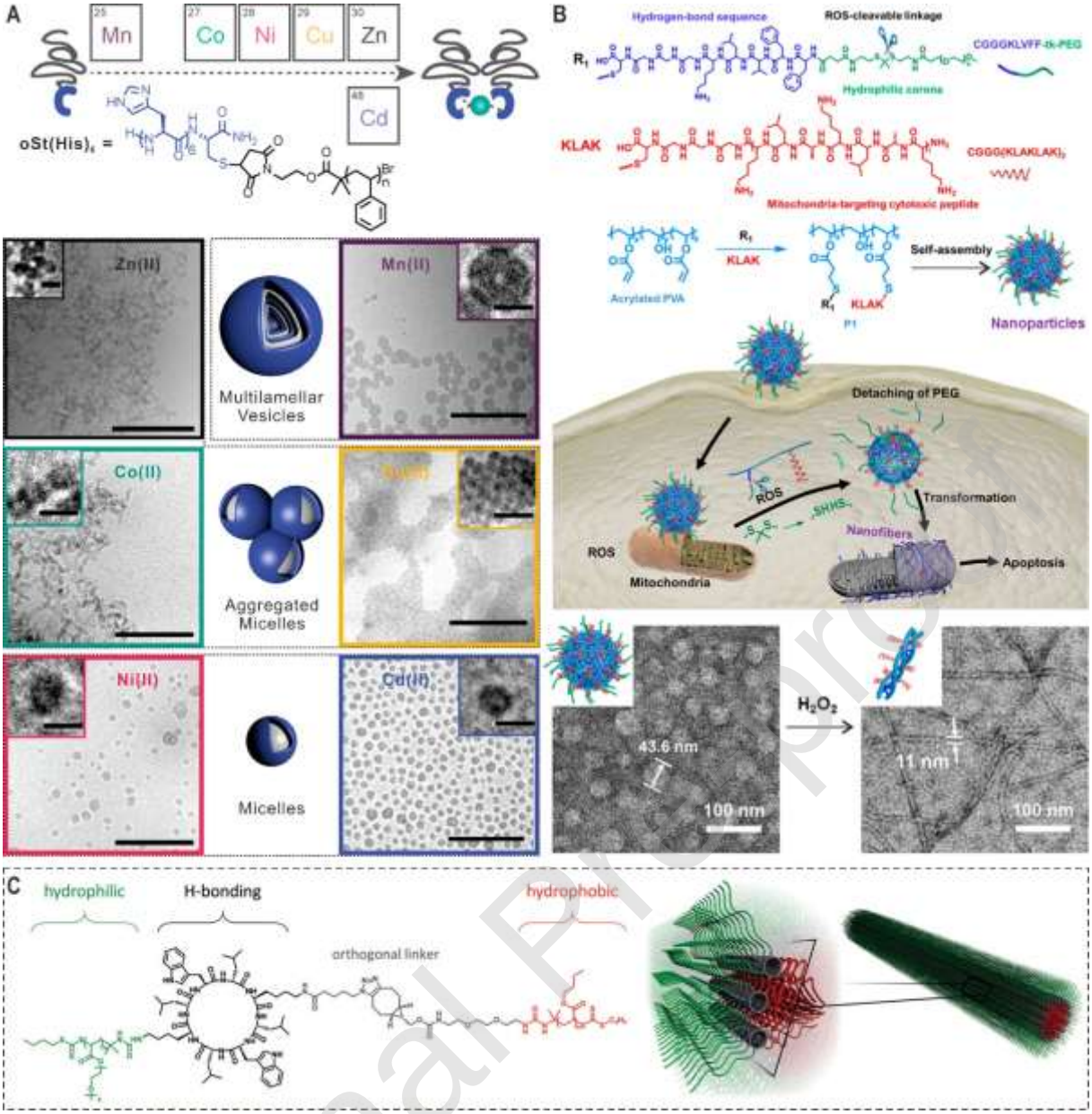

Fig. 8. Self-assembly of peptide–polymer conjugates. (A) Metal ion coordination as driving force for the self-assembly of amphiphile oSt(His)$_6$ into various morphologies. Scale bars in cryogenic transmission electron microscopy (cryo-TEM) images represent 200 nm (larger image) and 20 nm (inset image); (B) Synthesis and self-assembly of ROS-responsive peptide–polymer conjugates and the shape transformation around mitochondria for enhanced antitumor efficacy. Bottom: transmission electron microscopy (TEM) images showing H_2O_2-induced shape change of the peptide–polymer conjugate; (C) Molecular structure of PBA-CP-PPEGA and its hierarchical self-assembly into tubisomes.
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(C) [298], Copyright 2018. Reproduced with permission from John Wiley and Sons Inc.

Long fibrous structures can be obtained through self-assembly of sequence-controlled oligopeptides that have a tendency to form β-sheet structures [268-270]. Not surprisingly, introducing β-sheet forming peptides into the structure may allow peptide-guided self-assembly of polymer conjugates into well-defined fiber-like structures. For instance, Börner et al. reported the formation of fibers with a maximum length of up to 1 μm by conjugating PEO

chains to template pre-organized oligopeptides [271]. These oligopeptides exhibited a high tendency to form β-sheet motifs due to the restriction of conformation freedom. Chen and coworkers investigated the self-assembly behaviors of a series of amphiphilic brush polymers with dendronized oligosaccharide and oligophenylalanine as side chains [272]. Depending on different ratios of sugar units to the oligopeptide, various self-assembled morphologies including compound micelles, nanowires, and nanoribbons were observed. Interestingly, the nanowire was formed via a hierarchical self-assembly process driven by the carbohydrate–carbohydrate interaction of the sugar units and the β-sheet forming tendency of oligophenylalanine.

In biological systems, peptides are dynamic materials and their conformations and biological activities are often regulated by changes in their direct surrounding. Synthetically, the introduction of switchable peptides into polymer bioconjugates would offer vast opportunities for the structural control over their assemblies. The Börner group reported a PEO–peptide conjugate based on a peptide sequence with five repeats of alternating valine and phosphorylated threonine [273]. This conjugate was soluble in aqueous solution. However, fiber formation was triggered by the enzymatic dephosphorylation of the peptide block due to conformation changes of the peptides into β-sheet structures [273]. Recently, in situ construction and shape transformation of peptide-based assemblies in specific physiological environments have been demonstrated as promising strategies for biomedical applications [274-277]. Wang, Qiao and coworkers reported reactive oxygen species (ROS)-responsive polymer–peptide conjugates, which undergo morphology changes inside tumor cells [278]. Possessing a mitochondria-targeting peptide KLAK and a β-sheet forming peptide KLVFF conjugated with PEG through a ROS-cleavable linker as side chains, these conjugates were able to self-assemble into spherical nanoparticles and target mitochondria after entering cells (Fig. 8B). Due to the high ROS concentration around mitochondria, PEG chains were detached, which induced in situ formation of nanofibers with exposure of KLAK peptides. This shape transformation enhanced the multivalent cooperative interactions between KLAK and mitochondria, leading to improved anticancer effects *in vitro* and *in vivo*.

In addition to linear peptides, β-sheet forming cyclic peptides that can self-assemble into well-defined nanotubes have received special attention in recent years. Polymer conjugation offers many advantages to these nanotubes such as improved solubility, a wide spectrum of functionalities, and additional control over the tube length [279]. This does not only allow for a better understanding of the self-assembly mechanism, but also significantly broadens applications of these nanotubes. Basically, polymer strands can be attached to peptide nanotubes both before and after assembly via either the *grafting to* or *grafting from* approach [280-282]. In 2005, Biesalski et al. reported

the first example of growing polymer chains from surface-immobilized initiators of cyclic peptide nanotubes via ATRP [283]. In addition, they demonstrated that length and diameter of the polymer–peptide nanotubes are highly affected by the molecular weight of grafted polymers [284]. Since the early studies, Perrier and collaborators have made significant contributions in this field by elucidating the tube structure [285, 286], tracking their assembly processes [287, 288], as well as exploring a wide variety of applications [289-291].

In order to control the self-assembly behavior and tube length, Perrier et al. have introduced different stimuli-responsive polymers to cyclic peptide nanotubes. For example, poly(2-ethyl-2-oxazolin) was successfully used to realize temperature-controlled reversible transformation from nanotubes to microparticles [292]. In addition, a series of pH-responsive polymers including PAA [293], poly(dimethylamino ethyl methacrylate) [294], poly[2-(diisopropylamino)ethyl methacrylate] [295] have also been conjugated into cyclic peptide nanotubes, which allow modulation of their self-assembly upon pH changes. Recently, host–guest interactions were also employed to switch the self-assembly of a cyclic peptide–PEG conjugate [296]. In this system, two phenylalanine groups as binding sites of cucurbit[7]uril were attached to the cyclic peptide, and the nanotube formation could be tuned by reversibly incorporating two bulky cucurbit[7]uril moieties via host–guest chemistry.

Cyclic peptide–polymer nanotubes have also been used as building blocks to construct well-defined higher order structures. For instance, Jolliffe et al. reported that hydrophobic cyclic peptide–polymer nanotubes with a Janus corona were able to self-assemble in artificial phospholipid bilayers and form transmembrane channels for a dye [297]. In collaboration with the Perrier group, they further designed an asymmetric cyclic peptide–polymer conjugate (PBA–CP–PPEGA) with a hydrophilic PPEGA chain on one side and on the opposite side, a hydrophobic poly(*n*-butyl acrylate) (PBA) chain [298]. This amphiphilic conjugate demonstrated a hierarchical self-assembly in aqueous solution by first forming amphiphilic Janus nanotubes via hydrogen bonds and then generating a superstructure, called tubisome based on terms liposome and polymersome, driven by the hydrophobic interactions (Fig. 8C). These tubisomes were able to fuse into the lipid bilayer of lysosomes in cells forming artificial pores. To identify the key factors to obtain tubisomes, a more detailed study was conducted with varied hydrophilic–hydrophobic ratios of the PBA–CP–PPEGA conjugate [299].

3.2.2. Self-assembly of protein–polymer conjugates

As a unique class of polypeptides with fully folded structures and globular shapes, proteins in most cases provide biological functions to protein–polymer conjugates. When hydrophobic polymers are attached to water-

soluble proteins, the amphiphilic conjugates self-assemble in a manner similar to that of low molecular weight surfactants and synthetic block copolymers in aqueous solution. Therefore, these protein-based amphiphiles can also serve as building blocks for the construction of a wide range of solution nanostructures. Early examples reported by Nolte and coworkers have demonstrated the self-assembly of protein–polymer conjugates into fibers [300], vesicles [301], and toroids [302]. In recent years, self-assembled nanoparticles based on protein–polymer conjugates have been intensively explored as carriers for delivery of anticancer drugs [303-306]. Due to the presence of proteins, these self-assembled nanostructures possess the special advantage of built-in bioactivity. For example, Thordarson et al. conjugated a maleimide-capped PNIPAM chain to the free cysteine residue of a GFP variant (amilFP497) [307]. The resulting conjugate PNIPAM-*b*-amilFP497 assembled into vesicles in aqueous solution upon heating to 37 °C. Fluorescent characteristics of amilFP497 were not affected during polymer conjugation, which allowed direct observation of vesicle formation using confocal microscopy. These vesicles were used as carriers to encapsulate doxorubicin and a fluorescent light-harvesting protein phycoerythrin 545 (PE545) [307]. Importantly, the location of payloads could be determined by combining fluorescence lifetime imaging microscopy and Förster resonance energy transfer (FRET), showing PE545 protein primarily located inside the vesicle membrane whereas doxorubicin was found both in the core and membrane.

Recently, *in situ* growth of an insoluble polymer block from solvophilic polymers in solution to generate self-assembled nanostructures has become a new trend in macromolecular self-assembly [308-311]. This technique, termed polymerization-induced self-assembly (PISA), has also been expanded to the field of protein–polymer conjugates. As a proof-of-concept experiment, Gao et al. site-specifically attached an ATRP initiator to the only free cysteine group (Cysteine 34) of HSA and then polymerized water-soluble 2-hydroxypropyl methacrylate (HPMA) from the initiator via ATRP [312]. The resulting amphiphilic conjugate HSA–poly(2-hydroxypropyl methacrylate) (PHPMA) could self-assemble to well-defined nanostructures with tunable morphologies including micelles, wormlike micelles, and vesicles (Fig. 9A). In order to construct a tumor microenvironment-responsive fluorescence probe, this approach has been used to prepare pH-responsive micelles by polymerizing 2-(diisopropylamino)ethyl methacrylate from HSA [313]. In a similar way, Huang, Liu and coworkers reported photoinitiated RAFT PISA to generate protein–polymer micelles via polymerization of HPMA from a multi-RAFT agent modified BSA [314].

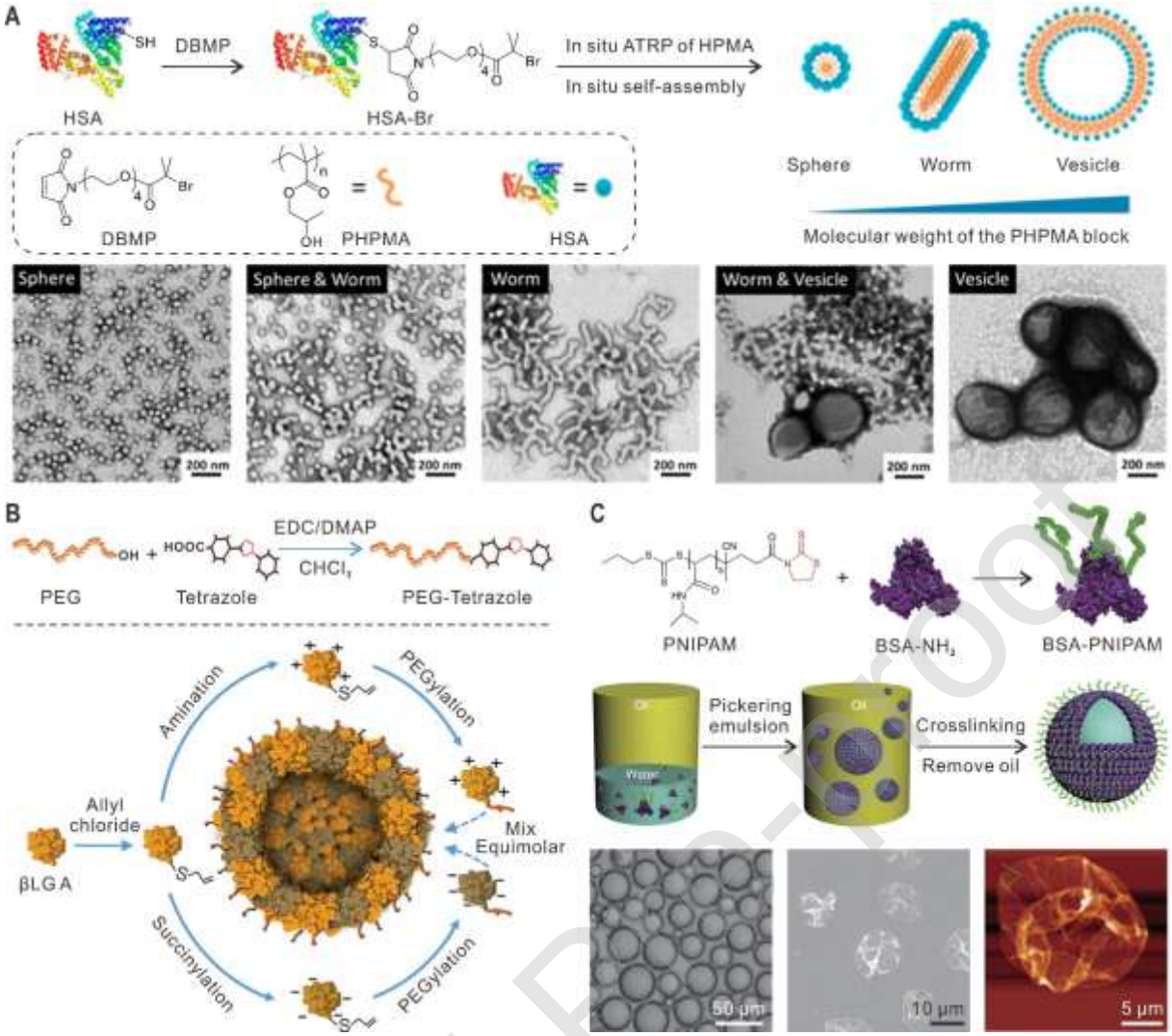

Fig. 9. Self-assembly of protein–polymer conjugates into high order nanostructures. (A) Schematic illustration and TEM images showing the in situ site-specific polymerization-induced self-assembly of HSA–PHPMA into tunable morphologies from spheres to worms and vesicles; (B) Synthesis of two oppositely charged βLG A–PEG conjugates and the preparation of nanocapsules by mixing the protein–polymer conjugates at equimolar ratio in solution; (C) Synthesis of BSA–PNIPAM and its self-assembly at the water droplet/oil interface to prepare proteinosomes. Bottom: optical microscopy
(A) [312], Copyright 2017. Reproduced with permission from the American Chemical Society.
(B) [315], Copyright 2017, Reproduced with permission from John Wiley and Sons Inc. image (left) of proteinosomes dispersed in oil, as well as SEM (middle) and atomic force microscopy (AFM, right) images of dried proteinosomes.
(C) [319], Copyright 2013. Reproduced with permission from Springer Nature.

As mentioned earlier, protein cages are monodispersed and highly organized protein architectures, which are formed based on the specific and directional interactions between protein subunits. Well-defined protein-based nanostructures can therefore be generated by an alternative approach using interactions between proteins to drive the self-assembly of protein–polymer conjugates. Nallani, Liedberg and coworkers designed and synthesized two oppositely charged β-lactoglobulin A–PEG (βLG A–PEG) conjugates and investigated their co-assembly behaviors [315]. As shown in Fig. 9B, the positively and negatively charged conjugates were obtained by amination or succinylation of βLG A followed by PEGylation via photoinduced click chemistry. Driven by electrostatic and

hydrophobic interactions between the proteins, spherical capsules with a diameter of 80–100 nm and a narrow size distribution could be obtained by mixing the two charged protein–polymer conjugates at equimolar ratio. These capsules were able to accommodate GFP and FITC-labelled dextran in their interior. On the other hand, the connection between proteins in protein–polymer conjugates could also be created and strengthened by a third component. For example, Cornelissen et al. observed an irreversible dissociation of cowpea chlorotic mottle virus capsids when they were conjugated with PEG chains [316]. However, the resulting protein subunit–PEG conjugates could then reassemble into much more stable virus-like particles in the presence of polystyrene sulfonate (PSS), due to the electrostatic interactions between PSS and the positively charged protein subunits.

In addition to self-assembly in aqueous solution, amphiphilic protein–polymer conjugates have also been reported to organize at water/oil interfaces for emulsion stabilization [250, 317, 318]. As shown in Fig. 9C, Mann et al. prepared hollow protein capsules termed proteinosomes by interfacial assembly of a protein–polymer conjugate [319]. The conjugate BSA–PNIPAM was synthesized by coupling mercaptothiazoline-capped PNIPAM chains with cationized BSA-NH_2. By emulsifying an aqueous solution of the conjugate in 2-ethyl-1-hexanol, a closely packed and continuous monolayer of protein–polymer conjugates could form at the interface generating proteinosomes with diameters in the range of 20–50 μm. The proteinosomes were stable in oil and were transferable to aqueous solution after crosslinking, which facilitates their application for guest molecule encapsulation, selective permeability, and as stimuli-responsive micro-reactors.

Owing to their potential applications in biosensors and heterogeneous catalysis, solid-state materials based on protein–polymer conjugates with well-defined nanostructures have attracted much interest in recent years [320, 321]. By solvent evaporation from concentrated solutions, protein–polymer conjugates have been observed by Olsen and coworkers to form ordered nanostructures including lamellae, perforated lamellae, and hexagonally packed cylinders [322, 323]. Particularly, they have intensively studied effects of various factors such as the chemistry of the polymer block [323, 324], protein surface charges [325, 326], and molecular topology of the conjugates [327] on the self-assembly behavior. For instance, the electrostatic repulsion of supercharged proteins has been found to severely affect the nanostructure formation and the degree of ordering was reduced in the self-assembled structures [326]. These studies expand our understanding on the bioconjugate self-assembly and may allow the structural control of protein–polymer conjugates in the solid state.

3.3. Well-defined protein/peptide–polymer conjugates on surfaces

Due to their robustness, versatility, and good processability, synthetic polymers have been used extensively to immobilize biomolecules including peptides and enzymes on various surfaces, which could find potential applications in biosensors, biotechnology, and biomedical devices [328, 329]. These functional surfaces with attractive biological activities such as antibacterial [330] and cell adhesion properties [331], can be constructed by either direct deposition of polymer bioconjugates or stepwise immobilization of polymers and biomolecules on surfaces. For example, Maynard et al. designed and synthesized a heterotelechelic biotin–maleimide polymer by RAFT polymerization, which site-specifically conjugated proteins and immobilized them onto streptavidin- or neutravidin-functionalized surfaces [197]. Due to the presence of a cleavable disulfide bond in the polymer, the protein–polymer conjugate could be detached from the surfaces under mild reducing conditions.

Similar to those studies in solution and in bulk, surface-deposited protein/peptide–polymer conjugates are able to self-assemble to well-defined nanostructures, forming novel materials combining unique features and bioactivities of polymers and biomolecules, respectively. Early example by Jenekhe et al. showed the self-assembly of triblock copolymers containing a central π-conjugated polymer and two polypeptide end blocks into spherical and fibrillar nanostructures [332]. He et al. reported the hierarchical self-assembly of block copolymers containing PEG and polypeptides with alkyl side chains on graphite [333]. Depending on the block copolymer concentration, diverse morphologies from island-like aggregates and monolayers to monolayers with larger nanorods or ring-shaped aggregates were observed. The self-assembly of globular protein–polymer conjugates into cylindrical nanostructures has been demonstrated by Olsen and coworkers [334]. The conjugate containing fluorescent protein mCherry and poly(oligoethylene glycol acrylate), was flow coated into thin films on PEG-grafted silicon surfaces. Long-range order could be achieved under high humidity in surrounding air with a high coating speed. Polymer bioconjugates can also be co-assembled with synthetic block copolymers leading to hierarchically structured functional biomaterials [335]. Xu et al. reported the simultaneous co-assembly of a PEO-conjugated heme protein and an amphiphilic diblock copolymers, polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO), into thin films with macroscale lateral ordering and regular nanoscale morphologies. Importantly, the protein structure and function were not affected during the film processing [335].

In addition to direct deposition of polymer bioconjugates on surfaces, proteins and peptides can be covalently immobilized onto polymer substrates via a wide range of conjugation chemistries [336, 337]. Polymer brushes, polymer chains covalently anchored to surfaces, are ideal substrates to precisely immobilize biomolecules because they can provide exceptional control over surface properties and functionalities [338]. Various functional groups of

polymer brushes such as epoxide, carboxylic acid, hydroxyl, aldehyde and amine groups have been employed to immobilize different biomolecules including peptides, proteins, and enzymes [329]. For instance, Popik, Locklin and coworkers reported functional polymer brushes containing photoreactive 3-(hydroxymethyl)naphthalene-2-ol (NQMP) moieties on silicon oxide surfaces [339]. Upon irradiation with 300 or 350 nm light, NQMP converts efficiently to *o*-naphthoquinone methide, allowing very fast Diels–Alder addition to vinyl ethers such as vinyl ether–biotin conjugate. FITC-functionalized avidin could then be immobilized to the polymer brushes with a significantly higher protein loading amount than that of self-assembled monolayer-based systems. Recently, antifouling polymer brushes containing alkene functional groups have also been used to immobilize cell adhesive peptides via thiol–ene radical coupling for the design of cell microarrays [340].

Very important is that patterned polymer brushes [341-344] can be easily prepared by emerging surface patterning techniques including photolithography, colloidal lithography, microcontact printing (μCP), electron-beam lithography (EBL), and scanning probe lithography (SPL), serving as a powerful platform to create well-defined surfaces and biochips with spatial control of biological functions. For example, μCP was successfully used to prepare patterned protein-resistant polymer brushes with nitrilotriacetic acid (NTA) groups that can selectively immobilize histidine-tagged proteins [345]. The protein resistance of NTA-functionalized POEGMA brushes was retained, which allowed the preparation of well-defined binary protein patterns. Yang et al. fabricated protein nanopatterns with different shapes including nanodot arrays, elliptical rings, microdiscs, triangles, and microgrids, by covalently conjugating proteins on hierarchical polymer brush patterns prepared by combining colloidal lithography and photolithography [346-348]. These protein patterns could promote cell adhesion and cell location. In contrast to μCP and colloidal lithography, EBL and SPL are writing techniques that can be used to fabricate arbitrary patterns at the nanometer scale. Maynard et al. employed EBL for the nanoscale arrangement of multicomponent two-dimensional (2D) single-layer or 3D multi-layer protein patterns [349]. Eight-arm PEGs modified with biotin, maleimide, aminooxy, or nitrilotriacetic acid were cross-linked onto Si surfaces using electron beams to form polymer patterns, which could be further used to site-specifically bind proteins with different functional moieties. Dip-pen nanodisplacement lithography (DNL) is a high resolution and program controllable SPL that is particularly suitable for constructing 2D and 3D patterned polymer brushes [350-352]. Zheng et al. employed DNL to create biomimicking nano-micro binary polymer brushes consisting poly(glycidyl methacrylate) (PGMA) and PNIPAM [353]. Gelatin was conjugated to PGMA brush nanolines, which offers the capability to regulate cell orientation.

3.4. Emerging applications based on the well-defined structure

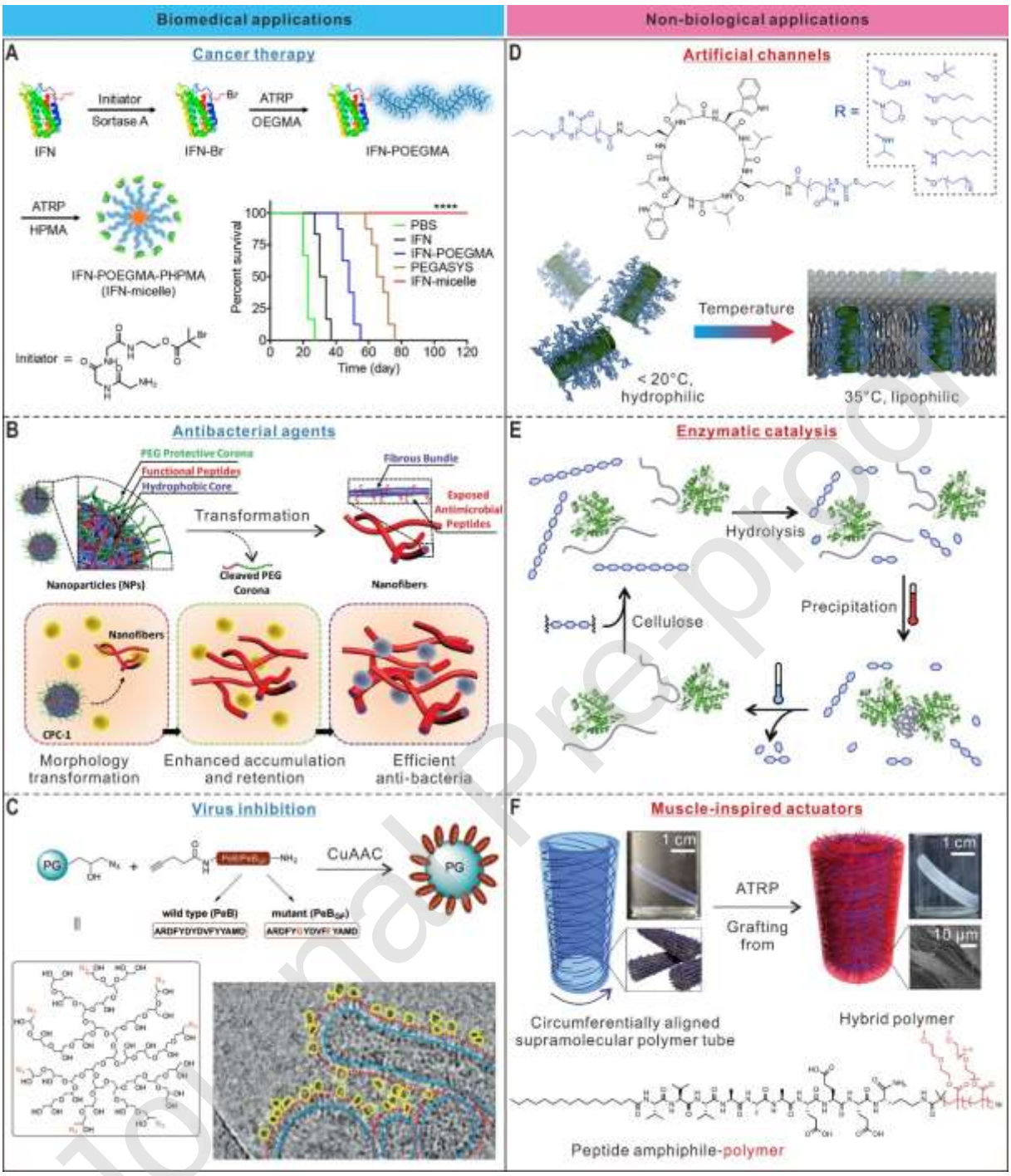

Fig. 10. Representative applications of protein/peptide–polymer conjugates. (A) Preparation of IFN–POEGMA-PHPMA micelle (IFN-micelle) by site-specific in situ PISA for cancer therapy. Bottom right: cumulative survival of mice showing the in vivo antitumor activity of IFN-micelle; (B) On-site morphology transformation of self-assembled nanoparticles based on a chitosan–peptide conjugate to nanofibers for treatment of bacterial infection; (C) Multivalent peptide conjugation of a dendritic polyglycerol for influenza A virus inhibition. The cryo-TEM image shows the interaction of the conjugate (yellow) with the virus corona (red dashed line); (D) Thermoresponsive cyclic peptide–polymer conjugates for the generation of well-defined phospholipid trans-bilayer channels; (E) Recyclable thermoresponsive polymer–endoglucanase conjugates for the enzymatic hydrolysis of cellulose; . (F) Preparation of muscle-inspired anisotropic actuators by grafting thermoresponsive polymers from the surface of a hydrogel tube made of aligned peptide amphiphile nanofibers. (A) [362], Copyright 2018. Reproduced with permission from the American Chemical Society.

By combining the precision structure and evolved functionality of biomolecules as well as the synthetic flexibility and stimuli-responsiveness of polymers in one platform, protein/peptide–polymer conjugates have demonstrated great potential for numerous applications particularly in biomedical fields. In addition, these hybrids with well-defined structures are also very promising from a materials perspective [18]. While some examples have been presented during the discussion on synthetic approaches and various structures, we highlight here representative systems in which the conjugate structure plays a critical role for their applications.

3.4.1. Biomedical applications

Protein/peptide–polymer conjugates have been intensively investigated and widely used for therapeutic applications. On one hand, polymer conjugation often imparts increased stability, tunable activity, and prolonged blood circulation time of the therapeutic proteins and peptides [76, 354]. For these systems, structural factors including conjugation site, grafting density, and length of polymers may have impact on the bioactivity and therapeutic effects of conjugates [355]. On the other hand, protein–polymer conjugates and their assemblies have also been used as delivery vehicles or coatings for various therapeutic agents and nanoparticles [356-360]. In these cases, the well-defined and hierarchical structure of polymer conjugates is the basis for their respective applications. Alexander et al. synthesized different conjugates of transferrin by grafting polymers either from specific cysteine residues of recombinant transferrin variants or from random amine sites on the surface of native proteins [361]. The self-assembly behavior of these conjugates and their ability to deliver anticancer drugs were investigated. In comparison to the hybrids prepared by nonselective conjugation, the engineered transferrin–polymer conjugates could form better-defined assemblies with enhanced performance in paclitaxel delivery. In addition, the self-assembled nanostructures of the protein–polymer conjugates were found as a key factor to achieve high delivery efficacy. As discussed earlier, PISA has been successfully used to prepare various assemblies from protein–polymer conjugates. To apply this approach for therapy, Gao et al. grafted an amphiphilic block copolymer site-specifically from the C-terminus of a therapeutic protein interferon-α (IFN) (Fig. 10A) [362]. The obtained conjugate IFN–POEGMA-PHPMA could self-assemble into spherical micelles with a diameter of 112 ± 23 nm. Very importantly, these micelles (IFN-micelle) demonstrated

significantly enhanced *in vitro* bioactivity and *in vivo* half-life than that of FDA-approved PEGylated IFN (PEGASYS). Moreover, IFN-micelle also showed superior tumor accumulation compared to IFN conjugates modified with hydrophilic PEG or POEGMA chains [362]. Remarkably, tumor growth could be fully inhibited by IFN-micelle and 100% animal survival was achieved after four months in a mouse model of ovarian cancer (Fig. 10A). This example clearly reveals the advantages of self-assembled nanostructures as future therapeutics.

It is well understood that the sizes and shapes of self-assembled nanoparticles are important structural features affecting their pharmacokinetics [363]. Especially, nanostructures with elongated shapes often display longer circulation times in the body and are internalized by cells through different uptake pathways [363, 364]. In this regard, Perrier et al. synthesized PHPMA-based cyclic peptide–polymer conjugates, which can self-assemble into well-defined nanotubes as anticancer carriers [365]. By introducing a pyridine-containing comonomer into the polymer, the conjugates could be functionalized with an organoiridium anticancer complex. Compared to free drugs and non-assembling drug-loaded polymers, drug-bearing nanotubes demonstrated higher toxicity toward human ovarian cancer cells. Moreover, cellular accumulation studies indicated that the increased activity could be ascribed to a more efficient action mode of the nanotube through a different drug partitioning profile into the cell organelles. To further explore cyclic peptide–PHPMA nanotubes as an effective drug delivery system, in vivo experiments were performed to compare their pharmacokinetics and biodistribution with non-assembling polymers [366]. After intravenous administration of samples to rats, nanotubes were found to circulate for more than 10 hours and the plasma exposure was 3-fold higher than that of the polymer control. Importantly, the conjugates could be ultimately cleared from the systemic circulation, which is likely due to the slow disassembly of nanotubes into small entities, making them a promising vector for *in vivo* drug delivery.

Apart from protein and drug delivery for tumor therapy, protein/peptide–polymer conjugates with well-defined structures have also been applied in the construction of other biomaterials such as fluorescence nanoprobes [367-369] and cell matrices [370]. Moreover, proteins and peptides provide a rich library of biofunctions, for example, cell targeting [371, 372] and antibacterial properties [373-376], to polymer bioconjugates broadening their application in countless fields. It is well-known that some intractable human diseases, including Parkinson's disease, are associated with the assembly of amyloid β peptides into fibrils. Moore et al. reported multivalent polymer–peptide conjugates as inhibitors to redirect the formation of amyloid β fibrils into discrete nanostructures through specific peptide interactions and multivalent effect [377]. Furthermore, they found that these conjugates of high molecular weights (166–224 kDa) could efficiently break down existing amyloid fibrils [378].

We have introduced above the on-site morphology transformation of nano-assemblies as a novel strategy for *in vivo* tumor therapy [276-278]. This strategy has also been used for bacterial infection treatment. Wang et al. reported shape-transformable nanostructures based on polymer–peptide conjugates containing a chitosan backbone and two peptide side chains, i.e. an antibacterial peptide and a PEG-terminated enzyme-cleavable peptide [379]. This conjugate self-assembled into spherical nanoparticles with diameter of 34 ± 5 nm. After exposure at the bacterial infection site, the nanoparticles underwent morphology transition spontaneously into nanofibers in the presence of gelatinase (Fig. 10B). During this process, the protecting PEG corona was removed through cutting off the cleavable peptide linker and the antibacterial peptide was exposed to the surface, leading to the multisite cooperative electrostatic binding to bacterial membranes. In addition, enhanced accumulation and retention of nanomaterials were demonstrated by in vivo experiments, which were ascribed to the formation of fibrous structures. Collectively, the chitosan–peptide conjugates exhibited highly efficient antibacterial activity [379]. In order to address infections caused by multidrug-resistant Gram-negative bacteria, novel well-defined antimicrobial agents with a dendrimer core and determined numbers of peptide side chains have been reported by Qiao, Reynolds and coworkers [380]. These star-shaped nanomaterials, termed "structurally nanoengineered antimicrobial peptide polymers", were synthesized via ROP of lysine and valine *N*-carboxyanhydrides from the terminal amines of second- and third-generation PAMAM dendrimers. Remarkably, they exhibited sub-μM antibacterial activity against all tested Gram-negative bacteria and displayed selectivity towards pathogens over mammalian cells.

Well-defined peptide–polymer conjugates were also used as multivalent platforms for virus inhibition. Our group has designed and synthesized a thiol-reactive poly(bis-sulfone) copolymer, which allowed multiple conjugation of an endogenous peptide that targets the C-X-C chemokine receptor type 4 [381]. The resultant polymer–peptide conjugate could self-assemble into narrowly dispersed nanoparticles and demonstrated enhanced antiviral activity on HIV infection. Herrmann and coworkers reported peptide–polymer conjugates based on a dendritic polyglycerol scaffold as non-toxic and high affinity multivalent inhibitors for the influenza A virus [382]. As illustrated in Fig. 10C, the conjugate was synthesized by CuAAC coupling of alkyne-containing peptides to an azido-polyglycerol. In vitro experiments demonstrated that the viral infection was significantly reduced by increasing the size of polyglycerol scaffold and tuning the peptide density. Binding of the conjugate with virus was directly observed by cryo-TEM (Fig. 10C). More importantly, in vivo experiments demonstrated that the conjugate provides the ability to efficiently protect mice from virus infection.

3.4.2. Non-biological applications

Because of their well-defined structures, protein/peptide–polymer conjugates and their assemblies have attracted rapidly growing interest in the materials community for non-biological applications such as nanomaterial synthesis, molecular separation and catalysis [18]. For instance, we have presented that PEG-conjugated denatured proteins can be used for templated synthesis of spherical and flower-like gold nanoparticle catalysts [224, 225]. Self-assembled PEG–oligopeptide conjugates have also been used as template for the controlled growth of silver nanoparticle arrays with high particle density [383]. In Nature, the internal interfaces of hierarchical composites are often regulated through peptide-based interface active molecules. Inspired by this, Börner et al. reported the application of peptide–PEG conjugates as specific compatibilizers for a model composite consisting of MgF_2 nanoparticles and PEO matrix, which offers enhanced composite stiffness and toughness at the same time [384]. In addition, Sharma et al. reported BSA–polymer conjugates as a water-less and universal solvent for various dry solutes of different sizes and surface chemistries even including micrometer-sized polystyrene beads [385].

When protein/peptide–polymer conjugates are self-assembled into membranes, they can form specific pores with controlled sizes and shapes for the separation of molecules and particles. Using interfacial self-assembly, Böker et al. fabricated ultra-thin membranes of protein–polymer conjugates with the cage protein ferritin immobilized in the polymer matrix as a sacrificial template [386]. After removal of ferritin by denaturation, uniform pores formed and their diameter was dependent on the protein size. This membrane with a thickness of 7 nm showed good stability when a transmembrane pressure up to 50 mbar was used. Importantly, the membrane was found to have a preferred permeability for gold nanoparticles below 20 nm. As discussed earlier, cyclic peptide–polymer conjugates formed well-defined nanotubes via self-assembly. These nanotubes were also introduced into different membranes for the selective transport of small molecules. For example, Xu et al. reported the co-assembly of block copolymers and cyclic peptide–polymer nanotubes forming porous thin films with high-density arrays of channels at the sub-nanometer scale for gas separation [387]. Furthermore, they performed a more detailed study on the kinetic pathway of the co-assembly process pointing out the key factors to increase the membrane quality [388]. Perrier, Jolliffe and coworkers reported the self-assembly of cyclic peptide–polymer conjugates in the phospholipid bilayer of large unilamellar vesicles to form artificial channels (Fig. 10D) [289]. Through synthesis of a series of conjugates based on different hydrophilic and hydrophobic polymers, the channel type and structure-channel formation relationship were elucidated and lipophilicity of the polymer block was found to be important for the formation of unimeric channels. Because the lipophilicity of PNIPAM can be tuned by temperature, thermoresponsive cyclic peptide–

PNIPAM conjugates were synthesized for the on-demand control over transbilayer channel formation (Fig. 10D). These transmembrane channels were used to transport cargoes between the cytosol and the extracellular media mimicking natural phospholipid membranes. In their subsequent work, a simple protocol to directly observe proton transport across the bilayer membrane has been developed [290]. Very recently, Perrier et al. reported the synthesis of cyclic peptide–polymer conjugates connected by a cleavable linker between peptide and polymer [291]. These conjugates could prevent undesired and unspecific interactions of self-assembled cyclic peptide–polymer nanotubes with lipid membranes, allowing the on-demand formation of membrane channels triggered by a stimulus in the environment.

Because of its efficiency and selectivity, enzymatic catalysis has been used for industrial productions in many areas such food, medicine, biofuel synthesis and biomass transformation [389]. However, the high cost of enzymes is often a barrier, which restricts the development of these fields. Polymer conjugation is a promising strategy to reduce enzyme costs by providing enhanced activity and recyclability to enzymes [320, 390, 391]. For example, Mackenzie and Francis reported a library of thermoresponsive polymer–endoglucanase bioconjugates as recoverable catalysts for hydrolysis of cellulose [392]. As shown in Fig. 10E, the bioconjugate is soluble in solution below the lower critical solution temperature (LCST) and can be used for the hydrolysis of cellulose. After the catalytic reaction, the bioconjugate is precipitated out when the temperature is increased above the LCST. By removing the oligosaccharide product and tuning back the temperature, the bioconjugate can be recovered and reused for several cycles of the catalytic depolymerization. Importantly, the authors have demonstrated the easy regulation of the material's LCST in the range of 20–60 °C through polymer structure design, enabling the application and recovery of enzymes at different temperatures.

More complex, hierarchical structures based on self-assembled peptide amphiphile fibers have also been developed by Stupp and coworkers showing interesting actuating properties and potential applications [393]. They firstly fabricated a macroscopic hydrogel tube by circumferentially aligning the supramolecular nanofibers within a tubular mold using weak shear forces, and then grafted thermoresponsive polymer chains from the tube surfaces by ATRP (Fig. 10F). These hybrid supramolecular tubes with different levels of ordered structures exhibited anisotropic contraction along the length of the tube upon heating. Macroscopic alignment of the supramolecular nanofibers and the covalent attachment of polymer chains were identified as two key factors for the anisotropic actuation. This work demonstrates the great opportunities to build smart soft actuators responsive to external stimuli based on well-defined peptide–polymer conjugates to realize complex applications.

4. Nucleic acid–based polymer conjugates

Nucleic acids represent the other class of precision biopolymers, which Nature has evolved specifically as the blueprint of life. In comparison to peptides and proteins, the interaction between the nucleotide pairs (A–T, G–C) are more streamlined in a way where the inter- and intramolecular forces are well-correlated in 3D space. Recognizing this as a powerful tool from the field of biotechnology to guide the structure of polymers and polymeric assemblies, the role of nucleic acids in modern polymer chemistry has recently seen a rising impact.

The combination of nucleic acids and synthetic polymers has shown distinct benefits based on the unique structural features oligonucleotides provide. The first involves the principle of complementarity of nucleic acid hybridization, where any sequence is programmed to recognize its complementary strand selectively. This allows any polymeric or self-assembled structure appended with ssDNA/RNA to possess an intrinsic bio-orthogonal handle coupled with sequence recognition. Secondly, nucleic acids can be bioactive in different forms (i.e. DNAzymes, aptamers, siRNA, etc.), thus imparting both structural and functional features for the design for sophisticated biohybrid materials.

4.1. Nucleic acid-templated synthesis of precision polymers

In DNA, the ubiquitous double helical structure is a pervasive structural component independent of the sequence combination. On the contrary, the macromolecular structure of polymers largely depends on the molecular constituents. In an exemplary situation, a PNIPAM grafted to a DNA oligonucleotide would very likely demonstrate very similar physical (self-assembly, LCST, etc.) and chemical behavior using any non-self-complementary sequence of the same length. Hence, the flexibility in sequence and the assurance that the oligonucleotide would possess similar physicochemical properties have fueled their widespread application ranging from precision materials, nanorobots, ultrasensitive sensors, molecular computers, medical diagnostics, and therapeutics.

In spite of these advantages, nucleic acids often require stringent conditions to remain stable, with RNA being more susceptible than DNA to hydrolysis due to intramolecular nucleophilic cleavage. For biomedical applications, oligonucleotides have poor pharmacokinetics and in vivo stability thus making them unattractive candidates as therapeutics [394]. Similarly, nucleic acids are likewise challenging to be used in materials science due to their limited scalability. However, likewise in protein–polymer conjugates, several of these drawbacks can be

addressed by synthetic methods and even made to surpass their individual capabilities within the field of application. Hence, in recent years, nucleic acid bioconjugates have played an emerging role in nanotechnology due to their unique sequence programming capabilities.

The methodologies to link oligonucleotides to polymeric materials have been summarized in Chapter 2 as well as in many excellent reviews [395, 396]. Hence, this section adopts a different perspective involving detailed considerations about the special role of oligonucleotides in macromolecular science by guiding precise assemblies at length scales ranging from molecular to nano-objects. On a molecular level, by exploiting the complementary interactions between base pairs, synthetic molecules can be arranged in a sequence specific fashion, coded by the oligonucleotide template. The first examples of this approach using DNA or peptide nucleic acids (PNAs) templates were shown by Liu's and Lynn's groups, respectively [397-399]. Short sequences of DNA/PNA were synthesized to investigate the capabilities of a step-growth oligomerization process that was guided by a continuous DNA template. By selecting the reductive amination as a distance-dependent reaction, these short sequences were shown to ligate spontaneously programmed by the code of the templates. Introduction of errors and mismatch sequences afford only minimal products, demonstrating the regio- and sequence specificity of the concept. In this first proof of concept, the extent of polymerization was accomplished up to a 40-base template, affording a PNA oligomer with a molecular weight of ~10,000.

In addition to sequence precision, oligonucleotides also provide distinct spatial 3D arrangements of two target functionalities to control their interactions. These reactions can take place within the grooves of the DNA double helix or in a micellar system formed by a DNA-*b*-PPO copolymer system [400, 401]. Within the minor groove of the double helix, polyamide hairpins find themselves arranged by the "pairing rules", which is presented as the exposed Watson–Crick base pairs for hydrogen bonding (Fig. 11A). This allows the hairpins to be arranged non-covalently according to the sequence of the DNA template, where subsequent click reactions with copper catalyzed azide–alkyne cycloaddition allowed these hairpins to be ligated [400]. While the internal features of the double helix are an attractive avenue to orient the formation of chemical bonds across large oligomeric molecules, spatial programming can be achieved simply by DNA hybridization. The 5'-end of the template strand and 3'-end of the complementary strand are brought in close vicinity, allowing a fluorogenic isoindole reaction to specifically take place [401].

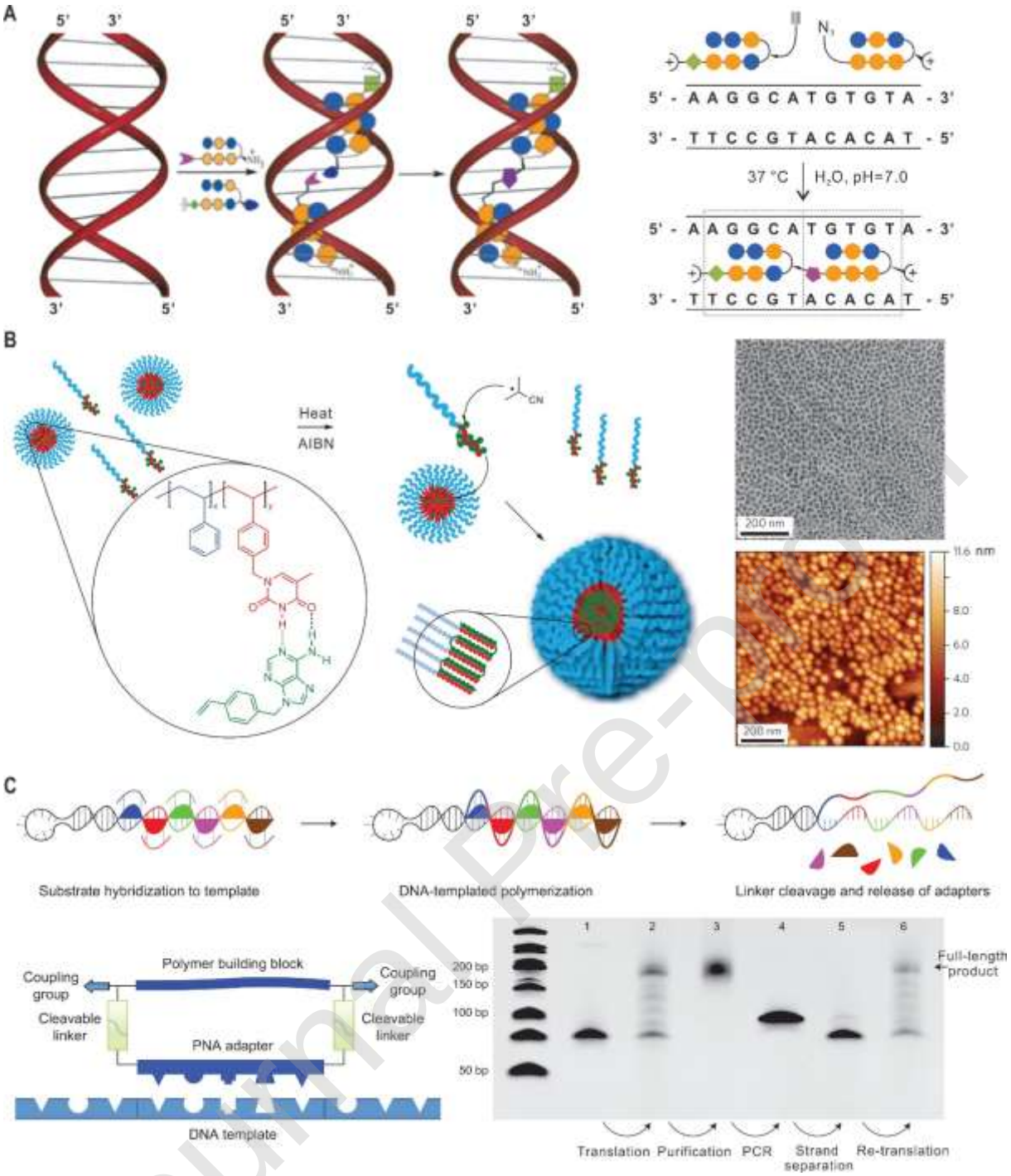

Fig. 11. Nucleic acid-templated synthesis. (A) Schematic illustration of DNA-templated tandem hairpin formation. Polyamides bind to contiguous match sites on DNA and their complementary reactive groups (alkyne and azide) are placed in close proximity forming a covalent triazole linker which is displayed as a purple pentagon; (B) A bioinspired approach to free radical polymerization of a VBA monomer in the presence of a monodisperse block copolymer micellar template with complementary PVBT cores. The right images are TEM and AFM characterization of micelles after the addition and polymerization of VBA; (C) Enzyme-free translation of nucleic acids into sequence-defined non-nucleic acid polymers. The bottom left scheme represents a macrocyclic substrate for the translation system and the bottom right gel image shows a complete cycle of translation, PCR amplification, strand separation and re-translation.
(A) [400], Copyright 2003. Reproduced with permission from the American Chemical Society.
(B) [402], Copyright 2012. Reproduced with permission from Springer Nature.
(C) [403], Copyright 2013. Reproduced with permission from Springer Nature.

By exploiting how DNA can position interacting molecules in space, one of the first examples using DNA to control synthetic polymer chemistry was reported by the group of O'Reilly [402]. In this seminal work, synthetic analogue of thymine (vinylbenzyl thymine, VBT) was block co-polymerized with styrene (St) to form the template PSt_{115}-*b*-$PVBT_{18}$ (Fig. 11B). This allowed the solubility of the block template in chloroform and thereby promoting the H-bond interaction between the thymine of the template with the target adenine. With the block template, vinylbenzyl adenine (VBA), which was insoluble in chloroform, became soluble through the formation of complementary interactions. Free radical polymerization was conducted on the pre-assembled VBA initiated by azobisisobutyronitrile (AIBN) to form the daughter polymer PVBA. Interestingly, monomodal high molecular daughter PVBA can be formed from just 18 units of PVBT in the block template. The result is a "hopping" feature where propagation of radicals between adjacent strands occurred within the micellar core of the block copolymer template. In contrast, polymerization without the template produced ill-defined, low molecular weight polymers, clearly demonstrating the potential of using DNA based interactions, albeit as a synthetic variant, to direct polymerization processes in a controllable fashion.

While the above methodology provides an elegant approach towards polymer synthesis, it is challenging to incorporate sequence information within the framework. In this respect, Liu's group encoded PEG, α-peptides, and β-peptides onto a "codon" defined by a sequence and arrangement of penta-nucleotide analogues [403]. Using a 5' hairpin as the DNA template, complementarity allows each codon to hybridize against the template in a sequence specific manner (Fig. 11C). The close vicinity of the codons subsequently facilitates the covalent coupling of the encoded synthetic fragments together into a polymer, preserving the sequence information. The release of the afforded polymer was achieved by installing a stimulus responsive linker, in this case a disulfide, between the coding region and the fragment. Liu's group further refined this strategy to utilize DNA ligase to catalyze the formation of up to 50 consecutive codes along a DNA template, accomplishing a biosynthetic pathway to form a fully customizable nucleic acid based polymeric scaffold [404, 405]. On a molecular level, the DNA code can act as a guide to direct polymerization reactions where, as a consequence, confer this information onto the newly created synthetic macromolecule. In another seminal methodology established by the Sleiman group, sequence identity from DNA can be imprinted into polymeric nanoparticles, creating a unique code that programs their assembly [406]. Using a DNA cube scaffold as a template with DNA–polymer amphiphiles flanking the sides, an internal hydrophobic pocket customized by the nature of the polymer can be cross-linked to form an imprinted nanoparticle. Upon hydrolysis of the template, a characteristic polymeric structure comprising of a DNA code ranging from divalent to hexavalent can

be precisely constructed. With these coded nanoparticles, self-assembly into various geometries can be exactly defined where features such as interparticle distances, angles and particle junctions are manipulated in a facile way. As a result, nanostructures with identities conferred by the particles were created likewise within a sequence but on a different length scale.

4.2. Precision polymer nanostructures programmed by DNA

While the complementarity and recognition of DNA has enabled programmable features involving the orienting chemical motifs in molecular space, its capabilities extents even further into the nanoscale. DNA sequences can be manipulated to form any arbitrary wire-frame structures as well as continuously folded nanoarchitectures, a collective concept spearheaded by Rothemund and Seeman et. al. known as DNA origami [407-409]. The assortments of different DNA shapes and sizes have exponentially grown over the past decade and have since proven to be the pinnacle of synthetic nanotechnology due to its customization potential. Therefore, the sheer possibility of "on-demand" customization and positioning of nanomaterials onto a singular precision platform have brought about new concepts in biophysics, nanomedicine and polymer synthesis.

The arrangement of DNA sequences in the assembly of these complex structures is inspired and derived from the way Nature recombines and shuffles genetic information in cells through the formation of Holliday junctions [410]. These junctions are interlocking multi-arm DNA forming the immobile and thus stable connections within most, if not all, DNA-based architectures. As these junctions are rigid with defined distances between each arm, positioning of nanomaterials in 3D space can be accomplished with great precision. At this length scale, the inclusion of polymers into DNA to confer hydrophobicity, stimulus responsiveness and/or synthetic functions within a defined 3D scaffold offers exciting new prospects in nanoscience.

In this context, Sleiman's group constructed minimalistic wire-frame DNA prisms and cubes appended with different hexaethylene glycol units to promote a controlled aggregation process [411, 412]. Micellar assemblies containing specific number of cubes and prisms can be tailored according to the polymer length and shape of the initial wire-frame DNA. Superscale assemblies ranging from 1–10 μm, containing micelles from these two different shapes, can also be achieved. This strategy was further developed to include hydrophobic 1,12-dodecanediol in both block and alternating format with hexaethylene glycol to better understand the motivation of assembly both inter- and intramolecularly [413]. Using this methodology, a new range of DNA nanostructures can be accessed from the same precursors but with sequence variation of the appended polymeric segment. Separately, the approach of

constructing superlattices of DNA to orientate macromolecular objects was further demonstrated by combining different shapes into a three-layer architecture where inter-object distances can be tuned in both nanometer and micrometer scale [414].

As DNA controls structure formation through sequence regularity and specific interconnections into nanometer size objects, its templating effect on the molecular order of synthetic polymers reaches another paradigm. In a seminal study, Gothelf's group demonstrates that a conjugated brush polymer, 2,5-dialkoxy-*p*-phenylene vinylene, can be routed individually on designated patterns of a DNA origami tile [415]. This was achieved through the attachment of ssDNA along the side chains of the polymer, which is complementary to the different patterns (i.e. S-shaped, U-shaped, O-shaped) extending out of the origami tile. The routing procedure was also demonstrated in 3D, by wrapping the single strand polymer around a cylindrical origami. Optical properties were investigated using polyfluorene as an energy transfer donor to poly(*p*-phenylene vinylene), which were both routed in close proximity onto the same origami tile [416]. Efficient inter-polymer energy transfer was observed only upon successful attachment whereas the introduction of a 4-(dimethylaminoazo)benzene-4-carboxylic acid (DABCYL) quencher would block the optical communication between the two polymer strands. Using DNA toehold displacement technology, the alignment of polymers along the origami tile was switched reversibly to form differently oriented tracks [417]. The kinetics of the nanomechanical switching was characterized by time-dependent FRET studies and shown that the complete transformation was achieved in about 30 min.

Beyond directing the conformation changes of synthetic polymers, DNA nanoscale structures can provide an opportunity to guide polymerization reactions to transfer the precise shape profile of DNA onto synthetic polymers. Our group arranged ATRP radical initiators in various shapes (i.e. lines, squares, crosses etc.) on DNA origami tiles, where polymers can subsequently be grafted from [418]. The polymerization reaction includes bis-acrylate cross-linkers to ensure that the growing polymer chains from the origami scaffold were stabilized through the interconnections (Fig. 12A). Degradation of the sensitive origami template was achieved to yield the patterned polymeric structures. The methodology was subsequently expanded to pattern catalytic DNA structures, known as DNAzymes, from which the controlled polymerization of dopamine can be promoted (Fig. 12B) [419]. As polydopamine has a strong propensity to adhere to any neighboring material, it aggregates directly at the catalytic sites and thus takes the shape aspect of the designated pattern. In this way, distinct polydopamine nanostructures were formed at the DNA template. In addition, both polymerization methods were subsequently conducted in sequence on 3D tube origamis to form polymers orthogonally located at the internal and external surfaces of the tube

(Fig. 12C) [420]. This opens interesting prospects for cross-sectional engineering of nanoscale objects with synthetic polymers.

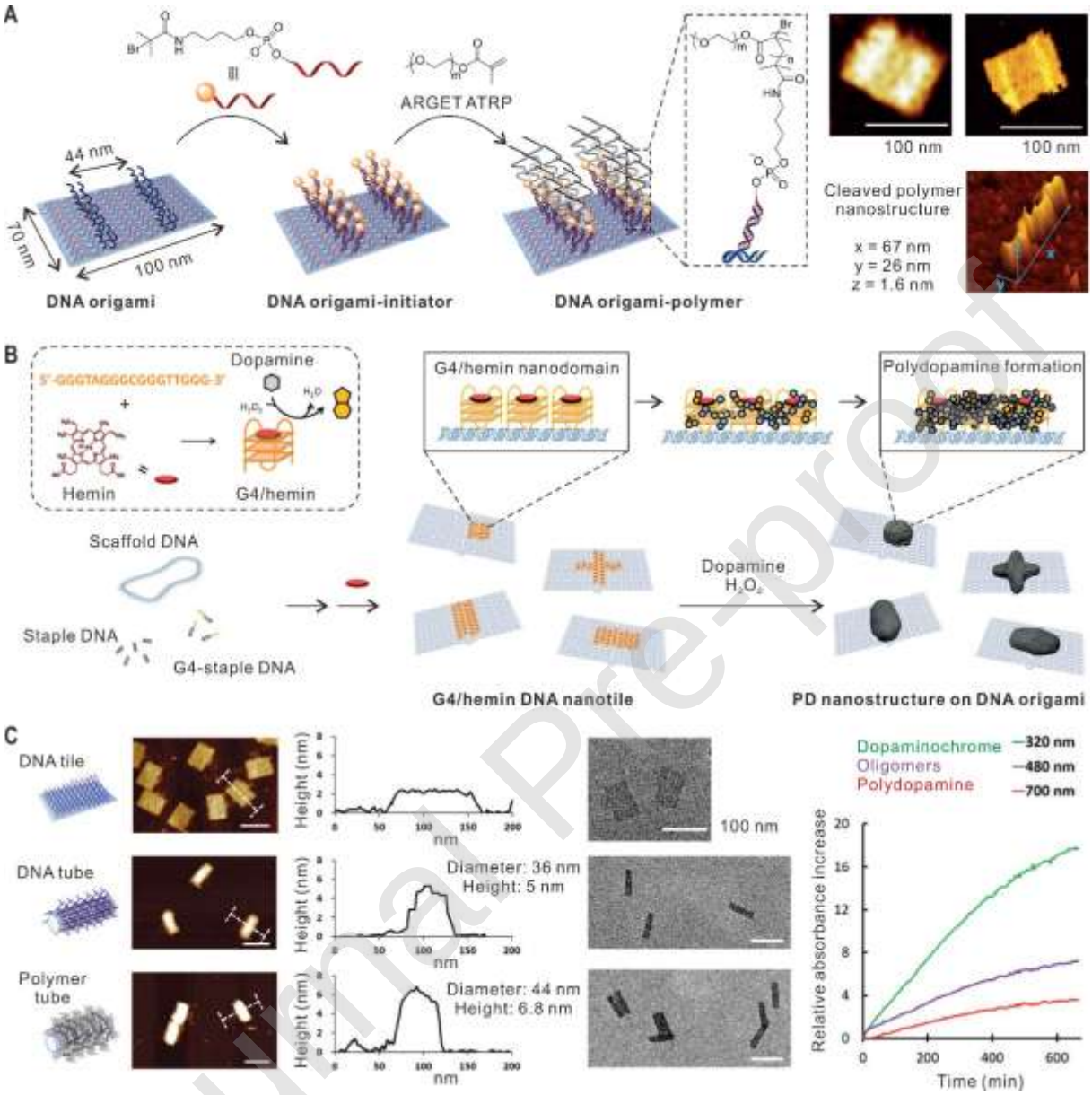

Fig. 12. DNA origami for templated synthesis of precision polymer nanostructures. (A) The fabrication of patterned polymer nanostructures on DNA origami by in situ ATRP. The 3D AFM image on the bottom right shows a cross-linked polymer structure extracted from the DNA origami template; (B) Schematic illustration of the process for constructing defined polydopamine nanostructures on DNA origami; (C) A 3D DNA tube transformed from a 2D DNA tile was used as a precise nanotemplate for ATRP from the surface and polydopamine formation in the interior cavity. The rightmost figure shows the kinetics of dopamine polymerization in the DNA–polymer hybrid tube;
(A) [418], Copyright 2016. Reproduced with permission from John Wiley and Sons Inc.
(B)[419], Copyright 2018. Reproduced with permission from John Wiley and Sons Inc.
(C) [420], Copyright 2018. Reproduced with permission from the Royal Society of Chemistry.

4.3. Applications of well-defined nucleic acid–polymer conjugates

Beyond its sequence identity, DNA is a functional molecule from both chemical and biological perspective. Chemically, the complementarity of DNA is essentially a biorthogonal handle where molecules or materials of interest have been shown to ligate seamlessly [421-423]. This aspect has been exploited liberally in all variations of DNA nanotechnology and applications ranging from photonics, therapeutics, sensing, and nanomaterials. Comparatively, the biological relevance of DNA is more self-explanatory, as nucleic acids often are used to affect genetic information or mediate biological functions through single-stranded DNA or RNA sequences that bind to specific target molecules known as aptamers. The attachment of polymers to such sequences typically takes the stage of increasing the stability of DNA within the biological system, acting as a vehicle to cross cellular membranes and/or as a combinatorial platform for multimodal medical applications [424, 425].

Recent advances in this area generally attempt to integrate multiple functions (i.e. stimulus and temporal control, targeting etc.) onto a polymeric scaffold to enhance the bioactivity of DNA and its pharmacological properties. In this respect, the groups of Sumerlin and Tan demonstrated the grafting of DNA aptamers onto a hyperbranched PEG using photo-responsive chemistry [426]. Loaded with the chemotherapeutic, doxorubicin, the drug delivery system exhibited aptamer mediated targeting simultaneously with photo-dependent release (Fig. 13A). Other than aptamers, different classes of biologically attractive nucleic acid sequences such as siRNA have found similar avenues within polymer science. Although RNA is intrinsically more hydrolytically labile, both *grafting to* [427] and *grafting from* [428] strategies work well to form the desired bioconjugates. The groups of Albertazzi and Dankers expanded the possibilities by integrating siRNA into a multicomponent supramolecular polymer platform [429]. The supramolecular polymer is built upon using a 1,3,5-benzenetricarboxamide (BTA) derivative into nanofibrillar architectures (Fig. 13B). By functionalizing the BTA end groups with positively charged amines, siRNA can be complexed along the fiber axis while the hydrophobic core of the fiber provides the possibility to load small organic molecules of interest. The resultant polymeric construct facilitates both intracellular transport and up to 41% gene silencing capabilities against ELAV1, an RNA-binding protein, messenger RNA expression of HK-2 cells after 48 h. Other examples of functionally active DNA include spherical nucleic acids (SNAs) in which the self-assembly into a core–shell architecture is driven by the attachment of a diblock copolymer onto an oligonucleotide [430]. Using different sequences for the SNA formation, cellular internalization, trafficking and gene knockdown effects were elucidated, demonstrating that these assemblies remain highly bioactive through their self-assembly processes.

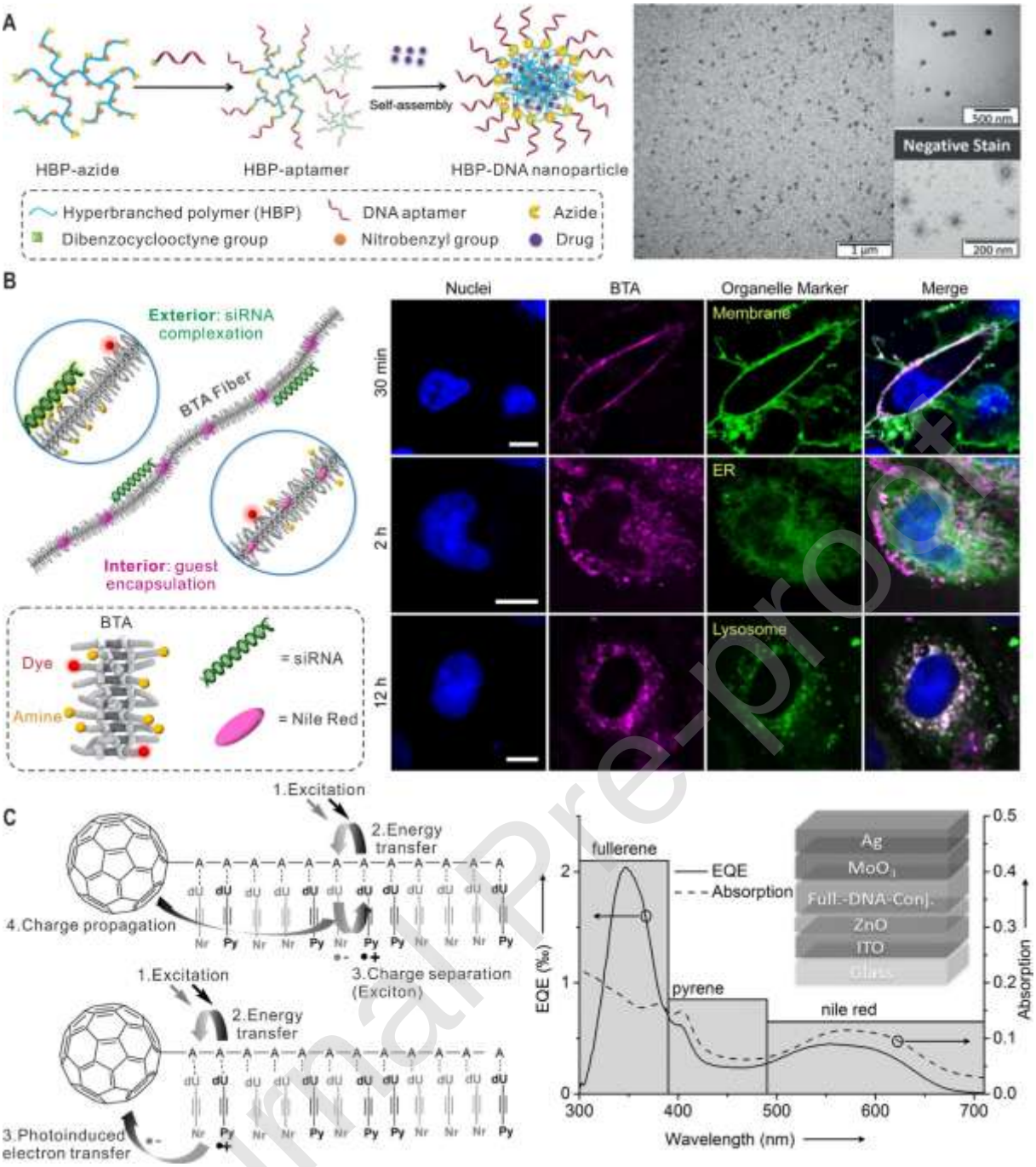

Fig. 13. Representative applications of nucleic acid–polymer conjugates. (A) Self-assembly and TEM images of nanocarriers based on aptamer-grafted hyperbranched polymers for targeted and photo-responsive drug delivery; (B) Multicomponent BTA supramolecular polymers with two functional compartments, small fluorescent molecules loaded in the hydrophobic core and siRNA immobilized on the hydrophilic exterior, were used as a modular platform for intracellular delivery. Confocal microscopy images on the right indicate the intracellular trafficking of BTA polymers. Scale bars: 10 μm; (C) Supramolecular assembly of two different chromophores (pyrene and Nile red) along a fullerene–DNA conjugate scaffold forming ordered and mixed assemblies, which were employed as a photo-active layer in solar cells. The right figure shows the broad spectral absorption of the photoactive layer and respective external quantum efficiency (EQE) of a typical solar cell.
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(B) [429],. Copyright 2016. Reproduced with permission from the American Chemical Society.
(C) [433], Copyright 2016. Reproduced with permission from John Wiley and Sons Inc.

In certain cases, the interest does not solely lie on the bioactivity of nucleic acids but rather the use of DNA interactions to enhance polymer derived functions i.e. fluorescence, optoelectronics. By conjugating oligonucleotides onto a semi-conducting polymer derivatized from polythiophene, the amphiphilicity of the DNA–polymer conjugate was the driving force for the observed vesicular assembly, and nanoribbons were formed by co-assembly with a PEGylated polythiophene [431]. This concept was also similarly demonstrated in light harvesting polymers where hydrophobic chromophore stacks containing oligo(*p*-phenylene-ethynylene) can be directed by DNA interactions to form fibrillar architectures [432]. Bringing such concepts into optoelectronic devices, Wagenknecht's group found that mixed arrays of pyrene and Nile red can be templated along a fullerene functionalized oligonucleotide consisting of 20 repeats of deoxyadenosine [433]. With various pyrene and Nile red ratios, exciton dissociation by electron transfer to the fullerene were manipulated to different extents (Fig. 13C). In addition, the three-component system was incorporated as a photoactive layer in solar cells and charge-carrier generation of the material was demonstrated.

5. Polymer conjugates based on other biotemplates

5.1. Carbohydrate–polymer conjugates

Carbohydrates, also known as saccharides, are composed of monosaccharides, disaccharides, oligosaccharides, and polysaccharides. In contrast to the biomolecules discussed so far, saccharides often reveal complex branching structures, and they interact with various biological target structures. Carbohydrates play many critical roles in living organisms including energy storage and as structural components. Due to their unique features such as biocompatibility, biodegradability, and multifunctionality, carbohydrates have attracted great interest in biomedical and materials fields. The conjugation of functional polymers to carbohydrates is an effective strategy to improve their properties and broaden the applications. For example, cellulose, which is a polysaccharide and the most abundant biopolymer on earth, has been modified by many modern polymerization techniques [434-437]. Malmström et al. conducted ATRP of methyl acrylate from cellulose fibers at ambient temperature, which is the first example of controlled radical polymerizations for polymer growth from cellulose [438]. Using hydroxyl groups on cellulose as initiators, biodegradable polymers such as poly(L-lactic acid) and poly(ε-caprolactone) can also be conjugated via ROP [439]. Other carbohydrates including chitosan, pullulan, dextran, starch, and hyaluronan have also been modified by these polymerization methods, yielding functional materials for a variety of applications such as drug and gene delivery [440, 441]. Unlike proteins and nucleic acids with absolute structures, most polysaccharides have

varied molecular weights and properties. Therefore, this section does not provide a full overview of all carbohydrate–polymer conjugates but some examples with well-defined structures are highlighted.

Well-defined carbohydrate–polymer conjugates can be prepared by introducing monosaccharides or oligosaccharides to a precision polymer scaffold. For instance, functional copolymers with 2-naphthol groups and a narrow molecular weight distribution were synthesized by ATRP, and α-mannoside was conjugated to the backbone using cucurbit[8]uril-based host–guest inclusion forming supramolecular glycopolymers [442]. Linhardt and Lee et al. prepared a series of well-defined conjugates by attaching 6'-sialyllactose (6SL) to different generation PAMAM dendrimers, which were used to inhibit influenza A viruses [443]. In spherical generation 4 and 5 scaffolds, the interligand spacing was found to be a more important factor than the number of ligands for the antiviral effect. Generation 4 6SL–PAMAM conjugates with a spacing of 3 nm between 6SL ligands demonstrated the highest binding to a hemagglutinin trimer and displayed the best effect to block H1N1 infection. The structure-based design of carbohydrate–polymer conjugates can therefore serve as an effective strategy to improve the antiviral efficacy of the bioconjugates.

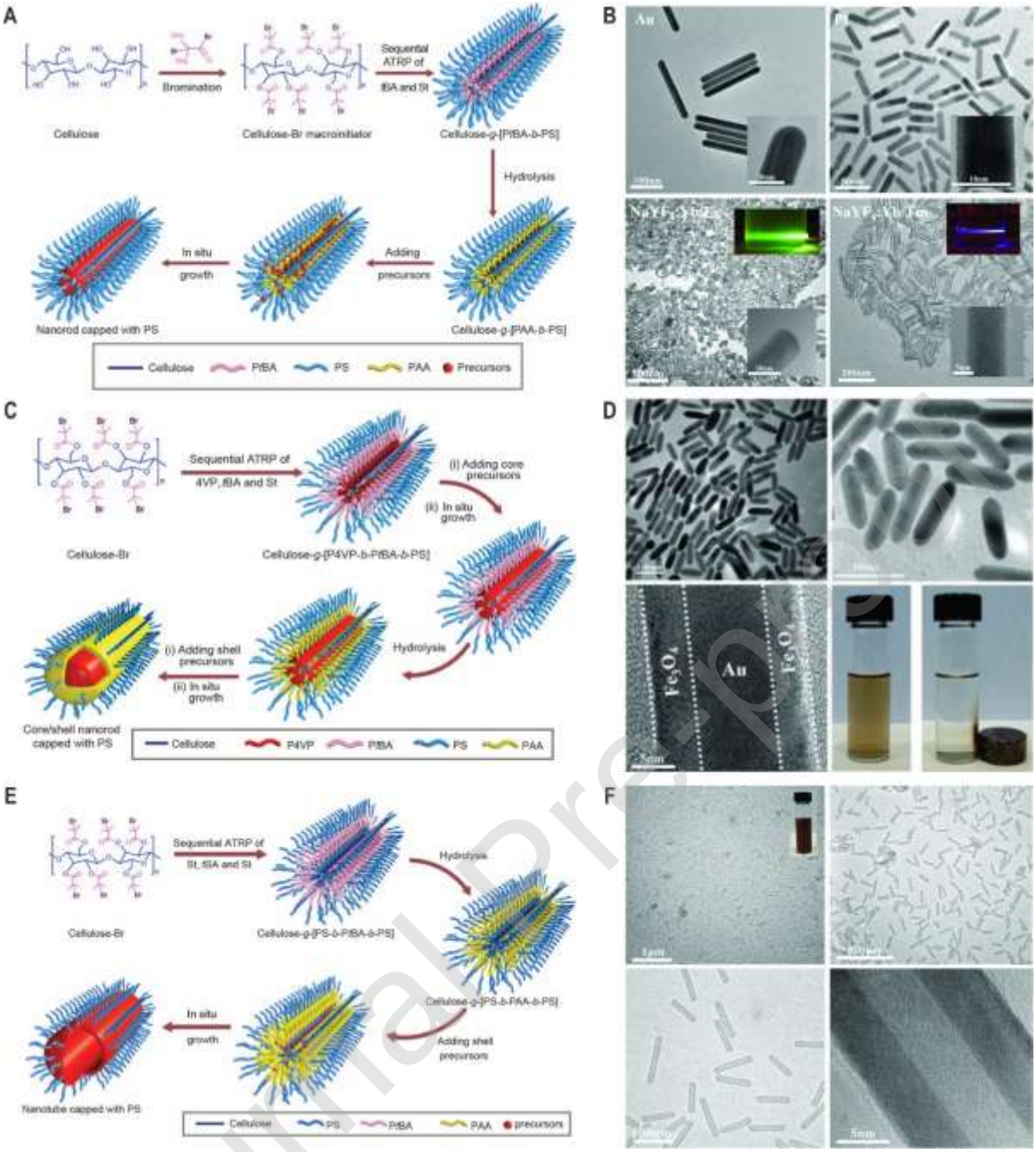

Fig. 14. Preparation of precision 1D nanocrystals by using cylindrical cellulose–polymer conjugates as nanoreactors. (A) Plain nanorods templated by cellulose-*g*-(PAA-*b*-PS). St, styrene; *t*BA, *tert*-butyl acrylate. (B) TEM images of a variety of plain nanorods. (C) Core–shell nanorods templated by cellulose-*g*-(P4VP-*b*-PtBA-*b*-PS). (D) TEM and digital images of Au-Fe_3O_4 core–shell nanorods. (E) Nanotubes templated by cellulose-*g*-(PS-*b*-PAA-*b*-PS). (F) TEM images of Au nanotubes at different magnifications.
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Carbohydrates have also been used as precision templates to grow polymers with controlled polymerization techniques, generating carbohydrate–polymer conjugates with well-defined architectures. β-Cyclodextrin (β-CD) is a cyclic oligosaccharide consisting of seven D-glucopyranoside units connected by α-1,4-glucosidic bonds. Each glucopyranoside unit has three hydroxyl groups, which can be functionalized with ATRP initiators. Lin et al. prepared

a β-CD macroinitiator by esterification of hydroxyl groups with 2-bromoisobutyryl bromide and pioneered the synthesis of 21-arm, star-like block copolymers using ATRP in combination with click chemistry [444, 445]. These star-like polymers can be used as unimolecular micelles for inorganic nanoparticle synthesis, as well as drug and gene delivery [446, 447]. In particular, they have demonstrated the preparation of nearly monodisperse colloidal nanocrystals with precisely controlled dimensions, compositions, and architectures by using the well-defined star-like polymers as nanoreactors [448]. Specifically, metallic, ferroelectric, magnetic, semiconductor, and luminescent colloidal nanocrystals with desired sizes and architectures were synthesized following this strategy. Because cellulose forms a rigid backbone, the strategy has been further extended to realize one-dimensional rod-like nanocrystals using cellulose–polymer conjugates as cylindrical unimolecular nanoreactors [449]. As a proof of concept for the preparation of plain nanorods, amphiphilic cellulose-*g*-(PAA-*b*-PS) {cellulose-*graft*-[poly(acrylic acid)-*block*-polystyrene]} was synthesized (Fig. 14A). The PAA blocks can accommodate and coordinate a large volume of inorganic precursors, allowing the nucleation and growth of inorganic nanorods (Fig. 14B). Importantly, the outer PS blocks impart solubility to the obtained nanorods in organic solvents, which facilitates their processing and applications. This approach was readily adaptable to more complex nanostructures such as core–shell nanorods (Fig. 14C and D), and nanotubes (Fig. 14 E and F) through rational design and synthesis of functional bottlebrush-like bioconjugates with different triblock copolymer side chains.

5.2. Lipid–polymer conjugates

In addition to proteins, nucleic acids and carbohydrates, lipids are the last member of the four major classes of biomolecules. Lipids can be hydrophobic or amphiphilic small molecules. A famous example are amphiphilic phospholipids, which possess unique self-assembly characteristics and are a major component of all cellular membranes. Early studies of lipid–polymer conjugates mainly focus on the PEGylation of lipids to enhance the stability and circulation time of lipid-based drug nanocarriers [450-452]. For example, Farokhzad and coworkers reported a lipid–polymer hybrid nanoparticle platform, which was composed of a biodegradable and hydrophobic polymeric core for drug loading, a lipid monolayer at the interface to promote drug retention, and a hydrophilic PEG layer that was covalently attached to the lipid layer to afford stealth properties [453]. The hybrid nanoparticle combines the advantages of polymeric nanoparticles and liposomes and can be prepared by self-assembly through a single-step nanoprecipitation method. In order to deliver siRNA, the same group later reported a hollow core–shell lipid–polymer–lipid hybrid nanoparticle system consisting of an outer lipid–PEG surface, a middle hydrophobic

polymer layer, and a positively charged lipid layer generating the inner hollow core [454]. Besides PEG, a range of other polymers have also been conjugated to lipids through various chemical strategies. Hennink et al. reported the attachment of biodegradable polypeptides to lipids for the design of long-circulating liposomes with drug-targeting capacity [455]. Hawker et al. prepared a variety of lipid–polymer conjugates with controlled molecular weights and narrow molecular weight distributions by photoelectron transfer RAFT polymerization [456].

Bioengineering techniques have also been developed to prepare well-defined lipid–polymer conjugates. Inspired by the post-translational modification of proteins in Nature, Chilkoti et al. reported the high efficiency synthesis of lipid–peptide polymer hybrids through an eukaryotic post-translation modification [457]. Myristic acid as a lipid was conjugated to an elastin-like polypeptide (ELP), and the resulting conjugate self-assembled into tunable micelles that can be applied to deliver anticancer drugs. By further introducing a short β-sheet-forming peptide in between of the lipid and the ELP block, three stimuli-responsive lipid–polypeptide conjugates were prepared, which exhibited temperature-triggered hierarchical self-assembly [458]. Very recently, this genetically encoded approach has also been employed to synthesize cholesterol-conjugated peptide polymers [459].

5.3. Engineering live cells via polymer conjugation

An exciting new research direction in polymer bioconjugation is direct engineering of living cells with polymers. One could envision that cell–polymer conjugates could provide improved *in vivo* compatibility as well as reduced immune responses and enzymatic degradation can be afforded to modified cells, suggesting entirely new perspectives for fundamental studies in cell biology as well as applications in transfusion, cell-based therapeutics, and tissue engineering [460]. For instance, Scott and coworkers pioneered the covalent conjugation of PEG to the red blood cell (RBC) membrane via cyanuric chloride coupling [461]. The conjugated polymer chains could block antibody mediated recognition of RBC surface antigens. Hyperbranched polyglycerol (HPG) has also been conjugated to RBC surfaces via an ester–amide linker and the *in vivo* circulation in mice indicated that more than half of HPG-grafted cells were functional and retained a normal circulation behavior [462]. Although the cell surface modification has been achieved in some cases, their low conjugation efficiency due to the repulsion between hydrophilic polymers and cell surfaces represents a major limitation. To address this issue, Kizhakkedathu et al. developed a universal technique to significantly improve cell surface modification by introducing nonreactive and cell-compatible polymers as additives [463]. Unprecedented enhanced polymer grafting by up to 10-fold was demonstrated using four different cell types. Pasparakis et al. synthesized two functional copolymers, which were conjugated to live cells to control

cell aggregation behaviors [464]. Recently, Gibson and coworkers reported that telechelic polymers bearing different functional groups prepared by RAFT polymerization can be site-specifically conjugated to metabolic glycans on cell surfaces using strain-promoted azide–alkyne click cycloaddition [465, 466].

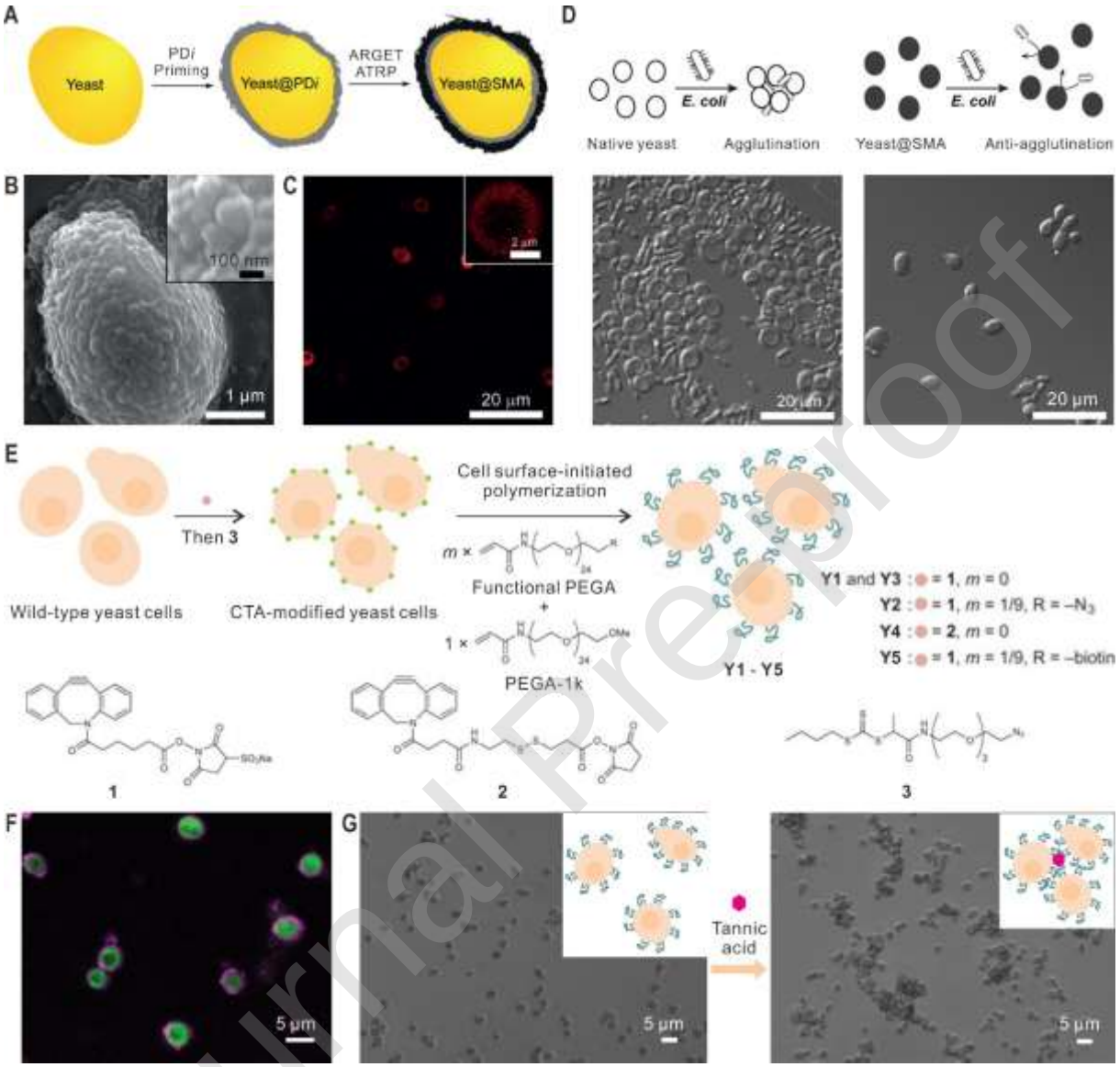

Fig. 15. Polymer grafting from live cell surfaces using cytocompatible controlled radical polymerization techniques. (A) Schematic illustration for polymer grafting from yeast cells via surface-initiated ARGET ATRP. (B) SEM images of SMA-coated yeast. (C) Confocal laser scanning microscopy images of azide-functionalized SMA-coated yeast after coupling with alkyne-linked Alexa Fluor 594. (D) Agglutination assay of yeast: (left) native yeast and (right) SMA-coated yeast. (E) Schematic illustration of polymer growth from yeast cells via surface-initiated RAFT polymerization. (F) Confocal fluorescent microscopy shows fluorescent labelling of treated yeast cells. Polymers on the surface were labelled with a derivative of Alexa Fluor 647, indicating the successful polymer growth at the cell surface. (G) Tannic acid which binds to PEG through hydrogen bonding was used to manipulate aggregation of polymer-grafted yeast cells
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In situ growth of functional polymers from live cell surfaces by controlled radical polymerizations has also be reported. Choi and Yang et al. selected ARGET ATRP to grow polymers from living cell surfaces because only

low concentrations of ATRP catalysts were required, and the reaction was conducted in the aqueous solution under atmospheric conditions [467]. Polydopamine-based ATRP initiators were firstly attached to yeast cells to prevent radical attack during ATRP process (Fig. 15A). A water-soluble and biocompatible monomer, sodium methacrylate (SMA), was then polymerized for a predetermined time. The successful polymer growth was confirmed by scanning electron microscopy (SEM, Fig. 15B) and confocal laser scanning microscopy (Fig. 15C) images. Moreover, poly(SMA)-coated yeast cells did not aggregate when they were mixed with *Escherichia coli*, which indicated that the binding between *E. coli* and yeast cells had been blocked by the polymer layer (Fig. 15 D). These results clearly demonstrated that highly dense polymers can be grafted onto live cell surfaces by ARGET ATRP using the *grafting from* strategy. Very recently, the *grafting from* ATRP strategy was also applied to attach thermoresponsive PNIPAM to specific proteins at the surface of living cells for isolation and analysis of membrane proteins [468]. Hawker and coworkers pioneered the *in situ* polymer growth from live yeast and mammalian cells via cytocompatible RAFT polymerization (Fig. 15E) [469]. Specifically, a visible light mediated RAFT process was developed, which allowed the polymerization of functional PEG monomers into narrowly distributed polymers (M_w/M_n < 1.3) at room temperature in 5 minutes. As a proof-of-concept experiment to introduce functional polymers to the surface of cells, copolymerization of methoxy-PEG acrylamide-1k (PEGA-1k) and ω-azido PEG acrylamide with a molar ratio of 9:1 was conducted after introducing RAFT CTAs to the surface of yeast cells. The obtained azide-containing yeast cells were further functionalized with a derivative of Alexa Fluor 647. As shown in Fig. 15F, strong fluorescence of Alexa Fluor 647 was only observed at the surface of yeast cells, indicating the successful surface-initiated growth of reactive polymers. Furthermore, tannic acid, a compound known to bind PEG through hydrogen bonding interactions, was added to a suspension of polymer-modified yeast cells. Considerable aggregation was observed after mild shaking for 1 h (Fig. 15G), indicating that the approach can be used to control cell–cell interactions. These examples impressively indicate the great potential of modern polymerization techniques for directly engineering live cell surfaces. It should be noted that the radical polymerization of biocompatible acrylic and methacrylic monomers inside living cells has also been reported [470]. A light-controlled polymerization method was successfully employed to generate polymers in complex intracellular environments. Therefore, we can expect even more complex and well-defined polymer bioconjugates prepared by conjugating live objects inside cells with synthetic polymers in the near future.

6. Summary and outlook

The development of polymer science and its connection to biology has evolved rapidly in recent years. The field has started as a concept to provide stability to biomolecules and improve their application as therapeutics. However, from the success of the first bioconjugates that moved into clinical phases, the impact of synthetic polymer bioconjugates became apparent not only in application driven research, but also found its place in newly developed areas of fundamental science such as supramolecular chemistry, precision polymer synthesis and self-organization. Specifically, these are instances where biomolecules have helped to achieve greater heights as well as diversity in macromolecular science. From polymer synthesis, the appreciation of the enzyme degassing system through glucose oxidase/sodium pyruvate has granted the access to *grafting from* controlled radical polymerizations at exceedingly low volumes and in ambient conditions. This important technical progress will enable technologies such as polymerization induced self-assemblies possible with biomolecules such as unnatural peptides or DNA, which have limited scalability. Therefore, it is important to recognize that technical breakthroughs at the synthesis level are essential to provide access to entirely new biohybrid architectures with designed functionalities. With the help of sophisticated enzyme design possibly through directed protein evolution, one could envision that enzymes could be programmed as synthetic polymerases to build polymers on demand.

While the bioactivity of biomolecules often represents the main reason for their applications, their perfect structure could be considered as an equally important feature. There is an emerging interest in the application of biomolecules to direct or template polymer syntheses and assemblies. In this context, the application of DNA has been the main focus where its complementary recognition has an unrivalled specificity. Significant efforts have been made to use DNA base pairs and to arrange a sequence order for synthetic oligomeric or polymeric fragments. While these technologies have already proven success, they are still quite laborious and costly given the quantities that can be fabricated. However, should these templated syntheses achieve directed amplification akin to the polymerase chain reaction, it would immensely broaden the applications DNA-polymer conjugates. To our mind, we are just at the beginning to apply Natures polymers as templates for precision polymer bioconjugates and hybrid materials. Meanwhile, the hierarchical self-assembly of polypeptides into defined nanostructures will create fast access tailored functional nanomaterials by supramolecular copolymerization. In addition, there is also an enormous potential to elucidate the structure of polypeptides and proteins at different levels of order, i.e. in the globular ordered, intrinsically disordered or denatured states. These studies could give entirely new insights into the structures and functions of intrinsically disordered proteins that are just being explored and one could already appreciate many similarities to the behavior of polymers.

Nonetheless, the mainstream applications of biomolecule-polymer conjugates in medicine will remain and we foresee significant developments in the future where treatments and diagnostics may become personalized. As there is typically very limited chemical space available at the target biomolecule, a conjugated polymeric component could impart new features such as enhanced specificity or pharmacokinetics that could be tailored for the individual patient to maximize *in vivo* efficacy. Here, there have been already important discoveries that incorporated synergistic combinations of stimulus responsive chemistry and dynamic self-assemblies to optimize the biological profile of the bioconjugate. We foresee that the evolution of these conjugates moves towards higher complexity and "intelligence" and, at certain stages, show semblance of primitive autonomous behavior. With the advent of modern chemical tools, it would be highly attractive to furnish an autonomous bioconjugate, where it can seemingly decide for itself to solve a targeted biological problem.

Collectively, every aspect of chemistry, from the synthetic tools that enable the bioconjugates to higher ordered assemblies have each found a new lease of life. Every bond formed and its significance will undoubtedly be increasingly featured in the coming years as the community unravels novel possibilities to create greater control of structures and structural complexity. While comparisons to Nature's capabilities are often discussed in the literature, one must not forget that the breadth of synthetic macromolecular chemistry far exceeds those found in the biology. However, what makes Nature unique and seemingly intelligent is the vast network of macromolecules working and communicating within a highly regulated self-sustaining system. Here, although the myriad of conjugates produced by synthetic chemistry has been consistently innovative, relationships between these novel macromolecules are rarely put together and studied within an artificially controlled environment. It could be envisioned that the future of synthetic bioconjugates would greatly lie in establishing the molecular principle of how these macromolecules can be customized to the extent of how an engineer builds a robot.

CRediT author statement

Chaojian Chen: Chapters 2, 3, 5. David Y.W. Ng: Introduction, Chapter 4, Outlook. Tanja Weil: Concept, Structuring, Reviewing, Corrections

Declaration of interests

☒ 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.

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