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Review Article

Long-circulating protein-polymer conjugates: Advancing beyond PEGylated proteins

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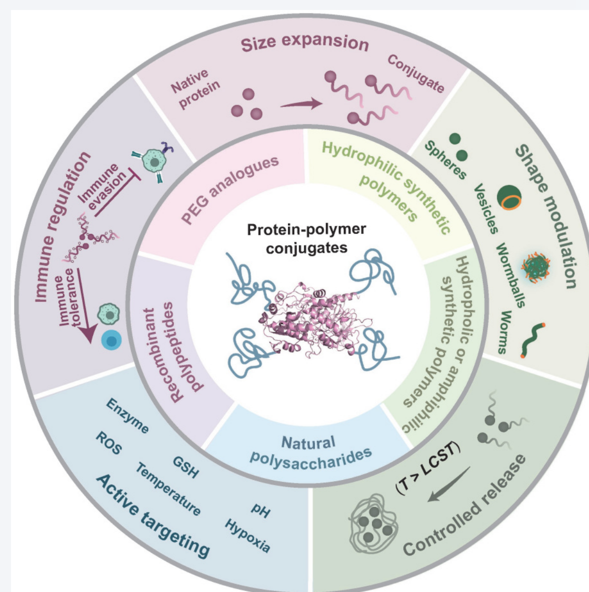
ABSTRACT: Protein-polymer conjugation has emerged as a powerful approach to prolong the *in vivo* half-life of therapeutic proteins, enabling reduced administration frequency and improved patient compliance. Among various conjugation methods, PEGylation has become the most successful strategy for more than 34 clinically approved drugs and have established the gold standard for long-acting biotherapeutics. Over the past decades, continuous advances in polymer chemistry and conjugation technologies have driven the innovation of alternatives to polyethylene glycol (PEG) to overcome its inherent limitations, such as non-biodegradability, limited functionality, and potential immunogenicity. In this review, we summarize the development and recent progress of long-circulating protein-polymer conjugates beyond PEGylation, focusing on emerging PEG alternatives, key design principles such as size expansion, shape modulation, controlled release, active targeting, and immunomodulation. Furthermore, the current challenges in large-scale manufacture, quality control, and clinical translation are discussed. This review provides insights into the emerging directions for next-generation long-circulating conjugates and charts a rational path toward the design of more effective long-acting therapeutics.



KEYWORDS: protein-polymer conjugates, long-circulation, polyethylene glycol (PEG) alternatives, construction principles and strategies

1 Introduction

Long-circulating protein drugs have been widely used in clinic, especially for cancer, diabetes, viral diseases and other severe diseases [1–3]. It is due to the following superiorities of long circulating protein drugs: better patient compliance owing to low-frequency administration, along with reduced side effects and improved final efficacy [4, 5].

Traditional and also the most common strategy of constructing long-circulating protein drugs is PEGylation invented in the 1970s, and its resulting products, PEGylated proteins, are the first kind of therapeutic protein-polymer conjugate [6]. PEGylation effectively enhances the pharmacological performance of therapeutic proteins by protecting them from degradation, aggregation, and denaturation, while reducing macrophage uptake and renal clearance, thereby prolonging their systemic circulation (Fig. 1) [1, 7]. After half a century of optimization and development, more than 34 PEGylated long-acting protein drugs have been approved for clinical treatment at present, and PEGylation has become the "gold standard" of preparing long-acting protein drugs. However, polyethylene glycol (PEG), the polymer part of PEGylated proteins, exists some intrinsic shortcomings such as non-degradability, limited functionalization and potential antigenicity and immunogenicity [8, 9].

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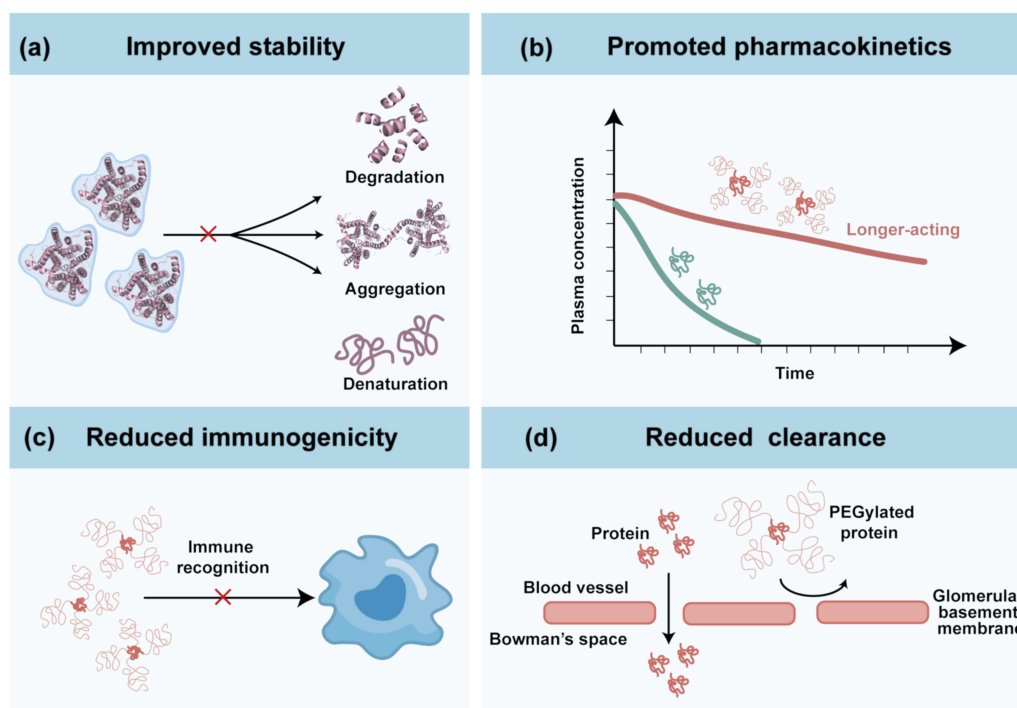

Figure 1 Advantages of PEGylation. PEGylation enhances the pharmacological performance of therapeutic proteins by (a) protecting them from degradation, aggregation, and denaturation; (b) prolonging their systemic circulation time; (c) reducing macrophage uptake; and (d) decreasing renal clearance.

Therefore, based on the molecular structure and chemical properties of PEG, a variety of novel polymers have been developed in the past few years as potential alternatives of PEG for constructing long-circulating protein-polymer conjugates in the future. Beyond that, emerging strategies are being designed by combining the principles of long-circulating and multi-functionalities of polymers. On the other side, there are also some issues waiting to be considered for these novel polymers and corresponding emerging strategies before being approved and applied in clinic, such as scale-up manufacture, quality control, clinical safety and comparative advantages toward other strategies.

In this review, we provide a comprehensive overview of advances in long-circulating protein-polymer conjugates beyond conventional PEGylation. Firstly, we outline the emerging PEG alternatives, which have been developed inspired from the specific molecular composition and corresponding properties of PEG. Then, we summarize the principles and emerging strategies of constructing long-circulating protein-polymer conjugate, including size expansion, shape modulation, controlled release, active targeting, and immuno-interaction. Moreover, we highlight key challenges and novel development in the manufacture and clinical development of long-circulating protein-polymer conjugates. Lastly, we compare polymer-conjugation strategies with alternative modalities for extending protein half-life, such as albumin fusion, Fc-fusion, and transferrin fusion, to clarify their respective advantages and limitations. Collectively, these insights provide an integrated and technological framework for the development of next-generation long-acting protein therapeutics.

2 Alternatives of PEGylation

Over the past decades, PEG has been widely regarded as a biocompatible and non-toxic polymer. However, after nearly fifty years of extensive research and clinical application, its inherent limitations have gradually become evident. One of the notable drawbacks lies in its non-degradability. PEG molecules are typically designed with molecular weights exceeding the renal clearance threshold to prolong the systemic circulation half-life of conjugated drugs. Nevertheless, the absence of metabolic degradation pathways for PEG can result in prolonged retention of PEGylated conjugates in circulation, leading to eventual hepatic clearance or undesired accumulation within various tissues [10, 11]. In addition, the immunogenicity of PEGylated formulations has emerged as another critical concern. Notably, approximately 25% of individuals have been reported to develop anti-PEG antibodies, including both IgG and IgM isotypes, which can significantly compromise the therapeutic efficacy of PEGylated drugs by inducing the accelerated blood clearance (ABC) phenomenon [9, 12, 13].

Driven by concerns over non-biodegradability, immunogenicity, and the risk of long-term accumulation, researchers have extensively explored alternative polymers to replace PEGylation. At the structural level, alternative polymers are engineered based on the ethylene glycol (EG) unit by reorganizing the backbone, functionalizing side chains, or incorporating degradable linkages. At the property level, one strategy mimics the highly hydrophilic and unstructured characteristics of PEG to form a random coil conformation and a denser hydration layer for enhanced stealth effects and steric stabilization, while another involves the construction of amphiphilic polymers to grant stealth functionality and self-assembly capabilities, facilitating long-circulating behavior and reduced immunogenicity. These emerging polymers not only replicate the beneficial properties of PEG but also overcome its inherent limitations. To systematically advance this field, five major types of PEG alternatives have been developed, including structural PEG analogues, hydrophilic synthetic polymers, hydrophobic or amphiphilic synthetic polymers, natural polysaccharides, and recombinant polypeptides, which are summarized in Fig. 2 and discussed in the following sections. By effectively mitigating the

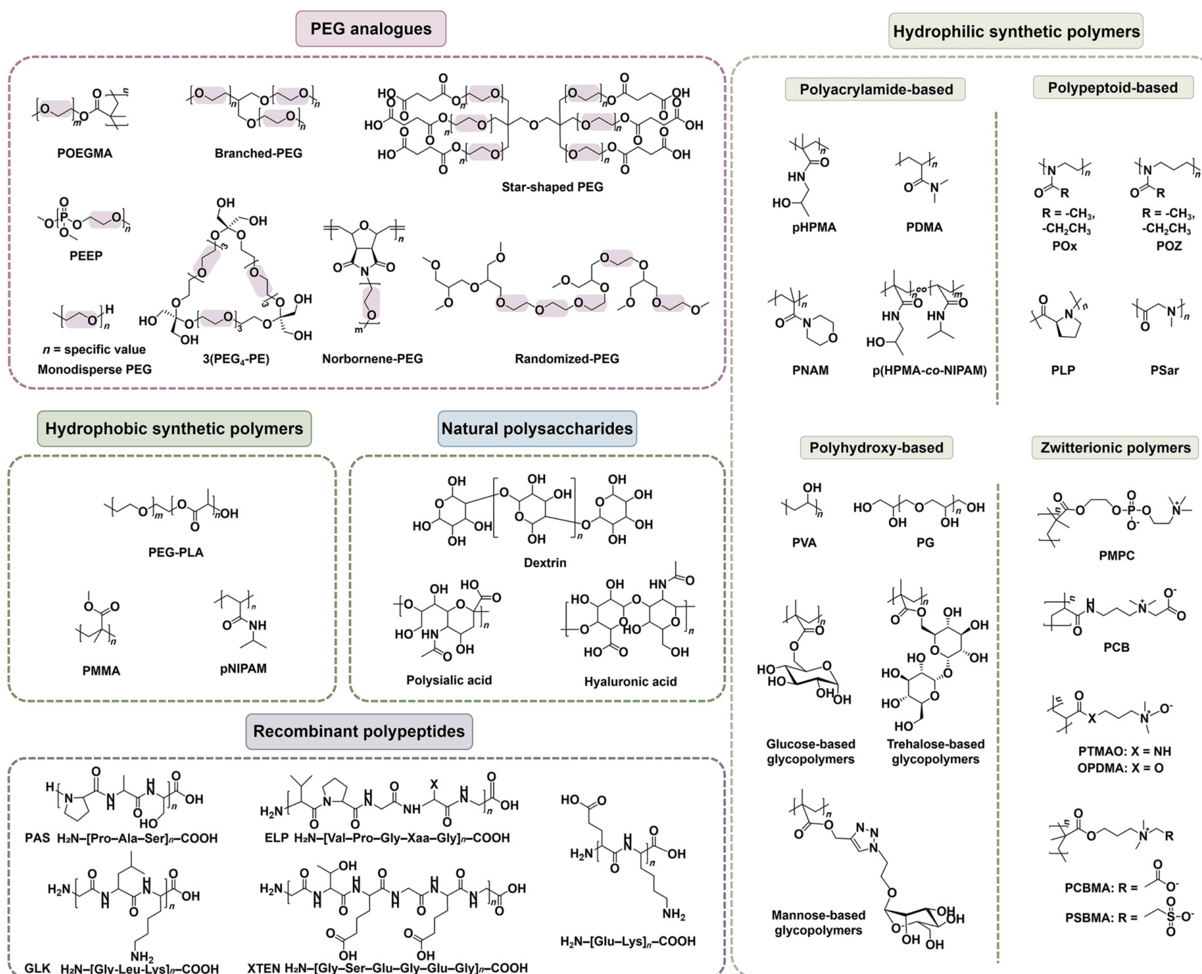

Figure 2 Chemical structures of novel polymers for long-circulating protein-polymer conjugates.

drawbacks of PEG, these new materials are poised to significantly enhance the safety and therapeutic performance of next-generation protein-polymer conjugates.

2.1 PEG analogues

PEG analogues refer to a class of polymers that strictly contain EG repeat units within their backbone or side chains, aiming to retain PEG's desirable properties while addressing its intrinsic limitations. These analogues maintain the hydrophilicity, flexibility, and protein-repellent features granted by the EG units, while also offering improved degradability, diverse structures, or enhanced functionalization capacity [14].

Poly(oligo(ethylene glycol)methacrylate) (POEGMA) is a comb-like polymer that serves as a structurally tunable alternative to linear PEG, consisting of a methacrylate backbone grafted with oligo(ethylene glycol) side chains. This architecture allows precise control over topology and surface grafting density, and more importantly, enables superior performance in bioconjugation [15–20]. POEGMA with different lengths of EG repeating units in the side chains can affect the pharmacological properties and immunogenicity of conjugated therapeutic proteins. Qi et al. investigated the impact of POEGMA with side chains containing either 3 or 9 EG units on the pharmacological properties of exendin-4. EG9 increased the half-life of exendin-4 from 0.7 to 6.2 h, markedly prolonging its glucose-lowering effect up to 120 h, although minor binding to anti-PEG antibodies was still observed. In contrast, EG3 extended the half-life to 9.0 h and completely abrogated the anti-PEG antibody response, while maintaining a sustained glucose-regulatory effect (approximately 96 h) [21]. Furthermore, variations in the end-group structure of POEGMA can significantly alter its impact on the functional performance of the conjugated protein. Ozer et al. and Moncalvo et al. grafted methoxy (-OCH_3)-terminated POEGMA to uricase and lysozyme, respectively, demonstrating that POEGMA modification, through tuning side-chain length, polymer stoichiometry, and architecture, can enhance protein stability, prolong circulation half-life, and reduce immunogenicity, highlighting its potential as a superior alternative to conventional PEGylation for therapeutic proteins [22, 23]. Notably, Joh et al. demonstrated that hydroxyl (-OH) end groups confer superior immune evasion, with -OH-terminated POEGMA exhibiting lower antigenicity than -OCH_3-terminated POEGMA [24].

Poly(ethyl ethylene phosphate) (PEEP) features a phosphate-based backbone composed of repeating ethylene phosphate units linked by phosphorus-oxygen (P-O) bonds, imparting inherent biodegradability via hydrolysis and enzymatic degradation [25, 26]. Its hydrophilic segments ensure water solubility comparable to PEG, supporting stealth behavior in circulation. Unlike PEG, PEEP is efficiently degraded *in vivo*. This inherent degradability reduces long-term polymer accumulation and mitigates immune recognition, thereby addressing issues like the ABC phenomenon

[25]. Steinbach et al. covalently modified several proteins, including bovine serum albumin (BSA) and catalase, with PEEP, which can be degraded by phosphodiesterase, providing protection similar to that of PEG while being biodegradable [27]. In brief, the combination of a phosphate backbone and hydrophilic segments make PEEP a functionally superior alternative to PEG for constructing biodegradable long-circulating conjugates.

Branched [28], also known as "**Y-shaped**" and **star-shaped** [29] **PEGs** are architectural modifications featuring multiple PEG chains extending from a central core, forming a compact, globular structure with a larger hydrodynamic radius than linear PEG. This reduces renal clearance by exceeding the glomerular filtration threshold (5–6 nm), prolonging systemic circulation. Their dense PEG shell provides strong steric stabilization, minimizes opsonization and mononuclear phagocyte system (MPS) recognition, and forms a hydration barrier that blocks protein adsorption, enhancing stealth properties and immune evasion. Peginterferon alfa-2a (PEGASYS), one of the U. S. Food and Drug Administration (FDA)-approved PEGylated biopharmaceuticals, employs a ~ 40 kDa Y-shaped branched PEG structure in which two PEG chains are covalently linked via a lysine residue to interferon α-2a (IFN-α2a) through stable amide bonds. The Y-shaped PEGylated IFN-α2a enables a once-weekly dosing regimen, achieving maximum plasma concentrations approximately 80 h post-administration, while substantial drug levels are already detectable as early as 3 to 8 h after dosing. Moreover, the plasma half-life of IFN-α2a is extended from 4–6 h to approximately 90 h without a significant increase in adverse effects compared with standard IFN therapy, demonstrating the distinct pharmacokinetic advantages of this branched PEGylation approach over conventional linear PEGylation. However, the spatial shielding effect of branched or star-shaped PEG on protein receptor binding site interferes with the interaction between protein and their receptors, leading to significantly reduced biological activity of proteins [30]. For example, star-shaped PEG-antimicrobial peptides conjugates possess intrinsic limitations, such as reduced solubility, a pronounced propensity for supramolecular aggregation, and restricted architectural tunability, which collectively hinder optimal peptide presentation and functional performance relative to comb-like counterparts [31].

Monodisperse PEG, precisely defines in chain length and topology through iterative or orthogonal synthesis, effectively overcomes the inherent heterogeneity of conventional polydisperse PEG. By eliminating conjugate mixtures and improving pharmacokinetic predictability, monodisperse PEG enables more reliable structure-activity evaluation and rational protein conjugate design [32]. Jiang's group developed monodisperse oligoethylene glycol (M-OEG) polyamides with molecular weights of 5.8 kDa and 11.4 kDa. Using human serum albumin (HSA) and bovine serum albumin (BSA) as models, they demonstrated that M-PEGylation produced a single, well-defined conjugate. Notably, M-PEG was fully degraded within 6 h, whereas 26%–32% of regular PEG remained even after 72 h, showing that monodisperse M-PEG overcomes the non-degradability of conventional PEG. Moreover, HSA-S-M-PEG11.4K exhibited comparable reductions in IgG and IgM immunogenicity to conventional PEGylation, collectively indicating that monodisperse, biodegradable M-PEG improves homogeneity, maintains or reduces immunogenicity, and holds great potential as a PEG alternative [33]. Likewise, Wang et al. developed discrete, chiral, monodisperse PEG-like polymers (2 kDa and 5 kDa) for site-specific insulin conjugation. These conjugates preserved insulin structure and aggregation, showed faster or longer-lasting glucose control than PEGylated insulin or Lantus *in vivo*, and exhibited no toxicity or immunogenicity, demonstrating that monodisperse PEG can precisely tune protein therapeutics [34]. Going a step further, structured monodisperse PEG incorporates precisely engineered molecular geometries alongside uniform chain length. An example is triangular PEG, which consists of PEG units connected by a pentaerythritol (PE) linker (3(PEG_4-PE)). These structured monodisperse PEGs exhibit enhanced hydration shell organization, directional shielding, and reduced intermolecular hydrophobic interactions. These properties contribute to predictable stability, controllable pharmacokinetic behavior, and immunomodulation, thereby facilitating the development of topologically defined and activity-tunable protein therapeutics [32, 35].

Norbornene-PEG, synthesized via ring-opening metathesis polymerization (ROMP) of norbornene or oxanorbornene monomers, provide a versatile platform for PEG analogues with precise control over chain length, architecture, and functionality. Church et al. conducted a study in which water-soluble polynorbornene (PNB) polymers were grown from lysozyme and Qβ coat proteins using a "grafting-from" ROMP approach. The resulting lysozyme conjugates retained nearly 100% enzymatic activity, and Qβ-PNB conjugates showed > 95% reduction in recognition by anti-PEG antibodies compared to Qβ-PEG, demonstrating that ROMP-derived PNB can function as a PEG alternative, preserving protein function while minimizing PEG-specific immunogenicity [36]. Similarly, Davis et al. reported poly(oxanorbornene)-protein conjugates prepared via a grafting-to ROMP strategy. Poly(oxanorbornene) (PONB) block copolymers were conjugated to lysozyme and urate oxidase. The conjugates retained protein activity, exhibited strong resistance to anti-PEG antibody recognition, and showed favorable aqueous solubility [37].

Randomized PEG (rPEG) is generated by copolymerizing ethylene oxide with glycidyl methyl ether (GME) to create a PEG-like polyether backbone bearing randomly distributed methoxy-methylene side chains. Dreier et al. synthesized such rPEGs via controlled anionic ring-opening copolymerization, achieving high end-group fidelity and narrowly dispersed polymers with precisely tunable GME incorporation. Compared with conventional PEG, rPEG induced no inflammatory cytokine secretion or complement activation and exhibited a drastic reduction in anti-PEG antibody recognition, establishing rPEG as a robust, low-immunogenicity PEG analogue [38].

In brief, while POEGMA and PEEP focus on enhancing structural tunability and backbone degradability, branched and monodisperse architectures prioritize spatial complexity and uniform chain length, respectively. These structural features allow for the extension of hydrodynamic volume and the modulation of molecular geometry, providing the physical foundation for the circulation-prolonging and morphology-dependent delivery strategies discussed in the following sections.

2.2 Hydrophilic synthetic polymers

Hydrophilic synthetic polymers possess high aqueous solubility and biocompatibility, making them invaluable for protein-polymer conjugation. They can be broadly categorized into several subclasses based on their backbone chemistry and functional features as following.

Polyacrylamide-based hydrophilic polymers have emerged as promising PEG alternatives for therapeutic protein conjugation due

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to their tunable architecture, favorable solubility, and low immunogenicity. **Poly(N-(2-hydroxypropyl) methacrylamide) (pHPMA)** is one of the most extensively investigated polymer platforms for drug conjugation. Vladimir et al. synthesized pHPMA-superoxide dismutase (SOD) conjugates, which demonstrated enhanced stability of SOD against temperature and hydrogen peroxide. Moreover, the pHPMA-SOD conjugates exhibited a significant reduction in immunogenicity compared with free SOD, highlighting the potential of pHPMA as a PEG alternative for prolonging circulation by decreasing immune clearance [39]. Souček et al. modified bovine seminal ribonuclease (BS-RNase) with pHPMA (S-BS) to reduce its rapid elimination. It was demonstrated that after 24 h, 40% of S-BS remained in the bloodstream, whereas 98% of the native BS-RNase had already been eliminated, leading to improved antitumor activity [40]. **Poly(N,N-dimethylacrylamide) (PDMA)** stabilizes proteins such as human galectin protein Gal3 (Gal3C) comparably to PEG, preserving thermal stability and unfolding profiles, especially with sufficient chain length for shielding and aggregation prevention [41]. **Poly(N-acryloylmorpholine) (PNAM)**, grafted onto lysozyme, enhanced solubility at high salt and low pH, with short monovalent chains even improving enzymatic activity-despite some shielding in divalent forms [42]. Lastly, **poly(N-(2-hydroxypropyl) methacrylamide-co-N-isopropylacrylamide) (p(HPMA-co-NIPAM))** conjugation offers trastuzumab Fab' fragment better plasma retention than PEG (9.8 vs 3.6 h) and lower *in vivo* immunogenicity [43].

Polypeptoid-based polymers, including true polypeptoids and related families, feature a neutral, hydrophilic tertiary amide backbone lacking hydrogen-bond donors, which minimizes nonspecific protein adsorption and confers low immunogenicity, excellent anti-fouling properties, and high solubility [44]. Their controlled polymerization enables precise tuning of molecular weight and architecture, allowing rational design of drug delivery systems with long circulation, thermos-responsive behavior, and enhanced permeability and retention (EPR), while avoiding anti-PEG antibody-related immune responses [45, 46]. **Polysarcosine (PSar)**, a prototypical polypeptoid composed of N-methylglycine units, is synthesized via N-carboxyanhydride (NCA) ring-opening polymerization (ROP), which enables precise control over chain length and dispersity. PSar exhibits exceptional hydrophilicity, ultra-low protein adsorption, and minimal immunogenicity. Hu et al. demonstrated that PSar can be efficiently conjugated to IFN-α2b with high site specificity and minimal structural perturbation. The resulting PSar-IFN maintained *in vitro* activity, extended the *in vivo* half-life from ~ 0.8 to ~ 4.8 h and elicited reduced anti-IFN antibody responses. These findings underscore Sar-NCA-derived PSar as a biodegradable, low-immunogenicity PEG alternative suitable for creating hydrophilic, stealth protein conjugates [47, 48]. **Poly(2-oxazoline)s (POx)** possess a tunable hydrophilic tertiary amide backbone, which can be adjusted through side-chain or block copolymer design to confer stealth properties, thermos-responsiveness, and amphiphilicity. These versatile physicochemical features enable the development of long-circulating and EPR-targeted drug carriers. Notably, poly(2-ethyl-2-oxazoline) (PEtOx)-based candidates, such as SER-214, have progressed to clinical evaluation (NCT02579473) [49]. As a structural evolution of POx, **poly(2-oxazine)s (POZ)** offer a broader range of hydrophobicity and a more readily adjustable lower critical solution temperature (LCST) near physiological temperatures. **Polyproline (PLP)** is a polypeptide composed of repeating proline residues, where each proline has a side chain cyclized to the backbone, imparting exceptional rigidity and a highly restricted conformational space. This unique structure forms a stable polyproline helix, making it a valuable model for protein folding studies and a robust, biocompatible scaffold in biomaterials. Zhao et al. developed uricase-poly(L-proline) (UOx-PLP) conjugates through an ultrafast, *in situ*, protein-initiated ring-opening polymerization of proline N-carboxyanhydride completed within 5 min (Fig. 3(a)). In a UOx knockout hyperuricemia mouse model, the UOx-PLP conjugates markedly reduced complement activation and decreased the production of anti-UOx and anti-PEG antibodies by more than 250-fold and 30-fold, respectively (Figs. 3(b) and 3(c)). Notably, UOx-PLP exhibited no signs of the ABC effect, which was attributed to the dense, rigid, spike-like PLP layer that repelled and prevented direct contact of UOx with proteases, antibodies, and immune cells (Figs. 3(d) and 3(e)) [50].

Polyhydroxy-based polymers, a broad class of polymers containing multiple hydroxyl groups, exhibit extensive hydrogen bonding with water, resulting in high hydrophilicity and excellent water solubility. **Poly(vinyl alcohol) (PVA)** is a biocompatible polymer with unique ice recrystallization inhibition (IRI) activity. However, unlike PEG, PVA is challenging to modify site-specifically due to the limited availability of mono-end-functional polymers. To address this limitation, a recent photocatalytic approach enabling orthogonal end-group reduction has been developed, allowing the synthesis of well-defined PVA-protein conjugates, including alkyne-, biotin-, and BG-tagged, while preserving protein bioactivity. This strategy opens new opportunities for PVA-based cryoprotective applications [51]. **Polyglycerol (PG)** offers excellent biocompatibility and flexible architecture. It was proved that compared to PEG, PG-conjugated anakinra retained structure, showed 2 °C higher thermal stability, and greater protease resistance. PG conjugates modestly reduced receptor binding but extended plasma half-life up to 2.3 h (4-fold), comparable to PEG, highlighting the potential of PG as a promising platform for constructing long-circulating conjugates [52, 53]. **Glycopolymers** are a class of multifunctional biomimetic polymers characterized by multivalency, controllable molecular weight and architecture. Protein-glycopolymer conjugates can even surpass the natural functions of glycoproteins, which play essential roles in molecular recognition and immune regulation [54, 55]. Heather D. Maynard and co-workers constructed trehalose glycopolymer-insulin conjugates employing both "grafting to" and "grafting from" approaches. At first, they coupled pre-synthesized ketone- or benzaldehyde-terminated trehalose glycopolymers to insulin via reductive amination [56]. Then, the team advanced to a grafting-from strategy, introducing an atom transfer radical polymerization (ATRP) initiator at LysB29 of insulin to enable controlled polymerization of trehalose methacrylate under mild aqueous conditions [57]. The resulting site-specific conjugates exhibited improved thermal stability and precise architecture, underscoring the advantages of controlled polymer growth from protein surfaces and highlighting the potential of trehalose glycopolymers as biocompatible PEG alternatives. Not only trehalose, but also mannose [58] or glucose [59] can be conjugated to proteins to achieve long circulation of therapeutic proteins.

Zwitterionic polymers, such as **poly(carboxybetaine) (PCB), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), trimethylamine N-oxide-based zwitterionic polymer (PTMAO), poly(carboxybetaine methacrylate) (PCBMA), or**

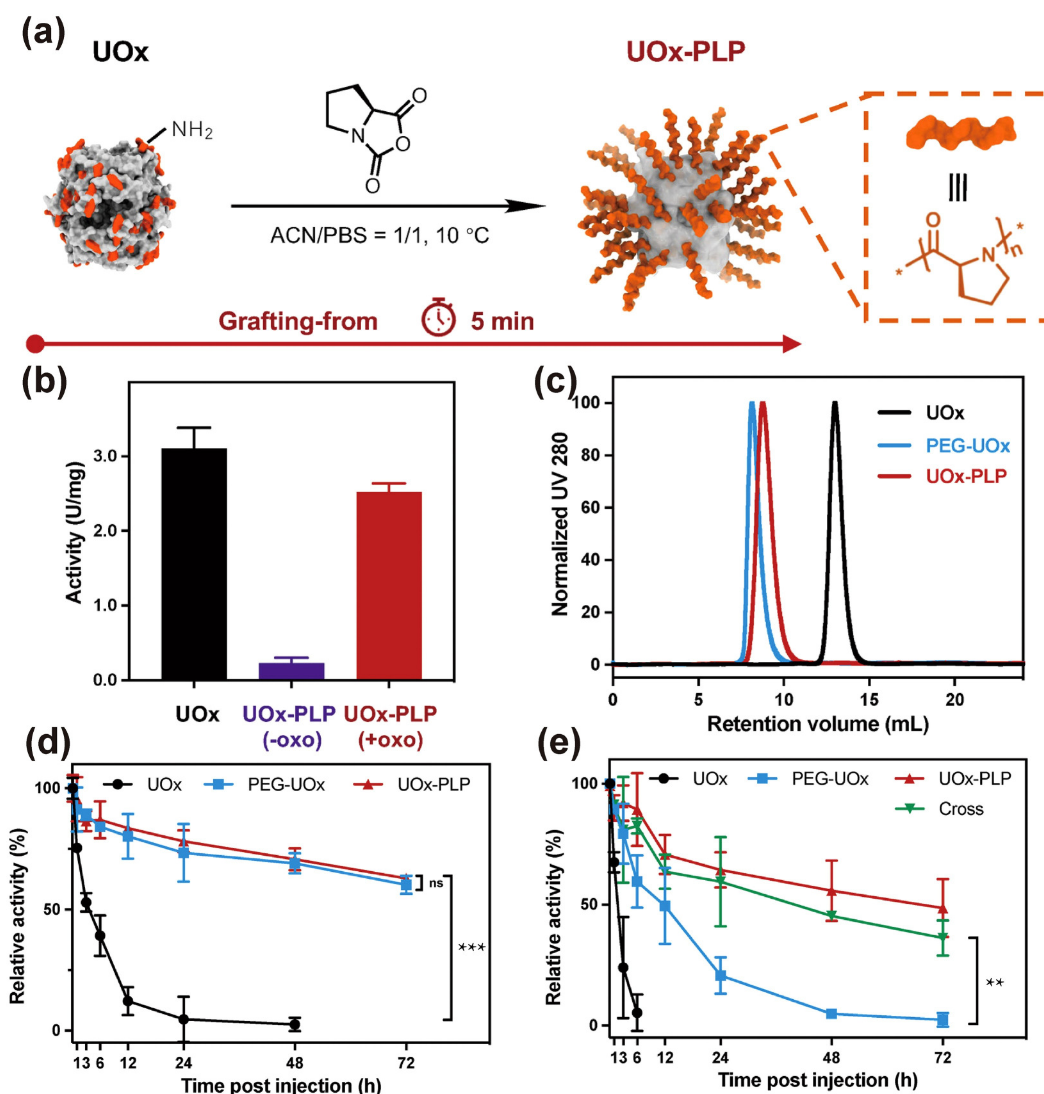

Figure 3 Nanourchin-like uricase-poly(L-proline) conjugate with retained enzymatic activity, mitigated immunogenicity, and sustained efficacy upon repeated administrations. (a) Synthetic Scheme of UOx-PLP using the grafting-from approach. (b) Enzymatic activity of UOx-PLP prepared without or with addition of xonic acid potassium salt (oxo). (c) Size exclusion chromatography (SEC) of UOx, UOx-PLP, and PEG-UOx. The PK profiles, determined by enzyme activity assay, of UOx and different UOx conjugates after the (d) 1st and (e) 3rd administrations. Reproduced with permission from Ref. [50], © Wiley-VCH GmbH 2025.

poly(sulfobetaine methacrylate) (PSBMA), mimic cell membranes by forming dense hydration shells through electrostatic interactions. This structural feature imparts superior hydrophilicity, ultra-low biofouling, and immune-evasive properties, offering clear advantages over PEG [60, 61]. PCB exhibits minimal immunogenicity even when conjugated to highly immunogenic carriers, such as keyhole limpet hemocyanin (KLH). In contrast, PEG can induce anti-polymer antibodies in a carrier-dependent manner due to its haptenic nature, whereas PCB elicits negligible immune responses, making it suitable for long-term therapeutic applications [62]. Similarly, PMPC adopts a compact conformation in water and, when conjugated to IFN via bis-thiol chemistry, preserves bioactivity while reducing antibody binding compared to PEG-IFN. Although PMPC-IFN and PEG-IFN have similar hydrodynamic sizes, PMPC-IFN displays prolonged absorption and elimination half-lives (51.6 h), likely due to its zwitterionic hydration shell, enhanced tissue retention, and reduced *in vivo* clearance [63]. Moreover, Li et al. reported that PTMAO effectively prevents surfaces from biofouling and exhibits undetectable immune recognition. Furthermore, PTMAO conjugation significantly enhanced the stability of uricase at high temperatures. The PTMAO-uricase conjugate demonstrated a consistently superior circulation half-life (19.1 h) compared to both native uricase (3.9 h) and PEG-uricase (16.2 h), without triggering systematic immune recognition [64]. Recently, Wei et al. developed a novel noninvasive transdermal insulin delivery approach by conjugating insulin to a skin-permeable, pH-responsive zwitterionic polymer, **poly[2-(N-oxide-N,N-diethylamino)ethyl methacrylate] (OPDMA)**, which belongs to the PTMAO family. The polymer binds to the acidic sebum layer and then rapidly diffuses through the intercorneocyte lipid lamellae. OPDMA then traverses the epidermis and dermis by "hopping" along cell membranes and finally enters the systemic circulation via the dermal lymphatic vessels. Conjugation with OPDMA increased the cumulative amount of insulin permeation over 24 h to 14.52 μg, which is a 9-fold and 5-fold increase compared to insulin alone or PEG-conjugated insulin, respectively [65].

Compared to PEG, these hydrophilic polymers excel in

providing a dense hydration shell and resisting immune recognition. Unlike conventional PEG, these materials maintain high stability and "stealth" properties, effectively minimizing non-specific biological interactions and preventing the ABC phenomenon. By preserving protein bioactivity and reducing overall antigenicity, these polymers provide a critical foundation for optimizing host-immune interactions during long-term therapy.

2.3 Hydrophobic or amphiphilic synthetic polymers

The conjugation of a hydrophobic polymer with a hydrophilic protein generates amphiphilic protein-polymer conjugates. These bioconjugates exploit amphiphilicity to undergo self-assembly into organized nanostructures such as micelles, vesicles, and bilayers [66]. Compared with PEG, which mainly provides a steric shielding effect, amphiphilic block copolymer-protein conjugates can deliver additional functionality, such as drug encapsulation, protection of hydrophobic therapeutics from hydrolysis or enzymatic degradation, controlled release, and improved bioavailability, while simultaneously reducing systemic toxicity. For example, Liu et al. employed site-specific *in situ* polymerization-induced self-assembly (SI-PISA) to directly grow an amphiphilic block copolymer, POEGMA-PHPMA, from the C-terminus of IFN-α (Fig. 4(a)). This approach produced a self-assembled IFN-micelle with a hydrodynamic radius of 64.9 nm, which was 28- and 9.4-fold larger than those of native IFN and IFN-POEGMA, respectively (Figs. 4(b) and 4(c)). As a result, the micelle exhibited significantly enhanced circulation half-life (83.8 h), tumor accumulation, and antitumor efficacy without systemic toxicity (Figs. 4(d) and 4(e)) [67]. **Poly(methyl methacrylate) (PMMA)**, an FDA-approved hydrophobic medical polymer, was conjugated with BSA to form BSA-PMMA conjugates. These conjugates self-assemble into macromolecular micelles and enhanced the antitumor activity of camptothecin as a drug delivery platform [68]. Moncalvo et al. site-specifically grafted hydrophobic **poly(N-isopropylacrylamide) (pNIPAM)** and hydrophilic mannose glycopolymer onto BSA using a rebridging agent. Above the LCST (32 °C), the pNIPAM blocks undergo a hydrophilic-to-hydrophobic phase transition, driving the formation of core-shell nanoparticles with a glycopolymer corona. Cooling below the LCST reverses this process, as the rehydration and conformational recovery of pNIPAM chains lead to complete nanoparticle disassembly back into monodisperse conjugates. The amphiphilic conjugates provide long-circulation potential while additionally enabling tunable self-assembly, multilayer formation, and stimuli-triggered disassembly, highlighting hydrophobic/amphiphilic polymers are key enablers for programmable protein-polymer conjugates [69].

The advantage of hydrophobic or amphiphilic polymers lies on their ability to undergo self-assembly via processes such as SI-PISA. By transitioning from single chains into higher-order nanostructures such as micelles or elongated assemblies, these polymers facilitate the physical encapsulation and site-specific presentation of protein. This macromolecular organization enables control over particle morphology and allows for temporal regulation of drug liberation at targeted sites.

2.4 Natural polysaccharides

Naturally sourced polysaccharides are increasingly recognized as viable alternatives to PEG due to their superior biocompatibility, controllable biodegradability, and versatile chemical functionalities. **Dextrin**, for example, a biodegradable polysaccharide, has been employed to functionalize recombinant human epidermal growth factor (rhEGF) through covalent conjugation. The dextrin shell not only enhanced rhEGF stability but also rendered the conjugate bio-responsive, as it can be selectively cleaved by α-amylase, an enzyme naturally present in physiological environments. In HEp2 carcinoma cell assays, α-amylase-mediated degradation of the dextrin component enabled controlled and sustained release of active rhEGF, which maintained proliferative activity for over 8 days, highlighting the potential of dextrin as a natural PEG alternative [70].

Polysialic acids (PAs), naturally hydrophilic polysaccharides composed of α2,8-linked sialic acid residues, such as colominic acid (CA), exhibit excellent hydrophilicity and have been widely applied in protein modification. Conjugation of CA to L-asparaginase via reductive amination significantly reduced immunogenicity in mice with pre-existing anti-asparaginase antibodies and extended the enzyme's circulation half-life by approximately 3- to 4-fold [71]. Similarly, insulin conjugates with CA of 22 kDa and 39 kDa molecular weights prolonged glycemic control durations by nearly twofold and threefold, respectively, in normoglycemic T/O mice [72].

Hyaluronic acid (HA), a biocompatible and biodegradable polysaccharide with multiple reactive sites, such as hydroxyl, carboxyl, acetamide, and aldehyde groups, has been widely used for therapeutic protein and peptide conjugation [73]. HA conjugation has been shown to extend the activity of bioactive peptides. For instance, Kong et al. developed a long-acting hyaluronate-exendin-4 (HA-Ex4) conjugate for the treatment of type 2 diabetes. The conjugation of exendin-4 to hyaluronic acid not only enhanced peptide stability but also significantly prolonged its glucose-lowering effect, maintaining efficacy for up to 72 h in diabetic db/db mice [74]. Similarly, Oh et al. synthesized an anti-Flt1 peptide-hyaluronate (HA-anti-Flt1) conjugate to inhibit pathological corneal neovascularization. The HA conjugation enhanced peptide stability and significantly prolonged tissue retention in the cornea, maintaining therapeutic levels for several days, which resulted in superior efficacy compared to both the free peptide and the clinically used angiogenesis inhibitor Avastin® [75].

Natural polysaccharides like HA and dextrin are distinguished by their inherent biodegradability and potential for biological recognition by endogenous receptors. These biological attributes allow them to engage with specific cellular components or be selectively cleaved by disease-related enzymes in pathological microenvironments. Such features facilitate enhanced penetration into dense tissues and support the development of conjugates capable of precise environmental activation and targeted tissue accumulation.

2.5 Recombinant polypeptides

Recombinant polypeptides have emerged as highly promising alternatives to PEG for extending the plasma half-life of therapeutic peptides and proteins, offering advantages such as genetic encodability, monodispersity, tunable length, complete biodegradability, and reduced immunogenicity. Among these, **PASylation** technology employs unstructured, hydrophilic sequences composed of Pro, Ala, and Ser to mimic the stealth and solubility effects of PEG while maintaining full receptor-binding activity [76–78]. PASylated proteins exhibit dramatically extended circulation half-lives *in vivo*, up to 94-fold in the case of human growth hormone (hGH), without eliciting immune responses.

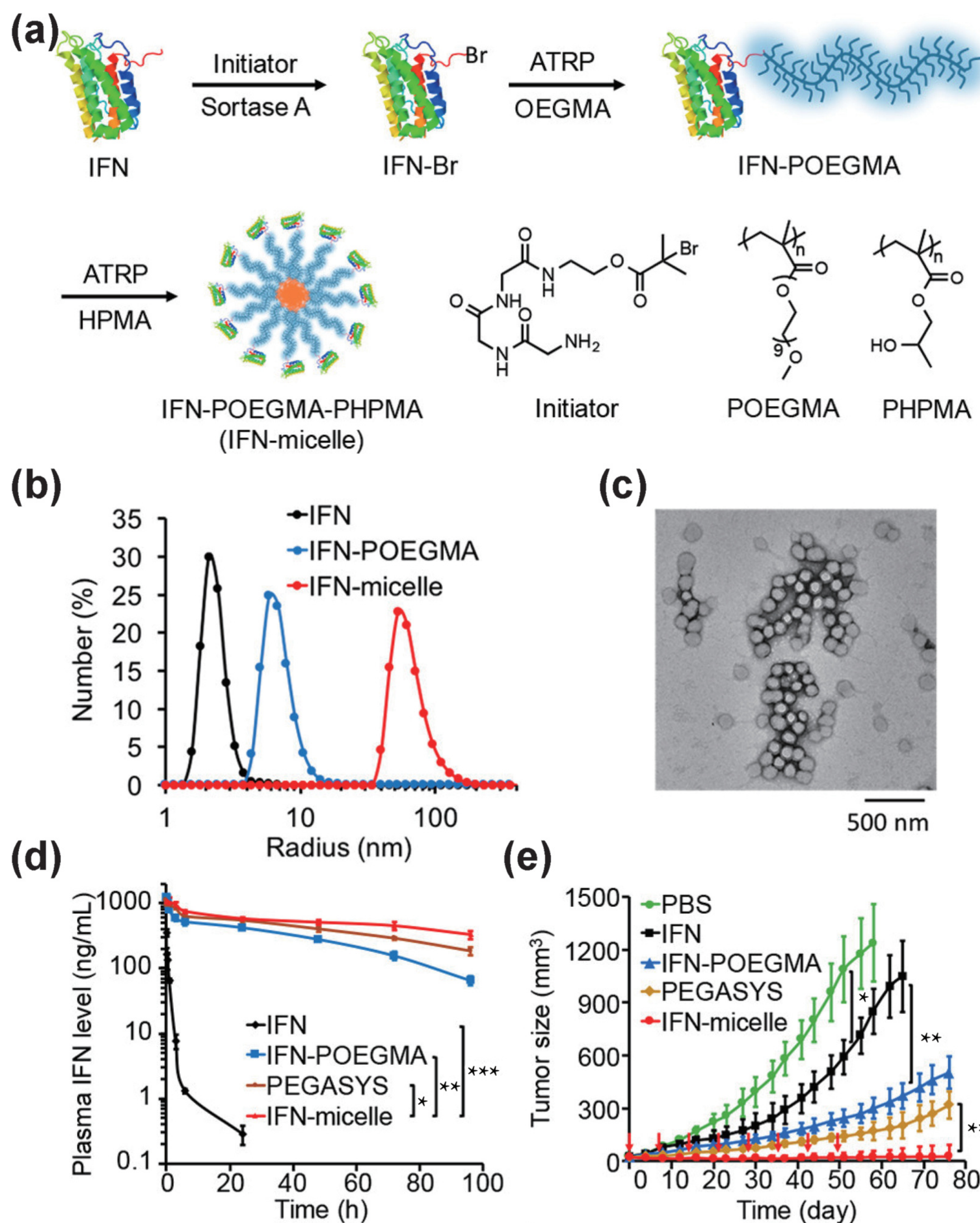

Figure 4 Polymerization induced self-assembly of a site-specific interferon α-block copolymer conjugate into micelles with remarkably enhanced pharmacology. (a) Site-specific *in situ* polymerization induced self-assembly (SI-PISA) for the synthesis of an IFN-POEGMA-PHPMA micelle (IFN-micelle) with greatly enhanced pharmacology for tumor therapy. (b) DLS analyses of IFN, IFN-POEGMA and IFN-micelle. (c) TEM image of representative IFN-micelle. (d) Plasma concentrations of IFN-micelle, PEGASYS, IFN-POEGMA, and IFN versus time post intravenous administration. (e) Cumulative survival of mice. Reproduced with permission from Ref. [67], © American Chemical Society 2018.

Moreover, the length of the PAS segment can be precisely controlled to optimize pharmacokinetic properties [79].

The unstructured recombinant polypeptide **XTEN**, comprising 864 amino acids, was developed through rational design and screening to markedly extend the plasma half-life of therapeutic proteins. This half-life extension capability is exemplified by exendin-XTEN, which demonstrated a half-life of 60 h in cynomolgus monkeys with a projected 139-h half-life in humans, all without observed toxicity or immunogenicity [80]. The translational promise of this platform is now being validated in clinical trials. The exendin-XTEN fusion (VRS-859) is advancing in a Phase I trial for type 2 diabetes with a biweekly dosing regimen, while the hGH-XTEN fusion (VRS-317) is being evaluated in a Phase II trial as a once-monthly therapy for growth hormone deficiency [81].

Elastin-like polypeptides (ELPs), composed of repeated Val-Pro-Gly-X-Gly motifs, exhibit LCST phase transition properties, allowing the design of thermos-responsive injectable depots for sustained drug release [82–84]. Fusions such as glucagon-like peptide-1 (GLP1)-ELP demonstrated glucose control for up to 5 days from a single subcutaneous injection in mice, and a protease-operated depot (POD) version extended efficacy to 120 h,

showcasing the depot-forming ability of ELPs as unique among PEG alternatives [85].

Gelatin-like polypeptides (GLKs), engineered from hydrophilic redesigns of natural gelatin sequences, have emerged as a promising PEG alternative for protein modification. GLKs combine biological inertness, low immunogenicity, and strong protein-stabilizing properties, making them particularly suitable for extending protein pharmacokinetics. Huang and co-workers demonstrated that fusion of GLKs to granulocyte-colony-stimulating factor (rGLK/G-CSF) was achieved by recombinant expression of the G-CSF coding sequence in-frame with the GLK sequence, followed by purification using standard chromatographic methods. The resulting fusion protein prevented aggregation, enhanced serum half-life (10 h for rGLK/G-CSF), and maintained superior *in vitro* bioactivity (140% of native G-CSF), outperforming PEG- and albumin-fused variants. These improvements were attributed to the expanded hydrodynamic radius, negative surface charge, and resistance to renal clearance and proteolysis conferred by the GLK moiety. Such findings highlight GLK fusions as a versatile strategy to improve the stability, circulation time, and therapeutic efficacy of protein therapeutics [86].

Lastly, zwitterionic **EKEKEK polypeptides**, constructed by alternating Glu and Lys residues in multilayers, mimic natural protein surfaces to generate dense, multilayer peptide cloaks. This EK-coating strategy eliminates both specific and non-specific interactions with biological media, extends systemic circulation, and significantly reduces immunogenicity, outperforming both traditional lysine-conjugation and single-site fusion technologies [87, 88].

In brief, recombinant polypeptide platforms offer a modular and clinically translatable alternative to synthetic polymers. Beyond their genetic precision and high-yield microbial production, these polypeptides can be fused to proteins to form monodisperse entities with customizable biophysical properties. Their unique thermo-responsive phase transitions and extended chain conformations are particularly effective for stabilizing protein and sustained-release depot, providing the specific material basis for the kinetic and distribution strategies discussed in Section 3.

Collectively, these rationally engineered polymers exhibit diverse chemical structures, degradability, immunogenicity, and pharmacokinetic profiles. PEG analogues and hydrophilic polymers excel in stealth and tunable architecture, while natural polysaccharides offer biodegradability and biocompatibility, and recombinant polypeptides provide genetically encoded, precisely tunable, and low-immunogenic platforms. Choice of polymer should consider protein size, therapeutic goal, and desired half-life, with opportunities to combine materials for optimized long-circulating protein-polymer conjugates. Table 1 has summarized various protein-polymers conjugates and their prolonged terminal half-lives, which offer options for tailoring the pharmacokinetic profiles of therapeutic proteins to specific clinical needs. These diverse polymers provide a chemical foundation for further functional engineering. By leveraging their unique properties, such as structural tunability and environmental responsiveness, various design principles can be applied to optimize the biological performance of the resulting protein-polymer conjugates. Accordingly, the following section will transition from these specific materials to the overarching strategies used to overcome biological barriers and enhance therapeutic efficacy.

3 Principles and emerging strategies

Building on the diverse PEG alternatives mentioned above, the rational design of protein-polymer conjugates entails integrating material properties into defined functional strategies. The *in vivo* performance of long-acting protein-polymer conjugates depends on circulation half-life, biodistribution, and immune recognition. Achieving prolonged circulation and favorable tissue distribution while minimizing immune clearance remains a key challenge, as conjugates often face rapid renal or hepatic elimination, non-specific uptake, and potential immunogenicity. To address these challenges, a wealth of research has emerged. Based on these, we induce general principles and categorize various current strategies into following five types according to their mechanism of action: **size expansion**, increasing particle size to evade renal filtration and prolong systemic circulation (Fig. 5(a)); **shape modulation**, tailoring the morphology of conjugates to influence circulation dynamics and cellular uptake (Fig. 5(b)); **controlled-release**, prolonging systemic exposure through depot formation (Fig. 5(c)); **active targeting**, enabling pathological tissues-specific delivery and responsive drug release (Fig. 5(d)); and **immune regulation**, minimizing clearance and immunogenicity through passive immune evasion and the induction of immune tolerance (Fig. 5(e)). Designed by these five strategies, protein-polymer conjugates can significantly prolong the circulation time of therapeutic proteins, enabling them to maintain a more stable and long-lasting plasma concentration within the therapeutic window, thereby reducing dosing frequency and improving overall therapeutic efficacy. In the following sections, each principle, design considerations, and representative examples are discussed in detail.

3.1 Size expansion via conjugation or self-assembly

Size expansion via conjugate or self-assembly refers to a strategy wherein therapeutic proteins are covalently conjugated with polymers thereby significantly increasing their hydrodynamic size and altering their pharmacokinetic profiles. This increased scale enlargement effectively reduces renal clearance by exceeding the glomerular filtration threshold (5–8 nm), enhances plasma stability by shielding proteins from proteolytic degradation, and facilitates passive accumulation at disease sites [89].

For example, IFN-α was conjugated to PMPC via site-specific conjugation, which increased its size from 2.3 to 9.7 nm. Notably, the PMPC conjugate exhibited a 194- and 158-fold increase in systemic exposure and tumor uptake, respectively, compared with native IFN-α, which was partly attributed to the size expansion of IFN-α [63]. Lately, the Li group conjugated the antioxidant enzyme SOD to amphiphilic block copolymers via ATRP, incorporating a hydrophilic POEGMA segment and an acid-sensitive poly(ethyl propyl amino)ethyl methacrylate (PEPA) block (Fig. 6). This design enabled SOD to self-assemble into protein-polymer micelles with markedly enlarged hydrodynamic diameters (~ 90 nm) compared to native SOD (~ 3.6 nm). The micelles also exhibited significantly enhanced enzymatic preservation during intracellular delivery, owing to efficient endo/lysosomal escape, and improved renal retention (over 60% at 48 h post-injection) [90]. In summary, polymer-induced self-assembly effectively enlarges the size of the protein and provides a protective polymer corona, thereby extending the circulation time and enhancing the therapeutic effect.

3.2 Shape modulation

Shape modulation is an effective design principle for prolonging the

Table 1 Long-circulating protein-polymer conjugates

Protein	Polymers	Protein modification strategy	Terminal ($t_{1/2\beta}$) half-lives/fold increase	Ref.
Exendin-4	POEGMA	ATRP	6.2 h, 8.9-fold	[21]
Uricase	POEGMA	ATRP	46.2 h, 21-fold	[22]
	PTMAO	ATRP	19.1 h, 4.9-fold	[64]
GFP	POEGMA	ATRP	60 h, 6-fold	[139]
	POEGMA	ATRP	26 h, 6.5-fold	[19]
IFN	POEGMA	ATRP	53.1 h, 47.9-fold	[140]
	POEGMA-PHPMA	ATRP	83.8 h, 100-fold	[67]
	PODMA	ATRP	48.9 h, 96-fold	[131]
	Polysarcosine	Cysteine-selective modification	4.8 h, 6-fold	[47]
	PMPC	ATRP	51.6 h, 34.6-fold	[63]
	ELP	Genetic fusion expression	8.6 h, 27.7-fold	[84]
	ELP	Genetic fusion expression	280 h, 116-fold	[105]
	ELP	Genetic fusion expression	9.9 h, 1.2-fold	[101]
	ELP	Genetic fusion expression	54.7 h, 124.3-fold	[141]
	Pro-Leu-Gly-Leu-Ala-Gly-ELP	Genetic fusion expression	8.9 h, 6.4-fold	[142]
	$P(EG_3Glu)_{20}$	Sortase A-mediated transpeptidation	6.3 h, 12.6-fold	[143]
	Albumin-binding domain (ABD)	Genetic fusion expression	6.9 h, 3.6-fold	[144]
SOD	P(OEGMA-b-DPA)	ATRP	48 h	[90]
HSA	PEPA	ATRP	3.44 h	[93]
HSA/CAT	PEPA	ATRP	72.1-fold	[130]
Transferrin protein	PMSEA	RAFT	9.3 h, 2.7-fold	[126]
Lysozyme	PCLA	Michael-addition reaction	10.8 h, 5-fold	[145]
	PMSEA	RAFT	25.6 h, 2.3-fold	[146]
BSA	P(DEGA-co-PEGA)	RAFT	30.9 h, 7.7-fold	[117]
	(O'20-b-M'20-b-O'20-b-H'20-b-H'20-b-M'20)	ATRP-based QOILP	18.53 h	[147]
Myoglobin	POEGMA	ATRP	18 h, 6-fold	[18]
	poly(carboxybetaine methacrylate)	ATRP	17 h, 5.5-fold	[148]
Anakinra	Linear polyglycerol	N-terminal reductive amination	10.1 h, 4.4-fold	[52]
Trastuzumab Fab'	p(HPMA-co-NIPAM)	RAFT	9.8 h	[43]
	PSA	Genetic fusion expression	28.19 h, 21-fold	[79]
Insulin	Trehalose glycopolymer	RAFT	22.7 min	[56]
Rituximab	ELP	Genetic fusion expression	109.5 min	[95]
L-asparaginase	ELP90	Genetic fusion expression	353.2 h, 116.8-fold	[103]
	ELP(HV)60	Genetic fusion expression	466.8 h, 109-fold	[102]
Clotting factor VIII	XTEN	Genetic fusion expression	7.8 h, 34-fold	[149]
Human growth hormone	XTEN	Genetic fusion expression	110 h, 16.18-fold	[81]
Granulocyte-colony-stimulating factor	GLK	Genetic fusion expression	12 h, 2.6-fold	[86]
Asparaginase	Poly-L-proline	N-carboxyanhydride ring-opening polymerization	26 h, 18-fold	[150]
	EK peptides	Amine amplification	18.9 h, 2.3-fold	[87]
	ELP	Genetic fusion expression	528.1 h, 132-fold	[104]

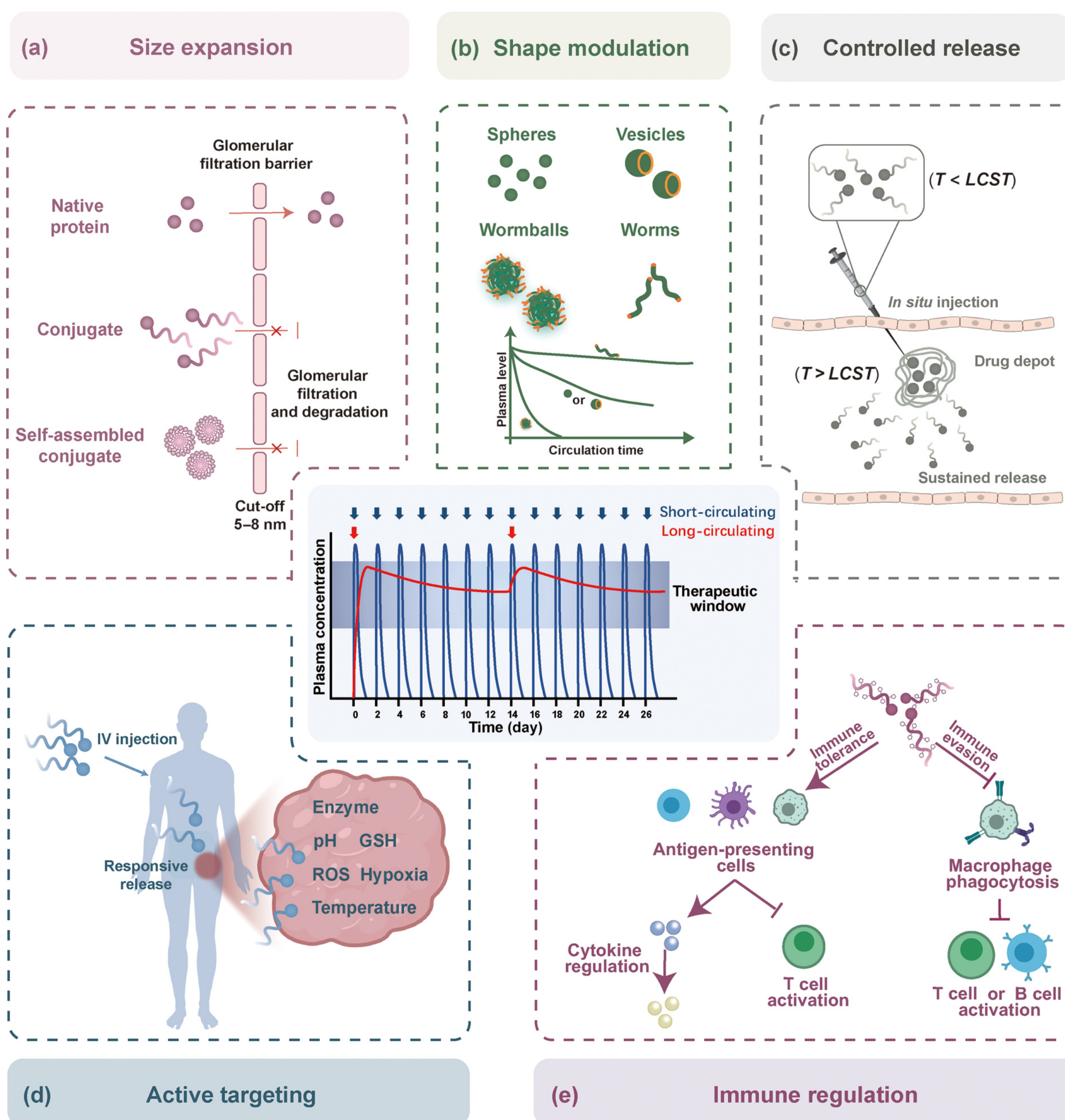

Figure 5 Principles and emerging strategies for long-circulation of protein-polymer conjugates. (a) Size expansion: Amphiphilic polymer conjugation drives protein self-assembly into nanoscale structures, increasing hydrodynamic size and reducing renal clearance. (b) Shape modulation: Altering protein-polymer nanostructures from spheres to worm-like architectures prolongs circulation by reducing MPS recognition. (c) Controlled release: Formation of localized drug depots gradually releases therapeutic proteins, prolonging systemic exposure. (d) Active targeting: Stimuli-responsive delivery systems release therapeutic agents in response to specific biochemical or physical cues, enabling long circulation and site-specific action. (e) Immuno-interaction: Protein–polymer conjugates modulate immune recognition through passive evasion and active induction of tolerance, reducing clearance and enhancing circulation for more effective therapeutics.

in vivo circulation of protein-polymer nanostructures by changing the particle morphology from spheres to anisotropic structures, such as nanoworms. Compared to spherical particles, which are readily recognized and rapidly cleared by the MPS, worm-like nanostructures exhibit prolonged blood retention due to their high aspect ratio and flexible, elongated shape, which reduces interactions with endothelial cells and phagocytes. This is because the hydrodynamic forces under flow align and pull them away from phagocytes, preventing rapid recognition and clearance by the MPS [91]. PISA is a highly versatile technique for the rational synthesis of colloidal dispersions of diblock copolymer [92]. By controlling the polymerization process, PISA enables precise morphological tuning of protein-polymer conjugate nanoparticles, allowing structures to evolve from spherical micelles to worm-like or vesicular assemblies. Such structural control can be achieved either through the *in situ* growth of hydrophobic polymer blocks directly from protein surfaces [93]. These methods exploit molecular curvature modulation and interfacial stabilization to achieve shape-controlled self-assembly under mild conditions, providing a versatile platform for designing functional nanostructures.

For example, Huang et al. engineered nanoworms by *in situ* growth of HSA-poly(tertiary amine)-doxorubicin (HSA-PEPA-

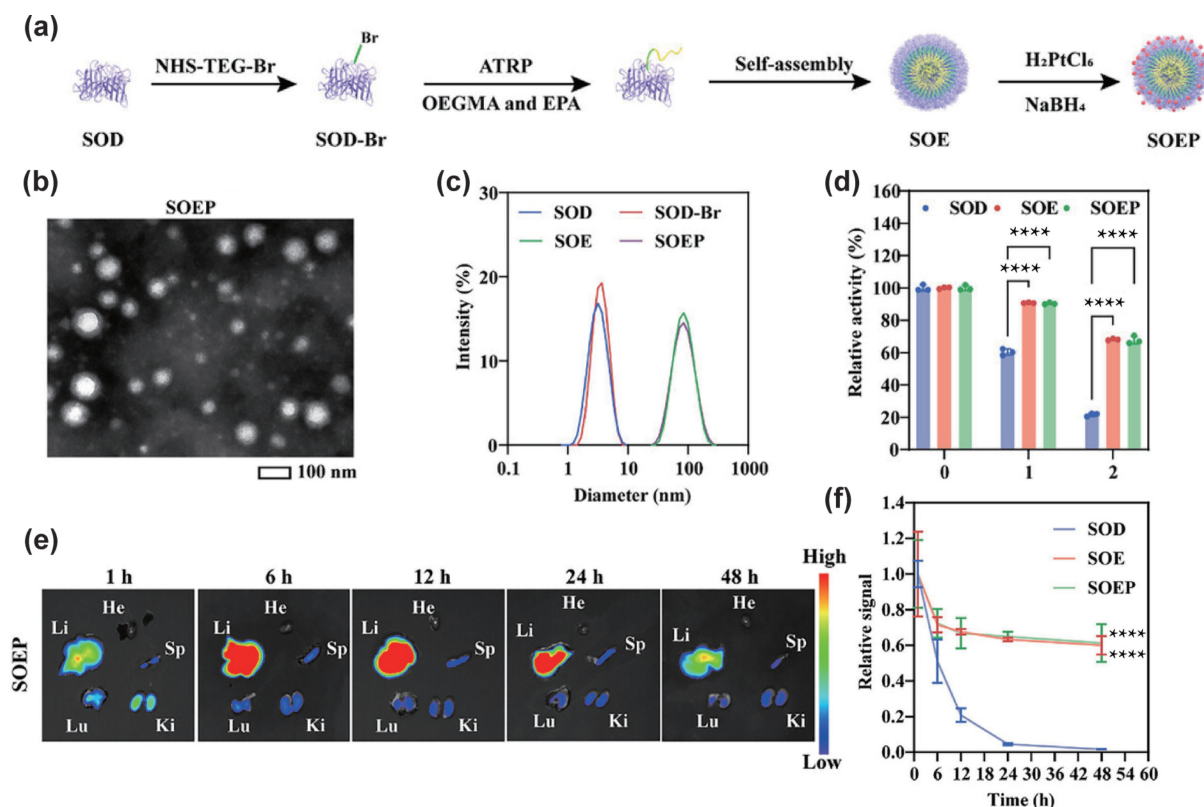

Figure 6 Polymer-conjugated SOD-Pt^0 micelles enhance ROS cascade scavenging to alleviate ischemia-reperfusion injury during kidney transplantation. (a) Synthetic route for the preparation of SOE and SOEP. (b) TEM image of SOEP. (c) DLS analysis of SOD, SOD-Br, SOE, and SOEP. (d) Thermal stability of SOD samples. (e) *Ex vivo* imaging of major organs, including kidney (Ki), liver (Li), lung (Lu), spleen (Sp), and heart (He) at 1, 6, 12, 24, and 48 h after a single intravenous injection of Cy7-SOEP. (f) Relative signal-time curves of kidneys treated with Cy7-SOEP, quantified from panel (e). Reproduced with permission from Ref. [90], © Wiley-VCH GmbH 2025.

DOX) conjugates, which exhibited lower uptake by normal endothelial cells than nanospheres or free DOX (Figs. 7(a) and 7(b)). The worm-like morphology also enhanced tumor accumulation and penetration, owing to acid-triggered dissociation into positively charged single isomers, thereby promoting endocytosis and transcytosis (Fig. 7(c)). Furthermore, the dual acid-sensitive design enabled efficient release of DOX, resulting in effective tumor suppression and prolonged survival in a triple-negative breast cancer model while minimizing systemic toxicity (Figs. 7(d)–7(f)) [94]. Aluri et al. engineered elongated protein nanoworms targeting the B-cell antigen CD20. They first created a building block by tandemly linking multiple single-chain variable fragments (scFvs). These multi-valent scFv proteins then spontaneously self-assembled into the final nanoworm architecture. The high-aspect-ratio structures promoted efficient CD20 clustering on lymphoma cells, thereby triggering apoptosis more effectively than the clinically used monoclonal antibody Rituximab. Notably, the nanoworms exerted their pro-apoptotic activity independent of Fc-mediated effector functions, highlighting a unique mechanism of action. Owing to their genetically encoded and biodegradable design, scFv-nanoworms offer precise sequence-level control, scalable recombinant production, and enhanced therapeutic efficacy, establishing a versatile platform for targeted protein nanomedicines [95].

Together, these findings suggest that shape modulation, from spheres to nanoworms, offers a promising strategy for developing long-circulating protein-based nanomedicines with enhanced bioavailability, tissue penetration, and therapeutic potency.

3.3 Controlled released by forming drug depot

Controlled-release strategies that form local drug depots and gradually release therapeutic proteins at the site of administration have become effective means to prolong systemic exposure and enhance therapeutic efficacy. To date, ELPs fusion proteins or thermo-gels are particularly promising because they can self-assemble to form *in situ* depots after administration and release proteins in response to physiological stimuli to prolong their circulation time *in vivo* [96–101].

ELP fusion proteins represent a key innovation in the development of *in situ* long-circulating biopeptides. For example, L-asparaginase (ASP), an important enzyme drug used to treat acute lymphoblastic leukemia, has been fused to ELP to generate thermos-responsive bioconjugates (ASP-ELP) due to the LCST phase transition properties of ELP. Upon subcutaneous injection, ASP-ELP undergoes a reversible phase transition above its LCST, forming a localized drug depot that gradually releases the enzyme into circulation. This depot-based delivery markedly prolongs the pharmacological activity of ASP, sustaining its release for over 500 h. Importantly, the ELP fusion design not only preserved the enzymatic activity of ASP but also mitigated its immunogenicity, leading to reduced anti-drug antibody production and improved tolerance *in vivo*. Furthermore, by introducing additional pH sensitivity into the ELP segment, the depot system enhanced tumor penetration and intra-tumoral retention, thereby further boosting antitumor efficacy in hematologic malignancy models [102–104]. Similarly, IFN-ELP, a fusion of IFN-α and temperature-sensitive

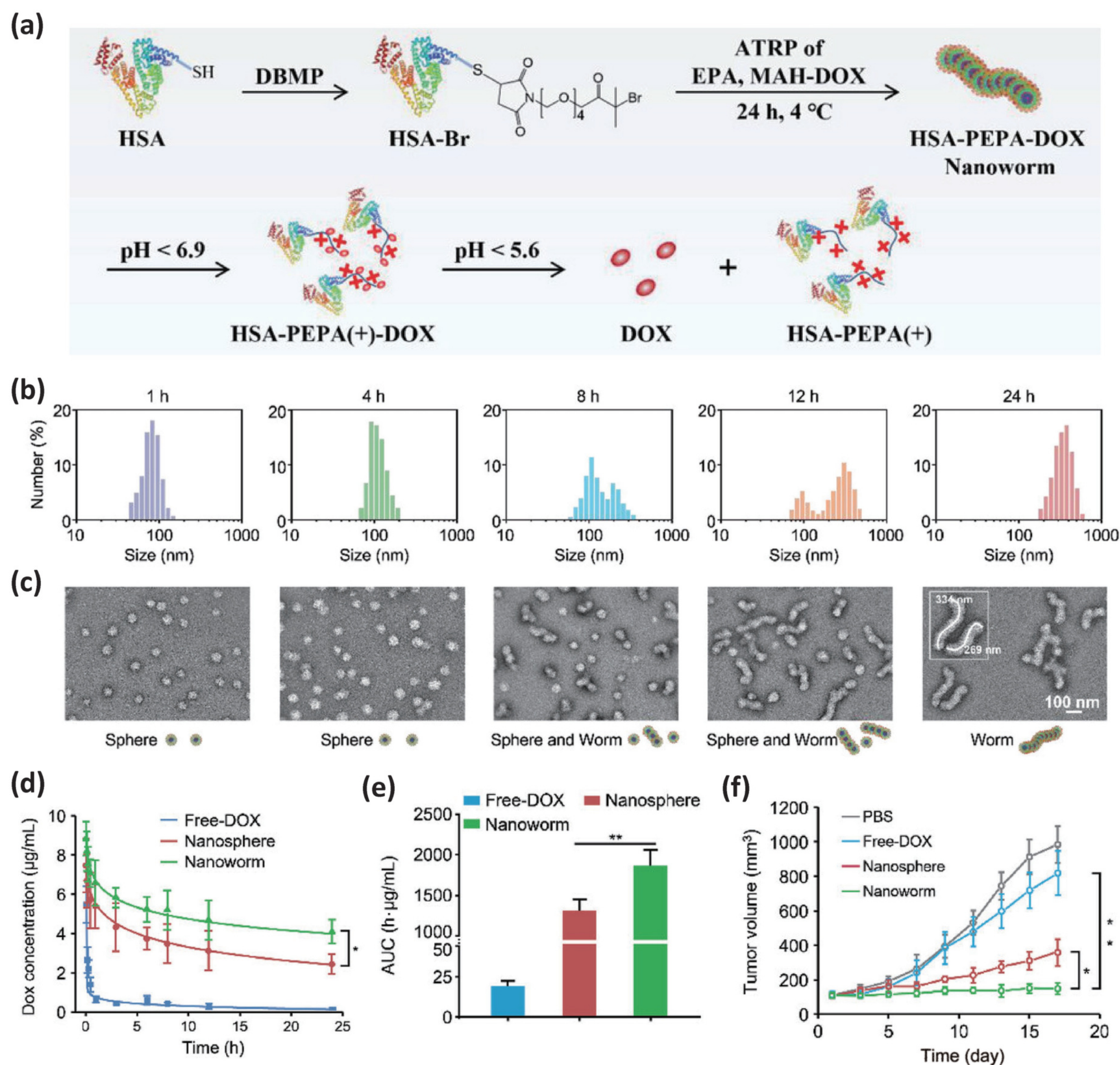

Figure 7 Spatiotemporally-programmed dual-acid-sensitive nanoworms of albumin-poly(tertiary amine)-doxorubicin conjugates for enhanced cancer chemotherapy. (a) Synthetic route of HSA-PEPA-DOX with dual-acid-sensitive properties. (b) Rh distribution of synthesized HSA-PEPA-DOX nanoparticles after different reaction times. (c) Typical TEM images showing morphological changes of HSA-PEPA-DOX nanoparticles as the reaction progressed. (d) Plasma DOX concentrations at different post-injection time points. (e) Area under the curve (AUC) values of HSA-PEPA-DOX. (f) The growth in tumor volume with time during the treatment period. Reproduced with permission from Ref. [94], © Wiley-VCH GmbH 2023.

ELP, forms an *in situ* drug depot when injected into the surgical cavity of glioblastoma (GBM) in mice (Fig. 8). This depot exhibits zero-order release kinetics that persist for over 3 weeks, allowing for sustained stimulation of antitumor immunity. Importantly, the combination of IFN-ELP with temozolomide (TMZ) resulted in a GBM-free survival rate of 60%, significantly superior to IFN alone (12.5%) [105].

Thermo-gels are a novel class of smart materials that utilize temperature as the sole triggering factor to induce a reversible transition from a fluid state to a gel state [106]. According to the International Union of Pure and Applied Chemistry (IUPAC) 2025 Top Ten Emerging Technologies in Chemistry report, they have been recognized as one of the representative examples. Thermo-gels can be engineered to form *in situ* depots for sustained release of therapeutic proteins, with the gel network acting as a diffusion-regulating reservoir that enables prolonged and near zero-order release kinetics [107, 108].

3.4 Active targeting by stimuli-responsiveness

Controlled-release strategies prolong protein retention by creating a physical barrier that limits their diffusion and release. This passive mode relies on intrinsic material properties, such as LCST or hydrophilicity, to regulate protein release kinetics. In contrast, stimuli-responsive delivery systems provide a versatile platform for achieving long-circulation and active targeting. These systems are designed to exploit specific cues within physiological and pathological microenvironments to release drugs, such as changes in pH, enzyme activity, reactive oxygen species (ROS), and glutathione (GSH) levels, as well as hypoxia. In this way, the systems can minimize ineffective drug loss under non-pathological conditions, thereby achieving precise and controllable drug delivery and effectively prolonging the duration of action of protein drugs *in vivo*.

pH-responsive protein-polymer conjugates have been rationally designed to exploit the acidic microenvironmental characteristics of

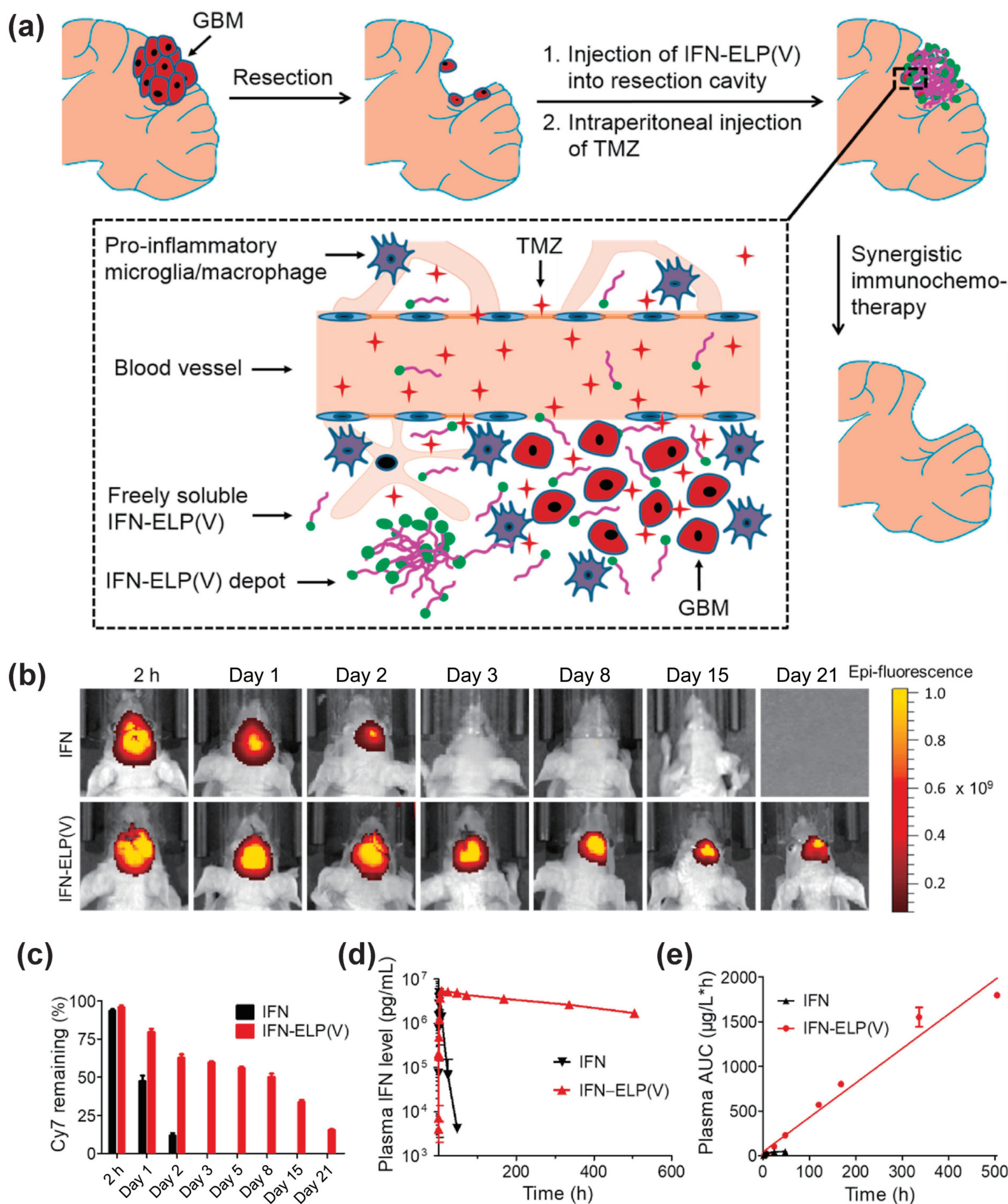

Figure 8 Spatiotemporal combination of thermosensitive polypeptide fused interferon and temozolomide for post-surgical glioblastoma immunochemotherapy. (a) Spatiotemporally-programmed combination of IFN-ELP(V) and TMZ for post-surgical synergistic immunochemotherapy of GBM. (b) Representative *in vivo* fluorescence imaging of Cy7-labelled IFN or IFN-ELP(V) after injection into resection cavity. (c) Fluorescence quantification of Cy7-labelled IFN or IFN-ELP(V) after injection into resection cavity (n = 3). (d) Plasma IFN level of IFN or IFN-ELP(V) as a function of time (n = 3). (e) Plasma AUC of IFN or IFN-ELP(V) as a function of time (n = 3). Reproduced with permission from Ref. [105], © Elsevier Ltd. All rights reserved 2020.

tumors, inflamed tissues, or intracellular organelles. Their tunable release behavior and intracellular trafficking are typically achieved through three major strategies: (1) Incorporation of ionizable polymer segments, such as poly(2-(diisopropylamino)ethyl methacrylate (PDPA), which remain neutral at physiological pH but protonate in acidic niches, enhancing secrecy in the blood circulation while promoting local activation [109–111]; (2) linking therapeutic proteins functional polymers via acid-labile bonds, which cleave under acidic conditions to enable controlled release of the protein payload [112]; and (3) designing conjugates with charge-reversible polymers, such as sulfonamide-derivatized blocks, that switch from neutral/negative to positive charge in acidic

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environments, thereby enhancing electrostatic interactions with cell membranes and improving site-specific uptake [94, 113, 114]. In summary, by integrating these three strategic elements, long-term circulation of pH-responsive conjugates can be achieved while simultaneously enabling precise local activation.

Enzyme-responsive protein-polymer conjugates enhance therapeutic precision by harnessing the elevated activity of disease-associated enzymes to achieve site-specific drug release and activation. This strategy relies on the incorporation of cleavable linkers that remain stable in circulation but are cleaved by specific enzymes at the target site [115]. The primary mechanisms involve two types of enzymatically cleavable linkers. A common approach uses protease-sensitive peptide linkers, which can be genetically encoded or chemically introduced. These linkers, cleaved by enzymes such as matrix metalloproteinases (MMPs) or cathepsin B, can not only control drug release but also promote cellular uptake through enzyme-triggered charge modulation [116–118]. Alternatively, small molecule substrate linkers can be employed. For example, ester bonds are stable in the blood but cleaved by intracellular esterases to activate the therapeutic protein. A notable application of this type is the use of azobenzene-based linkers that are selectively cleaved by gut microbial azoreductases, enabling colon-targeted delivery [119, 120]. By leveraging these enzyme-responsive designs, protein-polymer conjugates can be tailored for intelligent, context-dependent behavior, significantly expanding their versatility and therapeutic precision.

Temperature-responsive protein-polymer conjugates utilize polymers with tunable LCST that undergo phase transitions in response to localized temperature changes often found in inflamed or pathological tissues. Polymers like poly(N-isopropylacrylamide (PNIPAM), poly(2-dimethylamino ethyl methacrylate) (PDMAEMA), and P(DEGMA-co-DPA) become hydrophobic above their LCST, destabilizing micelles and triggering drug release [121–123]. Adjustments via copolymer blending and cross-linking allow fine-tuning of the transition temperature for site-specific responsiveness.

ROS-responsive protein-polymer conjugates are designed to exploit the distinct ROS gradient between the circulation and disease sites to efficiently release protein drugs at target sites. Polymers incorporating moieties such as phenylboronic acid or thioketone remain stable in normal tissues but undergo ROS-induced oxidation in pathological sites, leading to backbone cleavage or hydrophilicity shifts that trigger payload release [124–126]. For example, Swierczynski et al. reported a one-step strategy for site-selective modification of BSA under mild conditions using boronic acid-functionalized polymers, successfully constructing BSA-polyarylboronate conjugates. This direct conjugation method allows for precise polymer grafting while preserving the structural integrity and bioactivity of BSA. A key advantage of this design lies in the utilization of ROS-responsive arylboronates. This mechanism involves oxidative cleavage of the chemical bond in a hydrogen peroxide environment. By exploiting this ROS-triggered linker degradation to control protein release, this strategy offers a new solution for active, targeted delivery of protein drugs and prolonged therapeutic duration *in vivo* [127].

GSH-responsive protein-polymer conjugates exploit the difference in GSH concentrations between intracellular and extracellular environments. Under disease conditions, intracellular GSH levels are significantly higher than those in the extracellular fluid. The disulfide bonds, such as cystamine linkages, within these conjugates remain stable during systemic circulation but are selectively cleaved by the elevated intracellular GSH concentration upon cellular entry, thereby enabling controlled therapeutic protein release [115]. For example, triblock copolymers containing pH-sensitive poly(L-histidine) and GSH-sensitive linkers have been developed to enable doxorubicin delivery with minimal premature release and targeted intracellular unloading [128]. What's more, Davis et al. reported a method for preparing a heterogeneous bifunctional protein-polymer conjugate. They used a bifunctional reversible addition-fragmentation chain transfer (RAFT) agent with a terminal pyridyl disulfide (PDS) group to site-specifically modify BSA. Subsequently, oligo(ethylene glycol) acrylate (OEGA) and N-(2-hydroxypropyl) methacrylamide (HPMA) were introduced via *in situ* polymerization. The disulfide bonds in the conjugate undergo reduction-responsive cleavage in response to GSH, mimicking the highly reducing environment within cells. The conjugate was structurally stable under normal extracellular conditions, but in the presence of millimolar GSH, it dissociated from the polymer chains and rapidly released the protein. This result validates the potential of this redox-responsive protein-polymer platform for controlled protein delivery within cells and effectively prolongs the duration of action of functional proteins [129].

Hypoxia-responsive protein-polymer conjugates are core components of tumor microenvironment-targeted delivery systems. They are specifically designed to be activated under the hypoxic conditions prevalent in solid tumors. A typical strategy involves conjugating bio-reducible polymers (such as poly-N-oxides) to therapeutic proteins, conferring long-circulating "stealth" properties. In a hypoxic environment, enzymatic reduction reactions convert these polymers into positively charged polyamines, triggering a critical charge reversal. This transformation significantly promotes deep drug penetration in tumor tissues by enhancing electrostatic interactions and transcytosis, thereby achieving hypoxia-responsive protein release [130]. For example, Zhang et al. developed an IFN-PODMA conjugate by polymerizing poly(2-(N-oxide-N,N'-dimethylamino)-2-ethyl methacrylate) (PODMA) from the C-terminus of IFN (Figs. 9(a)–9(d)). The hypoxic tumor microenvironment reduces the poly(N-oxide) side chain to a polytertiary amine, which is then protonated in an acidic environment (Fig. 9(b)). This property, combined with an extended circulation half-life (51 h), gives the conjugate enhanced tumor penetration and antitumor efficacy superior to that of PEG-IFN (Figs. 9(e)–9(g)) [131]. Thus, by directly responding to hypoxia signals, IFN-PODMA transforms the pathological environment into a unique trigger that precisely activates protein release, prolongs *in vivo* circulation time, and enhances intra-tumoral distribution.

Collectively, these diverse stimuli-responsive mechanisms enhance the stability and circulation time of drug carriers while enabling precise, environment-specific activation across a broad spectrum of diseases and tissues. This versatile approach represents a powerful strategy for next-generation long-circulating and actively targeted drug delivery systems.

3.5 Immune regulation

Protein-polymer conjugates can be rationally designed to modulate interactions with the immune system, a key determinant of their *in vivo* fate. By influencing immune recognition, these conjugates reduce clearance and improve pharmacokinetics, providing a

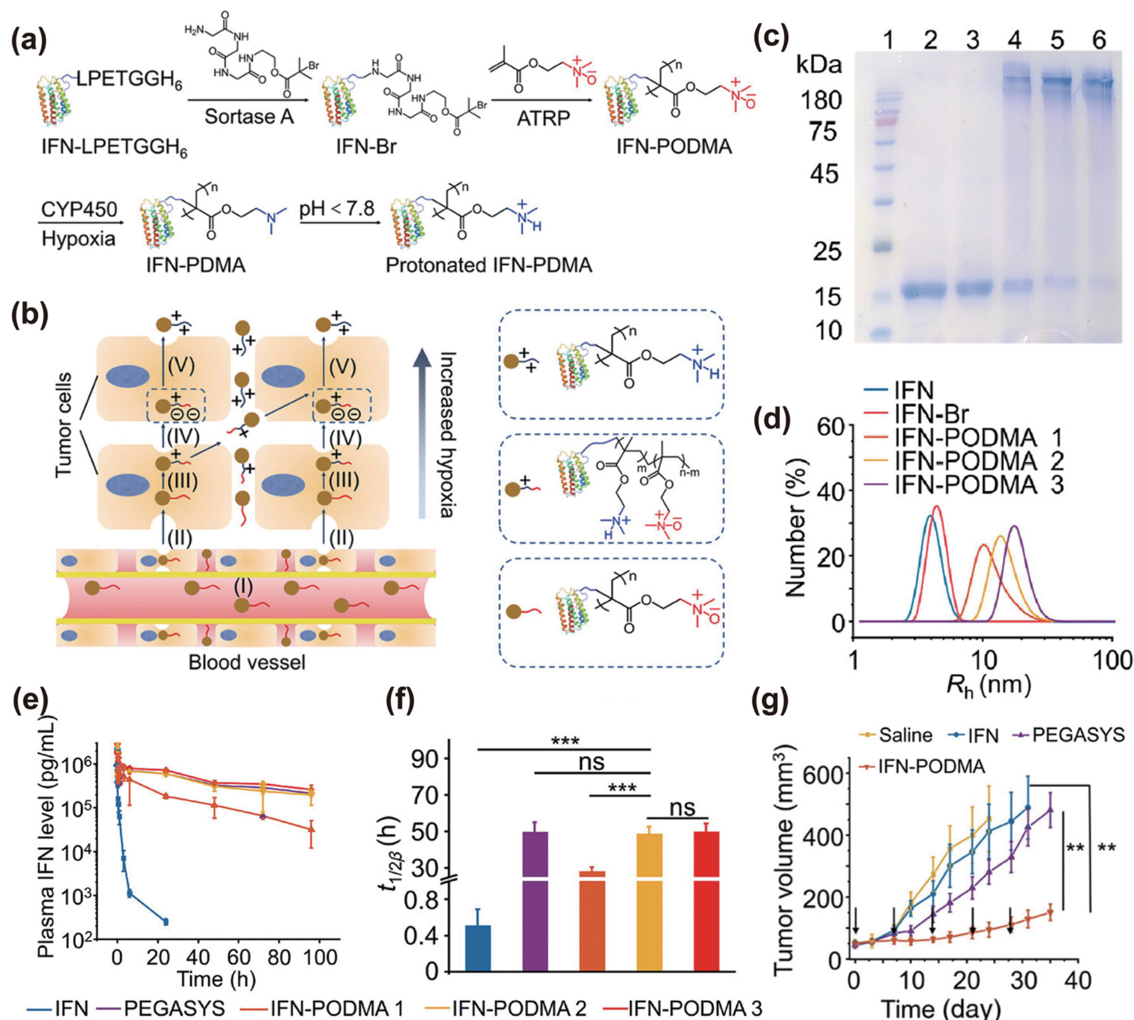


Figure 9 Hypoxia-triggered bioreduction of poly(N-oxide)-drug conjugates enhances tumor penetration and antitumor efficacy. (a) Sortase A-mediated protein ligation and ATRP are used to generate C-terminal IFN-PODMA conjugates. Under hypoxia conditions, CYP450 enzymes catalyze the reduction of IFN-PODMA to IFN-PDMA, which can be further protonated under acidic conditions. (b) IFN-PODMA can circulate in the blood for a long time after intravenous injection (I); however, once it enters a tumor (II), it can be reduced into IFN-PDMA by CYP450 enzymes overexpressed in the hypoxia region of the tumor (III). The electrostatic attraction of the protonated IFN-PDMA (IV) and negatively charged tumor cells mediates the transcytosis of the conjugate (V), which makes it feasible for the conjugate to reach the deeper layers of the tumor. (c) Gel electrophoresis analysis of the preparation of IFN-PODMA. Lane 1: protein marker; lane 2: IFN-LPETGGH$_6$; lane 3: IFN-Br; lane 4: IFN-PODMA 1 after purification; lane 5: IFN-PODMA 2 after purification; lane 6: IFN-PODMA 3 after purification. (d) DLS curves of IFN, IFN-Br, IFN-PODMA conjugates. (e) Plasma concentrations of IFN-PODMA, PEGASYS, and IFN vs time (n = 3). (f) Terminal half-life ($t_{1/2\beta}$) values of IFN-PODMA, PEGASYS, and IFN. (g) Tumor growth curves after the treatments at a dosage of 20 μg of IFN equivalent per mouse (n = 6–8). The black arrow in the figure represents the time point of administration. Reproduced with permission from Ref. [131], © American Chemical Society 2023.

platform for long-circulating and more effective therapeutics. The following sections will detail how polymer modifications achieve this through mechanisms such as passive immune evasion and active induction of immune tolerance [132].

For prolonged systemic circulation, protein-polymer conjugates can be rationally designed to achieve passive immune evasion. Conjugation of hydrophilic polymers, such as PEG or zwitterionic polymers, to the protein surface creates a steric "stealth" shield that reduces IgG opsonization and recruits the protein, thereby minimizing macrophage uptake and clearance. This stealth effect synergistically extends plasma half-life while maintaining protein bioactivity, providing a general approach to enhance the pharmacokinetic profile of therapeutic proteins [133]. For example, Ozer et al. used a three-ethylene glycol-long side-chain (EG3) yielding hydroxyl-functional POEGMA, because EG3 is neither recognized by anti-PEG antibodies nor undergoes phase transition at body temperature, making it an ideal "PEG-like" polymer with low immunogenicity (Fig. 10(a)). They synthesized POEGMA-uricase conjugates extended uricase's circulation from 2.2 to 46.1 h. Notably, POEGMA-uricase did not induce any secretion of anti-POEGMA IgM or IgG antibodies (Figs. 10(b) and 10(c)). This immune escape mechanism enabled prolonged systemic circulation and improved pharmacokinetics compared to unmodified proteins and traditional PEGylated counterparts, highlighting the potential of brush polymer conjugation to enhance long-circulating protein therapeutics [22].

Beyond passive immune evasion, protein-polymer conjugates can also be rationally designed to actively induce immune tolerance. Unlike "stealth" coatings that simply reduce immune recognition, tolerance-inducing systems utilize the interaction between glycans and immune receptors to regulate host immune responses. For example, glycopolymers with sialic acid motifs can bind to Siglec

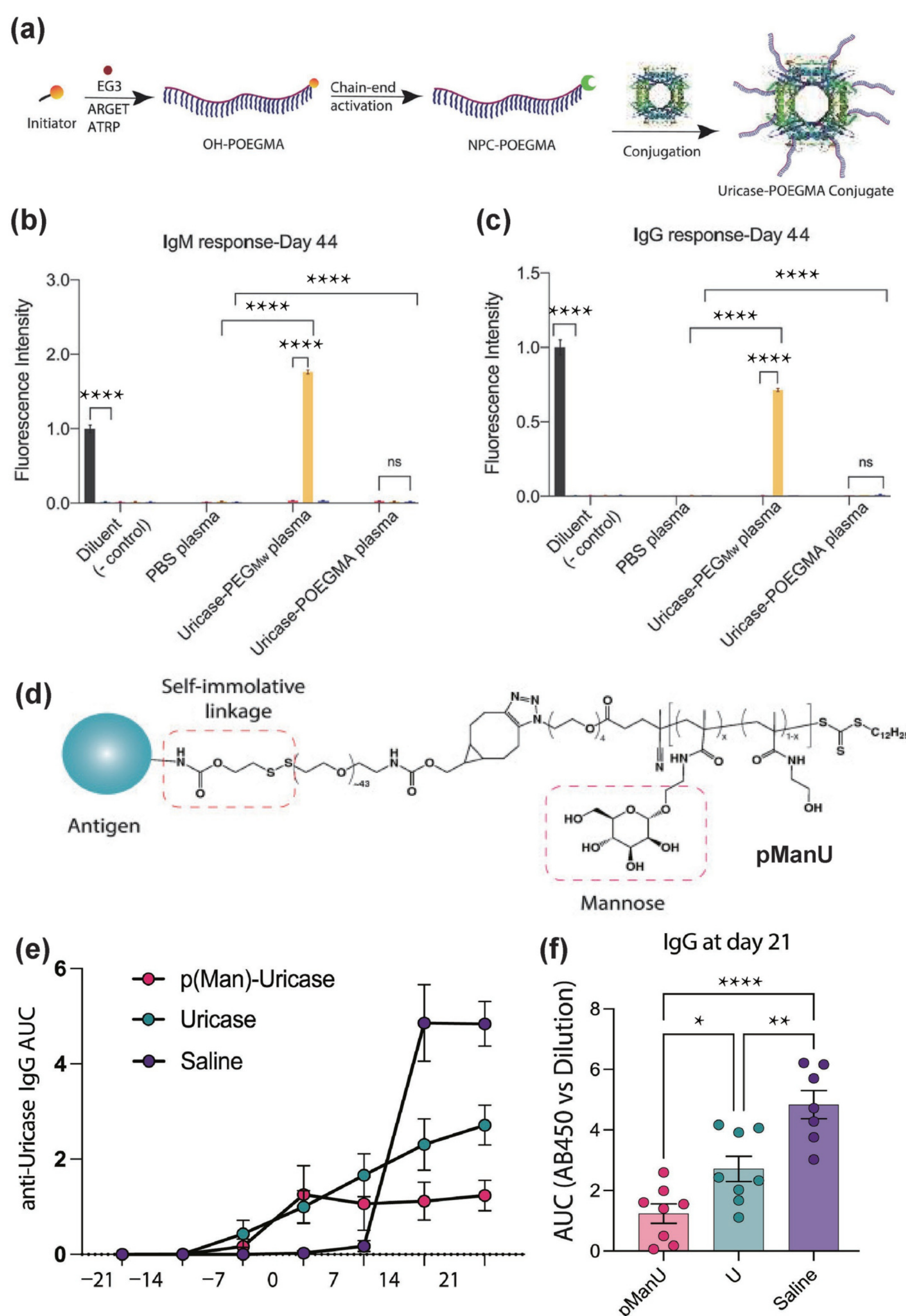


Figure 10 Protein-polymer conjugates can achieve long circulation through passive immune evasion and active induction of immune tolerance. (a) OH-functional POEGMA was synthesized by ARGET-ATRP of an EG3 monomer and subsequently activated to an NPC-terminated polymer, which was then conjugated to the uricase tetramer to yield uricase-POEGMA. (b) IgM response on day 44. (c) IgG response on day 44. (d) Structure of the p(Man) polymer conjugated to protein antigen. (e) Time course of uricase-specific IgG response represented as the area under the curve of absorbance vs. log-transformed dilution (AUC). Symbols represent mean across n = 8 mice. (f) Uricase-specific IgG response at day 21 represented by AUC. Reproduced with permission from Ref. [22], © Ozer, I. et al. Advanced Science published by Wiley-VCH GmbH 2022.

receptors on dendritic cells, triggering immunoreceptor tyrosine-based inhibitory motifs (ITIM) phosphorylation and Src homology region 2 domain-containing phosphatases-1/2 (SHP-1/2) recruitment. This inhibitory signaling drives tolerogenic programming, thereby suppressing antigen-specific immune responses and promoting long-term tolerance [134, 135]. Similarly, polymers functionalized with mannose can target C-type lectin receptors (such as DC-SIGN) on antigen-presenting cells, regulate cytokine secretion, and promote cell differentiation towards a tolerant phenotype (Fig. 11) [136]. In addition to regulating cytokines, targeting mannose receptors can also directly affect the antigen presentation process. For example, Jeffrey Hubbell's group has long been dedicated to the design and development of protein-polymer conjugates aimed at modulating immune responses [54, 137]. One of their works used a synthetic mannosylation strategy to modify highly immunogenic therapeutic proteins and synthesized mannosylated antigens (SMAs) (Fig. 10(d)). The results of their study showed that SMA changed

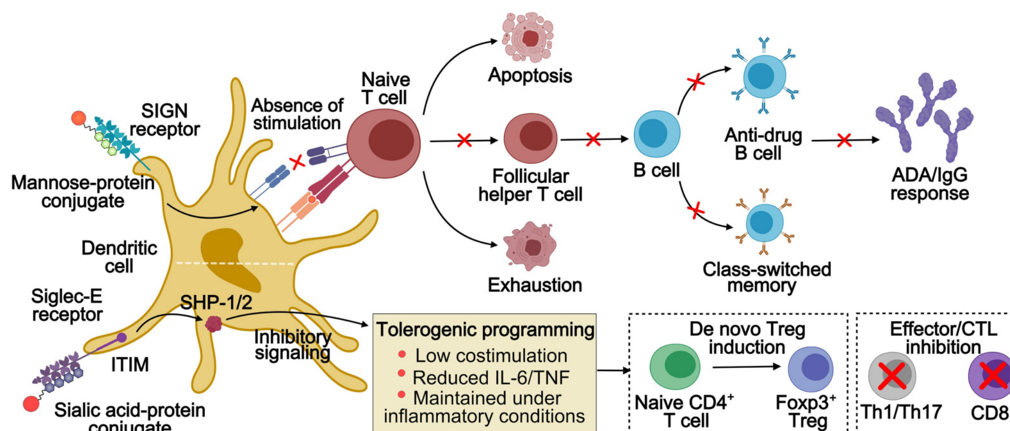


Figure 11 Mechanism of glycopolymers-protein conjugates mediated immune regulation via DC-SIGN and Siglec-E receptor pathways. Mannose-protein conjugates are recognized by the SIGN receptor. In the absence of stimulation, this pathway blocks follicular helper T cell differentiation, thereby inhibiting anti-drug B cells and the subsequent ADA/IgG response. This process promotes immune tolerance through T-cell apoptosis or exhaustion. Sialic acid-protein conjugates bind to the Siglec-E receptor, triggering ITIM phosphorylation and SHP-1/2 recruitment. This Inhibitory signaling drives tolerogenic programming (low co-stimulation, reduced IL-6/TNF), leading to de novo Foxp3$^+$ Treg induction and the inhibition of inflammatory Th1, Th17, and CD8$^+$ CTL expansion [135, 138].

the antigen processing and presentation pathway and T cell activation state by binding to the mannose receptor on the surface of dendritic cells, ultimately inducing antigen-specific immune tolerance. In animal models, SMA modification significantly reduced the formation of anti-drug antibodies (ADA) against biologics (Figs. 10(e) and 10(f)). This work highlights the potential of glycan-based innate immune recognition regulation in inducing immune tolerance and provides a promising strategy for reducing the immunogenicity of therapeutic proteins and prolonging their circulation time *in vivo* [138].

In summary, uniquely designed polymers enable protein therapeutics to evade immune clearance or actively induce tolerance, thereby enhancing long-term circulation, offering a promising strategy to improve safety and efficacy of biologics.

4 Superior performance of PEG alternatives in specific diseases

The transition from conventional PEGylation to advanced polymer alternatives is fundamentally driven by the need to overcome clinical obstacles in specific therapeutic areas. By integrating material innovations with sophisticated design principles, these alternatives provide customized solutions to meet the specific requirements of different diseases, effectively addressing the functional limitations of traditional PEGylation.

4.1 Cancer therapy

In oncology, while conventional linear PEG-protein conjugates primarily rely on passive accumulation via the EPR effect, they often suffer from poor intratumoral penetration and a lack of site-specific activation. PEG alternatives excel by enhancing the delivery of therapeutic proteins into malignant niches. To prevent the ABC phenomenon, a common drawback observed with repeated doses of PEGylated proteins, biodegradable PEEP-protein conjugates [25, 27] and low-immunogenicity rPEG-modified proteins [38] have been developed to ensure consistent pharmacokinetics during multi-cycle chemotherapy. Furthermore, unlike spherical PEGylated proteins that are rapidly cleared, morphology-enhanced systems such as IFN-α nano-assembly [67, 94] achieve prolonged retention and superior tumor penetration. Moreover, whereas traditional PEGylation provides only static shielding of the protein payload, stimuli-responsive systems like hypoxia-activated PODMA-IFN conjugates [131] or GSH-reducible conjugates [129] ensure that the therapeutic protein is selectively "unlocked" and released only within the tumor microenvironment, maximizing efficacy while minimizing systemic toxicity.

4.2 Metabolic diseases

The management of chronic metabolic disorders is often hindered by the polydispersity and immunogenicity of traditional PEGylated proteins, which can lead to unpredictable blood glucose control. Recombinant fusion proteins, such as XTEN-exendin-4 and PAS-hGH, have surpassed conventional PEGylation by significantly prolonging circulation half-lives while offering superior sequence-level precision [80, 81]. The clinical performance of these bioconjugates is further refined by the adoption of monodisperse PEG. This approach eliminates the molecular weight heterogeneity inherent in polydisperse PEGylated insulin, thereby ensuring a more predictable and stable glucose-lowering effect [33, 34]. Innovation also extends to non-invasive administration. For instance, zwitterionic OPDMA-insulin conjugates facilitate effective transdermal delivery, a challenge for bulky PEGylated insulin, substantially enhancing skin permeation while preserving the protein's essential regulatory function [65].

4.3 Inflammatory diseases

The treatment of inflammatory diseases requires protein-polymer conjugates capable of navigating the enzyme-rich and oxidative microenvironments characteristic of inflamed tissues. Conventional PEGylated proteins, such as PEGylated catalase or SOD, often fail to achieve localized therapy because the chemically inert PEG shell lacks the ability to respond to pathological signals, which may also lead to undesirable polymer accumulation. In contrast, PEEP-modified antioxidant enzymes represent a more sophisticated strategy for managing both acute and chronic inflammation. For instance, PEEP-catalase conjugates [27] leverage their phosphate-based backbones to undergo selective cleavage by phosphodiesterases, which are significantly overexpressed at

inflammatory sites. This mechanism enables the "on-demand" release of active enzymes to neutralize ROS precisely where needed. Similarly, in the management of hyperuricemia and gouty inflammation, zwitterionic PTMAO-uricase conjugates [64] offer a superior hydration shell compared to PEG. This structural advantage more effectively preserves enzymatic activity and significantly extends systemic circulation beyond the duration provided by conventional PEGylation, all while maintaining an immune-silent profile that avoids systemic recognition. Furthermore, trehalose glycopolymer-insulin conjugates have demonstrated enhanced systematic stability, shielding therapeutic proteins from potential environmental fluctuations of chronic inflammatory states while ensuring the preservation of precise bioactivity [56, 57].

4.4 Other diseases

For rare diseases, the primary challenge is the rapid renal clearance of small therapeutic proteins, which necessitates frequent and painful injections. For growth hormone deficiency (GHD), while conventional linear PEGylation has been explored, it often leads to unpredictable pharmacokinetics and reduced efficacy. VRS-317 [81] and hGH-XTEN fusion, have surpassed these limitations by providing a once-monthly therapy option currently being validated in clinical trials, whereas traditional PEGylated versions typically require more frequent dosing. Regarding the treatment of Hepatitis C, although branched Y-shaped PEGASYS [30] reduces renal clearance, it incurs a significant loss of bioactivity due to the massive steric hindrance exerted by the PEG branches. PEG alternatives such as PASylated IFN [79] or comb-like POEGMA-IFN conjugates [21, 22] resolve this issue by expanding the hydrodynamic volume while maintaining flexible architectures that preserve high receptor-binding affinity. Furthermore, in the treatment of acute lymphoblastic leukemia, a rare hematologic malignancy, ASP-ELP fusions [102] create *in situ* drug depots that sustain the release of active ASP for over 500 h. This approach provides a significantly longer therapeutic window and lower immunogenicity than the clinically used PEG-ASP, effectively overcoming the functional ceiling inherent in traditional PEGylation platforms.

5 Challenges

Recently, the emergence of numerous PEG alternatives has greatly expanded the application landscape of long-circulating protein-polymer conjugates. These polymers retain the pharmacokinetic advantages conferred by PEG while overcoming several of its inherent limitations, such as non-biodegradability, potential immunogenicity, and recognition by anti-PEG antibodies. Nevertheless, despite these remarkable advantages, translating such complex conjugates into clinical and industrial applications still faces considerable challenges.

5.1 Manufacture process

Although numerous studies have clearly demonstrated the potential of long-circulating protein-polymer conjugates to improve pharmacokinetics, harsh reaction conditions, such as pH, temperature, and oxygen, during the polymerization of protein-polymer conjugates pose significant challenges for scale-up, quality control, and standardization [151, 152]. While these parameters are easily controlled in small-scale experiments, transitioning to pilot or commercial scale often introduces local concentration gradients and temperature control failures. Traditional PEGylation is limited to the "grafting to" approach, where pre-formed PEG chains are attached to proteins. This method suffers from random attachment, heterogeneous conjugate mixtures, reduced protein activity, low conjugation efficiency, and high cost. In contrast, the "grafting from" strategy introduces polymerization-initiating groups on the protein surface and grows polymers *in situ*, offering high efficiency, precise control, and high product purity, while enabling site-selective protein modification to precisely control the number and location of polymer chains.

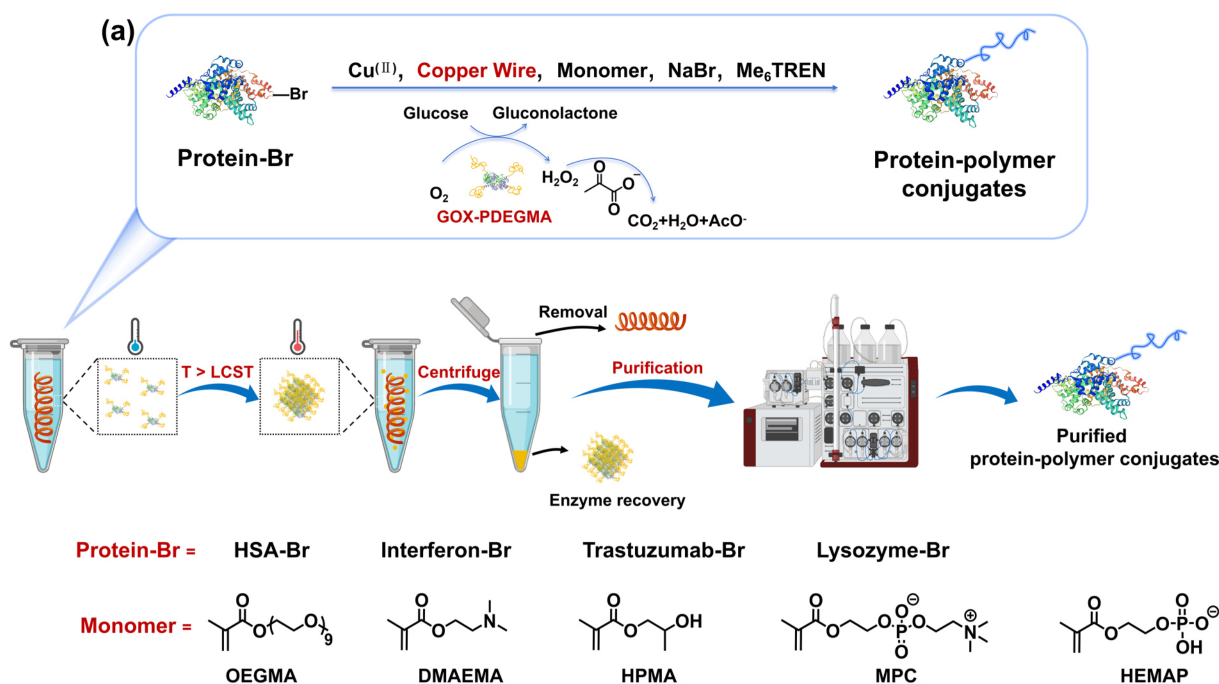
ATRP has been used in the "grafting-from" method to achieve controlled synthesis of protein-polymer conjugates. However, traditional ATRP requires stringent deoxygenation procedures, which severely limit the scalability and throughput of protein-polymer conjugate synthesis, making large-scale application challenging [153, 154]. Oxygen-tolerant polymerization strategies have been developed to overcome these constraints [155]. Our group developed a universal, application-oriented strategy for the synthesis and purification of protein conjugates. This approach employs a glucose oxidase-poly(di(ethylene glycol) methyl ether methacrylate) conjugate (GOX-PDEGMA) as a thermosensitive deoxygenation agent and copper wire as a reducing agent, achieving open-air polymerization with facile control over reaction timing. Moreover, the thermos-responsive property of GOX-PDEGMA allows its recyclability, thereby reducing costs (Fig. 12(a)). The system exhibits excellent scalability, accommodating reaction volumes ranging from 10 μL to 100 mL, and demonstrates universal compatibility with therapeutic proteins such as IFN, Herceptin, and lysozyme (Figs. 12(b)–12(d)) [156].

Besides, the water-assisted polymerization method was developed by Hu et al. to address the challenges of conventional N-carboxyanhydride (NCA) ring-opening polymerization, which typically requires harsh conditions and is difficult to scale up. Specifically, they introduced a mixed acetonitrile/water (ACN/H_2O) solvent system and employed amine initiators under ambient conditions, without the need for strictly anhydrous or oxygen-free environments. This system significantly lowered the reaction energy barrier (by approximately 7.1 kcal/mol) and reduced the polymerization time of proline NCA (ProNCA) from about one week to 2–5 min, yielding well-defined poly(L-proline) (PLP) with controlled number-average molecular weight (Mn) and dispersity (Đ). The mechanism enabled a rapid, controllable, and biocompatible polymerization process without inert protection, thereby greatly enhancing the scalability and industrial potential of the reaction system [150].

Besides oxygen-tolerant and water-assisted polymerization systems, researchers have developed several complementary strategies to improve the scalability and biocompatibility of protein-polymer conjugation, such as photo-controlled polymerizations that enable low-temperature and neutral-pH conditions [157] and continuous-flow or microreactor platforms [158] that provide precise control over reaction kinetics and reproducibility. Overall, simplifying and alleviating reaction constraints during the manufacturing process is indispensable for enhancing scalability and facilitating large-scale production.

5.2 Clinical challenges

Protein-polymer conjugates represent a transformative strategy for



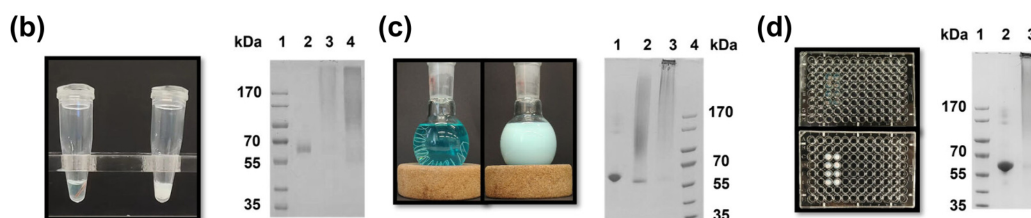


Figure 12 Efficient synthesis of protein-polymer conjugates with open-air fabrication and facile purification driven by the thermoresponsive protein-polymer conjugate. (a) Efficient preparation of protein-polymer conjugates via open-air fabrication and thermoresponsive purification. (b) Photograph and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis showing the polymerization of OEGMA or HPMA, initiated by HSA-Br, in a small reaction volume of 10 μL. (c) Photograph and SDS-PAGE analysis showing the polymerization of HPMA, initiated by HSA-Br, in a large reaction volume of 100 mL. (d) Preparation and SDS-PAGE analysis of HSA-PHPMA in 96-well plates. Reproduced with permission from Ref. [156], © Chen, B. L. et al. Published by American Chemical Society 2025.

enhancing the therapeutic profile of proteins by improving pharmacokinetics, stability, and targeting. Despite their promise, clinical translation hinges on overcoming several interconnected challenges.

Safety profiles of these conjugates require rigorous assessment. The covalent attachment of synthetic polymers can alter the protein's native biocompatibility, potentially inducing aggregation, off-target toxicity, or unforeseen immunogenic reactions [159]. A particular concern is the long-term accumulation of non-biodegradable polymers upon repeated administration. Even for biodegradable systems, the biological safety of their degradation byproducts must be thoroughly evaluated [160].

Therapeutic efficacy is a key trade-off. While polymer conjugation generally prolongs plasma half-life by reducing proteolytic degradation, it can simultaneously sterically mask the active site of the protein, thereby impairing receptor binding and bioactivity. For example, PEGylation can prolong half-life but can reduce enzyme activity and therapeutic response in some leukemia patients due to steric hindrance. Clinical studies have shown that activity is sometimes lower than that of native ASP, requiring dosage adjustments [161]. Achieving an optimal balance requires precise control of conjugation site specificity, polymer molecular weight, and structure [162].

Immunogenicity remains a major obstacle to the clinical translation of protein-polymer conjugates. Both the protein itself and the polymer shell may induce immune responses, leading to accelerated blood clearance and hypersensitivity reactions. The emergence of anti-PEG antibodies demonstrates that even polymers such as PEG, which are considered "invisible", are not completely immunologically inert [163]. Therefore, the key direction for developing the PEG alternatives is to minimize the body's immune response, which is crucial for prolonging the circulation time of therapeutic proteins in the body and avoiding related side effects [164].

5.3 Competition with other strategies

To extend the *in vivo* half-life of therapeutic proteins, strategies based on various biological mechanisms have been developed. Protein-polymer conjugation, the focus of this review, is a classic chemical modification method. By covalently attaching PEG or its alternatives to the protein surface, it significantly increases the drug's hydrodynamic volume, effectively slowing renal filtration. Furthermore, the "steric barrier" formed by the polymer chains partially shields the protein, reducing protease degradation and immune recognition.

Furthermore, gene fusion strategies cleverly exploit the body's endogenous protein recycling pathways. Fc fusion technology fuses the target protein to the Fc fragment of IgG, which specifically

binds to the neonatal Fc receptor (FcRn), which is abundant in endothelial cells. In this intracellular storage pathway, FcRn captures the fusion protein in the acidic endosomal environment and returns it to the cell surface for release into the circulation at physiological pH, completing a "rescue" process and achieving a long half-life comparable to that of antibodies [165, 166]. Albumin fusion follows a similar principle. Because the fusion target, HSA, is itself a key endogenous ligand for FcRn, it can similarly leverage this recycling system to achieve long-term circulation [167].

Transferrin fusion (Tf-fusion) is a biodesign strategy that covalently fuses a therapeutic protein to transferrin (Tf) (usually through genetic fusion to form a single-chain polypeptide). Tf is a plasma glycoprotein that naturally binds and transports iron ions (Fe^{3+}). Its long half-life *in vivo* (7–10 days) is primarily dependent on reversible binding to the transferrin receptor 1 (TfR1) and intracellular recycling [168]. Leveraging this receptor-mediated transport property, Tf-fusion not only effectively prolongs the circulation of therapeutic proteins *in vivo* but also enables cell- or tissue-specific delivery. Furthermore, because its receptor is highly expressed on endothelial cells of the blood-brain barrier, it provides a potential targeted delivery vehicle for therapeutic proteins into the brain [169, 170].

Finally, lipidation represents a minimalist yet highly effective chemical strategy. This method covalently modifies proteins with fatty acid chains (such as palmitate) at specific sites, enabling them to reversibly bind to long-circulating albumin *in vivo*. This allows the modified protein to "hitch a ride" on albumin, indirectly utilizing the FcRn-mediated recycling pathway and significantly extending its retention in the bloodstream [171, 172].

Table 2 systematically compares the mechanisms of action, key advantages, and major limitations of the five aforementioned strategies. The choice of a specific protein modification strategy depends on the ultimate goal of the experiment to achieve optimal research results.

6 Summary and outlook

Protein-polymer conjugates have evolved from a primary goal of extending half-life to a complex strategy for designing multifunctional biotherapeutics. While PEGylation established the paradigm, its limited biodegradability and potential immunogenicity have driven the development of new polymers and strategies aimed at overcoming its inherent limitations. Currently, the design objectives of these conjugates are undergoing a fundamental paradigm shift, from passive, pharmacokinetic-driven goals to active, function-driven strategies. However, the rapidly expanding diversity of polymers, conjugation chemistries, and functional design parameters makes exhaustive experimental screening increasingly time-consuming, costly, and difficult to systematize, highlighting the growing importance of data-driven

Table 2 Competitions between various long-circulating strategies

Strategy	Mechanism	Advantages	Limitations	Clinical applications	Ref.
Protein-polymer conjugates	The conjugation of polymer chains prolongs the protein's half-life by mitigating renal clearance and proteolysis, while simultaneously reducing opsonization and immune recognition.	• Well-established, versatile platform; • Significant half-life extension (days); •Improved stability and solubility; • Reduced immunogenicity of the core protein	• Possible loss of activity; • Anti-PEG antibodies accumulation of non-biodegradable polymers	Pegaspargase (Oncaspar®, PEG–asparaginase, NCT00057862); Pegfilgrastim (Neulasta®)	[151, 173, 174]
Albumin fusion	Genetic fusion to HSA leverages the protein's intrinsic, long circulatory half-life, which is primarily mediated by the FcRn recycling pathway.	• Inherently stable and soluble; • Low immunogenicity risk (human protein); • High plasma concentration	• Very large fusion partner can significantly impair bioactivity; • May hinder receptor binding and tissue access; • Complex production of large fusion proteins	Albiglutide (Tanzeum®, GLP-1-HSA fusion, NCT00838903)	[175, 176]
Fc-fusion	Fusion to the IgG Fc fragment leverages both FcRn-mediated recycling and natural dimerization to achieve prolonged plasma half-life.	• FcRn recycling is a highly efficient, natural mechanism; • Dimerization can enhance target affinity; • Simplifies purification (Protein A/G)	• Large fusion partner (~ 50 kDa) can alter protein pharmacology and hinder tissue penetration; • May elicit effector functions; • Risk of immunogenicity from the Fc domain	Etanercept (Enbrel®, TNF-α inhibitor, NCT00005094); Dulaglutide (Trulicity®, GLP-1-Fc fusion, NCT01644500)	[168, 177]
Transferrin-fusion	Genetic fusion to transferrin harnesses the transferrin receptor (TfR) recycling pathway to achieve prolonged plasma circulation.	• Leverages endogenous Tf biology; • Potential to cross BBB via TfR	• Less mature technology platform; • Fewer approved drugs; • Complex biology due to iron-binding cycle; • Half-life extension can be less predictable than FcRn-based methods.	Tf-Factor IX fusion in hemophilia models (preclinical/early clinical)	[178, 179]
Lipidation	Site-specific attachment of a lipid chain to the protein confers an extended half-life by mediating reversible binding to circulating albumin.	• Minimal increase in molecular size, preserving bioactivity and tissue penetration; • Efficient half-life extension via albumin's FcRn pathway; • Well-defined, site-specific chemistry; • Low immunogenicity risk	• Limited modification sites; • Risk of reduced solubility; • Variability with albumin levels	Liraglutide (Victoza®, GLP-1 analog, NCT00331844); Semaglutide (Ozempic®/Rybelsus®, GLP-1 analog, NCT01595789)	[180, 181]

and machine learning-assisted approaches for guiding rational conjugate design.

In recent years, artificial intelligence (AI) and machine learning (ML) have emerged as supportive tools in the development of protein-polymer conjugates, primarily by enabling systematic analysis of relationships between polymer modification parameters and protein behavior *in vivo*, rather than replacing conventional biochemical experiments [182]. In these systems, polymer molecular weight, topology, conjugation sites, and chain length distribution strongly influence protein stability, biological activity, and pharmacokinetic performance. However, the complex and often non-linear interplay among these parameters limits optimization based solely on empirical approaches, motivating the adoption of data-driven strategies [183]. Machine learning has therefore been applied to datasets of polymer-modified proteins to predict circulation half-life and clearance behavior [184]. For example, analyses of PEGylated proteins have revealed quantitative correlations between polymer molecular weight, modification density, and blood circulation time [185]. More broadly, multiparametric models integrating intrinsic protein properties with polymer features have been explored to compare pharmacokinetic outcomes across conjugation strategies. At present, AI and ML primarily serve to extract design-relevant patterns from experimental data, while structural validation and functional evaluation remain reliant on established biochemical and *in vivo* studies.

While these collaborative efforts across computational modeling and automated bioconjugation are crucial for closing the loop between design and validation, navigating the subsequent regulatory and policy hurdles remains equally vital. The regulatory pathway for these diverse PEG alternatives can be significantly informed by the extensive precedents established by the 34+ approved PEGylated drugs. However, regulatory bodies now demand more granular data on the unique risks of novel polymers, such as the potential for immunogenicity. Furthermore, safety assessments should leverage cross-industry benchmarks from the food and cosmetic sectors as well as materials with established regulatory approval in medical devices, such as PMPC and HA, to streamline toxicology evaluations. Finally, clinical application must be underpinned by stringent good manufacturing practice (GMP) compliance tailored to specific manufacturing scenarios. Regulatory policies must therefore evolve to define precise quality-by-design (QbD) metrics for polydispersity and conjugation efficiency. Addressing these technological and policy challenges is critical to ensuring that the next generation of conjugates can successfully transition from rational design to effective, long-acting clinical therapeutics.

Based on the above analysis of current design strategies and translational challenges in protein-polymer conjugates, future research should focus on addressing several key technical bottlenecks that limit their clinical development. A primary challenge lies in structural heterogeneity, as broad polymer dispersity and poorly controlled conjugation stoichiometry complicate structure-activity relationship analysis, pharmacokinetic predictability, and regulatory evaluation. Continued advances in site-specific conjugation chemistries, together with the development of monodisperse or sequence-defined polymers, will be essential for improving batch-to-batch consistency and enabling more rational, reproducible conjugate design. In parallel, scalable manufacturing and robust quality control remain major obstacles for next-generation protein-polymer conjugates, particularly those employing complex architectures or biodegradable polymer backbones. Future efforts should prioritize synthetic routes compatible with GMP production and establish clear QbD criteria, including acceptable limits for polymer dispersity, conjugation efficiency, and long-term stability. Addressing these interconnected challenges will be critical for translating increasingly sophisticated protein-polymer conjugates from rational design concepts into clinically reliable and commercially viable biotherapeutics.

Overall, this review summarizes recent advances in protein-polymer conjugates with an emphasis on long-circulation principles and emerging strategies beyond conventional PEGylation. We discuss how polymer architecture, and characterization influence circulation half-life, biological activity, and *in vivo* behavior. Particular attention is given to novel long-circulation strategies of protein-polymer conjugates. Together, this review provides an integrated framework to support the rational design and translational development of long-circulating protein therapeutics.

Data availability

Not applicable.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

S. L.: Conceptualization, data curation, visualization, writing – original draft. X. L. D: Project administration, supervision, funding acquisition. X. Y. L.: Conceptualization, writing – original draft, writing – review and editing, visualization, project administration, supervision, funding acquisition. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

The authors acknowledge the use of OpenAI's ChatGPT-4 for grammar checks. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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