



Review

Structure–function insights of poly(β -amino ester)s for efficient nucleic acid deliveryZihnil A.I. Mazrad^{a,b,c,*}, Seema Agarwal^{a,d,*}^a Advanced Sustainable Polymers, Macromolecular Chemistry II, University of Bayreuth, Bayreuth 95440, Bayern, Germany^b Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia^c Materials Science and Engineering, Monash University, Clayton, VIC 3800, Australia^d Bavarian Polymer Institute, University of Bayreuth 95440 Bayreuth, Germany

ARTICLE INFO

Keywords:

PBAEs
Gene delivery
Stimuli-responsive
Polymer design
Non-viral vector

ABSTRACT

Nucleic-acid therapeutics hold great promise but face delivery barriers such as enzymatic degradation, poor cellular uptake, and rapid clearance. Poly(β -amino esters) (PBAEs) have emerged as a highly versatile and modular platform for non-viral gene delivery, addressing many limitations of conventional cationic polymers such as polyethyleneimine (PEI), including cytotoxicity, poor biodegradability, and lack of stimuli-responsiveness. Their tunable chemical architecture, comprising hydrolyzable ester backbones, protonatable amines, and functionalizable end-groups, enables precise control over nucleic acid condensation, endosomal escape, intracellular trafficking, and cytosolic release. Rational modification of backbone structure, branching, end-groups, and stimuli-responsive motifs has enhanced transfection efficiency, stability, and targeting. This review summarizes recent advances in “smart” PBAEs that enable stimuli-responsive, targeted delivery of nucleic acids. We discuss design strategies (linear $\rightarrow$ branched $\rightarrow$ hyperbranched architectures), functionalization including PEGylation, zwitterions, and lipid conjugation, and incorporation of stimuli-responsive motifs such as pH, redox, reactive oxygen species (ROS), and light. Hybrid systems, including in lipid nanoparticles (LNPs) and hydrogel composites, are examined for improved stability, organ selectivity, and therapeutic efficacy. Finally, we outline current challenges, including scalability, long-term safety, and formulation stability, and propose future directions to enhance clinical translation. PBAEs thus offer a highly adaptable platform for next-generation, safe, and precisely controlled gene-based therapeutics.

1. Introduction

Nucleic acid-based therapeutics, including messenger RNA (mRNA), small interfering RNA (siRNA), plasmid DNA (pDNA), antisense oligonucleotides, and gene-editing components such as CRISPR-Cas9, represent a transformative class of medicines capable of addressing diseases at their genetic root [1]. Unlike conventional small-molecule drugs or protein-based biologics, these nucleic acids offer transient, tunable, and programmable modulation of gene expression offering new treatment paradigms for cancer, genetic disorders, infectious diseases, and autoimmune conditions [1]. Despite their therapeutic potential, clinical translation of nucleic acids remains constrained by substantial delivery barriers. Naked nucleic acids are inherently unstable in physiological environments due to enzymatic degradation [2], poor cell permeability [3], and rapid renal clearance [4]. As a result, the development of safe,

efficient, and targeted delivery systems is urgently required to realizing the full promise of nucleic acid therapeutics [5,6].

Various types of (cationic) formulation materials, including natural and synthetic lipids and polymers, have been explored to facilitate complexation of the nucleic acid payload by electrostatic interaction with positively charged excipients into nanoparticles that have an appropriate size to be internalized by cells and to guide intracellular trafficking of exogenous nucleic acids [7,8]. Lipid-based drug delivery systems, particularly lipid nanoparticles (LNPs), are currently the gold standard for gene delivery due to their high transfection efficiency and established clinical use [9,10]. LNPs typically comprise ionizable lipids, cholesterol, PEG-lipids, helper lipids, and a therapeutic payload (e.g., mRNA, siRNA, or DNA) [11]. Despite these advances, several limitations remain. Many lipid components exhibit limited biodegradability, which can contribute to immune activation and potential long-term toxicity

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<https://doi.org/10.1016/j.eurpolymj.2026.114893>

Received 22 April 2026; Received in revised form 10 June 2026; Accepted 12 June 2026

Available online 12 June 2026

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[10]. In addition, LNPs often display restricted tissue tropism, with predominant accumulation in the liver, alongside challenges related to complex manufacturing, formulation-dependent stability, and suboptimal endosomal escape efficiency [12]. Furthermore, lipid-based carriers frequently require precise compositional optimization for each specific nucleic acid cargo, limiting their generalizability across therapeutic applications. Practical challenges also persist, as current LNP-based mRNA formulations require stringent cold-chain storage conditions, resulting in logistical and economic constraints [13]. These limitations have driven growing interest in alternative non-viral delivery systems, particularly polymer-based vectors, which offer enhanced chemical tunability, structural diversity, and opportunities for modular design. In parallel, hybrid lipid-polymer delivery systems have emerged as a promising strategy that combines the favorable biophysical properties of lipids [14,15] with the versatility of polymers, enabling improved stability, biodistribution, and delivery efficiency.

Polymers have emerged as promising alternatives to lipid-based carriers for nucleic acid delivery, offering advantages such as structural diversity, synthetic adaptability, and enhanced stability [16]. These features are particularly beneficial for forming stable nucleic acid-polymer complexes. The effectiveness of polymeric carriers is largely governed by tunable properties, including charge density, pKa, molecular weight, branching architecture, biodegradability, and interactions with cellular membranes, which can be precisely engineered to optimize delivery performance [17]. Polyethylenimine (PEI) has long served as the benchmark polymer for nucleic acid delivery owing to its high cationic charge density, which enables efficient condensation of nucleic acids into nanoscale complexes [18,19]. However, despite its strong transfection efficiency, PEI is non-biodegradable and induces significant cytotoxicity via necrosis and apoptosis [16,20]. High-molecular-weight PEI exhibits particularly severe toxicity due to its excessive positive charge, which disrupts cellular membranes and promotes nonspecific interactions with serum proteins [21]. In addition, native PEI lacks intrinsic responsiveness to biological stimuli, limiting its capacity for controlled or targeted delivery in complex physiological environments [16,19]. Importantly, recent advances have mitigated many of these limitations. Strategies such as the use of low-molecular-weight PEI, incorporation of biodegradable linkages, and development of modified or hybrid PEI-based formulations have demonstrated improved biocompatibility while maintaining effective nucleic acid delivery, including for RNA therapeutics [22]. Despite these advances, key challenges remain in balancing delivery efficiency, toxicity, and tissue specificity across polymeric systems.

In contrast, poly(β -amino ester)s (PBAEs) overcome many of these limitations by providing efficient gene delivery through protonatable amines that encapsulate nucleic acids and hydrolyzable ester bonds that promote timely release [23–29]. Their structural flexibility supports a wide range of functional modifications, from stimuli-responsiveness to ligand conjugation, enabling context-specific delivery, enhanced transfection efficiency, and reduced cytotoxicity [23,24,30–32]. Importantly, their biodegradable nature makes them suitable for repeated dosing with minimal long-term toxicity, making them highly attractive non-viral vectors for delivering a wide range of nucleic acids, including pDNA, siRNA, and mRNA.

This review provides a comprehensive overview of recent advances in the design, synthesis, and application of “smart” PBAE-based delivery systems for nucleic acid therapeutics, with a particular focus on structure-function relationships, responsive behavior, and emerging biomedical applications.

2. Overview of PBAEs

PBAEs are a class of biodegradable, cationic polymers first reported in 1983 and later advanced by Langer's group in the early 2000s for gene delivery applications [33].

PBAEs are synthesized via straightforward Michael addition

chemistry (Fig. 1), allowing rapid production without byproducts [32,34]. Their synthetic flexibility has enabled the generation of high-throughput combinatorial libraries encompassing hundreds of structurally diverse polymers, with structural variations dictated by monomer selection, reaction conditions (e.g., temperature and solvent), and end-group composition, all of which critically affect physicochemical properties and biological performance [35]. Structure modifications to the backbone, such as varying carbon chain length or introducing architectures (linear, branched, hyperbranched), [25,36] as well as incorporating stimuli-responsive linkers (e.g., ester, disulfides or nitrobenzene groups), further expand the functional scope of PBAEs. Additionally, side chains bearing hydroxyl groups enhance water solubility, reduce surface charge, and minimize cytotoxicity, further optimizing cellular compatibility [24,30,35]. These characteristics enable the rational design of “smart” PBAE-based nucleic acid delivery applications that respond to physiological cues, such as pH, redox environments, or reactive oxygen species (ROS), making them adaptable platforms for a wide range of clinical applications. Moreover, PBAEs possess protonatable amine groups that enable strong electrostatic interactions with negatively charged nucleic acids, forming polyplexes that facilitate cellular uptake and protect nucleic acid from degradation. Their ester-containing backbone promotes hydrolysis under physiological conditions, generating non-toxic byproducts that can be readily cleared through normal metabolic and excretory pathways. Furthermore, the polymers exhibit pH-responsive solubility and a proton sponge effect that enhances endosomal escape, key for efficient cytosolic delivery [37,38]. Although the proton sponge effect is the most commonly proposed mechanism for endosomal escape in PBAE systems due to the presence of amine moieties, recent studies suggest that lipophilic PBAEs may also promote endo-lysosomal escape through membrane fusion and pore formation. In this mechanism, lipophilic PBAEs destabilise endo-lysosomal membranes and interact with anionic lipids, leading to membrane inversion or disruption [39]. In addition, cationic PBAEs can form charge-neutral ion pairs with anionic membrane lipids, facilitating membrane destabilisation and the release of therapeutic cargo into the cytoplasm. This fusion process is driven by electrostatic interactions between positively charged polyplexes and negatively charged cellular or endo-lysosomal membranes, resulting in local lipid bilayer destabilisation and enhanced cytosolic delivery [40].

Building on this conceptual framework, the next section delves into the molecular architecture of PBAEs, the influence of backbone composition, end-group functionalization, and polymer topology on nucleic-acid complexation and release behaviour.

3. Design Strategies for Functional “smart” PBAEs

The performance of PBAEs in nucleic acid delivery hinges on the careful design of their molecular architecture. Because PBAEs are synthesized via modular, step-growth polymerization reactions (e.g., Michael addition), they allow for extensive chemical customization. This has led to the development of “smart” PBAE systems that respond to physiological or external stimuli, optimize nucleic acid release, enhance endosomal escape, and improve tissue targeting.

3.1. Structural Modifications of PBAEs

The chemical versatility of PBAEs enables precise manipulation of their backbone architecture, side chains, and terminal groups to optimize delivery performance (Fig. 1). These structural modifications govern critical features such as polyplex stability, nucleic acid encapsulation, and transfection efficiency. Table 1 provides a summary of the most widely explored PBAE design strategies, highlighting their structural features, key properties, and biological outcomes.

Below are key structural strategies used to enhance gene delivery:

a. Linear, Branched, and Hyperbranched Architectures

The molecular architecture of PBAE plays a central role in dictating

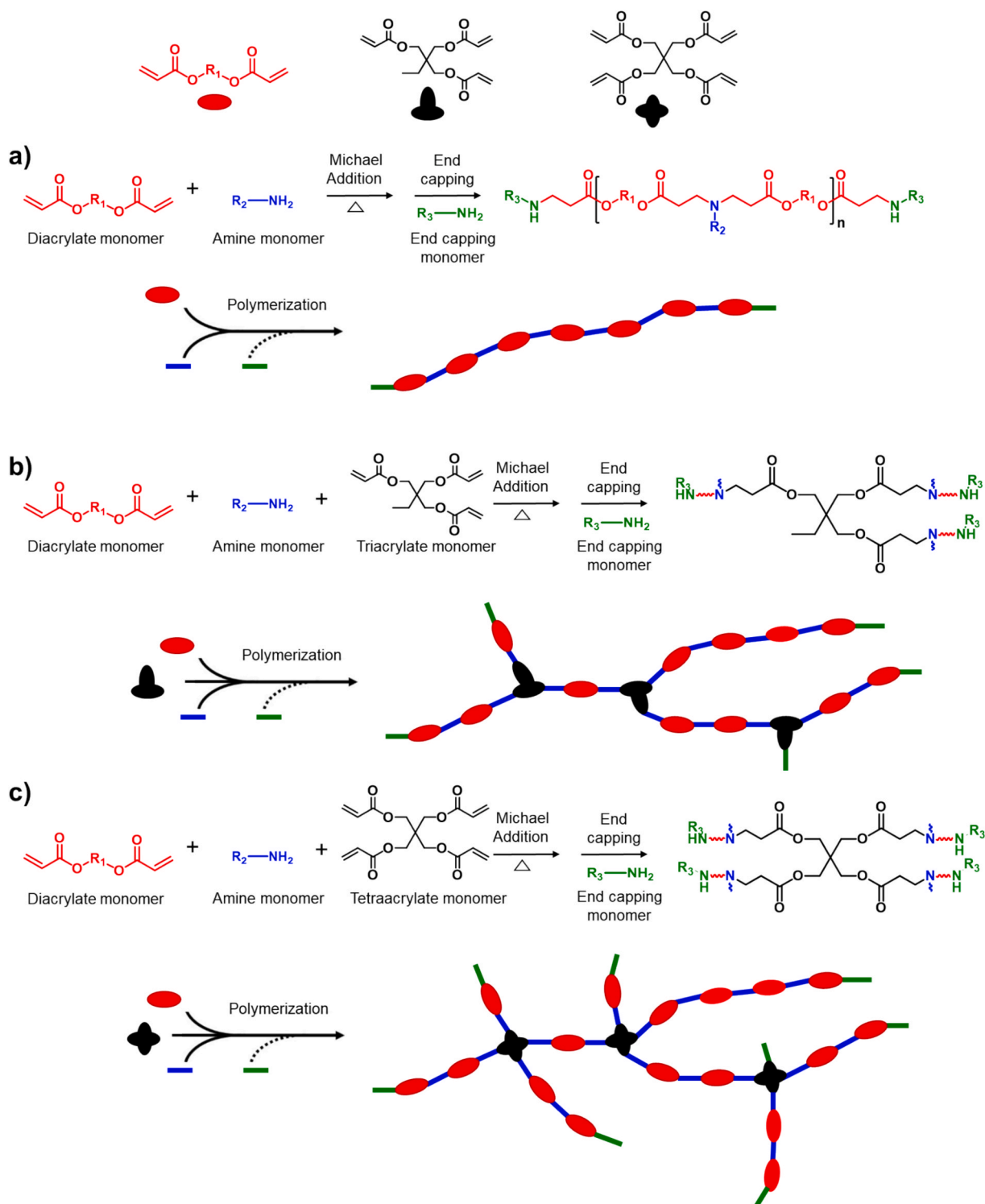


Fig. 1. Synthesis scheme of PBAEs through Michael addition chemistry. a) Linear PBAEs and b) branched PBAEs through triacrylate and c) tetraacrylate monomers. Linear PBAEs contain a backbone synthesized via polymerization of a diacrylate monomer and amine side chain. Branched PBAE backbones contain a ratio of diacrylate and triacrylate monomers or diacrylate and tetraacrylate monomers polymerized with an amine side chain. Adapted from [41].

their physicochemical properties and gene delivery performance. Linear PBAEs, typically synthesized from diacrylate and diamine monomers, are the simplest form and have been widely studied as non-viral vectors due to their biodegradability and ease of synthesis. Their favorable features include synthetic simplicity, hydrolytic degradability, and lower cytotoxicity relative to PEI [32,42–46]. However, their linear topology results in relatively low charge density and protonatable amine

availability, which limits buffering capacity and weakens endosomal escape, and reduced protection against serum nucleases [47], leading to modest transfection efficiencies in vitro and in vivo [44,48,49]. These limitations motivated the development of branched and hyperbranched variants that enhance multivalent charge presentation.

Branched PBAEs are synthesized by incorporating tri-functional monomers, such as triacrylates, via Michael-type addition (Fig. 1b)

Table 1
Summary of PBAE architectures and chemical modifications with biophysicochemical properties.

No	PBAE architecture/ topology	Chemical modification	Apparent pKa / buffering capacity	Nucleic acid Cargo	Particle size after complexation	Zeta potential after complexation	Relative transfection efficiency	Target Organ/tissue	Safety Notes	Ref
1	Linear	Amine end-group modification	pH 6.0 to 6.5	pDNA, mRNA	90–200 nm	−10 to − 11.02 mV	Higher than Lipofectamine in mouse embryonic fibroblasts	Intraperitoneal injection: stomach, liver, lung, spleen, and kidney; higher expression than DNA alone	Increased amine content increased toxicity; cell viability decreased to below 80%	[47,92]
2	Linear	Methylpiperazine end- group modification	pH 5.1 to 7.4	pDNA	~150 nm	+21 to + 29 mV	Higher than PEI in COS-7 cells	Not reported	>90% cell viability	[44],
3	Branched with pentaerythritol tetraacrylate	Methylpiperazine end- group modification	Not reported	pDNA, CRISPR/ Cas9	~200 nm	~+30 mV	100% cellular uptake and 76% transfection in CFBE41o- cells; 12-fold higher than Lipofectamine	Lung	4-fold higher cell viability than Lipofectamine	[55]
4	Branched with trimethylolpropane triacylate	Amine end-group modification	Not reported	mRNA	100–300 nm	+15 to + 60 mV	All amine end-group variants showed high transfection efficiency in HeLa and SiHa cells	End-group-dependent organ distribution, mainly lung, spleen, and liver	No liver toxicity based on ALT/AST levels; no pathological changes observed	[53,56]
5	Linear	Lipid modification and hybridisation with PEG polymer	Not reported	mRNA and DNA	~160 nm	+26.6 to + 23.8 mV	Enhanced pDNA and mRNA transfection efficiency in vitro and in vivo compared with unmodified PNP/PBAE formulations	Spleen targeting for immunisation	Increased positive charge in hybrid nanoparticles increased toxicity	[15]
6	Linear	Fluorinated end-group modification	Not reported	DNA	~150 nm; smaller particles observed when the polymer/ DNA ratio was increased to 50	~+20 mV	Fluorination increased transfection efficiency in HEK-293 T cells	Increased lung targeting	No liver toxicity based on ALT/AST levels	[64]
7	Linear	Incorporation into standard LNP formulation	Not reported	mRNA	<200 nm	−10 to 0 mV	Optimal transfection efficiency of PBAE-LNPs was comparable to standard LNPs	Liver and Lung	Terminal amino groups showed noticeable cytotoxicity in vitro and in vivo	[67,66]
8	Branched with trimethylolpropane triacylate	Aminolysis modification	Not reported	mRNA	~70–400 nm, depending on monomer combination	+16 to + 20 mV	Higher aminolysis degree produced transfection efficiency comparable to MC3-LNPs	Intravenous injection: mainly spleen	No in vivo toxicity reported	[79,80]
9	Linear	Spermine modification	Not reported	SiRNA	<100 nm	Positive; exact value not reported	Spermine increased GFP knockdown efficiency in H1299 cells	Lung cancer	Increasing N/P ratio increased toxicity due to higher positive charge	[83]
10	Linear	Lipid and spermine conjugation	Not reported	SiRNA	<100 nm	Positive range, depending on N/ P ratio	Lipid PBAEs polyplex with spermine achieve more than 90% GFP knockdown in H1299/eGFP cells	Not reported	Lipid modification caused toxicity similar to hyperbranched PEI; biocompatible up to 50 µg/mL	[85,84]

[41,42]. This structural diversification significantly increases local charge density and cationic site multiplicity, which improves nucleic acid condensation into compact polyplexes, enhances cellular uptake, and facilitates endosomal escape via the proton sponge effect [25,32,48,50]. Specifically, abundant protonatable amine groups buffer endosomal acidification, promoting proton and chloride ion influx followed by osmotic water uptake. This process induces endosomal swelling and membrane destabilization, ultimately facilitating cytosolic release of the polyplexes. This was explored by Dezhong Zhou, et al. [42], who did the comparative studies, and found that branched PBAEs exhibit markedly higher transfection efficiencies than their linear analogues and widely used commercial reagents such as PEI, SuperFect, and Lipofectamine 2000, suggesting that increasing multivalent charge interactions enhances cellular uptake and endosomal escape.

More recent work has expanded this concept through the design of branched PBAE libraries incorporating trifunctional and tetrafunctional branching (Fig. 1c) within equivalent PBAE backbones. Optimal

transfection was observed with moderately branched (~30%) structures, which enabled complete DNA encapsulation, rapid nanoparticle uptake, and robust gene expression at low polymer and DNA doses [41]. Importantly, the study also demonstrated a clear relationship between branching degree and cytotoxicity, where increasing branching degree from 30% to 60% resulted in higher toxicity. In addition, Wang et al. [51] identified branch unit distribution (BUD) as a critical determinant of transfection capability. It is demonstrated that highly branched PBAEs with a more uniform BUD, characterized by a higher proportion of branch units in low-molecular-weight fractions and a lower proportion in high-molecular-weight fractions, exhibited better gene delivery performance compared to linear polycation polymer as well as commercial reagents (e.g., Lipofectamine 3000 (Lipo3000), jetPEI, and Xfect). These findings underscore the importance of structural control in advancing branched PBAEs as high-performance gene delivery vectors. Moreover, sustainable synthetic strategies have also been explored. Özgümüş and colleagues [52] reported the first microwave-assisted

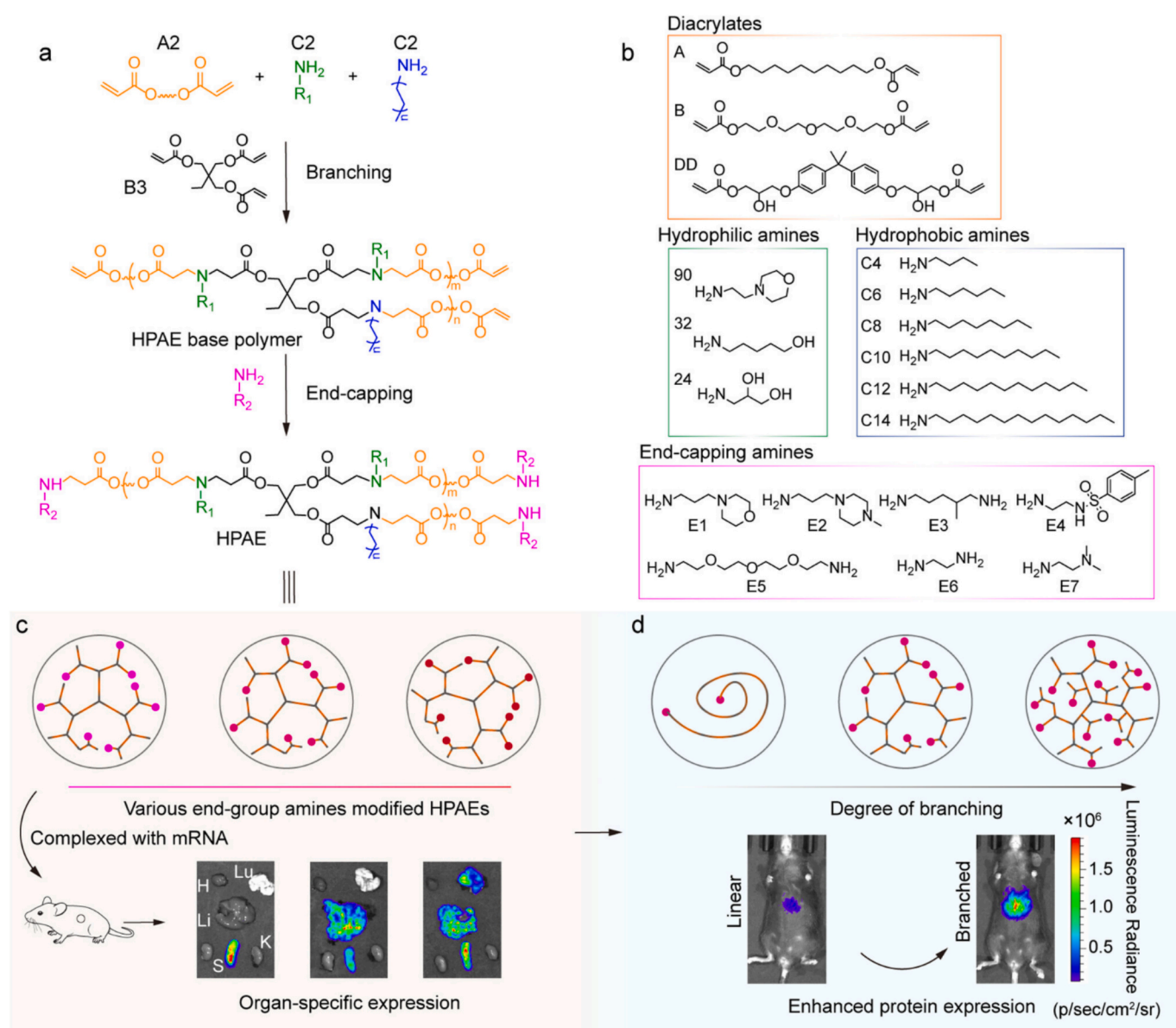


Fig. 2. Optimization of HPBAEs architecture. a) The synthetic route and b) chemical structures of monomers for HPBAE synthesis. c) Schematic illustration of HPBAEs with varying terminal groups for organ-specific mRNA delivery. d) The branched topological structure of HPBAEs significantly enhances mRNA expression in vivo. Schematic illustration of increasing degrees of branching, from linear to highly branched structures, and their impact on mRNA delivery efficiency, as demonstrated by enhanced in vivo protein expression with increasing branching density. Reproduced from [53] with permission Copyright © 2024 American Chemical Society.

synthesis of branched PBAEs, which preserved transfection efficiency while reducing reaction time from 48 h to 10 min and energy consumption from 1.5 kWh to 0.032 kWh, providing a greener and more scalable route to high-performance polymers.

Building on these developments, further increases in branching complexity have led to the emergence of hyperbranched PBAEs (HPBAEs), which exhibit extensive branching and a globular, dendrimer-like topology. Compared with linear or lightly branched analogues, HPBAEs offer increased charge multivalence, improved serum stability, and reduced aggregation. Their dense architecture also provides a high degree of functional group tunability, enabling terminal modifications for organ-specific targeting [25,53–56]. For example, Yong and colleagues developed a combinatorial library of 219 HPBAEs with diverse chemical compositions and topological structures in an “A2+B3+C2” Michael addition reaction platform through a one-pot copolymerization of a branching monomer TMPTA, a functional diacrylate (A, B, or DD), a hydrophilic amine (90, 32, or 24), and a hydrophobic amine (C4–C14). Subsequently, the polymers were end-capped with a third amine (E1–E7) (Fig. 2) to form three-dimensional, highly branched polymer networks, enabling organ-selective mRNA delivery through precise control of chemical composition and topology [53]. By tuning the relative ratios of A2, B3, and C2 components, the degree of branching and overall polymer architecture can be systematically controlled, thereby modulating delivery performance. The extent of branching can be tuned by adjusting the stoichiometric ratios of A2, B3, and C2 components, thereby modulating polymer structure and physicochemical properties [53]. Overall, the branched structures of HPBAEs conferred polyplexes with compact size, higher zeta potential, improved endosomal escape ability, and rapid mRNA release, enhancing serum resistance and achieving high transfection efficacy both in vitro and in vivo by up to 20.0-fold compared to linear polycationic polymer. However, despite the diverse hydrophilic and hydrophobic amines present in these HPBAEs library, no clear correlation was observed between transfection efficiency and the types of amines used but mostly by the end-group functionalities. For example, 30% branching degree showed optimal transfection with E6 end group around 4 to 9-fold compared to the linear counterparts and significantly reduced polyplex size. Importantly, terminal amine chemistry was shown to dictate organ selectivity: morpholine favored liver targeting, ethylenediamine enabled spleen delivery, and methylpentane facilitated mRNA expression in liver, spleen, and lungs simultaneously [53,55]. This work provides a comprehensive structure–activity framework for HPBAEs, elucidating how polymer topology and terminal chemistry govern organ selectivity and in vivo mRNA delivery, and establishing design principles for next-generation organ-targeted nucleic acid therapeutics [53].

Looking ahead, precise control over branching degree, branch unit distribution, and terminal functionality, combined with scalable and sustainable synthesis strategies, will be central to advancing PBAEs as next-generation, clinically translatable gene delivery platforms.

b. PEGylation and Zwitterionic modifications

Poly(ethylene glycol) (PEG) functionalization (PEGylation) is widely employed across a range of non-viral delivery systems, including LNPs, polymeric carriers, and hybrid lipid–polymer platforms, to improve colloidal stability, reduce nonspecific protein adsorption, and prolong systemic circulation [57]. PEGylation, the covalent attachment of PEG, is a widely employed strategy to shield cationic surfaces, reduce aggregation in biological fluids, and minimize immune recognition [58].

Surface unmodified PBAE/DNA nanoparticles often aggregate under physiological conditions, resulting in reduced stability and poor transfection efficiency, particularly in serum-containing environments and following intravenous administration [59]. In contrast, PEGylated PBAEs exhibit enhanced colloidal stability and reduced nonspecific protein adsorption due to steric shielding of the cationic surface, leading to improved circulation behavior and in vivo performance. This balance between stability and biological activity is evident across multiple studies. For example, Kim *et al.* employed a PEG-thiol molecule to end-

cap acrylate PBAEs through thiol–ene Michael addition reaction, resulting redox stimuli responsiveness. Demonstrated that DNA complexed with this PBAEs-PEG improved nanoparticle diffusion through brain tissue and enhanced tumor penetration compared to non-PEGylated counterparts in an orthotopic xenograft model of human glioblastoma in mice [60]. Similarly, PEG incorporation was shown to reduce the positive surface charge of PBAE nanoparticles, and improves serum stability, but excessive PEG density may compromise intracellular delivery efficiency. These findings highlight that PEGylation must be carefully optimized rather than maximized [23,61–63]. Additionally, fluorinated amphiphilic PBAE nanoparticles end-capped with PEG have been developed for targeted pulmonary gene delivery [64]. The combined incorporation of PEG end groups and fluorinated ligands enhances transfection efficiency by improving nanoparticle stability and cellular interactions. The optimized formulation achieved endothelial targeting efficiencies exceeding 90%, demonstrating the synergistic effect of PEGylation and fluorination.

From a structure–property–function perspective, PEG overall structure governs nanoparticle behavior. Hybrid systems further illustrate how the PBAE behaviour can further be modulated. Beyond simple PEGylation, hybrid nanoparticle systems have further expanded the utility of PEG in the PBAE delivery system. Lipid-modified PBAEs (LPBAEs), generated via esterification of hydroxyl-bearing side chains with variety of lipid acids (Fig. 3a), have been developed to introduce hydrophobic alkyl functionalities that enhance transfection potency and improve nanoparticle stability under physiological conditions [65]. When combined with PEG–PLGA, these polymers self-assemble into well-defined nanostructures capable of efficiently encapsulating DNA and mRNA nanovaccines with size around 160 nm (Fig. 3b–c). In these systems, PEG improved DNA and mRNA delivery efficiency both in vitro and in vivo, while PLGA contributed to sustained gene release and exceptional long-term stability, maintaining transfection efficacy after storage for at least 12 months at -20°C following lyophilisation [15]. Similarly, PEGylated PBAEs have been incorporated into LNPs (PBAE-PEG/LNPs) to form hybrid system for lung cell–specific gene delivery, resulting in enhanced mRNA delivery to lung epithelial cells, including alveolar type II cells, compared with non-PEGylated formulations [66,67].

Overall, PEGylation enhances nanoparticle stability, circulation time, and targeting efficiency, while its integration into more complex delivery systems, such as LNPs, further improves serum resistance and colloidal stability [23,28,68]. These advances highlight the importance of rational PEGylation strategies in optimizing the pharmacokinetics and therapeutic efficacy of PBAE-based nucleic acid delivery systems.

However, repeated administration of PEGylated nanoparticles can trigger immune responses, including anti-PEG antibody generation, hypersensitivity reactions, and accelerated blood clearance, potentially reducing efficacy and raising safety concerns [69–71]. Alternative hydrophilic polymers, such as poly(zwitterions) (e.g., phosphorylcholine- or sulfobetaine-based materials), and polysarcosine (PSar), provide stealth properties without eliciting anti-PEG immune responses; polysaccharides such as hyaluronic acid and dextran, provide biocompatibility and targeting potential; and polypeptide- or protein-based coatings can enhance biodegradability and cellular interactions. In addition, stimuli-responsive or cleavable PEG and PEG conjugates have been developed to retain the circulation benefits of PEG while enabling its shedding at the target site to improve cellular uptake [72,73].

These potentials have motivated the development of potential PEG alternatives in PBAE-based gene delivery systems. For example, pSar, a nonionic polypeptoid with excellent biocompatibility, has been conjugated to PBAEs to form pSar–PBAE–pSar triblock copolymers [74]. Mixed formulations of polysarcosinyllated PBAEs enable stable nanoparticle formation ($<200\text{ nm}$), efficient self-amplifying mRNA encapsulation, enhanced delivery in 3D colorectal cancer organoids, and reduced cytotoxicity compared with unmodified PBAEs [74]. These results demonstrate that zwitterionic and PSar-based modifications can

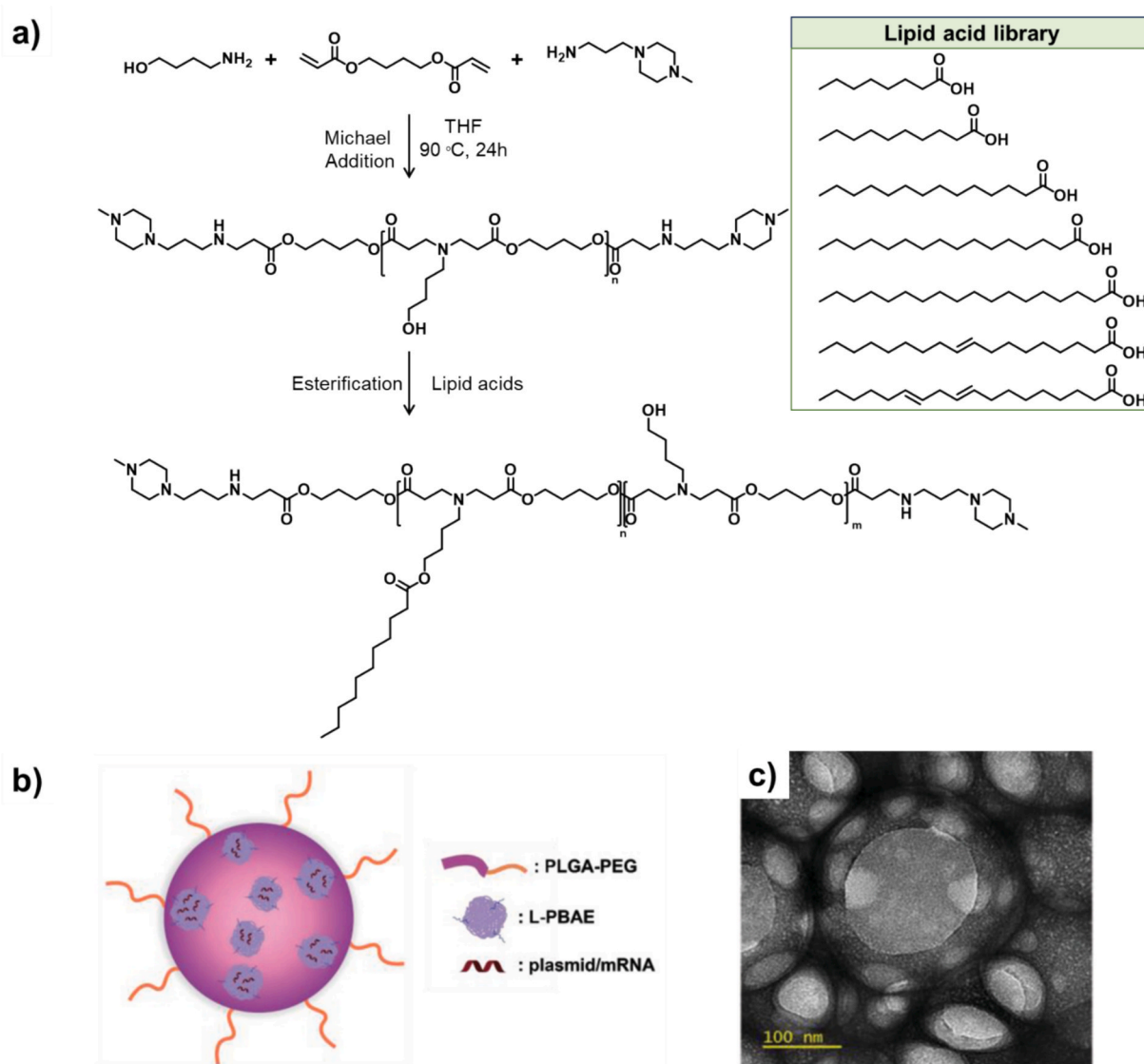


Fig. 3. Lipid-PBAEs hybrid “particle-in-particle” gene delivery platform. a) The chemical synthesis route of L-PBAEs under CALB enzyme-assisted esterification using library of lipid acids. b) A general schematic of L-PBAE and PEG-PLGA hybrid nanoparticles consisting of three components: i) L-PBAE/gene nanocomplexes embedded throughout PLGA-PEG nanoparticles, ii) The PLGA layer which provides sustained release and protection of embedded L-PBAE/gene nanocomplexes, iii) An outer PEG surface. c) Representative TEM image of the hybrid system. Reproduced from [15] with permission. Copyright © 2022 Wiley.

achieve PEG-like stealth properties while maintaining intracellular release efficiency, representing a promising strategy for the next generation of PBAE-based vectors in gene therapeutics. However, research on alternative polymers in the PBAE context is still in its infancy. We believe that designing next-generation PBAE gene vectors with novel hydrophilic coronas is a critical direction for the field. Such PEG-free stealth coatings could combine high delivery efficiency with enhanced safety. In summary, developing and optimizing new biocompatible polymers as PEG substitutes, with tunable pharmacokinetics and efficient intracellular release, is a promising path to improve the performance of gene delivery systems.

Collectively, these studies establish that PEGylation is not merely a stabilizing modification but a critical design parameter that regulates the trade-off between nanoparticle stability, circulation, and intracellular delivery. Rational optimization of PEG characteristics or even finding their alternative is therefore essential for maximizing the therapeutic efficacy of PBAE-based gene delivery systems.

c. Post polymerization modification

Functionalization of PBAEs with hydrophobic chains and diverse

functional groups has emerged as a powerful strategy to enhance gene delivery by modulating key physicochemical properties. Compared with unmodified PBAEs, these modifications can simultaneously improve nucleic acid condensation, nanoparticle stability, cellular uptake, and endosomal escape, although their effects depend strongly on the type and extent of functionalization.

For instance, incorporation of hydrophobic alkyl chains (e.g., C6 and C8) into PBAE side chain increases polymer hydrophobicity, which enhances intermolecular interactions and promotes the formation of more compact and stable polyplexes. This structural change improves cellular membrane interactions and uptake, resulting in high gene silencing efficiency (>80% siRNA knockdown) while maintaining low cytotoxicity.

[35,75–77] However, excessive hydrophobicity may lead to aggregation or reduced solubility, indicating that an optimal hydrophobic balance is required. In contrast, functionalization with polar or bioactive groups, such as silane or amino acid moieties, enhances transfection through different mechanisms, including improved biocompatibility, specific cellular interactions, and potentially enhanced intracellular interaction [78].

More recently, increasing cationic functionality within HPBAEs without altering branching degree has provided further insight into structure–property relationships (Fig. 4) [79,80]. Introducing additional amine groups increases charge density, leading to higher zeta potential, smaller nanoparticle size, and stronger nucleic acid binding. These changes enhance cellular uptake and transfection efficiency, as demonstrated by a 2.41-fold increase in mRNA expression relative to parent HPBAEs [80]. However, similar to trends observed in branched systems, excessive charge density may strengthen polymer–nucleic acid interactions to the extent that cytotoxicity is increased, again emphasizing the need to balance binding strength with biocompatibility.

Synthetic methodology further influences these structure–property–function relationships. Although Michael addition are commonly used to synthesize PBAEs, the resulting polymers often exhibit polydisperse molecular weight distributions, which complicates structure–property correlations and limits reproducibility [81]. In contrast, precisely defined PBAEs with controlled molecular weight, sequence, and topology exhibit more predictable behavior and enable clearer interpretation of how structural modifications influence delivery outcomes. To address this, tertiary amine and hydroxyl groups have been incorporated into the repeating units of precisely synthesized polyesters, enabling the generation of precise PBAEs with well-defined molecular weights, sequences, end groups, and topologies.[24] Furthermore, post-modification of these precise PBAEs with a guanidine end group markedly enhances their ability to encapsulate and deliver plasmid DNA at an exceptionally low molecular weight (~1 kDa). Such high transfection efficiency at low molecular weight represents a distinct advantage over conventional random PBAEs, which typically require higher molecular weights (6–8 kDa) to achieve similar activity, likely due to the more complex degradation pathways and immunogenic responses associated with high-molecular-weight random PBAEs.[24] These findings establish functional group engineering as a key design strategy for tuning PBAE performance across diverse delivery applications.

d. Spermine modification

In addition to hydrophobic chains and new functional group conjugation strategies, polyamine modification has emerged as a

complementary approach to enhance PBAE performance. Spermine, a naturally occurring polyamine with four protonatable amine groups, is particularly effective for PBAE modification.[82] Its multiple amines can enable strong electrostatic interactions with nucleic acids to improve complexation, while facilitating endosomal escape via the proton sponge effect. These features have made spermine-modified PBAEs particularly promising for applications such as mRNA vaccines, tumor-targeted gene delivery. First system focused on Boc-protected and deprotected spermine-based PBAEs (Sp-PBAEs) (Fig. 5a-b), synthesized via step-growth Michael addition polymerization with 1,4-butanediol diacrylate.[83] Both polymers demonstrated enhanced cellular uptake compared with branched PEI, with Sp-PBAEs showing decreased endosomal entrapment and achieving 60% eGFP knockdown in vitro. When applied to KRAS-mutated lung cancer cells, Sp-PBAEs polyplexes mediated 35–45% inhibition of KRAS expression and 33–55% reduction in cell motility, indicating their potential for therapeutic siRNA delivery. Further work on this PBAEs system have moved toward on hydrophobic modification. PBAEs synthesized from spermine and 1,4-butanediol diacrylate were further modified with diacylamine (Fig. 5b). This lipid modification formed stable polyplexes at N/P ratios ≥ 5 , achieving around 90% gene silencing in H1299-eGFP cells while exhibiting superior biocompatibility and cellular delivery compared with branched PEI.[84] Spermine-based PBAEs with varying oleylamine side chains (Fig. 5c) showed that increased hydrophobicity enhanced gene silencing, achieving over 90% GFP knockdown, while cellular uptake was modulated by the interplay of charge and hydrophobic interactions, likely facilitating endosomal escape.[85] Spray-drying of these polyplexes enabled pulmonary delivery and preserved siRNA activity after three months at room temperature, demonstrating potential for inhalable formulations.[85]

e. Hybrid Nanostructures

Recent advancements have led to the development of hybrid nanostructures that integrate PBAEs with other components. The combination of PBAEs with lipid components have been explored to generate hybrid system or defined nanostructures and to exploit the advantages of both systems. For instance, pH-responsive PBAE cores have been enveloped in lipid bilayers composed of 1,2-dioleoyl-3-

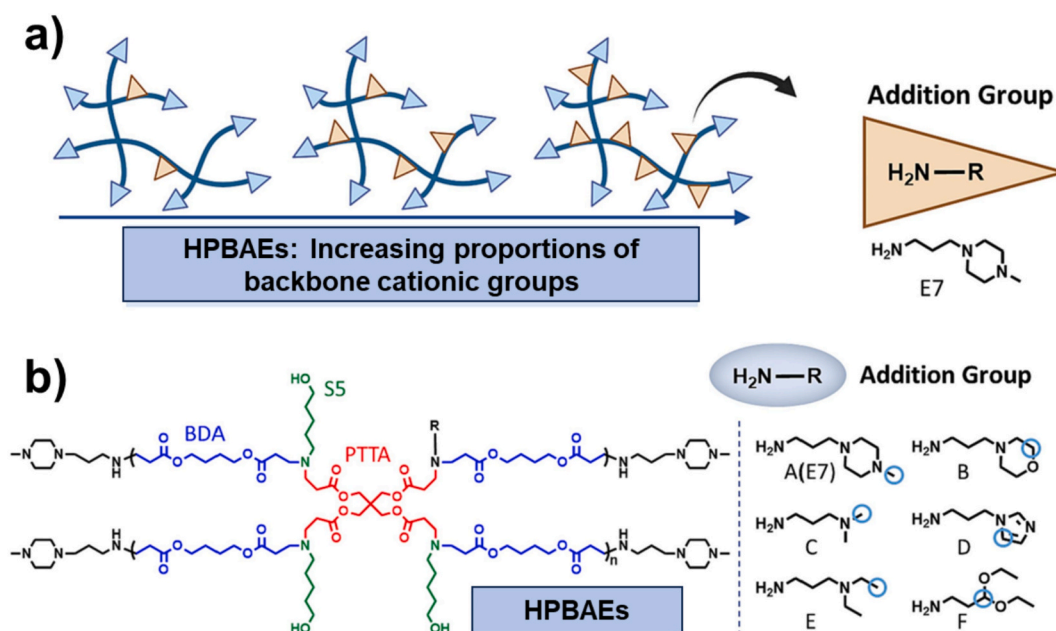


Fig. 4. Post polymerization modification of HPBAEs backbone with additional cationic amine groups. a) Schematic illustration of the HPBAE polymer structural changes b) Polymer and monomer chemical structures of HPBAEs. Additional tertiary amines structure: A(E7), 1-(3-aminopropyl)-4-methylpiperazine; B(MPA), 3-Morpholinopropylamine; C, 3-(Dimethylamino)-1-propylamine; D, 1-(3-Aminopropyl)imidazole; E, 3-(Diethylamino)propylamine; F, 4-Aminobutyraldehyde diethyl acetal. Reproduced from [80] with permission Copyright © 2024 Elsevier.

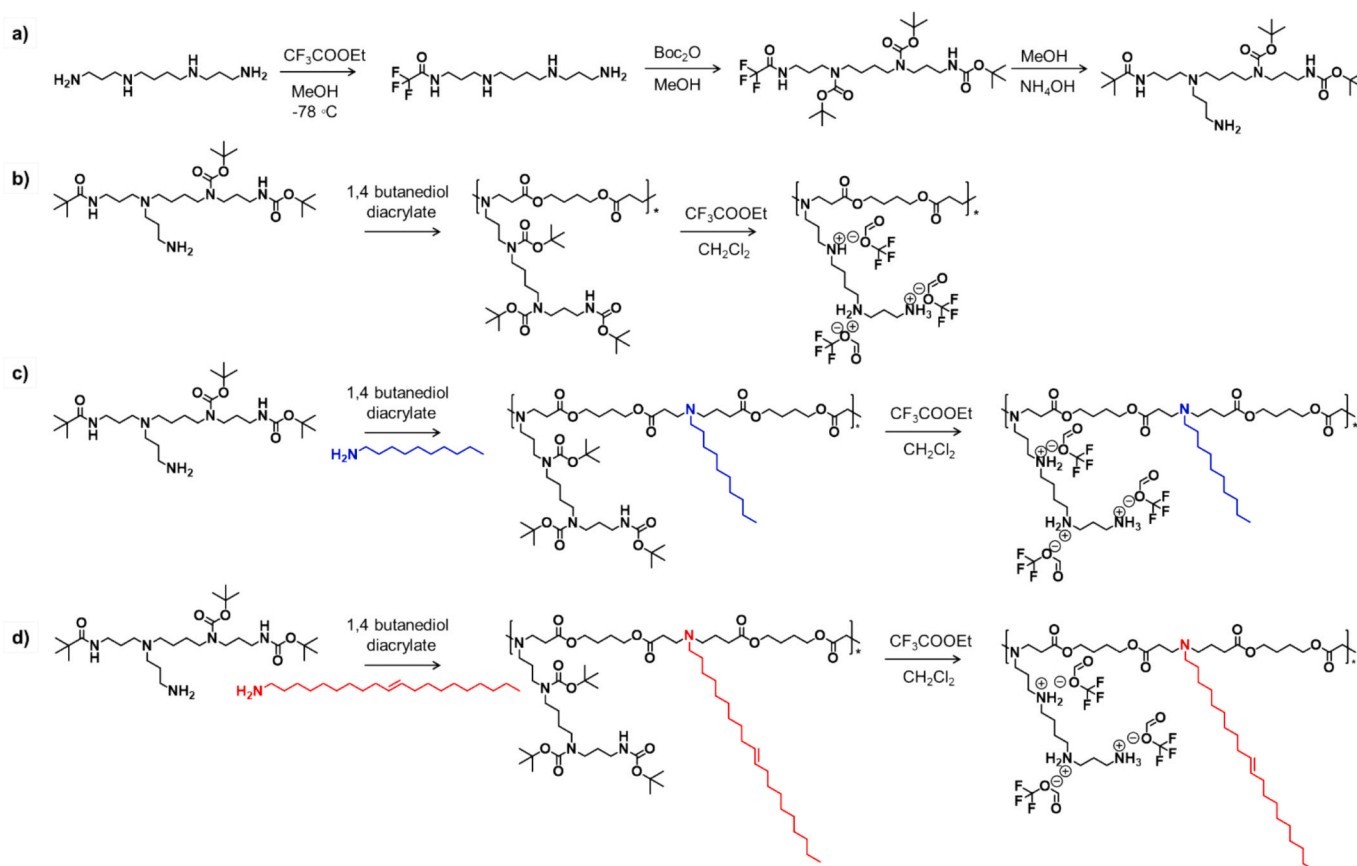


Fig. 5. Synthesis route of Spermine-based PBAEs with lipid modification. [83–85].

trimethylammonium-propane (DOTAP), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and DSPE-PEG.[86] Further optimization of

hybrid nanoparticles has been achieved through the incorporation of more complex lipid compositions, including cholesterol and helper

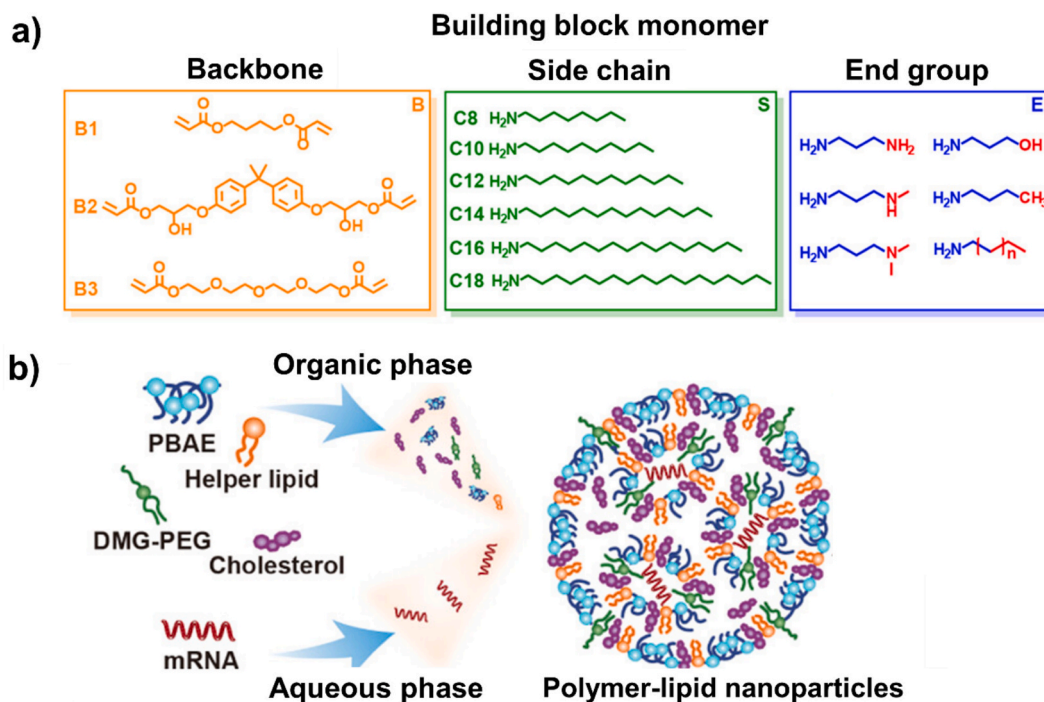


Fig. 6. Example of PBAEs hybrid nanostructures. a) PBAEs building block using a combination of different backbones, side chains, and end caps for incorporating with LNPs formulation. b) Construction of polymer-lipid nanoparticles using PBAEs along with three additional components using rapid mixing and ethanol dilution method. Reproduced from with permission [67] Copyright © 2024 American Chemical Society.

lipids, similar to those used in standard LNPs, together with additional PBAEs to modulate properties such as surface charge and internal hydrophobicity. For instance, PBAEs constructed with varying backbone hydrophobicity, side-chain length, and amine end-groups (Fig. 6a), similar to those prepared by Yong *et al.* (Fig. 2a), have been investigated to fine-tune LNP properties. These PBAEs were further formulated with other excipients, including helper lipids, cholesterol, and PEGylated lipids, to form polymer–lipid nanoparticles using standard rapid mixture techniques (Fig. 6). [67] In general, the PBAE core has good responsiveness in an acidic environment, which could allow the lipid-enveloped PBAE LNPs to disassemble to promote endosomal disruption and lead to higher mRNA expression, demonstrating the potential of hybrid nanoparticles for gene delivery applications. [66,87]

In addition to lipid-based hybrids, PBAEs have been incorporated into thermoresponsive and multifunctional hydrogels to enable localized and sustained therapeutic release. For example, PBAEs were chemically modified to make them thiol-reactive and star-shaped 4-arm-PEG-SH was used as a cross-linker to form the hydrogel network *in situ*. A composite synthetic hydrogel showed prolonged release of PBAE/nucleic acid nanoparticles, protecting the cargo and efficiently transfecting primary human dermal fibroblasts. [88] Beyond nucleic acid delivery, multifunctional PBAE hydrogels have been engineered to co-release bioactive molecules such as nitric oxide (NO) and L-arginine (L-Arg), promoting angiogenesis, collagen synthesis, and macrophage reprogramming. Methacrylate poly-L-Arg-functionalized PBAE hydrogels (PAMA-PBAE) demonstrated enhanced fibroblast proliferation, anti-inflammatory cytokine secretion, and accelerated wound closure (~98% in full-thickness skin defects), outperforming commercial dressings. [27] Additionally, the development of amphiphilic conetwork (APCN) gels combining PBAEs with poly(amido amines) or poly(caprolactone) enables precise control over composition, degradability, and multi-responsive drug release. These APCN gels exhibit triggered degradation under acidic, basic, enzymatic, and mildly reducing conditions while remaining stable under normal physiological conditions, highlighting their potential for controlled drug release, tissue engineering, and hemocompatible biomedical applications. [89,90] In more recent work, PBAE nanoparticles have been dispersed into poly(ethylene glycol)-poly(caprolactone)-poly(ethylene glycol) (PEG-PCL-PEG) triblock copolymer hydrogels for the site-specific delivery of interferon alpha (IFN- α 2b) in the treatment of ocular surface tumors. [91] This system combines the protective and pH-responsive properties of PBAEs with the sustained release capabilities of thermoresponsive hydrogels, offering a promising approach for localized gene therapy. Although not yet widely applied for nucleic acid delivery, such PBAE-based hydrogels could potentially be adapted for gene delivery applications.

The development of hybrid and composite nanostructures incorporating PBAEs has significantly advanced gene delivery systems. By combining the unique properties of PBAEs with LNPs or hydrogels, these nanostructures offer enhanced stability, targeted delivery, and controlled release of nucleic acids. Continued research and optimization of these systems hold great promise for the effective treatment of various diseases through gene therapy.

3.2. Stimuli-Responsiveness

An effective strategy to improve the therapeutic efficacy of genetic compounds is to deliver them to the right sites without resulting in undesired effects, with the goal of achieving strong encapsulation and precise release. Gene delivery systems generally rely on the intrinsic physical and biological properties of specific environments to control the cargo release, but this approach has clear limitations. Disease environments are often heterogeneous and vary depending on the patient's condition, making it difficult to predict or control how these factors will influence release kinetics and dosing after administration.

Stimuli-responsive delivery systems provide an attractive strategy for applications requiring controlled or on-demand therapeutic release.

In recent decades, responsive nanoparticles have been widely explored to exploit physiological differences between normal and diseased tissues. For example, normal intracellular pH is typically maintained around 7.2–7.4, whereas endosomal compartments are more acidic, with pH values near 5.0. Tumor tissues also commonly exhibit a mildly acidic extracellular environment, usually around pH 6.5–6.9, which can be used as a trigger for selective drug or gene release. In addition to pH gradients, redox differences provide another important biological cue. Glutathione (GSH), a non-protein thiol involved in cellular processes such as proliferation, differentiation, and apoptosis, is present at much higher concentrations inside cells than in the extracellular environment. Intracellular GSH levels are generally in the range of 0.5–10 mM, compared with only 2–20 μ M in plasma. Moreover, cancer cells often contain elevated GSH concentrations, approximately 2–10 mM, which may be several-fold higher than those in normal cells. [93] By exploiting such physiological differences, stimuli-responsive PBAEs are designed to undergo structural or chemical changes in response to specific biological conditions. Below are the most explored stimuli-responsive PBAEs for nucleic acid delivery (Table 2):

a. pH-Responsive Systems

A comparative study investigated how PEI and PBAEs respond to changes in pH. To examine their role in endosomal escape, extracellular pH modulation assays were used to evaluate the influence of polymer buffering capacity and effective pKa on plasmid DNA delivery. [26] The results showed that PBAE-mediated transfection is highly dependent on the effective pKa of the polymer. In acidic environments, PBAE transfection efficiency was significantly reduced, whereas PEI remained largely unaffected. At neutral to slightly basic pH, PBAEs exhibited strong buffering capacity, leading to markedly enhanced transfection efficiency. Notably, cellular uptake of the system was unchanged across different pH levels, indicating that the acidic microenvironment influences intracellular delivery rather than initial uptake. This study showed clearly pH responsiveness of PBAEs.

In 2015, Li and colleagues developed a rational strategy that combined pH-responsive PBAEs with PEI to co-deliver paclitaxel (PTX) and two siRNAs for the inhibition of breast cancer growth and metastasis. [18] In this design, micelles were formed through the self-assembly of PEI-PBAE and PEG-PBAE conjugates, both synthesized via the reaction of amines from PEI or PEG with diacrylate monomers. PEG serves as a non-ionic, inert, and stealth-forming component, providing low immunogenicity to the micellar corona and thereby enhancing the performance of the drug-delivery system. This dual-conjugate system enabled efficient encapsulation of PTX and siRNA within nanoparticles of small size (80–140 nm) and positive surface charge (16–36 mV), attributed to the hydrophobic PBAE core and the cationic contribution of PEI and PBAE. The micelles demonstrated pH-responsive behaviour, effectively releasing siRNA under acidic conditions (pH 5.0) or in the presence of heparin, consistent with polymer degradation. Importantly, PEI-PBAEs/PEG-PBAEs micelles exhibited high transfection efficiency, likely due to the lysosomal escape capacity of PBAEs and the efficient siRNA release triggered by the acidic microenvironment. [18] From a structure–property–function perspective, this dual-conjugate system demonstrates how different polymer segments contribute complementary delivery functions. Compared with purely cationic PEI-based carriers, the incorporation of degradable PBAE and PEG segments may improve biocompatibility and formulation stability. Thus, the high transfection efficiency of PEI-PBAE/PEG-PBAE micelles likely arises from the combined effects of nanoscale size, moderate positive charge, pH-responsive degradation, and efficient intracellular siRNA release rather than from a single structural feature.

More recently, histidine (His) was incorporated into PEG-PBAE conjugates to produce inhalable, pH-responsive, charge-reversal polymer–siRNA polyplexes (Fig. 7a). [68] The rational design of this combination integrates PEG with dual positively-charged PBAE and His to encapsulate siRNA payloads through enhanced electrostatic complexations. The dual integration of PBAE and His improved electrostatic

Table 2
Stimuli-responsive PBAEs for nucleic acid delivery.

No	PBAEs architectures: Building blocks		End cap	Branching	Size after complexation	Zeta potential after complexation	Stimulus	Nucleic acid cargo	Biological properties	Ref
	Backbone	Side chain								
1	1,4-butanediol diacrylate	4-amino-1-butanol	(1-(3-aminopropyl)-4-methylpiperazine)	—	pH dependent size; 100–1000 nm	pH dependent charges; +10 to +30 mV	pH	DNA	Improved transfection efficacy compared to PEI	[26]
2	1,4-butanediol diacrylate	5-amino-1-pentanol	PEG-NH ₂	—	80–140 nm at different mass ratio	+16 to +36 mV	pH	siRNA	More significant anti-metastasis effect	[18]
3	1,4-butanediol diacrylate	4-amino-1-butanol	PEG acrylate	—	120–160 nm depend on N/P ratio	+15 to +30 mV depend on N/P ratio	pH	siRNA	Improves siRNA stability and gene transfection compared to PEI	[68]
4	2,2'-disulfanediylbis (ethane-2,1-diyl) bisphenol-A	4-amino-1-butanol	1-(3-aminopropyl)-4-methylpiperazine (diethylenetriamine)	Trimethylolpropane triacrylate	~150 nm	~+15 mV	Redox	plasmid DNA	Improved DNA binding and transfection compared to the linear Redox-responsive PBAE polymers enhance gene delivery efficiency in cancer cells.	[75]
5	ethoxylate diacrylate	—	—	—	102.0 nm to 212.2 nm	+27.3 to +47.4 mV	Redox	plasmid DNA	Redox-responsive PBAE polymers enhance gene delivery efficiency in cancer cells.	[94]
6	1,4-butanediol diacrylate	4-amino-1-butanol	Cystamine	—	<100 nm depends on weight ratio	Not reported	Redox	siRNA	Efficiently released siRNA upon exposure to a reducing environment	[95]
7	disulfanediylbis (ethane-2,1-diyl) diacrylate	—	ethylene-dioxy-bis-ethylamine	—	200 to 450 nm		Redox	siRNA	Easily print-formulated into effective in vitro nucleic acid delivery	[96]
8	Disulfanediylbis (ethane-2,1-diyl) diacrylate	4-amino-1-butanol	N-(2-Hydroxyethyl) ethylenediamine	—	81 nm	+20 to +30 mV	Redox	mRNA	Enable targeted mRNA delivery to splenic DCs following systemic administration without the need for targeting ligands.	[97]
9	Diselandediylbis (hexane-6,1-diyl) Diacrylate	dodecylamine	N,N-Dimethylethylenediamine		200 nm	+10 mV	Redox	mRNA	Reduced internal hydrophobicity and improved cellular uptake, mediating potent mRNA delivery	[98]
10	2,20 -(propane-2,2-diylbis(sulfanediyl)) diacrylate	dodecylamine	Diethylamine	—	<200 nm	−10 mV	ROS	mRNA	Target splenic APCs, enhance mRNA expression	[92]
11	1,4-butanediol diacrylate	5-amino-1-pentanol	1-(3-aminopropyl)-4-methylpiperazine and Dithiothreitol (DTT)		625.16 to 140.58 ± 3.09 nm with increasing polymer ratios of DNA binding	+22.51 ± to +40.91 mV with increasing polymer ratios of DNA binding	ROS	CRISPR/Cas9 DNA	Could effectively edit target genes in HEK 293 T (MUC2) and SiHa (HPV16 E7) cells, and inhibit cancer cell proliferation both in vitro and in vivo.	[99]
12	(1,3-phenylene)bis (methylene) diacrylate	4-amino-1-butanol	—	—	~200 nm	~+35 mV	Light	DNA	Effective intracellular gene delivery, while upon UV irradiation post-transfection,	[100]

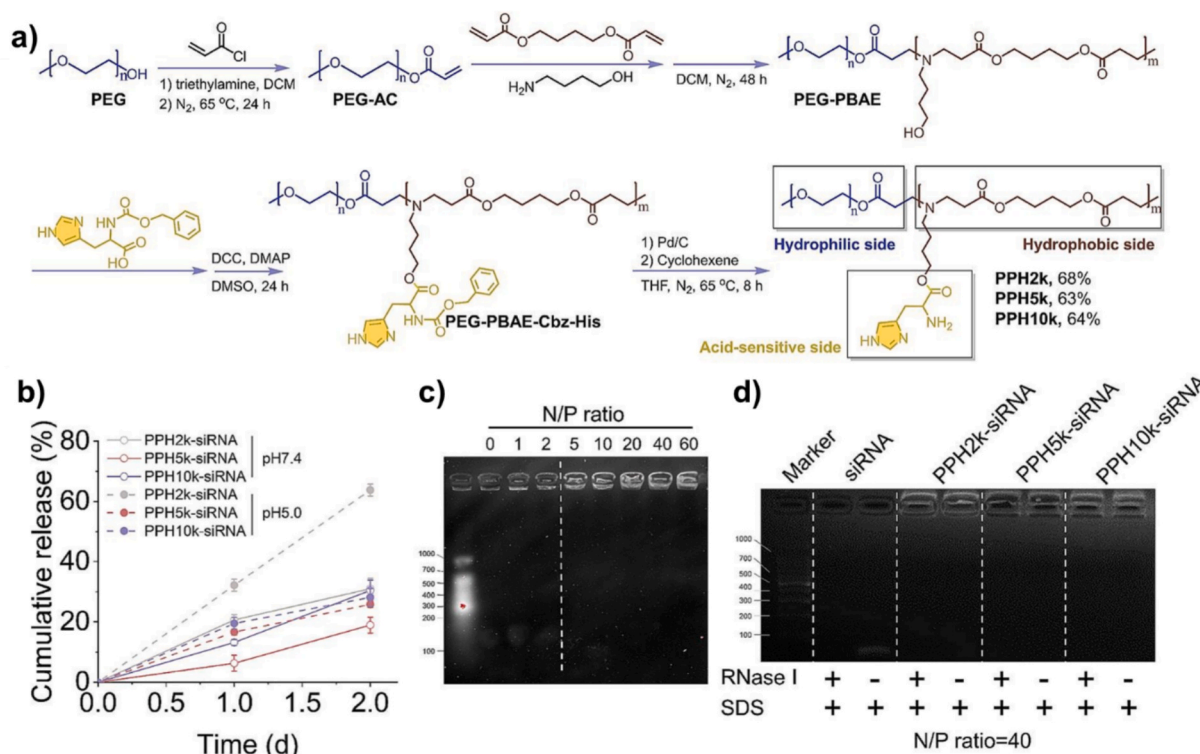


Fig. 7. The rational design of pH sensitive PBAEs. a) The synthesis route of histidine (His) to incorporate into PEG-PBAE conjugates b) Cumulative siRNA release profiles of PPH, with an evident accelerated release at acidic pH (dotted lines) compared to neutral pH (solid lines). c) Electrophoretic mobility shift assays (EMSA) of PPH2k-siRNA at N/P ratios from 1 to 60 at 20 min of separation. d) EMSA in the presence of RNase I to evaluate siRNA degradation. Reproduced from [68] with permission Copyright © 2025 Elsevier.

encapsulation and enhanced pH sensitivity (Fig. 7b-d). At low pH, increased cationic density induced polymer chain repulsion, triggering siRNA release, whereas at higher pH, gradual deprotonation and charge neutralization resulted in compact polyplexes.[101] This design preserved siRNA stability in circulation while facilitating endosomal escape and efficient intracellular release. In contrast, PEG-PBAE systems without His exhibited weaker pH responsiveness and less efficient acidic release, confirming the importance of dual PBAE/His modification for enhanced delivery performance.

b. Redox-Responsive Systems

While pH-responsive PBAEs exploit acidic microenvironments to trigger endosomal escape, redox-responsive designs harness intracellular GSH gradients to achieve cytosolic release. These complementary mechanisms broaden the versatility of PBAE carriers across diverse biological contexts. Redox-sensitive systems exploit the intracellular reducing environment, where the glutathione GSH concentration (0.5–10 mM) is orders of magnitude higher than extracellularly (2–20 μ M). Disulfide linkages incorporated into polymers remain stable extracellularly but are rapidly cleaved inside cells, triggering cargo release. PBAEs naturally degrade via ester hydrolysis over hours to days; however, the inclusion of disulfide bonds introduces a rapid, “on-demand” degradation mechanism for efficient intracellular nucleic acid release.[25,94]

Early studies demonstrated that cystamine-terminated PBAEs formed stable nanoparticles (<150 nm) that tightly bound siRNA at low polymer/siRNA ratios, yet rapidly released their cargo in the cytosolic, GSH-rich environment.[95] Later, disulfide bonds were introduced directly into the PBAE backbone using monomers such as 2,2'-disulfanediybis(ethane-2,1-diyl) and hexane-1,6-diyl, generating bio-reducible polymers with similar nanoparticle sizes and faster degradation kinetics, achieving complete cleavage within minutes under 5 mM GSH.[102] These materials exhibited rapid siRNA release within minutes under 5 mM GSH, demonstrating fast disulfide cleavage

compared to slower ester hydrolysis. This approach underscores the synergy between PBAE chemistry and disulfide incorporation to achieve controlled nucleic acid delivery.

While PBAEs are generally less cytotoxic than conventional cationic vectors,[32] their molecular weight, structural features, and supramolecular organization strongly influence both cytotoxicity and transfection efficiency.[51] Highly stable polymer-nucleic acid complexes are desirable during extracellular transport, yet intracellular nucleic acid release is accelerated when the polymer degrades into smaller, less charged fragments.[96] Building on this principle, β -aminoester oligomers (OBAEs) further improved biocompatibility, forming polyplexes (200–450 nm) that disassembled rapidly (from ~100 to ~10 nm in 2 h) in 10 mM GSH while maintaining high transfection efficiency and low cytotoxicity in A549 cells.[96] End-group modification with lipoic acid produced nanoparticles (102–212 nm, +27–47 mV) stable in dispersion for six weeks, yet capable of rapid disassembly upon dithiothreitol (DTT) treatment, significantly enhancing transfection in HeLa and HEK293T cells.[94] This example demonstrates that redox-responsive PBAEs can decouple extracellular stability from intracellular release, improving cargo protection before uptake while enabling rapid unpacking under cytosolic reducing conditions.

The spatial distribution of cationic charges plays a crucial role in nucleic acid binding, and multivalency has been widely adopted to enhance condensation and cellular uptake.[42,103] Incorporation of disulfide bonds into branched backbones (BPAE-SS), endowing the polymers with redox responsiveness. BPAE-SS condensed siRNA into compact polyplexes with high binding affinity yet underwent rapid disassembly in the reductive intracellular environment due to GSH-mediated cleavage of disulfide bonds. This release promoted efficient ICAM-1 knockdown while minimizing post-transfection cytotoxicity. In vivo, BPAE-SS/siRNA polyplexes achieved therapeutic efficacy in a rat myocardial ischemia-reperfusion model, highlighting the dual role of multivalency and redox sensitivity in safe and effective gene delivery.

[75]

Recent studies have focused on further structural modifications into redox sensitive PBAEs to enhance systemic delivery, transfection, and organ-specific targeting. For example, introducing lipid moieties into PBAEs nanoparticles is massive topic in gene delivery due to the success of LNPs.[11,14,15] Green et al. reported lipid moieties incorporated into disulphide PBAEs (Fig. 8a) improve nanoparticle condensation and mRNA encapsulation through hydrophobic interactions, as observed in combinatorial libraries targeting dendritic cells (DCs).[97] Variations in alkyl side-chain length (Fig. 8b) modulate particle size and encapsulation efficiency. This is likely due to increased hydrophobic interactions with the mRNA cargo forming more condensed nanoparticles. The trend

of improved cellular uptake with increased lipophilicity supports the higher in vitro transfection efficiency observed for lipophilic PBAEs-mRNA complexation.[97] Similarly, diselenide-containing PBAEs formed polyplex nanoparticles with moderate positive charge, optimal size, and enhanced cellular uptake and endosomal escape compared to non-diselenide analogues (Fig. 8d). A library of 180 PBAEs revealed that hydrophobic amine backbones with ionizable cationic end caps are critical for efficient mRNA packaging and delivery. These nanoparticles exhibited robust redox responsiveness, improved internalization, and effective mRNA delivery, achieving splenic-targeted delivery in mice via tail vein injection without the need for helper lipids. Such diselenide-containing PBAEs offer a promising platform for mRNA-based

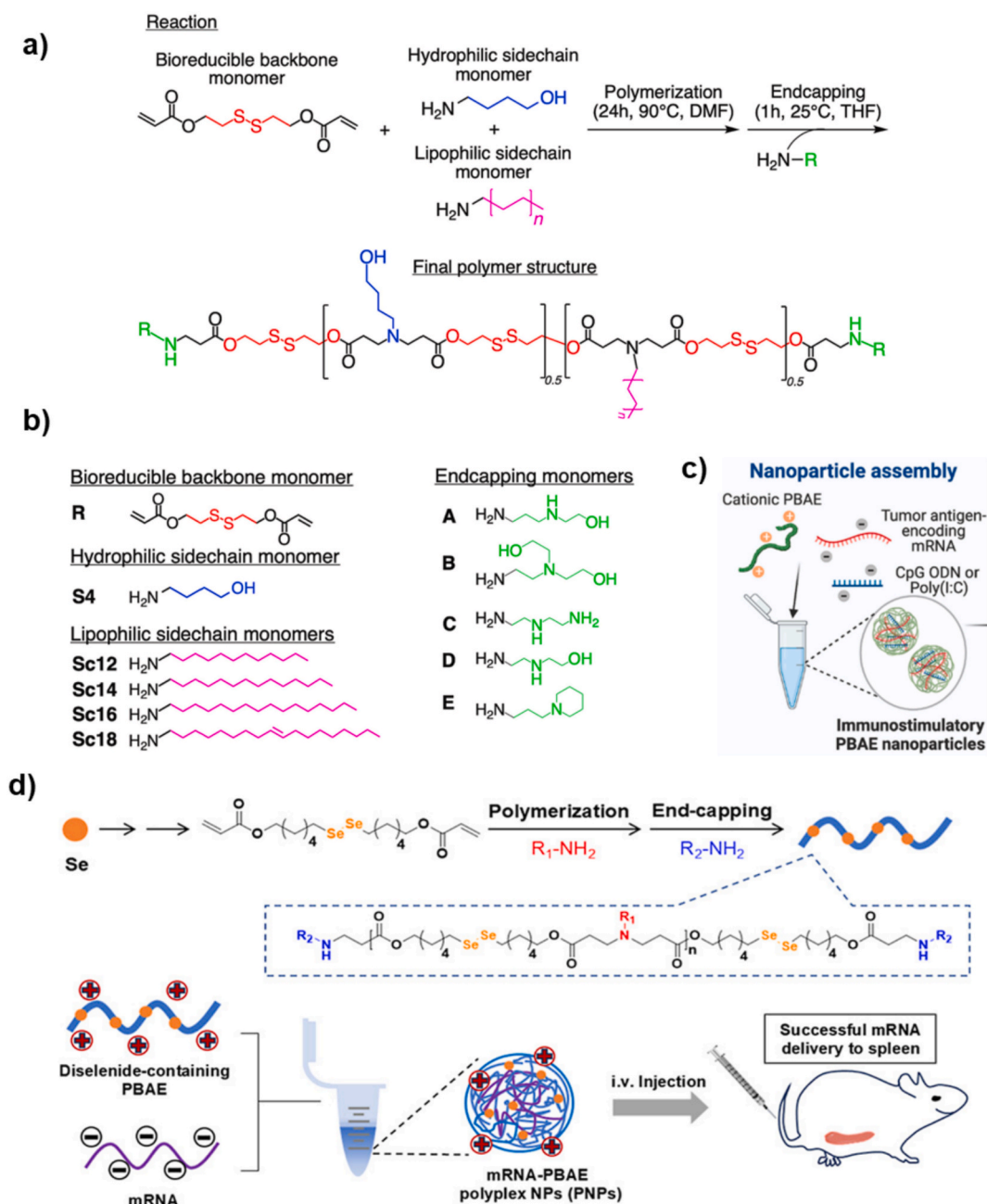


Fig. 8. Structural modifications into redox sensitive PBAEs. a) Reaction scheme for bioreducible lipophilic PBAEs. The bioreducible diacrylate backbone monomer (R) is polymerized with a 1:1 mixture of a hydrophilic amine sidechain monomer (S4) and a lipophilic amine side chain monomer (Sc12-18) via Michael addition. The obtained diacrylate-terminated random copolymer is end capped with an amine-containing monomer (A-E) to form the final polymer structure. b) Monomers used in the combinatorial library synthesis to form bioreducible lipophilic PBAEs. c) Disulphide PBAEs assembly with mRNA. d) Synthesis of diselenide-containing acrylate and PBAEs to for polyplex after complexation with mRNA. Reproduced from [97,98] with permission. Copyright © 2023 National Academy of Sciences and Copyright © 2025 American Chemical Society.

immunotherapies, highlighting the potential of redox-sensitive polymeric vectors for organ-specific nucleic acid delivery.[98]

These examples demonstrate that redox-responsive PBAEs can decouple extracellular stability from intracellular release, improving cargo protection before uptake while enabling rapid unpacking under cytosolic reducing conditions.

c. ROS-Responsive Systems

Beyond the redox balance, ROS-sensitive PBAEs have emerged as another adaptive strategy, particularly relevant in inflammatory or tumor tissues where oxidative stress is elevated. ROS levels are typically elevated in inflamed or diseased tissues, providing an opportunity to engineer ROS-cleavable PBAEs for targeted and controlled gene delivery.[99,104] Jiang et al. developed a ROS-responsive PBAEs that enhances mRNA expression in splenic antigen-presenting cells (APCs) while reducing hepatic accumulation, thereby promoting Th1-biased immune responses after intravenous injection.[92] The polymer was optimized through a three-step process involving adjustments to the combined diacrylate block containing ROS-responsive diacrylates, specifically 2,2'-(propane-2,2-diylbis(sulfanediyl))diacrylate (TKA) and bisphenol A glycerolate diacrylate (BGA), as well as modifications to the side-chain and end-cap amines to maximize spleen targeting (Fig. 9). In vivo studies demonstrated selective mRNA expression in splenic APCs with 10% TKA in the PBAE system, particularly in macrophages and dendritic cells, resulting in robust OVA-specific T-cell activation and IgG2c-dominant antibody production. In addition, addition DTT moieties into linear PBAEs demonstrated improved gene editing of RISPR/Cas9 for tumor inhibition about 67% in cervical cancer xenograft models.[99]

Similarly, thioether bonds were also introduced into the DTT containing PBAE backbone to form PBAE β -thioether ester copolymers (PBAE-TE). After optimization of monomer ratio and reaction time, PBAE-TE achieved high CRISPR/Cas9 DNA transfection efficiencies of 75.0% and 78.7% in multiple cell lines (HeLa, Me180, C666-1, and HEK 293T) cells, respectively, outperforming traditional PBAEs and inhibit cancer cell proliferation in vivo.[99] This improvement suggests that thioether incorporation can modulate backbone properties to enhance gene transfection while also conferring notable ROS-scavenging capability. The integration of ROS elimination with gene delivery capacity highlights ROS-responsive PBAEs as promising and adaptable platforms for next-generation gene therapy and mRNA vaccine applications. However, systematic comparisons of physicochemical properties and toxicity are still needed to identify which structural changes primarily drive the improved transfection performance.

d. Light-Responsive Systems

In addition to chemical stimuli, PBAEs can be engineered to respond to biological or external triggers such as enzymatic cleavage or light

irradiation, offering spatiotemporal control over gene release. For example, light-responsive PBAEs offer precise spatiotemporal control over polymer degradation and nucleic acid release. Incorporation of 2-nitrobenzene moieties into PBAE backbones enables UV-triggered cleavage of ester linkages, resulting in rapid polymer degradation and DNA release.[100] These photo-responsive PBAEs were synthesized via polyaddition of (2-nitro-1,3-phenylene)bis(methylene) diacrylate and bifunctional amines, ensuring each repeating unit contained a light-sensitive site. Upon UV irradiation, the ester linkages cleaved within minutes, promoting intracellular DNA unpacking and reducing toxicity. High-molecular-weight cationic PBAEs mediated efficient gene delivery, and post-transfection UV exposure further enhanced DNA release and cell viability across multiple mammalian cell types. This system demonstrates that integrating light-sensitive domains into PBAEs allows precise external control over degradation kinetics, gene release, and cytocompatibility, offering a powerful approach for controlled, non-viral gene delivery.

Despite these advantages, light-sensitive PBAEs have been relatively underexplored. This is likely due to several practical challenges: the limited tissue penetration of UV light restricts in vivo applications, potential phototoxicity can damage cells or tissues, and the synthetic incorporation of light-sensitive moieties can be chemically demanding and costly. These factors make researchers cautious, favoring more easily implementable stimuli such as pH or redox responsiveness, which are more straightforward to apply in biological system.

e. Dual and Multi-Stimuli Systems

Complex biological environments often require responsive systems that integrate multiple triggers. Multifunctional PBAEs have also been designed for combinatorial cancer therapy. For instance, dual stimuli-responsive nanoparticles were developed for the co-delivery of siRNA and doxorubicin (DOX), employing strain-promoted azide-alkyne cycloaddition (SPAAC) to attach tripeptide end-chains and thiol-disulfide exchange to conjugate DOX (Fig. 10), combined with a hydrazone linker for dual pH- and redox-responsive release.[105] In multidrug-resistant GLC-4/ADR cells, this platform effectively down-regulated BCL-2, enhancing the cytotoxic effect of DOX while minimizing off-target leakage.

PBAEs have firmly established themselves as a highly adaptable and modular platform for stimuli-responsive gene delivery, demonstrating remarkable versatility across a broad spectrum of biological contexts. While pH-responsive and redox PBAEs are most explored due to their robust performance, light-responsive PBAEs currently limited in number, highlight the potential for externally controlled, spatiotemporal regulation of polymer degradation and gene release, a concept that could be transformative for applications requiring precise temporal and spatial control. The relative underdevelopment of light-responsive systems likely reflects practical challenges. Overcoming these hurdles, potentially through longer-wavelength light activation, more biocompatible chromophores, or simplified synthetic routes, represents a promising avenue for future research.

Beyond single-stimulus designs, dual- and multi-stimuli PBAEs exemplify the sophistication achievable through rational polymer engineering. By integrating multiple responsive linkages, such as pH, redox, and ROS sensitivity, into a single construct, these systems can dynamically adapt to complex intracellular or tissue microenvironments, achieving sequential or combinatorial release profiles. However, study on PBAEs with multi stimulus are still limited, leaving considerable room for the development of new smart PBAEs with optimized gene delivery performance.

Critically, the field still faces translational challenges. While in vitro performance of PBAEs is exceptional, in vivo applications require careful consideration of polymer stability, tissue penetration, immunogenicity, and clearance pathways. Addressing these issues will likely benefit from an integrated approach combining rational chemical design with advanced delivery strategies, such as targeting ligands, responsive coronae, and organ-specific accumulation. Moreover, systematic studies

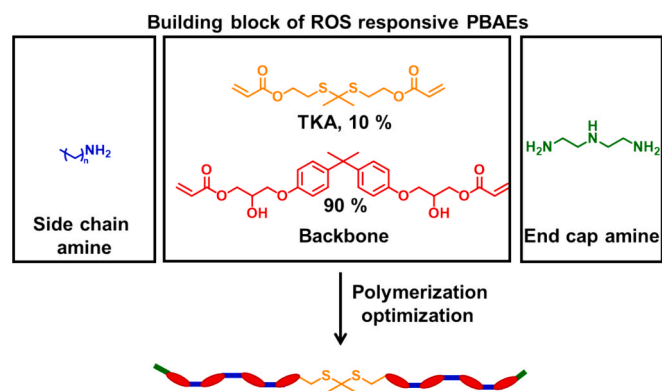


Fig. 9. Design of ROS responsive PBAEs. a) Schemes for optimizing backbone containing 2,20 -(propane-2,2-diylbis(sulfanediyl))diacrylate (TKA) and bisphenol A glycerolate diacrylate (BGA), side chain and end cap amine in PBAEs. Adapted from [92].

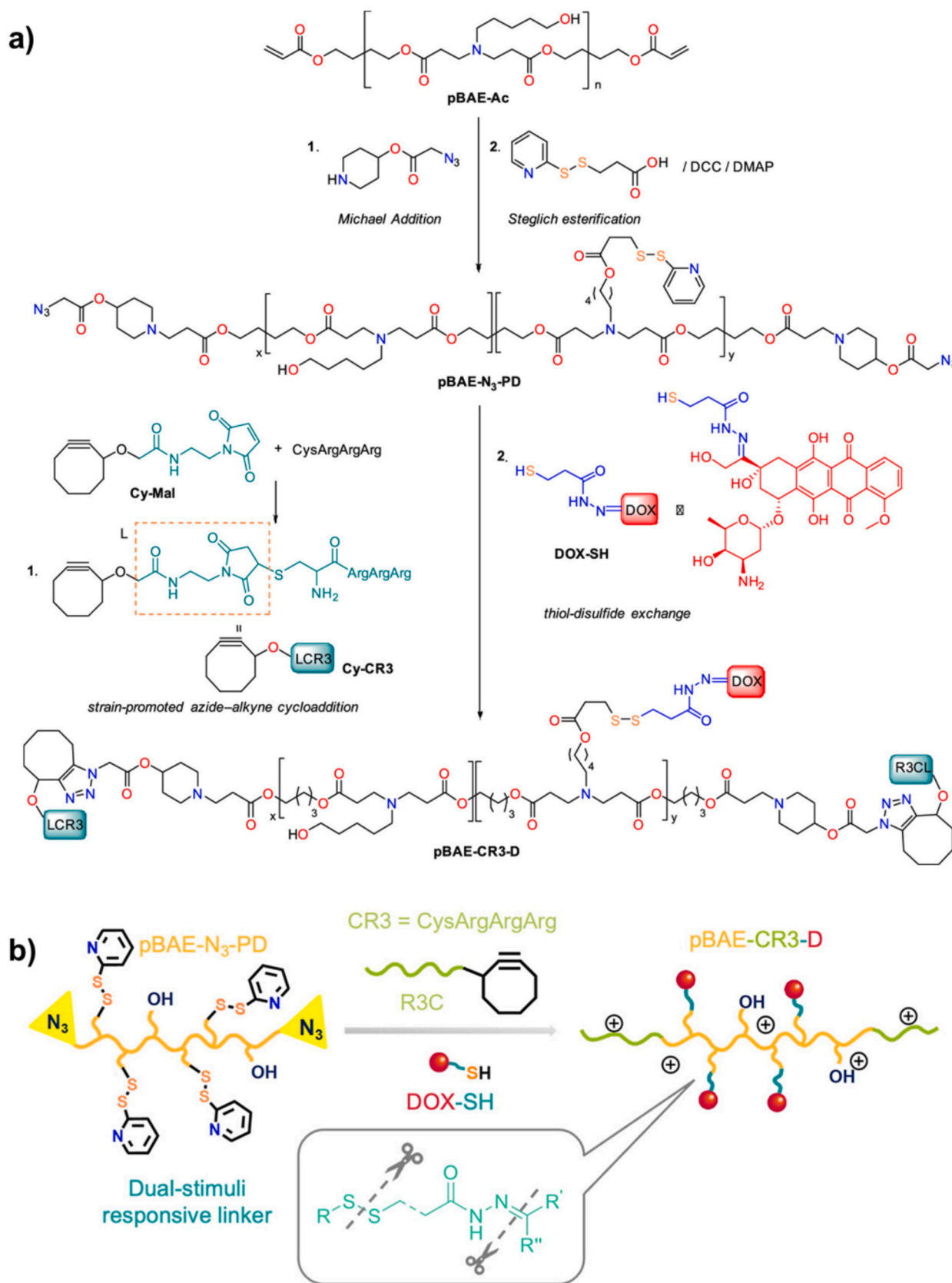


Fig. 10. Design of dual responsive PBAE containing redox and pH sensitive linkers. a) Scheme for the synthesis of PBAE-CR3-PD or PBAEs containing disulfide and hydrazone bond and b) Schematic representation of the prepared dual responsive PBAEs. Reproduced from [105] with permission. Copyright © 2025 American Chemical Society.

comparing the efficiency, cytotoxicity, and biodistribution of single versus multi-stimuli systems, as well as light-activated versus conventional stimuli-responsive PBAEs, are needed to guide rational design and facilitate clinical translation.

3.3. Targeted PBAEs

Beyond stimuli-responsiveness and structural tuning, targeted PBAEs plays a crucial role in improving delivery precision and minimizing off-target effects.

a. Ligand-Free Organ Targeting

Interestingly, certain PBAE structures inherently favor delivery to specific organs based on their surface charge, hydrophobicity, and terminal chemistry, even without targeting ligands modification. In a comprehensive structure–activity relationship study [53], branching degree was shown to strongly influence spleen accumulation after intravenous injection. Compared with non-branched PBAEs, 20% and 30% branched PBAEs produced higher luciferase expression in spleen, indicating that moderate branching can enhance *in vivo* transfection.

The identity of the terminal amine group also emerged as a dominant determinant of the selectivity of mRNA delivery to the lungs, liver, and spleen. HPBAEs terminated with morpholine preferentially drove mRNA expression in the liver, whereas polymers capped with ethylenediamine favored accumulation and gene expression in the spleen and lung. [106] Moreover, HPBAEs end-capped with methylpentane achieved broad biodistribution, delivering mRNA effectively to the liver, spleen and lungs. [53] In addition, ligand free PBAEs with long alkyl side chain and dialkyl amine termination demonstrated efficient transfection of primary human airway epithelial cells derived from both healthy donors and individuals with cystic fibrosis. [58] Upon intravenous administration in BALB/c and C57BL/6 mice, these optimized end-capped PBAE nanoparticles achieved high levels of lung-specific mRNA expression without the use of targeting ligands. [58] Similarly, hybrid PBAE-lipid nanoparticles incorporating cationic lipids like DOTAP have been engineered to enhance mRNA delivery to the spleen and lungs, with lipid composition modulating surface charge, internal hydrophobicity, and apparent pKa to improve cellular uptake and endosomal release. [87] By intentionally increasing the feed ratio and reaction time during

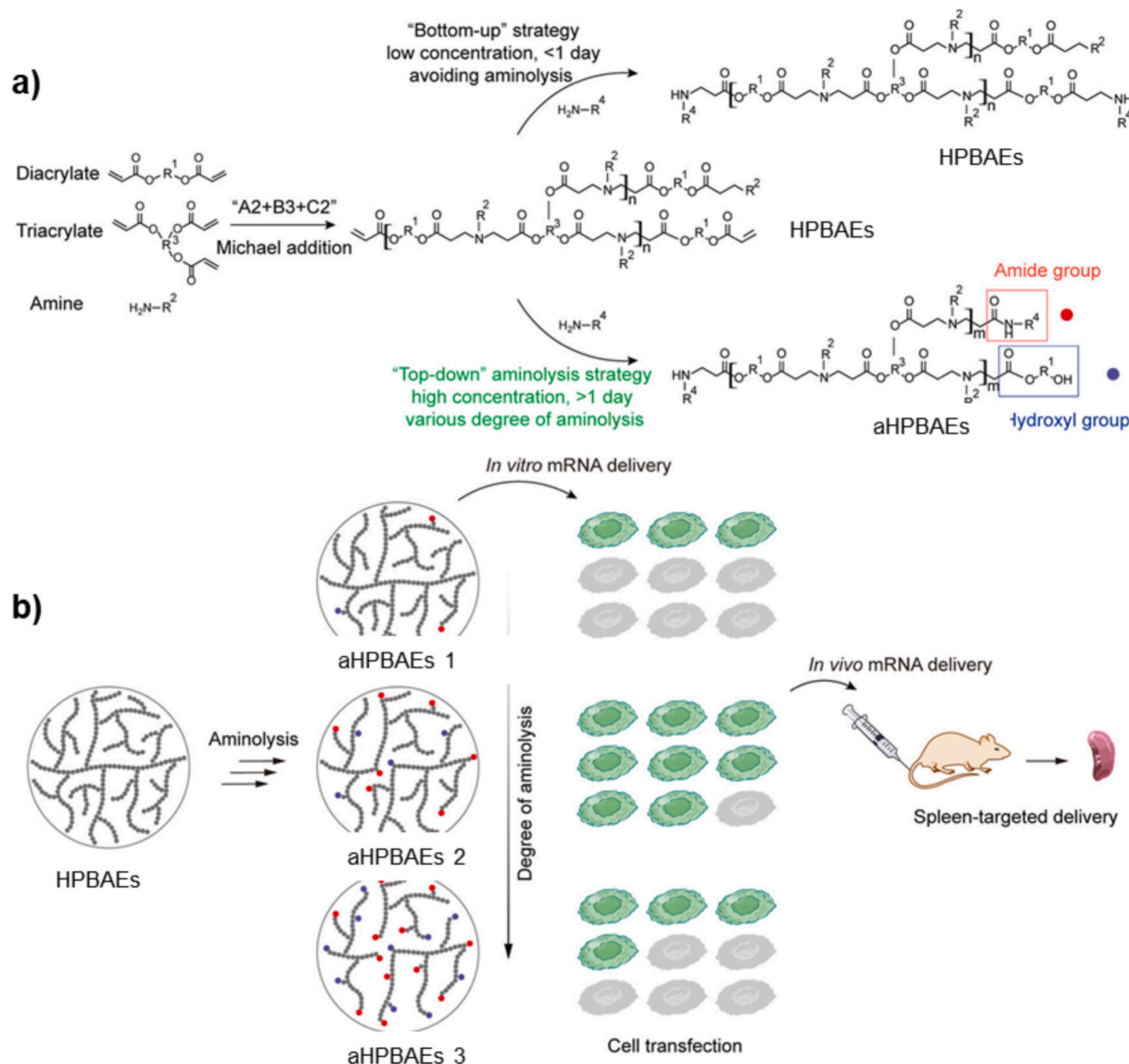


Fig. 11. Synthesis of aHPBAEs with superior mRNA transfection efficiency through “top-down” aminolysis. a) HPBAEs are synthesized via traditional “bottom-up” strategy, while aHPBAEs with additional amide and amine end groups are synthesized through the “top-down” aminolysis of HPBAEs. b) schematic representation of aHPBAEs with varying degrees of aminolysis (1–3) and the top candidate exhibits spleen selective mRNA delivery. Reproduced from [79] with permission. Copyright © 2025 American Chemical Society.

end-capping, the amines can both terminate vinyl groups and partially aminolyze the ester bonds of HPBAEs (Fig. 11a). This “top-down” aminolysis approach generates aminolyzed HPBAEs (aHPAEs) with reduced molecular weight and additional amide and amine functionalities, which critically influence polymer charge density, terminal chemistry, and overall transfection behavior.[79] Remarkably, the aHPAE with the highest concentration of aminolysis achieved selective accumulation in the spleen following systemic administration, demonstrating that structural and compositional tuning alone can enable organ-specific targeting without conjugated ligands (Fig. 11b).[79]

b. Mannose and Peptide Modifications

Mannose decoration of delivery vectors has emerged as a robust strategy to target antigen-presenting dendritic cells (DCs) through the mannose receptor CD206, a C-type lectin abundantly expressed on the surface of DCs and certain macrophages. By exploiting this receptor's endocytic pathway, mannose-functionalized carriers enhance selective uptake by DCs, thereby improving delivery of vaccine components, immunostimulatory RNA and nucleic acid payloads. Although many of the early studies employed PEI or PEG/PEI polyion complexes bearing mannose ligands (e.g., Man-PEG-b-PEI systems showing 2-3-fold higher gene delivery into CD11c⁺ DCs in vivo)[107], the same targeting paradigm is highly relevant to PBAE systems. In principle, incorporating mannose or mannose-mimetic moieties at the end-group, side chain or nanoparticle surface of PBAEs would confer DC tropism, permitting the design of ligand-free yet receptor-targeted gene delivery. Such mannose-modified PBAEs would combine the advantages of PBAEs (biodegradability, low cytotoxicity, efficient nucleic acid release) with enhanced DC-specific uptake and antigen presentation. This integration of carbohydrate receptor targeting with biodegradable polymer vectors opens promising avenues for next-generation mRNA vaccine carriers, especially when tuning of end-group chemistries and branching architectures of PBAEs is employed to optimise DC delivery and immunogenicity.

Early work on mannosylated PBAEs demonstrated improved in vitro transfection of antigen-presenting cells and enhanced immunogenicity versus non-mannosylated controls.[108] Typically, mannose residues are introduced into PBAEs through covalent conjugation between terminal amines of PBAEs with allyl- α -D-mannopyranoside to create mannose-capped PBAEs. More recent studies have synthesized

α -mannose-functionalized diacrylate monomers, which undergo Michael addition polymerization to produce mannosylated PBAEs (Man-PBAEs) bearing mannose moieties along their side chains.[109] These α -mannose PBAEs form stable, surface-decorated mRNA nanoparticles (~150–200 nm) that exhibit enhanced selective accumulation and transfection of splenic APCs following systemic administration, highlighting their potential as promising mRNA vaccine carriers.

Cell-penetrating peptides (CPPs) and tissue-homing peptides offer powerful means to enhance the functionality of PBAE delivery systems by improving intracellular delivery and promoting tissue-specific accumulation.[110] CPPs, typically short sequences of 5–30 amino acids rich in cationic residues such as arginine and lysine [110], can be either electrostatically complexed or covalently conjugated to polymeric vectors, facilitating membrane translocation via direct penetration or endocytosis.[111] When such CPPs are grafted onto PBAEs, they enhance cellular uptake of the PBAE-nucleic acid complex, thereby improving transfection efficiency, especially in challenging cell types. For example, PBAE nanoparticles surface-conjugated with the VHPK peptide (Fig. 12), Val-His-Pro-Lys amino acids [112], achieved by end-modifying an acrylate-terminated polymer with thiol-terminated oligopeptide were designed to target VCAM-1 on inflamed endothelial cells. These VHPK-functionalized PBAE nanoparticles selectively delivered anti-miR-712 to inflamed endothelium both in vitro and in vivo, demonstrating enhanced target specificity and therapeutic efficacy.[113] In another approach, CPPs such as mTAT, bPrPp and MPG were grafted onto PBAE/PLGA blend nanoparticles via PEG-phospholipid linkers, leading to 3- to 4.5-fold higher plasmid DNA loading and nearly 10-fold greater transfection efficiency in CFBE41o- cells compared with unmodified particles.[114] These findings emphasize that peptide modification of PBAEs permits both tissue-level targeting (via homing peptides) and enhanced intracellular delivery (via CPPs), broadening the versatility of biodegradable polymeric gene delivery systems.

c. Antibody Fragments (scFvs) modification

Despite breakthroughs in cancer treatment and research, developing a novel vector for site-specific therapeutic nucleic acid delivery remains a challenge. Single chain variable fragments (scFvs) of monoclonal antibodies are a promising candidate for targeting a specific tumor or desired cells due to their small size and high binding accuracy.[115]

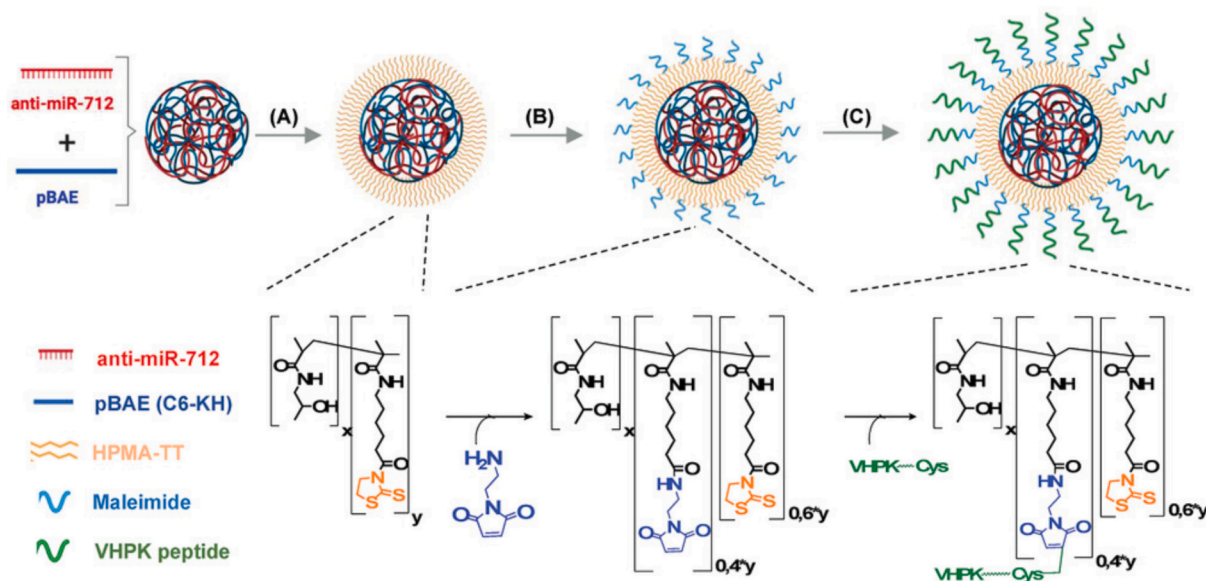


Fig. 12. Preparation of VHPK-conjugated PBAE nanoparticles. a) PBAE nanoparticles were coated with PHPMA-TT. The TT groups in the PHPMA-TT copolymer react with amine groups from PBAE nanoparticles. b) 40% of TT groups were modified using amino-maleimide linker while 60% of TT groups were used to react with PBAE nanoparticles. c) Maleimide groups were used to conjugate VHPK peptides via their terminal cysteine groups. Reproduced from [113] with permission. Copyright © 2021 Wiley.

Nanoparticles modified with scFvs can deliver the therapeutic nucleic acids to target tumor cells *in vivo*. [116] scFvs need to attach to the PEG on the surface of nanoparticles. Predominantly, a synthetic scFv is manufactured in the laboratory because we need to add C-terminal cysteine (scFv-Cys) at its carboxy end. [116] This extra cysteine provides an accessible SH group that can bind to sulfhydryl affinity reagents or resins, facilitating purification, or can participate in disulfide bond formation by mild oxidation and make ready the scFv for the reduction process to bind to the PEG effectively. [116] PBAE have also been modified with CD3e scFv to deliver the DNA (Fig. 13a) [117] and mRNA (Fig. 13b) [118] encoding artificial receptors, CARs, to the circulating T cells. To the best of our knowledge, these studies of PBAE are the pioneering research that made PBAE nanoparticles as a site-specific nucleic acid nanocarrier for *in vivo* cell manipulation in mice models. We are very aware that a subtle change in PBAE structure or physiological parameters can significantly affect the overall efficacy of systemic nanocarrier; thus, it is apparent that many challenges still need to be addressed when *in vivo* gene delivery approach comes to the attention.

d. Dual targeting modification

Dual-targeting strategies PBAEs have emerged as a powerful approach to enhance site-specific delivery of nucleic acids and therapeutics while minimizing off-target effects. Bioinspired oligopeptide-modified PBAEs, such as lysine-/histidine-functionalized C6-KH nanoparticles, have been optimized for RNAi delivery to vascular endothelial cells, achieving selective accumulation in the arterial endothelium,

lungs, and kidneys, while sparing the liver and heart. [119] PBAEs has also been applied for brain-targeted delivery, enabling accumulation in injured brain tissue and organelle-specific localization, such as mitochondria, through electrostatic interactions. [120] Specifically, two site-selective functional groups were introduced into the PBAEs using bio-orthogonal chemistry. Allyl groups were incorporated through end-capping, and alkyne groups were installed on the polymer side chains. The allyl end groups were conjugated with the thiol-containing CYIGSR peptide via a thiol-ene reaction to promote mitochondrial targeting, while the alkyne side chains were functionalized with nerve growth factor (NGF) mimetic peptides using azide-alkyne cycloaddition to facilitate blood-brain-barrier penetration. [120] Although this particular PBAE system has not yet been applied for gene delivery, it shows great potential for such applications.

Ligand conjugation should be considered a tunable structure-function parameter rather than a simple targeting add-on. Although ligand-modified PBAEs have shown improved receptor-specific uptake and transfection, many studies remain focused on targeting efficiency, while the effects of ligand density, orientation, spacer length, conjugation site, and surface accessibility are still insufficiently explored. These factors can strongly influence receptor recognition, serum behavior, biodistribution, cellular uptake, and intracellular delivery. Future work should therefore systematically optimize ligand presentation to achieve selective targeting without compromising nanoparticle stability, safety, or formulation reproducibility.

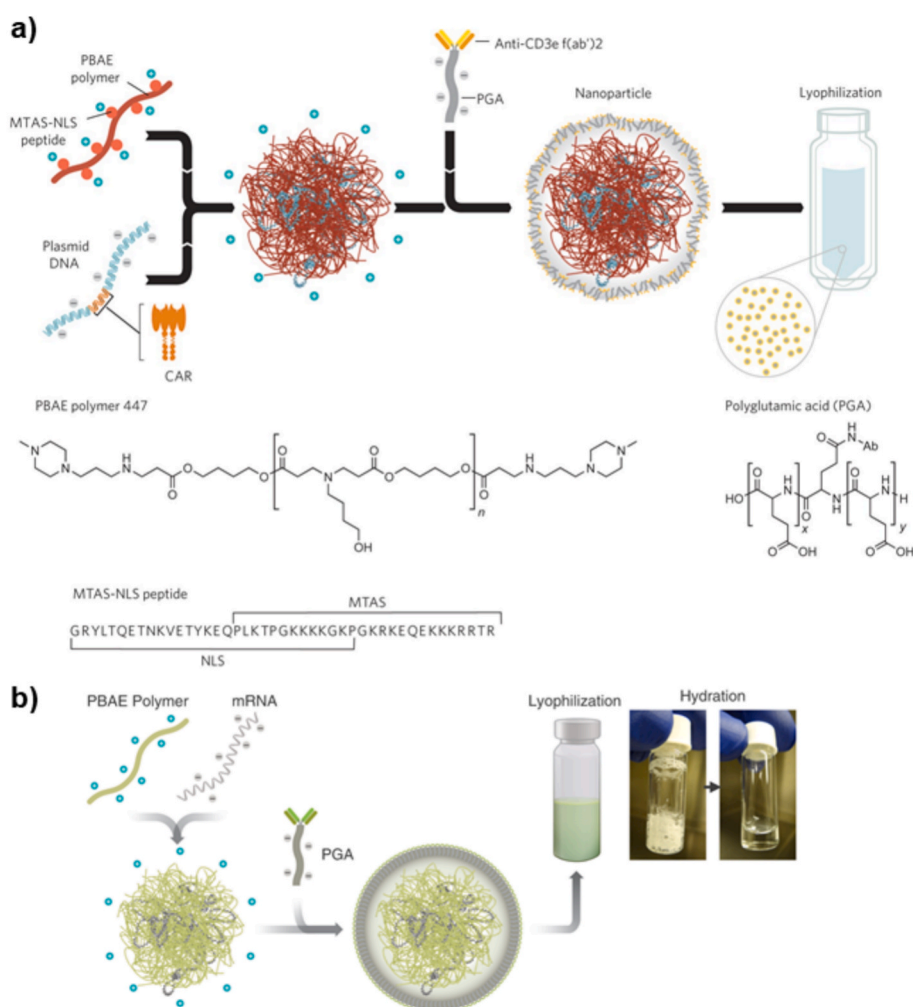


Fig. 13. A) Diagram describing the fabrication of the targeted PBAE nanoparticles with DNA. Also shown are the chemical structures of the PBAEs and polyglutamic acid. Reproduced from [117] with permission. Copyright © 2017 Springer Nature Limited. b) Fabrication the targeted PBAE nanoparticles with mRNA. Reproduced from [118] with permission. Copyright © 2020 Springer Nature Limited.

4. Structure–property–function relationships

All reported PBAE systems indicate that biological performance is governed by the interplay between polymer architecture, physicochemical properties, and biological barriers, rather than by any single structural parameter. Therefore, rational PBAE design requires balancing nucleic acid binding, nanoparticle stability, intracellular release, and biological compatibility.

Branching degree and polymer architecture strongly influence nucleic acid condensation, endosomal escape, cytotoxicity, and *in vitro*/*in vivo* transfection. Across multiple studies, it has been consistently demonstrated that linear PBAEs favor biocompatibility but exhibit limited delivery efficiency compared to branching version. Increasing branching degree systematically elevates charge density and multivalency, which strengthens nucleic acid condensation as well reduces polyplex size. However, excessive branching can lead to potentially increasing cytotoxicity, indicating the existence of an optimal intermediate branching level (e.g., ~30%). End-group chemistry emerges as a dominant determinant of biological specificity, particularly in hyperbranched systems. Variations in terminal amines influence not only surface charge and buffering capacity but also protein corona formation and organ biodistribution, enabling organ-selective delivery.

Charge densities are directly linked to nucleic acid condensation, surface potential, cellular uptake, and toxicity. For example, aminolysis of branched PBAEs increases the number of protonatable amine groups, thereby elevating charge density and strengthening nucleic acid binding, which has been associated with improved lung-targeted delivery. Similarly, addition charge through spermine modification also increases the transfection ability of PBAEs. However, excessive charge density often increases cytotoxicity. In contrast, insufficient charge density may result in unstable particles, incomplete encapsulation, premature cargo release, and poor transfection. Therefore, an optimal PBAE formulation should provide sufficient charge for condensation and endosomal escape while limiting the toxicity.

Backbone hydrophobicity is one of the most influential structural parameters governing nanoparticle formation and cellular interactions in PBAE-based delivery systems. A common strategy to modulate this property involves the incorporation of long alkyl chains or lipid-like moieties into the polymer backbone or side chains. Compared with less hydrophobic analogues, increasing backbone hydrophobicity strengthens polymer–nucleic acid interactions, leading to more compact polyplex formation, improved nanoparticle stability, and enhanced resistance to dissociation under physiological conditions. These changes also promote stronger interactions with cellular membranes, thereby increasing cellular uptake and transfection efficiency. However, this effect is not unidirectional. Excessive hydrophobicity can reduce aqueous dispersibility, promote aggregation, and negatively impact biocompatibility, highlighting a critical trade-off between stability and solubility. Across multiple studies, moderately hydrophobic backbones, typically incorporating alkyl chains in the C8–C20 range, have been shown to provide an optimal balance, improving intracellular delivery without compromising formulation stability. This trend is further supported in hybrid systems, where PBAE–lipid nanoparticles demonstrate composition-dependent performance. For instance, formulations incorporating C12 lipid chains in PBAEs exhibit particularly favorable properties for nucleic acid vaccine delivery, achieving efficient cellular uptake while maintaining adequate colloidal stability. In addition, PBAEs containing lipid moieties can be integrated into LNP, which remain among the most clinically validated systems for mRNA delivery. However, other PBAE architectures remain comparatively underexplored in the context of LNP hybridization. For example, branched or hyperbranched PBAEs could potentially enhance mRNA encapsulation, nanoparticle compactness when incorporated into LNP-like systems. Therefore, systematic investigation of non-linear PBAE architectures in hybrid LNP formulations represents an important future direction for improving mRNA delivery efficiency and expanding the design space of

next-generation gene delivery systems.

Stimuli-responsive motifs add another layer of control by linking cargo release to specific biological microenvironments. pH-responsive PBAEs often exploit endosomal acidification to promote polymer protonation, swelling, membrane destabilization, and endosomal escape. Other stimuli-responsive designs, including redox- and ROS-responsive PBAEs, can also be engineered to enhance the release of genetic cargo depending on the targeted application. For example, redox-responsive linkers can remain relatively stable in the extracellular environment but undergo degradation in the reducing cytosolic milieu, thereby supporting intracellular cargo release. Compared with externally triggered systems such as light-, ultrasound-, or magnetic field-responsive platforms, endogenous stimuli-responsive PBAEs are generally more favorable for systemic gene delivery because they do not require external devices, avoid issues related to limited tissue penetration, and are more compatible with physiological delivery routes. Nevertheless, external stimuli may still be useful for localized or superficial applications where spatial and temporal control is required. A notable advantage of PBAEs is that these responsive linkers can be incorporated not only into linear polymers but also into branched architectures. In branched PBAEs, stimuli-responsive linkages promote the formation of compact polyplexes with stronger gene binding and improved intracellular release compared with analogous linear responsive systems. This architectural flexibility highlights the potential of smart PBAEs to integrate efficient nucleic acid condensation, environmental responsiveness, and controlled release within a single delivery platform. PBAE design should be guided by balanced optimization rather than maximization of a single structural feature. Effective systems require sufficient charge density, branching, and hydrophobicity to condense nucleic acids, form stable nanoparticles, promote cellular uptake, and enable endosomal escape, but excessive values can increase aggregation, serum instability, and cytotoxicity. Therefore, optimal PBAEs should combine moderate hydrophobicity, controlled branching, tunable end-group chemistry, biodegradable backbones, and context-appropriate stimuli-responsive linkers to balance delivery efficiency with biocompatibility and translational robustness.

5. Challenges and Future Directions

One major translational limitation of smart PBAE systems is batch-to-batch variability, as their biological performance is highly sensitive to monomer ratio, molecular weight, dispersity, branching degree, end-group conversion, residual monomers, and degradation state. Even small changes in polymer synthesis, purification, or storage conditions can substantially alter nanoparticle pKa, buffering capacity, size, surface charge, cargo binding, degradation kinetics, serum stability, toxicity, and transfection efficiency both *in vitro* and *in vivo*. The scalability and reproducibility of polymer synthesis remain significant hurdles, especially when working with highly functionalized, branched or hyperbranched architectures, ligand-functionalized, lipid-modified, or multi-stimuli-responsive PBAEs, where small deviations in monomer ratio or reaction conditions may lead to major performance changes.^[32,34] Although complex architectures may improve biological performance, they can also increase synthetic complexity, purification burden, quality-control requirements, and regulatory uncertainty. Moreover, long-term formulation stability and storage performance remain insufficiently evaluated across different PBAE designs, including systematic assessment of their biodegradation behavior.

Another challenge in establishing general structure–function rules is the limited comparability of physicochemical parameters across different PBAE modifications, batches, formulation methods, and biological models. For example, pKa and buffering capacity strongly influence protonation behavior, endosomal escape, intracellular release, and transfection efficacy, however, these values are not always reported (Table 1) after modifications to the backbone, branching degree, end-group chemistry, lipid incorporation, PEGylation, or stimuli-

responsive linker design. This is problematic because previous studies have shown that end-group chemistry and amine content can alter pKa and buffering capacity, while recent physicochemical analyses further demonstrate that effective pKa and buffering behavior are closely linked to PBAE delivery efficiency.[121] This makes difficult to determine whether improved biological performance arises primarily from the intended structural modification itself or from associated changes in charge density.

In some cases, reported optimal parameters appear to cluster within characteristic but system-dependent ranges. Effective PBAE systems often exhibit pKa values close to the endosomal pH range, approximately 5.8–6.8, which can support proton sponge activity while limiting excessive cytotoxicity. Weight-average molecular weights are commonly optimized within the range of approximately 5–30 kDa to balance transfection efficiency, nanoparticle stability, biodegradability, and toxicity. Similarly, optimal N/P ratios often fall between 5:1 and 20:1, depending on the nucleic acid cargo, polymer architecture, and formulation conditions. Higher N/P ratios can improve nucleic acid condensation and cellular uptake, but they may also increase cytotoxicity and nonspecific interactions. These overlapping trends suggest that PBAE performance is governed not by a single parameter, but by coupled physicochemical design spaces. Therefore, future studies should routinely report pKa, buffering capacity, molecular weight, dispersity, N/P ratio, particle size, zeta potential, degradation kinetics, serum stability, and toxicity under standardized conditions to enable more reliable structure–property–function comparisons across PBAE platforms.

Stimuli-responsive PBAEs are highly relevant to cancer immunotherapy because tumor tissues provide several endogenous triggers, including acidic pH, elevated ROS, and altered redox balance microenvironments. For cancer immunotherapy, stimuli-responsive PBAEs should be designed not only to enhance nucleic acid delivery, but also to translate tumor-specific biological cues into controlled immune activation, antigen presentation, and durable therapeutic responses. However, this area remains insufficiently explored. Future work should therefore connect PBAE stimulus responsiveness with immune function, not only gene delivery efficiency, to better establish their potential as next-generation cancer immunotherapy platforms.[122] Moreover, there is limited data on repeated administration, where may be required to sustain antigen expression, immune activation, or therapeutic gene silencing.

Long-term toxicity and immune compatibility remain insufficiently explored for many PBAE systems. Although PBAEs are generally less cytotoxic than conventional cationic polymers, most studies focus on short-term cytotoxicity or single-dose in vitro and in vivo evaluation. More systematic studies are needed to assess repeated-dose safety, chronic inflammation, complement activation, cytokine release, organ toxicity, and clearance of polymer degradation products. For example, activation of antigen-presenting cells by PBAE nanoparticles has been reported, indicating that immune stimulation should be evaluated carefully depending on the intended therapeutic context.[32]

In parallel, although PEGylation can improve serum stability and circulation, PEG-containing systems may elicit anti-PEG IgM and IgG responses in some populations.[70,123–126] This issue has become increasingly relevant following the clinical use of PBAE systems in the future. PEG alternatives (e.g., pPSar [74], poly(2-oxazoline)s (POx) [14,127,128], zwitterionic polymers) remain important but underexplored strategies for improving the translational safety of PBAE-based delivery systems.

For clinical translation, PBAE systems must show acceptable safety profiles, including low cytotoxicity, minimal hemolysis, limited complement activation, reduced inflammatory responses, and efficient clearance of both polymer and degradation products. Repeated-dose toxicity and immune compatibility are particularly important for applications such as mRNA therapeutics, siRNA therapy, gene editing, and cancer immunotherapy. Another key requirement is improved correlation between in vitro and in vivo performance. PBAEs that perform well

in simplified cell culture may fail in vivo because of protein corona formation, extracellular matrix barriers, immune clearance, organ-specific biodistribution, and tissue heterogeneity. Therefore, future studies should include serum-containing assays, primary cells, three-dimensional models, organoids, ex vivo tissues, and relevant animal models to better predict clinical behavior. Finally, translation will require standardized characterization and quality-control criteria.

6. Conclusion

PBAEs have emerged as versatile non-viral vectors for gene delivery because they combine biodegradability, tunable cationic charge, and structural flexibility. Unlike conventional cationic polymers such as PEI, PBAEs contain hydrolyzable ester backbones, protonatable amines, and functionalizable end-groups, enabling control over nucleic acid condensation, endosomal escape, intracellular release, and toxicity. Continued refinement of PBAE chemistry through rational design of backbone structure, branching, end-group modification, and stimuli-responsive motifs has substantially improved stability, targeting, and transfection efficiency both in vitro and in vivo. However, effective PBAE design requires balancing multiple interconnected parameters rather than maximizing a single property. Increased charge density, hydrophobicity, or branching can improve nucleic acid binding, compactness of polyplex, charges, cellular uptake, and endosomal escape, but may also increase cytotoxicity, aggregation, or nonspecific interactions. Similarly, surface shielding can improve serum stability and biocompatibility, but may reduce uptake or cargo protection if not properly optimized. Stimuli-responsiveness is another important design feature of PBAEs, enabling cargo release in response to specific biological cues. pH-responsive PBAEs promote endosomal escape under acidic conditions, while redox- and ROS-responsive systems support intracellular or disease-site-specific release. Light-responsive PBAEs offer external spatiotemporal control, but their in vivo use is limited by poor tissue penetration and potential phototoxicity. Although dual- or multi-stimuli systems can improve release precision and targeting, their increased synthetic complexity and scalability challenges must be carefully considered. Therefore, future PBAE development should focus on establishing clearer structure–property–function relationships supported by standardized reporting of physicochemical properties, biological performance, and safety outcomes. With sustained interdisciplinary effort, smart PBAE systems are poised to play a central role in next-generation gene-based therapeutics.

CRediT authorship contribution statement

Zihnil A.I. Mazrad: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Seema Agarwal:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Acknowledgments

S.A. and Z.A.I.M. gratefully acknowledge and thank support from the Alexander von Humboldt Foundation through a Humboldt Research Fellowship.

Data availability

No data was used for the research described in the article.

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