HHS Public Access

Author manuscript

Biochemistry. Author manuscript; available in PMC 2022 April 06.

Published in final edited form as:

Biochemistry. 2021 April 06; 60(13): 966–990. doi:10.1021/acs.biochem.0c00860.

Disulfide Bridging Strategies in Viral and Non-viral Platforms for Nucleic Acid Delivery

Kingshuk Dutta[1], **Ritam Das**[1,4], **Jewel Medeiros**[1,4], **S. Thayumanavan**[*,1,2,3,4]

[1]Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, United States

[2]Department of Biomedical Engineering, University of Massachusetts, Amherst, Massachusetts 01003, United States

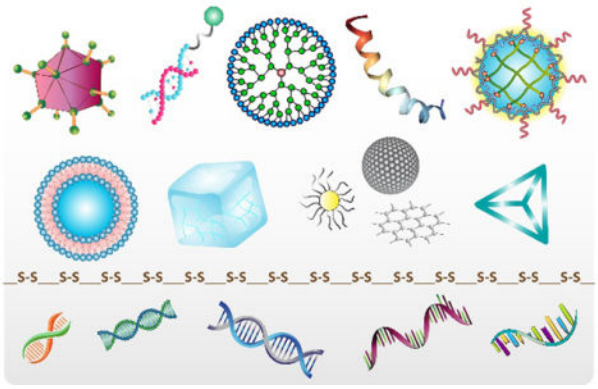
[3]Molecular and Cellular Biology Program, University of Massachusetts, Amherst, Massachusetts 01003, United States

[4]The Center for Bioactive Delivery- Institute for Applied Life Sciences, University of Massachusetts, Amherst, Massachusetts 01003, United States

Abstract

Self-assembled nanostructures that are sensitive to environmental stimuli are promising nanomaterials for drug delivery. In this class, disulfide containing redox-sensitive strategies have gained enormous attention for its wide applicability and simplicity in nanoparticle design. In the context of nucleic acid delivery, numerous disulfide-based materials have been designed relying on covalent or non-covalent interactions. In this review, we highlight major advances in disulfide-containing materials designs for nucleic acid encapsulation, including covalent nucleic acid conjugates, viral vectors or virus-like particles, dendrimers, peptides, polymers, lipids, hydrogels, inorganic nanoparticles and nucleic acid nanostructures. Our discussion will focus on the context of materials design and their impact on addressing the shortcomings of the current know-hows in the intracellular delivery of nucleic acids.

Graphical Abstract



[*]**Corresponding Author:** S. Thayumanavan, thai@chem.umass.edu.

1. Introduction

Advances in understanding biochemical pathways at cellular level have brought new insights into the possibility of treating challenging diseases with nucleic acid therapeutics.[1–3] Multiple nucleic acid candidates are either identified or chemically synthesized to intervene cellular processes for modulating gene expressions. Antisense oligonucleotides (ASO), plasmid DNA (pDNA), short interfering RNA (siRNA), microRNA (miRNA) and messenger RNA (mRNA) have become the most promising candidates for gene therapy.[4–8] While ASO, siRNA and miRNA act to repress translation via binding to mRNA and stop production of undesired proteins, pDNA and mRNA function to increase synthesis of a requisite protein in cells. Thus, with various nucleic acid design modalities it is now possible to expand the portfolio of biologic drugs with a promise to overcome severe side-effects and toxicity from small molecule drugs.

Similar to many other biologic candidates, however, nucleic acid therapeutics must reach to the target cellular site without alterations in their chemical structure and biological function. Although the conceptual basis for this field is quite mature, the bottleneck of intracellular delivery of nucleic acid still remains to be the major hurdle in successful translation to clinic.[3, 9] The nucleic acid cargo faces a variety of environmental threats like nuclease-mediated cleavage, opsonization and aggregation. Additionally, their inherent physical properties such as size, shape, molecular weight and hydrophilicity also deter them from crossing the hydrophobic cell membrane to reach the target work-site. Notably, the endocytosis process that traffics the molecules into intracellular space traps the therapeutics in a vesicle like structure, endosome. Release from the endosome is one of the prevailing hurdles in any macromolecular drug delivery. These practical challenges have inspired the development of suitable strategies to fill up the quintessential requirements for nucleic acid delivery.

Generally, two tactics are elucidated in literature: i) structural modifications of nucleic acid via chemical conjugation of functionalities; and ii) encapsulation into delivery vectors to resist effects from adverse environments and boost cellular delivery at target localization.[10, 11] Also, spaciotemporal control on the delivery activity is instrumental to circumvent the barriers producing desired biological response, which might be addressable by both tactics. Advances in synthetic and materials chemistry have offered numerous options for nucleic acid delivery. Newly developed bioconjugation techniques along with smart design of materials consisting polymers, peptides, lipids and inorganics have enabled to strategize a successful delivery route.[12, 13] One of the heavily explored materials design include structural manipulations that can be sensitized against environmental cues to trigger functional responses. In multi-step nucleic acid delivery perspective, responsive materials that sense changes in physiological conditions such as pH, redox, ATP, enzyme concentration, inflammation, and hypoxic conditions could be helpful. To this end, redox-based strategies can be of particular importance due to the ~3 log differences in redox-potentials between intra- and extracellular milieu, which is often more substantial between healthy and diseased cells.[14, 15] The thiol-disulfide based molecular redox-controllers such as glutathione (GSH), cysteine and thioredoxin precisely maintain complex cellular redox homeostasis.[16] Attracted by this phenomena, fascinating disulfide based redox-sensitive

systems have been delineated in literature to conduct drug delivery inside diseased cells. This concise review will specifically highlight disulfide-containing systems for nucleic acid delivery (Figure 1). We will discuss various designs of delivery systems from a materials perspective emphasizing both covalent and non-covalent approaches for nucleic acid encapsulation.

2. Covalent modifications on nucleic acids: redox-responsive *carrier-free* approaches

Some of the key hurdles that limit successful development of nucleic acid therapeutics are poor stability and pharmacokinetic properties of the biomacromolecules during systemic circulation. Although attractive designs of drug carriers are available to address this setback, a straightforward yet powerful strategy would be to attach various ligands that can provide desired biodistribution characteristics without altering the therapeutic outcome. Bioconjugation of specific ligands to nucleic acids can enhance cellular uptake, provide stability and targeting ability.[17–19] Compared to carrier-based systems that include complex, multistep and often difficult-to-control processes, carrier-free techniques can provide attractive incentives in case of distinct molecular characterizations and quality control.

2a. Conjugation to small molecule, lipid and polymer.

Attaching hydrophobic small molecules has often perceived as a notable strategy to carry nucleic acids across hydrophobic lipid bilayers of the cell membrane. Consistent with this thought, cholesterol conjugated non-coding RNAs were synthesized for repairing mitochondrial DNA mutations.[20] However, compared to a disulfide bridged conjugate, a pH-responsive hydrazine bearing RNA conjugate performed better in cellular transfection and mitochondrial genome manipulation. Another study looked at linker modification on cholesterol-conjugated anti-PCSK9 antisense oligonucleotide including disulfides for improving hepatic accumulation.[21] Further, the effect of cholesterol conjugation was tested in a study that compared gene silencing and immunogenic effects of TAT (48–60)-, penetration- and cholesterol-conjugated siRNA acting on p38 MAP kinase.[22] Similar to the co-delivery strategy, hydrophobic drug doxorubicin was conjugated to the sense strand of an siRNA via a self-immolative linker containing disulfide bond for targeting EWS/Fli1 protein relevant for Ewing's sarcoma.[23] Although hydrophobizing with lipids or small molecules had served as a typical procedure to enhance cellular penetration, this could lead to nanoassembly formation due to amphiphilicity of the oligonucleotide conjugates in aqueous media leading to reduced efficacy and voiding the original purpose. Inspired by the recent progress in disulfide-based systems showing interactions with cell surface thiols for direct cellular internalization,[24, 25] antisense DNA and siRNA molecules were tagged with multiple disulfide moieties at the chain end via phosphoramidite chemistry (Figure 2a).[26] The strategy enabled ultrafast delivery of therapeutics (within 10 min) along with silencing of ApoB expression relevant for hypercholesterolemia. Building on this, the cationic charge of the amino groups from the linker was masked in another study with the help of disulfide units, which was due to be unmasked under reducing environment to deliver nucleic acids for gene silencing.[27] Apart from the above examples, covalent conjugates of DNA and dendron bearing disulfide bridges have been also pursued for gene delivery.[28]

Unlike covalent conjugation, polyelectrolytic complexation of nucleic acids, specifically with smaller candidates like siRNA and miRNA, might not be conducive to incur sufficient stability in biological environment. Thus, adapting the idea of antibody-drug conjugates, multiple siRNA constructs were covalently bonded via cleavable disulfide linkages to the PLL chain of a RGD tagged PEG-PLL polymer.[29] The intracellular delivery and release of siRNA from the conjugate was confirmed from FRET studies and silencing of luciferase gene. An interesting strategy to further improve the covalent conjugation of nucleic acids includes the concept of 'dynamic polyconjugates' (Figure 2b).[30] Here, the amphipathic poly(vinyl ether) polymer backbone was attached with masking PEG chains for circulation stability and hepatocyte targeting ligand N-acetylgalactosamine (NAG or GalNAc) through pH-responsive carboxylated dimethyl maleamate groups that was amenable to cleavage in endosome by exposing cationic amines inducing destabilization of endosomal membrane.[30] The siRNA was tagged to the chain end with the help of disulfide bonds for redox-responsive cleavage. Also, this reversible covalent conjugation strategy efficiently demonstrated siRNA-mediated knock-down of apoB and ppara genes in mouse liver. The concept of polyconjugate was extended in another similar concept, wherein an endosomolytic poly(amido amine disulfide) polymer was synthesized to attach siRNA through bio-reducible disulfide linkages for silencing apoB gene.[31]

2b. Peptide nucleic acid (PNA).

PNAs are artificially synthesized DNA analogues wherein the deoxyribose phosphate backbone is replaced with N-(2-aminoethyl)glycine units.[32] For therapeutic purposes, PNAs are considered to have antisense activity for binding to specific mRNA halting gene expression.[33] However, like other nucleic acids PNAs are cell-membrane impermeable owing to their hydrophilicity. A simple approach was taken to address this issue, wherein a hydrophobic triphenylphosphonium (TPP) cation was tethered to a PNA via disulfide-linkage for GSH-mediated release in cytosol.[34] The delivered PNA, complementary to mRNA of mouse *Pax2* gene, was able to downregulate the Pax2 protein by >85%, whereby the non-disulfide control PNA could not produce any antisense effect. Releasing the PNA in original form without any remnant of the linkers is also critical for preserving its function. Built on this aim, a self-immolative strategy was conceived to link PNA with TPP cation via carbamate-disulfide bond.[35] The conjugates were shown to undergo GSH triggered thiol-disulfide reshuffling to free PNA. The system utilized a 16-mer PNA for intracellular delivery and showed anti-HIV activity via targeting TAR region of HIV-1 gene. To further aid in the cellular delivery, a covalently conjugated PNA to mesoporous silica nanoparticle (MSNP) was employed targeting Bcl-2 gene.[36] The disulfide-bridged PNA showed redox-mediated release and reduced Bcl-2 protein expression to 30% compared to untreated cells.

No doubt that covalent modifications are interesting strategies that have the potential to be implemented in the clinic. In fact, covalently modified siRNA is now a feasible product in pharmaceutical market (ONPATTRO® or patisiran).[37] Moving forward, recent studies in disulfide bridging approaches could serve as potential therapies to address many existing challenges with safety and biodegradability concerns.

3. Nucleic acid carriers with disulfide-sensitive functionalities

While conjugation of small molecules to nucleic acids offered certain advantages, the cargo itself remains exposed to degradative nucleases. Carrier-based approaches remain attractive for the protection of the nucleic acids from harsh biological environment and trafficking to the target location, which are otherwise difficult to achieve solely via carrier-free strategies. Major players in this sub-group are discussed below.

3a. Modified viral vectors or virus-like particles.

Viral vectors or virus-like particles (VLPs) are one of the primarily tested approaches to deliver therapeutics mimicking the viral mechanism of infection.[38–41] These are composed of either the original viral capsids or artificially assembled capsid proteins transformed into spherical hollow structures with the desired choice of nucleic acids encapsulated inside these.[41, 42] As the original genetic materials are removed from the capsids, those are supposed to lack infectious properties. In the construction of artificial mimics, disulfide crosslinking is heavily used only to impart structural stability to the capsid in VLPs,[43, 44] however evidences of disulfide chemistry in delivery is rare. A recent study constructed VLPs via reversibly crosslinking different capsid variants through a homobifunctional cross-linker DTSSP (3,3′-dithiobis (sulfosuccinimidylpropionate)) to stably encapsulate tRNA and siRNA up to 74% and 45%, respectively.[45] The construct not only showed silencing of the targeted luciferase gene, but also provided protection of the nucleic acids from enzymatic degradation. Although non-disulfide-based strategies are reported on viral vectors or its mimics, the systems still need to provide rigorous mechanistic insights on the delivery pathway and in vivo efficacy without toxicity issues. Also, viral vectors and VLPs are not completely devoid of eliciting immune responses; rather VLPs have beneficial effects for utilization in vaccine delivery. Incorporation of disulfide bonds might become effective to reduce the burden of immunogenic concerns for viral vectors, albeit needs further investigations.

3b. Dendrimers.

In the context of non-viral vectors, dendritic gene delivery agents have been of great research interest due to its tunable structural features to generate low-to-high generation molecules for optimizing electrostatic interactions with nucleic acids. While high generation dendrimers are perfectly suitable for nucleic acid complexation owing to their high cationic charge density, they are found to impart cytotoxicity. In contrary, low generation molecules are relatively safe while less efficient in gene silencing. To reach a practical solution to this paradox, research approaches have always been weighing to balance the two outcomes. In the past, poly(amidoamine) (PAMAM),[46, 47] poly(L-lysine) (PLL),[48, 49] PAMAM-PEG-PLL,[50] PLL-PEG-PLL[51] or PLL-pLac-PLL[52] (pLac: poly(L-lactide) based dendrimers or dendritic polycations had been explored for complexation of nucleic acids. Due to the higher pK_a (~10) of primary terminal amines incorporated into the dendron structure, they can complex with anionic nucleic acids at physiological pH. However, higher order structures raise concerns on cytotoxicity and immunogenicity issues from high cationic charge density. In an effort to reduce toxicity via designing smaller degradable dendrons, disulfide-bridged lysine-based dendritic structures were utilized in synergistic combination with cationic

lipids to complex DNA.[53] Similarly, lipoic acid-derived crosslinkable dendritic amphiphiles were also optimized for optimal self-assembly with siRNA.[54] Higher generation PAMAM dendrimers are considered to be as efficient as polyethyleneimine (PEI) in gene transfection, but their synthesis is labor intensive and thus costly. As a remedy, a second generation (G2) PAMAM dendrimer was synthesized and crosslinked via a disulfide based (3,3′-dithiodipropionic acid-di(N-succinimidyl ester), DSP) linker (Figure 3a).[55] The disulfide crosslinked G2 PAMAM dendrimer showed significantly higher transfection of pDNA for eGFP and luciferase genes in comparison to G2 or G5 control PAMAM dendrimers (Figure 3b). Although there are commercial transfection agents based on dendrimers, such as Superfect and Priofect, their clinical translation is limited by the increased cationic charge-induced cytotoxicity. While disulfide-based approaches in dendrimers have elucidated a plausible solution to the toxicity concerns, the research interest seems to be lacking in recent time.

3c. Cell penetrating peptides (CPPs).

Protein transduction domains or CPPs are short amino acid sequences that are membrane active. Traditionally, CPPs like TAT, HIV-TAT, oligoarginine, melittin, penetratin, transportan, are known to promote cellular uptake including macromolecular cargoes.[56–60] In this class, utilization of specific amino acids could serve three purposes: (i) complexation with nucleic acids by charge complementarity; (ii) increased interaction with cell membrane via both electrostatic and covalent interactions; (iii) influence direct uptake or endosomal escape via ‘proton sponge’ effect. The introduction of disulfide based CPPs have become popular recently to control stimuli-responsive release in biological milieu, impart biodegradability and thereby reducing toxicity. Following this concept, a combination of amino acids, viz. arginine, histidine and cysteine, were molded into a CPP along with a hydrophobic stearyl moiety (Figure 4a).[61] Cysteine served the purpose to crosslink the nanoparticle and introduce redox-responsive release of the luciferase-siRNA under in vitro and in vivo conditions. Disulfides had also been strategically incorporated into cyclical helical polypeptide, PPABLG-SH, to impart complex stability via crosslinking, specifically for small siRNAs where initial complexation had suffered due to rod-like conformationally rigid structure of the peptide.[62] In another scenario, disulfide constrained L-isomers of cyclic amphiphatic peptides, $Ac{-}C(FKFE)_2CG{-}NH_2$ and $Ac{-}C(WR)_4CG{-}NH_2$, were found efficient in silencing TTF1 gene in A549 cells and mouse lung cells presumably due to reduction and proteolytic degradation of the peptides to effectively unpack siRNAs inside cytosol.[63] Inspired by the viral capsid stability via covalent crosslinking based on disulfides to protect the genomic material from harsh environment, a dendritic disulfide bridged lipopeptide containing arginine clusters and hydrophobic oleyl double-tails was designed mimicking the viral envelope.[64] The non-viral vector was shown to transfect better than conventional PEI (~380 fold) and efficacies at lower N/P ratios compared to analogous non-disulfide control lipopeptides (N/P 10 vs. N/P 40). Although CPPs are one of the oldest materials utilized in drug delivery for more than three decades,[65] disulfide-based approaches in CPPs are rather new. As disulfide bonds seem to hold multi-dimensional advantages in gene delivery imparting stimuli-responsiveness and presumably influencing cellular uptake and endosomal escape as well, integration of disulfide into CPPs hold promises and needs further exploration.

3d. Polymers.

Nanoparticles constructed with polymeric materials have two functional advantages, tunable physicochemical properties and robust structure, which inherently promise better control in nucleic acid encapsulation and stability in systemic circulation. In this class of materials, both synthetic and natural polymers have been widely used for nucleic acid complexation. While natural polymers represent biocompatible materials with satisfactory encapsulation stability, synthetic versions offer a large arena of customizable materials that suits the choice of applications and critical environmental factors. In this context, disulfide-based approaches in synthetic polymer domain bring in long-demanding biodegradability feature addressing the key safety concerns with the artificial polymeric materials. Several disulfide bridged redox-sensitive strategies have been explored with polymers (Figure 5) and are classified below based on their structures.

Polyethylenimine (PEI).—Considered as the gold-standard for nucleic acid transfection, various PEI polymers have been exploited with a range of molecular weight distributions and structural parameters. Although high MW and branched structure in PEI are considered for better efficacy, those also bring in cytotoxic effects in comparison to low MW or linear polymers.[66–68] As much of the toxicity issues arise from charge-interactions with sub-cellular components, cellular processes and non-biodegradability of the PEI, a simple yet robust approach would be to incorporate disulfide-based PEI to introduce polyplex degradability under reducible cellular environment at a reasonable timescale (Figure 6a).[69, 70] In an effort to introduce degradability, low MW PEI was reversibly crosslinked with two disulfide based crosslinker dithiobis(succinimidylpropionate) (DSP) and dimethyl-3,3’-dithiobispropionimidate.2HCl (DTBP) to achieve efficient transfection.[71] Afterwards, dithiodipropionic acid or cystine crosslinked low MW (< 4.6 kDa) linear PEI was developed to reveal high transfection efficacy (≈60%) along with high cellular survival (>90%) across a wide range of cell types.[68] A similar strategy to develop disulfide containing linear poly(ethylenimine sulfide) (l-PEIS) with variable amine densities also provided evidence of this nanoparticle design over gene delivery.[72]

Earlier results showed that the sulfhydryl-activated pore-forming endosomolytic protein Listeriolysin O (LLO) attached with protamine via disulfide linkage could efficiently transfect pDNA in multiple cell lines.[73, 74] Extending this to PEI-based systems, LLO-S-S-PEI construct was designed to reveal a comparable transfection of pDNA and better cell viability with low MW PEI (1.8 kDA) compared to the high MW variant (25 kDA PEI).[75] Moreover, peptide conjugated bio-reducible PEI derivatives were explored for targeted delivery to the brain. Mimicking the viral transfection method, rabies virus glycoprotein (RVG) peptide was attached to PEG-PEI polymer encapsulated with pDNA to cross the blood-brain-barrier targeting acetylcholine receptor (nAchR) of brain neuronal cells.[76] In an interesting study, branched thiolated PEI (25 kDa) was compared with disulfide crosslinked derivatives via reacting with DTT.[77] Optimized crosslinking was found to transfect DNA better presumably due to elevated interactions with cell membrane and efficient unpackaging of nucleic acids inside cytosol, whereas either low or high crosslinking degree under- or over-stabilize the polyplexes leading to diminished chances of success in gene delivery. In an attempt to temporally control the delivery of co-encapsulated siRNA (for P-gp) and

doxorubicin in a dual stimuli-responsive scaffold, a redox-sensitive C16-alkyl grafted PEI (C16-S-S-PEI) nanoparticle was assembled with hydrophobized light-sensitive doxorubicin and PLGA.[78] The optimized system demonstrated sequential release of the drugs to provide chemotherapeutic effect in multi-drug resistance phenomena. The drug delivery outcome can be ameliorated via synergistic effect of sequential dual therapeutics delivery. Recently, a study demonstrated that delivery of miRNA-21 to ovarian cancer cells through a redox-sensitive branched PEG2k-PEI polymer system could help eliminate the cis-platin resistance through upregulation of tumor suppressor PDCD4 and downregulation of apoptosis inhibitor c-IAP2.[79, 80]

Polylysine (PLL) and poly(oligo-arginine).—Early examples of DNA transfection agents in PLL based systems with redox-sensitive moieties include PLL polymers with decorated surfaces by protective stimuli-responsive sheath of crosslinked disulfide bonds for ‘lateral stabilization’ as well as cytosolic delivery at high concentrations of glutathione (GSH).[81] Recent advances in PEGylation strategies helped to ameliorate circulation half-life, obstruct protein opsonization, and maintain nanoparticle stability through repulsive interactions.[82–84] Following this, a mPEG-S-S-PLL catiomer construct was developed responsive to intracellular redox conditions to detach the mPEG unit for delivery of pDNA expressing luciferase gene.[85] Further developments of dual stimuli-based PLL systems involves mPEG-S-S-PLL-glutaraldehyde star catiomer incorporated with acid labile imine and redox-sensitive disulfide units. The pH sensitive imine crosslinker in the star PLL architecture was hypothesized to undergo cleavage under acidic tumor or endosomal environment releasing the decrosslinked smaller mPEG-SS-PLL fragment to be processed by the reducing cytosolic environment to release pDNA cargoes for eGFP and luciferase gene expressions.[86]

Other potential amino acid-based strategies involve poly(oligoarginine)s wherein the cell penetrating capability of these positively charged protein transduction domain (PTD) mimics had been explored for nucleic acid transport inside cells. Oligoarginines hydrophobized with cholesterol (Chol-R9) had been developed to deliver siRNA targeting VEGF protein.[87] To reduce toxicity of the system, disulfide reduction induced fragmentation was introduced through a cysteine based oligomeric structure, Cys-(D-R9)-Cys, to deliver pDNA for luciferase expression in mice lungs, siRNA for VEGF gene suppression under in vivo tumor model and pDNA of HO-1 gene in ischemia/reperfusion (I/R)-induced brain stroke animal models.[88–90]

Poly(β-amino ester) (PAE).—Amongst hydrolytically degradable polymers, PAEs comprise a promising class of polymeric scaffold for nucleic acid encapsulation.[91] To introduce flexibility of incorporating additional targeting functionality, a pyridyldithio moiety was incorporated in polymer side chain.[92] This strategy had demonstrated integrin targeted delivery of pDNA.[93] In another scenario, disulfide moieties were incorporated in the PAE polymer backbone instead, showing redox-sensitive chain degradation as well as efficient pDNA transfection.[94] Another major purpose of incorporation of disulfide moieties is to provide a complementary yet faster degradation kinetics which was otherwise unachievable with PAEs undergoing slow hydrolysis kinetics.[95–97] These dual-responsive

systems had been able to co-deliver either doxorubicin or methotrexate and pDNA targeting hepatomas.[96, 98] PAE polymer was further engineered to introduce a dendritic structure aiming at better nucleic acid condensation and protection compared to the linear variations. A hyperbranched PAE was developed incorporating degradable disulfide units for gene transfection in adipose-derived stem cells and astrocytes with a transection efficacy of 52–77% (Figure 6).[99] Furthermore, the strategy was tested for clinically suitability with minicircle DNA delivery to elicit neurite outgrowth from model cell line.

Poly(amido amine) (PAA).—The cationic nature of PAAs, originated from the tertiary amine linkages in the linear amide polymer backbone, had been employed to capture nucleic acids.[100–102] Unlike poly(amino ester)s, the stability of PAAs under hydrolytic condition is governed by the stable amide bond relative to the ester. In an effort to introduce stimuli-sensitivity and bio-reducibility in the gene delivery vector, a series of PPAs were synthesized via Michael-addition of various primary amines with cystamine bis-acrylamide (Figure 6c).[103, 104] The amine candidates containing histamine or hydrophobic side chains, e.g., amino butanol, amino pentanol and methoxypropylamine showed higher pH buffering capacity for efficient endosomal disruption and transfection efficacy in comparison to PEI.[103, 105, 106] When this strategy was extended to investigate PAA polymers with oligoamine side chains with variable spacer lengths, aminoethylene incorporated polymers were found to perform better for pDNA transfection in COS-7 cells compared to aminopropylene polymers owing to the higher buffering capability of aminoethylenes.[107] In order to further improve, a library PAAs with 34 different amine side chains were chosen based on learnings from an earlier study with poly(amino ester)s.[108, 109] When tested for pDNA transfection efficacy of BMP-2 genes in tonsil-derived mesenchymal stem cells, bis(3-aminopropyl)piperazine incorporated PAA showed effective nucleic acid condensation, improvements of osteogenic potentials and expressions of related genes.[109] Processing of internalized polyplexes inside cytosol through GSH mediated decrosslinking have direct consequences in genetic delivery. Levels of intracellular GSH can influence the rate and efficacy of this process. An interesting study reported complexation of four different nucleic acids, e.g., antisense oligonucleotide, siRNA, mRNA and pDNA with disulfide-bearing PAA containing 1-(2-aminoethyl)piperazine to study the effect of cellular GSH levels towards gene delivery.[110] While reducible PAA transfect better compared to a non-reducible one in various pancreatic cancer cell lines with variable surface thiol and cellular GSH contents, only pDNA delivery was found to be improved with an increase in GSH levels in the cell lines.[110] In addition, spatiotemporal control of nucleic acid delivery is also critical to avoid premature leakage of the therapeutic drug, specifically when utilizing disulfide based redox systems due to its general sensitivity to variable GSH concentrations. To control biodegradation kinetics under different GSH levels, increased steric crowding was introduced via incorporation of methyl groups adjacent to disulfide bonds in the PAA polymers.[111] While at low GSH concentration (0.001–0.01 mM) 2-methyl cystamine disulfide PAA variant remained stable, stimuli-responsive release was observed for higher concentration (1–10 mM), along with higher pDNA transfection efficacy with reduced toxicity. Amongst other approaches, utilization of hyperbranched PAA for targeted delivery of pDNA via hyaluronic acid decoration or EGFR siRNA delivery via iRGD peptide functionalization were also notable.[112, 113]

Poly(disulfide).—Another class of redox-sensitive biodegradable material, exploited for nucleic acid encapsulation, is polydisulfides.[25, 114] In an early example of poly(disulfide) synthesis for nucleic acid complexation, a solid phase reaction was utilized to first synthesize ionizable or cationic dithiol monomer which was subsequently polymerized oxidatively in DMSO.[115] Both pDNA and siRNA for luciferase genes were successfully delivered with this molecular construct. In another effort, poly(cystaminebisacrylamide-diaminohexane) modified with arginine was reported to enhance cellular uptake and silence VEGF genes via siRNA delivery.[116] In order to study the effect of side chain pendant cationic units, three polydisulfides were synthesized with diamino-ethane, -butane and -hexane groups.[117] All of these structures showed efficient pH buffering capacity between pH 7.4–5.1, while the hexaethylene spacer performed 7-fold better in pDNA expression for luciferase gene compared to PEI 25 kDa polymer. As polydisulfides can be made to mimic polyarginines, it was hypothesized that these could also possess efficient cell-penetrating behavior to be utilized for therapeutic delivery directing into cytosol avoiding natural endosomal pathway.[25, 118] For nucleic acid delivery, polydisulfide modified mesoporous silica nanoparticles were encapsulated with antagomir and small molecule inhibitors for miR-21 to demonstrate the cellular uptake and GSH-mediated controlled release manipulating gene expressions (Figure 7a).[119] As co-delivery of small molecule drugs and biologics therapeutics have shown promising therapeutic outcome, a polydisulfide conjugated star polymer with β-cyclodextrin core was designed to encapsulate miRNA-203 and camptothecin drug (Figure 7b).[120] The polymer showed efficient cytosolic delivery of the therapeutics with synergistic reducing of cancer cell viability and silencing of surviving gene expression.

Amphiphilic random and block copolymers.—Self-assembly of polymeric materials has enabled to achieve difficult encapsulation goals.[14, 121] Tunable architectures of polymeric materials have been utilized to perform several challenging and wide-spread applications, e.g., catalysis, tissue engineering, encapsulation and stabilization of actives and drug delivery.[122–124] In the context of nucleic acid encapsulation, polymeric nanoparticles have widened the scope and opportunity to target nucleic acids with different structural features and sizes through numerous flexible design options. One of the interesting strategies in redox-responsive polyplex design involve ‘non-cationic’ encapsulation of nucleic acids. The logical approach for complexation of negatively charged nucleic acids involve designing cationic materials, which in turn promotes cytotoxicity owing to the interactions with proteins and sub-cellular components under in vivo conditions. A meaningful solution towards addressing this issue could be alteration or removal of cationic charge of the materials without hampering the encapsulation stability, which forms the inspiration for designing a N-methylated random copolymer of PEG and pyridyldisulfide (PDS) to encapsulate RNA.[125] A disulfide based crosslinking reaction was employed to shed-off the cationic moiety while stably holding the nucleic acid cargo.[125, 126] This supramolecular approach demonstrated successful delivery of dsTuba1a RNA in sensitive mouse embryo cells with minimal toxicity. In order to boost encapsulation efficacy and biocompatibility, a reverse-emulsion type approach was envisaged with cationic N-methylated p(PDSMA-co-DodecylMA) random copolymer (Figure 8).[14] The final nanoparticle decorated with zwitterionic lipids showed efficient delivery and silencing efficacy for eGFP, PLK1 and

MDR1 genes. Similar disulfide mediated delivery strategy was further explored for gene editing purposes via CRISPR-Cas9 delivery.[127]

One of the interesting candidates in block copolymers for nucleic acid encapsulation is polyionic complex (PIC) micelles made out of PEG and poly(aspartamide) blocks having N-(2-aminoethyl)-2-aminoethyl groups (PEG-P[Asp-(DET)]), which undergoes pH dependent protonation to exert 'proton-sponge' effect overcoming the barriers of endosomal entrapment.[128] Although incorporation of PEG in original P[Asp-(DET) polymer format enabled stability and longevity in systemic circulation, PEG palisade impeded gene transfection efficacy.[129] To overcome this issue, the polymer was incorporated with detachable PEG units via introduction of disulfide linker to form PEG-S-S-P[Asp(DET)] copolymer.[129] Even though luciferase gene expression with the disulfide-based system was slightly delayed in comparison to P[Asp-(DET) due to the processing time of disulfide bonds inside cytosol, pDNA transfection efficacy was found to be comparable to PEI.

In an effort to universally deliver multiple nucleic acids like DNA, mRNA, and Cas9 RNP, a cationic block copolymer (P[Asp-AED-ICA]-PEG) was synthesized incorporating imidazole groups for helping endosomal escape and disulfide bonds for intracellular glutathione responsiveness.[130] To deliver CRISPR-Cas9 systems, this approach was further modified to generate cationic disulfide bridged p(BAC-TET)-based polymers crosslinked via adamentane-β-CD chemistry along with non-crosslinked version (Figure 9a).[131] Both crosslinked and non-crosslinked versions revealed superior gene delivery along with genome-editing efficacy.

Combination therapy has the ability to circumvent many delivery barriers and increase the therapeutic window. In a recent approach, amphiphilic PEG-lipid decorated polyprodrug (polyHCPT, HPCT: 10-hydroxycamptothecin) nanoparticle was designed for nucleic acid encapsulation (Figure 9b).[132] The polymeric nanoparticle was also surface decorated with lactobionic acid for selective targeting through asialoglycoprotein receptors and simultaneously deliver HPCT drug and siRNA for Bcl-2 protein silencing to induce apoptosis.

Natural polymers.—In order to impart high colloidal stability, low toxicity and targeting ability, a natural dextran-based polymer with similar benefits like PEG was chemically modified to incorporate disulfide linked 1,4-bis(3-aminopropyl)piperazine (BAP) groups conjugated with folate ligand.[133] This cationic dextran conjugate with 30 BAP residues not only showed high pDNA delivery efficacy even in serum containing media, they could also successfully target SKOV-3 tumors under in vivo conditions to express LacZ gene. Extending the portfolio of natural polymers for gene delivery, a stearic amine tagged chitosan-based nanoparticle equipped with disulfide linkages was designed to co-deliver paclitaxel and Bcl-2 siRNA.[134] Similarly, another redox-responsive cationic glycopolymer based on galactose, P(LAEMA-st-AEMA-st-BMAC), was synthesized to encapsulate siRNA for EGFR silencing in HeLa cells.[135]

With a rich history of available chemistry and evolving strategy, polymeric materials represent one of the most promising as well as highly explored field for drug delivery

applications. With several encouraging results with disulfide containing polymers, it would be interesting to see successful clinical translations of these materials for nucleic acid delivery.

3e. Lipid-based nanoparticles.

Drug delivery systems based on lipids are well explored in the context of nucleic acid delivery in cellulo and in vivo due to their favorable biocompatible properties.[136–139] As cell membranes are comprised of phospholipids, lipidic nanocarriers have a natural tendency to interact with the physiological membrane due to structural similarity to facilitate uptake of nucleic acids. Moreover, owing to their relatively lower molecular weight compared to many other conventionally used synthetic materials, lipid-based delivery vectors display a lower risk of undesirable immunogenic effects. Because of these factors lipid-based delivery systems are well accepted in clinical trials for nucleic acid based therapies.[136] However, one of the major issues encountered by these systems is related to the transfection efficacy of the nucleic acid therapeutics, which can be mainly attributed to the inefficient release of the encapsulated payload due to strong binding interactions between the nucleic acids and the nanocarrier even after internalization in cells. This, in turn, limits the available number of therapeutic molecules to achieve desired gene silencing efficiency. An attractive solution could be designing smart lipid-based delivery vehicles which can readily release the encapsulated payload in response to distinct intracellular stimulus such as redox potential.[110]

Liposomes, vesicular structures comprised of lipid molecules, have gained significant interest in the field of nucleic acid delivery because of the relatively modular and straight forward formulation procedures.[136, 140] Developments in bioreducible liposomes have provided interesting examples for nucleic acid encapsulation wherein disulfide bonds are used as spacers to introduce rapid lipid degradation in reducing environment in the cytosol.[141] Early reports discussed the development of disulfide containing lipids, 1’, 2’-dioleoyl-sn-glycero-3’-succinyl-2-hydroxyethyl disulfide ornithine conjugate (DOGSDSO) or cholesteryl hemidithiodiglycolyl tris(aminoethyl)amine conjugate (CHDTAEA) for pDNA (pGL3-luciferase) delivery, revealing up to 50 times higher transgene expression compared to non-disulfide controls.[142, 143] In another approach, a new redox sensitive liposome comprised of triazine-based disulfide-bridged *gemini* surfactant SS14 had been utilized alongside supporter lipids DOPE and DOPC for delivering pDNA (pEGFP-N1 and pCMV-GLuc).[144, 145] Unsaturated dioleoyl groups of the *gemini* surfactant and phosphatidylethanolamine moiety of the DOPE lipid helped boost transfection efficacy. The study demonstrated a 3-fold higher gene transfection efficiency compared to the commercially available Lipofectamine 2000 for the DOPC/DOPE/SS14 formulation with ~50% (mol/mol) SS14 content. Similarly, another disulfide bridged H-shaped *gemini* like cationic lipid was synthesized for delivering GFP, EGFR and VEGF siRNA to investigate the gene silencing efficiency.[146, 147] In comparing with non-disulfide containing cationic lipid controls, the study showed superior efficacy of the disulfide-bridged lipoplexes towards endosomal escape and gene silencing, which could be the result of cellular break-down and disassembly of the lipoplex to monomeric units leading to release of siRNA. To improve the stability of an antisense oligonucleotide, a thiol-modified phosphorylated 18mer oligodeoxyribonucleotide (ODN, G3139) was conjugated with DOPE to generate DOPE-S-

S-ODN, which was then encapsulated in a liposomal composition consisting DOTAP, PC and TPGS lipids.[148] The antisense effect of hydrophobized ODN in the prepared liposomes was evaluated by Bcl-2 gene down regulation in KB cells which showed a reduction of Bcl-2 protein by ~58% in comparison to ~64% efficiency for positive control oligofectamine.

In the context of biodegradable or bioreducible lipids, a comprehensive understanding of the structure-property relationship towards improving gene delivery efficacy is highly desirable. In an interesting study, six bioreducible lipidoids were synthesized via Michael addition of aliphatic amines with acrylates incorporating degradable disulfide bonds.[149] Control lipidoids were also designed by replacing the disulfide bond with C-C bond to recognize the effect of bioreducibility. The lipoplex prepared with disulfide containing C16 alkyl chain bonded to N,N-dimethyl-1,3-propanediamine showed suppression of GFP and PLK1 gene expressions down to 28% and 25%, respectively, whereas non-bioreducible lipidoids could not show discernable silencing. Furthermore, the combinatorial library of bioreducible lipids was extended to include multiple primary and secondary amines serving as the lipidoid head group for the delivery of Cas9:single-guide (sg)RNA complex.[150] In this study, lipidoids with twelve different ionizable cationic headgroups were designed and screened for efficient delivery of negatively supercharged GFP-Cre and Cas9:sgRNA complexes in cells. With promising results in delivering active GFP-Cre to >90% cells via lipids containing 14,16 or 18C tails, the efficiency of the designed lipids were further investigated to deliver Cas9:sgRNA complex into EGFP expressing cells. Results showed ~70% loss in EGFP expression using lipids with 14C hydrophobic tails and three distinct amine head groups. However, a key challenge in broadening the spectrum of CRISPR/Cas9-based genome editing techniques is unwanted off-target effects and mutagenesis. To address this problem, bioreducible lipidoid nanoparticles had been utilized to efficiently administer Cas9 mRNA and sgRNA simultaneously into cells (Figure 10).[151] Compared to Cas9 plasmid, the corresponding mRNA was found to be relatively safer option to minimize insertional mutagenesis.[151–153] First, the gene transfection efficiency of the prepared liposomes, containing disulfide-tagged lipids, cholesterol, DOPE, DSPE-PEG2000 and nucleic acids, was thoroughly screened using luciferase knock out to identify promising lipidoid candidate.[151] The selected lipidoid containing disulfide showed significantly higher endosomal escape capability than the non-bioreducble control group. Further studies revealed 40% GFP knockout within 24 h that increased up to 90% after 36 h of treatment. In vivo studies showed PCSK9 down regulation to 20% upon treatment with lipidoid nanoparticle with Cas9 mRNA/sgPCSK9 nucleic acids.

Lipid based systems represent a favorable candidate for drug delivery in pharmaceutical industry. Similarity in structure and property of lipids with their natural siblings have raised their chances of success in drug delivery applications. Although there are marketed nucleic acid drugs that utilize liposomal vector, disulfide-based approaches are yet to be rigorously explored in clinic.

3f. Hydrogel.

While many nucleic acid delivery agents are reported to have efficient encapsulation and circulation stability, those often fall short in robust targeting mechanism and require

multiple dosages. This reduces patient compliance and increases risk of cytotoxicity. A local biodegradable material like hydrogel that can deliver therapeutics through a controlled mechanism could aid in resolving these issues. The three-dimensional crosslinked network in hydrogel had served to host therapeutics ranging from small molecule drugs to biomacromolecules.[154–157] In an effort to precisely control the physicochemical properties of hydrogels, a particle replication in nonwetting template (PRINT) technology was developed for encapsulation of siRNA through both electrostatic and covalent encapsulation methods.[158] To ensure redox-mediated release of the nucleic acid from PEG-modified cationic 2-aminoethyl methacrylate-based hydrogel, siRNA was covalently modified with a disulfide containing self-immolative linker before encapsulation into hydrogel matrix. While the transfection efficacies were comparable for all hydrogel systems, the disulfide-containing one showed significant luciferase gene silencing compared to non-disulfide and naked siRNA containing hydrogel systems. Another example of covalently attaching siRNA for controlling homogeneous distribution, release kinetics and minimization of toxicity from transfection agent include a dextran hydrogel system.[159] The thiol modified siRNA was attached to photo-crosslinkable mono(2-acryloyloxyethyl)succinate–modified dextran (DEX-MAES) hydrogel. The release of siRNA was facilitated by both hydrolysis and redox-triggered cleavage of disulfide bonds to silence GFP expression. Although there is a growing interest in hydrogel materials for biomacromolecules delivery, disulfide containing materials are only being explored recently. Combining unique physicochemical properties and redox-sensitive feature, hydrogels could offer many promising applications via nucleic acid delivery which might not be otherwise attainable conveniently.

3g. Inorganic nanoparticles.

Inorganic nanomaterials consisting gold, iron oxide, quantum dots, carbon and mesoporous silica have been well known for their unique physicochemical properties leading to promising applications in cellular imaging and therapeutics delivery.[160–162] Although several approaches have shown successful delivery and cellular internalization of nucleic acids, the release of the encapsulated cargo from the packed vehicle presents a potential challenge in obtaining desired extent of gene transfection.[163] Extension of stimuli-responsive materials platform to inorganic nanoparticles has broadened the possible scope of therapeutics development. Continuing our discussion, designing redox-responsive nanomaterials has manifested encouraging results in the context of nucleic acid transfection and safety profile.[161, 164, 165]

Au nanoparticles (AuNPs).—AuNPs have gained significant research interest in controlled delivery of genes due to the ease of synthesis, unique surface plasmon resonance and thermal ablation capabilities.[166–169] To design a GSH-responsive gene delivery vehicle, cationic trimethylammonium-modified mixed monolayer protected gold clusters (MMPCs) have been synthesized for delivery of functional DNA.[170] Large differences of glutathione concentrations in extra- and intra-cellular spaces[171] had been utilized to release the DNA from MMPC through the suggested charge based interactions, which was validated through ethidium bromide assay with a 37mer DNA in a dose-dependent manner. The GSH-mediated release of bound DNA showed transcription recovery T7 RNA polymerase. Inspired from these results, MMPCs with 2 nm Au core decorated with a tetra(ethylene

glycol)-containing cationic ligand and a Bodipy dye had been investigated for enhanced cellular uptake and GSH-sensitive release of the Bodipy dye.[15] As the Au-core served as a quencher, dye fluorescence remained quenched immediately upon cellular internalization, after which de-quenching started within several hours of post-processing via cellular GSH. A similar concept was tested in a subsequent study, wherein amino acid functionalized AuNPs were designed in order to obtain an enhanced gene transfection efficiency by tuning the surface charge on the nanoparticles.[172] DNA delivery via the lysine dendron modified AuNP resulted ~28 times superior activity of expressed reporter β-gal gene compared to polylysine transfection agent with no apparent cytotoxicity.

Though redox-responsive AuNPs have garnered significant amount of success as powerful gene carriers, the transfection efficiencies of those suffer from lack of specificity to target cells. Simple introduction of oligoarginine moieties can greatly enhance the cellular uptake of genes both in cancer and normal cells, causing undesired off-target effects and wastage of therapeutic genes.[173] Therefore, in an elegant approach bio-reducible AuNP-pDNA complex was coated with TAT polypeptide (pTAT) and hyaluronic acid for targeted gene delivery.[174] Notably, pTAT had been used for its excellent efficiency in cellular uptake and hyaluronic acid (HA), a ligand of CD44 receptors, had been coated to AuNP to achieve cell specific targeting. The delivered nanoplexes showed significantly enhanced transfection efficiency and luciferase (by pGL3 pDNA) expression after incubation with hydrophobic cell penetrating glutathione analogue GSH-OEt, which suggested the influence of intracellular GSH over gene delivery efficacy. In another study, EGFR targeting peptide GE11[175] and cell penetrating octaarginine (R8)[176] peptide were conjugated onto AuNPs through Au-S bonds enabling redox-mediated cleavage and cargo release (Figure 11a).[177] The gene transfection efficiency of prepared nanoplexes was monitored for pDNAs (pGL3 and pGFP) encoding luciferase and GFP genes, which revealed synergism of GE11 and R8 in transfection. Further, in vivo studies for shEGFR delivery via the nanoplexes also showed promising anti-tumor activity. With an aim to target nucleic acid delivery in soild tumor, a unimer polyion complex (uPIC) nanostructure with tunable size was designed via coating AuNP with charged matched PEG-PLL/siRNA complex.[178] The study investigated luciferase gene silencing using both in vitro and in vivo models.

Mesoporous silica nanoparticles (MSNs).—High surface area, control on morphology, pore size distribution and available chemistries for surface functionalization have made MSNs as one of the highly researched drug carriers, including nucleic acids.[179–181] Given the fact that many drug carriers have serious side effects, controlled delivery systems that enable stimulated release of cargo are getting popular. In this context, redox-responsive MSNs modified with suitable functionalities for stimulated release of nucleic acid drugs are quite interesting. In a recent study, multilayered nanocomplexes (MLNs) were designed consisting TAT-MSN as inner cationic core with anionic poly(allylamine hydrochloride)-citraconic anhydride (PAH-Cit) and cationic galactose modified trimethyl chitosan (GTC) sequentially coated on top of it.[182] The nanoparticle was utilized for encapsulation of DOX and siVEGF was complexed with the surface cationic charges. Reductive cleavage of the GTC layer was hypothesized for the liberation of siRNA inside cells. Dual delivery of DOX and siVEGF resulted ~84% gene

silencing in vitro and potent anti-tumor activity in vivo. In another study, PEI immobilized MSNs were further crosslinked with redox responsive dithiobis(succinimidyl propionate) (DSP) for delivering siVEGF.[183] In this design, short chain PEI was utilized as a capping agent after loading siRNA onto MSNs and crosslinked to secure the nanoparticle from cargo leakage. In vitro studies showed a significant downregulation of protein expression by ~75% compared to ~40% with non-crosslinked MSN-siRNA/PEI nanocomposites. The results were further evaluated by monitoring the VEGF protein and mRNA levels in the treated cells, which showed a significant inhibition of both by ~46% and ~55% respectively.

Controlled release of drugs associated with degradation of delivery vector serves as an encouraging lead towards development of biodegradable carriers with reduced toxicity.[184] Similarly, to realize simultaneous release of drugs and nucleic acid, a self-destructive MSN nanocarrier was designed using a one-pot method for encapsulation of DOX and pDNA (Figure 11b).[185] In vitro gene transfection studies via luciferase assay revealed increasing transfection efficiency with increasing N/P ratio of the nanocomplex, which was attributed to the increased pDNA condensation. Encouraged by the promising results of in vitro studies these systems were further evaluated by in vivo studies. Experiments on c6 glioma in vivo model substantiated an efficient and simultaneous delivery of DOX and P53 to achieve better anti-tumor efficacy. Similar synergistic co-delivery approaches include chitosan modified MSNs (MSN-SS-CP) or PDS-MSNs as redox responsive nanocarriers for simultaneous delivery of DOX-P53 gene or DOX-Bcl-2 siRNA into cells inducing apoptosis.[186, 187]

Nanoparticle based delivery systems often experience undesired off target effects attributed to lack of specificity and targeting ability.[188, 189] Addressing this AS1411 aptamer, known to strongly bind to cell surface nucleolin and inhibit anti apoptotic pathway in cancer cell via blocking of NF-kB signaling, was utilized in conjugation with gelatin-siloxane nanogels (GS-NGs) to deliver luciferase targeting siRNA sensitized by redox-environment selectively in A549 and 3T3 fibroblast cells.[190] The study showed enhanced uptake of AS1411 conjugated nanogels compared to the control non-targeted nanogel.

Miscellaneous redox-responsive inorganic nanoparticles.—Apart from Au and silica, various other inorganic nanoparticles modified with redox responsive functionalities for triggered release of nucleic acids have been designed and studied. One of such studies discussed the design of disulfide modified single walled carbon nanotubes (SWNTs) and their application as a potential gene delivery vector.[191] The functionalized nanotubes were prepared by stably suspending short SWNTs in aqueous medium with the aid of surface adsorbed PEG-functionalized phospholipids via hydrophobic and van der Waals forces. Thiol modified biomolecules were incorporated into SWNTs via a heterobifunctional crosslinker containing disulfide. Studies with cy3-labeled DTT showed rapid release of fluorescently labeled DNA upon reduction of the disulfide bonds, while excellent gene silencing efficiency with siRNA encoding lamin A/C protein was observed upon delivery through SWNTs. Similarly, PEI conjugates of SWNT bearing disulfides were found to show higher transfection efficacy of luciferase reporter gene compare to only PEI.[192] The study with 15 different vectors based on conjugation of SWNT and PEI through different spacers concluded the cysteine linker modified PEI (1.8 kDa)-SWNT conjugate was the most efficient in gene transfection showing >800 folds increase compared to PEI 25 kDa.

Apart from SWNT, carbon dots (CDs) have also gained significant interest in imaging and therapeutic platforms due to their biocompatibility, uniform size distribution and impressive quantum yield.[193, 194] However, unlike SWNT, reports on redox-responsive carbon nanodots as gene carriers are very limited. In a recent study, multi-functionalized CDs had been designed to efficiently deliver therapeutic siRNA to non-small cell lung cancer cells.[195] In this study, the CDs prepared by microwave pyrolysis of glycerol and PEI were treated with a disulfide containing crosslinker for imparting redox-sensitivity, which was further decorated with folic acids for targeting purposes. In vitro studies substantiated efficient release of two complexed siRNAs (cyclin B1 and EGFR) in a reductive environment resulting in reduction of H460 lung cancer cell viability up to ~30% in comparison to only ~60% while using single siRNA, revealing a combinatorial synergistic effect in play. Studies in mice model showed a dramatic decrease in tumor size as well as ~50% reduction in luciferase expression in tumor cells lasting for around 2 weeks.

Besides carbon nanotubes, iron oxide nanoparticles (ION) are also considered as gene carriers for its biocompatibility, degradability and unique magnetic properties.[196] In a recent study, IONs coated with redox responsive linker modified PEI (IONs@rPEI) were designed for high gene transfection efficiency with low cytotoxicity and also as a MRI contrast agent.[197] In vitro studies with the prepared nanocomposite material having pGL3 DNA showed significantly higher transfection efficiency compare to PEI (600 Da). This difference can be attributed to efficient redox responsive DNA release from IONs@rPEI nanocomposites, while higher polymer to DNA ratio in control PEI polymer-based systems retard the DNA release due to persistent electrostatic interactions.

Unlike MSNs, usage of silicon nanowires (siNW) in nucleic acid encapsulation is rather limited. In the context of redox sensitive systems, a PEI-modified renewable siNW surface with disulfides were introduced for encapsulation and delivery of pDNA.[198] The strategy not only showed recyclability and disulfide mediated delivery, but also proposed a different non-lysosomal delivery pathway.

Robust structural features and suitable functionalization methodologies have made inorganic nanomaterials stand out of other nucleic acid delivery platforms. Though disulfide mediated nucleic acid delivery has shown attractive opportunity, the overall degradability of the materials remains as a challenge that need rigorous attention to be able to move-forward these approaches further in clinical trials.

3h. Nucleic acid-based carriers.

DNA nanostructures are made up of strands of nucleic acids linked together and folded into shapes.[199] Moreover, DNA can be used to not only form linear structures but also to create junctions linked together by using sticky strands.[200] This is now thought to be a plausible method for drug delivery due to their high degree of biocompatibility.[201] DNA origami methods have enabled the community to make DNA nanostructures with void pockets for possible drug loading capabilities. However, DNA-modified delivery agents are often tedious to achieve without any established protocol for controlled release of encapsulated drugs. Incorporation of disulfide as a responsive unit could be beneficial for triggered release of nucleic acid drugs from these nanostructures. To this end, a DNA nanohydrogel Y-gel-Apt

was constructed with three nucleic acid monomers, each connected via disulfide bonds to enable GSH-mediated degradation (Figure 12a).[202] With the help of a S6 aptamer tagged with the nanostructure, the system was shown to be selectively internalized into A549 cells, instead of control untargeted HeLa cells. To check gene delivery efficacy, an antisense oligonucleotide for c-raf-1 mRNA and a DNAzyme targeting MMP-9 were conjugated into the DNA nanogel structure. In effect, the Y-gel-Apt system showed reduction in cell proliferation and silencing of target mRNA for MMP-9 protein only in A549 cells.

Non-cationic approaches for nucleic acid delivery are attractive as they promise attenuation of cytotoxicity from cationic charge of the carrier. Conceptualizing this idea, an siRNAsome was designed consisting of hydrophilic siRNAs linked via disulfide bonds to poly(N-isopropylacrylamide) (pNIPAM) polymer to create a core-shell type structure.[203] Understandably, the median shell was sensitive to redox-environment and temperature. The hydrophilic aqueous interior and hydrophobic median layer enabled the vesicular structure to simultaneously encapsulate both hydrophobic drug doxorubicin and hydrophilic derivative doxorubicin hydrochloride (Dox·HCl) and bovine serum albumin. The construct was also reported to deliver Pgp-siRNA for silencing Pgp mRNA in multi drug resistant (MDR) cells. Similar to this co-delivery strategy of multiple-drugs through same biodegradable nanoparticle, another nanocarrier was developed for nucleic acid delivery relying on DNA-origami derived delivery vector (Figure 12b).[204] An aptamer functionalized antisense oligonucleotide construct encapsulated with doxorubicin (Apt-Dox-origami-ASO) was prepared for controlled release of hydrophobic drug and ASOs in response to GSH. Two different ASOs targeting Bcl2 and P-gp genes were delivered in MDR cancer cell lines to achieve therapeutic benefit. Another instance of utilizing triangular DNA-nanostructures for treating MDR was reported to develop a disulfide-responsive system targeting both P-gp and surviving genes through RNAi (Figure 12c).[205] The siRNAs and DOX were delivered through this engineered origami structure attached with a targeting MUC1 aptamer to achieve gene silencing in vitro and in vivo. DNA-origami can be utilized for plug-and-play approach to assimilate multifunctional components to generate a better drug carrier. Similarly, a double-bundle DNA tetrahedron structure was designed conjugating ASOs targeting proto-oncogene *c-raf* via disulfide linkages and nuclear localization sequence (NLS)-tagged DNA.[206] The nanostructure revealed inhibition of cellular proliferation affecting viability through silencing *c-raf* gene expression.

Apart from these examples, other redox-responsive nucleic acid-derived nanocarriers are developed, but those are yet to be explored for nucleic acid drug delivery applications. Some of the interesting cases are discussed in brief. Inspired by the natural process of disulfide crosslinking to protect folded proteins, DNA strands were reversibly connected together through disulfide bridges to make a stable structure under denaturing conditions.[207] As the structures are responsive to reducing conditions in the cytosol, those were proposed to be utilized as redox-responsive drug carriers. Similarly, reversibly crosslinked DNA nanostructures were prepared using disulfides between 2’-deoxyuridine or 2’-deoxycytidine.[208] Out of four different motifs, the one containing eight helixes form a tube-like channel that could be used for future applications in drug delivery. A pre-requisite for an efficient drug delivery vehicle is to incorporate a smart release mechanism of the loaded therapeutic. Based on this, DNA nanostructures were designed that could be

formed and deformed with kinetic redox control via disulfide bonds.[209] Another switchable DNA self-assembly was created via cystamine and cysteamine interconversion.[210] The assembly and disassembly processes were controlled by the addition of peroxide and GSH, respectively which could be used as a stimuli-responsive cargo delivery system. Creation of a disulfide-containing molecular sticker (DSMS) on a DNA nanostructure was reported to aid in cellular uptake process.[211] DSMS was attached to the DNA nanostructures through salt bridges formed by the cationic guanidinium groups and the disulfide-containing tail helped in direct delivery to cells.

Nucleic acid-based nanostructures are one of the youngest candidates in nucleic acid delivery field. So, there is still a lot to learn about these materials in terms of toxicity, degradability, stability in circulation, half-life or renal clearance time, biodistribution pattern, and cargo release kinetics. Though incorporation of disulfide units has answered some of the questions raised above, the potential of this delivery platform is yet to be fully unfolded.

4. Conclusions and future directions

Biodegradability has become one of the key requirements in today's carrier-based nucleic acid delivery approaches to mitigate nanotoxicity concerns. Many outstanding research efforts have been devoted creating drug delivery platforms to comply with the ever-rising demands of the clinical trials. No surprise that the nuanced balance between the gene delivery efficacy and cytotoxicity is still difficult to achieve. In this context, stimuli-triggered materials that can respond towards subtle changes in environmental conditions are one of the most promising approaches to develop smart nano-carriers for systemic drug delivery. Disulfide based systems are particularly promising due to the stark differences in redox-potentials inside and outside cellular spaces. This specific criterion has been lucrative enough to develop numerous drug delivery strategies as discussed in this review. Though other stimuli like pH, temperature, light, enzyme and ROS are also promising, disulfide-based systems are probably the most appealing ones due to its simplicity, ease of functionalization and general applicability of the systems amid most in vivo conditions. Moreover, disulfide bond not only introduces biodegradability and provides a mechanism of drug release, but the functionality could also influence amphiphilicity and polarizability during nucleic acid condensation,[212, 213] favorably manipulate the pK_a of the tertiary amines when present at the β-position,[212] and contribute to cellular uptake and endosomal escape mechanisms.[214, 215]

Recent literature on disulfide-based systems showed both direct cellular uptake and endocytosis pathway for trafficking therapeutics.[216, 217] However, the precise role of disulfide in cellular internalization processes is yet to be fully unraveled by designing proper control systems. Also, limited studies have been performed on redox-sensitive strategies for mRNA and miRNA mediated gene therapy. In the context of endocytosis pathway, escape form the endosome is one of the prerequisites for designing any drug delivery agent. A rather unconventional way to address this challenge is the utilization of fusogenic delivery aids to either surpass the endocytic uptake or help evade endosome via fusion with cell and endosomal membranes, respectively. These fusogenic materials, e.g., fusogenic lipids

(DOPE, DMPC and DOTAP) or fusogenic peptide (KALA), not only directed cytosolic trafficking, those also helped overcome the structural limitations of liposomal systems when coated on rigid delivery vectors like polymers or inorganic nanomaterials.[14, 218–222] As fusogenic delivery platforms have shown improvement of efficacy for nucleic acid carriers, it might be an interesting strategy to couple these materials with redox-sensitive approaches to further enhance therapeutic efficacy.

Though nucleic acid therapeutics are still relatively new compared to other small molecule therapeutics, recent approvals of Onpattro, Tegsedi, Givlaari and Vyondys 53 are encouraging.[223–225] Even though the field has accomplished this feat, improvements are necessary to reach their full potential as therapeutics. Currently, the methods of nucleic acid delivery in clinics primarily comprise of lipid nanoparticles, conjugation systems like GalNAc conjugates, and a few polymeric systems based on PEG or PLGA polymers. Also, in order to reach the point of clinicals evaluations, suitable in vivo models need to be established for each specific disease. Few promising technologies, like LODER (Local Drug EluteR) and KRAS-LODER, utilize biodegradable PLGA polymer to deliver siRNA slowly over months.[226, 227] Lipid nanoparticles consisting ionizable lipids were used in potential therapeutics to deliver mRNAs for prophylactic (including SARS-CoV-2) and viral (Zika and Chikungunya) vaccines, cancer treatments, autoimmune and heart diseases.[228, 229]

Based on the recent developments, the progress in nucleic acid therapeutics is quite encouraging. However, going by the substantial unexplored pathways, it would be worthwhile to extend and explore existing disulfide-based redox sensitive strategies to various nucleic acid therapeutic candidates. With the lack of sufficient translatable technologies and historically poor clinical success rates in the nucleic acid delivery, the simple yet powerful disulfide bridging strategies could help to keep and deliver promises made.

Acknowledgements

We are thankful for the support from the U.S. Army Research Office (W911NF-15-1-0568) and partial support from the National Institutes of Health (GM-136395).

Abbreviations:

PAMAM
poly(amidoamine)

PLL
poly(L-lysine)

PLL-PEG-PLL
poly(L-lysine)-poly(ethylene glycol)- poly(L-lysine)

PLL-pLac-PLL
(pLac:poly(L-lactide)

PEI

polyethyleneimine

DSP
3,3′-dithiodipropionic acid-di(N-succinimidyl ester)

PPABLG-SH
poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl)-Lglutamate)-r-poly(γ-(4-((2-mercaptoethyl)aminomethyl)benzyl)-Lglutamate)

TTF-1
thyroid transcription factor-1

LLO
listeriolysin O

eGFP
enhanced green fluorescence protein

PAA
poly(amido amine)

GSH
glutathione

miR
microRNA

VEGF
vascular endothelial growth factor

PTD
protein transduction domain

BAP
1,4-bis(3-aminopropyl)piperazine

HO-1
heme oxygenase-1

P(Asp-AED-ICA)−PEG
poly(aspartic acid-(2-aminoethyl disulfide)-(4-imidazolecarboxylic acid))−poly(ethylene glycol)

p(BAC-TET)
poly(N,N′-bis(acryloyl)cystamine-co-triethylenetetramine)

P(LAEMA-st-AEMA-st-BMAC)
poly(2-lactobionamidoethylmethacrylamide-st-2-aminoethylmethacrylamide-st-N,N′-bis(methacryloyl)cystamine)

PLGA
poly (lactic-co-glycolic) acid

HPCT
10-hydroxycamptothecin

PDS
pyridyldisulfide

PLK1
polo-like kinase-1

MDR1
multidrug resistant-1

NAG
n-acetylgalactosamine

apoB
apolipoprotein B

ppara
peroxisome proliferator-activated receptor alpha

DSP
dithiobis(succinimidylpropionate)

DTBP
dimethyl-3,3’-dithiobispropionimidate.2HCl

RVG
rabies virus glycoprotein (RVG)

nAchR
acetylcholine receptor

DOGSDSO
1’, 2’-dioleoyl-sn-glycero-3’-succinyl-2-hydroxyethyl disulfide ornithine

CHDTAEA
cholesteryl hemidithiodiglycolyl tris(aminoethyl)amine conjugate

DOPE
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine

DOPC
1,2-dioleoyl-sn-glycero-3-phosphocholine

GLuc
gaussia luciferase

ODN
oligodeoxyribonucleotide

DOTAP
1,2-dioleoyl-3-trimethylammoniumpropane

PC
phosphatidylcholine/α-tocopheryl polyethylene glycol 1000 succinate

CRISPR/Cas9
clustered regularly interspaced short palindromic repeat associated protein 9

Dox·HCl
doxorubicin hydrochloride

pNIPAM
poly(N-isopropylacrylamide)

P-gp
P-glycoprotein

Bcl2
B-cell lymphoma 2

DSMS
disulfide-containing molecular sticker

TPP
triphenylphosphonium

MMPC
monolayer protected gold clusters

uPIC
unimer polyion complex

PEG-PLL
poly(ethylene glycol)-b-poly(L-lysine)

MLNs
multilayered nanocomplexes

PAH-Cit
poly(allylamine hydrochloride)-citraconic anhydride

GTC
galactose modified trimethyl chitosan

DSP
dithiobis(succinimidyl propionate)

SWNTs
single walled carbon nanotubes

ION
iron oxide nanoparticles

CD
carbon dots

DMPC
1,2-dimyristoyl-sn-glycero-3-phosphocholine

LODER
Local Drug EluteR

References

(1). (2019) Delivering the Promise of RNA Therapeutics. Nat. Med 25, 1321. [PubMed: 31501598]

(2). Hamann A, Nguyen A, and Pannier AK (2019) Nucleic Acid Delivery to Mesenchymal Stem Cells: A Review of Nonviral Methods and Applications. J. Biol. Eng 13, 7. [PubMed: 30675180]

(3). Jiang Z, and Thayumanavan S (2020) Noncationic Material Design for Nucleic Acid Delivery. Adv. Ther 3, 1900206.

(4). Crooke ST (2017) Molecular Mechanisms of Antisense Oligonucleotides. Nucleic Acid Ther. 27, 70–77. [PubMed: 28080221]

(5). Bennett CF, and Swayze EE (2010) RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform. Annu. Rev. Pharmacol. Toxicol 50, 259–293. [PubMed: 20055705]

(6). Juliano RL (2016) The Delivery of Therapeutic Oligonucleotides. Nucleic Acids Res. 44, 6518–6548. [PubMed: 27084936]

(7). Shin H, Park S-J, Yim Y, Kim J, Choi C, Won C, and Min D-H (2018) Recent Advances in RNA Therapeutics and RNA Delivery Systems Based on Nanoparticles. Adv. Ther 1, 1800065.

(8). Hajj KA, and Whitehead KA (2017) Tools for Translation: Non-Viral Materials for Therapeutic mRNA Delivery. Nat. Rev. Mater 2, 17056.

(9). Nguyen J, and Szoka FC (2012) Nucleic Acid Delivery: The Missing Pieces of the Puzzle? Acc. Chem. Res 45, 1153–1162. [PubMed: 22428908]

(10). Gallas A, Alexander C, Davies MC, Puri S, and Allen S (2013) Chemistry and Formulations for siRNA Therapeutics. Chem. Soc. Rev 42, 7983–7997. [PubMed: 23857524]

(11). Khvorova A, and Watts JK (2017) The Chemical Evolution of Oligonucleotide Therapies of Clinical Utility. Nat. Biotechnol 35, 238–248. [PubMed: 28244990]

(12). Ni R, Feng R, and Chau Y (2019) Synthetic Approaches for Nucleic Acid Delivery: Choosing the Right Carriers. Life 9, 59.

(13). Li Y, Maciel D, Rodrigues J, Shi X, and Tomas H (2015) Biodegradable Polymer Nanogels for Drug/Nucleic Acid Delivery. Chem. Rev 115, 8564–8608. [PubMed: 26259712]

(14). Dutta K, Bochicchio D, Ribbe AE, Alfandari D, Mager J, Pavan GM, and Thayumanavan S (2019) Symbiotic Self-Assembly Strategy toward Lipid-Encased Cross-Linked Polymer Nanoparticles for Efficient Gene Silencing. ACS Appl. Mater. Interfaces 11, 24971–24983. [PubMed: 31264399]

(15). Hong R, Han G, Fernández JM, Kim B. j., Forbes NS, and Rotello VM (2006) Glutathione-Mediated Delivery and Release Using Monolayer Protected Nanoparticle Carriers. J. Am. Chem. Soc 128, 1078–1079. [PubMed: 16433515]

(16). Moriarty-Craige SE, and Jones DP (2004) Extracellular Thiols and Thiol/Disulfide Redox in Metabolism. Annu. Rev. Nutr 24, 481–509. [PubMed: 15189129]

(17). Winkler J (2013) Oligonucleotide Conjugates for Therapeutic Applications. Ther. Deliv 4, 791–809. [PubMed: 23883124]

(18). Stasińska AR, Putaj P, and Chmielewski MK (2019) Disulfide Bridge as a Linker in Nucleic Acids' Bioconjugation. Part I: An Overview of Synthetic Strategies. Bioorg. Chem 92, 103223. [PubMed: 31494329]

(19). Stasińska AR, Putaj P, and Chmielewski MK (2020) Disulfide Bridge as a Linker in Nucleic Acids' Bioconjugation. Part Ii: A Summary of Practical Applications. Bioorg. Chem 95, 103518. [PubMed: 31911308]

(20). Dovydenko I, Tarassov I, Venyaminova A, and Entelis N (2016) Method of Carrier-Free Delivery of Therapeutic RNA Importable into Human Mitochondria: Lipophilic Conjugates with Cleavable Bonds. Biomaterials 76, 408–417. [PubMed: 26561937]

(21). Wada S, Yasuhara H, Wada F, Sawamura M, Waki R, Yamamoto T, Harada-Shiba M, and Obika S (2016) Evaluation of the Effects of Chemically Different Linkers on Hepatic Accumulations, Cell Tropism and Gene Silencing Ability of Cholesterol-Conjugated Antisense Oligonucleotides. J. Controlled Release 226, 57–65.

(22). Moschos SA, Jones SW, Perry MM, Williams AE, Erjefalt JS, Turner JJ, Barnes PJ, Sproat BS, Gait MJ, and Lindsay MA (2007) Lung Delivery Studies Using siRNA Conjugated to TAT(48–60) and Penetratin Reveal Peptide Induced Reduction in Gene Expression and Induction of Innate Immunity. Bioconjugate Chem. 18, 1450–1459.

(23). Gauthier F, Bertrand J-R, Vasseur J-J, Dupouy C, and Debart F (2020) Conjugation of Doxorubicin to siRNA through Disulfide-Based Self-Immolative Linkers. Molecules 25, 2714.

(24). Chuard N, Gasparini G, Moreau D, Lorcher S, Palivan C, Meier W, Sakai N, and Matile S (2017) Strain-Promoted Thiol-Mediated Cellular Uptake of Giant Substrates: Liposomes and Polymersomes. Angew. Chem. Int. Ed 56, 2947–2950.

(25). Fu J, Yu C, Li L, and Yao SQ (2015) Intracellular Delivery of Functional Proteins and Native Drugs by Cell-Penetrating Poly(Disulfide)s. J. Am. Chem. Soc 137, 12153–12160. [PubMed: 26340272]

(26). Shu Z, Tanaka I, Ota A, Fushihara D, Abe N, Kawaguchi S, Nakamoto K, Tomoike F, Tada S, Ito Y, Kimura Y, and Abe H (2019) Disulfide-Unit Conjugation Enables Ultrafast Cytosolic Internalization of Antisense DNA and siRNA. Angew. Chem. Int. Ed 58, 6611–6615.

(27). Shu Z, Ota A, Takayama Y, Katsurada Y, Kusamori K, Abe N, Nakamoto K, Tomoike F, Tada S, Ito Y, Nishikawa M, Kimura Y, and Abe H (2020) Intracellular Delivery of Antisense DNA and siRNA with Amino Groups Masked with Disulfide Units. Chem. Pharm. Bull 68, 129–132.

(28). Bell SA, McLean ME, Oh SK, Tichy SE, Zhang W, Corn RM, Crooks RM, and Simanek EE (2003) Synthesis and Characterization of Covalently Linked Single-Stranded DNA Oligonucleotide-Dendron Conjugates. Bioconjugate Chem. 14, 488–493.

(29). Bai QF, Liu J, Tang JN, Li Z, Zheng XJ, and Chen QX (2019) Redox-Responsive Polymeric RNAi Based on Multivalent Conjugation of siRNA for Improved Intracellular Delivery. Bioconjugate Chem. 30, 2777–2781.

(30). Rozema DB, Lewis DL, Wakefield DH, Wong SC, Klein JJ, Roesch PL, Bertin SL, Reppen TW, Chu Q, Blokhin AV, Hagstrom JE, and Wolff JA (2007) Dynamic Polyconjugates for Targeted in Vivo Delivery of siRNA to Hepatocytes. Proc. Natl. Acad. Sci. U. S. A 104, 12982–12987. [PubMed: 17652171]

(31). Parmar RG, Busuek M, Walsh ES, Leander KR, Howell BJ, Sepp-Lorenzino L, Kemp E, Crocker LS, Leone A, Kochansky CJ, Carr BA, Garbaccio RM, Colletti SL, and Wang W (2013) Endosomolytic Bioreducible Poly(Amido Amine Disulfide) Polymer Conjugates for the in Vivo Systemic Delivery of siRNA Therapeutics. Bioconjugate Chem. 24, 640–647.

(32). Nielsen PE, Egholm M, Berg RH, and Buchardt O (1991) Sequence-Selective Recognition of DNA by Strand Displacement with a Thymine-Substituted Polyamide. Science 254, 1497–1500. [PubMed: 1962210]

(33). Wang G, and Xu XS (2004) Peptide Nucleic Acid (PNA) Binding-Mediated Gene Regulation. Cell Res. 14, 111–116. [PubMed: 15115611]

(34). Filipovska A, Eccles MR, Smith RA, and Murphy MP (2004) Delivery of Antisense Peptide Nucleic Acids (PNAs) to the Cytosol by Disulphide Conjugation to a Lipophilic Cation. FEBS Lett. 556, 180–186. [PubMed: 14706847]

(35). Mehiri M, Upert G, Tripathi S, Di Giorgio A, Condom R, Pandey VN, and Patino N (2008) An Efficient Biodelivery System for Antisense Polyamide Nucleic Acid (PNA). Oligonucleotides 18, 245–256. [PubMed: 18707540]

(36). Ma X, Devi G, Qu Q, Toh DF, Chen G, and Zhao Y (2014) Intracellular Delivery of Antisense Peptide Nucleic Acid by Fluorescent Mesoporous Silica Nanoparticles. Bioconjugate Chem. 25, 1412–1420.

(37). Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, and Liang X-J (2020) Therapeutic siRNA: State of the Art. Signal Transduction Targeted Ther. 5, 101.

(38). Thomas CE, Ehrhardt A, and Kay MA (2003) Progress and Problems with the Use of Viral Vectors for Gene Therapy. Nat. Rev. Genet 4, 346–358. [PubMed: 12728277]

(39). Wang D, Tai PWL, and Gao G (2019) Adeno-Associated Virus Vector as a Platform for Gene Therapy Delivery. Nat. Rev. Drug Discovery 18, 358–378. [PubMed: 30710128]

(40). Rohovie MJ, Nagasawa M, and Swartz JR (2017) Virus-Like Particles: Next-Generation Nanoparticles for Targeted Therapeutic Delivery. Bioeng. Transl. Med 2, 43–57. [PubMed: 29313023]

(41). Nayak S, and Herzog RW (2010) Progress and Prospects: Immune Responses to Viral Vectors. Gene Ther. 17, 295–304. [PubMed: 19907498]

(42). Jeong H, and Seong BL (2017) Exploiting Virus-Like Particles as Innovative Vaccines against Emerging Viral Infections. J. Microbiol 55, 220–230. [PubMed: 28243941]

(43). Bundy BC, and Swartz JR (2011) Efficient Disulfide Bond Formation in Virus-Like Particles. J. Biotechnol 154, 230–239. [PubMed: 21536082]

(44). Lu Y, Chan W, Ko BY, VanLang CC, and Swartz JR (2015) Assessing Sequence Plasticity of a Virus-Like Nanoparticle by Evolution toward a Versatile Scaffold for Vaccines and Drug Delivery. Proc. Natl. Acad. Sci. U. S. A 112, 12360–12365. [PubMed: 26392546]

(45). Pretto C, and van Hest JCM (2019) Versatile Reversible Cross-Linking Strategy to Stabilize Ccmv Virus Like Particles for Efficient siRNA Delivery. Bioconjugate Chem. 30, 3069–3077.

(46). Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J, and Smith P (1985) A New Class of Polymers - Starburst-Dendritic Macromolecules. Polym. J 17, 117–132.

(47). Bielinska A, KukowskaLatallo JF, Johnson J, Tomalia DA, and Baker JR (1996) Regulation of in Vitro Gene Expression Using Antisense Oligonucleotides or Antisense Expression Plasmids Transfected Using Starburst PAMAM Dendrimers. Nucleic Acids Res. 24, 2176–2182. [PubMed: 8668551]

(48). Ohsaki M, Okuda T, Wada A, Hirayama T, Niidome T, and Aoyagi H (2002) In Vitro Gene Transfection Using Dendritic Poly(L-Lysine). Bioconjugate Chem. 13, 510–517.

(49). Yamagata M, Kawano T, Shiba K, Mori T, Katayama Y, and Niidome T (2007) Structural Advantage of Dendritic Poly(L-Lysine) for Gene Delivery into Cells. Bioorg. Med. Chem 15, 526–532. [PubMed: 17035030]

(50). Patil ML, Zhang M, and Minko T (2011) Multifunctional Triblock Nanocarrier (PAMAM-PEG-Pll) for the Efficient Intracellular siRNA Delivery and Gene Silencing. ACS Nano 5, 1877–1887. [PubMed: 21322531]

(51). Choi JS, Joo DK, Kim CH, Kim K, and Park JS (2000) Synthesis of a Barbell-Like Triblock Copolymer, Poly(L-Lysine) Dendrimer-Block-Poly(Ethylene Glycol)-Block-Poly(L-Lysine) Dendrimer, and Its Self-Assembly with Plasmid DNA. J. Am. Chem. Soc 122, 474–480.

(52). Li Y, Cui L, Li QB, Jia L, Xu YH, Fang Q, and Cao A (2007) Novel Symmetric Amphiphilic Dendritic Poly(L-Lysine)-b-Poly(L-Lactide)-b-Dendritic Poly(L-Lysine) with High Plasmid DNA Binding Affinity as a Biodegradable Gene Carrier. Biomacromolecules 8, 1409–1416. [PubMed: 17458996]

(53). Hardy JG, Love CS, Gabrielson NP, Pack DW, and Smith DK (2009) Synergistic Effects on Gene Delivery -Co-Formulation of Small Disulfide-Linked Dendritic Polycations with Lipofectamine 2000 (TM). Org. Biomol. Chem 7, 789–793. [PubMed: 19194595]

(54). Tschiche A, Thota BNS, Neumann F, Schäfer A, Ma N, and Haag R (2016) Crosslinked Redox-Responsive Micelles Based on Lipoic Acid-Derived Amphiphiles for Enhanced siRNA Delivery. Macromol. Biosci 16, 811–823. [PubMed: 26847397]

(55). Liu HM, Wang H, Yang WJ, and Cheng YY (2012) Disulfide Cross-Linked Low Generation Dendrimers with High Gene Transfection Efficacy, Low Cytotoxicity, and Low Cost. J. Am. Chem. Soc 134, 17680–17687. [PubMed: 23050493]

(56). Borrelli A, Tornesello AL, Tornesello ML, and Buonaguro FM (2018) Cell Penetrating Peptides as Molecular Carriers for Anti-Cancer Agents. Molecules 23, 295.

(57). Guidotti G, Brambilla L, and Rossi D (2017) Cell-Penetrating Peptides: From Basic Research to Clinics. Trends Pharmacol. Sci 38, 406–424. [PubMed: 28209404]

(58). Xie J, Bi Y, Zhang H, Dong S, Teng L, Lee RJ, and Yang Z (2020) Cell-Penetrating Peptides in Diagnosis and Treatment of Human Diseases: From Preclinical Research to Clinical Application. Front. Pharmacol 11, 697. [PubMed: 32508641]

(59). Blacklock J, Handa H, Soundara Manickam D, Mao G, Mukhopadhyay A, and Oupicky D (2007) Disassembly of Layer-by-Layer Films of Plasmid DNA and Reducible TAT Polypeptide. Biomaterials 28, 117–124. [PubMed: 16962657]

(60). Roy R, Jerry DJ, and Thayumanavan S (2009) Virus-Inspired Approach to Nonviral Gene Delivery Vehicles. Biomacromolecules 10, 2189–2193. [PubMed: 19722558]

(61). Tai Z, Wang X, Tian J, Gao Y, Zhang L, Yao C, Wu X, Zhang W, Zhu Q, and Gao S (2015) Biodegradable Stearylated Peptide with Internal Disulfide Bonds for Efficient Delivery of siRNA in Vitro and in Vivo. Biomacromolecules 16, 1119–1130. [PubMed: 25686200]

(62). Zheng N, Song Z, Liu Y, Zhang R, Zhang R, Yao C, Uckun FM, Yin L, and Cheng J (2015) Redox-Responsive, Reversibly-Crosslinked Thiolated Cationic Helical Polypeptides for Efficient siRNA Encapsulation and Delivery. J. Controlled Release 205, 231–239.

(63). Welch JJ, Swanekamp RJ, King C, Dean DA, and Nilsson BL (2016) Functional Delivery of siRNA by Disulfide-Constrained Cyclic Amphipathic Peptides. ACS Med. Chem. Lett 7, 584–589. [PubMed: 27326331]

(64). Chen XB, Yang J, Liang H, Jiang Q, Ke BW, and Nie Y (2017) Disulfide Modified Self-Assembly of Lipopeptides with Arginine-Rich Periphery Achieve Excellent Gene Transfection Efficiency at Relatively Low Nitrogen to Phosphorus Ratios. J. Mater. Chem. B, 5, 1482–1497. [PubMed: 32264639]

(65). Green M, and Loewenstein PM (1988) Autonomous Functional Domains of Chemically Synthesized Human Immunodeficiency Virus TAT Trans-Activator Protein. Cell 55, 1179–1188. [PubMed: 2849509]

(66). Kafil V, and Omidi Y (2011) Cytotoxic Impacts of Linear and Branched Polyethylenimine Nanostructures in A431 Cells. BioImpacts 1, 23–30. [PubMed: 23678404]

(67). Kunath K, von Harpe A, Fischer D, Peterson H, Bickel U, Voigt K, and Kissel T (2003) Low-Molecular-Weight Polyethylenimine as a Non-Viral Vector for DNA Delivery: Comparison of Physicochemical Properties, Transfection Efficiency and in Vivo Distribution with High-Molecular-Weight Polyethylenimine. J. Controlled Release 89, 113–125.

(68). Breunig M, Lungwitz U, Liebl R, and Goepferich A (2007) Breaking up the Correlation between Efficacy and Toxicity for Nonviral Gene Delivery. Proc. Natl. Acad. Sci. U. S. A 104, 14454–14459. [PubMed: 17726101]

(69). Gosselin MA, Guo WJ, and Lee RJ (2001) Efficient Gene Transfer Using Reversibly Cross-Linked Low Molecular Weight Polyethylenimine. Bioconjugate Chem. 12, 989–994.

(70). Xia W, Wang P, Lin C, Li Z, Gao X, Wang G, and Zhao X (2012) Bioreducible Polyethylenimine-Delivered siRNA Targeting Human Telomerase Reverse Transcriptase Inhibits HepG2 Cell Growth in Vitro and in Vivo. J. Controlled Release 157, 427–436.

(71). Gosselin MA, Guo W, and Lee RJ (2001) Efficient Gene Transfer Using Reversibly Cross-Linked Low Molecular Weight Polyethylenimine. Bioconjugate Chem. 12, 989–994.

(72). Lee Y, Mo H, Koo H, Park JY, Cho MY, Jin GW, and Park JS (2007) Visualization of the Degradation of a Disulfide Polymer, Linear Poly(Ethylenimine Sulfide), for Gene Delivery. Bioconjugate Chem. 18, 13–18.

(73). Portnoy DA, and Jones S (1994) The Cell Biology of Listeria Monocytogenes Infection (Escape from a Vacuole). Ann. N. Y. Acad. Sci 730, 15–25. [PubMed: 8080169]

(74). Saito G, Amidon GL, and Lee KD (2003) Enhanced Cytosolic Delivery of Plasmid DNA by a Sulfhydryl-Activatable Listeriolysin O/Protamine Conjugate Utilizing Cellular Reducing Potential. Gene Ther. 10, 72–83. [PubMed: 12525839]

(75). Choi S, and Lee K-D (2008) Enhanced Gene Delivery Using Disulfide-Crosslinked Low Molecular Weight Polyethylenimine with Listeriolysin O-Polyethylenimine Disulfide Conjugate. J. Controlled Release 131, 70–76.

(76). Son S, Hwang DW, Singha K, Jeong JH, Park TG, Lee DS, and Kim WJ (2011) RVG Peptide Tethered Bioreducible Polyethylenimine for Gene Delivery to Brain. J. Controlled Release 155, 18–25.

(77). Aravindan L, Bicknell KA, Brooks G, Khutoryanskiy VV, and Williams AC (2013) A Comparison of Thiolated and Disulfide-Crosslinked Polyethylenimine for Nonviral Gene Delivery. Macromol. Biosci 13, 1163–1173. [PubMed: 23749760]

(78). Wu M, Li J, Lin X, Wei Z, Zhang D, Zhao B, Liu X, and Liu J (2018) Reduction/Photo Dual-Responsive Polymeric Prodrug Nanoparticles for Programmed siRNA and Doxorubicin Delivery. Biomater. Sci 6, 1457–1468. [PubMed: 29770812]

(79). Jauanmardi S, Tamaddon AM, Aghamaali MR, Ghahramani L, and Abolmaali SS (2020) Redox-Sensitive, PEG-Shielded Carboxymethyl PEI Nanogels Silencing Microrna-21, Sensitizes Resistant Ovarian Cancer Cells to Cisplatin. Asian J. Pharm. Sci 15, 69–82. [PubMed: 32175019]

(80). Chan JK, Blansit K, Kiet T, Sherman A, Wong G, Earle C, and Bourguignon LYW (2014) The Inhibition of miR-21 Promotes Apoptosis and Chemosensitivity in Ovarian Cancer. Gynecol. Oncol 132, 739–744. [PubMed: 24472409]

(81). Oupicky D, Carlisle RC, and Seymour LW (2001) Triggered Intracellular Activation of Disulfide Crosslinked Polyelectrolyte Gene Delivery Complexes with Extended Systemic Circulation in Vivo. Gene Ther. 8, 713–724. [PubMed: 11406766]

(82). Harris JM, and Chess RB (2003) Effect of Pegylation on Pharmaceuticals. Nat. Rev. Drug Discovery 2, 214–221. [PubMed: 12612647]

(83). Veronese FM, and Mero A (2008) The Impact of PEGylation on Biological Therapies. BioDrugs 22, 315–329. [PubMed: 18778113]

(84). Belen LH, Rangel-Yagui CO, Beltran Lissabet JF, Effer B, Lee-Estevez M, Pessoa A, Castillo RL, and Farias JG (2019) From Synthesis to Characterization of Site-Selective PEGylated Proteins. Front. Pharmacol 10, 1450. [PubMed: 31920645]

(85). Cai XJ, Dong HQ, Xia WJ, Wen HY, Li XQ, Yu JH, Li YY, and Shi DL (2011) Glutathione-Mediated Shedding of PEG Layers Based on Disulfide-Linked Catiomers for DNA Delivery. J. Mater. Chem 21, 14639–14645.

(86). Cai X, Dong C, Dong H, Wang G, Pauletti GM, Pan X, Wen H, Mehl I, Li Y, and Shi D (2012) Effective Gene Delivery Using Stimulus-Responsive Catiomer Designed with Redox-Sensitive Disulfide and Acid-Labile Imine Linkers. Biomacromolecules 13, 1024–1034. [PubMed: 22443494]

(87). Kim WL, Christensen LV, Jo S, Yockman JW, Jeong JH, Kim YH, and Kim SW (2006) Cholesteryl Oligoarginine Delivering Vascular Endothelial Growth Factor siRNA Effectively Inhibits Tumor Growth in Colon Adenocarcinoma. Mol. Ther 14, 343–350. [PubMed: 16765648]

(88). Won YW, Kim HA, Lee M, and Kim YH (2010) Reducible Poly(Oligo-D-Arginine) for Enhanced Gene Expression in Mouse Lung by Intratracheal Injection. Mol. Ther 18, 734–742. [PubMed: 20029398]

(89). Won YW, Yoon SM, Lee KM, and Kim YH (2011) Poly(Oligo-D-Arginine) with Internal Disulfide Linkages as a Cytoplasm-Sensitive Carrier for siRNA Delivery. Mol. Ther 19, 372–380. [PubMed: 21081902]

(90). Hyun H, Won YW, Kim KM, Lee J, Lee M, and Kim YH (2010) Therapeutic Effects of a Reducible Poly (Oligo-D-Arginine) Carrier with the Heme Oxygenase-1 Gene in the Treatment of Hypoxic-Ischemic Brain Injury. Biomaterials 31, 9128–9134. [PubMed: 20828810]

(91). Green JJ, Langer R, and Anderson DG (2008) A Combinatorial Polymer Library Approach Yields Insight into Nonviral Gene Delivery. Accounts Chem Res 41, 749–759.

(92). Zugates GT, Anderson DG, Little SR, Lawhorn IEB, and Langer R (2006) Synthesis of Poly(β-Amino Ester)s with Thiol-Reactive Side Chains for DNA Delivery. J. Am. Chem. Soc 128, 12726–12734. [PubMed: 17002366]

(93). Majumder P (2018) Integrin-Mediated Delivery of Drugs and Nucleic Acids for Anti-Angiogenic Cancer Therapy: Current Landscape and Remaining Challenges. Bioengineering 5, 76.

(94). Lin C, Lammens TM, Zhong ZY, Gu H, Lok MC, Jiang X, Hennink WE, Feijen J, and Engbersen JFJ (2006) Disulfide-Containing Poly(β-Amino Ester)s for Gene Delivery. J. Controlled Release 116, e79–e81.

(95). Zhang S, Guo N, Wan G, Zhang T, Li C, Wang Y, Wang Y, and Liu Y (2019) pH and Redox Dual-Responsive Nanoparticles Based on Disulfide-Containing Poly(β-Amino Ester) for Combining Chemotherapy and Cox-2 Inhibitor to Overcome Drug Resistance in Breast Cancer. J. Nanobiotechnol 17, 109.

(96). Wang H, Wan G, Liu Y, Chen B, Chen H, Zhang S, Wang D, Xiong Q, Zhang N, and Wang Y (2016) Dual-Responsive Nanoparticles Based on Oxidized Pullulan and a Disulfide-Containing Poly(β-Amino) Ester for Efficient Delivery of Genes and Chemotherapeutic Agents Targeting Hepatoma. Polym. Chem, 7, 6340–6353.

(97). Liu Y, Li S, Feng L, Yu H, Qi X, Wei W, Li J, and Dong W (2016) Novel Disulfide-Containing Poly(β-Amino Ester)-Functionalised Magnetic Nanoparticles for Efficient Gene Delivery. Aust. J. Chem 69, 349–356.

(98). Zhang S, Wang D, Li Y, Li L, Chen H, Xiong Q, Liu Y, and Wang Y (2018) pH- and Redox-Responsive Nanoparticles Composed of Charge-Reversible Pullulan-Based Shells and Disulfide-Containing Poly(ß-Amino Ester) Cores for Co-Delivery of a Gene and Chemotherapeutic Agent. Nanotechnology 29, 325101. [PubMed: 29761789]

(99). Liu S, Gao Y, Zhou D, Zeng M, Alshehri F, Newland B, Lyu J, O'Keeffe-Ahern J, Greiser U, Guo T, Zhang F, and Wang W (2019) Highly Branched poly(β-Amino Ester) delivery of Minicircle DNA for Transfection of Neurodegenerative disease Related Cells. Nat. Commun 10, 3307. [PubMed: 31341171]

(100). Lin C, Zhong Z, Lok MC, Jiang X, Hennink WE, Feijen J, and Engbersen JF (2006) Linear Poly(Amido Amine)s with Secondary and Tertiary Amino Groups and Variable Amounts of Disulfide Linkages: Synthesis and in Vitro Gene Transfer Properties. J. Controlled Release 116, 130–137.

(101). Richardson SCW, Pattrick NG, Stella Man YK, Ferruti P, and Duncan R (2001) Poly(Amidoamine)s as Potential Nonviral Vectors: Ability to Form Interpolyelectrolyte Complexes and to Mediate Transfection in Vitro. Biomacromolecules 2, 1023–1028. [PubMed: 11710005]

(102). Christensen LV, Chang CW, Kim WJ, Kim SW, Zhong ZY, Lin C, Engbersen JFJ, and Feijen J (2006) Reducible Poly(Amido Ethylenimine)s Designed for Triggered Intracellular Gene Delivery. Bioconjugate Chem. 17, 1233–1240.

(103). Lin C, Zhong Z, Lok MC, Jiang X, Hennink WE, Feijen J, and Engbersen JF (2007) Novel Bioreducible Poly(Amido Amine)s for Highly Efficient Gene Delivery. Bioconjugate Chem. 18, 138–145.

(104). Ou M, Xu R, Kim SH, Bull DA, and Kim SW (2009) A Family of Bioreducible Poly(Disulfide Amine)s for Gene Delivery. Biomaterials 30, 5804–5814. [PubMed: 19615739]

(105). Lin C, Zhong ZY, Lok MC, Jiang XJ, Hennink WE, Feijen J, and Engbersen JFJ (2007) Random and Block Copolymers of Bioreducible Poly(Amido Amine)s with High- and Low-Basicity Amino Groups: Study of DNA Condensation and Buffer Capacity on Gene Transfection. J. Controlled Release 123, 67–75.

(106). Vader P, van der Aa LJ, Engbersen JFJ, Storm G, and Schiffelers RM (2011) Disulfide-Based Poly(Amido Amine)s for siRNA Delivery: Effects of Structure on siRNA Complexation, Cellular Uptake, Gene Silencing and Toxicity. Pharm. Res 28, 1013–1022. [PubMed: 21181546]

(107). Lin C, Blaauboer CJ, Timoneda MM, Lok MC, van Steenbergen M, Hennink WE, Zhong ZY, Feijen J, and Engbersen JFJ (2008) Bioreducible Poly(Amido Amine)s with Oligoamine Side Chains: Synthesis, Characterization, and Structural Effects on Gene Delivery. J. Controlled Release 126, 166–174.

(108). Green JJ, Langer R, and Anderson DG (2008) A Combinatorial Polymer Library Approach Yields Insight into Nonviral Gene Delivery. Acc. Chem. Res 41, 749–759. [PubMed: 18507402]

(109). Jeong H, Lee ES, Jung G, Park J, Jeong B, Ryu KH, Hwang NS, and Lee H (2016) Bioreducible-Cationic Poly(Amido Amine)s for Enhanced Gene Delivery and Osteogenic Differentiation of Tonsil-Derived Mesenchymal Stem Cells. J. Biomed. Nanotechnol 12, 1023–1034. [PubMed: 27305823]

(110). Manickam DS, Li J, Putt DA, Zhou QH, Wu C, Lash LH, and Oupicky D (2010) Effect of Innate Glutathione Levels on Activity of Redox-Responsive Gene Delivery Vectors. J. Controlled Release 141, 77–84.

(111). Elzes MR, Akeroyd N, Engbersen JF, and Paulusse JM (2016) Disulfide-Functional Poly(Amido Amine)s with Tunable Degradability for Gene Delivery. J. Controlled Release 244, 357–365.

(112). Gu J, Chen X, Ren X, Zhang X, Fang X, and Sha X (2016) CD44-Targeted Hyaluronic Acid-Coated Redox-Responsive Hyperbranched Poly(Amido Amine)/Plasmid DNA Ternary Nanoassemblies for Efficient Gene Delivery. Bioconjugate Chem. 27, 1723–1736.

(113). Guo Z, Li S, Liu Z, and Xue W (2018) Tumor-Penetrating Peptide-Functionalized Redox-Responsive Hyperbranched Poly(Amido Amine) Delivering siRNA for Lung Cancer Therapy. ACS Biomater. Sci. Eng 4, 988–996. [PubMed: 33418781]

(114). Morelli P, Bartolami E, Sakai N, and Matile S (2018) Glycosylated Cell-Penetrating Poly(Disulfide)s: Multifunctional Cellular Uptake at High Solubility. Helv. Chim. Acta 101, e1700266.

(115). Wang XL, Jensen R, and Lu ZR (2007) A Novel Environment-Sensitive Biodegradable Polydisulfide with Protonatable Pendants for Nucleic Acid Delivery. J. Controlled Release 120, 250–258.

(116). Kim SH, Jeong JH, Kim TI, Kim SW, and Bull DA (2009) Vegf siRNA Delivery System Using Arginine-Grafted Bioreducible Poly(Disulfide Amine). Mol. Pharm 6, 718–726. [PubMed: 19055368]

(117). Ou M, Wang XL, Xu R, Chang CW, Bull DA, and Kim SW (2008) Novel Biodegradable Poly(Disulfide Amine)s for Gene Delivery with High Efficiency and Low Cytotoxicity. Bioconjugate Chem. 19, 626–633.

(118). Gasparini G, and Matile S (2015) Protein Delivery with Cell-Penetrating Poly(Disulfide)s. Chem. Commun 51, 17160–17162.

(119). Yu C, Qian L, Ge J, Fu J, Yuan P, Yao SC, and Yao SQ (2016) Cell-Penetrating Poly(Disulfide) Assisted Intracellular Delivery of Mesoporous Silica Nanoparticles for Inhibition of miR-21 Function and Detection of Subsequent Therapeutic Effects. Angew. Chem. Int. Ed 55, 9272–9276.

(120). Yang WH, Yu CM, Wu CX, Yao SQ, and Wu SZ (2017) Cell-Penetrating Poly(Disulfide)-Based Star Polymers for Simultaneous Intracellular Delivery of Mirnas and Small Molecule Drugs. Polym. Chem, 8, 4043–4051.

(121). Khomein P, Dutta K, Gnanasekaran K, Gianneschi NC, and Thayumanavan S (2019) Spatiotemporal Control over the Host–Guest Characteristics of a Stimulus-Triggerable Trifunctional Polymer Assembly. Polym. Chem, 10, 1423–1430.

(122). Liechty WB, Kryscio DR, Slaughter BV, and Peppas NA (2010) Polymers for Drug Delivery Systems. Annu. Rev. Chem. Biomol. Eng 1, 149–173. [PubMed: 22432577]

(123). Dhandayuthapani B, Yoshida Y, Maekawa T, and Kumar DS (2011) Polymeric Scaffolds in Tissue Engineering Application: A Review. Int. J. Polym. Sci 2011, 290602.

(124). Shifrina ZB, Matveeva VG, and Bronstein LM (2020) Role of Polymer Structures in Catalysis by Transition Metal and Metal Oxide Nanoparticle Composites. Chem. Rev 120, 1350–1396. [PubMed: 31181907]

(125). Jiang ZW, Cui W, Prasad P, Touve MA, Gianneschi NC, Mager J, and Thayumanavan S (2019) Bait-and-Switch Supramolecular Strategy to Generate Noncationic RNA-Polymer Complexes for RNA Delivery. Biomacromolecules 20, 435–442. [PubMed: 30525500]

(126). Jiang ZW, Cui W, Mager J, and Thayumanavan S (2019) Postfunctionalization of Noncationic RNA-Polymer Complexes for RNA Delivery. Ind. Eng. Chem. Res 58, 6982–6991.

(127). Liu B, Ejaz W, Gong S, Kurbanov M, Canakci M, Anson F, and Thayumanavan S (2020) Engineered Interactions with Mesoporous Silica Facilitate Intracellular Delivery of Proteins and Gene Editing. Nano Lett. 20, 4014–4021. [PubMed: 32298126]

(128). Kanayama N, Fukushima S, Nishiyama N, Itaka K, Jang W-D, Miyata K, Yamasaki Y, Chung U. i., and Kataoka K (2006) A PEG-Based Biocompatible Block Catiomer with High Buffering Capacity for the Construction of Polyplex Micelles Showing Efficient Gene Transfer toward Primary Cells. ChemMedChem 1, 439–444. [PubMed: 16892379]

(129). Takae S, Miyata K, Oba M, Ishii T, Nishiyama N, Itaka K, Yamasaki Y, Koyama H, and Kataoka K (2008) PEG-Detachable Polyplex Micelles Based on Disulfide-Linked Block Catiomers as Bioresponsive Nonviral Gene Vectors. J. Am. Chem. Soc 130, 6001–6009. [PubMed: 18396871]

(130). Chen G, Ma B, Wang Y, and Gong S (2018) A Universal GSH-Responsive Nanoplatform for the Delivery of DNA, mRNA, and Cas9/sgRNA Ribonucleoprotein. ACS Appl. Mater. Interfaces 10, 18515–18523. [PubMed: 29798662]

(131). Wang Y, Ma B, Abdeen AA, Chen G, Xie R, Saha K, and Gong S (2018) Versatile Redox-Responsive Polyplexes for the Delivery of Plasmid DNA, Messenger RNA, and CRISPR-Cas9 Genome-Editing Machinery. ACS Appl. Mater. Interfaces 10, 31915–31927. [PubMed: 30222305]

(132). Li S, Saw PE, Lin C, Nie Y, Tao W, Farokhzad OC, Zhang L, and Xu X (2020) Redox-Responsive Polyprodrug Nanoparticles for Targeted siRNA Delivery and Synergistic Liver Cancer Therapy. Biomaterials 234, 119760. [PubMed: 31945619]

(133). Song Y, Lou B, Zhao P, and Lin C (2014) Multifunctional Disulfide-Based Cationic Dextran Conjugates for Intravenous Gene Delivery Targeting Ovarian Cancer Cells. Mol. Pharm 11, 2250–2261. [PubMed: 24892216]

(134). Hu Y, Liu N, Cheng B, Tan Y, Wen L, Yuan H, and Hu F (2016) Sequential Delivery of Therapeutic Agents Using a Rationally Designed Disulfide-Linked Glycolipid-Like Nanocarrier. Oncotarget 7, 83258–83269. [PubMed: 27825127]

(135). Peng YY, Diaz-Dussan D, Kumar P, and Narain R (2019) Tumor Microenvironment-Regulated Redox Responsive Cationic Galactose-Based Hyperbranched Polymers for siRNA Delivery. Bioconjugate Chem. 30, 405–412.

(136). Barba AA, Bochicchio S, Dalmoro A, and Lamberti G (2019) Lipid Delivery Systems for Nucleic-Acid-Based-Drugs: From Production to Clinical Applications. Pharmaceutics 11, 360.

(137). Parashar D, Rajendran V, Shukla R, and Sistla R (2020) Lipid-Based Nanocarriers for Delivery of Small Interfering RNA for Therapeutic Use. Eur. J. Pharm. Sci 142, 105159. [PubMed: 31747618]

(138). Majumder P, Bhunia S, Bhattacharyya J, and Chaudhuri A (2014) Inhibiting Tumor Growth by Targeting Liposomally Encapsulated CDC20siRNA to Tumor Vasculature: Therapeutic RNA Interference. J. Controlled Release 180, 100–108.

(139). Majumder P, Bhunia S, and Chaudhuri A (2018) A Lipid-Based Cell Penetrating Nano-Assembly for RNAi-Mediated Anti-Angiogenic Cancer Therapy. Chem. Commun 54, 1489–1492.

(140). Li W, and Szoka FC Jr. (2007) Lipid-Based Nanoparticles for Nucleic Acid Delivery. Pharm. Res 24, 438–449. [PubMed: 17252188]

(141). Meng T, Lu B, Shao S, Yuan M, Liu X, Yuan H, Huang X, and Hu F (2017) Sequential Therapy with Redox-Responsive Glucolipid Nanocarrier Separately Delivering siRNA and Doxorubicin to Overcome Multidrug Resistance. Int. J. Pharm 534, 368–377. [PubMed: 29051118]

(142). Tang FX, and Hughes JA (1999) Use of Dithiodiglycolic Acid as a Tether for Cationic Lipids Decreases the Cytotoxicity and Increases Transgene Expression of Plasmid DNA in Vitro. Bioconjugate Chem. 10, 791–796.

(143). Tang F, and Hughes JA (1998) Introduction of a Disulfide Bond into a Cationic Lipid Enhances Transgene Expression of Plasmid DNA. Biochem. Biophys. Res. Commun 242, 141–145. [PubMed: 9439625]

(144). Candiani G, Pezzoli D, Ciani L, Chiesa R, and Ristori S (2010) Bioreducible Liposomes for Gene Delivery: From the Formulation to the Mechanism of Action. PLoS One 5, e13430. [PubMed: 20976172]

(145). Candiani G, Frigerio M, Viani F, Verpelli C, Sala C, Chiamenti L, Zaffaroni N, Folini M, Sani M, Panzeri W, and Zanda M (2007) Dimerizable Redox-Sensitive Triazine-Based Cationic Lipids for in Vitro Gene Delivery. ChemMedChem 2, 292–296. [PubMed: 17191293]

(146). Ma XF, Sun J, Qiu C, Wu YF, Zheng Y, Yu MZ, Pei XW, Wei L, Niu YJ, Pang WH, Yang ZJ, Wang JC, and Zhang Q (2016) The Role of Disulfide-Bridge on the Activities of H-Shape Gemini-Like Cationic Lipid Based siRNA Delivery. J. Controlled Release 235, 99–111.

(147). Zheng Y, Guo YJ, Li YT, Wu Y, Zhang LH, and Yang ZJ (2014) A Novel Gemini-Like Cationic Lipid for the Efficient Delivery of siRNA. New J. Chem 38, 4952–4962.

(148). Weecharangsan W, Yu B, Liu S, Pang JX, Lee LJ, Marcucci G, and Lee RJ (2010) Disulfide-Linked Liposomes: Effective Delivery Vehicle for Bcl-2 Antisense Oligodeoxyribonucleotide G3139. Anticancer Res. 30, 31–37. [PubMed: 20150614]

(149). Wang M, Alberti K, Varone A, Pouli D, Georgakoudi I, and Xu Q (2014) Enhanced Intracellular siRNA Delivery Using Bioreducible Lipid-Like Nanoparticles. Adv. Healthcare Mater 3, 1398–1403.

(150). Wang M, Zuris JA, Meng F, Rees H, Sun S, Deng P, Han Y, Gao X, Pouli D, Wu Q, Georgakoudi I, Liu DR, and Xu Q (2016) Efficient Delivery of Genome-Editing Proteins Using Bioreducible Lipid Nanoparticles. Proc. Natl. Acad. Sci. U. S. A 113, 2868–2873. [PubMed: 26929348]

(151). Liu J, Chang J, Jiang Y, Meng X, Sun T, Mao L, Xu Q, and Wang M (2019) Fast and Efficient CRISPR/Cas9 Genome Editing in Vivo Enabled by Bioreducible Lipid and Messenger RNA Nanoparticles. Adv. Mater 31, 1902575.

(152). Liang X, Potter J, Kumar S, Zou Y, Quintanilla R, Sridharan M, Carte J, Chen W, Roark N, Ranganathan S, Ravinder N, and Chesnut JD (2015) Rapid and Highly Efficient Mammalian Cell Engineering Via Cas9 Protein Transfection. J. Biotechnol 208, 44–53. [PubMed: 26003884]

(153). Sahin U, Kariko K, and Tureci O (2014) mRNA-Based Therapeutics--Developing a New Class of Drugs. Nat. Rev. Drug Discovery 13, 759–780. [PubMed: 25233993]

(154). Vermonden T, Censi R, and Hennink WE (2012) Hydrogels for Protein Delivery. Chem. Rev 112, 2853–2888. [PubMed: 22360637]

(155). Fliervoet LAL, Engbersen JFJ, Schiffelers RM, Hennink WE, and Vermonden T (2018) Polymers and Hydrogels for Local Nucleic Acid Delivery. J. Mater. Chem. B, 6, 5651–5670. [PubMed: 32254973]

(156). Majumder P, Zhang Y, Iglesias M, Fan L, Kelley JA, Andrews C, Patel N, Stagno JR, Oh BC, Furtmüller GJ, Lai CC, Wang Y-X, Brandacher G, Raimondi G, and Schneider JP (2020) Multiphase Assembly of Small Molecule Microcrystalline Peptide Hydrogel Allows Immunomodulatory Combination Therapy for Long-Term Heart Transplant Survival. Small 16, 2002791.

(157). Majumder P, Baxa U, Walsh STR, and Schneider JP (2018) Design of a Multicompartment Hydrogel That Facilitates Time-Resolved Delivery of Combination Therapy and Synergized Killing of Glioblastoma. Angew. Chem., Int. Ed 57, 15040–15044.

(158). Dunn SS, Tian S, Blake S, Wang J, Galloway AL, Murphy A, Pohlhaus PD, Rolland JP, Napier ME, and DeSimone JM (2012) Reductively Responsive siRNA-Conjugated Hydrogel Nanoparticles for Gene Silencing. J. Am. Chem. Soc 134, 7423–7430. [PubMed: 22475061]

(159). Nguyen MK, Huynh CT, Gilewski A, Wilner SE, Maier KE, Kwon N, Levy M, and Alsberg E (2019) Covalently Tethering siRNA to Hydrogels for Localized, Controlled Release and Gene Silencing. Sci. Adv 5, eaax0801. [PubMed: 31489374]

(160). Mura S, Nicolas J, and Couvreur P (2013) Stimuli-Responsive Nanocarriers for Drug Delivery. Nat. Mater 12, 991–1003. [PubMed: 24150417]

(161). Xiao Y, Shi K, Qu Y, Chu B, and Qian Z (2019) Engineering Nanoparticles for Targeted Delivery of Nucleic Acid Therapeutics in Tumor. Mol. Ther.--Methods Clin. Dev 12, 1–18. [PubMed: 30364598]

(162). Scaletti F, Hardie J, Lee Y-W, Luther DC, Ray M, and Rotello VM (2018) Protein Delivery into Cells Using Inorganic Nanoparticle–Protein Supramolecular Assemblies. Chem. Soc. Rev 47, 3421–3432. [PubMed: 29537040]

(163). Guo X, Cheng Y, Zhao X, Luo Y, Chen J, and Yuan W-E (2018) Advances in Redox-Responsive Drug Delivery Systems of Tumor Microenvironment. J. Nanobiotechnol 16, 74.

(164). Loh XJ, Lee TC, Dou Q, and Deen GR (2016) Utilising Inorganic Nanocarriers for Gene Delivery. Biomater. Sci 4, 70–86. [PubMed: 26484365]

(165). Wen H, and Li Y Redox Sensitive Nanoparticles with Disulfide Bond Linked Sheddable Shell for Intracellular Drug Delivery. Med. Chem 4, 748–754.

(166). Mendes R, Fernandes AR, and Baptista PV (2017) Gold Nanoparticle Approach to the Selective Delivery of Gene Silencing in Cancer-the Case for Combined Delivery? Genes 8, 94.

(167). Huang X, and El-Sayed MA (2010) Gold Nanoparticles: Optical Properties and Implementations in Cancer Diagnosis and Photothermal Therapy. J. Adv. Res 1, 13–28.

(168). Sharma H, Mishra PK, Talegaonkar S, and Vaidya B (2015) Metal Nanoparticles: A Theranostic Nanotool against Cancer. Drug Discovery Today 20, 1143–1151. [PubMed: 26007605]

(169). Ding Y, Jiang Z, Saha K, Kim CS, Kim ST, Landis RF, and Rotello VM (2014) Gold Nanoparticles for Nucleic Acid Delivery. Mol. Ther 22, 1075–1083. [PubMed: 24599278]

(170). Han G, Chari NS, Verma A, Hong R, Martin CT, and Rotello VM (2005) Controlled Recovery of the Transcription of Nanoparticle-Bound DNA by Intracellular Concentrations of Glutathione. Bioconjugate Chem. 16, 1356–1359.

(171). Anderson ME (1998) Glutathione: An Overview of Biosynthesis and Modulation. Chem.-Biol. Interact 111–112, 1–14.

(172). Ghosh PS, Kim C-K, Han G, Forbes NS, and Rotello VM (2008) Efficient Gene Delivery Vectors by Tuning the Surface Charge Density of Amino Acid-Functionalized Gold Nanoparticles. ACS Nano 2, 2213–2218. [PubMed: 19206385]

(173). Heitz F, Morris MC, and Divita G (2009) Twenty Years of Cell-Penetrating Peptides: From Molecular Mechanisms to Therapeutics. Br. J. Pharmacol 157, 195–206. [PubMed: 19309362]

(174). Yu F, Huang J, Yu Y, Lu Y, Chen Y, Zhang H, Zhou G, Sun Z, Liu J, Sun D, Zhang G, Zou H, and Zhong Y (2016) Glutathione-Responsive Multilayer Coated Gold Nanoparticles for Targeted Gene Delivery. J. Biomed. Nanotechnol 12, 503–515. [PubMed: 27280248]

(175). Klutz K, Schaffert D, Willhauck MJ, Grünwald GK, Haase R, Wunderlich N, Zach C, Gildehaus FJ, Senekowitsch-Schmidtke R, Göke B, Wagner E, Ogris M, and Spitzweg C (2011) Epidermal Growth Factor Receptor-Targeted 131i-Therapy of Liver Cancer Following Systemic Delivery of the Sodium Iodide Symporter Gene. Mol. Ther 19, 676–685. [PubMed: 21245850]

(176). Khalil IA, Kogure K, Futaki S, Hama S, Akita H, Ueno M, Kishida H, Kudoh M, Mishina Y, Kataoka K, Yamada M, and Harashima H (2007) Octaarginine-Modified Multifunctional Envelope-Type Nanoparticles for Gene Delivery. Gene Ther. 14, 682–689. [PubMed: 17268535]

(177). Yan XX, Li SR, Qu YC, Wang WL, Chen BG, Liu SR, Ma XJ, and Yu XF (2020) Redox-Responsive Multifunctional Polypeptides Conjugated with Au Nanoparticles for Tumor-Targeting Gene Therapy and Their 1+1 > 2 Synergistic Effects. ACS Biomater. Sci. Eng 6, 463–473. [PubMed: 33463244]

(178). Kim HJ, Takemoto H, Yi Y, Zheng M, Maeda Y, Chaya H, Hayashi K, Mi P, Pittella F, Christie RJ, Toh K, Matsumoto Y, Nishiyama N, Miyata K, and Kataoka K (2014) Precise Engineering of siRNA Delivery Vehicles to Tumors Using Polyion Complexes and Gold Nanoparticles. ACS Nano 8, 8979–8991. [PubMed: 25133608]

(179). Zhou Y, Quan G, Wu Q, Zhang X, Niu B, Wu B, Huang Y, Pan X, and Wu C (2018) Mesoporous Silica Nanoparticles for Drug and Gene Delivery. Acta Pharm. Sin. B 8, 165–177. [PubMed: 29719777]

(180). Torney F, Trewyn BG, Lin VSY, and Wang K (2007) Mesoporous Silica Nanoparticles Deliver DNA and Chemicals into Plants. Nat. Nanotechnol 2, 295–300. [PubMed: 18654287]

(181). Darvishi B, Farahmand L, and Majidzadeh-A K (2017) Stimuli-Responsive Mesoporous Silica Nps as Non-Viral Dual siRNA/Chemotherapy Carriers for Triple Negative Breast Cancer. Mol. Ther.--Nucleic Acids 7, 164–180. [PubMed: 28624192]

(182). Han L, Tang C, and Yin C (2015) Dual-Targeting and pH/Redox-Responsive Multi-Layered Nanocomplexes for Smart Co-Delivery of Doxorubicin and siRNA. Biomaterials 60, 42–52. [PubMed: 25982552]

(183). Sun L, Liu YJ, Yang ZZ, and Qi XR (2015) Tumor Specific Delivery with Redox-Triggered Mesoporous Silica Nanoparticles Inducing Neovascularization Suppression and Vascular Normalization. RSC Adv. 5, 55566–55578.

(184). Zhang SL, Chu ZQ, Yin C, Zhang CY, Lin G, and Li Q (2013) Controllable Drug Release and Simultaneously Carrier Decomposition of Sio2-Drug Composite Nanoparticles. J. Am. Chem. Soc 135, 5709–5716. [PubMed: 23496255]

(185). Zhang Q, Shen CN, Zhao N, and Xu FJ (2017) Redox-Responsive and Drug-Embedded Silica Nanoparticles with Unique Self-Destruction Features for Efficient Gene/Drug Codelivery. Adv. Funct. Mater 27, 1606229.

(186). Lin JT, Liu ZK, Zhu QL, Rong XH, Liang CL, Wang J, Ma D, Sun J, and Wang GH (2017) Redox-Responsive Nanocarriers for Drug and Gene Co-Delivery Based on Chitosan Derivatives Modified Mesoporous Silica Nanoparticles. Colloids Surf., B 155, 41–50.

(187). Zhao S, Xu MM, Cao CW, Yu QQ, Zhou YH, and Liu J (2017) A Redox-Responsive Strategy Using Mesoporous Silica Nanoparticles for Co-Delivery of siRNA and Doxorubicin. J. Mater. Chem. B, 5, 6908–6919. [PubMed: 32264340]

(188). Zhang PH, An KL, Duan XM, Xu H, Li F, and Xu F (2018) Recent Advances in siRNA Delivery for Cancer Therapy Using Smart Nanocarriers. Drug Discovery Today 23, 900–911. [PubMed: 29373841]

(189). Lin A, Giuliano CJ, Palladino A, John KM, Abramowicz C, Yuan ML, Sausville EL, Lukow DA, Liu LW, Chait AR, Galluzzo ZC, Tucker C, and Sheltzer JM (2019) Off-Target Toxicity Is a Common Mechanism of Action of Cancer Drugs Undergoing Clinical Trials. Sci. Transl. Med 11, eaaw8412. [PubMed: 31511426]

(190). Zhao XQ, Xi YY, Zhang YM, Wu QY, Meng RY, Zheng B, and Rei L (2019) Redox-Sensitive Gelatin/Silica-Aptamer Nanogels for Targeted siRNA Delivery. Nanoscale Res. Lett 14, 273. [PubMed: 31414279]

(191). Kam NW, Liu Z, and Dai H (2005) Functionalization of Carbon Nanotubes Via Cleavable Disulfide Bonds for Efficient Intracellular Delivery of siRNA and Potent Gene Silencing. J. Am. Chem. Soc 127, 12492–12493. [PubMed: 16144388]

(192). Nia AH, Eshghi H, Abnous K, and Ramezani M (2017) The Intracellular Delivery of Plasmid DNA Using Cationic Reducible Carbon Nanotube — Disulfide Conjugates of Polyethylenimine. Eur. J. Pharm. Sci 100, 176–186. [PubMed: 28104471]

(193). Mohammadinejad R, Dadashzadeh A, Moghassemi S, Ashrafizadeh M, Dehshahri A, Pardakhty A, Sassan H, Sohrevardi SM, and Mandegary A (2019) Shedding Light on Gene Therapy: Carbon Dots for the Minimally Invasive Image-Guided Delivery of Plasmids and Noncoding RNAs - a Review. J. Adv. Res 18, 81–93. [PubMed: 30828478]

(194). Feng T, Ai X, Ong H, and Zhao Y (2016) Dual-Responsive Carbon Dots for Tumor Extracellular Microenvironment Triggered Targeting and Enhanced Anticancer Drug Delivery. ACS Appl. Mater. Interfaces 8, 18732–18740. [PubMed: 27367152]

(195). Wu YF, Wu HC, Kuan CH, Lin CJ, Wang LW, Chang CW, and Wang TW (2016) Multi-Functionalized Carbon Dots as Theranostic Nanoagent for Gene Delivery in Lung Cancer Therapy. Sci. Rep 6, 21170. [PubMed: 26880047]

(196). Shen LZ, Li B, and Qiao YS (2018) Fe3o4 Nanoparticles in Targeted Drug/Gene Delivery Systems. Materials 11, 324.

(197). Peng S, Wang QY, Xiao X, Wang R, Lin J, Zhou QH, and Wu LN (2020) Redox-Responsive Polyethyleneimine-Coated Magnetic Iron Oxide Nanoparticles for Controllable Gene Delivery and Magnetic Resonance Imaging. Polym. Int 69, 206–214.

(198). Liu Y, Wang J, Huang X, Zhao Y, Sun Y, Zhang S, Wang H, Yuan L, and Chen H (2019) Glutathione-Sensitive Silicon Nanowire Arrays for Gene Transfection. ACS Appl. Mater. Interfaces 11, 46515–46524. [PubMed: 31746585]

(199). Voigt NV, Torring T, Rotaru A, Jacobsen MF, Ravnsbaek JB, Subramani R, Mamdouh W, Kjems J, Mokhir A, Besenbacher F, and Gothelf KV (2010) Single-Molecule Chemical Reactions on DNA Origami. Nat. Nanotechnol 5, 200–203. [PubMed: 20190747]

(200). Seeman NC (1982) Nucleic Acid Junctions and Lattices. J. Theor. Biol 99, 237–247. [PubMed: 6188926]

(201). Chi Q, Yang Z, Xu K, Wang C, and Liang H (2020) DNA Nanostructure as an Efficient Drug Delivery Platform for Immunotherapy. Front. Pharmacol 10, 1585–1585. [PubMed: 32063844]

(202). Li J, Zheng C, Cansiz S, Wu C, Xu J, Cui C, Liu Y, Hou W, Wang Y, Zhang L, Teng IT, Yang HH, and Tan W (2015) Self-Assembly of DNA Nanohydrogels with Controllable Size and Stimuli-Responsive Property for Targeted Gene Regulation Therapy. J. Am. Chem. Soc 137, 1412–1415. [PubMed: 25581100]

(203). Zheng M, Jiang T, Yang W, Zou Y, Wu H, Liu X, Zhu F, Qian R, Ling D, McDonald K, Shi J, and Shi B (2019) The siRNAsome: A Cation-Free and Versatile Nanostructure for siRNA and Drug Co-Delivery. Angew. Chem. Int. Ed 58, 4938–4942.

(204). Pan Q, Nie C, Hu Y, Yi J, Liu C, Zhang J, He M, He M, Chen T, and Chu X (2020) Aptamer-Functionalized DNA Origami for Targeted Codelivery of Antisense Oligonucleotides and Doxorubicin to Enhance Therapy in Drug-Resistant Cancer Cells. ACS Appl. Mater. Interfaces 12, 400–409. [PubMed: 31815420]

(205). Liu J, Song L, Liu S, Zhao S, Jiang Q, and Ding B (2018) A Tailored DNA Nanoplatform for Synergistic RNAi-/Chemotherapy of Multidrug-Resistant Tumors. Angew. Chem. Int. Ed 57, 15486–15490.

(206). Yang J, Jiang Q, He L, Zhan P, Liu Q, Liu S, Fu M, Liu J, Li C, and Ding B (2018) Self-Assembled Double-Bundle DNA Tetrahedron for Efficient Antisense Delivery. ACS Appl. Mater. Interfaces 10, 23693–23699. [PubMed: 29963858]

(207). De Stefano M, and Vesterager Gothelf K (2016) Dynamic Chemistry of Disulfide Terminated Oligonucleotides in Duplexes and Double-Crossover Tiles. ChemBioChem 17, 1122–1126. [PubMed: 26994867]

(208). Wolfrum M, Schwarz RJ, Schwarz M, Kramer M, and Richert C (2019) Stabilizing DNA Nanostructures through Reversible Disulfide Crosslinking. Nanoscale 11, 14921–14928. [PubMed: 31360975]

(209). Del Grosso E, Prins LJ, and Ricci F (2020) Transient DNA-Based Nanostructures Controlled by Redox Inputs. Angew. Chem. Int. Ed 59, 13238–13245.

(210). Wang B, Song L, Jin B, Deng N, Wu X, He J, Deng Z, and Li Y (2019) Base-Sequence-Independent Efficient Redox Switching of Self-Assembled DNA Nanocages. ChemBioChem 20, 2743–2746. [PubMed: 31100196]

(211). Yang W, Liu X, Li H, Zhou J, Chen S, Wang P, Li J, and Yang H (2020) Disulfide-Containing Molecular Sticker Assists Cellular Delivery of DNA Nanoassemblies by Bypassing Endocytosis. CCS Chem. 2, 1178–1186.

(212). Lin C, and Engbersen JF (2009) The Role of the Disulfide Group in Disulfide-Based Polymeric Gene Carriers. Expert Opin. Drug Delivery 6, 421–439.

(213). Meylan WM, and Howard PH (1995) Atom/Fragment Contribution Method for Estimating Octanol-Water Partition Coefficients. J. Pharm. Sci 84, 83–92. [PubMed: 7714751]

(214). Yang J, Chen H, Vlahov IR, Cheng J-X, and Low PS (2006) Evaluation of Disulfide Reduction During Receptor-Mediated Endocytosis by Using Fret Imaging. Proc. Natl. Acad. Sci. U. S. A 103, 13872–13877. [PubMed: 16950881]

(215). Torres AG, and Gait MJ (2012) Exploiting Cell Surface Thiols to Enhance Cellular Uptake. Trends Biotechnol. 30, 185–190. [PubMed: 22260747]

(216). Abegg D, Gasparini G, Hoch DG, Shuster A, Bartolami E, Matile S, and Adibekian A (2017) Strained Cyclic Disulfides Enable Cellular Uptake by Reacting with the Transferrin Receptor. J. Am. Chem. Soc 139, 231–238. [PubMed: 28001050]

(217). Gasparini G, Bang EK, Molinard G, Tulumello DV, Ward S, Kelley SO, Roux A, Sakai N, and Matile S (2014) Cellular Uptake of Substrate-Initiated Cell-Penetrating Poly(Disulfide)s. J. Am. Chem. Soc 136, 6069–6074. [PubMed: 24735462]

(218). Torchilin VP (2005) Recent Advances with Liposomes as Pharmaceutical Carriers. Nat. Rev. Drug Discovery 4, 145–160. [PubMed: 15688077]

(219). Ozpolat B, Sood AK, and Lopez-Berestein G (2014) Liposomal siRNA Nanocarriers for Cancer Therapy. Adv. Drug Delivery Rev 66, 110–116.

(220). Kim B, Sun S, Varner JA, Howell SB, Ruoslahti E, and Sailor MJ (2019) Securing the Payload, Finding the Cell, and Avoiding the Endosome: Peptide-Targeted, Fusogenic Porous Silicon Nanoparticles for Delivery of siRNA. Adv. Mater 31, e1902952. [PubMed: 31267590]

(221). Kim B, Pang HB, Kang J, Park JH, Ruoslahti E, and Sailor MJ (2018) Immunogene Therapy with Fusogenic Nanoparticles Modulates Macrophage Response to Staphylococcus Aureus. Nat. Commun 9, 1969. [PubMed: 29773788]

(222). Li X, Chen Y, Wang M, Ma Y, Xia W, and Gu H (2013) A Mesoporous Silica Nanoparticle – PEI – Fusogenic Peptide System for siRNA Delivery in Cancer Therapy. Biomaterials 34, 1391–1401. [PubMed: 23164421]

(223). Garber K (2018) Alnylam Launches Era of RNAi Drugs. Nat. Biotechnol 36, 777–778. [PubMed: 30188543]

(224). (2020) Second RNAi Drug Approved. Nat. Biotechnol 38, 385.

(225). Roberts TC, Langer R, and Wood MJA (2020) Advances in Oligonucleotide Drug Delivery. Nat. Rev. Drug Discovery 19, 673–694. [PubMed: 32782413]

(226). Ramot Y, Rotkopf S, Gabai RM, Zorde Khvalevsky E, Muravnik S, Marzoli GA, Domb AJ, Shemi A, and Nyska A (2016) Preclinical Safety Evaluation in Rats of a Polymeric Matrix Containing an siRNA Drug Used as a Local and Prolonged Delivery System for Pancreatic Cancer Therapy. Toxicol. Pathol 44, 856–865. [PubMed: 27147553]

(227). Golan T, Khvalevsky EZ, Hubert A, Gabai RM, Hen N, Segal A, Domb A, Harari G, David EB, Raskin S, Goldes Y, Goldin E, Eliakim R, Lahav M, Kopleman Y, Dancour A, Shemi A, and Galun E (2015) RNAi Therapy Targeting Kras in Combination with Chemotherapy for Locally Advanced Pancreatic Cancer Patients. Oncotarget 6, 24560–24570. [PubMed: 26009994]

(228). Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, McCullough MP, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott A, Flach B, Doria-Rose NA, Corbett KS, Morabito KM, O'Dell S, Schmidt SD, Swanson PA, Padilla M, Mascola JR, Neuzil KM, Bennett H, Sun W, Peters E, Makowski M, Albert J, Cross K, Buchanan W, Pikaart-Tautges R, Ledgerwood JE, Graham BS, and Beigel JH (2020) An mRNA Vaccine against Sars-Cov-2 — Preliminary Report. N. Engl. J. Med 383, 1920–1931. [PubMed: 32663912]

(229). Servick K This Mysterious $2 Billion Biotech Is Revealing the Secrets Behind Its New Drugs and Vaccines, https://www.sciencemag.org/news/2017/02/mysterious-2-billion-biotech-revealing-secrets-behind-its-new-drugs-and-vaccines (accessed 2 Dec, 2020).

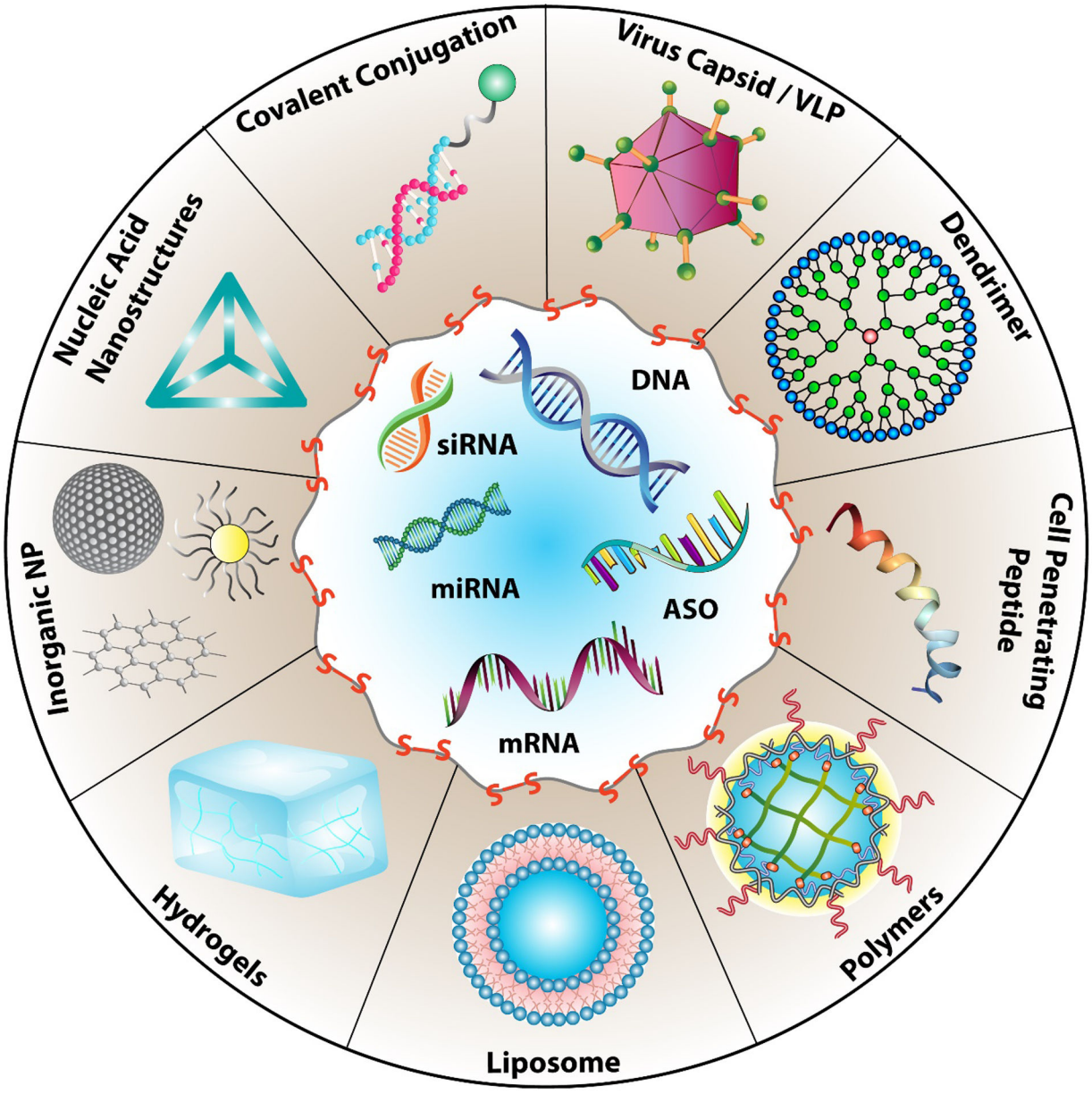


Figure 1.

Schematic for available drug delivery materials that rely on disulfide-bridges to enable nucleic acid delivery.

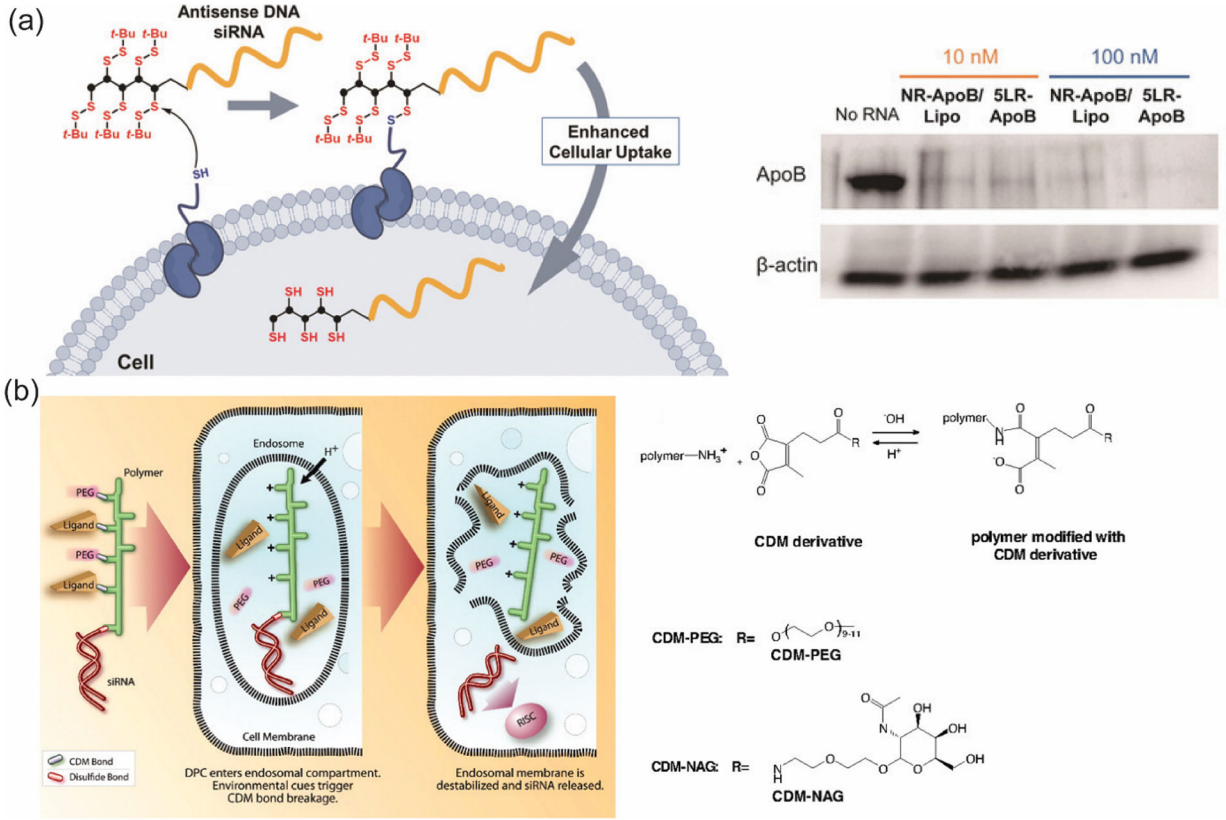


Figure 2.

(a) *left:* Ultrafast delivery of nucleic acids via disulfide conjugation to antisense DNA or siRNA; *right:* Western blot showing knockdown of ApoB gene via delivery of siRNA. Reprinted with permission from reference 26. Copyright 2019 Wiley-VCH Verlag GmbH & Co. (b) Design of 'dynamic polyconjugate' construct for delivery of siRNA into hepatocytes; siRNA is conjugated to the polymer via disulfide linkage with a conjugation efficiency of ~70–90%. Reprinted with permission from reference 30. Copyright 2007 The National Academy of Sciences of the USA.

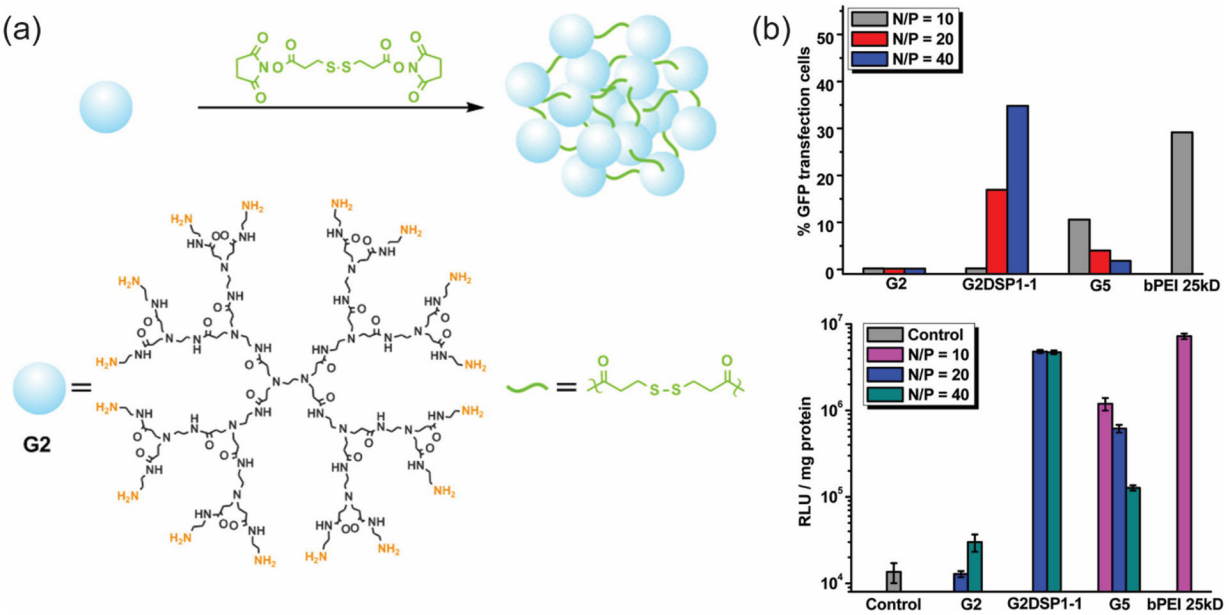


Figure 3.

(a) Strategy for disulfide-mediated crosslinked G2DSP dendrimer for pDNA complexation; (b) Comparison of eGFP (top) and luciferase (bottom) gene expressions with G2DSP PAMAM dendrimers and controls including G2, G5 dendrimers and branched PEI (25 kDa). Reprinted with permission from reference 55. Copyright 2012 American Chemical Society.

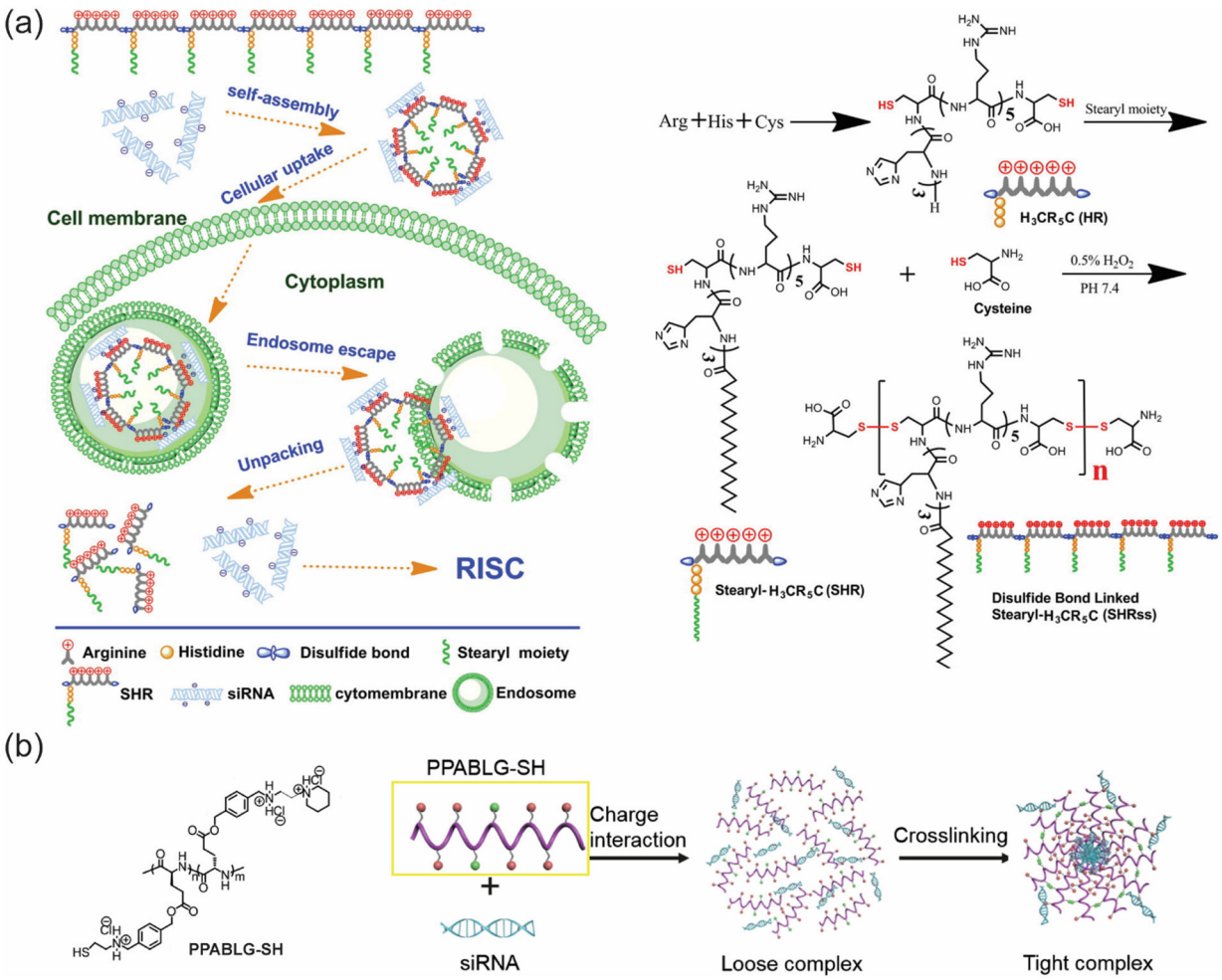
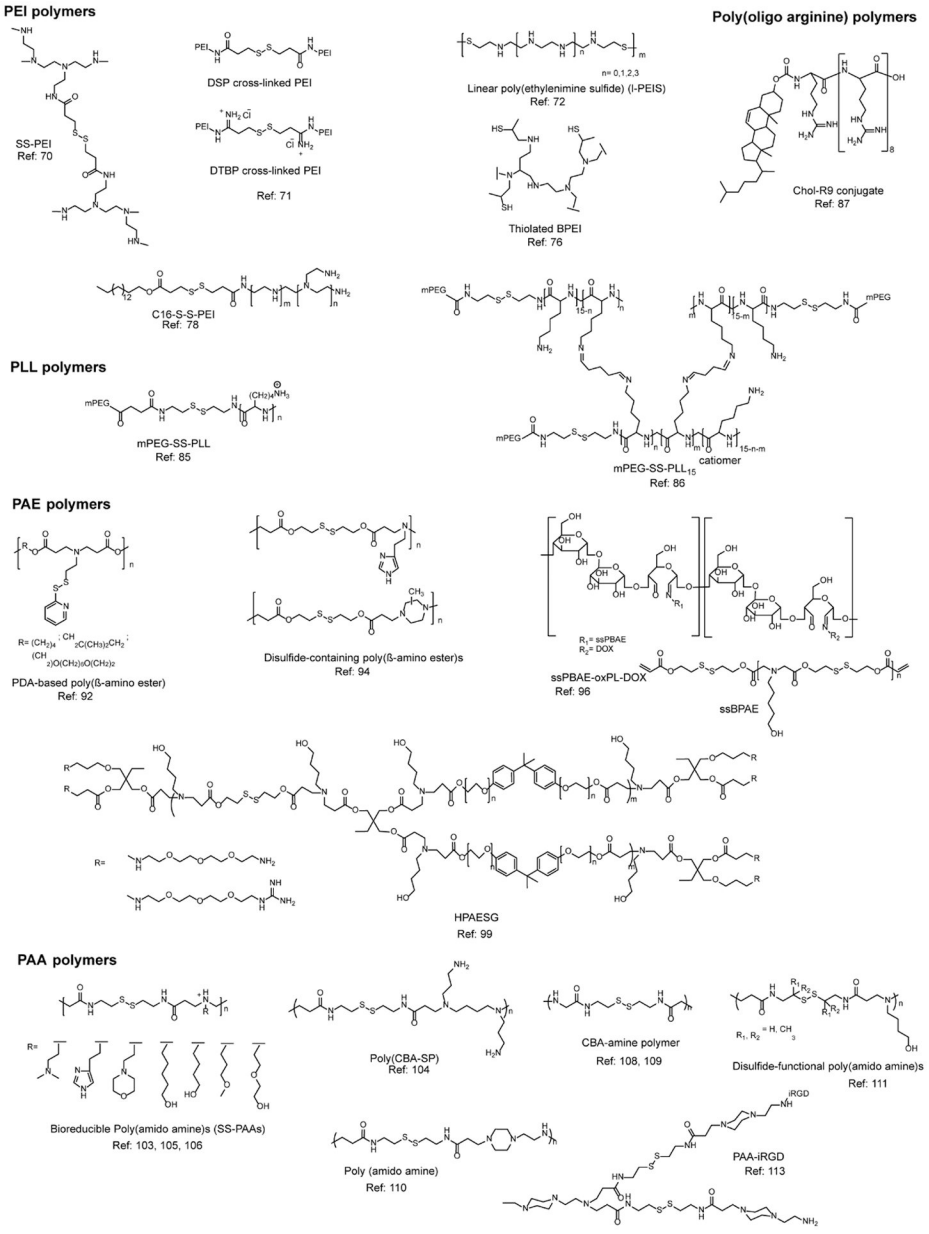


Figure 4.

(a) Design and synthesis for disulfide-crosslinked stearylated peptide conjugates for siRNA delivery. Reprinted with permission from reference 61. Copyright 2015 American Chemical Society. (b) Redox-responsive thiolated helical polypeptide for siRNA condensation. Reprinted with permission from reference 62. Copyright 2015 Elsevier.



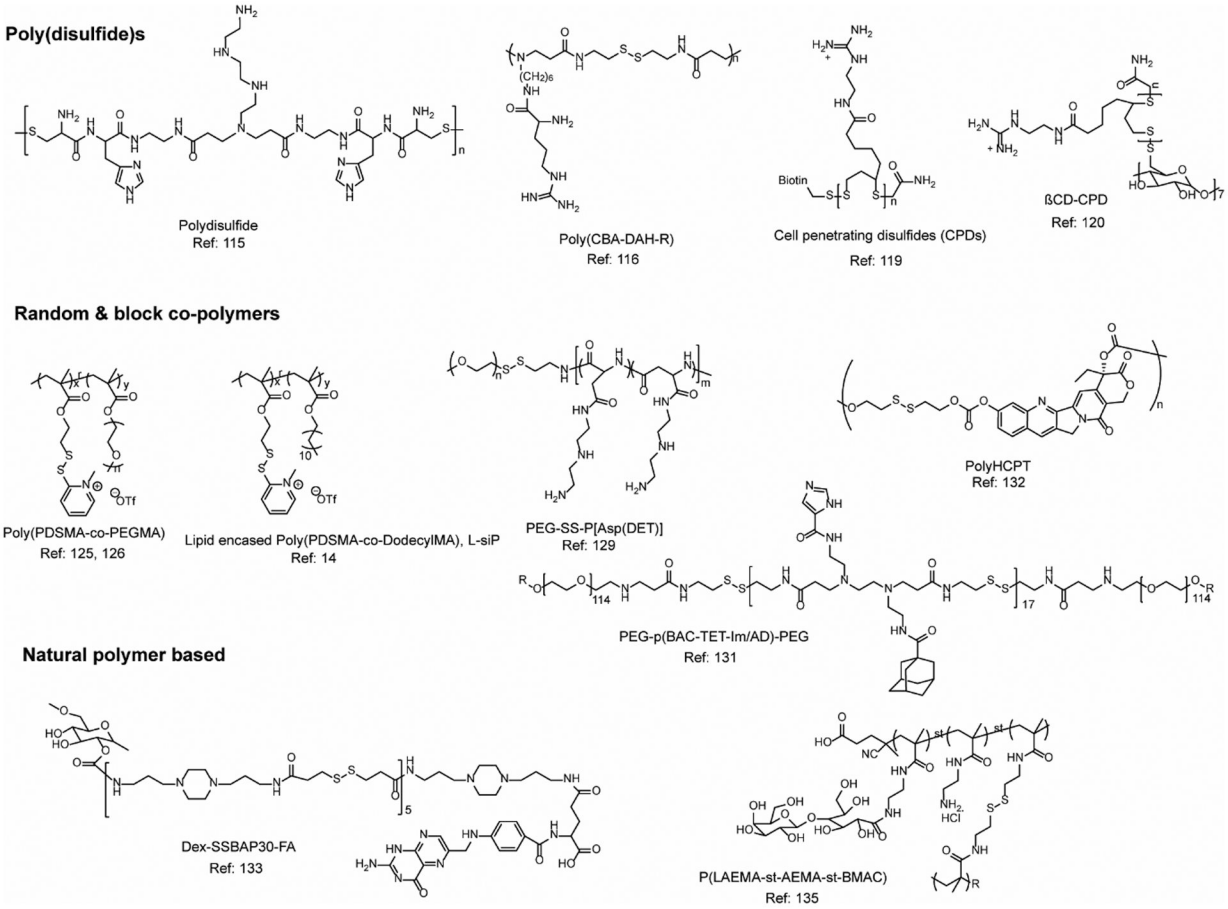


Figure 5.

Chemical structures of key disulfide bridged polymers, discussed in this review, for complexation and delivery of nucleic acids.

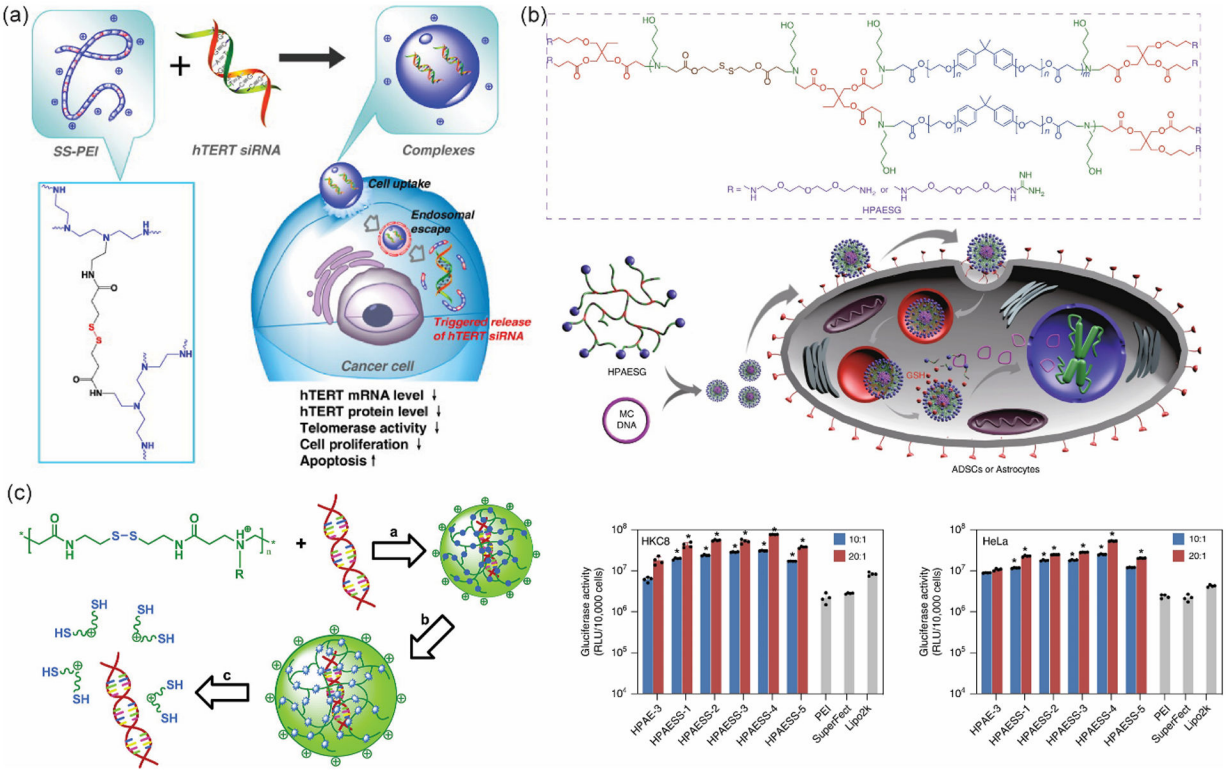


Figure 6.

(a) Bioreducible PEI for delivery of hTERT siRNA. Reprinted with permission from reference 70. Copyright 2011 Elsevier. (b) *top:* Structure of hyperbranched PAE and its delivery of minicircle DNA into stem cells and astrocytes; *bottom:* higher transfection of Gluciferase gene was observed for disulfide containing PAE nanocomplexes in HKC8 and HeLa cell lines. Reprinted with permission from reference 99. Copyright 2019 Nature Publications. (c) Condensation of DNA with PAA and intracellular release via cleavage of disulfide bonds. Reprinted with permission from reference 103. Copyright 2007 American Chemical Society.

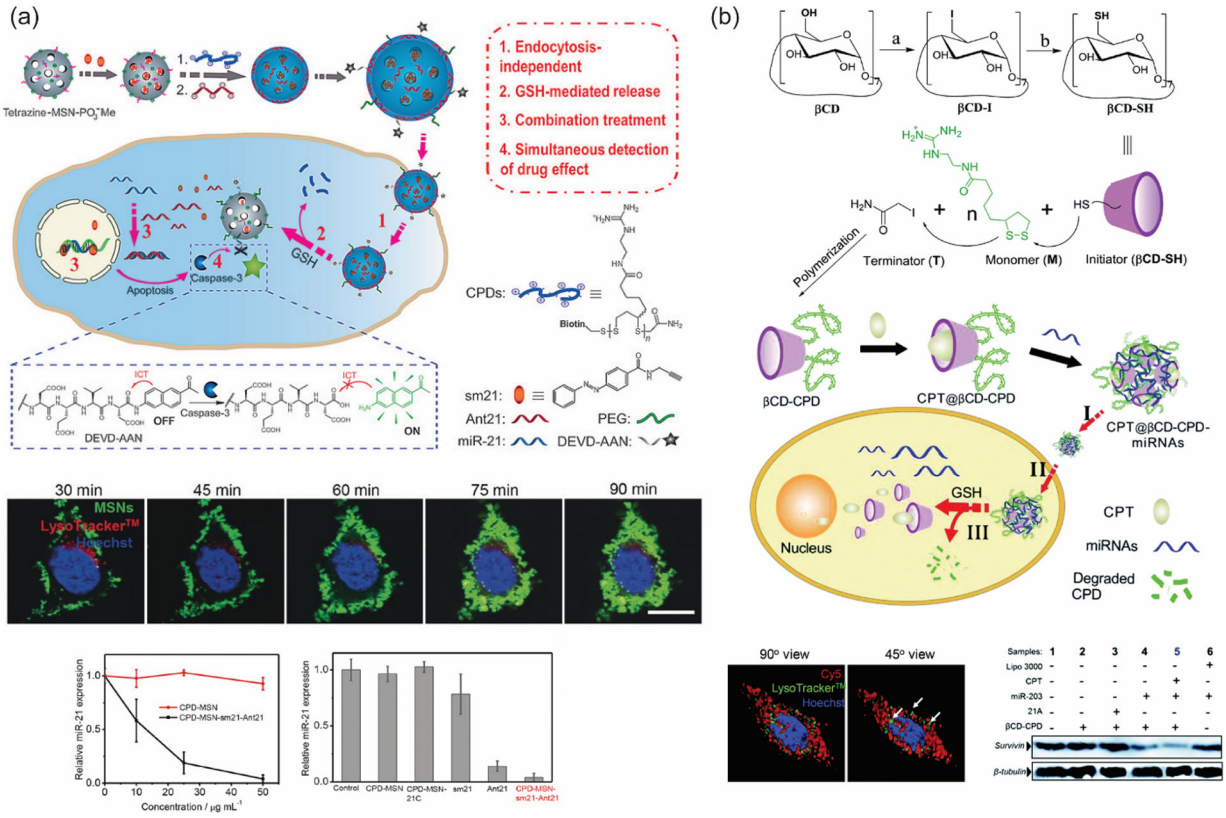


Figure 7.

(a) *top to bottom*: Strategy showing cell penetrating poly(disulfide) assisted delivery of miRNA for gene regulation; time-dependent efficient cellular uptake in HeLa cells; and qPCR analysis of miR-21 expression levels after treatment with nanoparticles. Reprinted with permission from reference 119. Copyright 2016 Wiley-VCH Verlag GmbH & Co.

(b) *top to bottom*: β-cyclodextrin decorated poly(disulfide) nanoparticle for codelivery of miRNA and camptothecin drug; efficient cytosolic distribution of miR-203^{cy5} and western blot analysis for inhibition of survivin protein expression. Reprinted with permission from reference 120. Copyright 2017 Royal Society of Chemistry.

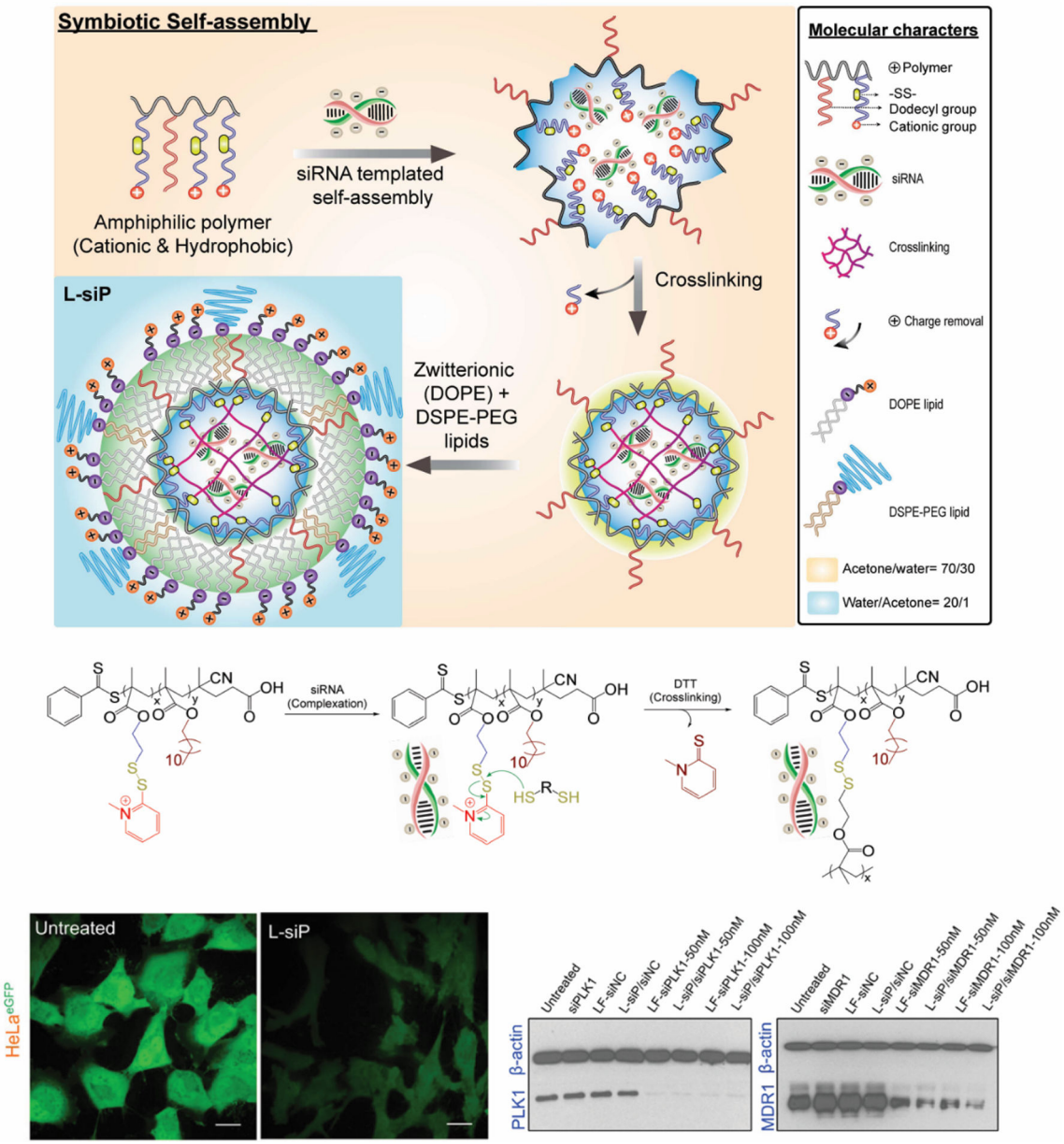


Figure 8.

Top to bottom: Symbiotic self-assembly of lipid decorated random copolymer and siRNA; reaction scheme of the non-cationic approach for siRNA complexation; and silencing of eGFP, PLK1 and MDR1 genes via intracellular delivery of siRNAs. Reprinted with permission from reference 14. Copyright 2019 American Chemical Society.

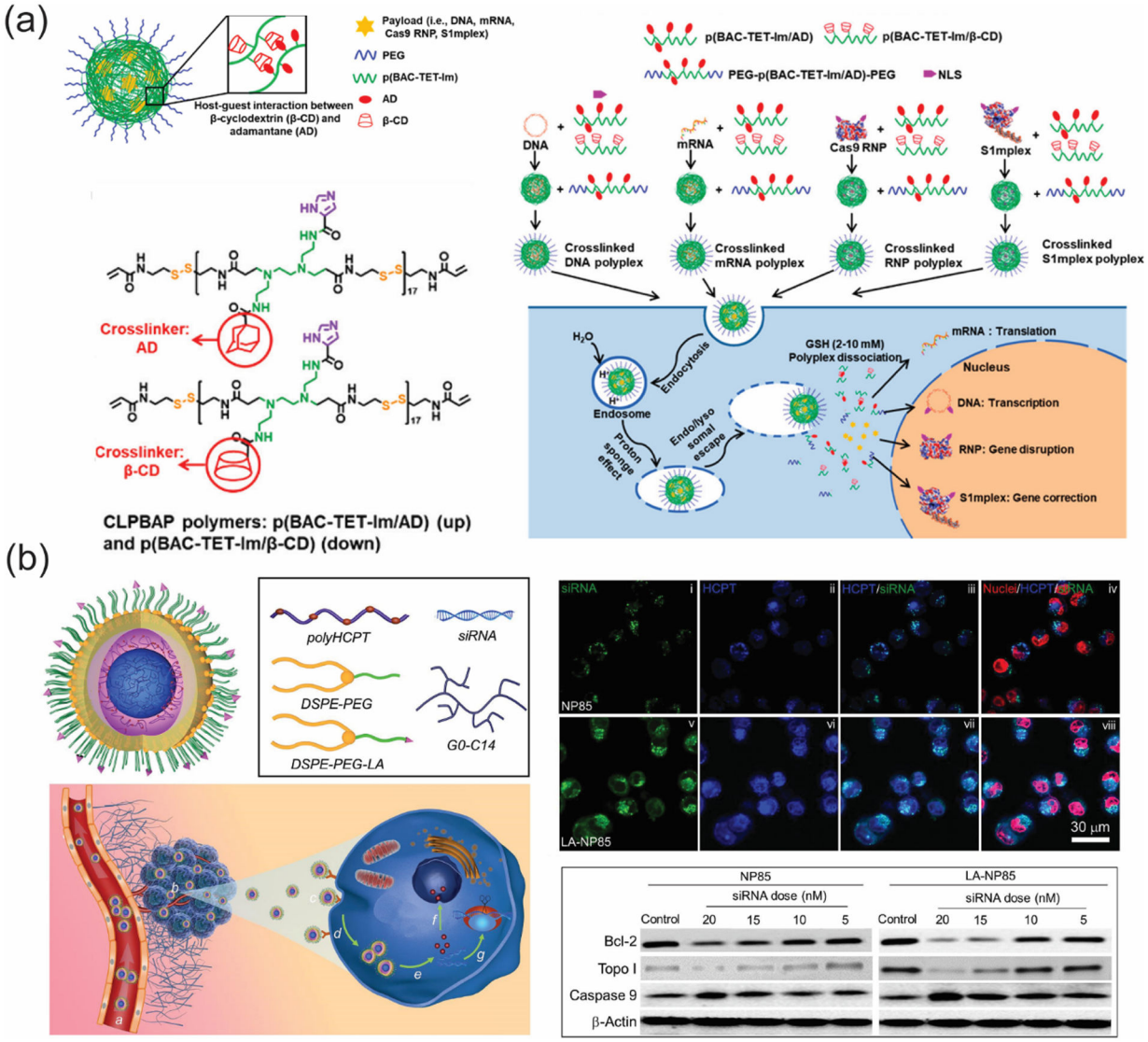


Figure 9.

(a) Redox-responsive polyplex design for delivery of multiple cargoes, including pDNA, mRNA, CRISPR-Cas9. Reprinted with permission from reference 131. Copyright 2018 American Chemical Society. (b) Redox-sensitive polyprodrug concept for targeted and synergistic delivery of siRNA and small molecule drug; confocal microscopy images showing dual cellular delivery of siRNA and polyHCPT; and western blot analysis of different protein expressions after treatment with nanoparticles loaded with siBcl-2. Reprinted with permission from reference 132. Copyright 2020 Elsevier.

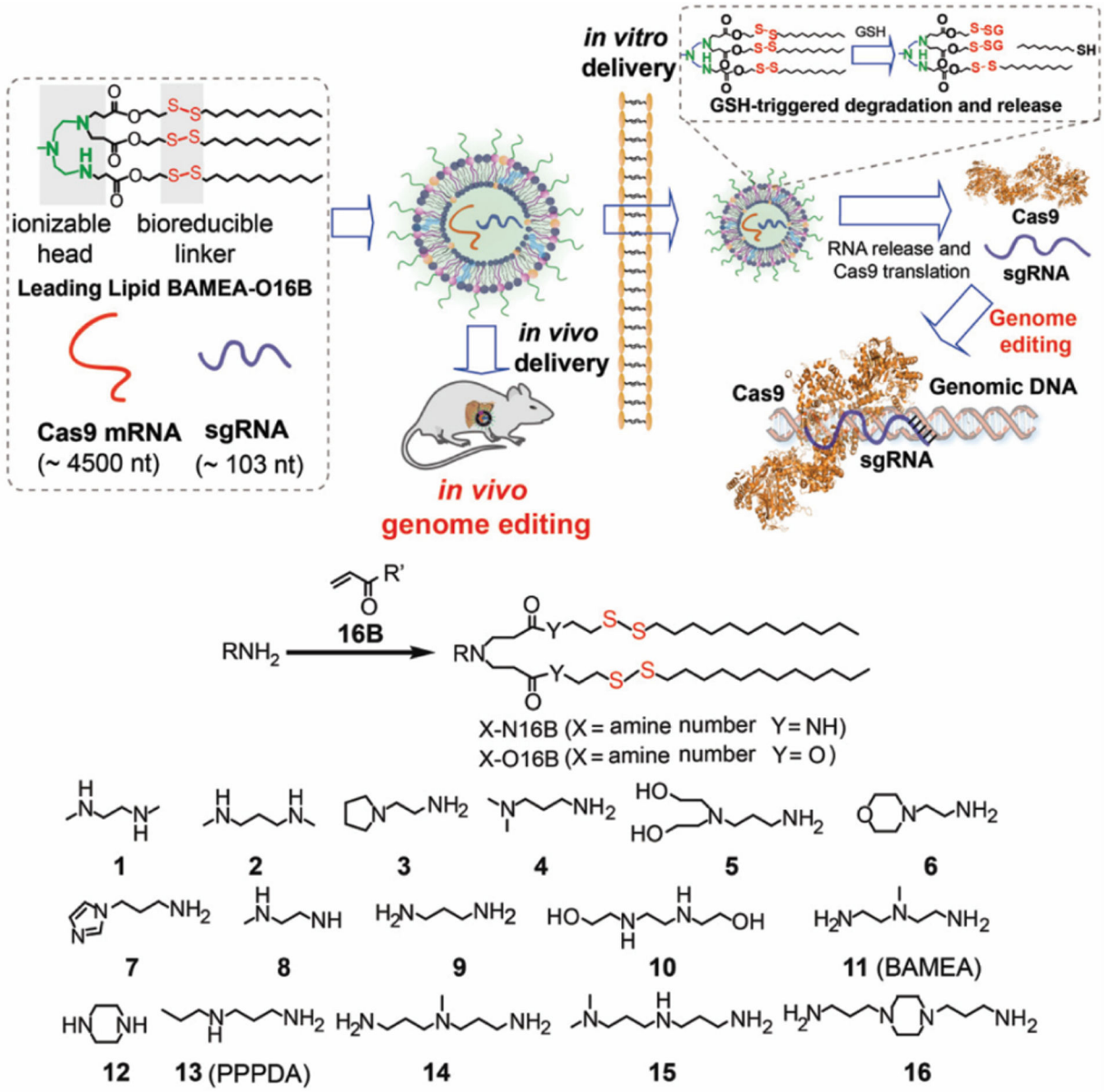


Figure 10.

Molecular designs of bioreducible lipids for Cas9 mRNA/sgRNA delivery in vitro and in vivo and various amine functionalities incorporated into the lipid structure. Reprinted with permission from reference 151. Copyright 2019 WILEY-VCH Verlag GmbH & Co.

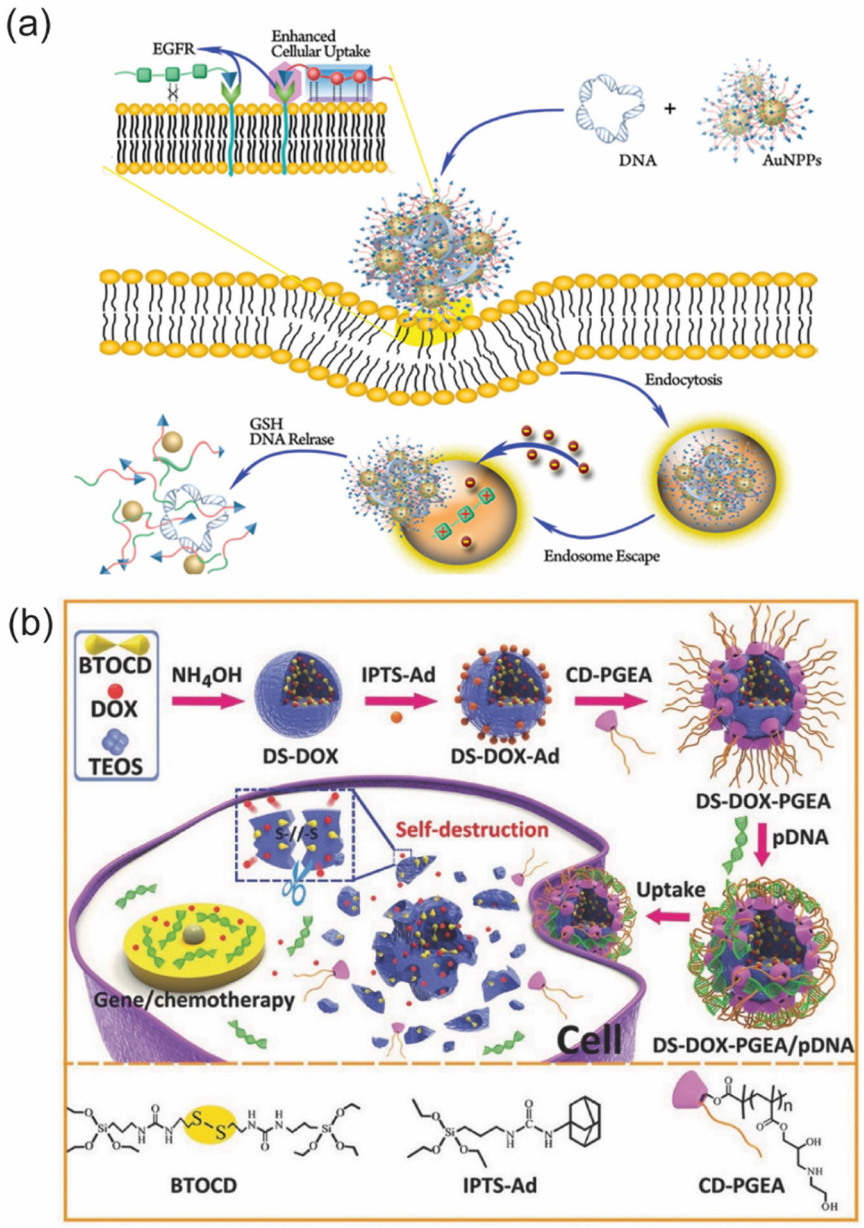


Figure 11.

(a) Polypeptide (cysteine, histidine and arginine) decorated AUNPs for intracellular transport of DNA. Reprinted with permission from reference 177. Copyright 2020 American Chemical Society; (b) Redox-sensitive mesoporous silica nanoparticle mediated co-delivery of drug and nucleic acid. Reprinted with permission from reference 185. Copyright 2019 WILEY-VCH Verlag GmbH & Co.

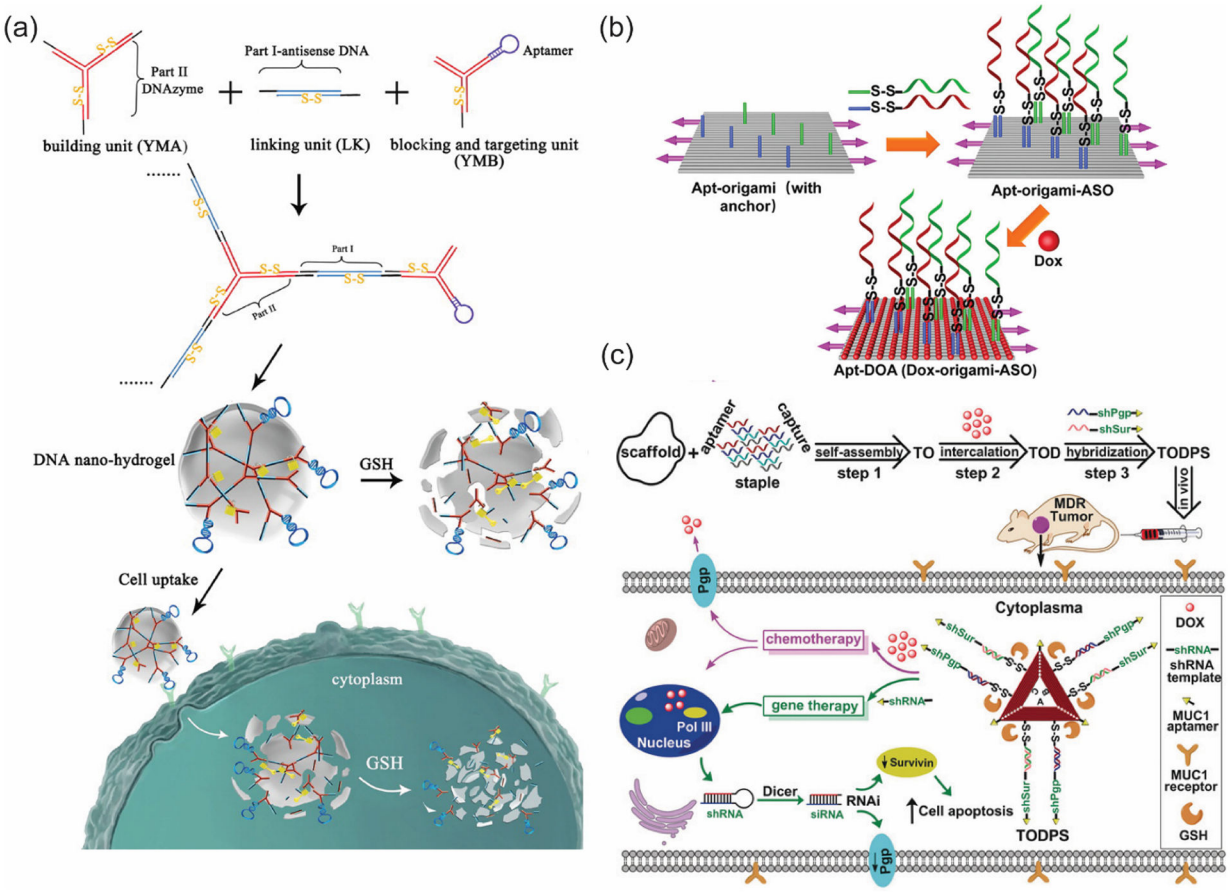


Figure 12.

(a) Redox-sensitive DNA nanohydrogel for targeted modulation of gene expression. Reproduced with permission from ref 202. Copyright 2015 American Chemical Society. (b) Aptamer functionalized disulfide mediated co-delivery of doxorubicin and ASO. Reprinted with permission from reference 204. Copyright 2020 American Chemical Society. (c) Synergistic delivery of chemotherapeutic drug and small hairpin RNA via DNA-origami based nanostructures. Reprinted with permission from reference 205. Copyright 2018 Wiley-VCH Verlag GmbH & Co.