Published in final edited form as:

*Curr Pharm Des*. 2015 ; 21(31): 4566–4573.

# siRNA Delivery by Stimuli-Sensitive Nanocarriers

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## Abstract

Since its discovery in late 1990s, small interfering RNA (siRNA) has become a significant biopharmaceutical research tool and a powerful option for the treatment of different human diseases based on altered gene-expression. Despite promising data from many pre-clinical studies, concrete hurdles still need to be overcome to bring therapeutic siRNAs in clinic. The design of stimuli-sensitive nanopreparations for gene therapy is a lively area of the current research. Compared to conventional systems for siRNA delivery, this type of platform can respond to local stimuli that are characteristics of the pathological area of interest, allowing the release of nucleic acids at the desired site. Acidic pH, abnormal levels of enzymes, altered redox potential and magnetic field are examples of stimuli exploited in the design of stimuli-sensitive nanoparticles. In this review, we discuss on recent stimuli-sensitive strategies for siRNA delivery and we highlight on the potential of combining multiple stimuli-sensitive strategies in the same nano-platform for a better therapeutic outcome.

### Keywords

Stimuli-sensitivity; nanoparticles; siRNA delivery; PEGylated nano-systems; combined therapy; multifunctional nanoparticles

## 1. INTRODUCTION

The design of nanoparticulate drug delivery systems is currently one of the main challenge for the pharmaceutical research to enhance the effectiveness of active agents for the treatment of different diseases. Nanoparticles (NPs) such as micelles, liposomes, solid lipid NPs, nanotubes, dendrimers, in a variety of matrixes (lipids, polymers, metals), often represent the real solution to overcome several problems associated with conventional and new drugs. Poor aqueous solubility, non-specificity and low stability in the biological fluids

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

are some of them [1-3]. Over the past decades, different “first-generation” therapeutic NP-based products are on the market. Liposomes, the first NPs platform used in the clinical setting, have widely demonstrated a significant improvement of the therapeutic benefit of clinically validated drugs by enhancing drug tolerability and/or efficacy [4]. Now, the research is focused on the design of more sophisticated NPs, aimed at addressing multiple challenges at the same time, such as overcome different physiological barriers and deliver a drug or different drugs simultaneously at a specific target tissue rather than unexpected sites. An ideal “multifunctional” NP should include four main proprieties: 1) long circulation time; 2) ability to incorporate and/or to co-incorporate sufficient amount of active agents; 3) a targeting moiety which function is to deliver the NPs to a desired targeted tissue or to bind to a particular biological target; 4) release the drugs or “de-shield” a protecting coating moiety in specific sites in response to stimuli [5, 6].

Stimuli-sensitive NPs, which can release the active agents in response to stimuli that are characteristic of the pathological area of interest, are a noteworthy and growing area of the pharmaceutical research. Temperature, abnormal pH, redox conditions, over-expression of certain molecules, such as enzymes, are examples of intrinsic stimuli that can be characteristics of a sought target site. External stimuli, such as magnetic field or irradiation (UV, infrared or visible) are also widely used (Fig. **1**).

In addition, imaging contrast moieties can be included in the NPs to “see and treat” a pathological area [1,7]. The development of a such complex system able to combine all these components in a nanosizer structure needs an accurate design criteria. In late 1975, Ringsdorf H, recognized the potential of polymers as a backbone to combine different moieties including therapeutic agents and targeting moiety [8]. The idea was to link a biocompatible and water soluble backbone to a therapeutic agent and to a targeting moiety via a spacer [8]. Poly(ethylene glycol) (PEG), a flexible and hydrophobic polymer, is one of the gold-standard backbone. The use of a PEG chain in the preparation of NPs offers advantages including long circulation time, a surface easily functionalized with targeting moieties such as monoclonal antibodies, folate and transferring, as well as the possibility of including stimuli-sensitive components in the formulation [6]. Recently, the possibility to introduce a stimuli sensitive moiety between the PEG chain and the nanocarrier has been exploited to overcome the “PEG dilemma”. It has been reported that the presence of a PEG corona on the surface of NPs results in low cellular uptake. Moreover, PEG can hinder the endosomal release of the carrier [9]. Furthermore, introducing stimuli-sensitive segments in the block of polymers, such as PEG, has the dual advantage of having long circulation NPs with environmentally controlled release mechanisms [5, 6].

In this review, we highlight on stimuli-sensitive nanopreparations. In particular, we focus on the recent advances on stimuli-sensitive NPs for gene delivery, where an appropriate delivery strategy is still a great challenge. Both internal stimuli-responsive NPs, including pH, redox, enzyme and external, such as magnetic stimuli will be discussed in the next sections. We will focus on those stimuli-sensitive nanopreparations that have significant therapeutic effect *in vitro* and *in vivo* and the potential of combing multiple stimuli-sensitivity in one “multifunctional” nanopreparation (Fig. **2**).

## 2. siRNA: DELIVERY CHALLENGES AND HURDLES

siRNA represent the “jewels in the crown” of the pharmaceutical research. siRNA inhibit the expression of “un-controllable” genes involved in human diseases which are un-targetable by conventional agents. The potency of siRNAs in knocking down the expression of specific genes has been widely demonstrated *in vivo* for the treatment of several diseases, such as hepatitis B virus (HBV) [10,11], human papilloma virus [12], ovarian cancer [13]. However, since naked siRNA are highly instable in the bloodstream and too large and negatively charged to cross the cellular membranes, to achieve successful gene inhibition, an effective and intact amount of siRNA has to reach the target cells. Then, once in the cells, intracellular barriers such as endosomal, lysosomal and nuclear barrier must be overcome [14]. In recent years, many efforts have been made to develop a valid delivery system able to translate the siRNA into the clinical setting. Physical methods, conjugation methods, viral or non-viral drug delivery systems are some of the proposed approaches. In addition, stimuli-sensitive NPs for gene delivery represent a promising new strategy that provides an ability to hold off the transfection function while the siRNA is in the bloodstream and to be active once at the targeted tissue cells. Abnormalities of pathological area, such as altered redox potential, different pH, up-regulated proteins are examples of stimuli that release siRNA at the desired target [5, 6, 14]. Several strategies used to prepare stimuli-sensitive based nanopreparations for siRNA delivery will be discussed individually below and are summarized in Table **1**.

## 3. pH-RESPONSIVE SYSTEMS

The pH-gradient is one of the most exploited stimulus to design stimuli-sensitive NPs for siRNA delivery in tumors. Solid tumors have an acidic environment caused by increased levels of metabolites, such as $CO_2$ and lactic acid. The extracellular pH in tumors can drop to 6.5 or less, and cancer cells have even more acidic pH in endosomes and lysosomes (pH 4-6). Three main pH responsive components can be used to design a pH-sensitive nanocarrier: protonizable, acid-labile and destabilizing compounds [5, 6, 15].

Poly-histidine, a polycationic peptide rich of imidazol groups, is one of the most effective pH-buffering agent. Histidine-rich polymers, peptides and lipids have been used as efficient carriers for gene delivery for their ability to readily form complexes with siRNA and to enhance cell-specific siRNA delivery [16]. The destabilization of the structure at a specific pH is due to the protonization of the imidazole ring of histidine, a weak base that at a pH below 6 acquire a cationic charge which results in membrane fusion and/or membrane permeation. In addition, the accumulation of histidine residues inside acidic vesicles can induce a “proton sponge” effect conferring endosomolytic property. The “proton sponge” effect refers to the accumulation of weak base in the acidic vesicles (endosome, lysosome) with a significant accumulation of protons, chloride ions and water in the vesicles. As a consequence of the increase of osmolarity, the vesicles swell and release their content in the cytosol. Many examples of different matrixes enriched with poly-histidine are reported in literature. Cell penetrating peptides (CPPs), short cationic peptides consisting of about 5-30 amino acids, have been used to enhance the cellular uptake of nucleic acid [17]. However, the CPPs/siRNA complexes show a low cellular internalization and/or inability to deliver

the siRNA in the cytosol by entrapment of the complex in the endosome. As recently reported by Chu D, *et al*., the modification of the CPPs oligoarginine with histidine groups significantly improved the internalization of siRNA by cells in comparison to the unmodified peptide [18]. Both proton sponge effect and pH-sensitive membrane disruption are the mechanisms underlying the increase of the siRNA delivery.

Another crucial pH-sensitive approach for siRNA delivery is the use of the cationic polymer polyethylenimine (PEI), a universal carrier for gene transfection. PEI contains protonable amines that at acidic pH act as weak acid inducing the “proton sponge” effect. The advantages of use PEI for gene delivery has been widely demonstrated both *in vitro* and *in vivo*. However, the use of high molecular weight PEI with un-doubted transfection proprieties, results in high toxicity that limit their use. To increase the buffering capacity of low molecular weight PEI, poly-histidine and poly-arginine have been coupled to PEI [19]. Arginine- and histidine-enriched PEI resulted in a substantial improvement in gene transfection abilities compared to unmodified PEI and in all the cases in low levels of cytotoxicity. Another strategy to increase the stability of the siRNA/PEI complexes as well as to reduce the carrier-mediated toxicity is to conjugate PEI to biodegradable lipids such as cholesterol [20] or poly-glutamic acids derivates [21].

Recently, the potential for the direct conjugation of dioleoylphosphatidylethanolamine (DOPE), a fusogenic lipid to a low molecular weight PEI (1.8 kDa) has been investigated [22]. Upon endocitosis, the DOPE melts with the endosomal membrane causing its destabilization. The incorporation of the DOPE in cationic liposomal formulations for gene delivery results in an improvement of the endosomal escape [23]. The idea to combine the fusogenic propriety, positive charge and buffering capacity of DOPE and PEI has lead to an improvement of the intracellular delivery of siRNA and lower toxicity compared to only PEI for the treatment of different diseases [22]. Moreover, the conjugate PEI/DOPE in aqueous solution self-assembly in a micellar core-shell structure which results in a better siRNA transfection efficiency [22]. Furthermore, the conjugate PEI/DOPE can be easily upgraded with PEG chain to confer long circulation propriety. Interestingly, in a animal model of multi-drug resistant breast cancer, the injection of PEG-DOPE-PEI containing a siRNA direct against P-glycoprotein (anti-P-pg) achieved a significant accumulation of the complex in tumor tissues via EPR effect followed by a significant silencing of the mRNA levels of P-gp in the treated tumors [24].

In line with this concept, a pH-sensitive polymer of PEG-grafted carboxymethyl chitosan has been synthesized to cover siRNA containing calcium phosphate NPs (CaP NPs) [25]. CaP NPs have been widely used for gene delivery [26, 27] because of their ability to highly condense nucleic acids together with advantages such as biodegradability [28, 29], low cost and non-toxic degradation products [30]. Moreover, the rapid dissolution of CaP particles in acidic endosomal or lysosomal vesicles leads to endosomal escape and release of siRNA in the cytoplasm [31, 32]. However, their application *in vivo* is limited by the formation of large agglomerates after preparation which can results in a destabilization of the intracellular calcium homeostasis with cell death [33]. Xie Y *et al*. showed the potential of combining a PEG derivative carboxymethyl chitosan (CMCS), a pH sensitive derivative chitosan, with CaP NPs [34]. Beside the stabilization of the CaP NPs, the carboxylic groups of CMCS can

be protonated at the acidic endosomes leading to dissociation of the carboxymethyl chitosan with the CaP. In addition, the reactive amine groups of the CMCS are suitable for PEGylation. The developed NPs showed an efficient delivery of siRNA into tumor cells, with a significant gene knockdown efficacy in cancer cells. In a HepG2 xenograft tumor model, the systemic administration of PEG-CMCS/CaP hybrid lead to a significant inhibition of the tumor growth by silencing hTERT gene in a non-toxic manner [34].

Another strategy is the introduction of an acid labile moiety between PEG chain and the nanocarrier to facilitate acidic pH-cause release of nucleic acids from endosomes. Carmona S *et al.*, proposed liposomes composed of DOPE and an aminoxy cholesteryl lipid, which allows the attachment of biocompatible polymers, such as PEG, on the surface of the NPs [35].The pH-sensitive moiety is an oxime bond, which is stable at physiological pH, but hydrolyzable at pH 5.5 and lower. When in the endosome, the detachment of PEG leads to a destabilization of the NPs followed by endosomal escape. The use of the proposed system for the delivery of a siRNA targeting HBV resulted in a significant suppression of viral replication in mouse hepatocytes [35]. In another study, a PEG-bpolycation block copolymer was electrostatically coated to the surface of cationic liposome to confer longevity and stability when in the bloodstream [36]. Once in the acidic endosomal vescicles, the PEG-b-polycation polymer was detached from the liposome surface, allowing the cationic liposomes to fuse with the anionic endosomal membrane and release the siRNA in the cytoplasm. When compared to Oligofectamine complexed siRNA and free siRNA, PEG-b-polycation coated liposomes showed a remarkable silencing activity which may be linked to their ability to evade the immune system and alter the biodistribution [36].

## 4. REDOX-SENSITIVE SYSTEMS

The marked difference in the redox potential between normal tissues and tumor tissues is another environmental trigger exploited to design tumor targeted NPs. Glutathione (GSH) is an ubiquitous small molecule involved in important cellular pathways such as in the maintenance of intracellular redox state [37]. In humans, extracellular GSH concentrations are estimated to be between 2 and 4 μM, while the intracellular concentrations are up to 10 mM [38]. Interestingly, in tumor tissues, the redox potential increases significantly with intracellular concentrations of reductive GSH 100-fold higher than the extracellular ones [7]. From here, the rational to design reduction-sensitive systems for tumor targeting as well as for intracellular delivery of payloads. Different class of cationic polymers and lipids containing disulfide linkages have been designed to prepare redox-triggered complexes with siRNA [39, 40]. The introduction of a disulfide linkage in the complex is aimed at increasing siRNA transfection efficiency for improved release of siRNA from the complexes and decreased toxicity for preferential release of the siRNA in cells with high levels of GSH, such as cancer cells. Recently, a PEG shielded poly(L-lysine) (PLL) and PEI has been synthesized for siRNA delivery introducing disulfide linkages to graft PEG (MW 2000) and PLL to a low molecular weight PEI [41]. The designed copolymer, has been further functionalized with a single chain of the monoclonal antibody Herceptin to increase the transfection activity of the copolymer/siRNA nanocomplex in ovarian cancer cell line. As result, the new biodegradable copolymer efficiently complexed siRNA and, the conjugation

with the targeting ligand Herceptin leaded to a superior therapeutic activity both *in vitro* and *in vivo*.

Musacchio T *et al.*, reported the potential of the direct conjugation of an anti-GFP siRNA to a phospholipid (PE) moiety via a disulfide bond [42]. The PE moiety allows the incorporation of the modified siRNA into a non-toxic delivery system, such as polyethylene glycol2000-phosphatidylethanolamine (PEG2000-PE)-based mixed micelles, where siRNA is protected from the nuclease degradation. *In vitro*, the mixed micelles were able to efficiently release the siRNA in endothelial cells leading to 50-fold more of GFP silencing compared to free siRNA [42].

## 5. ENZYME-RESPONSIVE SYSTEMS

The altered levels of certain local enzymes in cancer tissues, such as matrix metallo proteinases (MMPs), human leukocyte elastase (HLE) and cancer-associated proteases (CAPs), have been the rationale to develop enzyme-triggered NPs for drug delivery [43]. Over-expressed levels of MMP2 and HLE are found in the tumor microenvironment, where they promote invasion, progression and metastasis of most human tumors by degrading the intercellular collagen matrix and extracellular matrix barrier, respectively [44-47]. Recently, Yingyuad P *et al.*, have successfully synthesized enzyme-triggered PEGylated siRNA NPs [48]. Sensitive amino acid sequences of MMP2 and HLE were introduced in PEG-lipid derivatives (MW 2000) and used to prepare PEGylated siRNA NPs. The idea is to “de-shield” the protective PEG function once in the tumor microenvironment, where the over-expressed levels of the proteolytic enzymes made the cleavage of the enzyme-sensitive linkage. After cleavage, the presence of residues of peptides on the surface of the siRNA NPs could promote cellular internalization by their N-terminal positive charges as well as allow proteins to coat the NPs surface increasing the transfection efficiency.

In line with this concept, we have proposed a multifunctional micellar platform based on a new self-assembly MMP2-sensitive copolymer for the co-delivery of siRNA and hydrophobic drugs, such as Paclitaxel [49]. The block copolymer is made of a long PEG chain, PEI and DOPE and contains a MMP2 sensitive peptide sequence between PEG and PEI. The advantage of the proposed system is to have in one NP several features including the possibility to efficiently co-incorporate different drugs, excellent physical characteristics and passive tumor targeting via the EPR effect. Besides those, the main novelty is to release the siRNA preferentially in tumors where up-regulated tumor levels of MMP2 triggered the de-shield of PEG and the exposure of the previously hidden PEI. All together leads to a significant improvement of the tumor targeting, tumor cell internalization, and synergistic effect of siRNA and Paclitaxel in a xenograft mouse model of lung cancer [49].

## 6. MAGNETIC-SENSITIVE SYSTEMS

Magnetic resonance imaging (MRI) techniques represent a great tool for monitoring the distribution and the therapeutic outcome of NP-based drug delivery systems [50]. Moreover, MRI-visible NPs have been used to deliver drugs at a specific site by exploiting an external magnetic field [51, 52]. Drug delivery systems can be magnetized either by incorporation of magnetite inside the NPs or by direct modification of biocompatible polymer used to prepare

NPs. Superparamagnetic iron oxide NPs (SPIONs), one of the most efficient T2 contrast agent for MRI, consist of cores made of iron oxides that can be targeted to a desired area through external magnetic stimuli. SPIONs are widely used in different biomedical applications either for their nanoscale size or for the absence of magnetic interaction when the external magnetic field is removed [53]. SPIONs are still undergoing optimization to improve their stability, biocompatibility and overcome non-specific distribution of the encapsulated drugs associated with side effects.

A valid approach is to modify the surface of SPIONs with biocompatible compounds, such as PEG, glutamic [54], dextran [55] and/or with active targeting moieties, such as folate for tumor targeting [56]. In addition, various stimuli-sensitive moieties have been used to link targeting moieties and/or therapeutic agents to the SPIONs for stimuli-triggered release of the NPs. Lee JH *et al.* designed PEGylated SPIONs functionalized with αvβ3-integrin-specific RGD (Arg-Gly-Asp) peptides, Cy5 fluorescent dye, and siRNA. The siRNA was anchored to the SPIONs via a disulfide linkage resulting in an efficient delivery of the siRNA in the cytoplasm and a significant gene silencing effect. Moreover, they showed a specific binding of the RGD-conjugated SPIONs to the αvβ3 integrin-positive cells, confirmed by both MR and near-infrared fluorescent imaging [57].

In another study, a block copolymer of PEG-g-PEI-SPION was synthesized and further functionalized with a neuroblastoma cell-specific ligand (scAbGD2) for the delivery of a Bcl-2 siRNA into tumors. In human neuroblastoma model, the functionalized PEG-g-PEI-SPIONs were more effective in delivering Bcl-2 siRNA than the non-targeting one with a significant suppression of the tumor growth [58]. A similar system containing RGD peptides was developed to deliver a siRNA direct against survivin specifically to Bel-7402 cells, human hepatocellular carcinoma cells. The RGD-modified PEG-g-PEI-SPION/siRNA compared to the non-targeted formulation, showed a 3-fold lower expression of survivin mRNA levels in Bel-7402 cells and a 4-fold decrease of the tumor volume in a Bel-7402 hepatoma animal model. Site-specific distribution was confirmed by a 47.3% of reduction in MR signal intensity observed in tumor tissues [59].

## 7. “MULTIFUNCTIONAL” STIMULI-SENSITIVE SYSTEMS

The use of combined therapy in the treatment of diseases, such as cancer, represent, one of the solution to achieve a better clinic outcome. “Multifunctional” NPs able to simultaneously incorporate different drugs, such as siRNA and conventional drugs, have been reported in many studies [60, 61]. As a next step, the upgrade of multifunctional NPs with two or more of the stimuli discussed above, could leads to a microenvironment-specific release of the active agents improving their therapeutic efficacy as well as reducing the non specificity-based side effects. For example, a ternary block copolymer, PEG-PAsp(AED)-PDPA, consisting of a combination of a pH-sensitive, poly(2-(diisopropyl amino) ethyl methacrylate) (PDPA) and a reduction-sensitive moiety, poly(N-(2,2'-dithiobis(ethylamine) aspartamide PAsp(AED)), shielded by a PEG chain has been synthesized for the co-delivery of Doxorubicin (DOX) and Bcl-2 siRNA in human ovarian cancer cells, SKOV-3 [62]. In aqueous solution, the dual stimuli-sensitive system self-assembles into a three-layered micellar structure where DOX is in the pH-sensitive core and Bcl-2 siRNA in the reduction-

sensitive interlayer. Once inside the cancer cells, the high concentrations of GSH efficiently cleaved the reduction-sensitive PAsp(AED) removing the protective PEG shell with consequent release of siRNA into the cytoplasm. At the same time, acidic lysosomes triggered the dissociation of the pH-sensitive PDPA with the consequent release of DOX. The simultaneous delivery of siRNA and DOX by using the designed dual sensitive micelles achieved a superior inhibition of the tumor growth and a significant increase of the survival rate compared to the treatment with the single agents in an animal model of SKOV3. Moreover, the treatment with PEG-PAsp(AED)-PDPA containing both agents induced a significant down-regulation of the expression of the anti-apoptotic Bcl-2 protein with consequent sensitization of the cancer cells to the chemotherapeutic agent, DOX.

In another study, the combination of reduction-sensitivity and SPIONs has been studied for siRNA [63]. A double layer NP containing an internal SPIO core and a disulfide-containing polyethylenimine (SSPEI) external layer, has been designed for reducible-triggered siRNA release in HepG2 cells (hepatocellular carcinoma cells). In *in vitro*, the theranostic NPs were able to efficiently complex the siRNA and to facilitated its release in the presence of 5-20 mM of dithiothreitol. In HepG2 cells, a significant knocking down of the hTERT protein expression was observed. Compared to other formulations made of SPIONs-PEI [64, 65] the proposed bioreducible formulation presents a relative low cytotoxicity also at high dose and a the ability to mediate intracellular gene release. From MR imaging study performed *in vivo*, the injection of SSPEISPIONs in mice bearing a HepG2 tumor, achieved a significant decreased of T2-weighted signal in tumor site, indicating the tumor accumulation of SSPEI-SPION confirming their applicability as theranostic NPs for gene delivery.

## CONCLUSION

The design of a nanoparticulate system able to efficiently incorporate siRNA and deliver it safe only in the cytoplasm of desired cells is still a great challenge of the pharmaceutical research. Stimuli-responsive nanopreparations, compared to conventional drug delivery systems, present superior ability to efficiently control the release and enhance the internalization of genes in the pathological area of interest. Despite the progress of stimuli-responsive nanopreparations, there are still some issues to resolve. Low target specificity and insufficient response to the local stimuli are some of them [66, 67]. Therefore, an accurate design and a better characterization of the stimuli-sensitive polymer/lipid conjugates together with a detailed evaluation of the intensity, distribution and activity of the chosen internal and external stimulus are needed. The combination of multiple stimuli-sensitive moieties as well as other strategies, such as the functionalization of the NPs with specific ligands could be a way to enhance the targetability and the efficacy of the nanopreparations. In addition to these features, the final product should be easy to prepare, cheap and stable under normal storage conditions, binging stimuli-responsive nanopreparations a step closer to clinics.

## ACKNOWLEDGEMENTS

This paper was supported by the NIH Grant (5U54CA151881) to V. Torchilin and CAPES Foundation Scholarship (BEX13275/13-5) to Daniel F. Costa. The authors thank Dr. William Hartner for helpful comments in the manuscrip preparation.

## Biography


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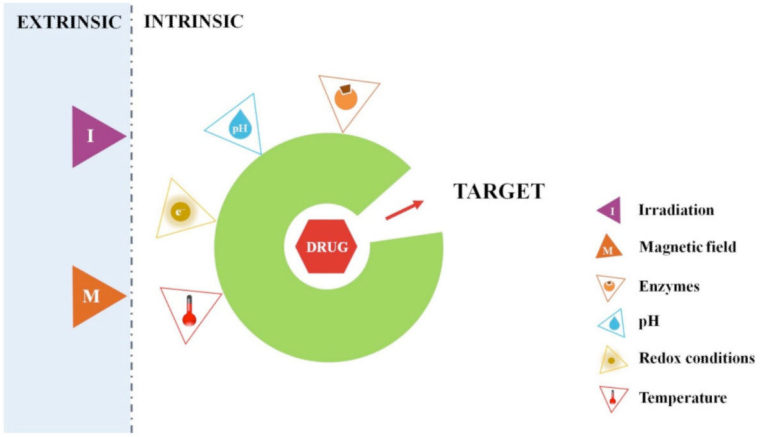


**Fig. (1).**
Schematic representation of extrinsic and intrinsic stimuli exploited to prepare stimuli-sensitive NPs.

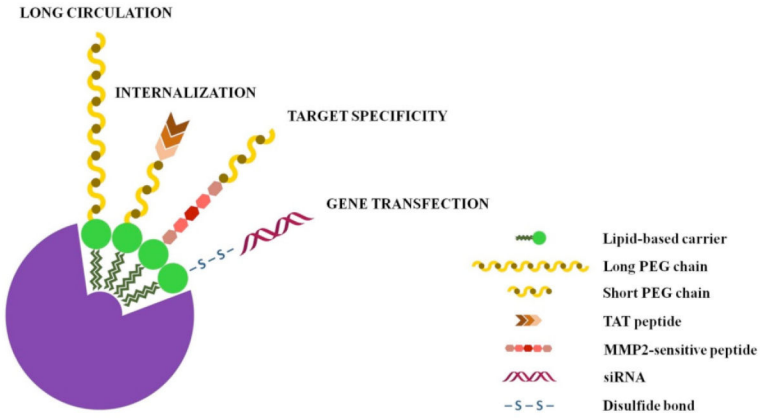


**Fig. (2).**
Combination of multiple stimuli-sensitive moieties and other strategies for siRNA delivery.

## Table 1

Stimuli-sensitive nanopreparations for siRNA delivery.

| Stimulus | Stimuli-sensitive system | Outcome | Refs. |
|---|---|---|---|
| **pH** | • Oligoarginine modified with<br>• oligohistidine and stearyl moieties | Improvement of siRNA transfection | [18] |
| | • Arginine-rich PEI derivative | Decrease of cytotoxicity<br>Improvement in siRNA transfection | [19] |
| | • DOPE–PEI | Decrease of cytotoxicity<br>Improvement in siRNA transfection | [22] |
| | • PEG-DOPE-PEI | Tumor target delivery<br>Enhanced gene silencing | [24] |
| | • Calcium phosphate PEG-CMCS hybrid | Tumor target delivery<br>Enhanced gene silencing | [25] |
| | • Calcium phosphate PEG-CMCS hybrid | Tumor target delivery<br>Enhanced gene silencing | [34] |
| | • PEGylated liposome | Suppression of viral replication | [35] |
| | • PEG-b-polycation coated liposome | Enhanced gene silencing | [36] |
| **Redox potential** | • Arginine-conjugated poly(CBA-DAH-R) | Enhanced gene silencing | [39] |
| | • Disulfide-based poly(amido amine) | Enhanced gene silencing | [40] |
| | • Ternary copolymer<br>• mPEG-b-PLL-g-(ss-lPEI) | Enhanced gene silencing | [41] |
| | • siRNA-S-S-PE/PEG-PE | Decrease of cytotoxicity<br>Enhanced gene silencing | [42] |
| **Enzyme activity** | • MMP2-sensitive PEG-lipid derivative | Enhanced gene silencing | [48] |
| | • MMP2-sensitive PEG-PEI-DOPE | Tumor target delivery<br>Enhanced gene silencing<br>Synergistic effect (siRNA/drug) | [49] |
| | • sPLA2-sensitive lipoplex | Improvement of siRNA transfection | [68] |
| **Magnetic field** | • PEG-RGD-SPION | Tumor target delivery<br>Enhanced gene silencing | [57] |
| | • PEG-g-PEI-SPION | Improvement of siRNA transfection | [58] |
| | • RGD-PEG-g-PEI-SPION | Tumor target delivery<br>Enhanced gene silencing | [59] |
| **Combined stimuli** | | | |
| **pH/enzyme** | • PEG-PAsp(AED)-PDPA | Tumor target delivery<br>Enhanced gene silencing<br>Synergistic effect (siRNA/drug) | [62] |
| **pH/magnetic** | • SSPEI-SPION | Tumor target delivery<br>Enhanced gene silencing | [63] |
| **pH/magnetic** | • pArg-PEG-SPION | Decrease of cytotoxicity<br>Enhanced gene silencing | [65] |